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Letters

SIGNIFICANCE OF BREAK IN ARRHENIUS PLOT OF RICE PARBOILING KINETICS

Bakshi and Singh (1980) have shown that rice parboiling can be simulated by a mathematical model describing simultaneous water diffusion and gelatinization of starch. They have done a commendable work by calculating from the model the diffusion coefficients and reaction rate constants for soaking of both rough rice and brown rice. We have, however, some comments on what they have stated about our work and also on the interpretation of their result of parboiling kinetics.

We first observed that there is a prominent break in the plot of an Arrehenius-type relationship for the soaking kinetics by analyzing the data of earlier investigators (Bandyopadhyay and Roy, 1975) and later confirmed the break in our work (Bandyopadhyay and Roy, 1976, 1978). Bakshi and Singh (1980), while discussing their results, did not mention that a similar break was observed in our work, and probably overlooked our latest publication in this field (1978), in which a unified approach on parboiling kinetics based on analysis of a large number of data of different workers was reported.

Since we evaluated an overall kinetic parameter, which incorporated both the diffusivity and intrinsic rate constant terms, it was possible only to obtain an apparent activation energy from the Arrhenius-type relationship. The range of values of apparent activation energy reported by us is approximately 23000–32000 cal/mol in the higher temperature region of 60–80°C and approximately 2600–7800 cal/mol in the lower temperature region of 30–60°C (Bandyopadhyay and Roy, 1978). The break points in the Arrhenius plot or the transition temperatures obtained with a large number of rice varieties fall within 60–69°C (Bandyopadhyay and Roy, 1978). It may be noted that the temperature range in which higher activation energy was obtained by us (1976) should be 62–80°C and not 70–85°C as reported by Bakshi and Singh (1980).

We have also concluded that the temperature range 60–69°C, where transition takes place, is in the vicinity of the gelatinization temperature range of rice varieties, reported as 58–80°C by the U.S. Department of Agriculture (1965). The break obtained by Bakshi and Singh (1980) at about 85°C did not correspond to our observation. Subsequently the values of activation energy obtained by them *were not* within the range reported by us as above, and therefore, the claim made by them in this regard becomes invalid.

On the basis of break in the Arrhenius plot we have first propounded a hypothesis on the mechanism of soaking rough rice (Bandyopadhyay and Roy, 1976). The break indicates a transition to predominance of chemical reaction at a higher temperature and a predominance of simple diffusion at a lower temperature. The conclusion drawn by Bakshi and Singh (1980) on the break in the Arrhenius plot appears to be similar, but differs in the temperature of break. Since this temperature plays a prominent role in parboiling kinetics by indicating the prevalence of two different mechanisms during soaking, a uniformity in the results of Bakshi and Singh (1980) and ours would have a better significance in parboiling kinetics.

We are of the opinion that the interpretation of the values of activation energy by Bakshi and Singh (1980), in the light of Satterfield's comment, is incorrect. According to Satterfield (1970), in a diffusion limited reaction the apparent activation energy drops to a value of one-half that of the intrinsic activation energy. The apparent activation energy is calculated from the Arrhenius plot of overall rate or rate constant, and it means the arithemetic average of the activation energies for the diffusional process and for the intrinsic chemical reaction, provided the reaction is simple and the intrinsic kinetics can be expressed by a power-law relationship (Satterfield, 1970). Bakshi and Singh (1980) did not consider an overall kinetic constant which incorporates both the diffusivity and chemical rate constant; instead they have evaluated the true activation energies for the moisture diffusion and gelatinization reaction separately. That is why their argument is not tenable here. Consequently it may be noted that the break in the Arrhenius plots of both moisture diffusion and reaction rate constant has not been adequately explained by them. Since the autors have plotted the Arrhenius relationships of true moisture diffusivity, we think, there should not have been any break in the plot, indicating two different mechanisms. The same argument may be put forward in the case of Arrhenius plot for intrinsic reaction rate constant, provided there are not two different chemical mechanisms operating above and below 85°C. In this connection, we suspect, since gelatinization is either instantaneous or nearly complete at a temperature of about 85°C (Bakshi and Singh, 1980), cooking of parboiled rice might have started at that temperature, and, therefore, cooking reactions such as depolymerization of starch into lower saccharides might have been responsible for a drop in activation energy. This reasoning is corroborated by Suzuki et al. (1977) who observed that the equivalent activation energy of the cooking reaction rate in the temperature region of 70–98.5°C was about 20,000 cal/mol. This value is less than that obtained by us in the temperature region of 60–80°C. Their results showed that the cooking reaction rate was mainly limited by the reaction of rice components with water at temperatures 70–98.5°C but also relatively influenced by the diffusion rate of water in the cooked rice layer at 98.5°C. Therefore, in the light of our argument and the observation of Suzuki et al. (1977), the conclusion drawn by Bakshi and Singh (1980) that diffusion of water in rice is the limiting factor for parboiling at temperatures above 85°C appears to be doubtful.

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. . . and the reply

Bandyopadhyay and Roy (1976) used Fick's law of diffusion to model the parboiling process. This model considers only diffusion and neglects the reaction of water with starch. The diffusion model was proposed by Becker (1959) to model wheat drying. Drying can be modeled as a diffusion process; however, drying per se does not involve chemical reaction. Thus, the diffusion model alone is inadequate to describe the parboiling process which involves both simultaneous diffusion and reaction. The kinetic parameters calculated by Bandyopadhyay and Roy when plotted against the inverse of

absolute temperature showed a break in the line at 68°C for Pankaj variety, 75°C for Sitasal, Thingasal and Rupsal varieties, and no break for Patnai and Aus varieties in the temperature range 65–85°C (Bandyopadhyay and Roy, 1975). As the rice, of Patnai and Aus varieties was not soaked at a temperature above 85°C, the soaking characteristics of these rice varieties above this temperature are not known. From the above data and our work that showed a break in the line at 85°C for a California grown *short grain* variety (S6), it can be concluded that soaking characteristics of rice are highly dependent upon the variety. Thus, a break in the Arrhenius plot for rice soaking may be observed at any temperature between 60–85°C depending upon the variety under consideration.

The break in the Arrhenius plot of reaction rate constant and diffusion coefficent can be explained as follows: There should not be any break in the Arrhenius plot for reaction rate constant if the reaction is a simple reaction. This is not true in the case of soaking of rice. One of the reactions involves absorption of reducing sugars from the bran layer and embryo during initial soaking, followed by reaction with amino acids to produce browning (Roberts et al., 1954). The absorption of reducing sugars is a function of time of soaking, temperature of soaking and moisture concentration. Another reaction which occurs during soaking is gelatinization, which itself involves three phases (Kerr, 1950). During the first phase water is slowly and reversibly taken up, and limited swelling occurs. The starch granules retain its characteristic appearance. In the third phase of swelling, the granules become almost formless sacs, swollen granule sacs become rigid gel on cooling which results in imparting hardness to the rice grain. Thus soaking of rice is not a simple reaction and a break in the Arrhenius plot for reaction rate constant was observed in our study. Suzuki et al. (1976) observed a similar break in the plot of cooking rate constant for rice at 110°C. The diffusion coefficient is a function of moisture concentration. However, in our study we considered it to be independent of concentration. The calculated value of diffusion coefficient can be viewed as apparent diffusitivity and not true diffusitivity as suggested by Bandyopadhyay and Roy in the above letter. As proposed by Kruckels (1973) at the beginning of the process, the moisture gradients inside the particles are high, the apparent or effective diffusitivity is low; in contrast, as the process advances, the gradients become low and apparent diffusitivity is high. Thus, again during rice soaking, the diffusion coefficient is not true diffusitivity but apparent diffusitivity, which is a function of moisture concentration in addition to temperature. It is also quite possible that the diffusitivity of gelatinized starch is different from the nongelatinized starch in a rice kernel. Depending on the extent of gelatinization in rice, we therefore expect a break in the Arrhenius plot for diffusion coefficient.

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MOISTURE MIGRATION IN FROZEN RAW BREADED SHRIMP

I read with interest the article on moisture loss in the frozen shrimp product [Moisture Migration in Frozen Raw Breaded Shrimp During 9 Months' Storage by S.K. Williams, R. Martin, W.L. Brown, and J.N. Bacus. J. Food Sci. 46(5): 1577-1581 (1981)] since very few studies of long duration have been done. The data were quite interesting but I was disappointed that the authors did not apply simple physical chemistry and engineering principles to evaluate and explain the data more fully.

Moisture loss from the breaded shrimp can occur in two directions. Firstly, there is moisture migration through the package (specified only as wax cartons). This follows the simple formula [see *Cereal Foods World,* 26(7): 335 (1981)] of:

$$\frac{\Delta W}{\Delta \theta} = \left(\frac{k}{x}\right) A\,(P_f - P_R) = \frac{k}{x} A\,P_f \left[1 - \frac{\%RH}{100}\right]$$

where $\Delta W/\Delta\theta$ = grams water lost per day; (k/x) = film permeability to water; A = area of box; P_f = vapor pressure of water in food; P_R = vapor pressure of water in freezer; %RH = relative humidity in the freezer.

Thus, the moisture loss depends on the type of package used. We (above reference) have published some data to show that film permeability rises as freezer temperature decreases. Thus, the box at the lower temperature used (warehouse freezer at −24.4°C) should have a higher permeability than at retail storage of −26.6°C. Secondly, the equation predicts that the loss depends on the area. If boxes are stacked the loss rate will go down. Thirdly, the equation says that the rate of loss depends on the vapor pressure difference between the food and the air in the freezer. The vapor pressure of a frozen food is solely determined by the temperature (see Fennema, 0., 1981. Water activity at subfreezing temperatures. In *"Water Activity Influences on Food Quality,"* Ed. L. Rockland. Academic Press, New York). Thus, at −24.4°C the vapor pressure of the food is 0.505 mm Hg and at −26.6°C it is 0.405 or 20% lower. Thus the retail pack should lose less if the freezer humidities are the same. No data are given for this value; however, Fig. 3 in the paper suggests that the retail freezer had a lower humidity than the warehouse freezer since it lost more weight. In fact, it would have to be lower by more than the 20% unless the difference is due to film permeability (small effect) or the cumulative effect of the higher defrost time/temperature sequence. The difference in freezing method should not affect the rate of moisture loss as is evident in Fig. 4, especially at warehouse conditions. Error could account for some of the differences since it is not mentioned in the methods how the authors prevented or accounted for any condensation taking place on the shrimp or packaging. The data in the tables suggest that this was a problem since it shows weight increases for some samples.

The second method of moisture migration is from the shrimp itself to the breading. This will occur because of

the difference in vapor pressure (or water activity) between the two, not because of a difference in moisture content as stated in the article. The a_w of any frozen material is solely determined by the frozen temperature if the product has ice in it (see Fennema article cited above). Thus at any freezer temperature, the breading has an a_w (at room temperature) below the theoretical (and actual) a_w for the frozen temperature, thus, water will migrate from the shrimp (it has an a_w of about 1 at room temperature and thus will form ice slightly below 0°C) to the breading. It is too bad the authors were not able to measure the a_w of the breading to confirm this. However, the data in Fig. 7 suggest that it was low enough to not freeze since the breading increases in moisture to a maximum which is the point where it gains enough water to freeze and achieves an a_w the same as the shrimp. Thereafter (in three cases) it decreases as the total breaded shrimp product loses to the atmosphere.

What is not understandable and not explained is the increase in % shrimp after 6–8 months storage. Of course since it is presented as a %, the shrimp may not be actually gaining moisture, but if the breading is losing moisture more rapidly to the outside air at the same time the shrimp is losing moisture to the breading, the % shrimp could go up. Of course this is important in light of regulations for a standard of identity for frozen breaded shrimp which requires more than 50% shrimp (21 CFR 161.175(b), not Sec 36.3(b) 21 CFR which was changed to the present number almost 4 yr ago). It is also interesting to note that the authors used the method for % shrimp promulgated by the National Marine Fisheries Service 50 CFR 262.21(s) rather than the required FDA method listed in 21 CFR 161.175(g). I am not sure both methods are the same.

I hope that this letter will clarify some of the interesting results in the authors' paper and shows how a little physical chemistry can explain things better.

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. . . and the reply

We appreciate that Dr. Labuza offered additional discussion and suggestions. We agree that the formula presented in his article in *Cereal Foods World* [26(7): 335 (1981)] would have been very beneficial in aiding in the discussion of moisture migration.

The fact that the methodology outlined in 50 CFR, 262.21 u (not 262.21 s as quoted by Dr. Labuza) was used to determine percent shrimp does not in any way change the results presented in the article as Dr. Labuza would lead one to believe. The precedures outlined in 50 CFR 262.21 u are the same as those outlined in 21 CFR 161.175 g.

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ERRATA NOTICE

• *J. Food Sci.* 46(6): 1635–1638. Atlantic mackerel (*Scomber scombrus,* L.): Seasonal variation in proximate composition and distribution of chemical nutrients by S-S. Leu, S.N. Jhaveri, P.A. Karakoltsidis and S.M. Constantinides. On page 1638, paragraph 3: change line 7 to read . . . 10.13 μg/100g and 2.46 μg/100g, respectively; change line 9 to read . . . 0.1–0.2 μg/g; change line 14 to read: iron levels averaged 79.51 μg/100g and 1.20 μg/100g, respectively.

• *J. Food Sci.* 46(6): 1874–1877 + 1880. Comparison of microwave and conventional baking of potatoes in relation to nitrogenous constituents and mineral composition by L.B. Klein and N.I. Mondy. On page 1880, delete the reference, Klein et al. (1980) and substitute the following reference: Klein, L.B., Chandra, S. and Mondy, N.I. 1980. The effect of phosphorus fertilization on the chemical quality of Katahdin potatoes. Amer. Potato J. 57: 259.

Influence of Cooking Method on Digestibility of Legume and Cereal Starches

S. E. FLEMING

ABSTRACT

The influence of processing on starch nutrition was evaluated. Cereal starches including wheat, normal corn and high amylose corn; legume starches including field pea, kidney bean and mung bean; and potato starch and glucose were evaluated as carbohydrate sources. Starches were fed raw, or cooked in a pressure kettle or autoclave, dried in a spray drier or freeze-drier, or simultaneously cooked and dried on a drum drier or by extrusion cooking. Except for potato and high amylose corn, cooking the starches provided no nutritional advantage, as evaluated by weight gain, feed efficiency ratio, or apparent protein digestibility. Spray-dried starches were less dense and appeared to influence growth by restricting food consumption.

INTRODUCTION

THE COOKING of starches has been generally considered to improve carbohydrate, protein and total dry matter digestibility of diets since the gelatinization process allows for more rapid attack of the starch granules by digestive enzymes. However, in most cases it is difficult to distinguish the effect cooking had on the starch from the corresponding effects on other dietary components since, in most cases, the starches have not been treated alone, but have rather been treated within a complex seed meal.

Several legume starches and wheat starch were previously shown to stimulate higher feed intakes when fed raw rather than cooked (Fleming and Vose, 1979). Additionally, the cooked starches showed the digestibility of casein protien to be lower. This is contrary to the popularly held belief that cooking renders starch more digestible. In particular, workers have reported accelerated in vivo hydrolysis following gelatinization by both extrusion cooking (Delort-Laval and Mercier, 1976) and autoclaving (Kumar and Venkataraman, 1976). However, Kayisa and Hood (1979) reported that the drying process following cooking reduced the susceptibility of cereal starches to enzyme hydrolysis.

From these results it appears that gelatinization increases the rate of in vivo starch hydrolysis and that the cooking and drying processes may influence in vitro digestibility of starch. However, it is not clear whether these processes all exert similar influences for all types of starches, or whether the processing always improves digestibility and growth performance. The present study was designed to further explore the influence of processing on starch nutrition. In addition, some efforts were made to explain the observed results.

MATERIALS & METHODS

THE SEEDS of two legumes were obtained from a local supplier and included smooth-seeded field peas (*Pisum sativum* L. var. Trapper) and mung beans (*Vigna radiata*). Starch was prepared from these seeds by a wet-milling process. The seeds were ground in a single disc mill [Bauer Brothers Co., Springfield, IL] to give a slurry which was defibered on a horizontal vibratory screen [Sweco Separator, Southwestern Engineering Co., Los Angeles, CA]. The starch was removed and purified by repeated centrifugation in a basket centrifuge [Fletcher Works Inc., Philadelphia, PA] operated at 1600 x *g*. A final purification was effected by use of a counter-current water-wash utilizing a block of 10 nylon, 1 x 10 cm liquid cyclone units [Dorr-Oliver Inc., Stanford, CN]. The starches (2.5–5.0% solids) were dried in a direct gas fired spray drier [Proctor-Schwartz Corp., Philadelphia, PA.] at an outlet temperature of 90°C. Wheat, potato and normal corn starch were obtained from a local supplier. High-amylose corn starch was obtained commercially [A.E. Staley, Decatur, IL].

The starches were cooked using several methods. In one case, aqueous slurries of starch (7% w/v) were heated in a stainless steel steam-jacketed kettle. The slurries were continuously stirred during heating at 121°C for 15 min. Starches were cooked in a similar fashion by conventional autoclaving, without agitation, at 121°C for 15 min. The slurries had been previously heated to 95°C with stirring in order to minimize separation and improve granule hydration. In a third cooking method, raw starch slurries were applied to a drum drier operated at a steam pressure of 75 psi. This process effected starch gelatinization as well as drying. In a fourth method, starches were cooked using a thermoplastics extruder [C.W. Brabender Co., Model No. 2003]. The screw was operated at 70 rpm and extrusion was made through a 3/16 inch die. Zones 1, 2 and 3 along the barrel were maintained at 65, 163 and 121°C, respectively. Wheat, field pea and mung bean starches were adjusted to 20–22% moisture, and potato starch was adjusted to 27% moisture, prior to extrusion cooking. The extrusion process effected cooking and drying, simultaneously.

With the exception of the extrusion-cooked starches, the cooked starch slurries were adjusted to 4% solids and dried. Three drying methods included freeze drying, drum drying and spray drying, and the methods were previously described.

The dietary starch components were analyzed using the dual-enzyme method (Banks et al., 1970). Nitrogen contents were determined using a CHN Analyzer and were converted to protein estimations by the calculation of %N x 6.25. Chromic oxide, added to diets to facilitate digestibility determinations, was measured by utilizing perchloric acid digestion (Bolin and Lockhart, 1960).

Diets were formulated to contain 55% starch, 10.5% ANRC casein [Humko Sheffield, Memphis, TN], 5% cellulose [Alphacel, ICN Pharmaceuticals, Inc., Montreal, Quebec], 4.5% minerals (Anon. 1977) [AIN mineral and vitamin mixes 76, ICN Pharmaceutical. Formula compositions are given in reference Anon., 1977], 1.5% vitamins, 0.2% choline bitartrate [ICN Pharmaceutical], 0.3% DL- methionine [ICN Pharmaceutical], 0.2% chromic oxide [Fisher Scientific Co.] and sufficient sucrose (15.0–15.5%) so that the dietary components totalled 100%.

Weanling, male, Wistar strain rats [Canadian Breeding Farm and Laboratories Ltd., St. Constant, Quebec] were housed in individual wire-bottomed cages permitting feces collection. They were maintained in an environment which was controlled for temperature, relative humidity and light. Diets and water were available ad libitum. Weight gains and feed intakes were recorded weekly and feces were collected during only the last week of the studies.

Two separate experiments were conducted. Experiment I served as a preliminary study and was continued for only 2 wk using four rats per treatment. Many cooking treatments were evaluated, but only wheat, field pea and kidney bean starches were included. Fewer cooking treatments were evaluated in experiment II, but wheat, potato, field pea, mung bean, normal corn and high amylose corn starches were included. In addition, the starch was replaced by glu-

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cose in on dietary treatment. Six rats were assigned to each treatment.

The experiments were analyzed using a completely random design. The data were subjected to one-way analysis of variance and Duncan's multiple range test (Duncan, 1955) to determine differences.

RESULTS & DISCUSSION

THE GROWTH RESULTS of Experiment I showed similar patterns following 1 and 2 wk of feeding (Table 1). However, due to the short time periods and small number of replicates per treatment, differences were not often significant. As previously reported (Fleming and Vose, 1979) wheat starches subjected to pressure kettle cooking and spray drying gave lower weight gains than did raw wheat starch, but had no significant effect on feed efficiency ratios. The lower numerical values for this processing treatment were not significantly different from the raw starches of field pea and kidney beans. Drum dried and cooked starches provided consistently higher weight gains than the other treatments but these values were not statistically higher than those obtained for the corresponding raw starches. Freeze-dried starches showed responses which varied with the type of starch being evaluated. Whereas the cooked, then freeze-dried, wheat and kidney bean starches showed weight gains equivalent to the raw starches, cooked and freeze-dried field pea starch gave values significantly lower than raw field pea starch. These variable results may reflect differences in response of these starches to the processing treatment, but may also represent variability due to the small number of rats used for each evaluation. The number of observations for each treatment was increased to six in the next experiment.

Of the six starches evaluated in Experiment 2, only high-amylose corn starch consistently showed significantly lower weight gains than the other starches (Table 2). However, this difference was not reflected in the feed efficiency ratio (FER) since feed intake on that diet was also low. Raw potato starch showed a high weight gain but also high feed intake, providing a significantly lower FER than the other starches. Previously, weight gains also were found to be significantly less when raw potato starch was fed (Fleming and Vose, 1979). Starches prepared by pressure cooking and spray drying showed consistently lower weight gains than the raw starches, but since feed intakes were also affected, FER's were not significantly different from the raw starches. Starches which were pressure cooked and then drum dried, or extrusion cooked showed no significant differences from the raw starches for weight gains or FER in most cases. However, drum-dried normal corn starch did provide a higher FER than the raw starch since feed intake was reduced. Weight gains were strongly dependent on feed intake, and these parameters were highly correlated ($r = +0.85$).

The high level of dietary glucose was included in an effort to establish the effect of carbohydrate availability on growth and digestibility parameters. However, the glucose treatment gave weight gains, feed intakes and FER's which were statistically equivalent to most of the raw starches or starches drum dried or extrusion cooked. This indicates that the rates at which carbohydrates are digested and absorbed did not have an influence on the growth parameters as measured.

The dietary treatments had relatively little influence on the digestibility of casein protein (Table 2). Raw potato starch and high amylose starch gave low values of 78 and 66%, respectivly, and spray-dried wheat starch gave a value of only 76.8%. However, in all other cases protein digestibility was 80–90%. These results are somewhat lower than those reported previously (Fleming and Vose, 1979), and may be accounted for by the minimal, 10% dietary protein, fed in this study compared to the 20% protein previously incorporated.

Several factors influence weight gains–nutrient composition, presence of toxicants or antinutritional factors, and feed intake. In our experiments, nutrient composition was carefully controlled and, since all diets were made from purified food ingredients, should not have contained any toxicants or antinutritional factors. However, feed intakes were variable. In particular, the pressure-kettle cooked and spray-dried starches showed consistently lower feed intakes and also lower weight gains. The very low density of these starches and, consequently, low density diet may have restricted feed intake by the rat. Additionally, gelatinization curves of the starches used in experiment 2 showed large variability in cold paste viscosity (data not presented). The spray-dried starches showed the highest viscosities indicating that the processing had caused swelling of the granules and partial gelatinization. Following rehydration, the dried granules were able to absorb water and become viscous. This process would have occurred soon after eating, and may have restricted food intake. The extruded and

Table 1–Influence of the starch processing method on growth response of rats (Experiment 1)

	Treatment method		Growth response			
			1 week		2 weeks	
Starch	Cooking	Drying	Weight gain (g)	FER[x]	Weight gain (g)	FER[x]
Wheat	None	None	$40.4^{y,a-c}$	0.538^{ab}	82.4^{a-d}	0.485^{ab}
	Drum heat	Drum dry	43.3^{ab}	0.625^{b}	91.1^{ab}	0.549^{cd}
	Pressure kettle	Drum dry	36.0^{b-d}	0.585^{b}	76.6^{cd}	0.510^{a-d}
	Pressure kettle	Freeze dry	37.4^{a-d}	0.578^{b}	79.5^{b-d}	0.515^{a-d}
	Pressure kettle	Spray dry	30.4^{d}	0.469^{a}	72.9^{d}	0.488^{a-c}
	Autoclave	Freeze dry	36.7^{a-d}	0.529^{ab}	79.0^{b-d}	0.483^{ab}
Field pea	None	None	43.2^{ab}	0.606^{b}	95.0^{a}	0.541^{b-d}
	Drum heat	Drum dry	43.3^{ab}	0.588^{b}	90.0^{ab}	0.518^{a-d}
	Pressure kettle	Drum dry	43.9^{a}	0.602^{b}	94.4^{a}	0.549^{cd}
	Pressure kettle	Freeze dry	35.2^{cd}	0.529^{ab}	71.0^{d}	0.461^{a}
	Pressure kettle	Spray dry	36.9^{a-d}	0.538^{ab}	76.8^{cd}	0.510^{a-d}
Kidney bean	None	None	38.8^{a-c}	0.578^{b}	89.2^{a-c}	0.524^{b-d}
	Pressure kettle	Spray dry	35.4^{cd}	0.546^{ab}	75.8^{cd}	0.495^{a-d}
	Autoclave	Freeze dry	36.5^{a-d}	0.541^{ab}	82.8^{a-d}	0.500^{a-d}
$s_{\bar{x}}$[z]			2.18	0.028	4.08	0.012

[x] FER refers to feed efficiency ratio and was calculated as weight gain divided by feed intake.
[y] Values are the means of n = 4 rats per treatment. Means not sharing a common superscript are significant at $P < 0.05$.
[z] $s_{\bar{x}}$ refers to standard error of the mean using 47 error degrees of freedom.

Table 2—Growth response of rats fed raw or cooked starches for 3 wk (Experiment 2)

Starch	Treatment method		Weight gain (g)	Feed intake (g)	FER[v]	Apparent protein digestibility[w] (%)
	Cooking	Drying				
Wheat	None	None	140.9[z,a]	359.5[z,ab]	0.396[z,ab]	89.9
	Pressure kettle	Spray dry	102.2[cd]	279.2[de]	0.367[a-c]	76.8
	Drum heat	Dry dry	121.6[a-c]	316.0[b-d]	0.385[ab]	89.0
	Steam	Extrusion	128.6[ab]	342.6[bc]	0.375[ab]	88.7
Potato	None	None	126.3[ab]	388.6[a]	0.325[cd]	77.9
	Pressure kettle	Spray dry	62.7[e]	216.7[f]	0.292[d]	90.5
	Drum heat	Drum dry	126.1[ab]	335.0[bc]	0.376[ab]	92.4
	Steam	Extrusion	132.2[ab]	338.9[bc]	0.391[ab]	86.1
Field pea	None	None	128.8[ab]	317.8[b-d]	0.404[a]	81.6
	Pressure kettle	Spray dry	114.3[bc]	295.5[c-e]	0.389[ab]	81.5
	Drum heat	Drum dry	129.0[ab]	333.3[bc]	0.388[ab]	88.9
	Steam	Extrusion	125.1[ab]	323.9[b-d]	0.388[ab]	81.7
Mung bean	None	None	117.3[a-c]	300.8[c-e]	0.388[ab]	85.4
	Drum heat	Drum dry	115.1[bc]	315.7[b-d]	0.365[a-c]	88.5
	Steam	Extrusion	130.0[ab]	344.3[bc]	0.376[ab]	81.9
Normal corn	None	None	126.7[ab]	336.9[bc]	0.285[d]	88.4
	Drum heat	Drum dry	121.9[a-c]	317.9[b-d]	0.384[ab]	88.3
High amylose corn	None	None	90.2[d]	317.6[b-d]	0.375[ab]	66.0
	Drum heat	Drum dry	91.2[d]	258.7[e]	0.354[bc]	89.3
Glucose	None	None	117.9[a-c]	337.3[bc]	0.351[bc]	91.0
$s_{\bar{x}}$[x]			7.01	14.4	0.014	
CV[y]			14.62	11.04	9.59	

[v] FER refers to feed efficiency ratio.
[w] Means of two values each from a pool of feces from three rats.
[x] $s_{\bar{x}}$ refers to standard error of the mean using 95 error degrees of freedom.
[y] CV refers to coefficient of variation.
[z] Values are the means of n = 6 rats per treatment. Means not sharing a common superscript are significant at $p < 0.05$.

drum-dried starches showed viscosity values intermediate between raw and spray-dried starches indicating a lower degree of granule swelling. Diets containing these starches gave weight gains and feed intakes which were, in most cases, equivalent to the same starch fed without a cooking treatment. This variability in diet density may have been largely responsible for the variation in feed intake and then weight gain. Consequently, the processing method should be paid particular attention when foods or feeds of high starch content are evaluated and nutritionally compared.

CONCLUSIONS

RAW POTATO and high amylose starches were less digestible and provided lower growth responses than the other cereal and legume starches which were tested. The spray-drying process which was used following pressure cooking of the starches adversely affected weight gains, but this may be due to its restricting effect on feed intake since FER and protein digestibility were not affected. Starch was not shown to be present in the feces of rats fed starches processed in this manner which indicates that the starch digestibility was not impaired by processing. It appears from these results that gelatinization, when achieved by drum drying or extrusion cooking has no adverse nutritional effect, but neither does this have any benefit. It may be functionally advantageous to cook the starches, or cooking may improve dry matter digestibility by influencing non-starch components. However, although gelatinization increases the rate at which enzymes may cause hydrolysis, no beneficial effect on growth or protein digestibility was evident from the results of this study.

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Kinetics of Nitrogen Solubility Loss in Heated Flour and Protein Isolates From Bean, Phaseolus vulgaris

A. M. PILOSOF, G. B. BARTHOLOMAI, and J. CHIRIFE

ABSTRACT

The effect of temperature, time and moisture content on the loss of nitrogen solubility of heated bean flour and protein isolates was studied. Solubility loss followed first-order reaction kinetics in almost all cases studied. The rate of solubility loss was greatly increased with increasing moisture content both for flour and protein isolates; protein isolate was far more sensitive to heat treatment than the flour. Activation energies for the first-order reaction constants were calculated and ranged between 20–30 kcal/mole.

INTRODUCTION

LEGUME SEEDS are an important source of protein in the diets of large population groups around the world. Dry bean (*Phaseolus vulgaris*) contains over 20% protein in addition to several essential minerals and vitamins. In most Central American countries beans provide a significant percentage of the protein in the diets of rural populations (Bressani et al., 1961). Legume seeds contain relatively high amounts of lysine and have been reported as a good complement to cereal proteins (Bressani et al., 1962).

In a previous publication (Pilosof et al., 1981) the effect of some selected heat-treatments on water sorption isotherms and nitrogen solubility of bean flour and protein isolates was studied; mainly as regarding to the interrelationship between changes experienced by both functional properties.

Nitrogen solubility levels are frequently used as indicators of protein functionality and potential end use (Johnson, 1970; Hutton and Campbell, 1977). Solubility data provide an indication of the innate solubility of a protein concentrate, the potential solubility in food systems and the degree of denaturation, to the extent that solubility may be used as a general indication of denaturation (Betschart, 1974; Wu and Inglett, 1974). For this reason various studies have been made on the effect of heat and moisture on the progressive insolubility of protein concentrates and isolates (Beckel et al., 1942; Pence et al., 1953; Fukushima, 1959; McWatters and Holmes, 1979; McWatters and Cherry, 1975).

Present work studies the effects of time, temperature and moisture content on the loss of solubility of bean flour and protein isolates.

EXPERIMENTAL

Materials

Crushed field beans (*Phaseolus vulgaris*) were ground to an 80 mesh size.

Bean flour was extracted with water and dilute NaOH (1:10 extraction ratio). The dispersion was centrifuged for 1 hr at 2,000 rpm in a laboratory centrifuge and the supernatant filtered. The filtrate was then adjusted to pH 3.7 (its approximate isoelectric point) with 0.1N HCl and centrifuged for 30 min at 2,000 rpm. The precipitate containing the protein isolate, was then washed twice with distilled water and centrifuged each time under similar conditions. The protein isolate was then frozen and freeze-dried at room temperature. Proximate composition of the bean flour was (dry basis) 23.6% protein, 2.7% oil, 55.1% starch and 8% total sugars. The composition of the protein isolate was (dry basis) 88.1%, protein, 2.5% lipids, 1.1% total sugars and 0.7% starch.

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Methods

Moisture content. Moisture content was determined gravimetrically by placing the samples in a vacuum oven at 70°C over magnesium perchlorate for 48 hr. Moisture content was expressed as g water/100g dry matter.

Soluble nitrogen. 8g of bean flour or 2g of protein isolate were added to 90 ml of distilled water, the pH adjusted to 10 and stirred for 1 hr at 25°C. The dispersion was centrifuged for 30 min at 2,000 rpm in a laboratory centrifuge and the supernatant filtered and brought to a volume of 100 ml. Extractable nitrogen was determined by dye-binding with C.I. acid orange 12 (Romo et al., 1975). For the construction of the calibration curve, solutions of different contents of bean flour protein or protein isolate were diluted to contain no more than 0.15% (w/v) crude protein (nitrogen x 6.25). 5 ml dilute protein solution were added to 10 ml dye-buffer reagent and the solutions were mixed and allowed to stand overnight to attain equilibrium. The supernatant was filtered through a glass fiber paper, suitably diluted and the absorbance of the solution measured at 482 nm in a Beckman DU-2 spectrophotometer. For the construction of the calibration curve the absorbance of the diluted supernatant was substracted from a blank determination and plotted against the crude protein content of the original protein solution determined by Kjedahl method. % soluble nitrogen was expressed as extractable nitrogen over total nitrogen.

Estimation of the error in the determination of nitrogen solubility. Ten replicates of nitrogen solubility in an unheated sample of protein isolate were performed and mean value and absolute error (at the 95% confidence level) were calculated; results were: mean value; 77.11% (expressed as % of total nitrogen which was soluble in water); and absolute error (expressed in the same units as mean value) was 3.25%.

"Dry" and "moist" heat treatments. Samples of nearly "dried" (≃1% moisture) bean flour or freeze-dried protein isolates were sealed in small (7 cm diameter, 4 cm height) enameled tin cans, placed in a temperature-controlled (± 1°C) oven and heated at the desired temperature. Following heat treatment, samples were removed from the oven, rapidly cooled and analyzed for soluble nitrogen. It was assumed that the time intervals required for temperature equilibration during heating and cooling were negligible as compared to heating periods studied.

Different moisture levels were obtained by direct addition of water. Flours or isolates were spread in thin layers and humidified by spraying water until the desired moisture content was obtained. The moistened samples were sealed in plastic pouches, placed in a refrigerator and allowed to equilibrate during 4 days. The moisture-equilibrated samples of varied moisture contents were then transferred to the cans, sealed and subjected to the desired heat treatment.

RESULTS & DISCUSSION

NITROGEN SOLUBILITY of both flour and protein isolates were reduced by the application of heat at various constant moisture levels, as shown in Fig. 1–6. It can be seen that in almost all cases solubility loss follows first-order reaction kinetics as indicated by the straight lines

obtained when plotting the logarithm of solubility versus heating time. The only exception to this behavior corresponds to the data for protein isolate humidified at the highest moisture level (30%) (Fig. 6); in this case the course of the reaction does not appear to fit first-order rate curves. Pence et al. (1953) also reported a first-order reaction for the loss of solubility in wet gum gluten heated at various temperatures, although they also noted that the course of the later phases of the reactions appeared to fit second-order rate curves best.

Two facts are apparent from the analysis of data shown in Fig. 1–6. One is that the rate of solubility loss was great-

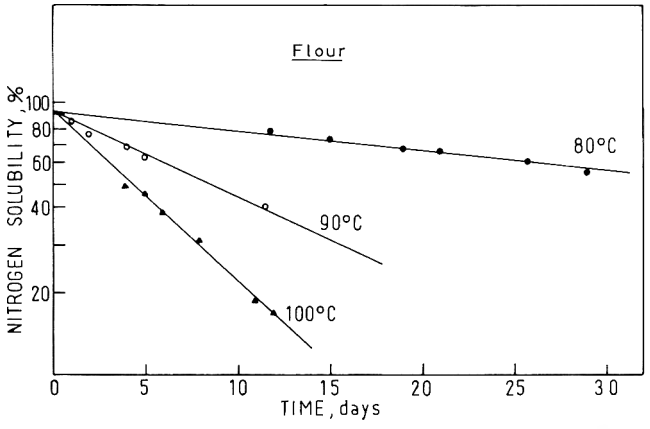


Fig. 1—Loss of nitrogen solubility in heated bean flours at ≃ 1% moisture content.

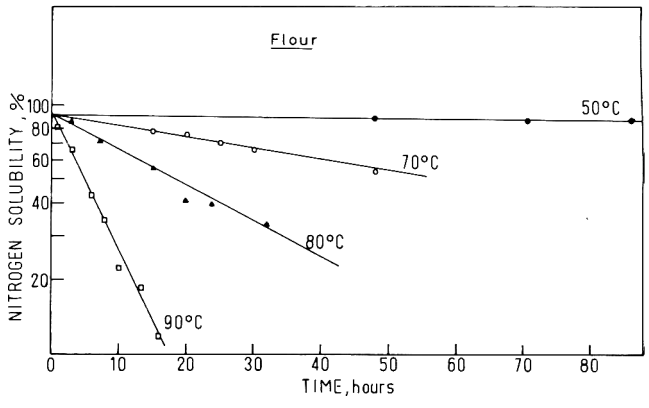


Fig. 2—Loss of nitrogen solubility in heated bean flours at 14.1% moisture content.

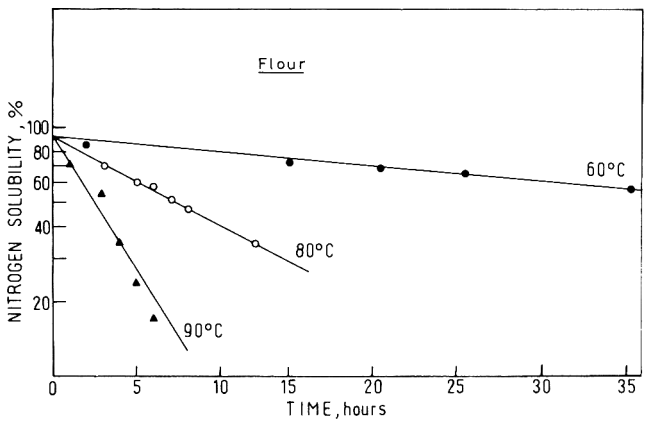


Fig. 3—Loss of nitrogen solubility in heated bean flours at 29.8% moisture content.

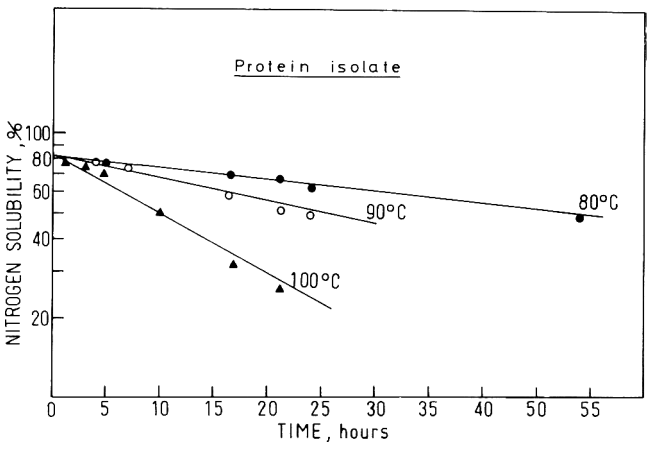


Fig. 4—Loss of nitrogen solubility in heated bean proteins isolates at ≃ 1% moisture content.

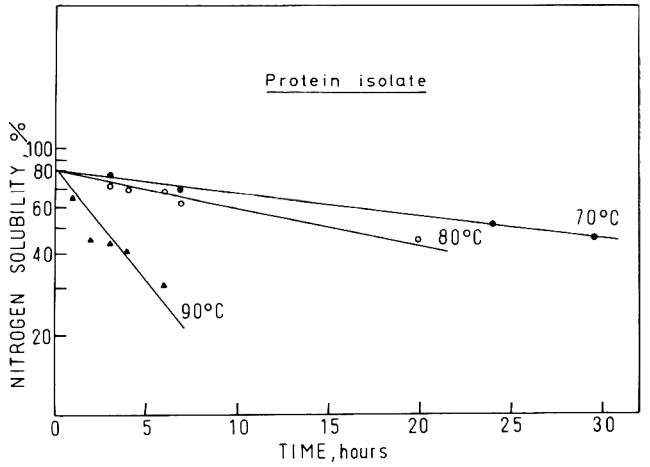


Fig. 5—Loss of solubility in heated bean protein isolates at 11.1% moisture content.

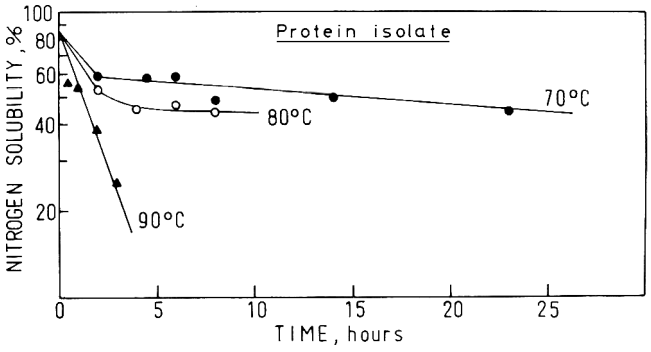


Fig. 6—Loss of solubility in heated bean protein isolates at 29.6% moisture content.

ly increased with increasing moisture content both for flour and isolates, and the other that protein isolate was far more sensitive to heat treatment than the flour. This can be best observed in Fig. 7 which compares the half-life, t½, (time for 50% loss of nitrogen solubility) for flour and protein isolates at various moisture contents. A logarithmic representation had to be used due to the extreme variation of t½. Half-life was calculated according to (Labuza, 1980),

$$k = \frac{0.693}{t_{1/2}} \quad (1)$$

where k is the first-order rate constant obtained by least-squares analysis of the straight-lines showed in Fig. 1–6. The dramatic influence of moisture content on rate of solubility loss can be made clear with an example: increasing moisture content from, i.e. 5% up to 20% decreases the half-life at 90°C of flour and isolates by a factor of 8.3 and 5.1 respectively. Pence et al. (1953) also found a similar and strong influence of moisture content on rates of solubility loss in heated gluten.

Fig. 8 and 9 show the curves obtained by plotting values of the first-order rate constants on a logarithmic scale against the reciprocal of absolute temperature and for the various moisture levels. It can be seen that plots conform to Arrhenius equation which permits the calculation of activation energies. Calculated values for flour ranged between about 23–32 kcal/mole and for protein isolates between about 21–28 kcal/mole depending on the moisture level. The magnitude of the values obtained indicates that temperature has a relatively important effect on the kinetics of solubility loss. Pence et al. (1953) reported an activation energy of 43.8 kcal/mole for solubility loss in heated wet gum gluten.

It is usual that activation energies for deteriorative reactions in low-moisture foods depend on the moisture level, and several literature results showed such correlations (Mizrahi et al., 1970, Labuza, 1980).

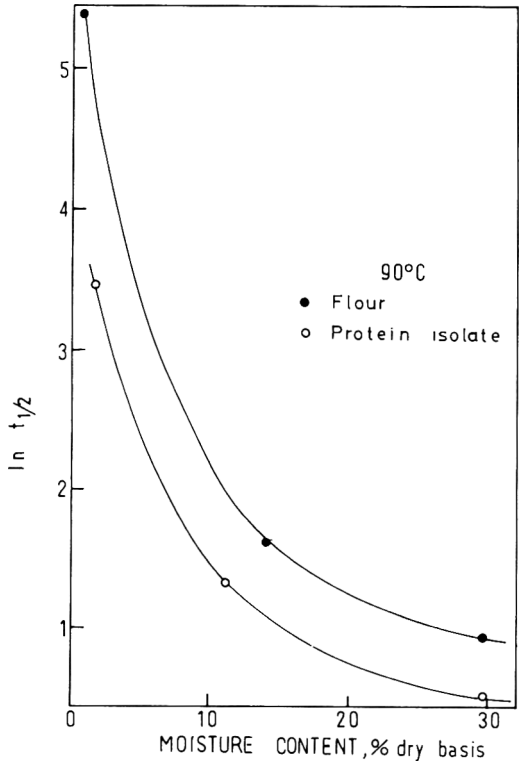


Fig. 7—Comparison of the time for 50% loss of nitrogen solubility (t½) in bean flour and protein isolates heated at 90° C.

However, before discussing any relationship between activation energy and moisture level in bean flour and protein isolates, it is useful to estimate, at least roughly, the errors associated with the determination of the temperature dependence of k through the use of the activation energy concept. This is done as follows (Hill and Grieger-Block, 1980).

The relative uncertainty in the rate constant may be calculated as

$$\left(\frac{\Delta k}{k}\right)^2 = \left(\frac{\Delta t_1}{t_2 - t_1}\right)^2 + \left(\frac{\Delta t_2}{t_2 - t_1}\right)^2 + \left[\frac{1}{\ln\left(\frac{C_{A1}}{C_{A2}}\right)}\right]^2 \left(\frac{\Delta C_{A1}}{C_{A1}}\right)^2 + \left[\frac{1}{\left(\frac{C_{A1}}{\ln\left(\frac{C_{A1}}{C_{A2}}\right)}\right)}\right]^2 \left(\frac{\Delta C_{A2}}{C_{A2}}\right)^2 \quad (2)$$

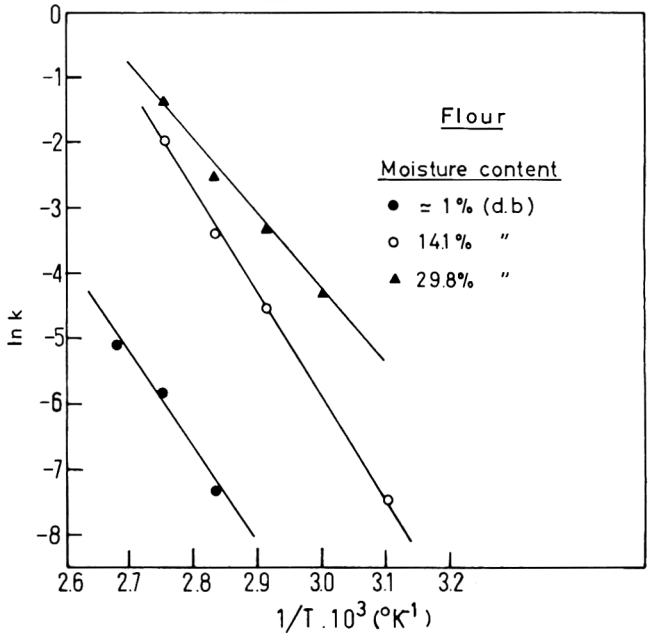


Fig. 8—Arrhenius plot for nitrogen solubility loss in bean flours heated at various moisture contents.

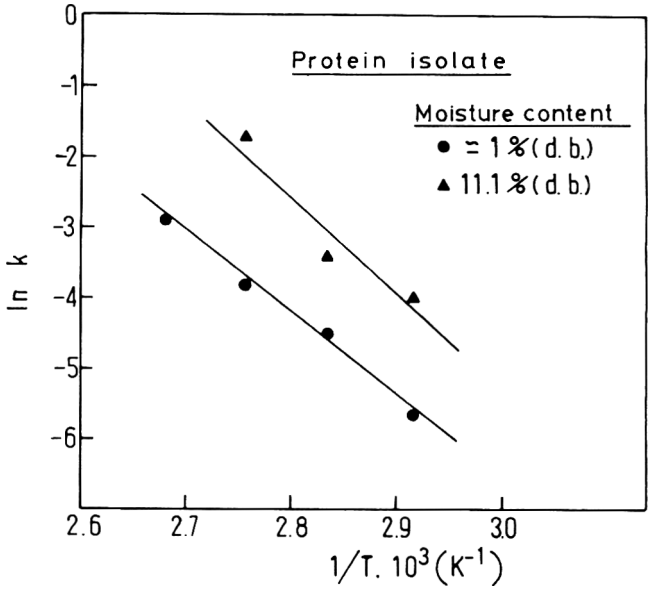


Fig. 9—Arrhenius plot for nitrogen solubility loss in bean protein isolates heated at different moisture contents.

where Δt_1 and Δt_2 are the uncertainty in each time measurement, $t_2 - t_1$ is the time interval chosen, and $\Delta C_{A1}/C_{A1}$, $\Delta C_{A2}/C_{A2}$, are the relative uncertainties in each solubility measurement.

The relative error in the Arrhenius activation energy is given by

$$\left(\frac{\Delta E_A}{E_A}\right)^2 = \left(\frac{T_2}{T_2 - T_1}\right)^2 \left(\frac{\Delta T_1}{T_1}\right)^2 + \left(\frac{T_1}{T_2 - T_1}\right)^2 \left(\frac{\Delta T_2}{T_2}\right)^2 + \left(\frac{1}{\ln k_2/k_1}\right)^2 \left[\left(\frac{\Delta k_1}{k_1}\right)^2 + \left(\frac{\Delta k_2}{k_2}\right)^2\right] \quad (3)$$

From Eq (3) it can be seen that the relative error in the activation energy is strongly dependent on the size of the temperature interval chosen. The uncertainties in both T_1 and T_2, as well in the rate constants also contribute to the uncertainty in the activation energy.

From experimental conditions and the analytical methods employed in the present work a rough estimate of the various uncertainties (solubility measurement, temperature, time, etc.) needed to calculate $\Delta k/k$ and $\Delta Ea/Ea$ might be made. Substitution of these values in Eq (2) and (3) gives the results shown in Table 1 and Table 2. For bean flours the precision achieved in the measurement of Ea is such that it does not allow to say that values at 1% and 14.1% moisture are different; however, the activation energy at the highest moisture level (29.8%) may be considered to be significantly lower than the value at the other moisture levels. For protein isolates, the uncertainties in Ea are such that it would be difficult to say if values at $\simeq$ 1% and 11.1% are significantly different.

CONCLUSIONS

USUALLY, the loss of solubility in heated proteins is associated with denaturation (Pence et al., 1953; Betschart, 1974), although it has to be noted that changes in solubility may result from the dual effects of protein denaturation (unfolding) and protein aggregation; the relative importance of one or the other depends on each particular system and heating conditions (Fennema, 1977).

The loss of nitrogen solubility in heated bean flour and protein isolates was found to follow in almost all cases, first-order reaction kinetics, a behavior which is also usually found for various deteriorative reactions in food systems (Labuza, 1980). The rate of solubility loss was greatly increased with increasing moisture content, both for flour and isolates; protein isolate was far more sensitive to heating (at all moisture levels) than the flour, probably due to the increased "concentration" of the protein reacting species. Various literature data concerning deteriorative reactions in foods at low moisture levels show that activation energy increases with decreasing moisture content (Vojnovich and Pfeifer, 1970; Mizrahi et al. 1970). The amount of data collected in present work as well as the precision in measured activation energies do not allow us to make definite statements on the effect of moisture content on Ea for solubility loss. It does appear, however, that at least for the highest moisture here studied, Ea for bean flour has a tendency to decrease. This means that the relative loss of solubility in heated bean flour is less affected by temperature changes at high moisture values than at low ones. Nevertheless, and for all moisture values, activation energies are big enough to conclude that small temperature changes will greatly affect the rate of solubility loss.

Table 1—Approximate errors in calculated rate constants and activation energies for solubility loss in heated bean flour

% moisture (dry basis)	T (°C)	Δk/k Eq (2)	ΔEa/Ea Eq (3)	Ea (kcal/mole)
≃1	80	0.133	0.108	28.6
	100	0.120		
14.1	70	0.131	0.104	32
	90	0.145		
29.8	60	0.13	0.076	23.1
	90	0.116		

Table 2—Approximate errors in calculated rate constants and activation energies for solubility loss in heated bean protein isolates

% moisture (dry basis)	T (°C)	Δk/k Eq (2)	ΔEa/Ea Eq (3)	Ea (kcal/mole)
≃1	80	0.145	0.135	20.9
	100	0.114		
11.1	70	0.140	0.108	27.8
	90	0.115		

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Functional Properties of the Great Northern Bean (*Phaseolus Vulgaris* L.) Proteins. Amino Acid Composition, *In Vitro* Digestibility, and Application to Cookies

S. K. SATHE, V. IYER, and D. K. SALUNKHE

ABSTRACT

The Great Northern bean flour proteins, albumins, and protein isolates were characterized by high acidic amino acid content while globulins and protein concentrates had high proportion of hydrophobic amino acids. Sulfur containing amino acids and leucine were first and second limiting amino acids in these proteins, respectively. The bean proteins were resistant to in vitro enzymatic attack. Heating improved in vitro susceptibility of these bean proteins to enzymatic hydrolysis. Moist heat was more effective than dry heat in improving in vitro digestibility of the bean proteins. Cookie diameter was negatively correlated to alkaline water retention capacity of the blends of wheat flour with bean flour ($r = -0.90$) and protein concentrates ($r = -0.93$).

INTRODUCTION

IN OUR EARLIER INVESTIGATIONS on the Great Northern beans (*Phaseolus vulgaris* L.), we examined the protein solubilization and electrophoretic characteristics (Sathe and Salunkhe, 1981a), certain hydrodynamic properties of bean proteins (Sathe and Salunkhe, 1981b), antinutritional and flatulence factors (Sathe and Salunkhe, 1981c), and effects of addition of the bean flour and protein concentrates on dough rheology and baking quality of bread (Sathe et al., 1981).

The purpose of the present investigation was to study the amino acid composition, in vitro digestibility, and evaluate cookie performance of the Great Northern bean proteins.

MATERIALS & METHODS

THE SOURCE of the Great Northern beans, preparation and composition of bean flour, albumins, globulins, protein concentrates, and protein isolates were reported earlier (Sathe and Salunkhe, 1981a). For in vitro digestibility studies, heat treatments were given to samples as follows. For dry heating, samples (dry) were heated in an oven (110°C) for 10 hr, cooled to room temperature (21°C) and used for enzymatic digestion. For moist heating, appropriate amount (described under in vitro digestibility) of sample was dispersed in 0.5M NaCl and heated on a water bath (95°C, 30 min), cooled to room temperature (21°C) and used for the enzymatic digestion.

Amino acid analyses

About 100 mg (accurately weighed) sample was hydrolyzed with 20 ml of 6N HCl for 24 hr at 110°C in an evacuated teflon screw capped test tube. To the resultant hydrolyzate, 10 ml of citrate buffer (0.2M, pH 2.20) was added, adjusted to pH 2.20 with dilute NaOH (0.1N) or HCl (0.1N) and made to 50 ml. Samples (100 µl) were analyzed in a Beckman model 118 Amino Acid Analyzer, with Norleucine as an internal standard. Amino acid contents were corrected for 100% recovery of proteins. Tryptophan content was determined by the spectrophotometric method (Bencze and Schmid, 1957). Proportion of total essential amino acids with total amino acids in the protein (E/T %), limiting amino acids, and chemical score were evaluated based on the provisional amino acid scoring pattern (WHO, 1973). Chemical score of protein was the lowest essential amino acid score. Amino acid score was calculated as follows.

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in WHO reference pattern}} \times 100$$

In vitro digestibility

Modified method of Romero and Ryan (1978) was used to determine the % peptide bond hydrolysis. A combination of equal amount of enzymes peptidase, trypsin (Sigma Chemical Co., St. Louis, MO), and α-chymotrypsin (P.L. Biochemicals, Milwaukee, WI) was used for protein hydrolysis (total enzymes to protein ratio was 1:10, w/w). To 0.5g sample, 25 ml 0.5M NaCl was added and blended for 15 min in a Sorvall Omni mixer, 0.08M sodium barbital buffer (pH 8.6) added, and pH adjusted to about 8.0. The slurry was incubated for 30 min at 37°C and mixture of enzymes (peptidase in distilled water, trypsin and α-chymotrypsin in distilled water containing 100 mM $CaCl_2$) added. Digestion was carried out for 4 hr at 37°C. The slurry was then heated in a boiling water bath (95°C) for 15 min to inactivate the enzymes, cooled to room temperature (21°C) and diluted to 100 ml with distilled water. Aliquots of this digest were used for trinitrobenzene sulfonic acid (TNBS) assay (Fields, 1972). The enzyme digests were centrifuged (5,000 x g, 15 min) and to an aliquot of the supernatant were added 0.1M borate buffer (pH 9.5) to make volume to 1.0 ml, and 20 µl TNBS (100 mg/0.2 ml distilled water, freshly prepared), mixed thoroughly, and incubated for 30 min at room temperature (21°C). The reaction was stopped by adding 2.0 ml 0.1 M sodium sulfate and optical density read at 420 nm in a Beckman DB-G Spectrophotometer. Appropriate blanks, prepared simultaneously, contained enzyme mixture or sample alone. The net optical density was calculated by subtracting the values of two controls from those of the digested samples. The % peptide bond hydrolysis was the ratio of the number of new α-amino groups in the digestion mixture to the total number of peptide bonds in protein. The number of new amino groups were calculated using 22,000 as the molar extinction coefficient for the TNP-α amino groups at 420 nm. The total number of peptide bonds in the protein were calculated by dividing g of protein in the digest by 113, the average molecular weight of amino acids. Means of triplicate determinations were reported.

Alkaline water retention capacity

The modified method of Yamazaki (1953) was used. To 1g of all-purpose wheat flour/blend (test tube weighed with its contents, W_1), 5 ml 0.1N $NaHCO_3$ was added, mixed for 30 sec in a Vari-Whirl mixer, and allowed to stand at room temperature (21°C) for 20 min. The samples were centrifuged (2,000 rpm, 15 min) and drained for 10 min at an angle of 10–15° with the horizontal. The test tubes were then weighed (W_2). Alkaline water retention capacity was calculated as follows.

$$\frac{\text{Alkaline water retention capacity (g)}}{\text{g blend}} = W_2 - W_1$$

Analyses were performed in triplicate and the means reported.

Preparation of cookies

The basic formula employed for the cookie preparation was:

All purpose wheat flour	60g
Vegetable shortening	50 g
Fine granulated sugar	25g
Salt	700 mg

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Ammonium bicarbonate	5g
Cardamom (powder)	200 mg
Nutmeg (powder)	200 mg
Water	10 ml

Cookies with bean flour and protein concentrates were prepared by replacing all purpose wheat flour at 10, 20, 30, 40, and 50% (by weight) in each case. The doughs were mixed (all in one) and allowed to equilibrate for 1 hr at 4°C. The doughs were divided into 15 ± 0.5g portions, shaped round (by hand), and baked on greased trays at about 149°C (300°F) for 25 min. Baked cookies were cooled to room temperature (21°C) and immediately evaluated for physical parameters.

Scanning electron microscopy

About 5g of the dough of the control and that with 10% protein concentrates were freeze-dehydrated and fractured in liquid N_2. A small piece of sample was placed on an aluminum stub (with double-stick tape) and coated with gold-palladium alloy in a Polaron E5000 Sputter Coater (U.K.). The specimens were viewed and photographed in an AMR 1000B Scanning Electron Microscope (Cambridge, MA) at an accelerating voltage of 20 KV and suitable magnification.

Cookie evaluation

Weight, height, and diameter measurements were performed on five representative cookies in each batch. Diameters were measured three times on each cookie. Mean weight, height, and diameter were reported.

Statistical analyses

Simple correlation coefficients for the alkaline water retention capacity and cookie diameter were calculated as described by Ostle (1974).

RESULTS & DISCUSSION

Amino acid analyses

Results of amino acid analyses are summarized in Table 1. The bean flour proteins, albumins, and protein isolates contained high amounts of acidic amino acids while globulins and protein concentrates were characterized by high hydrophobic amino acids content. Essential amino acid to total amino acids ratio (E/T %) was highest for protein concentrates followed by globulins, albumins, protein isolates, and the bean flour proteins in decreasing order. Protein isolates had the lowest chemical score (18.9) among all samples. In all the samples, sulfur-containing amino acids (met + cys) and leucine were first and second limiting amino acids, respectively. Pusztai et al. (1979) have reported sulfur-containing amino acids to be limiting amino acids in the Great Northern beans. Yadav and Liener (1977) also reported the sulfur-containing amino acids were limiting in navy beans (*Phaseolus vulgaris*).

In vitro digestibility

In vitro digestibility as measured by TNBS assay, of the Great Northern bean flour proteins, albumins, globulins, protein concentrates, and protein isolates was 9.30, 2.73, 10.09, 13.60, and 28.59% respectively (Table 2). Moist heat improved the digestibility more than did dry heat. Romero

Table 1—Amino acid content of the Great Northern bean proteins[a]

Amino acid	Bean flour proteins	Albumins	Globulins	Protein conc	Protein isolates
Aspartic acid	164.5	143.8	98.7	74.1	204.4
Threonine	43.1	54.8	51.6	63.9	41.2
Serine	47.3	55.4	57.4	40.8	53.7
Glutamic acid	224.4	197.8	133.9	138.1	169.8
Proline	trace	trace	trace	trace	trace
Glycine	6.9	7.5	1.5	7.8	0.7
Alanine	66.2	68.4	63.9	68.8	60.6
Half cystine	7.7	6.4	7.5	6.9	5.3
Valine	56.5	72.9	92.3	180.7	57.2
Methionine	2.7	1.6	1.4	1.3	1.3
Leucine	27.6	42.9	41.2	38.1	38.9
Isoleucine	51.9	53.3	71.1	46.2	55.2
Tyrosine	29.1	38.7	52.5	45.8	43.3
Phenylalanine	55.0	59.8	89.7	81.8	77.1
Histidine	52.0	34.4	39.5	31.7	28.4
Lysine	67.9	78.9	68.6	72.4	72.6
Ammonia	8.9	5.8	11.3	10.7	8.3
Arginine	67.5	64.8	103.1	77.1	68.6
Tryptophan	20.8	12.6	14.7	13.7	13.0
Classified distribution of amino acids (% of total)					
Hydrophobic	28.1	31.2	37.4	43.1	30.3
Uncharged polar	13.4	16.3	17.1	16.5	14.4
Basic	18.7	17.8	21.1	18.1	17.0
Acidic	38.9	34.2	23.3	21.2	37.4
E/T (%)[b]	36.2	42.2	49.1	55.1	40.5
Chemical score[b]	29.7	22.9	25.4	23.4	18.9
Limiting amino acids[b]					
First	Met+Cys (29.7)	Met+Cys (22.9)	Met+Cys (25.4)	Met+Cys (23.4)	Met+Cys (18.9)
Second	Leu (39.4)	Leu (61.3)	Leu (58.9)	Leu (54.4)	Leu (55.6)

[a] Amino acid contents expressed as mg/g protein, corrected for 100% protein recovery.
[b] Evaluated with WHO-UN (1973) provisional amino acid pattern as reference. The figures in parentheses correspond to amino acid scores.

and Ryan (1978) have reported resistance of major protein of *Phaseolus vulgaris* L. (var. Tendergreen) to enzymatic hydrolysis. They also observed improvement in susceptibility of this protein to enzymatic hydrolysis on heating. Recently, Chang and Satterlee (1980) reported isolation of a major protein of the Great Northern beans and found that the native protein had a compact structure. They attributed the resistance of protein to enzymatic attack to this compactness of structure. They further reported that the protein unfolding followed by aggregation on heat denaturation occurred, leading to improved in vitro protein digestibility. Iyer et al. (1980) have also reported an improvement in in vitro digestibility of the Great Northern, kidney, and pinto bean proteins on cooking (moist heat). The improved in vitro digestibility of proteins in the present investigation may improve the nutritional quality of these less expensive proteins.

Table 2—Susceptibility of the Great Northern bean proteins to in vitro enzymatic hydrolysis as measured by trinitrobenzene sulfonic acid (TNBS) assay[a]

Sample	% Peptide bond hydrolysis		
	Control	Dry heat (110°C, 10 hr)	Moist heat (95°C, 0.5 hr)
Bean flour	9.30 ± 0.13	68.58 ± 0.13	79.51 ± 0.16
Albumins	2.73 ± 0.10	24.05 ± 0.22	72.31 ± 0.16
Globulins	10.09 ± 0.14	29.67 ± 0.26	65.27 ± 0.19
Protein conc	13.60 ± 0.20	24.05 ± 0.52	37.14 ± 0.38
Protein isolates	28.59 ± 0.17	34.31 ± 0.09	49.48 ± 0.19

[a] Mean ± standard deviation of triplicate determinations

Table 3—Correlation between the alkaline water retention capacity and cookie diameter

Blend (% wheat flour replaced)	Alkaline water retention capacity[a] (g/g blend)	Cookie diameter[b] (cm)	Correlation coefficient
Bean flour			
10	0.95 ± 0.001	7.03 ± 0.27	
20	1.05 ± 0.017	7.09 ± 0.35	
30	1.11 ± 0.005	6.47 ± 0.24	−0.90
40	1.17 ± 0.014	6.47 ± 0.18	
50	1.27 ± 0.030	6.21 ± 0.17	
Protein conc			
10	1.09 ± 0.038	6.41 ± 0.18	
20	1.29 ± 0.022	5.69 ± 0.13	
30	1.59 ± 0.044	4.52 ± 0.18	−0.93
40	1.93 ± 0.044	4.39 ± 0.09	
50	2.28 ± 0.043	4.04 ± 0.13	

[a] Mean ± Standard deviation of triplicate determinations
[b] Mean ± Standard deviation of 15 determinations

Cookie Dough Mixing, Baking, and Scanning Electron Microscopy

During dough mixing, it was observed that as the proportion of the bean flour or protein concentrates increased, doughs became progressively stiffer. This effect was more pronounced in case of protein concentrates than the bean flour addition. This effect may have been due to increased water absorption capacity of the blends (Table 3). During baking, collapse occurred more in case of doughs containing added bean flour than those with added protein concentrates, which was reflected in the final diameter of cookies (Fig. 1). These observations suggest that in addition to proteins, starch may also have an important role in structural characteristics of cookies.

Scanning electron microscopic observations on representative doughs (control and with 10% protein concentrates) were made for comparative purposes (Fig. 2). Addition of protein concentrates altered dough characteristics. The noticeable change appeared to be in the gluten network of dough. These changes may have been due to the competition between the wheat flour proteins and the added bean proteins for available water.

Cookie evaluation

Data on physical parameters and protein content of cookies are summarized in Table 4. In general, the cookie spread ratio decreased with increase in protein content of the cookies. The mechanism by which cookie spread is reduced by certain wheat flour supplements is not completely understood. It is reported that rapid partitioning of free water to hydrophilic sites during mixing increases dough viscosity thereby limiting cookie spread and top grain formation during baking (McWatters, 1978). The reduction in spread ratio was more pronounced in the case of protein concentrates addition than for bean flour addition. This may have been partially due to higher hydrophobicity (43.1% hydrophobic amino acid residues) of protein concentrates compared to that of the bean flour (28.1% hydrophobic amino acid residues). It was also noted that

Table 4—Physicochemical data on cookies prepared from blends of all purpose wheat flour with bean flour and protein concentrates

Blend (% wheat flour replaced)	Protein content[a] (%)	Increase in protein content[b] (%)	Cookie			
			Weight (g)	Diameter (cm)	Height (cm)	Spread ratio
Control	4.63	00.00	13.47	7.77	0.60	12.95
Bean flour						
10	5.15	11.23	13.41	7.03	0.80	8.79
20	5.66	22.25	13.15	7.09	0.86	8.24
30	6.12	32.18	13.73	6.47	0.96	6.74
40	6.69	44.49	13.28	6.47	0.98	6.60
50	7.21	55.72	13.32	6.21	1.04	5.97
Protein concentrates						
10	7.54	62.85	13.66	6.41	0.74	8.66
20	10.45	125.70	13.66	5.69	1.05	5.42
30	13.36	188.55	13.45	4.52	1.44	3.14
40	16.26	251.19	13.30	4.39	1.50	2.93
50	19.18	314.25	14.14	4.04	1.80	2.24

[a] Calculated protein content per 100g cookies on dry weight basis
[b] Increase (%) with reference to protein content of the control cookies

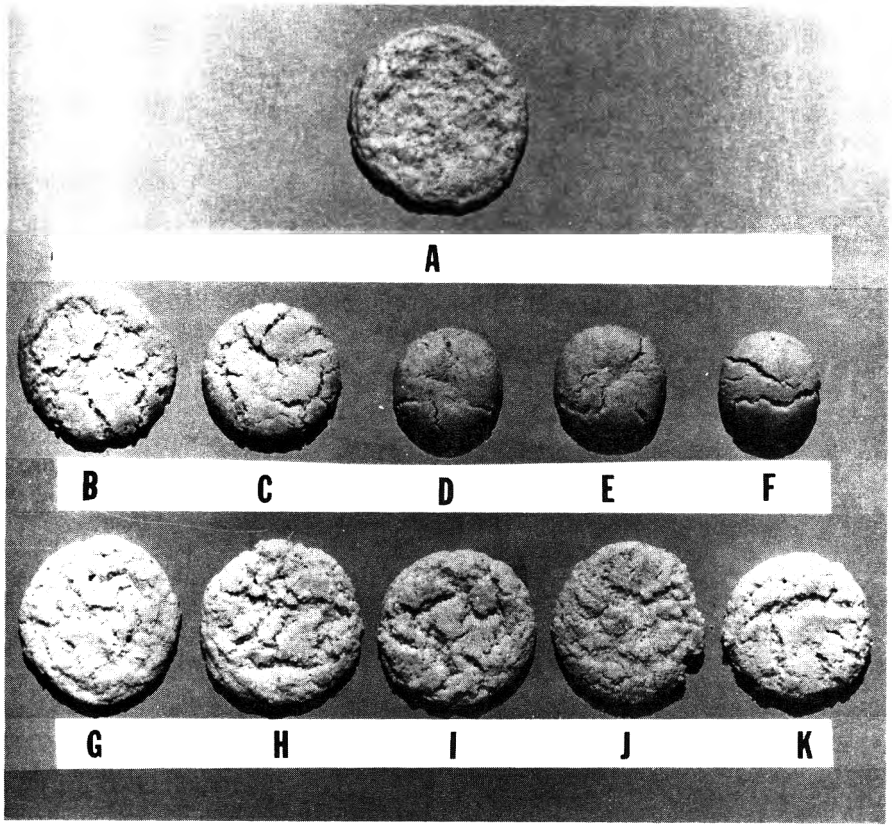


Fig. 1—Cookies prepared from all purpose wheat flour and its blends with the Great Northern bean flour and protein concentrates: (A) control; (B, C, D, E, and F) wheat flour replaced with 10, 20, 30, 40, and 50% (by weight) protein concentrates, respectively; (G, H, I, J, and K) wheat flour replaced with 10, 20, 30, 40, and 50% (by weight) Great Northern bean flour, respectively.

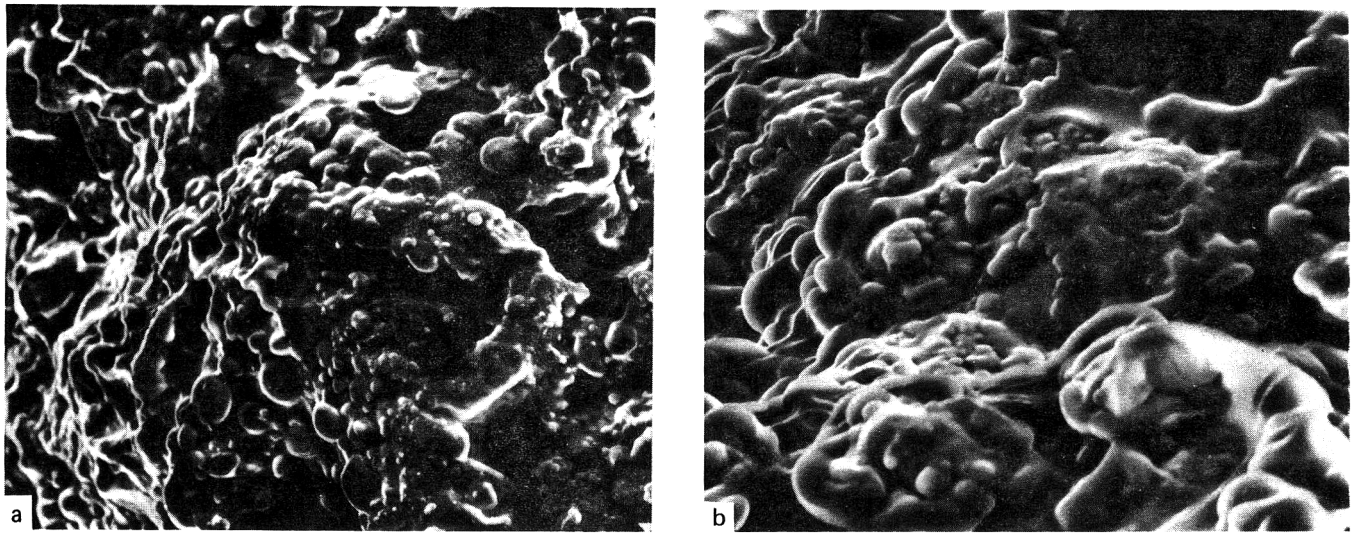


Fig. 2—Scanning electron photomicrograph of cookie doughs: (a) control (1,000X); (b) with 10% replacement of the all purpose wheat flour by the Great Northern bean protein concentrates (2,000X).

the cookie diameter was negatively correlated to the alkaline water retention capacity of the blend (Table 3) with correlation coefficients of −0.90 for the bean flour blends and −0.93 for the blends containing protein concentrates. Yamazaki et al. (1977) reported similar correlation between alkaline water retention capacity and cookie diameter for several wheat flours. Observations in the present investigation suggest that the correlation between alkaline water retention capacity and cookie diameter occurs for composite blends as well.

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Flatulence Activity of the Smooth-Seeded Field Pea as Indicated by Hydrogen Production in the Rat

S. E. FLEMING

ABSTRACT

The objective of this study was to evaluate the distribution of flatulence-causing components within the seeds of smooth-seeded field peas. Fractionation studies showed that separated components could be re-combined without significantly changing the quantity of hydrogen produced by the laboratory rat. Hydrogen production was closely associated with the quantity of oligosaccharides remaining in the seed meal. However, the flatulence activity of the whole seed appears to be equally due to the indigestible oligosaccharides, and components of the cell-wall fiber.

INTRODUCTION

PRODUCTION of intestinal gas is one factor limiting the consumption of legume seeds by humans (Protein Advisory Group, 1973). This has nutritional implications throughout the world since, in many countries, legume seeds and cereals are the major sources of dietary protein (Bressani, 1972). Consumption of legume seeds by infants, children and the elderly is limited because of the difficulties associated with the expulsion of intestinal gases. In addition, the social embarrassment resulting from the expulsion of intestinal gas is a factor limiting consumption of legume seeds in the Western world (Murphy et al., 1972). Increasingly, processed legume seeds and seed components are used as functional ingredients in foodstuffs and as lower cost alternatives to traditional foods. Awareness of the flatulence activity of these alternative ingredients is helpful in predicting any impact on the flatulence activity of the processed food.

The quantity of hydrogen excreted by the laboratory rat has been shown to correlate highly with the quantity of flatus excreted by humans (Wagner et al., 1977). The laboratory rat provides a less expensive and less time-consuming alternative to using human subjects. Using the rat model, flatulence of the California small white bean appeared to be distributed between the indigestible oligosaccharides including raffinose, stachyose, and verbascose, and some unidentified component in the residue of seeds previously extracted with 70% aqueous ethanol (Wagner et al., 1976; Olson et al., 1975). Using seven types of legume seeds, hydrogen production was reported to be significantly and positively correlated to oligosaccharide and acid-hydrolyzable pentosan contents, but negatively correlated to starch and lignin contents (Fleming, 1981). Of these seed components, we know that the oligosaccharides are not digested by intestinal enzymes, but are fermented by the microorganisms within the large intestine to cause gas production. Therefore, we can speculate that the oligosaccharides and acid-hydrolyzable pentosans generally represent the flatulent fractions in legume seeds.

The smooth-seeded field pea has received considerable interest as a grain legume crop in the Prairie regions of Canada (Vose, 1977). This crop is agronomically superior to the soybean and horsebean (Warnock, 1976) for this geographical region and also is free of toxic constituents. Consequently, the agricultural industry has been encouraged to cultivate the field pea for livestock feed, and some processing industries have been established to process mature pea seeds for human consumption. These processing industries produce seed fractions selected to be rich in protein, starch or, in some cases, fiber. Previously, whole, smooth-seeded field peas were shown to be less flatulent than many beans (Fleming, 1981). This study was designed to evaluate the distribution of flatulence-causing components within the seeds of field peas. Fractionation studies were used to more accurately predict the flatulence activity of processed seed components.

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EXPERIMENTAL

Assay for hydrogen production by rats

The rate and total production of hydrogen by rats was determined by the procedure of Gumbmann and Williams (1971) and modified by Fleming (1980). In particular, rats were housed in individual, gas-tight chambers. At hourly intervals, gas samples were removed and analyzed for hydrogen concentration using gas chromatography. A typical plot of hydrogen concentration taken over a 12-hr time interval is represented in Fig. 1. The "maximum rate" of hydrogen production is calculated from the region of greatest slope on the curve; the total "accumulation" of hydrogen is taken at the time when the slope of the curve returns to the rate at which hydrogen accumulates when the rat is fed a basal diet. Values calculated by these two measures are reported.

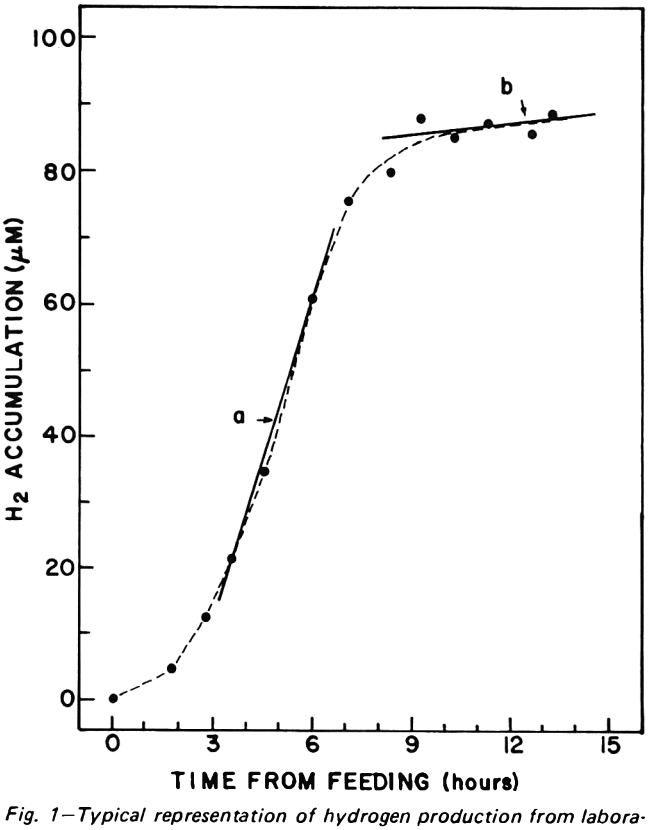


Fig. 1—Typical representation of hydrogen production from laboratory rats fed a legume seed diet.

During evaluations, 5-wk old male Wistar strain rats (Canadian Breeding Farm and Laboratories Ltd., St. Constant, Quebec) were fasted for 12 hr and then were fed 3.0g of test substances alone or combined with basal diet. Rats were evaluated over 12-hr periods and at 7-day intervals as previously described (Fleming, 1981). In most cases, ten diets were evaluated in three replicates per week for a total of 6 wk so that a 10 x 6 factorial design with three replicates was used to determine statistical significance of the results. Differences were determined by Duncan's multiple range test (Duncan, 1955). When not being evaluated, the animals received the basal diet ad libitum. The basal diet consisted of 55% wheat starch, 20% ANRC casein (Humko Sheffield, Memphis, Tennessee), 8% corn oil, 5% cellulose (Alphacel, ICN Pharmaceuticals Inc., Montreal, Quebec), 4.5% AIN mineral mix 76 (ICN Pharmaceuticals, Inc.) 1.5% AIN vitamin mix 76 (ICN Pharmaceuticals, Inc.), 0.2% choline bitartrate, 0.3% DL-methionine (ICN Pharmaceuticals, Inc.) and 5.5% sucrose.

Preparation of test diets

Mature, dry smooth-seeded field pea (*Pisum sativum* L. var. Trapper) seeds were obtained from a local supplier. Whole seeds or fractions were prepared for evaluations.

Whole seeds were prepared by soaking in distilled water (seed: water weight of 1:4) at 2°C for 16 hr and autoclaving at 121°C for 15 min. The pulp and liquor were cooled, ground and freeze dried. The dried meal was combined with basal diet and evaluated at doses of 1.0, 2.0, and 3.0g of field pea meal.

Seeds were fractionated on a pilot-plant scale to prepare products rich in cell-wall fiber and starch. In particular, dehulled seeds were ground by a wet-milling process in a single disc mill (Bauer Brothers Co., Springfield, IL) to give a slurry. Cell-wall fiber was separated on a horizontal vibratory screen (Sweco Separator, Southwestern Engineering Co., Los Angeles, CA). The fiber fraction was dried on a drum drier. The starch was removed from the remaining slurry, and purified by repeated centrifugation in a basket (32 cm diam) centrifuge (Fletcher Works Inc., Philadelphia, PA) operated at 1600 x *g*. A final purification was effected by use of a counter-current water wash utilizing a block of 10 nylon, 1 x 10 cm liquid cyclone units (Dorr-Oliver Inc., Stanford, CT). The starches were dried in a direct gas-fired spray dryer (Proctor Schwartz Corp., Philadelphia, PA) at an outlet temperature of 90°C.

Table 1—Proximate composition of legume seeds and seed components (moisture-free basis)

Seed or component	Crude protein (N x 5.7)%	Crude fat (%)	NDF (%)	Ash (%)
Field pea seeds	19.04	1.52	7.36	3.49
Components of field peas:				
Cell-wall fiber	15.90	–	10.97	–
Starch	0.30	0	5.61	0.08

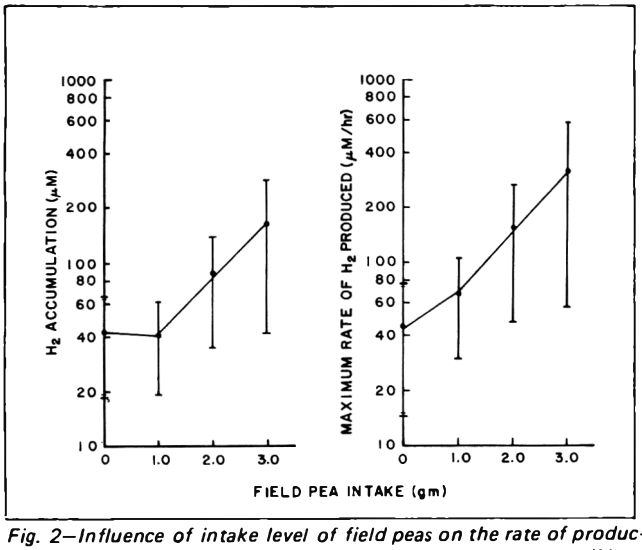


Fig. 2—Influence of intake level of field peas on the rate of production and accumulation of hydrogen gas by the laboratory rat (Mean ± 1 S.D.).

Oligosaccharides were removed from the seed meals by exhaustive extraction with hot (60–70°C), 80% aqueous ethanol (ethanol:H_2O of 80:20). The extracts and residues were freeze dried and weighed to determine the yield of each fraction. Oligosaccharides were also extracted to varying degrees by using a boiling-soaking method similar to that described by Olson (1978). In particular, whole seeds were boiled in water (1:4, 0–4 hr) and soaked (0–24 hr) in combinations designed to extract progressively more oligosaccharides. The seeds and liquor were separated, dried and analyzed for proximate composition and oligosaccharides. Residues and extracts were evaluated for their abilities to induce hydrogen production by laboratory rats.

Chemical analyses

Proximate principles including fat and ash were determined by AOAC (1975) procedures and neutral detergent fiber was quantified by AACC approved method 32–20 (1977). Nitrogen content was determined using a CHN Analyzer (Hewlett Packard 195B). Protein was calculated as %N x 5.7. Starch was analyzed by a modification of the dual enzyme semi-micro method (Banks et al., 1970) using α-amylase and glucoamylase (Tenase and Diazyme, respectively, purchased from Miles Laboratories Inc., Elkhart, IN). Oligosaccharides were identified using the gas chromatography method previously described (Fleming, 1981) and were quantified using an electronic integrator.

RESULTS & DISCUSSION

Proximate composition of legume seeds and their components

Fractionation of the field pea seeds provided components widely differing in composition (Table 1). The cell-wall fiber fraction was rich in neutral-detergent fiber (NDF) and ash whereas the starch fraction contained starch and small quantities of NDF. The starch content of the fiber fraction was determined to comprise 49%.

Hydrogen production in response to intake of field peas

Field peas fed in 1.0-g increments from 0–3.0g per dose provided an increase in hydrogen production by the laboratory rat (Fig. 2). Essentially, a straight-line logarithmic relationship was observed for both the maximum rate of production and total accumulation of hydrogen. These results are somewhat different than those reported by Gumbmann and Williams (1971). California small white beans were fed at doses ranging from 1–10g and those workers reported mostly a linear relationship. However, the shape of the curve at low intakes was indeterminate and may have been an exponential function if expanded. The nature of this relationship is of importance when feeding seed fractions and when making meaningful comparisons. In order to maintain some consistency, seed fractions were fed at the level determined to be present in 2.0g of whole seeds, and basal diet was added to provide 3.0g total feed in each case. This design made it unnecessary to transform data, and the 3.0g of feed ensured complete consumption within a short time period.

Influence of the fractionation of field pea seeds on hydrogen production

Efforts to identify the seed components responsible for hydrogen production in rats or flatulence in humans have been made by seed fractionation. However, in order for these studies to yield meaningful results, two criteria must be satisfied. The separated fractions must be able to be quantifiably reassembled to provide no measurable loss or gain in activity. Secondly, components must be able to be tested independently to provide meaningful results even though there may be synergistic relationships.

Field pea seeds were fractionated and reassembled to evaluate the resulting activity in two separate studies (Table 2). In study #1, the recombined fractions following alcoholic

extraction gave values which were lower but not statistically different from values of the unfractionated field pea diet. One must question whether these are truly equivalent. However, the sum of the rates of hydrogen produced for the solubles and residues fractions was 22.84 μmoles/hr following alcoholic extraction, and 21.64 μmoles/hr following aqueous extraction compared to 23.41 μmoles/hr for the unfractionated treatment. These results were verified in study #2 since the recombined fractions generally provided values similar to the unfractionated treatments. We can conclude that the processes of fractionation did not alter the hydrogen-producing potential of the components of field peas.

The two fractionation processes were primarily designed to separate the oligosaccharides from the residue. Neither process effected the sole removal of these sugars. In fact, the solubles fraction contained all mono- and disaccharides, and varying proportions of protein and ash. However, at least 85% of the protein, most of the starch and virtually all of the other complex carbohydrates remained in the residue. From these data (Table 2), it appears that the two fractions caused equal quantities of hydrogen to be produced.

Using the aqueous boiling and soaking technique, processing conditions were chosen to effect incremental extractions of oligosaccharides from field pea seeds. In addition, 5–15% of protein and 25–45% of ash were simultaneously removed from the seeds. No analyses were conducted for losses of B-vitamins.

Each data point on Fig. 3 represents a separate set of extraction conditions, and the quantity of hydrogen excreted by the rat when the residue fraction of that extraction was fed. The best-fit curves indicate a positive relationship between oligosaccharide content and hydrogen excretion. Although the standard deviations were large, the quantity of oligosaccharides (degrees of polymerization of 3–5) remaining in the seed and, therefore, ingested was highly correlated with rate of hydrogen production (r = +0.89). This same relationship was observed by Olson (1979) when California small white beans were extracted using a similar method.

It is interesting to note that the oligosaccharide-free field pea residue gave somewhat higher values than the basal diet which was free of all field pea constituents. In particular, the basal diet and oligosaccharide-free diet gave values for rate of hydrogen production of 3.81 and 8.06 μmoles/hr, respectively, and values for accumulated hydrogen of 31.47 and 52.75 μmoles, respectively, One-way analysis of variance (n = 18 for each diet) showed statistical significance between these two diets (F-values significant at $P < 0.05$). These data, and that presented in Table 2, indicate that removal of the oligosaccharides did not eliminate flatulence from the field pea. Therefore, some component of the residue must be indigestible by the rat and fermented by the gut microorganisms to produce gases. Olson (1979) has noted this same phenomenon for the California small white bean.

To further evaluate this observation, the cell-wall fiber of the field pea was concentrated for evaluation. Although not significantly different from the basal diets, the fiber caused nearly a twofold increase in hydrogen production (Table 3). The large degree of variability among rats must be reduced in order to differentiate values of this magnitude. A different experimental design may be helpful, or manipulation of the rats' environment may reduce diurnal fluctuations. The aqueous alcoholic extract, rich in oligosaccharides,

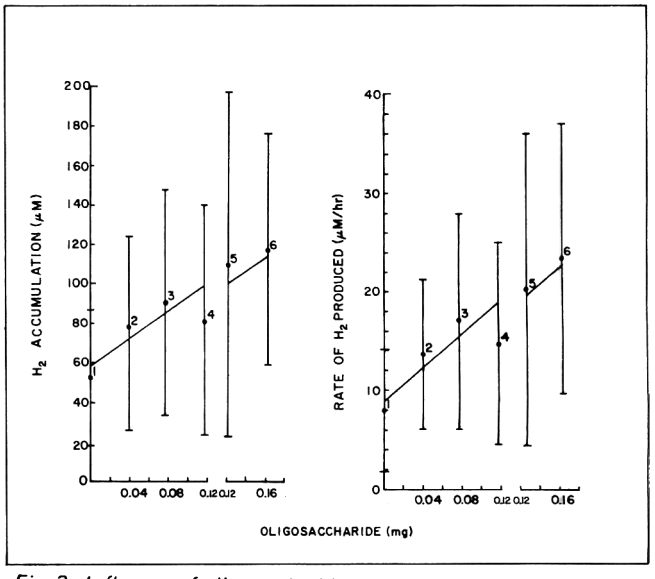


Fig. 3—Influence of oligosaccharide removal on hydrogen production when feeding the residual field pea meal (Sample 1 was extracted with 80% aqueous ethanol to effect complete removal of oligosaccharides; samples 2–5 were water-extracted to effect incremental extraction of oligosaccharides; sample 6 represents the whole seed meal. The oligosaccharide intake represents the quantity remaining in the residue fraction from 2.0g of seed).

Table 2—Influence of seed fractionation and reconstitution on hydrogen production

Diet Fraction	Extraction medium	Hydrogen production: Maximum rate (μmoles/hr) Study No. 1[a]	Maximum rate (μmoles/hr) Study No. 2[b]	Accumulation (μmoles) Study No. 1[a]	Accumulation (μmoles) Study No. 2[b]
Basal	–	3.81d[c]	5.67c	31.5c	47.5c
Field pea[d]					
Unfractionated	None	23.4ab	35.7ab	117.7a	171.4b
Solubles	80% aq ethanol	14.8bc		77.1ab	
Residue	80% aq ethanol	8.06cd		78.7ab	
Solubles & Residues	80% aq ethanol	13.6b-d	34.7ab	81.6ab	244.9a
Solubles	Water	7.99cd		65.9bc	
Residue	Water	13.6b-d		78.7ab	
Solubles & Residue	Water		44.1a		173.1b
$s_{\bar{x}}$		3.25	5.33	13.4	24.4

[a] Significance of differences were determined using a 10 x 6 factorial design with 3 replicates. The data reported represent only a portion of the total experiment.
[b] Significance of differences were determined using a two-way analysis of variance with diets and animals as variables. The data reported represent only a portion of the total experiment.
[c] Means connected by common lower case letters are not significantly different at $P \leq 0.05$.
[d] Values were determined for consumption of 2.0g field pea flour or, in the case of seed components, the quantity determined to be present in 2.0 g field peas. Sufficient basal diet was provided to total 3.0g.

Table 3—Fractionation of the carbohydrates in field pea seeds and their contribution to hydrogen production

Diet[a]	Hydrogen production	
	Maximum rate (μmoles/hr)	Accumulation (μmoles)
Basal	6.0d[b]	44.1d
Basal + fiber[c]	11.8cd	70.1cd
Basal + fiber + alcohol solubles[d]	30.7b	146.4b
Basal + fiber + alcohol solubles +H_2O-solubles[e]	24.1bc	133.1bc
$s_{\bar{x}}$ (70 error df)	5.80	25.6

[a] Field pea fractions were evaluated at quantities determined to be present in 2.0g field peas. Sufficient basal diet was provided to total 3.0g.
[b] Differences were determined using a two-way analysis of variance with diets and animals as variables. The data reported represent only a portion of the total experiment. Means connected by lower case letters are not significantly different at $P < 0.05$.
[c] Fiber represents the cell-wall fraction isolated by use of the Sweco vibratory screen.
[d] The fraction removed by exhaustive extraction with 80% aqueous ethanol, 60°C.
[e] The fraction removed by exhaustive extraction with water at 60°C, following extraction with 80% aqueous ethanol.

caused a statistically significant increase in hydrogen production when combined with the cell-wall fiber. Addition of the remaining water-soluble materials, rich in pentosans, pectins and water-soluble glucans and proteins, caused no further increase in hydrogen production. These data indicate that of the water-soluble carbohydrates, only the oligosaccharides contribute to hydrogen production by rats.

CONCLUSIONS

FLATULENCE ACTIVITY was predicted by measuring hydrogen production by laboratory rats. The flatulence activity of smooth-seeded field peas appears to be equally due to indigestible oligosaccharides and cell-wall fiber constituents. The oligosaccharides include raffinose, stachyose and verbascose, and have been claimed to cause flatulence in other legume seeds. In particular, these components have been claimed to be solely responsible for the flatulence in soybeans. The cell-wall fiber constituents of legume seeds have not been well identified. However, the water-insoluble fraction appears to be the major contributor to flatulence in the field pea. Further enzymatic and hydrolyses procedures may be helpful in elucidating the nature of these carbohydrates.

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Isolation and Identification of Objectionable Volatile Flavor Compounds in Defatted Soybean Flour

OLIVER A. -L. HSIEH, A. -S. HUANG, and STEPHEN S. CHANG

ABSTRACT

The volatile flavor constituents of defatted soy flour were isolated by a specially designed apparatus. The isolated volatiles were fractionated by gas chromatography. The fractions with characteristic beany, grassy, and green odors were identified by infrared and mass spectrometry. A total of 25 compounds was identified, including nine alcohols, six aldehydes, nine ketones, and 2-pentyl furan. Among the identified compounds, 2-pentyl furan and ethyl vinyl ketone are probably the key compounds for the beany and grassy odors of the soy flour. All the compounds identified in this study can be postulated as autoxidative decomposition products of soy lipids. Therefore, the presence of these compounds in soy flour might be due to the incomplete removal of lipids in soy flour.

INTRODUCTION

SOYBEANS are potentially the most abundant and economical source of food proteins. In order to develop new food items with soybean protein products, it is desirable to first eliminate the objectionable flavor of soybean protein products in order to obtain a truly bland base. The desirable flavor for the food item can then be added. There are two major groups of objectionable flavors in soy protein products. One is the volatile compounds responsible for the green, beany, and grassy odors, and the other is the nonvolatile compounds responsible for the bitter and astringent tastes.

A number of investigators have reported on the volatile flavor compounds identified from raw soybean (Arai et al., 1966b; Arai et al., 1967; Badenhop and Wilkens, 1969; Mattick and Hand, 1969; Honig and Rackis, 1975), soybean milk (Wilkens and Lin, 1970), soy flour (Fujimaki et al., 1965; Arai et al., 1966a; Sessa et al., 1969), soy protein concentrate (Arai et al., 1970), and soy protein isolate (Qvist and von Sydow, 1974). The present paper reports the isolation and identification of volatile flavor compounds from a defatted soy flour. The study of the nonvolatile flavor compounds will be reported in a separate paper.

EXPERIMENTAL

Material used

Four commercially available defatted soy flours, two nontoasted and two toasted, were submitted to sensory evaluation by a trained eight-member panel. The samples were made into 2% dispersions in distilled water. Fifty ml of each of the samples were served to the panels in a 70 ml weighing bottle which was kept inside holes drilled in a heated aluminum block. The odor strength, odor preference, flavor strength, and flavor preference of each sample were evaluated at 60°C. A scale of 1–9 was used for each of these properties. The higher score indicated either the higher strength or the higher preference. Odor response included beany, bitter, green chalky, musty, corn meal, and toasted. The flavor description included all the above, plus astringent. One of the nontoasted soy flour samples which had the strongest objectionable odor and flavor, as determined by sensory evaluation, was used for this study.

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Isolation of volatile flavor compounds (VFC)–continuous process

The apparatus described by Herz and Chang (1966) was used. It utilizes the principle of flash evaporation followed by vaporization from a continuous thin heated film under vacuum. The soy flour was made into a water slurry at the ratio of 7:1 (water:flour) using a Waring Blendor. The slurry was maintained at room temperature in a reservoir and fed into the apparatus at 2 liters per hour. The temperature of the water in the heating jacket was 75°C. The whole system was evacuated to a vacuum of 0.3–0.5 Torr. A total of 50 lb of soy flour was used. The distillate collected in the cold traps was combined, saturated with reagent grade sodium chloride, and extracted with anhydrous ethyl ether. The ethyl ether extract was first concentrated to a volume of 50 ml by the use of a 30-plate Oldershaw distillation column and finally concentrated to approximately 5 ml. by the use of a spinning band still (Kontes Glass Co., Vineland, NJ). During distillation the ether extract was maintained at 40–45°C and the column at room temperature.

Isolation of VFC–Batch process

The apparatus used is shown in Fig. 1. It utilized the slow evaporation of FVC from soy flour at an elevated temperature under vacuum. Five pounds of soy flour were put into each of the 12 liter flasks (A-1, A-2, and A-3) which were maintained at 50–60°C. in water baths. Trap B at room temperature was designed to retain a trace amount of the fine soy flour powder. The traps (C-1 through C-5) were cooled with a slurry of dry ice and acetone. The whole system was slowly evacuated to a vacuum of 0.01 Torr. Gradual increase of vacuum was necessary and it was achieved by decreasing the leakage through the flow meter (E). A trap (D) cooled with dry ice and acetone was used to remove any volatile materials from the air. The ultimate vacuum was reached after 3 hr of pumping. This slow evacuation prevented soy flour in the sample flasks from distrubance during isolation. The condensates collected in the cold traps after 1 wk were combined. The soy flour after the treatment did not yield any rancid odor. A total of 120 lb of soy flour was used.

The ethyl ether extract of the condensate was concentrated to a final volume of 5 ml in the same manner as the continuous process.

Sensory evaluation of the VFC

The odor of both the ethyl ether extracts of the VFC isolated by the continuous process and the batch process were evaluated by a trained four-member sensory panel. A drop of the extract was put on the tip of a perfumers' paper stick. After the ethyl ether had evaporated, the odor of the VFC was sniffed. Both extracts had the characteristic beany and grassy odor except that the VFC isolated by the batch process had a stronger odor. Since they both had the target odors and since they both were isolated from the same soy flour, they were combined to increase the amount of VFC in order to facilitate the identification.

Gas chromatographic fractionation of VFC

The VFC isolated from the soy flour was first separated into 25 broad fractions with the use of a Varian Aerograph 202 fitted with a 12 ft. x 1/8 in. O.D. stainless steel column packed with 10% OV-17 on 70/80 mesh Chromosorb W, AW, DMCS, and a thermal conductivity detector (Fig. 2). The helium flow rate was 30 ml/min and the column temperature was programmed from 40° to 150°C at a rate of 2°C/min, then from 150° to 230°C at a rate of 4°C/min. The injection was repeated until the sample was exhausted. Each frac-

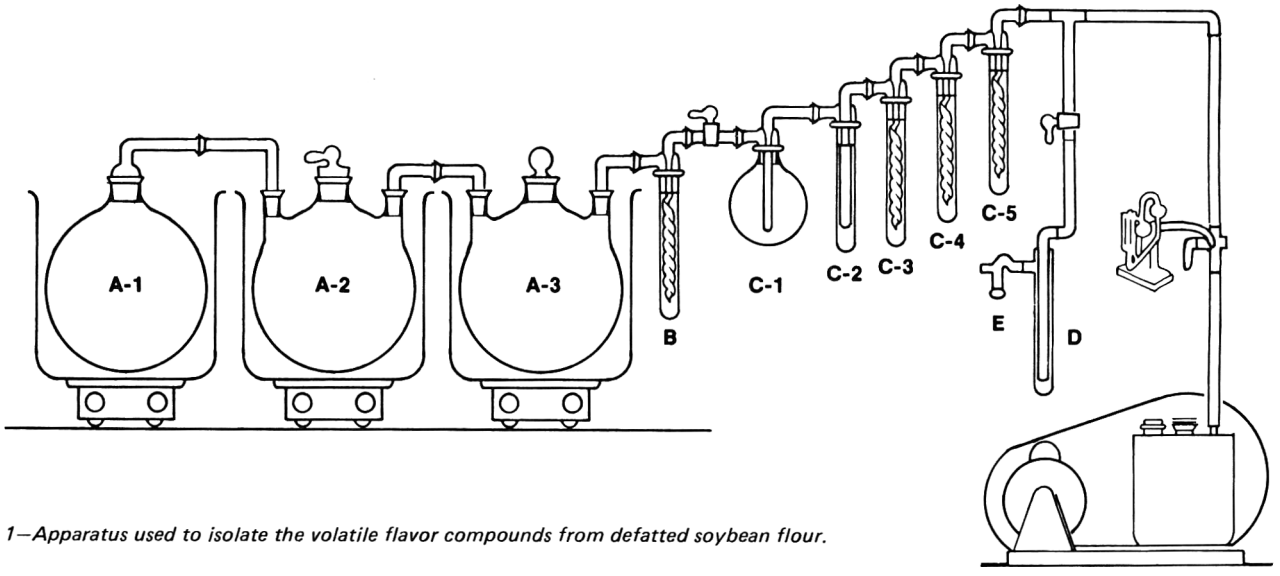


Fig. 1—Apparatus used to isolate the volatile flavor compounds from defatted soybean flour.

tion was accumulatively collected in a "hairpin" cold trap according to the method of Thompson et al. (1978). Fractions 5–15, which had the characteristic beany and grassy odors were rechromatographed to obtain subfractions using a Perkin-Elmer SIGMA 3 gas chromatograph which was equipped with a 10:1 column effluent splitter and a flame ionization detector. A 15 ft x 1/8 in. o.d. stainless steel column packed with 10% SP-1000 on 80/100 mesh Chromosorb W, AW, DMCS was used. The helium flow rate was 30 ml/min. and the column temperature was programmed in a manner to obtain resolution of each fraction. Some of the subfractions which had the characteristic beany and grassy odor of soy flour and which had a sufficient amount of sample, were further rechromatographed for a third time with the Perkin-Elmer SIGMA-3, using a 12 ft x 1/8 in. o.d. stainless steel column packed with 10% OV-101 on 80/100 mesh Chromosorb W, AW, DMCS.

Identification of the gas chromatographic fractions

The pure gas chromatographic fractions were analyzed by infrared and mass spectrometry. The infrared spectra were recorded on a Beckman Aculab-4 spectrophotometer with micro-attachments. The mass spectra were analyzed by a Du Pont Model 21-490 mass spectrometer with a jet separator interfaced with a Varian Moduline 2700 gas chromatograph. A 12 ft x 1/8 in. o.d. stainless steel

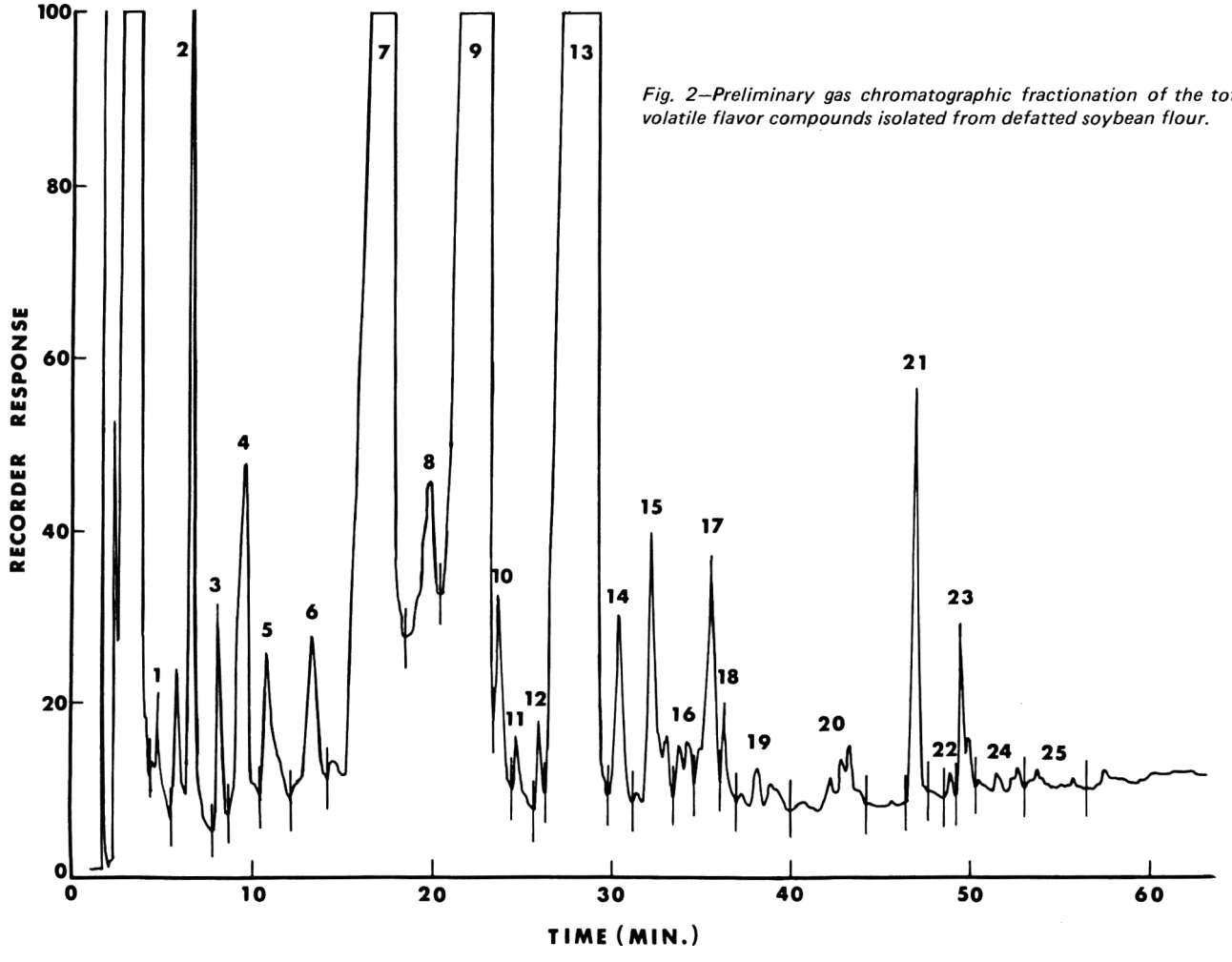


Fig. 2—Preliminary gas chromatographic fractionation of the total volatile flavor compounds isolated from defatted soybean flour.

column packed with 10% OV-101 on 80/100 mesh Chromosorb W, AW, DMCS was used. The helium flow rate was 30 ml/min and the column temperature was programmed to provide maximum resolution of each fraction. Conditions for the mass spectra scans were: ionization voltage: 70 eV; ion source pressure: $<10^{-6}$ Torr; ion source temperature and jet separator temperatures were at 270°C and 250°C, respectively.

RESULTS & DISCUSSION

THE GAS CHROMATOGRAPHIC fractions of the VFC isolated from a defatted soy flour were identified by infrared and mass spectrometry. From the fractions with beany, grassy and green odors, a total of 25 compounds was identified as shown in Table 1. Nine of these compounds have never been reported before in soy protein products.

Among the identified compounds, 1-pentanol, 1-hexanol, 1-octen-3-ol, and hexanal, were represented by relatively large gas chromatographic peaks. Pentanol and hexanol were identified in raw soybean and soybean milk by Arai et al. (1967), and Wilkens and Lin (1970), respectively. Hexanol which possesses a harsh grassy odor at high concentrations, has been described as one of the alcohols contributing to the green-beany odor of raw soybeans. Goosens (1974), in a review of soy protein food, indicated that among the alcohols a little ethanol will not ruin the flavor of soy, but some pentanol, hexanol, and 1-octen-3-ol will do so. Badenhop and Wilkens (1969) reported that significant quantities of 1-octen-3-ol were produced during the soaking of soybeans in water as a pretreatment for soy milk manufacture. They also concluded that the mode of formation of 1-octen-3-ol, in this case, was enzymatic. The preparation of a water slurry of soy flour for the continuous VFC isolation process might contribute to the large quantity of 1-octen-3-ol, in the total VFC of soy flour.

Table 1—Compounds identified in the volatile flavor compounds isolated from defatted soy flour.

Fraction[a] no.	Identified as	Identified By IR	Identified By MASS
	Alcohols		
7-4-1	3-Methyl butanol[b]	x	x
7-6-1	1-Pentanol	x	x
9-5-2	1-Hexanol	x	x
7-4-2	2-Hexanol	x	x
7-4-2	3-Hexanol[b]	x	x
9-5-1	t-2-Hexen-1-ol[b]	x	x
13-9-1	t-2-Hepten-1-ol[b]	x	x
13-7-2	1-Octen-3-ol	x	x
13-9-6	2-Octen-1-ol	–	x
	Aldehydes		
5-2	Pentanal	x	x
7-2	Hexanal	x	x
9-4-3	t-2-Hexenal	x	x
9-3-3	Heptanal	–	x
15-7-1	2, 4-Heptadienal	x	x
15-4-2	t-2-Octenal	x	x
	Ketones		
5-3	Ethyl vinyl keton	x	x
9-3-1	2-Methyl cyclopentanone[b]	x	x
14-4-2	Methyl ethyl cyclopentanone[b]	x	x
9-4-5	3-Methyl-2-cyclopentenone[b]	x	x
9-3-2	2-Heptanone	x	x
13-5-7	2,6-Dimethyl-3-heptanone[a]	x	x
13-3-1	3-Octanone	x	x
15-3-2	3-Octen-2-one[b]	x	x
13-4-1	Amyl vinyl ketone	x	x
	Furan		
12-4-2	2-Pentyl furan	x	x

[a] The first, second and third numerals indicate the position of the GC fraction during the first, second and third chromatographies, respectively.
[b] First reported in soy protein products

The compound, 3-octen-2-one, was synthesized according to the method of Sheikh et al. (1970) and the mass spectrometric identification of this compound was confirmed. This compound was found to have an odor threshold of 10 ppm in water (50% panel detectable). It had a pungent, sandalwood odor at high concentration, while at low concentration (10 ppm in water) it had a green, grassy note.

Hexanal is known to be found in the volatiles of all soy bean-related products. Fujimaki et al. (1965), reported that n-hexanal in raw soybean amounted to approximately 10 ppm which is, owing to its extremely low flavoring threshold, likely to be one of the main components of green bean flavor. Wilkens and Lin (1970) also reported that hexanal comprised about 25% of the volatiles of soybean milk. Sessa et al. (1969) reported that hexanal, acetaldehyde, and acetone represented the major volatile carbonyl compounds in both full-fat and defatted soybean flakes. Arai et al. (1970) studied the mechanism of formation of hexanal in soy protein concentrate and concluded that the formation of hexanal during the process of soy protein concentration was due mainly to lipoxygenase. They also stated that n-hexanal content in defatted soy flour might be attributed in part to the lipoxygenase activity.

The key compounds responsible for the beany, grassy and green odors in soy flour might be ethyl vinyl ketone and 2-pentyl furan. Ethyl vinyl ketone with its odor threshold as low as 0.02 ppm in oil (Evans et al., 1971) has been reported by Mattick and Hand (1969) as being responsible for the greenbean odor and flavor in soaked soybean volatiles. This compound was also isolated from soybean oil in the early stages of autoxidation by Hill and Hammond (1965). 2-Pentyl furan was first reported by Chang et al. (1966) as being responsible for the beany, grassy flavor of reverted soybean oil. It was later also isolated from soybean oil milk (Wilkens and Lin, 1970) and commercial soy protein isolate (Qvist and von Sydow, 1974). In a review, Sessa and Rackis (1977) suggested that 2-pentyl furan, 3-*cis*-hexenal, and ethyl vinyl ketone be considered key flavor compounds for the beany character of soybeans and reverted soybean oil. However, 3-*cis*-hexenal was not positively identified in this study.

Three cyclic ketones, namely, 2-methyl cyclopentanone, methyl ethyl cyclopentanone, and 3-methyl-2-cyclopentenone, were identified. They might be autoxidative decomposition products from linolenic acid through the mechanism of formation of endoperoxides which are formed in a free radical cyclization process proposed by Pryor et al. (1976).

All the compounds identified in this study can be postulated as autoxidative decomposition products of soy lipids. Therefore, the presence of these compounds in soy flour might be due to the incomplete removal of lipids in soy flour.

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Characterization of the Nonvolatile Minor Constituents Responsible for the Objectionable Taste of Defatted Soybean Flour

AN-SHUN HUANG, OLIVER A. -L. HSIEH, and STEPHEN S. CHANG

ABSTRACT

A scheme was developed for the separation of the nonvolatile minor constituents from soy flour which are responsible for the astringent an bitter tastes. The scheme involved solvent extraction, freeze-drying, and fractional crystallization. Two fractions, collected by semipreparative HPLC, had the characteristic bitter and astringent tastes. Further fractionation by reverse phase HPLC produced three pure compounds which were identified by UV, NMR spectrometry and elemental analysis, as daidzein, glycitein 7-β-O glucoside, and genestin. Preliminary sensory evaluation showed that these isoflavones might contribute additively to the undesirable bitter and astringent tastes of soy protein products.

INTRODUCTION

SOY PROTEIN PRODUCTS which can be produced in vast amounts with good functionality for uses in foods are a potential solution to world hunger. However, the incorporation of soy flour, concentrate, isolate, and textured products into foods is hindered by their bitter and astringent tastes.

Arai et al. (1966) and his co-workers identified a series of nine pheolic acids from defatted soy flour. These phenolic acids may have some influence on soybean flavor since they possess sour, bitter and astringent tastes. Honig et al. (1971), isolated ethyl-α-D-galactopyranoside and L-tryptophan as bitter components in soy flakes produced by hexane-ethanol extraction. Evidence showed that galactoside was an artifact formed during extraction.

In 1976, Sessa et al., isolated three oxidized phosphatidylcholines which all possessed keto and hydroxy fatty acid esters. Based upon sensory evaluation and flavor threshold, they concluded that oxidized phosphatidylcholines were largely responsible for the bitter taste of soy protein products. Further experiments suggested that a bound oxidized fatty acid in the lecithin molecule, instead of the phosphocholin moeity is responsible for the bitter taste (Sessa, 1977).

This paper reports an attempt to characterize the minor constituents of soy flour which are responsible for its bitter and astringent tastes.

EXPERIMENTAL

Material used

Four commercially available defatted soy flours, two nontoasted and two toasted, were sensory evaluated by a trained eight-member panel. The samples were made into 2% dispersions in distilled water. Their odor strength, odor preference, flavor strength, and flavor preference were evaluated at 60°C. In order to maintain the sample temperature during the evaluation period, the samples were served to the panelist in the glass weighing bottles which were set in holes especially drilled into a heavy aluminum block preheated to 60°C. A scale of 1 to 9 was used for the scoring of both strength and preference. The higher score indicated either the higher strength or the higher preference. The odor response included beany, bitter, green, chalky, musty, corn meal, and toasted. The flavor description included all the above, plus astringent. One of the nontoasted soy flours which had the strongest objectionable odor and flavor, as determined by sensory evaluation, was used for this study.

Isolation and fractionation of nonvolatile minor constituents with objectionable taste

The procedures used for the isolation and fractionation of the nonvolatile flavor constituents with objectionable tastes are outlined in Fig. 1.

Nontoasted, defatted soy flour (1.5 kg) was extracted with 7500 ml of 60% aqueous ethanol in a 5-gal stainless steel tank. The soy flour slurry was constantly stirred with a TALBOY 101 stirrer at room temperature for 2 hr. After standing at −20°C for 24 hr, the ethanol slurry was filtered through Whatman No. 1 filter paper and the residue was discarded. The filtrate was concentrated to approximately 1200 ml (I) with a Buchler rotary evaporator under reduced pressure (approx 27 mm Hg) at a temperature not exceeding 40°C.

Two hundred ml of the extract (I) were extracted in a 600 ml separatory funnel successively with two 100 ml portions of ethyl ether. This procedure was repeated six times in order to process all 1200 ml. of the aqueous extract. The aqueous phase (III) containing the nonlipid fraction was collected. The ether phase (II) containing the lipid fraction was freed from solvent with a rotary evaporator under vacuum, and was dissolved in 70 ml of hexane (Baker Analyzed Reagent, J.T. Baker Chemical Co., Phillipsburg, NJ). The hexane solution was poured slowly into 350 ml of 0°C acetone (Baker Analyzed Reagent, J.T. Baker Chemical Co., Phillipsburg, NJ) with constant stirring. The precipitate, after filtration, was washed twice with two 35 ml portions of 0°C acetone.

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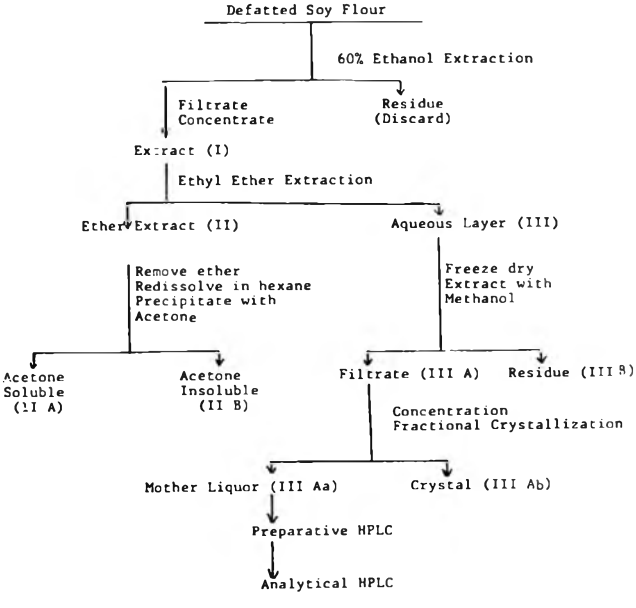


Fig. 1—Isolation and fractionation of defatted soy flour.

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The acetone filtrates and washings, both containing the neutral lipid fraction, were combined and freed from solvent to yield fraction IIA. The precipitate which contained polar lipids, after being freed of acetone, was stored in an amber glass bottle under nitrogen at −20°C (Fraction IIB).

The aqueous layer (III) was freeze-dried with a LABCONCO® FREEZE-DRY 12 freeze-drier (Labconco Corp., Kansas City, MO). The freeze-dried solids were extracted with ten times their weight of methanol at room temperature for 2 hr. The mixture, after being stored at −20°C for 24 hr, was filtered through Whatman No. 1 filter paper. The methanol insoluble residue (IIIB) was dis carded after organoleptic evaluation. The filtrate (IIIA) was submitted to fractional crystallization by reducing the methanol/sample ratio to 1:1 with a rotary evaporator. After sorting the solution at −20°C. for 24 hr, the white crystals formed were removed by filtration. The mother liquor (IIIAa), after concentration, was diluted to 42 mL with methanol for further fractionation.

Sensory evaluation

All fractions obtained above, after being carefully freed from solvent with a mechanical vacuum pump, or after freeze-drying, were tasted for bitter and astringent flavor by a sensory panel constituting three experienced persons. The water soluble fraction was made into a 2% solution in distilled water and sensory evaluated. The water insoluble fraction was put on the tongue of the panel members, together with 2 ml of warm, distilled water. The mouth was thoroughly rinsed with warm water before and after tasting each sample.

HPLC fractionation of fraction IIIAa

Fraction IIIAa which was identified by the sensory panel as having a bitter and astringent taste was further fractionated by preparative HPLC with a Waters 6000A high performance liquid chromatograph (Waters Associates, Inc., Milford, MA) equipped with an M-660 solvent programmer and an M-440 UV absorbance detector. The conditions used in the preparative HPLC are given in Table 1. Thirty injections of 100 μl each were made for the preparative HPLC of Fraction IIIAa. The seven fractions obtained, P1 through P7, were individually and accumulatively collected. They were then freeze-dried and sensory evaluated.

Table 1—Conditions used for preparative HPLC of fraction IIIAa

Column:	Two 7.8 mm. i.d. x 61 cm. Stainless Steel columns, connected in series, packed with BONDAPAK C_{18}/PORASIL B; particle size: 37–75μ
Solvent:	A —Water, pretreatment with active carbon and PORAPAK^R Q column B —Methanol, absolute; "PHOTREX" reagent; J.T. Baker Chemical Co., Phillipsburg, N.J.
Gradient:	I —30% Solvent B isocratically over 38 min. II —30% B → 45% B, follows M-660 solvent programmer curve 10, over 10 min. III —45% B isocratically over 18 min. IV —45% B → 100% B, follows M-660 solvent programmer curve 6, over 5 min. V —100% B isocratically over 30 min.
Flow Rate:	—3.5 ml/min, A+B
Detector:	—UV 254 nm at sensitivity 1.0 a.u.f.s.

Table 2—Conditions used for HPLC of subfraction P34S

Column:	— μ BONDAPAK C_{18} Column
Solvent:	A — 75% water/25% methanol isocratically for 20 min B — 75% water/25% methanol to 50% water/ 50% methanol follows M-660 solvent programmer curve 6 over 20 min C — 50% water/50% methanol isocratically
Flow Rate:	— 1.0 ml/min, methanol + water
Detector:	— UV 254 nm at sensitivity 0.1 a.u.f.s.

The two HPLC fractions, P3 and P4, which were sensory evaluated as having bitter and astringent tastes, were combined and coded as P34. This fraction was extracted with twice its weight of methanol at room temperature. The methanol-soluble fraction (P34S) and the methanol-insoluble fraction (P34I) were each freed from solvent and tasted by the sensory panel.

Analytical HPLC of fraction P34S into pure compounds

Fractions P34I and P34S, after being freed from solvent under high vacuum, were redissolved in twice their weight of water and methanol, respectively. Both fractions were chromatographed with a BONDAPAK C_{18} column, according to the conditions listed in Table 2. Mixtures of Fractions P34I and P34S, at different ratios, viz 2:1, 1:1, and 1:10 (v:v), were then injected according to the conditions used for the chromatography of Fraction P34S.

The fraction with a strong bitter and astringent taste, P34S, was separated into five subfractions by analytical HPLC. One hundred and forty five injections of 5 μl each were made. Each subfraction, A through E, was individually and accumulatively collected for chemical characterization.

Identification of subfractions, P34S-C, P34S-D, and P34S-E

Elemental analysis. All elemental analyses were performed by Robertson Microanalytical Laboratory, Florham Park, NJ.

Color reaction. Samples were first individually spotted on Whatman 3MM chromatographic paper. The colors of individual spots were then observed by different methods which included: (a) observation under ordinary daylight; (b) ultraviolet light; (c) daylight after concentrated ammonia fume treatment; (d) ultraviolet light after concentrated ammonia fume treatment; and (e) ordinary daylight after ferric chloride solution treatment.

Ultraviolet absorption spectrometry. All spectra were measured on a Varian Techtron Model 635 spectrophotometer. The wavelength calibration of the spectrophotometer was carried out with a Holmium oxide filter which has λ max's at 279.3, 287.6, 333.8, and 360.8. Spectroscopic methanol was used as a reference and as a solvent. The effects of NaOMe, $AlCl_3$-HCl, and NaOAc on the UV spectrometer were examined as described by Mabry et al. (1970).

Nuclear magnetic resonance (NMR) spectroscopy. The sample was first converted into trimethylsilyl ether derivatives according to the procedure developed by Waiss et al. (1964). NMR spectra of trimethylsilyl ether derivatives of P34S-C, and P34S-E were determined with a Varian Associates Model T-60 NMR spectrometer.

RESULTS & DISCUSSION

Yield and flavor characteristics of preliminary fractions

The yield and flavor characteristics of the preliminary fractions of the soy flour are shown in Table 3.

The total crude thanol extract (I) of the soy flour had a bitter, sweet, and biting taste. Both its lipid (II) and nonlipid fractions (III) showed bitter and astringent notes. However, the nonlipid fraction possessed a much stronger bitter and astringent taste than the lipid fraction.

By acetone precipitation, the lipid fraction was separated into a polar lipid fraction (IIB) and a neutral lipid fraction (IIA). The neutral lipids possessed a weak bitter and astringent taste with oily and waxy characteristics. The polar lipid fraction, which included phosphatides, had a plastic and cornmeal-like taste. These results, therefore, cannot confirm the report that oxidized phosphatidylcholine is responsible for the bitter flavor of soy protein products (Sessa et al., 1974).

The methanol extract of the nonlipid fraction (IIIA) tasted bitter, astringent and sweet. After the removal of the sucrose crystals (IIIAb), fraction IIIAa had an intense bitter and astringent taste. It was further fractionated by HPLC.

HPLC fractionation of Fraction IIIAa

Fraction IIIAa with an intense bitter and astringent taste was fractionated by preparative HPLC into seven fractions (Fig. 2). A reverse phase C_{18} BONDAPAK column was chosen due to the high solubility of the sample in polar

solvents. Gradient elution was employed because Fraction IIIAa contained components with a wide range of polarity. The early separation was done isocratically at a high water concentration (70%). This relatively high polar mobile phase improved the separation between the early elution peaks (Region 1). Decreasing the water concentration progressively (down to 55% vol) reduced the fractionation time without sacrificing the resolution between the late elution peaks (Region III). In the final stage of fractionation, pure methanol was used to ensure that all the material was eluted out of the column (Region V).

The ultraviolet absorption detector used is a selective detector which does not respond to compounds without absorbance or with low absorbances at the monitoring wavelength used. Therefore, area collection, by which each succeeding fractional collection started right after the preceding fractional collection ended, was performed to cover the entire fractionation as shown in Fig. 2. Continuous area collection ensured that all components, which included compounds without absorbance at the monitoring wavelength, were collected for organoleptic evaluation.

The flavor characteristics of the seven fractions collected by HPLC are shown in Table 4. Both Fractions P3 and P4 had bitter and astringent tastes. Whether this was due to overlapping between the two adjacent fractions or to different bitter and astringent substances, was difficult to ascertain. Therefore, Fractions P3 and P4 were combined as Fraction P34 for further fractionation.

Analytical HPLC of Fraction P34

Methanol extraction of Fraction P34 yielded 57% of insoluble materials (P34I) and 43% of soluble materials (P34S). The methanol-soluble fraction tasted bitter and astringent, while the insoluble fraction did not (Table 5). Since Fraction P34 originated from Fraction IIIAa, which was methanol-soluble, these results indicated that fractionation of trace amounts of flavor constituents of soy flour depends not only upon the solvent employed, but also upon other factors, including concentration, temperature, and mutual solubility of other constituents.

Both Fraction P34 and Subfraction P34S were analyzed by an analytical reverse phase BONDAPAK C_{18} column under identical conditions. The chromatograms of P34 and P34S are shown in Fig. 3 and 4, respectively. By injecting several different mixtures of various ratios of P34 and P34S, the three major peaks, C, D, and E, in P34S were confirmed as the same components as peaks C, D, and E in Fraction P34. Using the same technique in comparing the chromatograms of P34I and P34S, small peaks A and B were confirmed as residual peaks from P34I. Since only P34S tasted bitter and astringent, Subfractions C, D, and E of P34S were collected and identified.

Table 3—Yield and flavor characteristics of the preliminary fractions of the extract of soy flour

Fraction	Yield(%)[a]	Flavor characteristics
I	6.4[b]	bitter, sweet, biting
II	2.3	oily, waxy, slight bitter and astringent
IIA	1.6	rye bread-like, oily, weak bitter and astringent
IIB	0.7	plastic, corn meal
III	4.1	bitter, sweet, mealy, weak astringent
IIIA	2.5	bitter, astringent, sweet
IIIAa	1.6	intense bitter, astringent lingering after taste
IIIAb	0.9	sweet
IIIB	1.6	mealy, hay-like

[a] Weight percentage based on starting material (1500g)
[b] Sum of Fraction II and Fraction III (by calculation)

Chemical characterization of the subfractions of P34S

The chemical structures of the three subfractions of P34S, as identified in this study, are shown in Fig. 5. They all imparted a bitter and astringent flavor when tasted. Due to the very limited amount of these samples obtained, they could not be sensory evaluated. All three isoflavones identified in this study have been previously identified in defatted soybean products or raw soybeans. However, they were studied because of their physiological and nutritional effects, particularly their estrogenic activity. No report could be found on the relationship of isoflavones with objectionable flavor of soybean products. Based upon the organoleptic evaluations in this study, these isoflavones, particularly, glycitein 7-β-O-glucoside which possess a herb-like astringency and bitterness, might contribute additively to the nonvolatile objectionable flavor of soy protein products. These isoflavones were considered to be naturally occurring components, originally existing in soybeans, which have not been removed during processing.

Compound P34S-C identified as Daidzein, 4′,7-dihydroxy isoflavone

Elemental analysis found C, 72.90; H, 4.18; and 0, 22.92, which suggested an empirical formula, $C_{15}H_{10}O_4$.

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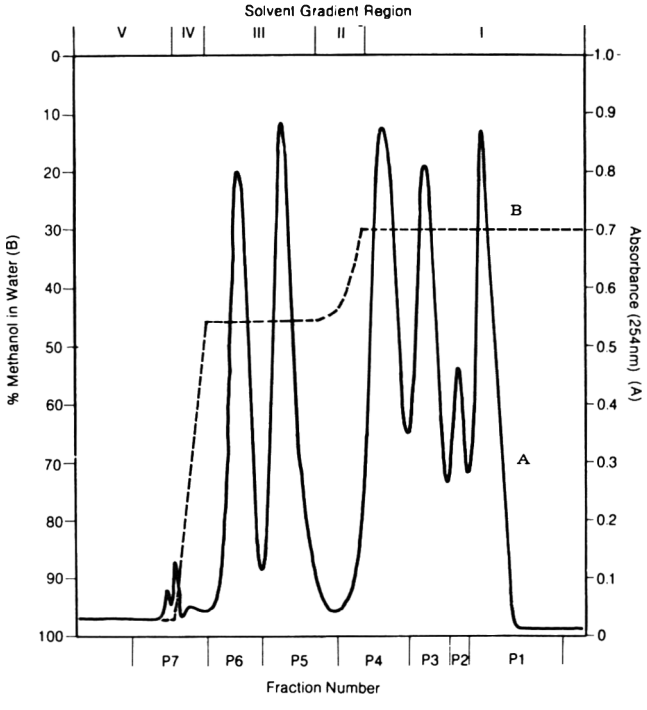


Fig. 2—Chromatography of the HPLC analysis of Fraction IIIAa.

Table 4—Flavor characteristics of the HPLC fractions of IIIAa

Fraction	Yield[a]	Flavor	Appearance
P1	15.7%	sweet, molasses	light yellow powder
P2	5.3%	woody, phenol-like	bright yellow powder
P3	17.5%	bitter, salty	light yellow powder
P4	24.0%	bitter, astringent	yellow green powder
P5	19.9%	woody, biting	light brown powder
P6	17.1%	tasteless	grey powder
P7	1.5%	tasteless	light brown powder

[a] Weight percentage based on Fraction IIIAa

These values are close to the theoretical value of Daidzein whose C%, H% and O% are 73.17, 4.06, and 22.76, respectively. Color reactions showed colorless under daylight, light yellow green under UV irradiation at 366 nm, colorless under daylight after ammonia fume treatment, yellowish blue under UV after ammonia fume treatment, violet under daylight after $FeCl_3$ treatment. These color reactions were compared with the data published by Seikel (1962) and Mabry et al. (1970).

Spectral analyses: UV λmax (MeOH) 237sh, 249, 259sh, 303sh; λmax (MeOH + MeONa) 259, 289sh, 325; λmax (MeOH + $AlCl_3$) 249sh, 249, 260sh, 300sh; λmax (MeOH + $AlCl_3$ – HCl) 240sh, 249, 262sh, 302sh. NMR (trimethylsilyl ether derivative) showed: δ7.9 singlet (H-2); δ7.40 doublet (H-2, H-5); δ7.1 doublet (H-6); δ6.9 singlet (H-8); δ6.7 doublet (H-3, H-5). These spectral data were compared to the standard spectra published by Mabry et al. (1970).

Table 5—Yield and flavor characteristics of the subfractions of P34

Fraction	Yield[a]	Appearance	Flavor
P34I	57%	pale yellow powder	woody, flour-like
P34S	43%	bright green yellow solid	bitter, astringent, weak phenol-like

[a] Weight percentage based on subfraction P34

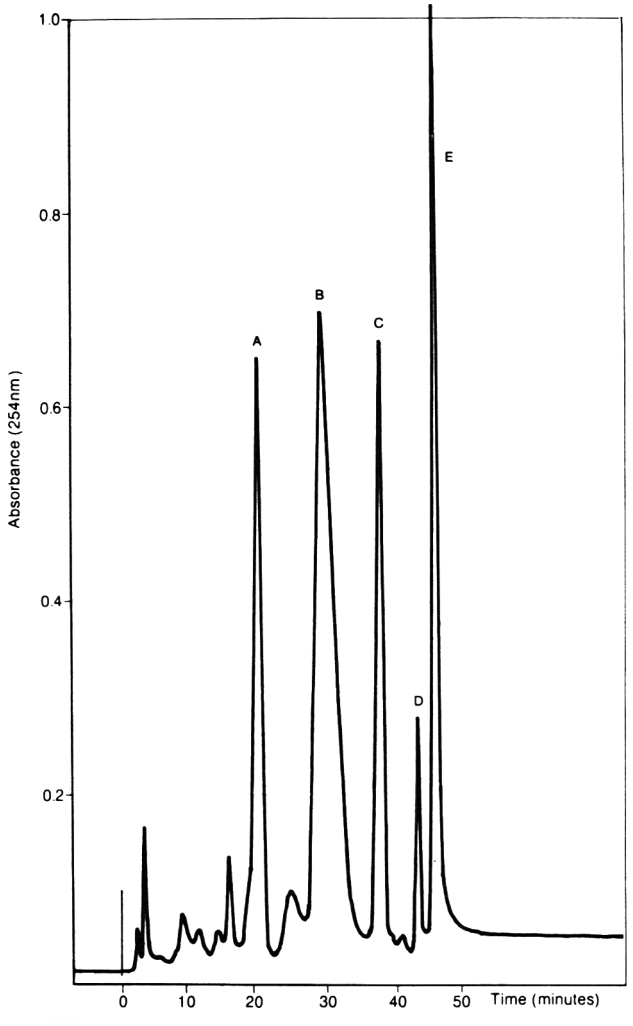


Fig. 3—Chromatogram of analytical HPLC analysis of Fraction P34.

Compound P34S-D identified as Glycitein 7-O-β-glucoside, 4′ hydroxy-6-methoxy-7-O-β-glucopyranosyl isoflavone

Elemental analysis found C, 58.42; H, 5.10; and O, 36.57, which suggested an empirical formula, $C_{22}H_{22}O_{10}$. These values are close to the theoretical values of glycitein 7-O-β-glucoside, whose C%, H%, and O% are 59.19, 4.92, and 35.87, respectively. Color reactions showed colorless under daylight, yellow green under UV irradiation at 366 nm, colorless under daylight after ammonia fume treatment, yellowish blue under UV after ammonia fume treatment, reddish violet under daylight after $FeCl_3$ treatment. These color reactions were compared with the data published by Seikel (1962) and Mabry et al. (1970).

Spectral analyses: UV λmax (MeOH), 228sh, 259, 319; λmax (MeOH + MeONa), 223, 271, 322sh; λmax (MeOH + $AlCl_3$) 228sh, 264, 312; λmax (MeOH + $AlCl_3$-

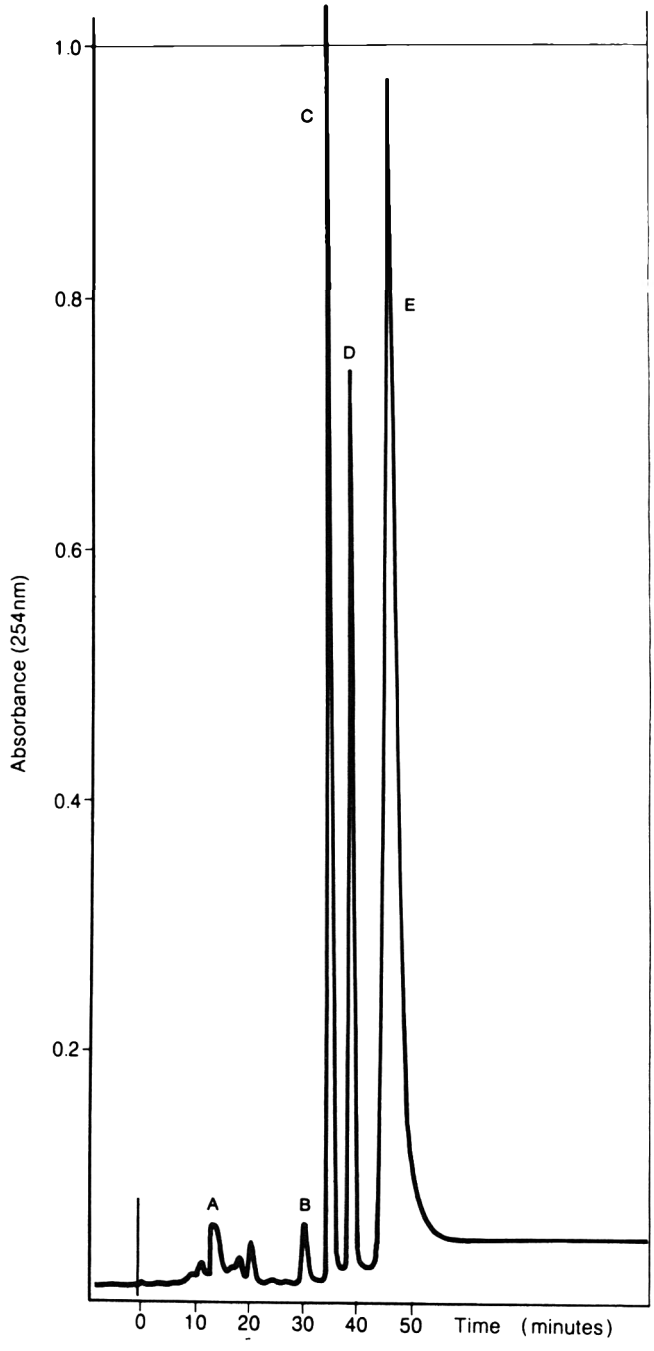
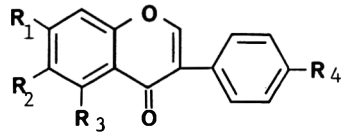


Fig. 4—Chromatogram of analytical HPLC analysis of Fraction P34S.

HCl) 228sh, 263, 312. These spectral data were compared to the data published by Naim et al. (1974).

Compound P34S-E identified as Genistin, 4'5-dihydroxy, 7-O-β-glucopyranosyl isoflavone

Elemental analysis found C, 58.0; H, 4.91; O, 37.09, which suggested an empirical formula, $C_{21}H_{20}O_{10}$. These values are close to the theoretical values of Genistin whose C%, H% and O% are 58.33, 4.62, and 37.03, respectively. Color reactions showed colorless under daylight, yellowish violet under UV irradiation at 366 nm, colorless under daylight after ammonia fume treatment, yellowish violet under UV after ammonia fume treatment, reddish violet under daylight after $FeCl_3$ treatment. These color reactions were compared with the data published by Seikel (1962) and Mabry et al. (1970).



	R_1	R_2	R_3	R_4
P34S-C	OH	H	H	OH
P34S-D	O-Glucosyl	OCH_3	H	OH
P34S-E	O-Glucosyl	H	OH	OH

Fig. 5—Chemical structures of subfractions P34S-C, P34S-D, and P34S-E.

Spectral analyses: UV λmax (MeOH), 260, 320sh; λmax (MeOH + MeONa), 270, 355sh; λmax (MeOH + $AlCl_3$), 272, 308sh, 375; λmax (MeOH + $AlCl_3$–HCl), 272, 307sh, 374; NMR (trimethylsilyl ether derivative) showed δ7.64 singlet (H-2); δ7.4 doublet (H-2', H-6'); δ6.8 doublet (H-3', H-5'); δ6.5 singlet (H-8); δ6.4 singlet (H-6); δ3.4–3.8 broad (glucosyl protons). These spectra were compared with and confirmed by the standard spectra published by Mabry et al. (1970).

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Phenolic Antioxidants of Soy Protein Hydrolyzates

DAN E. PRATT, CARMINE DI PIETRO, WILLIAM L. PORTER, and J. WALTER GIFFEE

ABSTRACT

Soy protein hydrolyzate (SPH) was investigated to identify the phenolic compounds responsible for antioxidant activity. Three isoflavones–genistein, daidzein, and glycitein–were found in SPH. These are the same isoflavones that have been identified in raw soybeans. No isoflavone glycosides were found in SPH. The phenolic acids found in SPH were caffeic, ferulic, p-coumaric, syringic, vanillic, gentisic, and p-hydroxybenzoic acids. The compounds were identified by TLC, GLC and UV spectra. The isoflavones were confirmed by their mass spectra.

INTRODUCTION

NUMEROUS PHENOLIC COMPOUNDS have been identified in soybeans and in soy flours and concentrates. Arai et al. (1966) found several phenolic acids, which included both substituted benzoic and cinnamic acids, in deffatted soy flour. Pratt and Birac (1979) identified chlorogenic, caffeic, ferulic, and p-coumaric acids as primary antioxidants in soybeans and soy products. Three isoflavones–genistein, daidzein, and glycitein–have been identified in soybeans and in soy flour (Naim et al., 1973). These isoflavones are responsible for a portion of the antioxidant acitivity attributed to soybeans and soy products (Pratt and Birac, 1979; Pratt, 1980). The antioxidant activity of soy flours, concentrates, and isolates have been summarized in an excellent review (Hayes et al., 1977).

Vegetable protein hydrolyzates are produced from several protein sources. They may be derived as the protein hydrolyzate of a single vegetable source or as a mixture of several vegetable sources. A number of low molecular weight phenolic compounds have been identified in soy protein hydrolyzates (SPH) by Manley and Fagerson (1970). Bishov and Henick (1972; 1975) reported primary and synergistic antioxidant activity in SPH. Hayes et al. (1977) spectulated that isoflavones and other phenolic compounds may be found in SPH which may contribute to their antioxidant activity. The present study was initiated to identify phenolic compounds of SPH that contribute to antioxidant activity.

EXPERIMENTAL

SOY PROTEIN HYDROLYZATE (SPH) (Vi-Zate 115 HVP powder, oil coated) was supplied by A.E. Staley Mfg. Co. (Protein Div) Chicago, IL. One kg of SPH was dissolved in 2L of deionized-distilled water. The oil coating and the residual lipids were removed by two extractions with 2L of hexane. Preliminary investigation demonstrated that oil extraction of the aqueous dispersion was as efficient as extraction of the dry powder. The scheme for isolating flavonoids and phenolic acids from SPH is presented in Fig. 1.

The concentrated methanolic extract (Fig. 1) was streaked on preparative (500μ) cellulose TLC plates and developed in the upper phase of l-butanol: acetic acid:water (5:1:4 v/v/v) (BAW). Bands were eluted in methanol, streaked on polyamide 6 TLC plates and developed in methanol:acetic acid:water (90:5:5). Bands were eluted in methanol and rechromatographed on polyamide, with ethanol:water (3:2) as the developing solvent.

Co-chromatography (TLC) in several developing systems, UV spectral analysis GLC, and GLC-mass spectrometer were used to identify phenolic acids and flavonoids.

UV spectral analyses were performed using a Beckman 26 spectrophotometer. Spectral analyses for flavonoids were conducted as outlined by Mabry et al. (1970). Spectral of phenolic acids were determined in methanol.

GLC analyses were conducted on trimethysilyl derivatives (TMS). Isolated bands eluted in methanol were evaporated to dryness. The residue was treated with 0.5 ml Bistrimethyl-silytrifluoroacetamide (BSTFA). The mixtures were tightly capped and heated for 30 min in a boiling water bath to facilitate derivatization. The gas chromatograph used was a Hewlett-Packard Model 5830A equipped with a FID detector. TMS derivatives were separated isothermally on a 6 ft x 1/8 in column of 3% S.E. 30 on 100/120 GCQ. The carrier gas (helium) flow rate was 20 ml per minute. The column temperatures were 240°C for isoflavones and 180°C or 200°C for the phenolic acids.

A Finnigan Automated GC/EI-CI Mass Spectrometer System was used to further confirm the identity of the isoflavones. Electron impact only was necessary due to the abundance of the parent ion. Probe analysis was conducted on isoflavones that had been further pruified by TLC on polyamide plates and developed in methanol: water (19:1). Bands were eluted in methanol and concentrated in vacuo. Samples (1–3 μl) were evaporated to dryness in a 5 μl vial. The vial was placed onto the probe and heated rapidly to 300°C while in contact with the ionizer region of the mass spectrometer. Conditions for the runs were: mass range, 20–27, 33–400 amu; integration time, 4 msec; scan rate, 2 sec/scan; filament current, 400 μA, electron energy, 70 eV; electron multiplier, 1500V; and ionizer temperature, 33°C.

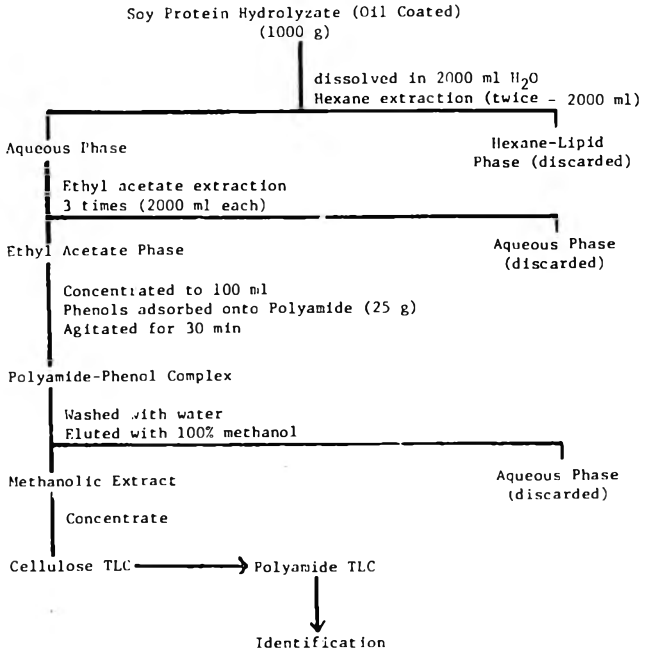


Fig. 1—Scheme for the separation of phenolic compounds from soy protein hydrolyzate.

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RESULTS & DISCUSSION

THIN-LAYER and gas-liquid chromatographic characteristics of isoflavones of SPH are given in Table 1. All isolated isoflavones were present as aglycones. This is presumably due to hydrolysis of the glycosides by the severity of the acid-heat treatment in preparation of SPH. The isoflavones-genistein (5,7,4′-trihydroxyisoflavone), daidzein (7,4′-dihydroxyisoflavone), and glycitein (7,4′-dihydroxy-6 methoxyisoflavone) are the same as those of soybeans and soy flour (Naim et al., 1973; Pratt and Birac, 1979).

The UV spectral characteristics of the isoflavones are shown in Table 2. Spectral analysis in methanol and with reagents to produce lathochromic wavelength shifts demonstrated that the compounds were genistein, daidzein, and glycetein. The wavelength shifts are practically identical with those reported by Mabry et al. (1970) and Pratt and Birac (1979).

Mass spectral data for the isoflavones are presented in Table 3. Spectra for two of the isolated isoflavones are nearly identical with those of authentic genistein and daidzein. No authentic sample of glycetein was available. Due to similarity of data for this isoflavone with chromatographic data and UV spectral data of Naim et al. (1973) and Pratt and Birac (1979) and mass spectral data presented, the compound was assumed to be glycetein. In addition to the three isoflavones shown in Table 3, the mass spectrometer analysis indicated a compound with a M.W. of 298. This is presumably a monohydroxy-demethoxy isoflavone. The compound was not present in sufficient quantity for further analysis.

Spectral and chromatographic characteristics of the phenolic acids of soy protein hydrolyzates are given in Table 4. The phenolic acids found in the hydrolyzate were essentially the same as Arai et al. (1964) reported for defatted soybean flour. The exceptions were chlorogenic and salicylic acids which were not found. It is not surprising that chlorogenic acid is not present. Due to the severity of the acid-heat treatment in the preparation of SPH, chlorogenic acid would be readily hydrolyzed.

From the data presented herein, it is obvious that flavonoids and phenolic acids are able to withstand the acid and heat treatments used in the preparation of SPH. Certain of these compounds are both primary and synergistic antioxidants and obviously contribute to the antioxidant activity of SPH. *Continued on page 35*

Table 1—Chromatographic characteristics of isoflavones of soy protein hydrolyzate

Compound	GLC[a]	TLC
Genistein		
Authentic	1.00	Co-chromatography in
Isolated	1.00	4 solvent systems (Same)
Daidzein		
Authentic	0.80	Co-chromatography in
Isolated	0.80	4 solvent systems (Same)
Glycitein		
Isolated	1.72	

[a] GLC analysis of TMS derivatives on 3% SE on GCQ at 240°C. Retention time relative to genistein.

Table 3—Mass spectral data for isoflavones isolated from soy protein hydrolyzate

Genistein[a]								
Mass to charge ratio	270[b]	153	133	269	51	271	50	89
Intensity[c]	100	40	28	28	24	15	14	12
Daidzein[a]								
Mass to charge ratio	254[b]	153	253	123	51	255	152	128
Intensity[c]	100	31	28	24	22	16	16	11
Glycitein[a]								
Mass to charge ratio	284[b]	283	166	123	69	285	167	18
Intensity[c]	100	61	31	18	15	15	11	11

[a] Isolated compound
[b] Parent Ion
[c] The intensities are normalized in respect to the parent ion.

Table 4—Phenolic acids of soy protein hydrolyzate

Acid	λmax[a] nm	Co-Chromatography[b] TLC[c]	GLC[d]	Concentration moles/kg
Caffeic	316, 285	5	1.00	3.6×10^{-3}
Ferulic	314, 287	5	0.80	1.5×10^{-4}
p-Coumaric	310, 286	5	0.43	Tr
Syringic	270	4	0.38	1.8×10^{-4}
Vanillic	260, 285	4	0.23	1.2×10^{-5}
Gentisic	328	4	0.25	Tr
p-Hydroxybenzoic	251	4	0.14	Tr

[a] λmax are the same for isolated and authentic compounds.
[b] Co-chromatography of isolated and authentic compounds
[c] Number of chromatographic systems used
[d] Retention time relative to caffeic acid

Table 2—UV spectral characteristics of isoflavones of soy protein hydrolyzate

Compound	λmax, nm MeOH[a]	NaOMe[b]	$AlCl_3$	Concentration moles/kg
Genistein				
Authentic	260, 330*	270, 355	272, 307*, 372	
Isolated	260, 331*	270, 355	273, 308*, 372	2.0×10^{-3}
Daidzein				
Authentic	237*, 247, 303*	259, 287*, 328	240*, 246, 301*	
Isolated	236*, 247, 302*	259, 285*, 326	241*, 246, 301*	0.6×10^{-3}
Glycitein				
Isolated	226*, 254, 316	254, 345	226*, 259, 310	0.3×10^{-3}

[a] Methanol
[b] Sodium methoxide
*Shoulder

Gelation Mechanism of Soybean 11S Globulin: Formation of Soluble Aggregates as Transient Intermediates

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ABSTRACT

Thermal association-dissociation behavior of soybean 11S globulin was investigated by sucrose density gradient centrifugation and polyacrylamide gel electrophoresis. Soluble aggregates with a molecular weight of 8 x 10^6 were formed when 0.5% and 5% protein solutions were heated for 1 min at 100°C. At the lower protein concentration, subsequent heating caused disappearance of the soluble aggregate followed by complete dissociation into acidic and basic subunits. At the higher concentration, however, subsequent heating caused formation of highly polymerized aggregates, and gel was formed after 5 min of heating. The soluble aggregates appear to be transient intermediates in the course of gel formation of 11S globulin.

INTRODUCTION

THE GEL-FORMING ABILITY of soybean proteins is of significance with respect to their usage in food systems. It has been reported that soybean protein dispersion forms a gel upon heating and cooling, and upon dialysis following alkali treatment. This has been adequately discussed by Hermansson (1978) and reviewed by Saio and Watanabe (1978) and Kinsella (1979), and extensive studies have established the basic factors affecting gelation of soybean proteins.

Heat-induced association-dissociation behavior of soybean proteins has also been studied by many workers (Mann and Briggs, 1950; Watanabe and Nakayama, 1962; Saio et al., 1968; Wolf and Tamura, 1969; Catsimpoolas et al., 1969, 1970; Aoki, 1970; Fukushima and Van Buren, 1970; Hashizume et al., 1975; Hashizume and Watanabe, 1979; Yamagishi et al., 1980). These studies demonstrated the fact that heating causes irreversible disruption of the quaternary structure of 11S globulin, the major component of soybean proteins, and subsequent dissociation into subunits (Wolf and Tamura, 1969; Catsimpoolas et al., 1969; Hashizume and Watanabe, 1979; Yamagishi et al., 1980). However, much less is known concerning the process of thermal aggregation of 11S globulin at the molecular level under conditions in which gel is formed.

In the present study, association-dissociation behavior of 11S globulin was investigated at early stages of heat-induced gelling process by sucrose density gradient centrifugation and polyacrylamide gel electrophoresis. This report concludes that the soluble aggregate, which has been seen by Wolf and Tamura (1969) when 11S globulin solution (0.5%) was heated at 100°C, is a transient intermediate in the course of gel formation.

MATERIALS & METHODS

Materials

DEAE-Sephadex A-50 was purchased from Pharmacia Co., Ltd. Sodium dodecyl sulfate (SDS), extra pure reagent, was obtained from Wako Pure Chemicals. 2-Mercaptoethanol, extra pure reagent, was obtained from Nakarai Chemicals. Soybeans (*Glycine max*, var. Tsuru-no-ko) were purchased from Mizuno Seed Co., Ltd.

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Methods

Preparation of 11S globulin. A crude 11S globulin fraction was prepared from soybeans according to the method of Thanh et al. (1975). Chromatographic fractionation of the crude 11S gloublin fraction was performed on a column of DEAE-Sephadex A-50 as described previously (Mori et al., 1979), where column was eluted with 35 mM potassium phosphate buffer (pH 7.6) containing 10 mM 2-mercaptoethanol, 0.02% NaN_3 and NaCl in linear gradient concentration of 0.25M to 0.5M.

Heating of 11S globulin. A portion of stock solution of purified 11S globulin in the eluting buffer was thoroughly dialyzed against 35 mM potassium phosphate buffer (pH 7.6) containing 0.4M NaCl just before its use. After dialysis, the protein solution was adjusted either to 0.5% or 5% protein concentration with the dialysing buffer. The protein solution in a 2.5 x 105 mm test tube (50 μl and 100 μl per tube for 5% and 0.5% protein solutions, respectively) covered with aluminum foil was heated in a water bath at 100°C for time periods as indicated in each Figure and Table. At the end of the heating period, the test tube was removed and rapidly cooled to room temperature by immersing in tap water. In experiments involving N-ethylmaleimide, 0.01M (final) N-ethylmaleimide was added to 5% globulin solution prior to heat treatment.

Centrifugation. The heat treated protein solution was centrifuged at 20°C either at 2,400 x *g* for 10 min (low speed centrifugation) or in 12 ml of 15–40% (w/v) linear sucrose gradient in the dialysing buffer at 152,000 x *g* for 60 min in a Hitachi RPS 40T rotor. The gradient was then divided into 0.6 ml fractions, and the absorbance measured at 280 nm with an ISCO density gradient fractionator. For sucrose gradient centrifugation, 2.5 mg of protein was used per experiment.

Electrophoresis. Each fraction from the low speed and the sucrose gradient centrifugation was analyzed by polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE following the method of Davis (1964) and Laemmli (1970), respectively. Electrophoreses were performed in 6.5% and 10% polyacrylamide gels according to the methods of Davis (1964) and Laemmli (1970), respectively, and the gels were stained with amido-black 10B as described previously (Mori and Utsumi, 1979). For SDS-PAGE and PAGE, 50 μg and 100 μg of protein were used, respectively.

Preparation of ribosome and tobacco mosaic virus. A ribosomal fraction was prepared from developing soybean seeds by differential centrifugation as described previously (Mori and Yokoyama, 1975). A tobacco mosaic virus (TMV, strain OM) fraction was prepared by differential centrifugation as described previously (Mori and Yokoyama, 1975).

Protein determination. Protein was determined by the method of Lowry et al. (1951).

RESULTS

THE 0.5% 11S GLOBULIN solution became turbid at and after 2 min of heat treatment and the protein began to precipitate. At 10 min of heating, 55.2% of total protein was precipitated as shown in Fig. 1. This result is consistent with that of Wolf and Tamura (1969) and Yamagishi et al. (1980). On the other hand, the 5% 11S globulin solution when heated became only faintly opalescent resulting in lesser precipitation. At 4 min of heating, 20.4% of total protein was precipitated as shown in Fig. 1. In the case of 5% protein concentration, a self-supporting gel was formed at and after 5 min. Therefore, insolubilization of 11S globu-

lin was examined up to 4 min in the case of high protein concentration. As shown by SDS-PAGE in Fig. 2-A, the concentrations of acidic and basic subunits in the supernatant and precipitate fractions, respectively, decreased as heating time increased. The supernatant and precipitate fractions at 10 min of heating consisted of the acidic and basic subunits, respectively, indicating that heating causes dissociation of 11S globulin almost completely into its acidic and basic subunits. This result again is compatible with that of Yamagishi et al. (1980). However, in the case of 5% protein concentration, such a marked change was not observed (Fig. 2-B). Both the supernatant and precipitate fractions at 4 min of heating consisted of the acidic and basic subunits (Fig. 2-B). This result indicates that 11S globulin does not dissociate into its subunits in the case of 5% protein concentration as much as it does in the case of 0.5%. When the supernatant fractions from the heat treated 11S globulin solutions (both 0.5% and 5%) were analyzed by PAGE (Fig. 3), bands were formed at the top of stacking gels. The band became faint with heating time in the case of 0.5% protein concentration (Fig. 3-A), while it remained unchanged in the case of 5% protein concentration (Fig. 3-B). These results could indicate the fact that heat-induced association-dissociation of 11S globulin differs depending on the protein concentration.

The 11S globulin solutions heated for varying times were analyzed by sucrose density gradient centrifugation. As shown in Fig. 4, heating for 1 min results in the formation of a slowly sedimenting fraction (Peak I) and soluble aggregates (Peak II) at both 0.5 and 5% protein concentrations. The molecular weight of these soluble aggregates was evaluated to be approximately 8×10^6 by comparing its sedimentation position with ribosome (4.3×10^6) and TMV (39.4×10^6). Heating for 2 min causes the soluble aggregates to decrease and the proportion of the faster sediment-

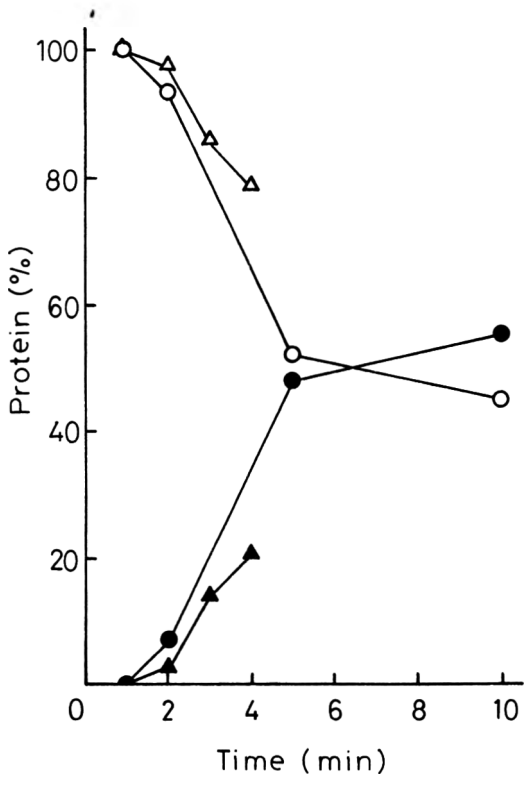


Fig. 1—Heat-induced insolubilization of 11S globulins. Protein concentrations: 0.5%, circles; 5%, triangles. Open and solid symbols indicate the supernatant and precipitate fractions from low speed centrifugation, respectively.

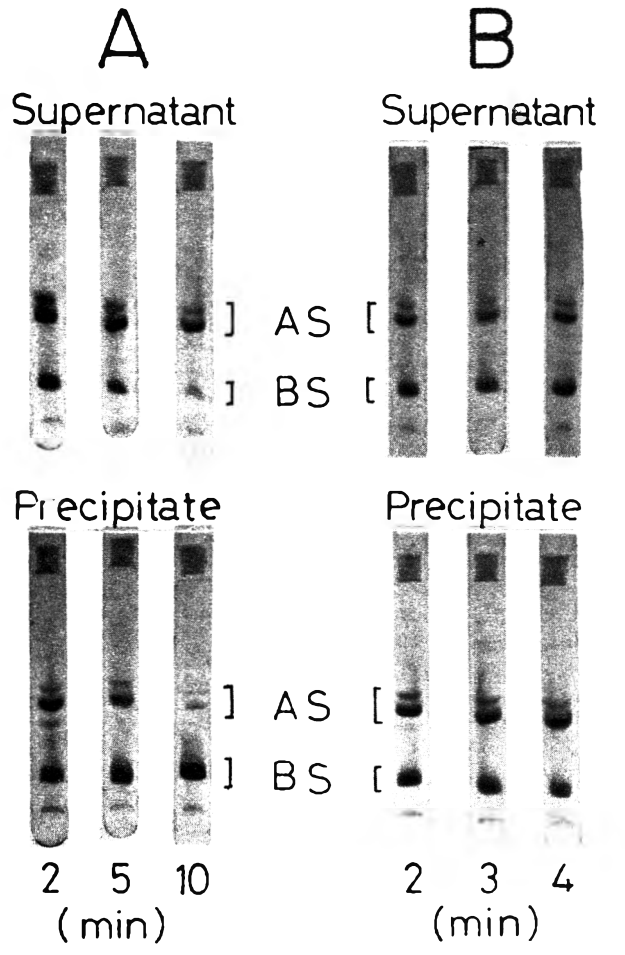


Fig. 2—SDS-PAGE in the presence of 2-mercaptoethanol of supernatant and precipitate fractions obtained by low speed centrifugation of heated 11S globulin solutions. Protein concentrations: 0.5%, A; 5%, B. AS and BS refer to acidic and basic subunits of 11S globulin, respectively.

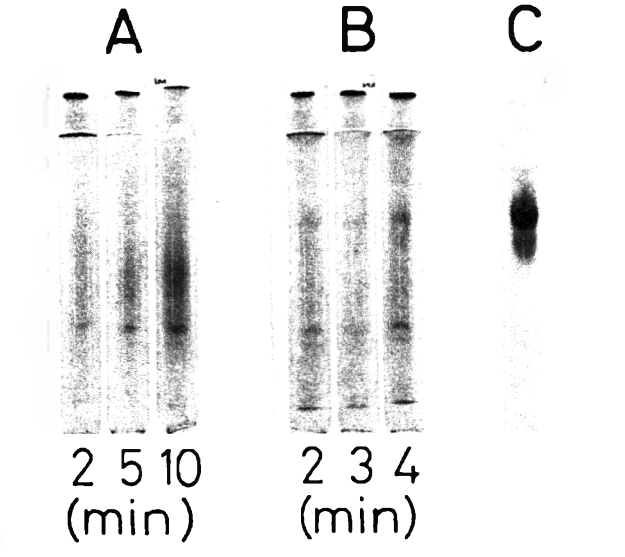


Fig. 3—PAGE of supernatant fraction obtained by low speed centrifugation of heated 11S globulin solutions. Protein concentrations: 0.5%, A; 5%, B. C refers to the unheated 11S globulin.

ing components (Peak III) to increase. This could be because of continued increase in particle size of the soluble aggregates. Longer periods of heating caused the disappearance of the soluble aggregate which consists of the acidic and basic subunits as shown in Fig. 5-A. The proportion of the acidic subunit of the soluble aggregate was slightly lower than that of native 11S globulin. The soluble aggregate disaggregated in the presence of SDS to an aggregate small enough to migrate into the stacking gel but not into the separating gel (compare Fig. 5-B and 5-C).

Table 1 shows changes in composition of protein solution as a function of heating time. Soluble macroaggregate in the bottom fraction represents highly polymerized soluble aggregates that are sedimentable by the sucrose gradient centrifugation but not by low speed centrifugation. The amount of soluble macroaggregate was estimated by subtracting the amounts of top and middle fractions and insoluble components from total amount centrifuged. Insoluble component in the bottom fraction corresponds to the precipitate shown in Fig. 1. It was observed that the soluble macroaggregates were formed at and after 2 min of heating with the concomitant decrease of the soluble aggregate. When the 11S globulin concentration was 0.5%, only a small amount of soluble macroaggregate was formed at 2 min and subsequently disappeared, whereas it continued to increase with heating time in the case of a 5% protein concentration. These results indicate that 11S globulin dissociates into its subunit form on heating at low protein concentration contrary to the case of higher protein concentration. This will be further discussed below.

Wolf and Tamura (1969) demonstrated that a soluble aggregate with an average size of 63S was formed when 11S globulin solution (0.5%) was heated at 100°C in the presence of N-ethylmaleimide (0.01M). This soluble aggregate was shown to remain substantially unchanged upon continued heating for 30 min. As shown in Fig. 6, sucrose gradient centrifugation of 11S globulin solution at 5% protein concentration heated in the presence of 0.01 M N-ethylmaleimide gave results which are consistent with those of Wolf and Tamura (1969). The molecular weight of the soluble aggregate thus formed was estimated to be approximately 4×10^6 by comparing its sedimentation position with ribosomes and TMV. This soluble aggregate did not change significantly on subsequent heating. This was also the case when the protein concentration was 0.5%. As shown in Fig. 7-A, the soluble aggregate formed in the presence of N-ethylmaleimide when electrophoresed on SDS-PAGE in the absence of 2-mercaptoethanol gave a main band corresponding to the intermediary subunits, disulfide-bonded acidic and basic subunits, which are the sub-structural components with which native 11S globulin is constructed (Kitamura et al., 1976). The soluble aggregate dissociated into the acidic and basic subunits in the presence of 2-mercaptoethanol as shown in Fig. 7-B. The proportion of the acidic subunit was lower than that of native 11S globulin and similar to that of the soluble aggregate which was formed in the absence of N-ethylmaleimide. In Fig. 7, disappearance of one of the acidic subunits (gel 2 in A) and appearance of two pale bands lying between the acidic and basic subunits (gel 2 in B) were observed. For these we have no clear explanation at present. The 5% 11S globulin solution when heated in the presence of N-ethylmaleimide does not form a gel which may be explained by above mentioned result that aggregation under this condition occurs upto a definite stage and then stops.

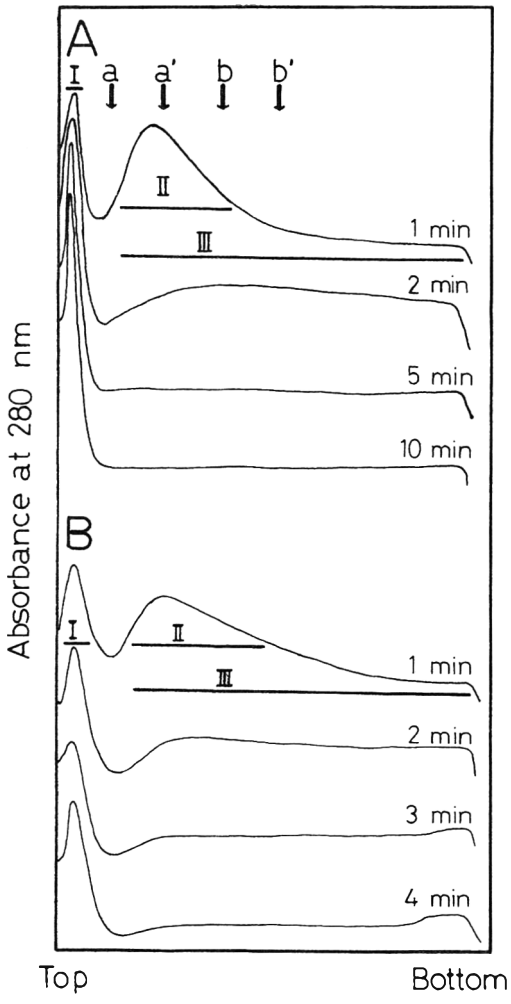


Fig. 4—Sucrose density gradient centrifugation of heated 11S globulin. Protein concentrations: 0.5%, A; 5%, B. The sedimentation positions of ribosome and TMV are indicated by arrows: a and a', monomer and dimer of ribosome, respectively; b and b', monomeric and dimeric forms of TMV respectively. The molecular weights of each monomeric form of ribosome and TMV are 4.3 x 10^6 and 39.4 x 10^6, respectively. The description of Peaks I, II and III is given in the text.

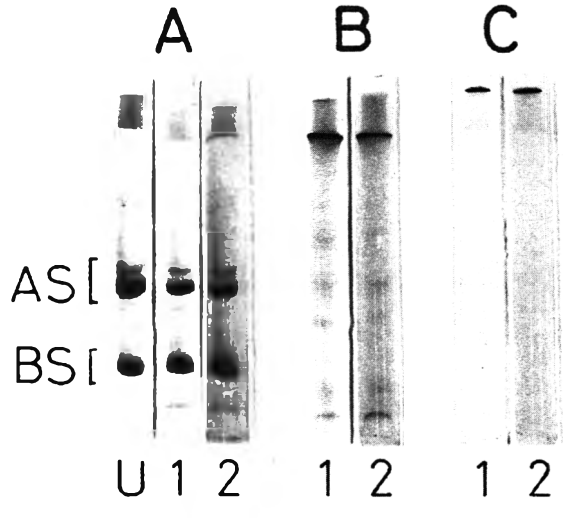
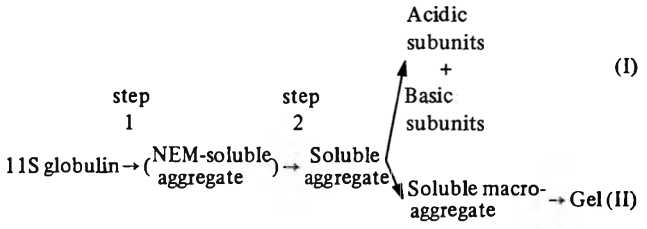


Fig. 5—Analysis of heat-induced soluble aggregates by gel electrophoresis. A and B are with and without 2-mercatoethanol in Laemmli system, respectively. C is in Davis system. U, unheated 11S globulin; Gel 1, peak II of Fig. 4-A; Gel 2, peak II of Fig. 4-B. AS and BS are similar to those in Fig. 2.

DISCUSSION

THERMAL ASSOCIATION-dissociation phenomena of 11S globulin at low protein concentration (≤ 0.5%) have been studied by Wolf and Tamura (1969) and Catsimpoolas et al. (1969, 1970), and subsequently by Hashizume and Watanabe (1979) and Yamagishi et al. (1980). They showed that 11S globulin solution become turbid on heating and that subsequent heating causes dissociation of 11S globulin into its acidic and basic subunits. In the present study, association-dissociation behavior of 11S globulin which was found to be homogeneous by ultracentrifugal and gel electrophoretic analyses was investigated at early stages of heat induced gelling process at both 0.5 and 5% protein concentrations. The results obtained conform and substantiate the previous findings regarding the behavior of soybean 11S globulin on heating. The overall scheme of the thermal change of 11S globulin is illustrated below:



A brief description of the proposed scheme is as follows: (1) Soluble aggregates are formed when 11S globulin solution is heated both at low and high protein concentrations. (2) On subsequent heating, the soluble aggregate disaggregates to acidic and basic subunits in the case of low protein concentration (process I), while in the case of high protein concentration it undergoes association resulting in gel formation (process II). Whether the heated protein solution undergoes disaggregation or gel formation is apparently governed by the concentration of the soluble aggregate. The soluble aggregate which is formed in the presence or absence of N-ethylmaleimide appear to be similar to those reported by Wolf and Tamura (1969), judging from their size and response to heat treatment.

The soluble aggregate seems to be stable at room temperature, since it can be isolated by fractionation on the sucrose gradient centrifugation as shown in Fig. 4. The isolated soluble aggregate at 0.05% protein concentration when heated again for 10 min disaggregated to give the acidic and basic subunits, and showed electrophoretic pattern similar to that shown in Fig. 2-A (gels of 10 min). This supports the proposed scheme showing that disaggregation proceeds through process I. Since the proportion of the acidic subunit of the soluble aggregate was lower than that of native 11S globulin (Fig. 5-A), release of acidic subunit may occur to some extent at steps 1 and/or 2 during formation of soluble aggregate. Soluble aggregate

Table 1—Effect of heating time on centrifugal composition of 11S globulin

Protein conc (%)	Time (min)	Fractions from sucrose density gradient centrifugation: Top[a]	Middle[a] (soluble aggregate)	Bottom[b] (Sol. macro-aggregate)	Bottom[b] (Insol. component)
		(%)			
0.5	1	28.6	71.4	0	0
	2	37.1	48.7	8.0	6.2
	5	38.9	12.1	0	49.0
	10	44.8	0	0	55.2
5.0	1	27.3	72.7	0	0
	2	32.5	48.8	16.1	2.6
	3	25.7	25.3	34.7	14.3
	4	20.0	21.4	38.2	20.4

[a] Top and middle fractions correspond to peaks I and III shown in Fig. 3, respectively. For each fraction the amount of protein was determined by the method of Lowry et al. (1951).
[b] Detailed descriptions are given in the text.

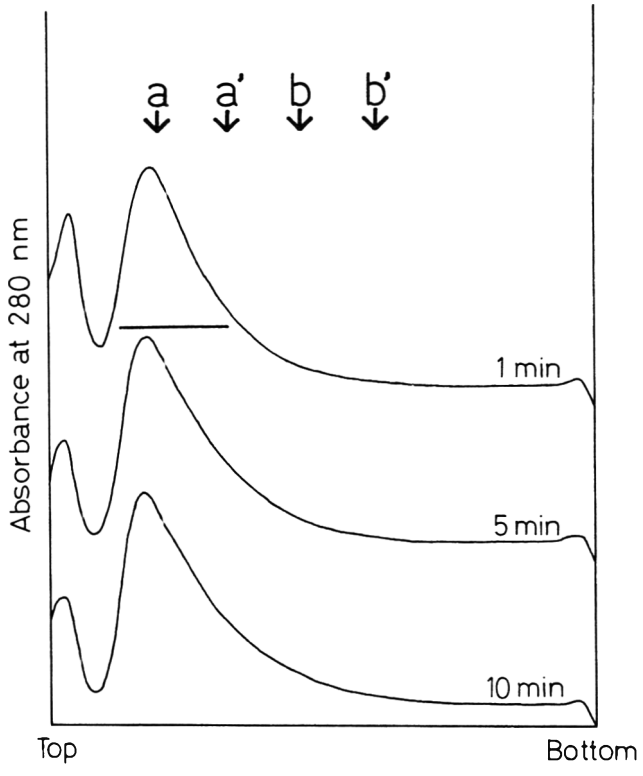


Fig. 6—Sucrose density gradient centrifugation of 11S globulin heated in the presence of N-ethylmaleimide. The indication of the sedimentation positions of ribosome and TMV is similar to that in Fig. 4.

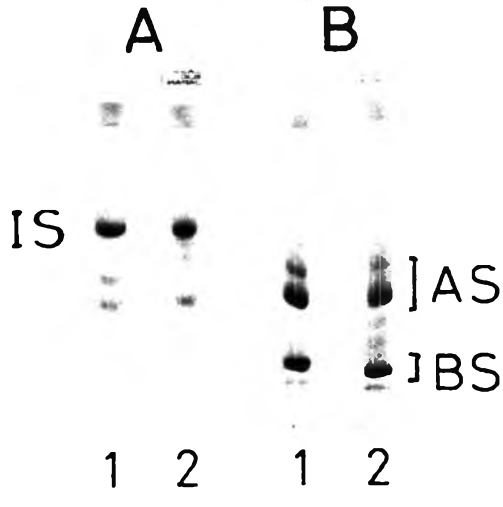


Fig. 7—SDS-PAGE of the soluble aggregate formed by heating in the presence of N-ethylmaleimide. A and B are with and without 2-mercaptoethanol, respectively. Gel 1, unheated 11S globulin; Gel 2, the underlined part of peak shown in Fig. 6. AS and BS are similar to those in Fig. 2. IS refers to the intermediary subunit of 11S globulin.

with a molecular weight of 4×10^6 was formed in the presence of N-ethylmaleimide and remained unchanged on subsequent heating (Fig. 6). The intermediary subunits in this soluble aggregate are bound together by non-covalent forces that are severed by SDS (Fig. 7-A). It seems that on heat treatment 11S globulin or its intermediary subunit prefers to associate together through such forces, possibly hydrophobic interaction. Therefore, such size of soluble aggregate derived on heat treatment of 11S globulin is shown as the postulated intermediate termed NEM-soluble aggregate tentatively in parenthesis in the scheme prior to the formation of the soluble aggregate which is formed in the absence of N-ethylmaleimide. In the absence of N-ethylmaleimide, a larger soluble aggregate (MW, 8×10^6) was formed. This soluble aggregate was found to contain disulfide and possibly hydrophobic bondings (Fig. 5-B and 5-C). Therefore, in the absence of N-ethylmaleimide, the intermolecular disulfide exchange reactions and hydrophobic interactions seem to occur during the formation of the soluble aggregate. It has been demonstrated that 11S globulin contains two sulfhydryl groups per mole of protein (Draper and Catsimpoolas, 1978). These sulfhydryl groups may play an important role in the disulfide exchange reactions at the initial stage.

At the higher protein concentration (5%) the soluble aggregate does not undergo disaggregation but undergoes further polymerization resulting in formation of soluble macroaggregates and finally gel formation through process II. When N-ethylmaleimide (0.01M) was added to 11S globulin solution after 1 min of heating when the soluble aggregate appears, gel was not formed in subsequent heating. This suggests that the disulfide bridge formation through disulfide exchange reaction is involved in the formation of soluble macroaggregate followed by subsequent gel formation.

Catsimpoolas and Meyer (1970) showed that soybean globulin dispersion is converted on heating to a progel state which is characterized by high viscosity and the gel is then obtained by cooling the progel. The gel can be converted to the progel state by heat which on cooling reverts to the gel form (Catsimpoolas and Meyer, 1970). It seems that the above mentioned progel state relates to the soluble aggregate and/or soluble macroaggregate observed in the present study. However, in the present study, when the 11S globulin solution (5%) was heated at 100°C for more than 5 min and then cooled to room temperature, self-supporting gel was obtained after a few minutes of cooling at room temperature. When the gel was placed in water in a test tube and heated again at 100°C, it remained unchanged up to 5 min indicating its heat-irreversibility. It may therefore be presumed that, aggregated components in the progel state are different from the soluble aggregate and/or soluble macroaggregate, with respect to the extent of disulfide bonding.

Further investigation is required in order to elucidate the thermal protein changes at an early stage of heating (steps 1 and 2 in the scheme shown above) and the forces involved in the structural formation of soluble aggregate and soluble macroaggregate. The information obtained from such investigations may shed some light on the mechanisms of gelation of soybean 11S globulin at molecular level.

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Changes of Aldehyde Levels in Defatted Soybean Extract

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ABSTRACT

Aldehyde levels in defatted soybean extracts were measured by using aldehyde dehydrogenase. The level increased during incubation of flour-water suspension and its centrifuged extract at pH 6.5, while it decreased markedly at alkaline pHs. This aldehyde-eliminating activity was observed in 1:5 flour:water ratio, but was not in 1:20 flour:water ratio. ^{14}C-labelled acetaldehyde added to 1:5 flour:water extract was converted into acetic acid. Fractionation of the extract indicated that a heat-labile protein and a heat-stable compound with low molecular weight were required for the aldehyde-eliminating activity.

INTRODUCTION

THE OBJECTIONABLE green bean flavor is one of the factors limiting the wide use of soybean protein as a foodstuff. Medium-chain aldehydes such as hexanal appear to be mainly responsible for the green bean flavor (Fujimaki et al., 1965; Qvist and von Sydow, 1974; Chiba et al., 1979a). The odor threshold levels of aldehydes are very low and therefore the complete removal of the aldehydes is required for efficient utilization of soybean protein as food. A number of reports on the removal of soybean flavor have appeared. Steaming of defatted soybean flakes and extraction with an organic solvent have been shown to be effective (Eldridge et al., 1971; Cowan et al., 1973). However, the interaction of the aldehydes with soybean protein made it difficult to find a way to remove the aldehydes completely (Arai et al., 1970; Noguchi et al., 1970; Gremli, 1974; Beyeler and Solms, 1974). The gradual release of protein-bound aldehydes during storage and cooking of soybean products develops an off-flavor. It has been reported that limited proteolysis of soybean protein releases the protein-bound aldehydes (Noguchi et al., 1970). Papers from this laboratory have demonstrated that incubation of soybean extract with aldehyde dehydrogenase in the presence of NAD^+ almost completely removes the green bean flavor through oxidation of aldehydes into corresponding acids (Takahashi et al., 1979a; Chiba et al., 1979a). We have also shown that aldehyde dehydrogenases from bovine liver and yeast oxidize soybean protein-bound aldehydes as well as free ones (Chiba et al., 1979b; Takahashi et al., 1979b). In the course of this investigation we have found that levels of aldehydes in defatted soybean extract change remarkably, depending on the conditions of protein extraction. The purpose of this paper is to demonstrate that defatted soybean extracts contain heat-labile protein and heat-stable compound which cause oxidation of aldehydes.

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MATERIALS & METHODS

Materials

Raw soybean flour defatted with hexane was a generous gift from Fuji Oil Co., Ltd. Yeast aldehyde dehydrogenase (Boehringer), β-NAD^+ (Kyowa Hakko Co., Ltd.), hexanal, acetaldehyde, and papain (Merck), [1,2-^{14}C] acetaldehyde (7.9 mCi/mmol) and AQUASOL-2 (New England Nuclear) were purchased from the indicated sources. Other chemicals were of reagent grade. Aldehyde solutions were prepared as described previously (Takahashi et al., 1979a). Bovine liver mitochondrial aldehyde dehydrogenase was prepared as described previously (Takahashi et al., 1979a).

Determination of aldehyde and protein

Aldehyde was measured enzymatically with yeast aldehyde dehydrogenase as described previously (Chiba et al., 1979b). Protein was determined by the method of Bensdoun and Weinstein (1976).

Preparation of defatted soybean flour-water suspension and centrifuged extract

Flour-water suspensions were prepared by suspending defatted soybean flour in five-fold (v/w of the flour) or in 20-fold distilled water. Centrifuged extracts were prepared by incubation of the flour-water suspensions at 25°C for 30 min, followed by centrifugation at 12,000 x *g* for 10 min.

Incubation of flour-water suspension

To explore dependency of aldehyde level in defatted soybean extracts on the conditions for protein extraction, three different conditions for extraction were set up: the 1:5 flour:water suspensions were incubated at pH 6.5 and at pH 7.5, and the 1:20 flour:water suspension was incubated at pH 7.5. Incubation was carried out at 40°C to simulate an industrial extraction condition. At intervals the suspensions were centrifuged at 12,000 x *g* for 10 min to prepare the supernatants. Aliquots of the 1:5 flour:water suspension incubated at pH 7.5 were diluted with distilled water to 1:20 flour: water ratio and after 10 min the diluted suspensions were centrifuged (expressed with 1:5 → 1:20 in Fig. 1). Aldehyde in the supernatants was measured immediately after their preparation.

Preparation of soybean protein isolate

The centrifuged extracts were acidified to pH 4.5 with 6N HCl to precipitate the protein. After standing for 15–20 min, the acidified extracts were centrifuged at 10,000 x *g* for 10 min. The precipitates were dissolved in distilled water by gradual addition of 6N NaOH. The solutions were adjusted to pH 7.0 and then their volume was made the same as the original with distilled water. The solutions were spray-dried at 120°C inlet/75°C outlet with a Yamato Minispray Model DL-21.

Sensory evaluation

Spray-dried protein isolates were dissolved in distilled water (3% solution). The solutions were warmed at 45°C and evaluated for green bean flavor. The odor score for the sample with the strongest green bean flavor was set at 5, and the score for distilled water was set at 0. Sensory panels consisting of 10 students and faculty who had been trained with hexanal solution compared the samples with respect to green bean flavor and the odor scores obtained from each panelist were averaged.

Experiment with radioactive acetaldehyde

The centrifuged 1:5 flour:water extract was adjusted to pH

8. The reaction mixtures contained, in a total volume of 3.0 ml, 2.9 ml of the extract, 0.1 ml of 3 mM acetaldehyde and 0.1 μCi ^{14}C-acetaldehyde. After incubation at 25°C for 0 hr and 3 hr, the mixture was deprotenized by adding 0.75 ml of 30% perchloric acid followed by centrifugation. To 2.0 ml of the supernatant, 1.0 ml of 1.66M K_3PO_4 was added to neutralize and precipitate perchloric ion as potassium perchlorate. After removal of the precipitate by centrifugation, the supernatant (1.0 ml) was applied on a column (0.7 x 10 cm) containing Dowex 1x8 (200–400 mesh). The column was washed with distilled water and then developed with a 20 ml-linear gradient of NaCl from 0–1.5M. The flow rate was 10 ml/hr. The eluate (1.1 ml/each fraction) was fractionated into tubes containing 0.2 ml of 1M sodium bisulfite to avoid vaporization of radioactive acetaldehyde. Radioactivity of 0.5 ml of each fraction was counted in 5 ml AQUASOL-2 with a Packard model 3255 scintillation spectrophotometer. When aldehyde dehydrogenase was used instead of soybean extract, the reaction mixtures contained, in a total volume of 3.0 ml, 1.5 ml of 200 mM Na-PPi buffer, pH 9.3, 0.1 ml of 10 mM NAD^+, 0.1 ml of 3 mM acetaldehyde, 0.1 μCi of ^{14}C-acetaldehyde and 1.5 unit of aldehyde dehydrogenase. After incubation at 25°C for 0 min and 60 min, the reaction mixture was taken out and treated as described above to detect radioactive acetaldehyde and acetic acid.

Fractionation of soybean extract by Sephadex G-50 column

The centrifuged 1:5 flour:water extract was incubated at pH 8.0 for 3.5 hr at 25°C to eliminate endogenous aldehyde. The incubated extract (200 ml) was applied on a Sephadex G-50 column (4.2 x 127 cm) which had been washed with distilled water. The column was developed with water. The flow rate was 2 ml/min. Fractions were 18 ml. Fractions were pooled and lyophilized. The lyophilized samples (each 200 ml) were dissolved in 1.0 ml of 20 mM Na-Pi buffer pH 7.5 and used for experiments.

Preparation of ammonium-precipitable fraction and perchloric acid-soluble fraction from soybean extract

The centrifuged 1:5 flour:water extract (440 ml) was fractionated with saturated ammonium sulfate solution. The materials precipitated with 43–62% ammonium sulfate were suspended in 10 ml of 20 mM Na-Pi buffer, pH 7.5. The suspension was dialyzed against the above buffer for 2 days. The insoluble material was removed by centrifugation. The centrifuged 1:5 flour:water extract (440 ml) was deprotenized by adding 100 ml of 1M perchloric acid. The supernatant (480 ml) was neutralized with 6N KOH and the resulting precipitate of potassium perchlorate was removed by centrifugation. The supernatant (450 ml) was spray-dried and the dry material (20g) was dissolved in 50 ml of 20 mM Na-Pi buffer, pH 7.5.

RESULTS

Aldehyde level during extraction

Dependency of aldehyde level in defatted soybean extracts on the conditions for protein extraction was investigated. Aldehyde in the centrifuged extract obtained after incubation of the flour:water suspensions under the various conditions was measured. As shown in Fig. 1, aldehyde levels greatly increase during 1:5 flour:water extraction at pH 6.5 and 1:20 flour:water extraction at pH 7.5. In contrast to these, a marked decrease in aldehyde level was observed during 1:5 flour:water extraction at pH 7.5. The dilution treatment of the 1:5 flour:water suspension just before preparation of the supernatant (expressed as 1:5 → 1:20 in Fig. 1) does not reverse the decrease in aldehyde level which has taken place in the 1:5 flour:water suspension. Measurements of protein in the supernatants indicate that protein extraction with 1:5 flour:water ratio is less efficient than that with 1:20 flour:water ratio, but this low efficiency is raised to the level with 1:20 flour:water extraction by the dilution treatment (Table 1). It is possible, therefore, to prepare a soybean extract containing less amount of aldehyde with a high yield of extracted protein.

Aldehyde level during incubation of centrifuged extract

The results described in the previous section suggest that in a defatted soybean flour suspension there is aldehyde-eliminating activity which is dependent on pH and flour concentration. The presence of this activity in centrifuged extracts was investigated. The centrifuged 1;5 flour:water extracts were incubated at various pHs and aldehyde levels in the extracts during incubation were measured. Fig. 2 shows the results. Incubation of the extract at pH 6.5 resulted in a striking increase in aldehyde. On the other hand, when the pHs of extracts were adjusted to 7.5, 8.5, and 9.3 with NaOH, aldehyde levels decreased to 10–30% of the initial ones after incubation for 5 hr at 25°C. Incubation of an extract at pH 6.5 in the presence of 100 mM NaCl did not affect the increase in aldehyde level found in the absence of NaCl, indicating that the aldehyde-eliminating activity observed at alkaline pHs was not due to sodium ion added. The possibility was also excluded that the aldehyde-eliminating activity was caused by aldehyde-assimilating activity of microorganisms in the extract, since the activity was found in an extract sterilized by filtering through a membrane filter with 0.45 μm pore size. The aldehyde-eliminating activity was also found in an extract of laboratory-made defatted flour from var. Tsuru-no-ko soybean grown in Japan.

The effect of concentration of centrifuged extract on the change in aldehyde level is shown in Fig. 3. Aldehyde levels in the extracts prepared with 1:20 flour:water ratio increased slightly, although these extracts were incubated at alkaline pHs where aldehyde in the 1:5 flour:water extracts decreased steeply.

The centrifuged 1:5 flour:water extracts were treated under three kinds of conditions prior to preparation of protein isolates by spray-drying. One of the extracts received no treatment. Another extract was adjusted to pH 9.3 and incubated for 3 hr at 25°C. The third extract was incubated for 3 hr at pH 9.3 in the presence of aldehyde dehydrogenase and NAD^+. The protein isolates prepared after these treatments were subjected to sensory evaluation with respect to the green bean flavor (Table 2). Although the green bean flavor of the isolate obtained after incubation at pH 9.3 was stronger than that of the isolate prepared from the aldehyde dehydrogenase-treated extract, it was significantly weaker than that of the isolate from the nontreated extract. Thus the aldehyde-eliminating activity functions in centrifuged extract of defatted soybean and contributes to decreased aldehydes having green bean flavor.

Aldehyde-eliminating activity in extract

To explore the mechanism by which aldehydes in soybean extract were eliminated during incubation at an alkaline pH, aldehydes were added to the extracts. Acetaldehyde and hexanal were added to the centrifuged 1:5 flour:

Table 1—Protein extraction from defatted soybean flour[a]

Incubation conditions of flour-water suspensions		Supernatant obtained (ml)	Protein in supernatant (mg/ml)	Protein extracted (g)
pH	flour:water	A	B	AxB
6.3	1.5	42	69.4	2.91
7.5	1.5	42	70.0	2.94
7.5	1:20	189	20.5	3.87
7.5	1:5→1:20[b]	189	19.8	3.74

[a] Supernatants were prepared by centrifugation of 10g defatted soybean flour:water suspension incubated at designated pH levels for 2 hr at 40°C.
[b] See caption to Fig. 1.

water extracts which had been preincubated at pH 8.0 to eliminate endogenous aldehydes, and then the mixtures were further incubated. Both aldehydes were found to decrease during the incubation (Fig. 4). No substantial decrease in either aldehyde level was observed in the absence of the extracts.

^{14}C-labelled acetaldehyde was used as a substrate to find the products of the aldehyde-eliminating reaction. After the labelled aldehyde was incubated with the centrifuged extract, the incubation mixture was deprotenized with perchloric acid and the protein-free supernatant was subjected to ion exchange column chromatography with Dowex 1x8. Fig. 5A shows chromatographic patterns of the supernatant before and after incubation of the labelled aldehyde with extracts. Before incubation, radioactivity was not adsorbed on the resin and appeared in the flow-through fractions; it was confirmed with nonlabelled acetaldehyde that the aldehyde does not bind to this resin (figure not shown). After incubation for 3 hr, about half of the radioactivity was adsorbed, and eluted with about 0.5M NaCl. Enzymatic quantification of residual acetaldehyde indicated that aldehyde level in the incubation mixture after 3 hr incubation decreased to approximately half of the initial value. The elution position of radioactivity was found to correspond exactly to that of acetic acid which was prepared by oxidizing the labelled acetaldehyde with aldehyde dehydrogenase in the presence of NAD^+ (Fig. 5B).

The centrifuged 1:5 flour:water extract was subjected to column chromatography with Sephadex G-50 to find the factors involved in the aldehyde-eliminating reaction. Fig. 6 shows a protein elution pattern. The fractions designated by letters in the figure were pooled and the pooled fractions were assayed with respect to their aldehyde-eliminating activity. As shown in Table 3, no significant activity was found in any individual fraction except C. The elution position of fraction C suggested that the activity in this fraction might be due to interaction of at least two substances, one with a relatively high molecular weight and the other, with a low molecular weight. All of these combinations of fractions A, B, D, and E, taken two at a time, were assayed, and high activity was found only in the combination of fraction B and D. It is clear from the results with dialysis and heating of these two fractions that a factor required for the activity in fraction B is a heat-labile, high molecular weight substance, while that in fraction D is a heat-stable, low molecular weight substance.

Requirement of two factors has also been found by using the ammonium sulfate-precipitable fraction (43–62% saturation of ammonium sulfate) and the perchloric acid-soluble fraction of the soybean extract. As shown in Fig. 7, the aldehyde-eliminating activity was undetectable in either fraction alone, but the activity appeared when both fractions were present.

—Continued on next page

Table 2—Sensory evaluation of soybean protein isolate.[a]

Incubation of extracts at 25°C			Aldehyde in extracts after incubation (μM)	Green bean flavor of protein isolates (odor score)
pH	Addition	Time (hr)		
6.5	–	0	40	5
9.3	–	3	12	2.7
9.3	ALDH[b] NAD^{+c}	3	undetectable	1

[a] The centrifuged 1:5 flour:water extracts were treated as indicated. After treatment aldehyde levels in the extracts were measured. Soy protein isolates were prepared as described in Materials & Methods.
[b] Bovine liver mitochondrial aldehyde dehydrogenase (0.3 unit/ml).
[c] 1 mM

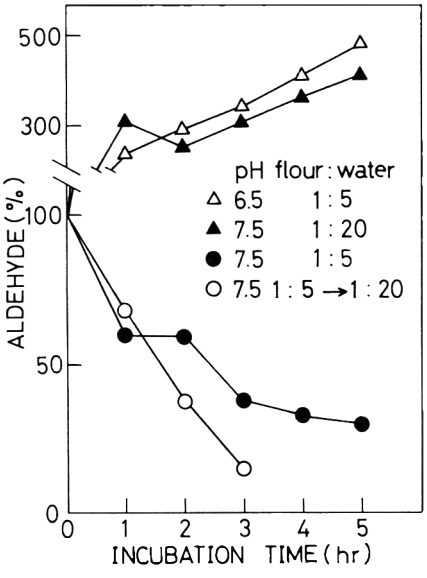


Fig. 1—Dependency of aldehyde levels in soybean extracts on extraction conditions. The 1:5 flour:water and 1:20 flour:water suspensions were incubated at 40°C under the conditions indicated in the figure. At intervals the suspensions were treated as described in text. The initial concentration of aldehyde was about 20 μM in 1:5 flour:water ratio and 5 μM in 1:20 flour:water ratio.

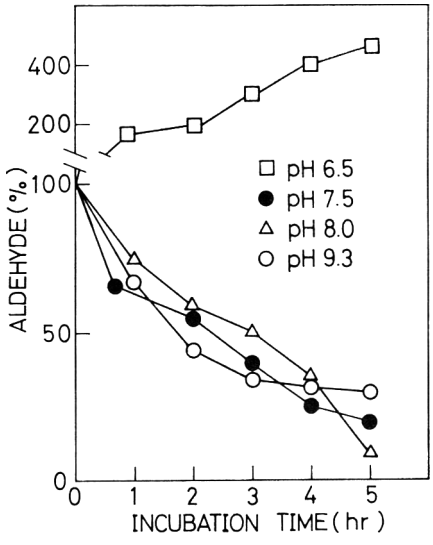


Fig. 2—Aldehyde levels in soybean extracts during incubation at various pHs. The centrifuged 1:5 flour:water extracts were adjusted to the indicated pHs 30 min after their preparation and the incubated at 25°C. Aliquots of the extracts were taken out during incubation to measure aldehyde. The initial aldehyde was approximately 45 μM.

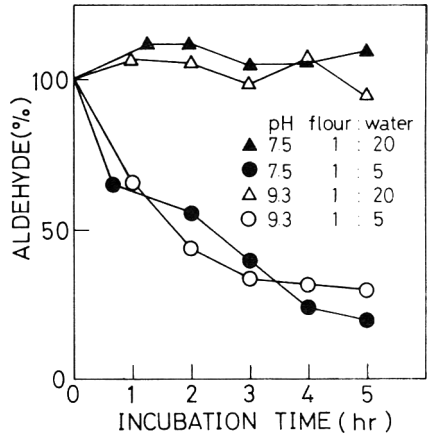


Fig. 3—Aldehyde levels in soybean extracts during incubation at different concentrations of extracts. The centrifuged extracts obtained with 1:5 flour:water and 1:20 flour:water extraction were treated as in Fig. 2. The initial aldehyde concentrations were 45 μM for the 1:5 flour:water extract and 11 μM for the 1:20 flour:water extract.

The aldehyde-eliminating activity in the soybean extract was repressed upon digestion with papain, suggesting that at least one factor with high molecular weight is a protein.

DISCUSSION

IT IS LIKELY from pH-dependency of aldehyde level in defatted soybean extracts (Fig. 1 and 2) that both aldehyde-producing and aldehyde-eliminating reactions proceed in flour-water suspensions and centrifuged extracts, offsetting each other to some extent. The aldehyde-producing activity appears to exceed the eliminating activity at acidic pH, but raising pH reverses the situation. In fact, the presence in soybeans of lipoxygenase, an enzyme probably responsible for aldehyde production, which has an optimum pH around 6, has been reported (Koch et al., 1958).

The aldehyde-eliminating activity was undetectable in the 1:20 flour:water suspension at pH 7.5 and in its centrifuged extract, although there was significant activity in 1:5 flour:water ratio (Fig. 1 and 3). Elucidation of the factors required for the activity is needed to explain this drastic disappearance of the activity by dilution.

Identification of acetic acid as product of the acetaldehyde-eliminating reaction argues against the possibility that a decrease in aldehyde levels in the extract at alkaline pH is attributable to binding of aldehydes with soybean protein so that the aldehyde dehydrogenase used for measuring aldehyde levels is unable to reach the substrate.

Table 3—The aldehyde-eliminating activity of soybean extract fractions obtained by Sephadex column chromatography[a]

Samples added					
A	B	C	D	E	Activity[b]
—	—	—	—	—	5
+	—	—	—	—	17
—	+	—	—	—	18
—	—	+	—	—	33
—	—	—	+	—	13
—	—	—	—	+	15
—	+	—	+	—	48
—	+[c]	—	+	—	19
—	+	—	+[c]	—	50
—	+[d]	—	+	—	39
—	+	—	+[d]	—	4

[a] The reaction mixture contained, in a total volume of 0.5 ml, 0.3 mM hexanal and 0.2 ml of each sample. After incubation at 25°C for 60 min, residual hexanal was measured.
[b] Decrease in aldehyde [(initial aldehyde) — (residual aldehyde)/(initial aldehyde) x 100].
[c] Samples B and D were heated at 80°C for 5 min and at 100°C for 10 min, respectively.
[d] Samples B and D were dialyzed against 20 mM Na-Pi buffer, pH 7.5, for one night.

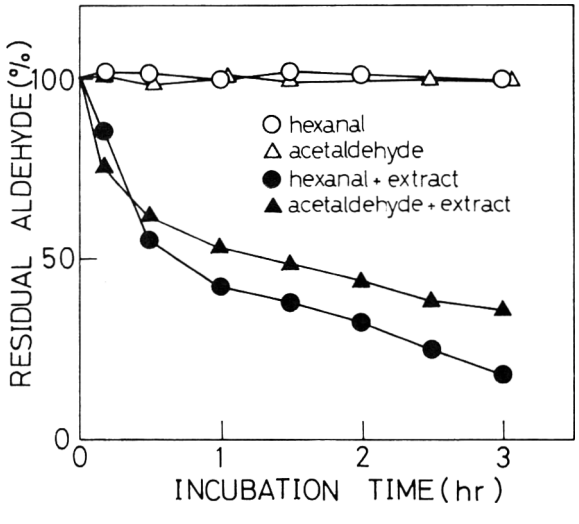


Fig. 4—The aldehyde-eliminating activity in soybean extracts acts on exogeneous aldehyde. The centrifuged 1:5 flour:water extract was preincubated at pH 8.0 for 3.5 hr at 25°C to eliminate the endogenous aldehyde. Hexanal and acetaldehyde were added to the preincubated extracts at 0.3 mM. The mixtures were incubated at 25°C and at intervals aliquots were taken to measure aldehyde.

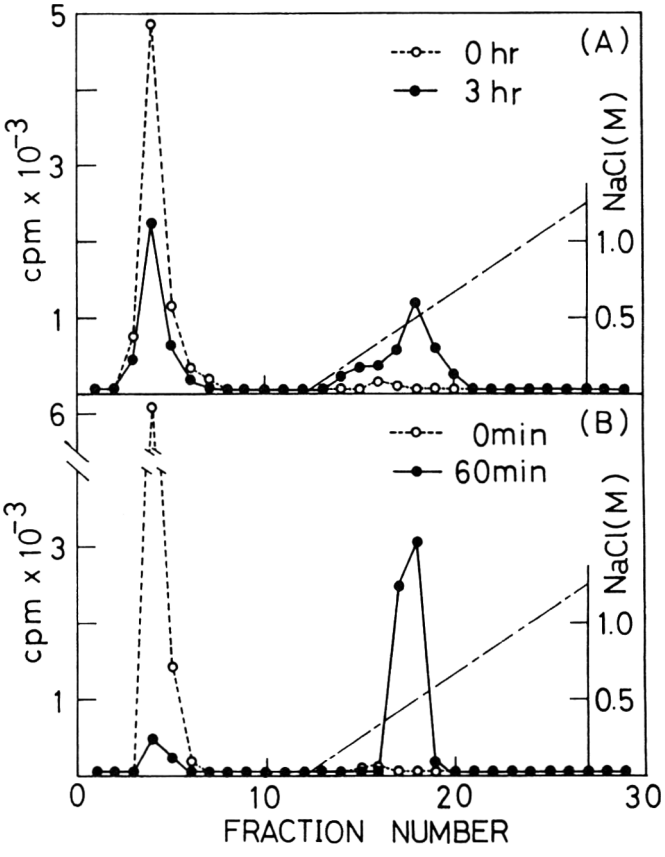


Fig. 5—Conversion of acetaldehyde to acetic acid by soybean extract (A) and yeast aldehyde dehydrogenase (B).

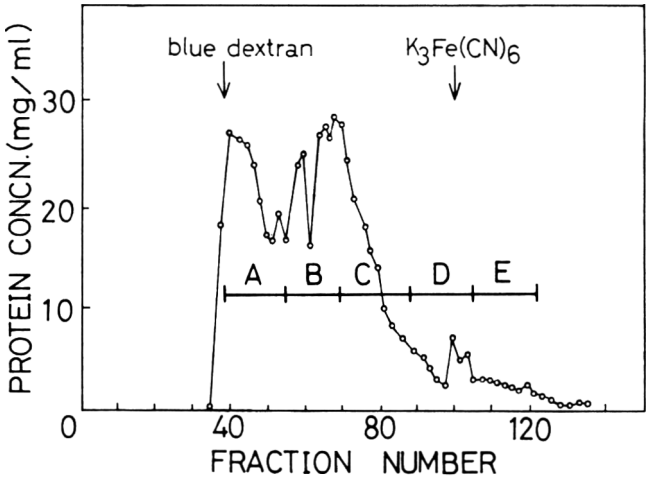


Fig. 6—Sephadex G-50 chromatography of soybean extracts.

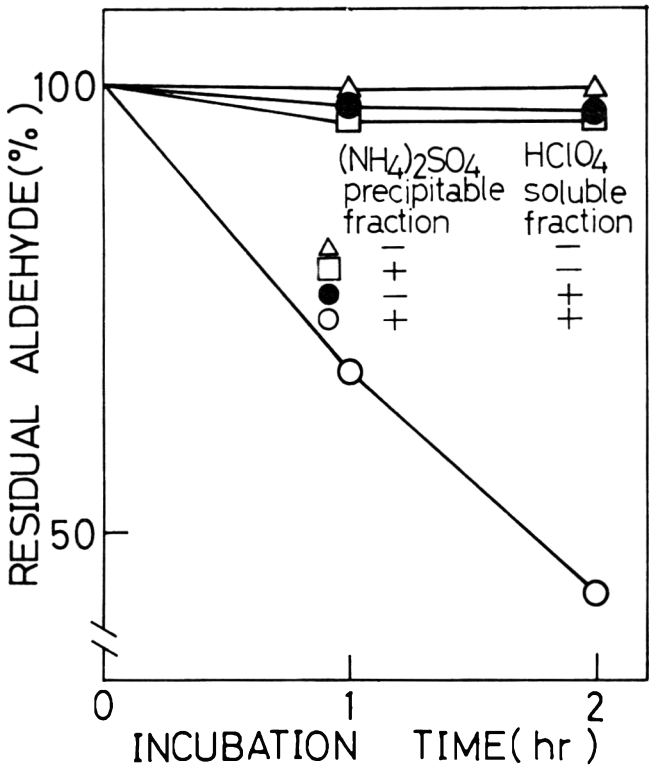


Fig. 7—Requirement of two factors for the aldehyde-eliminating activity. The reaction mixture contained, in a total volume of 9.0 ml, 6.3 ml of the ammonium sulfate-precipitable fraction, 1.8 ml of perchloric acid-soluble fraction and 0.3 mM acetaldehyde. The mixture was incubated at 25°C and at intervals aliquots were taken out to measure aldehyde.

It is likely that the aldehyde-eliminating reaction is catalyzed by an enzyme which requires a low molecular weight substance as cofactor. One might expect that aldehyde dehydrogenase is responsible for the eliminating activity and the low molecular weight of cofactor is NAD^+. However, alcohol was a main product when soybean extract was incubated with NAD^+ and acetaldehyde (Sasaki et al., in preparation). Thus participation of aldehyde dehydrogenase (which requires NAD^+) in the eliminating reaction is unlikely. Purification of the factors involved in this reaction is in progress in this laboratory.

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Fatty Acid Composition and Triglyceride Structure of Corn Oil, Hydrogenated Corn Oil, and Corn Oil Margarine

ANTONINO STROCCHI

ABSTRACT

Corn oil, hydrogenated corn oil and corn oil margarine were compared on the basis of fatty acid composition and triglyceride structure, including configurational isomerism of unsaturated fatty acids. Hydrogenated corn oil contained trans unsaturation and positional isomers not naturally found in corn oil. The β-position of triglycerides of hydrogenated corn oil was 85% occupied by trans octadecenoic acids (trans-18:1); that of corn oil was 70% occupied by linoleic acid. The proportion of cis octadecenoic acids (cis-18:1) in the β-position of the triglycerides was distinctly less in the hydrogenated corn oil than in corn oil, indicating a hydrogenation and/or a geometrical isomerization of cis-18:1 to trans-18:1 preferentially in the β-position, as compared with the α-positions. Chemical composition of corn oil margarine was intermediate between that of corn oil and that of hydrogenated corn oil.

INTRODUCTION

HYDROGENATION in the liquid phase of vegetable oil has been practiced since the discovery by William Normann (Normann, 1903), and it is now a vital process for both edible fat products and fatty chemical industries. In 1975 in the U.S., some 7.3 billion pounds of vegetable oil were hydrogenated for use in margarines, salad oils, cooking fats and shortenings (Allen, 1978). Edible oil hydrogenation occurs necessarily in a three-phase system: solid catalyst, nonvolatile liquid triglycerides and hydrogen gas with a limited solubility in the oil. Nickel is used almost exclusively as selective catalyst, except for some special use (Coenen, 1976; Allen, 1978). As a result of the reaction, part of the double bonds is eliminated, while a significant proportion of the remaining double bonds is isomerized through cis-trans conversion and positional shift in the fatty acid chain. Selective hydrogenation is characterized by high trans unsaturation development with minimum iodine value drops (Coenen, 1976; Wiedermann, 1978). As a result of these chemical changes, a change in two important quality aspects occurs: the melting range is shifted to higher temperatures and the stability to autoxidation and flavor deterioration is improved. Originally the former aspect was the primary aim of hydrogenation. Partially hydrogenated vegetable oils contain trans unsaturation, positional isomers and conjugation not naturally occurring in vegetable oils. The extent of the chemical transformations undergone by the hydrogenated oil can be expressed, even though in somewhat limited terms, by the content of trans unsaturated fatty acids (TUFA), especially as trans positional isomers of octadecenoic acid (trans-18:1 positional isomers), and in small quantity by trans isomers of octadecadienoic acid (cis-trans- and trans-trans-18:2 positional isomers). In the consumer available margarines in Italy we have analyzed, the quantity of TUFA varied from 7–35% (Strocchi, 1977; Strocchi and Bonaga, 1979); while even greater quantities have been reported by researchers in other countries (Zalewski and Kummerow, 1968; Carpenter and Slover, 1973; Parodi, 1976).

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In recent years there has been an increasing awareness of the influence of diet on human well-being, especially the effect of dietary fat on heart disease. Although the major emphasis has been on the fatty acid composition of food fats, other characteristics have been implicated. These include triglyceride structure (Elson et al., 1966; Yamamoto et al., 1969) Karney and Dhopeshwarkar, 1979) and configurational and positional isomers (Kummerow, 1974, 1975; Emken et al., 1979; Lawson and Kummerow, 1979; Wood, 1979). As a consequence of this awareness there have been numerous proposals that limitations be put on both the kind and amount of dietary fat. One of the results of these proposals is that it has become increasingly imperative that more information on the nature of food fats be available. There is a need for an overall characterization of a hydrogenated oil. This paper reports data on the natural and altered fatty acid composition and triglyceride structure of corn oil, as a result of the technological hydrogenation process used to produce corn oil margarine.

MATERIALS & METHODS

Samples

Three samples of edible oil products representative of the industrial production cycle of corn oil margarine have been analyzed, relative to their fatty acid composition and triglyceride structure. In the diagram of the production cycle (Fig. 1), the samples studied were those of (i) refined and bleached oil, (ii) hydrogenated oil (hydrogenated fat), and (iii) mixed oil (refined and hydrogenated oils) after deodorization. The last sample represents the fatty fraction of the margarine, and from now on will be referred to as corn oil margarine.

Determination of the fatty acid composition

The fatty acid composition, including the configurational isomers of octadecenoic and octadecadienoic acids (18:1 and 18:2), of the three samples of oils has been determined by GLC and TLC techniques. The analytical procedure involved, initially, the transformation of the triglycerides into methyl esters: a small part of them was then analyzed directly using gas chromatography (GLC-EGSS-X) to determine the fatty acid composition of the triglycerides; the remaining part was fractionated, based on the degree of unsaturation and configurational isomerism of the double bonds, using thin-layer chromatography with silica gel containing silver nitrate (TLC-$AgNO_3$). Gas chromatography (GLC-EGSS-X) was then used for the analysis of the individual fractions recovered from the TLC-$AgNO_3$ plates, with an internal standard, methyl heptadecanoate (17:0), to determine the cis:trans ratio for each unsaturated fatty acids in the triglycerides.

Determination of the triglyceride structure

The distribution of the fatty acids, including the configurational isomers of octadecenoic and octadecadienoic acids (18:1 and 18:2), in the α and β positions of the triglycerides, was determined by deacylation of the triglycerides with ethyl magnesium bromide. The analytical procedure involved (i) the partial deacylation of the triglycerides in such conditions that the maximum yield of diglycerides is obtained; (ii) the separation of the α-, β-diglycerides from the reaction mixture using thin-layer chromatography with silica gel containing boric acid (TLC-H_3BO_3); (iii) the transformation of the α-, β-diglycerides into the methyl esters; and (iv) the TLC-$AgNO_3$ and GLC-EGSS-X analyses of the methyl esters to determine, in this case, the fatty acid composition and the cis-trans ratio for each unsaturated fatty acid in the α-, β-diglycerides.

The analytical procedure is simpler for the corn oil and even more so, in general, for all the fatty oils which do not contain TUFA; in these instances it is sufficient to determine the fatty acid composition of the triglycerides and of the α-, β-diglycerides. The experimental conditions used for the preparation of the α-, β-diglycerides have been reported (Strocchi, 1980).

Argentation thin layer chromatography (TLC-$AgNO_3$)

20 cm x 20 cm glass TLC plates coated to a thickness of 0.3 mm with Kieselgel G containing 16% (w:w) silver nitrate were used. The plates were developed using the solvent system hexane:diethyl ether = 98.2 (v/v), air dried, sprayed with 0.1% 2.7-dichlorofluorescein in ethanol, and visualized with UV light. The zones containing cis-monoene, trans-monoene, cis, cis-diene and cis, trans plus trans, cis-diene were scraped from the plates, eluted with anhydrous diethyl ether and freed of solvent in a stream of N_2.

Gas liquid chromatography (GLC-EGSS-X)

Methyl esters were prepared from triglycerides and α-, β-diglycerides with H_2SO_4-methanol (AOAC, 1965), analyzed isothermally at 180°C on a 2m x 2 mm i.d. glass column packed with 10% EGSS-X on 100–120 mesh Gas Chrom P. Injector and detector temperatures were 230°C and 250°C, respectively. Carrier gas flow rate was 25 ml/min.

RESULTS & DISCUSSION

THE BASIC technological processing steps for vegetable fats and oils necessary to produce a bland stable finished edible oil are essentially operations for the removal of minor components which accompany the triglycerides, the material that we wish to purify (Cowan, 1976). These operations serve to remove the phosphatides (degumming), the free fatty acids (refining), the pigments to same degree (bleaching), and other objectionable materials which contribute to odor and flavor of the finished oil (deodorization). The modifications in the fatty acid composition caused by these operations are mainly represented by the formation of conjugated fatty acids (Guillaumin and Pertuisot, 1968) and of dimers and polymers from the polyunsaturated fatty acids (Waltking and Zmachinsky, 1970; Waltking et al., 1972; Evans et al., 1965; Rost, 1963). The quantity of these compounds formed in the oil processing steps is however small and, generally, has practically no influence on the fatty acid composition of the oil. In contrast, hydrogenation changes the fatty acid composition and triglyceride structure of the oil. These changes can be seen for the corn oil by comparing the fatty acid composition and the triglyceride structure for the corn oil and the corresponding hydrogenated corn oil (Table 1).

–Continued on next page

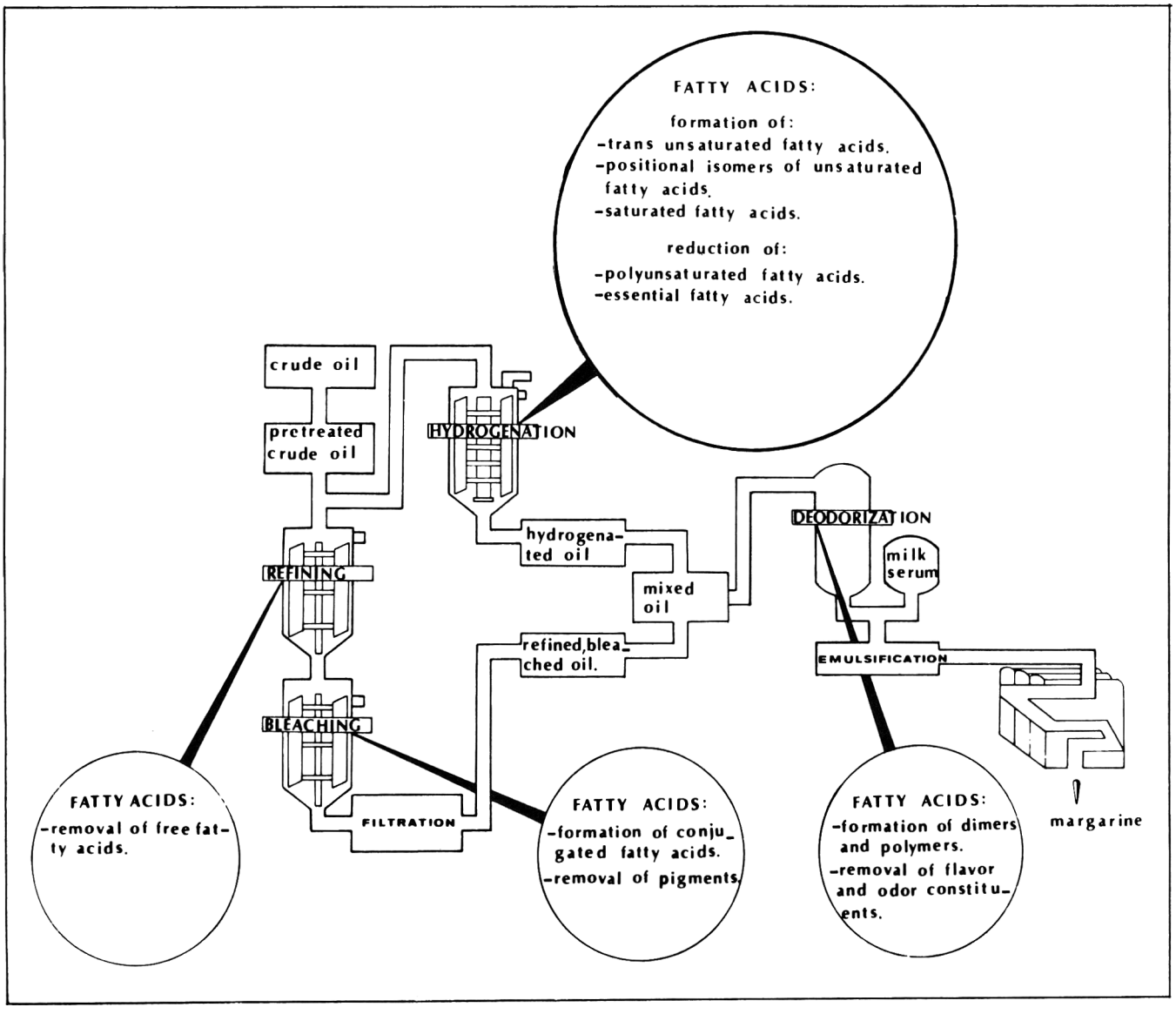


Fig. 1–Corn oil margarine processing plant.

Fatty acid composition

Eighty-six out of 100 of the moles of fatty acids in the triglycerides of the corn oil was C_{18} fatty acids, in the following proportions: cis,cis,cis-18:3 (linolenic acid: cis-9, cis-12, cis-15 octadecatrienoic acid) = 1%, cis,cis-18:2 (linoleic acid: cis-9, cis-12 octadecadienoic acid) = 55.2%, cis-18:1 (oleic acid: cis-9 octadecenoic acid) = 27.8%, and 18:0 (stearic acid) = 2%. In the hydrogenated corn oil the mole percent of the C_{18} fatty acids was nearly the same, 86.3%, but the proportions of the individual fatty acids were quite different: total 18:2 = 0.8% (cis,cis-18:2 = 0.6%; cis, trans-18:2 = 0.2%; trans,trans-18:2 = traces), total 18:1 = 78.2% (trans-18:1 = 59.1%, and cis-18:1 = 19.1%) and 18:0 = 7.2%.

From these data it can be seen that hydrogenation of corn oil, under the usual conditions employed in the technology of processes aimed at the production of table margarines, results in the almost complete reduction of the 18:2 to the 18:1, above all as trans-18:1. A small part of the 18:1 is in turn reduced to 18:0 since nickel has a good but not particularly high selectivity I (S_I). This expresses the preference for linoleic acid over oleic acid hydrogenation. If this preference is high, hydrogenation of linoleic acid goes stepwise and formation of stearic acid is limited until linoleic acid has almost been eliminated. The elimination of linoleic acid and the formation of monoenoic and saturated acids can be described approximately with two consecutive pseudo first-order reactions:

$$18{:}2 \xrightarrow{K1} 18{:}1 \xrightarrow{K2} 18{:}0$$

Thus, selectivity I can be defined as $S_I = K_1/K_2$.

Scheme 2 (Fig. 2) which is an extension of the half hydrogenated state mechanism first proposed by Horiuty and Polanay (1934) for the hydrogenation of ethylene, has been developed to account for the configuration and positional 18:1 isomers formed and for the isomerization reactions occurring during hydrogenation of methyl linoleate (Koritala et al., 1973a, b).

Triglyceride structure

Only two experimental values are necessary for the determination of the distribution of the fatty acids in the α and β positions of a triglyceride using the method of deacylation with ethyl magnesium bromide: the fatty acid composition of the triglycerides and that of the α-, β-diglycerides. Starting from these data one can calculate the distibution (mole percent) of the esterified fatty acids in the α positions (sn − 1 + sn − 3) and the β position (sn − 2) using the following formulas (Yurkowski and Brockerhoff, 1966):

$$\alpha\text{-Fatty acids} = 3 \times \text{triglyceride} - 2 \times \alpha\text{-, }\beta\text{-diglyceride}$$
$$\beta\text{-Fatty acids} = 4 \times \alpha\text{-, }\beta\text{-diglyceride} - 3 \times \text{triglyceride}$$

In the corn oil, 97.6% of the fatty acids in the β position of triglycerides were C_{18} fatty acids in the following proportions: 18:3 = 2.2%, cis,cis-18:2 = 70%, cis-18:1 = 25.4%. Once again the mole percent of the C_{18} fatty acids in the hydrogenated corn oil was nearly the same 97.2%, but with quite different proportions of the individual acids: the cis,cis-18:2 decreased to 1.4%, the cis-18:1 to 7.5%, and the trans-18:1 increased to 86.3%. The β position of the triglycerides in the corn oil was 70% occupied by the cis,cis-18:2, whereas in the hydrogenated corn oil it was 86% occupied by the trans-18:1. It also should be noted that in the corn oil, 31% of the cis-18:1 was present in the β position while in the hydrogenated corn oil only 13% of the cis-18:1 was in the β position. This indicates hydrogenation and/or geometrical isomerization of the cis-18:1 preferentially in the β position as compared with the α positions.

With regard to the corn oil margarine, the values of the cis,cis-18:2, cis-18:1 and trans-18:1 acids in the triglycerides, those in the α-, β-diglycerides and their corresponding values in the α- and β-fatty acids were indicative of a chemical composition intermediate between that of corn oil and hydrogenated corn oil. This is what would be expected since corn oil margarine is a mixture of these two kinds of oils.

In both the hydrogenated corn oil and margarine, the trans-18:1 isomers were found preferentially in the β position as compared with the α positions, in the proportion 46–49% in β as compared with the statistical value of 33%. This occurs because of the preferential placement of cis,cis-18:2 in the β position in the original vegetable oil (Brockerhoff, 1971) and then hydrogenation yields trans-monoenes in the β position. In contrast, trans-18:1 acids occurring naturally in animal fats are preferentially located in the α positions of the triglycerides (Duncan and Gartan, 1967; Woodrow and De Mann, 1968).

Table 1—Fatty acid composition and triglyceride structure of corn oil, hydrogenated corn oil and corn oil margarine

			Fatty acids[d] (mole %)											
Product	Compound	Positions	14:0	16:0	16:1	18:0	18:1 C	18:1 T	18:2 C-C	18:2 C-T	18:2 T-T	18:3 C-C-C	20:0	20:1
Corn oil	Triglyceride	α,β,α	0.1	13.1	0.2	2.0	27.8	tr.	55.2	–	–	1.0	0.5	0.1
	α,β-Diglyceride	α,β	0.1	10.3	0.2	1.5	27.2	tr.	58.9	–	–	1.3	0.4	0.1
	α-Fatty acids, (calc)[a]	α	0.1	18.7	0.2	3.0	29.0	tr.	47.8	–	–	0.4	0.7	0.1
	β-Fatty acids, (calc)[b]	β	0.1	1.9	0.2	0	25.4	tr.	70.0	–	–	2.2	0.1	0.1
	Proportion, % (calc)[c]	β	33	5	33	0	31	0	42	0	0	73	7	33
Hydrogenated corn oil	Triglyceride	α,β,α	0.1	13.0	0.1	7.2	19.1	59.1	0.6	0.2	tr.	0.1	0.4	0.1
	α,β-Diglyceride	α,β	0.1	10.2	0.1	6.0	16.2	65.9	0.8	0.2	tr.	0.1	0.3	0.1
	α-Fatty acids, (calc)[a]	α	0.1	18.6	0.1	9.6	24.9	45.5	0.2	0.2	tr.	0.1	0.6	0.1
	β-Fatty acids, (calc)[b]	β	0.1	1.8	0.1	2.4	7.5	86.3	1.4	0.2	tr.	0.1	0	0.1
	Proportion, % (calc)[c]	β	33	5	33	11	13	49	78	33	0	33	0	33
Corn oil Margarine Hydrogenated corn oil + corn oil	Triglyceride	α,β,α	0.1	13.0	0.1	4.3	23.8	26.6	31.1	0.1	tr.	0.4	0.4	0.1
	α,β-Diglyceride	α,β	0.1	10.4	0.1	3.6	22.2	29.1	33.5	0.1	tr.	0.5	0.3	0.1
	α-Fatty acids, (calc)[a]	α	0.1	18.2	0.1	5.7	27.0	21.6	26.3	0.1	tr.	0.2	0.6	0.1
	β-Fatty acids, (calc)[b]	β	0.1	2.6	0.1	1.5	17.4	36.6	40.7	0.1	tr.	0.8	0	0.1
	Proportion, % (calc)[c]	β	33	6	33	11	24	46	44	33	0	67	0	33

[a] 3 x Triglyceride − 2 x α,β-Diglyceride
[b] 4 x α,β-Diglyceride − 3 x Triglyceride
[c] (β-Fatty acids/3 x triglyceride) x 100
[d] C = Cis; T = Trans

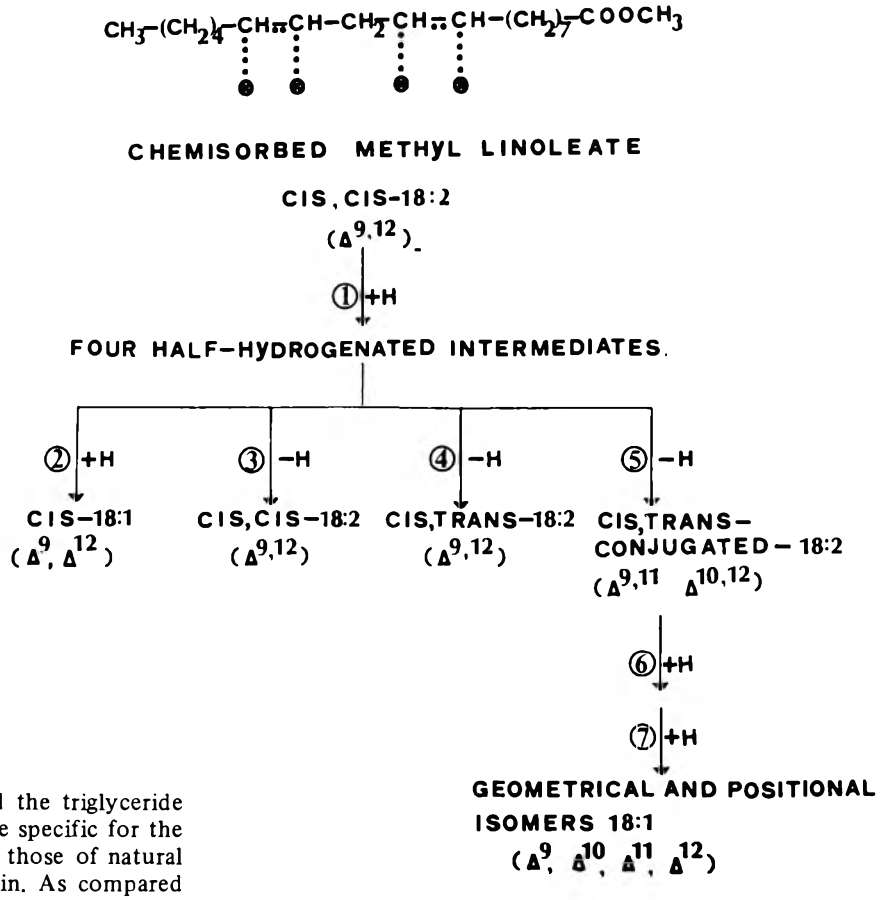


Figure 2—Half hydrogenated state mechanism for the hydrogenation of methyl linoleate.

CONCLUSION

THE FATTY ACID COMPOSITION and the triglyceride structure of the hydrogenated corn oil are specific for the hydrogenated oil and not comparable to those of natural lipids of either vegetable or animal origin. As compared with the vegetable oils, the hydrogenated corn oil contained double bond configurational and positional isomers; as compared with animal fats, the hydrogenated corn oil contained higher net quantities of TUFA (up to 59.1%), and had them preferentially located in the β position of the triglyceride.

Corn oil margarine, just as any edible oil product obtained by mixing natural and hydrogenated vegetable oils, should be considered a product which has undergone partial chemical transformations, with all the resulting consequences from both the biological and nutritional point of view and food labeling requirements.

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Anthocyanins of Sunflower (*Helianthus annuus*)

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ABSTRACT

The presence of anthocyanins in the achenes of some varieties of sunflower was ascertained for the first time. The two major pigments were identified as Pn-3-divanillylsambubioside and Cy-3-vanillylsambubioside. For two other pigments present in smaller quantities, a tentative identification awaiting confirmation was made and the constituents were identified. Traces were found of five other pigments for which chromatographic data are presented.

INTRODUCTION

ONE OF THE PROBLEMS facing the food industry today is the replacement of the natural coloring of foodstuffs that is destroyed during processing, preservation, or both. With regard to red colorants, in the last few years there has been a gradual abandonment of synthetic products (Botnra, 1974; Italian Ministry of Health, 1976), because of their potential toxicity in favor of natural pigments. One can therefore predict a rapid spread in the use of natural colorants for foodstuffs, especially those colorants of vegetable origin. Interest in new sources of natural pigments is therefore justified, especially if there is the possibility of obtaining large quantities at high concentrations and low production costs.

This paper deals with the identification of the anthocyanin pigments found in the achenes of some varieties of sunflower (*Helianthus annuus*) with a view towards using this colorant source on an industrial scale (Pifferi, 1979). There is nothing in the literature on the sunflower as a possible source of anthocyanins (Dupaigne, 1974; Francis, 1975), with the exception of one paper (Bottazzi, 1949), in which, however, the identity of such compounds was not investigated. In this paper a positive correlation between the presence of pigment in the leaf stems and in the parts of reproductive organs was established by statistical analysis, as well as the action of modifying factors on a gene conditioning the pigmentation.

EXPERIMENTAL

LABORATORY GRADE REAGENTS were used for the extractions and preliminary treatments of the material; at least analytical grade reagents were used for the other phases. Samples of the seeds of over 200 varieties of sunflower from all parts of the world were analyzed. Four seeds of each variety were treated with 10 ml of 1% HCl in CH_3OH. The qualitative composition of the colored extracts was determined by two-dimensional paper chromatography on Whatman 3MM using the solvents *n*-butanol:butylacetate:formic acid (99%):water (BBAW, 13:5:2:3) (Vaccari and Pifferi, 1978) and acetic acid:conc HCl:water (AAW, 15:3:82). A comparative analysis of the pigment quantities in the different varieties was made by recording the UV-visible spectra with a model 254 Perkin Elmer spectrophotometer after 1:5 dilution with 1*M* HCl.

The pigments were extracted with HCl in CH_3OH ($25 \cdot 10^{-4} M$) yielding a solution that was so rich in pigment that further concentration was not required. This extract was fractionally precipitated with a mixture of ethyl ether and petroleum ether (60–80°C) (1:1 v/v) with ratios of 1:3, 1:6, and 1:12 (v/v).

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The last fraction, which had the usual characteristics of free anthocyanin precipitates, was separated by paper chromatography (Du et al., 1975) with BBAW, and the pigments were recovered with 1% HCOOH in CH_3OH. Where necessary, the final separation was obtained by rechromatographing the extracts of the unresolved bands with AAW.

The individual pigments were precipitated with an excess of ethyl ether:ethyl acetate (10:2 v/v) (Pifferi et al., 1972), repeating the operation until complete purification, which we tested for by using two-dimensional paper chromatography with BAW (12:3:5) and AAW, with chromogenic reagents for acids (Roberts and Wood, 1951) and sugars (Smith, 1960). Finally, the pigments were vacuum-dried.

The pigments were identified by following the standard chromatographic and spectroscopic procedure fully described in the literature (Harborne, 1967). Alkaline and acid hydrolysis was used to determine the constituents of the pigments (Francis and Harborne, 1966; Chen and Luh, 1967). We used thin-layer chromatography to identify the sugars (Ovodov et al., 1967; Taludker, 1971; Hansen, 1976; De Zeeuw and Dull, 1975) and acids (Pifferi, 1966; Jangaard, 1970; Bhatia and Bajaj, 1970; Hotellier and Delaveau, 1970; Rapp an Ziegler, 1973).

RESULTS & DISCUSSION

OF THE DIFFERENT VARIETIES examined, only 5% contained anthocyanins. With the exception of the Airelle variety, these all showed the same qualitative spectrum, with the presence of nine different pigments, four of which predominated, differing only quantitatively. For simplicity, all successive operations were carried out on one cultivar of sunflower we isolated in the course of our research. The total amount of red pigments in the husks was 2.2% (w/w).

Of the nine pigments present in the sunflower extracts (Table 1), only the major pigments (No 1, 2, 4, and 5) were tentatively identified.

Pigment 1

This was the second major anthocyanin in the husk extract. Partial acid hydrolysis of this pigment yielded three intermediates ($R_f \times 100$ in BAW 34, 37, and 41, respectively), the first with a mobility similar to authentic Cy-3-sambubioside (Du and Francis, 1973) and the second to Cy-3-glucoside (Ribéreau-Gayon, 1958). Complete acid hydrolysis demonstrated the presence of the aglycone cyanidin, the sugars glucose and xylose, and vanillic acid, the last-mentioned also confirmed by alkaline hydrolysis (Table 2). The vanillic acid was identified examining the TLC plates under UV-radiation, before and after exposure to ammonia vapor, and then spraying with bromcresol green 0.1% (w/v) in ethanol and after with diazotized p-nitroaniline reagent (Sakellariades and Luh, 1974) or aqueous 2% $FeCl_3$ solution, acidified with 1 ml of 2M HCl (Pifferi, 1965).

To our knowledge this is the first time that vanillic acid has been identified as acyl moiety of anthocyanins, even though its presence has been noted in the phenolic fractions of some varieties of seeds (el-Basyouni and Towers, 1964; Méndez and Lojo, 1971). Spectral data (Table 1) led us to exclude glycosidation of position 5.

The UV-visible spectrum of the pigment appears to confirm acylation with vanillic acid. This acid, in fact, has

a UV spectrum with absorption maxima and molar absorptivity (ε) (Lemon, 1947; Englis and Manchester, 1949; Mikawa et al., 1956) such as to justify the absence of a peak in the zone characteristic of anthocyanins acylated with cinnamic acids (Harborne, 1967; Chen and Luh, 1967), which, in addition, have ε values that are quite higher (Kamlet, 1960). Vice versa, the fact its spectrum overlaps that of the anthocyanin justifies the width of the UV band of the anthocyanin, which has a shoulder at ca 250 nm and an intensity that reaches values near those of its maximum in the visible spectrum, unlike that which has been reported by Harborne (1967) and Ribéreau-Gayon (1968) for nonacylated anthocyanins. On this basis, the pigment was identified as Cy-3-vanillylsambubioside.

Pigment 2

The spectral data showed a free position 5 and a large absorption in the UV region at a wavelength below 300 nm already discussed above. Complete acid hydrolysis yielded malvidin and probably xylose and arabinose, in addition to vanillic acid, also identified by alkaline hydrolysis. The number and sequence of the pigments resulting from partial acid hydrolysis led us to assume that the pigment was an acylated Mv-3-diglycoside. The small quantity of starting pigment together with the lack of suitable reference standards prevented us from making a complete, confident identification.

Pigment 4

For this pigment also, it was possible, on the basis of

Table 1—Characteristic data of Sunflower anthocyanins

		Pigments								
		1	2	3	4	5	6	7	8	9
R_f x 100	BBAW	19	23	27	29	38	51	65	63	69
	AAW	24	30	21	22	31	31	40	59	64
	BAW	38	46	–	47	53	58	68	77	76
	BuHCl[a]	25	38	–	30	45	–	–	–	–
	1% HCl[b]	9	11	–	11	10	–	–	–	–
UV fluorescence		neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
λ_{UV} max (nm)		281	280	–	273	240	–	–	–	–
$\lambda_{vis.}$ max. (nm)		530	528	–	510	520	–	–	–	–
$\Delta\lambda_{vis.}$ Max with $AlCl_3$ (nm)		27	0	–	34	0	–	–	–	–
$E_{440}/E_{\lambda vis.\ max}$		0.26	0.31	–	0.33	0.27	–	–	–	–

[a] BuHCL, n-butanol:2N HCl (1:1, top layer)
[b] 1% HCl, water:conc HCl (97:3)

Table 2—Chromatographic mobilities of hydrolysis products

	R_f x 100											
Sample	BAW	AAW	Forestal[b]	CMF[c]	BDA[d]	KCl 20%[e]	AMW[f]		EMBW[g]		BAcW[h]	
Aglycones:												
# 1	66	10	49									
# 2	59	7	60									
# 4	68	10	47									
# 5	72	12	65									
Acid:												
# 1				65	70	50						
# 2				65	69	50						
# 4				66	69	49						
# 5				67	68	48						
Sugars:												
# 1							34	68	14	37	14	27
# 2							70	51	38	27	29	20
# 4							35	53	13	23	13	20
# 5							35	70	14	36	14	27
Standards:												
Cyanidin[a]	68	11	49									
Malvidin[a]	58	9	60									
Peonidin[a]	71	12	30									
Vanillic acid				64	70	52						
Glucose							36		14		13	
Xilose							71		38		27	
Arabinose							53		25		20	

[a] Mullick and Brink (1967)
[b] Forestal, acetic acid:conc. HCl:water (30:3:10)
[c] CMF Chloroform:methanol:formic acid (85:10:5) (Pifferi,1965)
[d] BDA, benzene:dioxane:acetic acid (90:25:5) (Hotellier and Delaveau, 1970)
[e] KCl 20%, aqueous 20% potassium chloride solution (Bhatia and Bajaj, 1970)
[f] AMW, acetone:methanol:water (80:15:5) (De Zeeuw and Dull, 1975)
[g] EMBW, ethyl acetate:methanol:n-butanol:water (16:3:3:2) (Talukder, 1971)
[h] BAcW, n-butanol:acetone:water (4:5:1) (Ovodov et al., 1967)

its UV-visible spectrum to exclude glycosidation of position 5 of the aglycone and to assume the presence of a benzoic-type acid. Chromatographic analysis of the products of partial acid hydrolysis demonstrated the presence of three intermediates (R_f x 100 in BAW 28, 36 and 42, respectively), the second of which was identified as Cy-3-glucoside by comparison with authentic sample (Ribéreau-Gayon, 1958). The glycidic residues were identified as glucose and arabinose, the acyl residue as vanillic, and the aglycone as cyanidin (Table 2). The small quantity of pigment available did not permit us to make a complete identification; it was possible to put forward only a tentative identification as Cy-3-vanillyl-glucosylarabinoside.

Pigment 5

This was the major pigment in the sunflower achenes, accounting for more than 50%. The $E_{440}/E_{\lambda vis\cdot max}$ value of 0.27 ruled out glycosidation of position 5 of the aglycone. The analysis of the products of complete acid hydrolysis led to the identification of the aglycone peonidin, the sugars glucose and xylose, and vanillic acid, confirmed also by alkaline hydrolysis.

The number of pigments yielded by partial acid hydrolysis was not decisive for identification. Additional information was gained by observing the sequence in which they appeared with increasing hydrolysis time. In fact, the original pigment was rapidly transformed into another having a lower R_f in BAW (45 instead of 53), which subsequently gave rise to three other pigments (R_f x 100 in BAW 35, 41, and 47, respectively), the first two identified as Pn-3-sambubioside (Harborne, 1960) and as Pn-3-glucoside (Ribéreau-Gayon, 1958) by comparison with standard anthocyanin samples.

It seemed reasonable to assume that the intermediate pigment was Pn-3-sambubioside monoacylated, and the original pigment could thus be identified as Pn-3-divanillylsambubioside. The chromatographic behavior of this pigment in the various solvents was consistent with this identification.

The UV-visible spectrum of the pigment (Fig. 1) confirmed this identification, in that, in addition to showing the previously discussed characteristics, it also showed two very distinct peaks below 300 nm, the first of which at the characteristic value of the more intense band of vanillic acid (Lemon, 1947; Englis and Manchester, 1949; Mikawa et al., 1956), considering also the bathochromic effect of the solvent. This behavior can be justified only on the basis of a greater acylation.

CONCLUSIONS

THE MAJOR anthocyanin pigments present in the seed of some varieties of sunflower were Cy-3-vanillylsambubioside and Pn-3-divanillylsambubioside. For another two pigments present in small quantities it was possible to gain some indications of their probable structure, which, however, necessitate confirmation. Another five pigments were present in trace amounts.

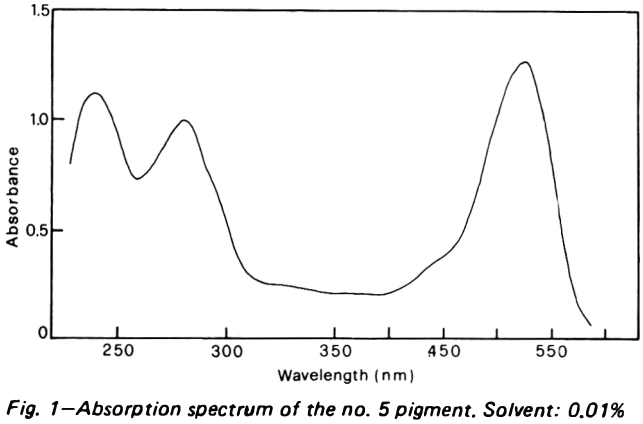


Fig. 1—Absorption spectrum of the no. 5 pigment. Solvent: 0.01% HCl in CH_3OH.

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Nutritional Properties and Metabolic Studies of Acylated Beef Heart Myofibrillar Proteins

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ABSTRACT

Beef heart myofibrils were acylated with several concentrations of acetic (AA) and succinic (SA) anhydride, and digestibility and utilization of the myofibrillar proteins were determined. Results for in vitro hydrolysis of the untreated and acylated proteins varied, depending on the enzyme(s) used in the analysis. Rat protein efficiency ratios and net protein ratios for untreated (PER = 2.83, NPR = 116) and acetylated (PER = 2.55, NPR = 110) proteins were greater than for casein (PER = 2.50, NPR = 100), whereas values for succinylated proteins (PER = 2.36, NPR = 87) were less than for casein. Most of the radioactivity recovered after 24 hr from rats fed ^{14}C-acylated myofibrillar proteins was in expired CO_2; 62.8% for ^{14}C-acetylated and 45.8% for ^{14}C-succinylated proteins. Rats acclimated to an acylated protein diet for 28 days showed improved metabolism of ^{14}C-acetylated protein and decreased metabolism of ^{14}C-succinylated proteins; 75.7% and 38.1% recovery as expired CO_2, respectively.

INTRODUCTION

ALTHOUGH CHEMICAL MODIFICATION of food proteins may be used for several purposes (Shetty and Kinsella, 1979), improvement in protein functional properties has been a major goal of most investigators. Studies on improving functionality of proteins from muscle sources have been limited to fish protein (Groninger, 1973; Chen et al., 1975; Groninger and Miller, 1975; Miller and Groninger, 1976; Hatano et al., 1979a, b) and beef heart protein (Eisele and Brekke, 1981). In these studies, modification with acylating agents resulted in improved protein functional properties such as solubility, emulsification parameters, and foaming. If use is to be made of acylated protein derivatives in food systems, information on their nutritional and metabolic properties must also be obtained and considered.

Bjarnason and Carpenter (1969) fed lysine-acylated bovine plasma albumin to lysine-deficient rats. Acylated protein gave a lower growth response than equivalent amounts of unmodified protein. Creamer et al. (1971) fed healthy rats diets of acylated casein and observed a decrease in protein efficiency ratio (PER). Although the rats survived, they were lighter in weight, had smaller litters, and had less depot fat than the control animals. Belikow et al. (1975) used a microbial assay and reported succinylation of amino groups reduced the biological value of the casein protein by 10%, whereas acylation of the same amino group with a stearyl radical actually enhanced the biological value by 15%.

Groninger and Miller (1979) fed acylated fish proteins to rats and found that highly acetylated (75% of the ϵ-amino groups of lysine) myofibrillar protein had a PER of 85% of the reference casein, and medium succinylated (50% of the ϵ-amino groups of lysine) myofibrillar protein had a PER of 73% of the reference casein. The researchers concluded that the influence of acylation on protein utilization and nutritional quality appears to be partially dependent on the type of protein, the amount of protein modification, and the type of acylating agent used.

Other methods of measuring protein nutritional quality involve the use of in vitro enzymatic proteolysis tests (Stahmann and Woldegiorgis, 1975). Groninger and Miller (1979) reported tryptic hydrolysis was less for both succinylated and acetylated fish protein compared to native protein. These findings were consistent with the results of Li and Bertch (1960) in that trypsin did not hydrolyze peptide bonds that involved acylated lysine. However, another proteolytic enzyme, pepsin, was shown to hydrolyze succinylated and acetylated protein at the same rate as unacylated protein (Groninger and Miller, 1979). Proteolytic hydrolysis of an acylated protein evidently depends on the enzyme used.

The study by Groninger and Miller (1979) also investigated the metabolism of acylated protein. They fed ^{14}C-succinylated fish protein to rats and noted that the majority of the radioactivity was recovered in the carbon dioxide expelled from the lungs and in the urine. Their findings indicated that the rat could digest, absorb, and metabolize ^{14}C-acylated protein. In general, ^{14}C-acetylated protein was utilized to a greater extent than was ^{14}C-succinylated protein, as evidenced by the greater amount of expired radioactive carbon dioxide from rats fed ^{14}C-acetylated protein. Intravenous administration of ^{14}C-succinyllysine and ^{14}C-acetyllysine resulted in less utilization than an oral dose of either derivative. Groninger and Miller (1979) suggested that the action of gut microorganisms may have distorted the actual utilization of the ^{14}C-acetylated protein by the rat. Nevertheless, rats were able to digest, absorb, and metabolize acylated protein.

It has been shown that acylating beef heart myofibrils with acetic or succinic anhydride improved protein solubility, emulsifying capacity, emulsion activity and emulsion stability (Eisele and Brekke, 1981). The objective of the work reported here was to examine the nutritional value and metabolic properties of acylated beef heart myofibrillar protein compared to unmodified protein.

EXPERIMENTAL

Isolation and acylation of myofibrils

Beef heart myofibrils were isolated and acylated as described in a previous report (Eisele and Brekke, 1981). Acylating agents were acetic (AA) and succinic anhydride (SA) at 0, 0.6, 1.5 and 5.0 mmol anhydride/g protein.

Determination of extent of chemical modification

Protein was measured by the biuret method (Paik and Kim, 1972). Reactive groups in modified (including the radioactive derivatives) and unmodified myofibrillar proteins were assayed as follows: available lysine with picryl sulfonic acid (Kakade and Liener, 1969); sulfhydryl groups with Ellman's reagent (Habeeb, 1972); and hydroxyl groups by deacylation with hydroxylamine (Hestrin, 1949).

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In vitro protein digestibility

A multienzyme system developed by Hsu et al. (1977) was used to evaluate in vitro digestibility of native, acetylated, and succinylated beef heart myofibrillar protein. Acylated protein has been reported to be less susceptible to trypsin hydrolysis (Groninger and Miller, 1979). Therefore, the multienzyme system of trypsin, chymotrypsin, and peptidase, as well as trypsin alone, was used. Enzymes were purchased from Sigma Chemical Company, St. Louis, MO: trypsin, type IX, from porcine pancreas; chymotrypsin, type II, from bovine pancreas, and peptidase, grade III, from hog intestinal mucosa.

Native and acylated beef heart myofibrillar proteins were precipated at pH 4.5, the pH at which native and acylated myofibrillar proteins were insoluble (Eisele and Brekke, 1981), and centrifuged at 10,000 x *g* for 15 min. The protein pellets were resuspended in water adjusted to pH 4.5 with HCl. This washing procedure, centrifugation and resuspension was repeated three more times. The final pH was adjusted to 8.0 with 0.01M KOH. Enzymes were added, and the pH was recorded automatically over a 10-min period using a recording pH meter. Regression analysis of 23 samples tested by Hsu et al. (1977) showed the correlation coefficient between pH at 10 min and in vitro apparent digestibility was 0.90.

In vitro pepsin hydrolysis

A modification of the procedure of Akeson and Stahmann (1964) for in vitro pepsin hydrolysis of proteins was used to evaluate hydrolysis rates of native, acetylated, and succinylated beef heart myofibrillar protein. Native and acylated myofibril suspensions were dialyzed against water for 48 hr to remove salts and excess acylating reagents. Three hundred milligrams of protein suspended in 100 ml distilled water, plus 30 mg pepsin (Sigma Chemical Co., St. Louis, MO) at pH 1.5, were incubated at 37°C for 3 hr. Aliquots of 0.2 ml were removed at periodic time intervals for determining α-amino groups with ninhydrin reagent. The increase in α-amino groups, expressed as nmol leucine/g protein, was plotted versus time.

Amino acid analysis

Amino acid analysis was performed on native, acetylated, and succinylated beef heart myofibrillar protein to determine whether acylation altered the amino acid composition. Single samples from two different preparations of each treatment were hydrolyzed by the procedure of Spackman et al. (1958) and analyzed on a Beckman 121 amino acid analyzer. Protein samples were dialyzed against water for 48 hr, then vacuum dried at 30°C prior to analysis.

Amino acid score

The amino acid score was calculated as a percentage of the lowest essential amino acid in the protein sample compared to the FAO/WHO (1973) amino acid reference pattern. The amino acid with the lowest amino acid score was judged the limiting amino acid.

Computed protein efficiency ratio (C-PER)

A rapid method of estimating protein quality that was developed by Satterlee et al. (1979), termed the computed protein efficiency ratio (C-PER), was used. The C-PER was reported to give reasonably accurate predicted protein efficiency ratio (PER) values in comparison to rat PER values for foods that ranged from 0.67–3.22 PER.

Rat protein efficiency ratio (PER) and net protein ratio (NPR)

The rat assay for protein efficiency ratio (PER) was conducted according to the method outlined in AOAC (1975) with several modifications. Vitamin-free casein, AIN Mineral Mix 79, and AIN Vitamin Mix 76 (ICN Pharmaceuticals, Inc., Cleveland, OH) were used in the diet as recommended by the Report of the AIN Ad Hoc Committee on Standards for Nutritional Studies (1977) rather than the ANRC reference casein, USP salt mix, and the AOAC vitamin mix. Rats were an average of 32 days old at the start of the experiment, rather than the recommended 21 to 28 days, and rats were acclimated in the test laboratory for 9 days rather than the recommended 3–7 days. The male Sprague-Dawley rats used were obtained from the Washington State University Laboratory Animal Resource Center.

Native and acylated beef heart myofibril suspensions were freeze-dried, then crushed into fine particles with a mortar and pestle. The freeze-dried portion was mixed with the other dry diet ingredients with an electric mixer, followed by gradual addition of corn oil, then water.

Ten rats per diet were fed at the 10% protein level for 28 days on the following test diets: casein, beef heart myofibrillar proteins, beef heart myofibrillar proteins modified with 0.6 mmol acetic anhydride/g protein and beef heart myofibrillar proteins modified with 1.5 mmol succinic anhydride/g protein. At the end of the feeding trial, rats were sacrificed, organs were removed, examined, and liver/body weight ratios calculated.

The diets and the test animals for the NPR assay were the same as described for the PER assay. The NPR was a 10-day assay which also included a no-protein group to estimate maintenance requirements (Janse, 1978). The NPR was calculated by adding the weight loss of the no-protein group to the weight gain of the test protein group divided by the weight of test protein consumed. The mean NPR for each test protein group was expressed as a percentage of the corresponding value for the casein control group.

Preparation of ^{14}C-labelled acylated proteins

Acetic anydride at 0.6 mole/g protein and 100 μCi [1-^{14}C] acetic anhydride (New England Nuclear, Boston, MA) were dissolved in methyl Cellosolve. The methyl Cellosolve, containing the labelled and unlabelled anhydride, was added dropwise to 80 ml of beef heart myofibrils at 25 mg protein/ml in 0.1M NaCl, 0.05M potassium phosphate, pH 8.0–8.5. The protein solution, at 2–5°C, was stirred constantly while maintaining the pH with 0.1N KOH. Succinic anhydride at 1.5 mmol/g protein and 100 μCi [1,4-^{14}C] succinic anhydride (New England Nuclear) were dissolved in methyl Cellosolve and used in a similar manner to synthesize the ^{14}C-succinylated beef heart myofibrillar protein.

The acetylated and succinylated ^{14}C-derivatives were purified by precipitation at pH 4.5, then centrifuged at 10,000 x *g* for 15 min. The protein pellet was resuspended in water adjusted to pH 4.5 with dilute HCl, and the procedure repeated until the supernatant had a radioactive count equal to background. The final pellet was suspended, with the use of a glass stirring rod, in 0.05M potassium phosphate, pH 7.4. The specific activity was 2800 dpm/mg protein for the ^{14}C-acetylated protein and 3300 dpm/mg protein for the ^{14}C-succinylated protein.

Metabolism of ^{14}C-labelled acylated protein

Eight rats, 6 wk old, were injected intragastrically (IG), using a syringe fitted with 20 cm of Teflon tubing, with 1.0 x 10^6 dpm of ^{14}C-labelled acylated beef heart myofibrillar protein per 100g body weight; four rats with the 0.6 mmol AA/g protein and four rats with the 1.5 mmol SA/g protein. The rats were on a standard lab chow diet (College of Veterinary Medicine, Washington State Univ., 1979) and were starved 24 hr before injection of the ^{14}C-labelled acylated protein. Immediately after injection, animals were placed in metabolism chambers (Clark, 1964; Nixon et al., 1977). Air was pulled through two 20% KOH traps; one as a pre-trap to remove incoming carbon dioxide and another to collect expelled ^{14}C-labelled carbon dioxide. Urine was allowed to flow into a glass tube, and feces were collected under a wire mesh screen. After the alloted time of 24 hr, animals were decapitated and as much blood as possible was collected into a funneled centrifuge tube.

Blood was centrifuged at 2000 x *g* for 15 min to separate it into serum and red blood cells. Volume of serum, red blood cells, and urine was recorded. The stomach, intestine, liver, spleen, kidneys, heart and lungs were removed, put on ice, blotted, and weighed. These organs, plus the urine, feces, and red blood cells were digested in 20% KOH for 24–48 hr at room temperature, and then bleached with hydrogen peroxide. Aliquots of the digested samples were analyzed for radioactivity in a scintillation counter.

The extent of recovery in the ^{14}C-labelled carbon dioxide and the radioactivity in the urine was used as a measure of the utilization of the ^{14}C-labelled acylated protein. The amount of radioactivity in the stomach, intestine, and feces was used as measure of nonabsorption of these same materials over the 24-hr period.

From the PER feeding trial, four rats from the diet modified with 0.6 mmol AA and four from the 1.5 mmol SA diet were subjected to the same metabolic experiment as previously described. The purpose was to determine if a rat on a particular diet for 28 days would adjust to that diet, and, hence, utilize or metabolize

acylated protein more efficiently. An increase in expired ^{14}C-labelled carbon dioxide and radioactivity in the urine would demonstrate that effect.

Statistical analysis

All data represent a mean value of a duplicate or triplicate analysis of two or more replications. For the chemical modification analysis and the nutritional evaluations, a replication represents one heart myofibril preparation. Means and standard deviations were calculated. Significance of difference between stated sets of data was determined by the "t" statistic (Steel and Torrie, 1960).

RESULTS & DISCUSSION

In vitro enzyme hydrolysis and in vitro digestibility

Several researchers have shown that succinylated and acetylated proteins impair in vitro trypsin hydrolysis, but have no effect on in vitro pepsin hydrolysis (Groninger and Miller, 1979; Li and Bertch, 1960). The results for tryptic hydrolysis of unacylated, acetylated, and succinylated beef heart myofibrillar protein are shown in Fig. 1. With increased modification of the ϵ-amino groups of both acetylated and succinylated proteins, hydrolysis was progressively less than for unacylated protein. Also, at any given extent of acylation, except at 5.0 mmol/g protein, tryptic hydrolysis was less for acetylated than for succinylated protein. This reflects the increased reactivity of lysine with AA compared to SA (Table 3). Trypsin is an endoenzyme specific for hydrolysis on the carboxyl side of lysine and arginine. As the ϵ-amino group of lysine was modified with increased acylation, the protein became unavailable to the tryptic enzyme. These results were in agreement with the work of Groninger and Miller (1979) on tryptic hydrolysis of acylated fish myofibrillar protein.

Pepsin hydrolysis was also evaluated with unacylated, acetylated, and succinylated beef heart myofibrillar protein (Fig. 2) by measuring the release of α-amino groups. Compared to unacylated protein, acetylated protein was less susceptible to pepsin hydrolysis while succinylation enhanced hydrolysis. These results are in disagreement with pepsin hydrolysis rates on an acylated fish protein reported by Groninger and Miller (1979). The difference may be due to the amount of modification and the type of protein used in this study.

The hydrolysis rates using the multienzyme method on unacylated, acetylated, and succinylated beef heart myofibrillar proteins are shown in Fig. 3. The hydrolysis of the modified proteins was less than the unacylated protein with the succinylated protein showing less hydrolysis than acetylated protein.

In vitro digestibility coefficients were calculated from the enzymatic hydrolysis rates by the method of Hsu et al. (1977) and are given in Table 1. Statistical analysis of the in vitro digestibility coefficients of unacylated, acetylated, and succinylated beef heart myofibrillar proteins derived

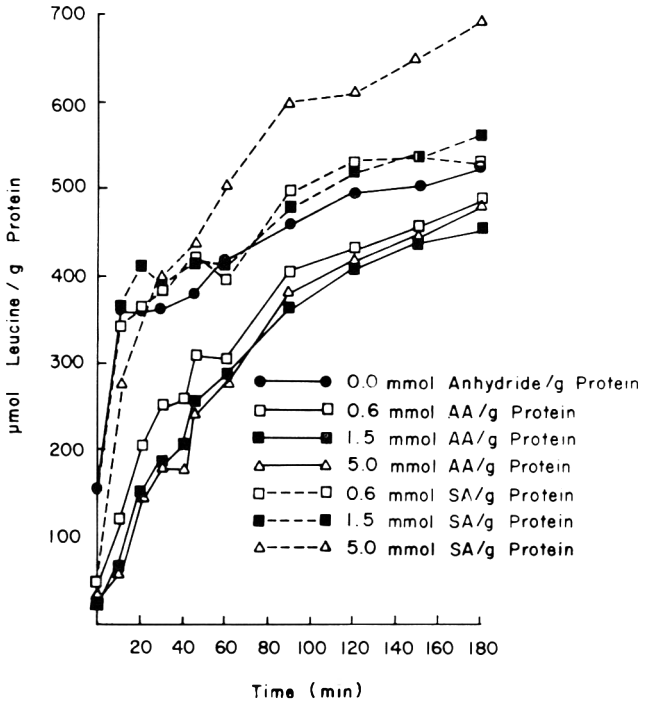


Fig. 2—Pepsin hydrolysis of unacylated, acetylated and succinylated beef heart myofibrillar protein expressed as leucine α-amino groups/g protein. Extent of acylation is given on a reactant weight basis.

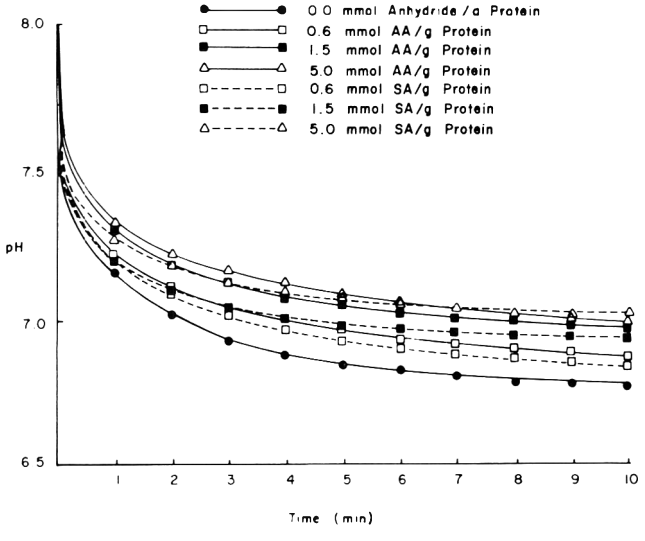


Fig. 1—Trypsin hydrolysis rates of unacylated, acetylated and succinylated beef heart myofibrillar protein expressed as change in pH. Extent of acylation is given on a reactant weight basis.

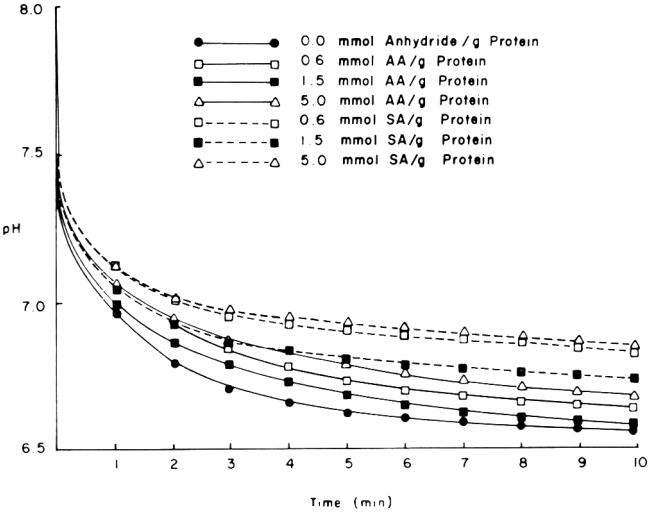


Fig. 3—Multienzyme hydrolysis rates of unacylated, acetylated and succinylated beef heart myofibrillar protein expressed as change in pH; enzymes: tryosin, chymotrypsin, and peptidase. Extent of acylation is given on a reactant weight basis.

from the multiple enzyme hydrolysis indicated no statistically significant difference among proteins. And the coefficients obtained were close to those of casein (87.6–90.5) reported by Hsu et al. (1977). However, there was a statistical difference in in vitro digestibility coefficients derived from trypsin hydrolysis when comparing acylated protein to unacylated protein. These results suggest that there may be in vivo tryptic digestibility problems with acylated protein.

Amino acid composition

The amino acid composition of unacylated, acetylated, and succinylated beef heart protein is given in Table 2. The amino acid totals, as well as each individual amino acid for succinylated and acetylated proteins, were lower than for the unacylated protein. The same observations were reported by Groninger and Miller (1979) with acetylated and succinylated fish myofibrillar protein. It may be that the modified proteins were hygroscopic and absorbed water between the vacuum drying and weighing procedures. There would be less protein on a weight basis; and, as a consequence, the amino acid total for the modified proteins would be less than the amino acid total for the unacylated protein. However, although not shown in Table 2, when each of the amino acids was expressed as a percentage of the respective total amino acid composition, no differeces in amino acid pattern among unacylated, acetylated or succinylated proteins were apparent.

Comparing the amino acid compositions of the proteins in Table 2 to the FAO/WHO (1973) standard, the limiting essential amino acid for all three protein preparations was leucine. The unacylated protein contained 78%, acetylated 71%, and the succinylated 72% of the leucine standard. These values are reflected in the amino acid score shown in Table 4. The second limiting essential amino acid was valine, followed by threonine.

The amino acids of greatest concern in human diets are lysine and the sulfur amino acids, methionine and cysteine (Jansen, 1978). It is noteworthy that chemical modification did not destroy these amino acids. In fact, Shetty and Kinsella (1979) indicated that succinylation of yeast homogenates prior to extraction of proteins protected some amino acids and did not reduce lysine concentrations. Acylation of a protein may actually protect some amino acids that usually are partially destroyed during extraction or heat processing.

Table 1—Effect of acylation of beef heart myofibrillar protein on in vitro digestibility

Treatment[a]	mmol Anhydride/g protein	In vitro digestibility[b] Multienzyme[c]	Trypsin
Unacylated	0.0	88.41 ± 1.55[d]	88.38 ± 0.38[e]
AA	0.6	87.45 ± 1.70	85.30 ± 0.13
	1.5	86.69 ± 2.06	84.76 ± 0.38
	5.0	87.10 ± 1.70	84.40 ± 0.13
SA	0.6	86.41 ± 1.58	86.30 ± 0.00
	1.5	86.88 ± 0.50	85.76 ± 0.52
	5.0	87.35 ± 0.99	83.31 ± 0.64

[a] AA: Acetic anhydride; SA: Succinic anhydride.
[b] Data expressed as an average of five or more values ± standard deviation.
[c] Trypsin, chymotrypsin, and peptidase.
[d] Means in the same column do not differ significantly ($P < 0.05$) from the unacylated protein by "t" statistic.
[e] Means in the same column differ significantly ($P < 0.05$) from the unacylated protein by "t" statistic.

Table 2—Amino acid composition of unacylated, acetylated, and succinylated beef heart myofibrillar protein

Amino acid[a]	Unacylated protein	Acetylated protein[b]	Succinylated protein[b]
	g Amino acid/100g protein		
Aspartic acid[c]	8.69	7.36	7.49
Threonine	5.41	4.22	4.39
Serine	4.57	3.73	3.82
Glutamic acid[d]	12.33	10.85	11.16
Proline	3.98	3.23	3.45
Glycine	3.84	3.11	3.13
Alanine	5.44	4.57	4.66
Valine	5.37	4.52	4.77
Methionine[e]	3.42	2.84	2.85
Isoleucine	8.47	7.12	7.22
Leucine	6.16	5.71	5.85
Tyrosine	4.85	3.80	3.85
Phenylalanine	5.03	3.86	3.93
Histidine	2.79	1.98	2.04
Lysine	8.75	7.14	7.11
Arginine	7.66	5.93	6.03
Cysteine[e]	1.68	1.67	1.47
Total	98.44	81.64	83.22

[a] Amino acid values are an average of an analysis on two different beef heart myofibrillar preparations.
[b] Extent of acylation, reactant concentration basis: 0.6 mmol acetic anhydride/g protein; 1.5 mmol succinic anhydride/g protein.
[c] Value includes aspartamine and aspartic acid.
[d] Value includes glutamine and glutamic acid.
[e] Methionine and cysteine values obtained by performic acid oxidation.

Rat feeding trials

The acylation levels of beef heart myofibrillar protein that were selected for evaluation of nutritional value were those shown previously (Eisele and Brekke, 1981) to be the most practical in enhancing protein functional characteristics without excessive modification. Rats were fed diets containing casein, unacylated beef heart myofibrillar protein, 0.6 mmol AA modified beef heart myofibrillar protein, or 1.5 mmol SA modified beef heart myofibrillar protein as the protein source. The amount of modification of the protein reactive groups is shown in Table 3. Approximately half (59%) of the ϵ-amino groups of lysine and half (55%) of the sulfhydryl groups were modified with acetic anhydride. Succinic anhydride modified a fourth (27%) of the ϵ-amino groups of lysine and a minimal amount (4%) of the sulfhydryl groups. AA modified some hydroxyl groups; SA did not react with hydroxyl groups.

The protein efficiency ratios (PER) and the net protein ratios (NPR) of the modified proteins were compared to unacylated beef heart myofibrillar protein and to casein (Table 4). The PER is the AOAC official method for measuring protein quality. However, the method is based solely on weight gain and does not consider either carcass composition or the need for tissue protein maintenance. The NPR overcomes this basic weakness by adding to the weight gain of rats fed the test protein diets the weight loss of rats fed a protein-free diet. Thus, the NPR takes into account the protein credited to body maintenance.

Beef heart myofibrillar protein had a PER of 2.83, which was considerably better than casein at 2.50. Acetylating beef heat myofibrillar protein lowered the PER to 2.55, and succinylating to 2.36. The NPR data showed the same trend as the PER data; beef heart myofibrillar protein and acetylated protein were higher than casein, and succinylated protein was lower than casein. The PER and NPR values for the modified proteins showed them to be comparable in nutritive value to lean beef (Satterlee et al., 1979) as well as to casein.

Groninger and Miller (1979) reported that acetylation of 50% of ϵ-amino groups of lysine resulted in little or no loss

Table 3—Amount of modification of ε-amino groups of lysine, sulfhydryl groups, and hydroxyl groups of beef heart myofibrillar protein used in rat PER and NPR feed trials, and ^{14}C-rat (metabolism) studies

Protein sample	Available lysine		Sulfhydryl groups		Hydroxyl groups
	mmol/g Protein	% Modified	mmol/g Protein	% Modified	mmol Acetylhydroxamate/g protein
Beef heart myofibrillar	446	0.0	55	0.0	0
0.6 mmol Acetylated beef heart myofibrillar	184	58.7	25	54.5	141
1.5 mmol Succinylated beef heart myofibrillar	327	26.7	53	3.6	0
0.6 mmol ^{14}C-Acetylated beef heart myofibrillar	165	62.6	25	54.5	170
1.5 mmol ^{14}C-Succinylated beef heart myofibrillar	301	31.7	51	7.3	0

in protein nutritive value when fed to rats. They also noted that acetylated protein gave a better growth response than succinylated protein. The results shown in Table 4 agree closely with their findings.

A method developed by Satterlee et al. (1979), termed the calculated protein efficiency ratio (C-PER), incorporates digestibility coefficients (Table 1) and amino acid composition (Table 2) of a test protein. The value obtained is then compared to that of casein in a set of complex calculations to arrive at a predicted PER value. The C-PER method was reported to be less time consuming than rat-PER feeding trials. The method worked for various proteins, but has not been evaluated on chemically modified proteins. Therefore, it was used in the present study to determine if the method is valid for use on acylated protein. Although tryptophan was not included in our amino acid analysis, it is known that it is not a limiting amino acid in animal products (Bogert et al., 1973), ranging from 1.1–1.4 g/100 protein (Rice, 1971). Therefore, an estimate of 1.1g tryptophan/100g protein was used in our calculations of C-PER. The C-PER data (Table 4) were comparable to rat-PER data, being slightly less than rat-PER for unacylated and acetylated myofibrillar protein. This difference in PER values was similar to those reported by Satterlee et al. (1979) for meat and egg proteins. The C-PER value for succinylated myofibrillar protein was greater than expected, compared to rat-PER data. Thus, although the C-PER for acylated protein gave a reasonably accurate prediction of PER, in agreement with the findings of Satterlee et al. (1979), rat-PER may still be necessary to ascertain small changes in PER caused by protein modification treatments.

At the conclusion of the PER feeding trial the rats were sacrificed. There were no apparent differences in liver/body weight ratios nor in overall depot body fat among rats fed the unacylated, acetylated, or succinylated beef heart myofibrillar protein diets. Gross examination of body organs also showed no difference due to diet. No acute toxic problems were noted as rats on all diets looked healthy and had good appetites throughout the experiment.

Table 4—Comparison of C-PER, rat-PER, rat-NPR, and amino acid score of acylated beef heart myofibrillar proteins, casein, and lean beef

Protein sample	C-PER[a]	Rat-PER[b]	Rat-NPR[c]	Amino acid score
Beef heart myofibrillar	2.62	2.83	116	78
0.6 mmol Acetylated beef heart myofibrillar	2.42	2.55	110	71
1.5 mmol Succinylated beef heart myofibrillar	2.48	2.36	87	72
Casein	2.50[a]	2.50	100	91[e]
Lean beef	2.20[a]	2.50[a]	78[d]	100[e]

[a] Method and values from Satterlee et al. (1979); C-PER = computed protein efficiency ratio.
[b] PER values corrected to that of casein of 2.50.
[c] NPR = net protein ratio.
[d] McLaughlan and Keith (1975).
[e] Bodwell (1977). Amino acid scores calculated using FAO amino acid pattern as the reference protein.

Metabolism of ^{14}C-acylated protein

Rats fed a standard diet were intragastrically administered carbon-14-labelled acylated beef heart myofibrillar protein. The amount of modification of the amino acid reactive groups for the ^{14}C-acylated beef heart myofibrillar protein was about equivalent to that of the PER diet acylated proteins (Table 3).

The distribution of radioactivity recovered in the various fractions, expressed as a percentage of radioactivity recovered, is shown in Table 5. The major portion of radioactivity for both ^{14}C-acylated protein preparations was recovered in the carbon dioxide, 68.2% for ^{14}C-acetylated and 45.8% for ^{14}C-succinylated. The amount of radioactivity in the urine was 4.9% for ^{14}C-acetylated protein and 9.3% for ^{14}C-succinylated protein. The radioactivity in the stomach and intestine for ^{14}C-succinylated protein was higher than for ^{14}C-acetylated protein, 28.6% versus 14.4%. Radioactivity was also found in other body organs, with the liver containing the greatest amount. The total amount of radioactivity recovered was 99% or greater of that administered.

The difference between ^{14}C-acetylated protein and ^{14}C-succinylated protein in total ^{14}C-carbon dioxide suggests that the rat can metabolize acetylated protein much better than succinylated protein. Also, the greater amount of radioactivity in the stomach and intestine from ^{14}C-succinylated protein compared to ^{14}C-acetylated protein may imply an absorption problem with succinylated protein. This may partially explain the difference in the C-PER and rat-PER values of succinylated protein (Table 4).

These data agree partially with the work of Groninger and Miller (1979), who fed ^{14}C-acetylated fish myofibrillar protein to rats. The majority of the radioactivity in their study for both ^{14}C-acetylated and ^{14}C-succinylated protein was in the urine, while in this study the majority of the radioactivity was in the carbon dioxide. The difference in radioactivity distribution in carbon dioxide and urine, between their study and this study, may be due to the difference in intestinal microorganisms in the rats. Both studies did show that ^{14}C-acetylated protein produced higher ^{14}C-carbon dioxide levels than ^{14}C-succinylated protein.

Numerous experiments have shown that modifying the fat, protein, or carbohydrate content of laboratory diets

Table 5—Twenty-four hour distribution of radioactivity in the rat injected intragastrically with carbon-14-labelled acylated beef heart myofibrillar protein

Item	Percentage of total radioactivity recovered[a]	
	0.6 mmol Acetic anhydride/g protein	1.5 mmol Succinic anhydride/g protein
Carbon dioxide	68.2 ± 1.8	45.8 ± 8.1
Stomach	1.7 ± 0.7	2.8 ± 0.9
Intestine	12.7 ± 1.6	25.8 ± 12.1
Feces	0.2 ± 0.5	2.1 ± 4.2
Urine	4.9 ± 1.6	9.3 ± 5.0
Liver	6.9 ± 1.0	7.3 ± 0.8
Spleen	0.8 ± 0.1	1.0 ± 0.2
Kidney	1.8 ± 0.5	2.0 ± 0.4
Heart	0.7 ± 0.5	1.0 ± 0.2
Lung	1.3 ± 0.3	1.4 ± 0.3
Red blood cells	0.8 ± 0.2	1.2 ± 0.5
Blood serum	b	b

[a] Data expressed as an average of four rats ± standard deviation.
[b] Less than 0.1%.

Table 6—Twenty-four hour distribution of radioactivity in the rat injected intragastrically with carbon-14-labelled acylated beef heart myofibrillar protein that was on the respective diet for 28 days

Item	Percentage of total radioactivity recovered[a]	
	0.6 mmol Acetic anhydride/g protein	1.5 mmol Succinic anhydride/g protein
Carbon dioxide	75.7 ± 3.1	38.1 ± 6.9
Stomach	0.8 ± 0.2	2.3 ± 1.5
Intestine	6.8 ± 1.2	29.8 ± 14.1
Feces	b	b
Urine	10.9 ± 3.4	20.9 ± 5.7
Liver	2.2 ± 0.1	3.4 ± 1.1
Spleen	0.3 ± 0.0	0.7 ± 0.5
Kidney	0.7 ± 0.1	1.2 ± 0.8
Heart	0.5 ± 0.2	0.5 ± 0.1
Lung	0.5 ± 0.2	1.3 ± 1.2
Red blood cells	1.6 ± 0.2	2.4 ± 1.0
Blood serum	0.1 ± 0.0	0.1 ± 0.0

[a] Data expressed as an average of four rats ± standard deviation.
[b] Less than 0.1%.

can affect the animal's ability to biotransform a variety of compounds (Clayson, 1975; Carroll, 1975). The change in diet or diet composition can cause an induction process which leads to new protein synthesis and may be manifested in increased enzyme levels and enzymatic activity (Conney and Perns, 1972).

To test for an induction effect on metabolism of acylated protein due to diet changes, rats fed acylated protein for 28 days on the PER diets were administered the ^{14}C-acylated protein in the same manner as those rats fed the lab chow diet. The results are shown in Table 6. Comparing ^{14}C-acetylated protein metabolism, rats on the acetylated protein diet had higher levels of radioactivity in the carbon dioxide (75.7%) and lower levels in the organs (Table 6) than the rats fed the lab chow diet (68.2% carbon dioxide, Table 5). These findings would suggest that rats fed acetylated protein could adjust to their respective diet. However, comparing ^{14}C-succinylated protein metabolism, rats on the succinylated diet had lower levels of radioactivity in the carbon dioxide (38.1%) and higher levels in the intestine (29.8%) and urine (20.9%) than rats fed the lab chow diet (45.8% carbon dioxide, 25.8% intestine, and 9.3% urine). The opposite effect took place comparing carbon dioxide, urine, and intestinal radioactivity in rats fed succinylated and acetylated protein diets. Succinylated protein fed to rats over an extended time period actually reduced ^{14}C-succinylated protein utilization (Table 6). Since succinylated protein reduced in vitro enzyme hydrolysis (Fig. 3) to a greater extent than did acetylated protein compared to unacylated protein, the lower utilization of succinylated protein was probably due to less susceptibility of in vivo proteolytic enzyme hydrolysis by succinylated ϵ-amino groups of lysine in the protein rather than to a possible "negative" induction effect.

Since the rat could metabolize some of the carbon-14 labelled modified protein to carbon dioxide, an enzyme must exist which is capable of deacylating the anhydride moiety from the protein. It is not known at this time where the deacylase enzyme is located. The enzyme could be located in the rat liver, other organs, or in the intestinal tract; or the enzyme may be located in the microbial flora of the gut.

These results indicated that succinylated or acetylated beef heart myofibrils can be utilized as a protein source. Since succinylated and acetylated beef heart myofibrils have been shown to have enhanced functional properties compared to unacylated protein (Eisele and Brekke, 1981), this research plus other literature suggests that chemically modified proteins are versatile and may be useful in appropriate food systems.

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Relationship of Mineral Content and Tenderness of Meat from Cattle Differing in Breed, Sex and Age

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ABSTRACT

The mineral contents and the shear values of raw and cooked meat, respectively, were determined using longissimus dorsi (LD) and semimembranosus (SM) muscles of 100 cattle, 80 of which were approximately 15 months of age and were classified by breed of sire and by sex and 20 of which were mature Shorthorn cows. Correlations between shear values and concentrations of the minerals Cu, Fe, Ca, Zn, Mg, Na and K, indicated that for the LD muscle no correlations were significant ($P > 0.05$) and for the SM muscle only correlations involving Ca and Fe were significant ($P < 0.05$). These correlation coefficients, however, were not of a magnitude to encourage the use of mineral concentrations of raw meat for the prediction of cooked beef shear value.

INTRODUCTION

ACCORDING to the consumer, tenderness continues to be one of the most important meat quality attributes (Jeremiah, 1978). At present, there is available no acceptable method for prediction of cooked meat tenderness from objective measures carried out on raw meat. Raw meat characteristics including histological and chemical properties (Pearson, 1963), tensile properties (Stanley et al., 1972), and resistance to shearing (Pearson, 1963; Anderson et al., 1972; Carpenter et al., 1972; Smith and Carpenter, 1972) have little potential although fresh muscle fragmentation (Olson et al., 1976; Reagan et al., 1975) may prove to be useful for the prediction of cooked meat tenderness.

Bovine muscle consists of three major fiber types as classified histochemically by Ashmore and Doerr (1971): βR (red), αR (intermediate) and αW (white). Melton et al. (1974, 1975) and May et al. (1977) found no correlation between tenderness and the muscle content of these fiber types. However, Calkins et al. (1981) recently determined that both panel tenderness and Warner-Bratzler shear values of the longissimus dorsi muscle varied with fiber type composition and these authors suggested a relationship between the oxidative capacity of this muscle and its cooked tenderness. Several minerals, including iron, copper, zinc, magnesium, manganese, calcium, sodium and potassium vary with muscle color and function (Cassens and Cooper, 1971; Wagner et al., 1976; Doornenbal and Murray, 1982). It is not unreasonable then to suggest that muscle mineral content may be related to tenderness. McClain and Mullins (1969) noted that more tender bovine longissimus dorsi muscles were higher in water-solubles total pigment and total iron contents. Vavak et al. (1976) reported that variation in the levels of the minerals iron, cobalt, zinc and copper accounted for essentially all of the variation in the tenderness of cooked bovine semimembranosus muscle and that the mineral levels of fresh raw meat could successfully predict cooked meat tenderness. However, these findings were disputed in a preliminary report (Whittaker et al., 1979).

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The present study was initiated to re-examine the relationship of cooked meat tenderness, as measured by a mechanical shear device, to muscle mineral content and to evaluate the potential use of muscle mineral content for the prediction of cooked meat tenderness. As part of this study the effects of breed of sire, sex, age and muscle on shear value and on the relationship between shear value and mineral content were investigated.

MATERIALS & METHODS

EIGHTY ANIMALS were classified by breed of sire (BOS) and sex as follows: Charolais–10 heifers and 10 steers; Simmental–10 heifers and 10 steers; Limousin–11 heifers and 9 steers; Chianina–10 heifers and 10 steers. The breed composition was a maximum of 50% from the BOS and 50% from F1 cows which were either from a Hereford X Angus control group or were produced by mating Charolais, Simmental and Limousin sires with Hereford, Angus and Shorthorn dams. These animals, which will be referred to as maturity I cattle, were slaughtered at weights ranging from 388–576 kg (Mean ± SE = 466 ± 4) and ages ranging from 421–492 days (Mean ± SE = 448 ± 2) after approximately 140 days on a high energy ration (1/2 to 2/3 barley and oats and 1/3 to 1/2 cereal silage on a weight basis including supplementary calcium). To determine the effect of age, 20 Shorthorn cows, which will be referred to as maturity II cattle, were slaughtered just after their calves were weaned and after having been maintained for approximately 5 months on grass. These cattle ranged in age from 3–12 years (Mean ± SE = 5.9 ± 0.6). Management practices (including feeding regimes) were similar for all animals within a particular maturity group.

After aging carcasses at 2°C for 6 days, two adjacent steaks, each 2.5 cm in thickness, were removed from the longissimus dorsi (LD) muscle near the 13th rib and from the central portion of the semimembranosus (SM) muscle. One steak of each pair was frozen in still air at −30°C and stored under the same conditions for subsequent analysis of the minerals copper (Cu), iron (Fe), calcium (Ca), zinc (Zn), magnesium (Mg), sodium (Na) and potassium (K) as described previously (Doornenbal and Murray, 1982). The other was cooked on the 6th day post-slaughter to a medium-well degree of doneness (internal temperature of 70–75°C) in a microwave oven as described by L'Hirondelle and Martin (1975) and then stored under a polyethylene film overnight at 2–4°C. Three cores were removed from each steak by coring perpendicular to the steak surface with a hand-held, 2 cm bore. The cores were sheared with an Ottawa Texture Measuring System fitted with a Warner-Bratzler heat (L'Hirondelle and Martin, 1975). With this method muscle fibers of the LD muscle were sheared at an angle of approximately 45° and those of the SM muscle at an angle of approximately 90°. Due to a technical problem shear values for the SM muscle were determined for only 71 of the 80 maturity I cattle.

Data were analyzed using SAS general linear model (analysis of variance) and correlation programs (SAS Institute Inc., 1979).

RESULTS & DISCUSSION

A SUMMARY of analysis of variance of shear values of maturity I cattle is presented in Table 1. The statistical model for the analysis included the main effects, muscle, breed of sire (BOS) and sex along with all possible interactions. Muscle × BOS and muscle × sex interactions were significant ($P < 0.05$). The nature of these interactions can most easily be understood by examination of the estimated least squares means (Table 2). The muscle × BOS interaction means indicate that Simmental was the only one of the

four breeds of sire for which the semimembranosus (SM) muscle did not have a higher shear value than did the longissimus dorsi (LD) muscle. The muscle x sex interaction indicated that the sexes differed in shear value for the LD muscle but not for the SM muscle. Comparison of the shear values of the two muscles must be done with caution in that the muscle fibers of the LD muscle were sheared at an angle of approximately 45° to their longitudinal axis whereas those of the SM muscle were sheared at approximately 90° to their longitudinal axis. Based on the results of Murray and Martin (1980) for the LD muscle, shearing this muscle at 90° to the direction of the muscle fibers would result in a shear value at least as high as for the SM muscle sheared at the same angle.

An analysis of variance of shear values for the Shorthorn cows ranging in age from 3–12 years is presented in Table 3. The linear model included muscle as a main effect, age as a covariate and the muscle x age interaction to test the homogeneity of the age affect with respect to muscle. The muscle x age term was significant ($P < 0.05$) indicating that muscles differed in the extent to which their shear values were affected by age. Introduction of the quadratic term (age x age) did little to further reduce the error mean square and therefore the age effect was assumed to be linear. Mean shear values (at a mean age of 5.9 years) as well as slopes of the regression of shear value on age are presented in Table 4. The shear value of the LD muscle was affected by age (increasing 0.22 kg/hr) while that of the SM muscle did not vary with age. The effect of age within the limited range of the maturity I cattle was not tested. Jacobson and Fenton (1956) and Tuma et al. (1963), however, have shown that the shear values of LD and SM muscles increased during the period between ages of 6 months and 3 years also.

Analysis of the effects of breed of sire, sex, age and muscle on mineral content have been presented in the previous paper (Doornenbal and Murray, 1982). Although for the present study nutritional regimes were kept constant, other researchers have shown that it is an additional source of variation in shear value (Bowling et al., 1977) and in muscle mineral content (Standish et al., 1971).

The relationship of shear values to mineral concentrations was examined by correlation analyses (Table 5). Analyses were carried out on each muscle because of the muscle differences reported above and to make the results more easily comparable to those of other researchers. Regression coefficients (not presented) were homogeneous with respect to BOS and sex for maturity I cattle and the correlation coefficients presented were derived by pooling within subclass, i.e. after adjustment for BOS and sex effects (maturity I) or for age effects (maturity II). Only Fe and Ca concentrations of the SM muscle of maturity I cattle and Ca concentration of the SM muscle of maturity II cattle were significantly related with shear value. Variation in concentrations of these minerals accounted for only an additional 10–20% of the variation in shear value. Because of the limited ability of the concentration of any one mineral to account for variation in shear value, a multiple correlation analysis, although completed, is not presented. Correlation coefficients, calculated by pooling over subclasses, that is, with no adjustment of BOS and sex effects (maturity I) or for age effects (maturity II), differed slightly in some cases from those discussed above. However they were too small to suggest the utilization of mineral concentration for segregation of carcasses or raw meat cuts into tenderness categories.

These conclusions differ from those presented by Vavak et al. (1976) who found that variation in concentrations of

Table 1—Analysis of variance of the shear value of maturity I (age 14–16 months) cattle

Source of variation	Degrees of freedom	Mean square
Muscle	1	26.2***
Breed of sire	3	0.3
Sex	1	4.0
Muscle x Breed of sire	3	6.1*
Muscle x Sex	1	30.4***
Breed of sire x Sex	3	3.9
Muscle x Breed of sire x Sex	3	3.4
Error	135	1.9

* $P \leq 0.05$ *** $P \leq 0.001$

Table 2—Estimated least squares means and standard errors for shear values of maturity I (age 14–16 months) cattle by muscle, by sex within muscle and by breed of sire within muscle

	N	Mean (kg)	SE	Muscles x BOS	N	Mean (kg)	SE
Muscle							
LD[a]	80	5.1	0.15	LD x Charolais	20	4.9	0.31
SM[a]	71	6.2	0.19	LD x Simmental	20	5.7	0.31
				LD x Limousin	20	5.1	0.31
Muscle x sex				LD x Chianina	20	4.7	0.31
LD x heifer	41	5.9	0.21	SM x Charolais	14	6.8	0.63
LD x steer	39	4.3	0.23	SM x Simmental	19	5.5	0.34
SM x heifer	37	5.8	0.26	SM x Limousin	20	6.1	0.31
SM x steer	34	6.6	0.30	SM x Chianina	18	6.4	0.38

[a] LD = longissimus dorsi; SM = semimembranosus

Table 3—Analysis of variance of the shear value of maturity II (age 3–12 yr) cattle with age as a covariate

Source of variation	Degrees of freedom	Mean square
Muscle	1	20.7***
Age	1	2.0
Muscle x Age	1	6.0*
Error	36	1.4

* $P \leq 0.05$ *** $P \leq 0.001$

Table 4—Mean shear values and slopes from the regression of shear values on age for maturity II (age 3–12 yr) cattle

Muscle	N	Mean (kg)[a] ± SE	Slope (kg/year) ± SE
Longissimus dorsi	20	6.7 ± 0.26	0.22 ± 0.09
Semimembranosus	20	8.1 ± 0.26	−0.06 ± 0.09

[a] At mean age of 5.9 years

Table 5—Correlation coefficients between shear value and the concentrations of several minerals after adjusting maturity I cattle for breed of sire and sex effects and maturity II cattle for age effect[a]

	Longissimus dorsi		Semimenbranosus	
	Maturity I	Maturity II	Maturity I	Maturity II
Cu	−0.17	0.24	−0.08	0.26
Fe	0.05	0.06	0.30**	−0.12
Ca	−0.18	−0.12	0.33**	−0.44*
Zn	−0.14	0.12	−0.20	0.10
Mg	0.16	0.23	0.19	−0.01
Na	−0.11	0.39	−0.15	0.18
K	−0.10	0.39	−0.07	−0.14

[a] N = 68–78 for maturity I and 20 for maturity II cattle.
* $P \leq 0.05$ ** $P \leq 0.01$

the minerals Fe, Co, Zn and Ca accounted for 97% of the variation in the shear value of the cooked SM muscle. However, the small sample of 10 animals were of choice, good and canner and cutter grades, indicating large differences in maturity and quality/palatability characteristics. Neither this nor other possible sources of variation were discussed by Vavak et al. (1976). Accordingly, their conclusions must be considered in light of the serious limitations of their experimental design. The findings of the present and the previous (Doornenbal and Murray, 1982) papers indicate that both shear value and mineral content may differ with differing breed, sex, age and muscle. However, no underlying relationship between shear value and mineral content was observed. Thus, concentrations of the minerals Cu, Fe, Ca, Zn, Mg, Na and K in fresh muscle are not useful for the prediction of shear values (tenderness) of cooked meat.

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Relationship Between TBA Numbers and Inexperienced Panelists' Assessments of Oxidized Flavor in Cooked Beef

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ABSTRACT

Fifty-two inexperienced sensory panelists rated a series of ground beef samples four or six times for intensity of oxidized flavor. Correlation coefficients for sensory scores versus TBA numbers were significant but low. Variability in panelist scoring appeared to account partly for the lower values. Of the 52 panelists tested, 28 were determined statistically to be consistent in their scoring of the treatment variables. Correlation coefficients were higher for this group of panelists. The initial range of oxidized flavor detection for this panel group was within a range of TBA numbers that closely resembled the previously determined threshold level for trained panelists.

INTRODUCTION

THE 2-THIOBARBITURIC ACID (TBA) test is the chemical test used most often for assessment of lipid oxidation in red meats, fish and poultry (Gray, 1978). Products of the lipid oxidation reaction are believed to be responsible for the oxidized flavor (also called rancid or warmed-over flavor) that develops in meat during storage. Tarladgis et al. (1960) found that TBA numbers (mg TBA reacting substances/kg tissue) were correlated highly with trained sensory panelists' scores for rancid odor in ground pork. These workers also found the TBA number at which a rancid odor first was perceived by the panelists to be between 0.5 and 1.0. This "threshold" has served as a guide for interpreting TBA test results. According to this study and those of others (Turner et al., 1954; Younathan and Watts, 1959) the TBA test can serve as an indicator of oxidized odor or flavor in meat when odor or flavor are judged by trained or experienced sensory panelists. It is not known if the TBA test is a useful indicator of consumer assessments of oxidized flavor.

The objectives of the present study were to determine whether the assessment of oxidized flavor in beef by inexperienced panelists could be correlated with TBA numbers and to establish the detection limit of the oxidized flavor.

EXPERIMENTAL

Preparation of samples

Beef semitendenosus muscles were purchased from a local supermarket. Meat was trimmed of cover fat and ground through a 1.5 cm followed by a 0.5 cm plate. A hamburger press was used to shape 50g aliquots into patties. Patties representing various increments in oxidized flavor intensity were microwaved 1 min on one side and 30 sec on the reverse side and stored at 4–6°C for either 6 hr, 1 day, 2 days, or 4 days. Patties representing samples having no detectable oxidized flavor were prepared with a combination of 0.05% ascorbic acid and 0.005% butylated hydroxy anisole. All treatment variables were then stored at −18°C until panel testing began. To prepare samples for sensory evaluation, the patties were microwaved for 1 min. Each pattie was cut into eight pie-shaped wedges for tasting.

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Procedures for sensory evaluation

A total of 52 panelists evaluated the beef samples for intensity of oxidized flavor. Evaluations were carried out by three different groups: Panel A was composed of 21 female home economics teachers whose ages ranged from 23–50 years. Sixteen females and four males from the student body and staff of the College of Home Economics comprised panel B. The age span was from 21–48 years. Panel C consisted of 11 males from the College of Agriculture whose ages ranged from 22–53 years.

While panelists were to be untrained, they were acquainted with the flavor characteristic referred to on the score sheet as "oxidized" by exposure to reference samples with high and low TBA numbers prior to a tasting session. While panelists were briefed on the tasting procedures and the score sheet, no formal training was given.

Panelists were seated in three-sided, neutral colored booths located in a room which was separately ventilated and free from outside noise and odor. At each session, panelists were presented with four or five coded samples arranged on coded white plates according to a randomized complete block design. Treatment variables always included a low TBA number (antioxidant-treated) sample, a high TBA number sample, and two or three samples which had TBA increments intermediate to the low and high. Panelists were instructed to chew an entire sample and then expectorate it. They were also requested to eat part of an unsalted cracker and to drink water after tasting each sample to avoid carry-over of flavor from one sample to the next. Panel sessions were conducted between the hours of 11:00 a.m. and 1:00 p.m., at the convenience of the panelists. A plate of samples was prepared for each panelist just prior to the stated time. Panel A attended four tasting sessions and panels B and C, six. With the exception of one individual on panel A who missed one session, every panelist was present at every session.

Measurement of lipid oxidation

The 2-thiobarbituric acid (TBA) test of Tarladgis et al. (1960) was used to assess lipid oxidation in all samples. Results are expressed as TBA number: mg TBA reacting substances/kg tissue. TBA tests were run on samples after initial preparation and after refrigerator storage in order to group the samples into the various TBA increments to be presented to panelists. Samples were analyzed again concurrent with each tasting session with aliquots taken randomly from the pie-shaped slices cut from each pattie. The latter TBA numbers were used in the statistical computations.

Analysis of data

A seven point category scale served as the scoring instrument, with a score of one denoting no oxidized flavor detectable and a score of seven denoting an extremely strong oxidized flavor. The Statistical Analysis System (Barr et al., 1976) procedures were used in the computations of analysis of variance, correlation coefficient and Duncan's Multiple range test.

RESULTS

Mean scores as affected by different panel groups

Mean TBA numbers and corresponding mean sensory scores for the samples presented to each of the three groups of panelists are given in Table 1. It can be seen from the data for panel A that as the TBA numbers increased, the mean sensory scores also increased. For the range of TBA numbers represented, panelists utilized a relatively small

range of the available terms on the score sheet. The ranges of scores assigned by panels B and C encompassed an even smaller portion of the category scale than those given by panel A. The treatment variables with the lowest and highest TBA numbers were scored correspondingly low and high. However, mean scores for treatment variables with the intermediate TBA numbers did not increase with increments in sample TBA numbers.

When the individual panelists' scores were examined, it was noted that some panelists had been more consistent than others in their scoring of the various samples. To determine if scores from those panelists who were consistent would be more closely associated with TBA numbers, an analysis of variance (ANOVA) was used to single out panelists who were consistent in scoring the samples over the four or six tasting sessions. Selection was based on an F-ratio significant at $P \leqslant 0.1$. This group was designated the "discriminating" (disc.) panel members. A total of 28 individuals fell into the sub-group; 13 from panel A, 10 from panel B and 5 from panel C. When mean scores given by disc. panel members were computed (Table 1), it became evident that a somewhat larger range of the score sheet was used by these panelists and that the extremes (samples 1 and 5) were more clearly noted when compared with scores from the total panels, A, B, and C. Scores from all three sub-groups got progressively higher as TBA numbers increased.

Total panels A, B, and C were not homogenous with regard to the mean scores assigned to each treatment variable. Data from these panel groups were treated separately in all ensuing statistical analyses. The three disc. sub-groups were homogenous in this respect. Data from these groups were treated separately and collectively.

Correlation of sensory scores with TBA numbers

Correlation coefficients between sensory scores and TBA numbers were calculated on individual observations by each member of the seven panel populations (total A, total B, total C, disc. A, Disc. B, disc. C, disc. A, B, C). Scores from all panel groups were correlated significantly ($P \leqslant 0.01$) with TBA numbers although r-values were low (see Table 2). Correlation coefficients were higher for the disc. panel groups than for the total panel groups. This finding is in agreement with that of Quinlan et al. (1974) who also selected discriminating judges but by a slightly different procedure. These data also agree with those of Turner et al. (1954) and Tarladgis et al. (1960) in that significant simple correlations were observed between TBA values and sensory scores. However, the above workers reported higher r-values than those obtained from the present data.

The r^2 is the coefficient of determination. It gives a measure of the percent variation in the objective test as reflected in the sensory evaluation (Szczesniak, 1968). When r^2 values were computed on the present data, only 2–28% of the variation was found to be due to the TBA test. Panelists' discriminating ability explains part of this as evidenced by the increase in r^2 for the disc. panel groups over the total panel groups (Table 2). Since none of the panelists in the present experiment was trained to associate certain stimuli with a given descriptive term, as would be the case with trained panelists (Ehrenberg and Shewan, 1953), a large portion of the variability may be explained by the nonstandardization of panelists' judgments. The idea that judgments by untrained panelists can introduce variability into an experiment has been documented previously (Amerine et al., 1965; Szczesniak, 1968).

Additional ANOVAs were computed to examine sources of variability relative to panelist performance. Sample treatments, judges, dates and interactions were tested. A summation of the results is presented in Table 3. There was a highly significant difference ($P \leqslant 0.01$) among scores for sample treatments given by all panel groups except total panel C, indicating that panelists did detect differences among samples with different TBA numbers. However, there was a significant difference ($P \leqslant 0.05$, disc. panel C; $P \leqslant 0.01$ all others) among scores given by the individual judges and a significant interaction between sample treatment and judge for all panel groups except disc. panels A and C. There was no significant difference in scores from

Table 1—TBA numbers and sensory scores for samples evaluated by total panels A, B and C and discriminating members of each panel

	Treatment variable	Mean TBA no.	Mean sensory score[a]	
			Total panel	Discriminating panel members
Panel A	1	0.4	2.2	1.6
	2	2.0	2.8	2.7
	3	4.0	3.2	3.3
	4	5.3	3.3	3.8
	5	9.5	4.3	4.8
Panel B	1	0.7	2.0	1.3
	2	2.0	2.8	2.9
	3	4.0	2.7	3.1
	4	5.3	2.7	3.2
	5	10.5	3.3	4.1
Panel C	1	0.7	2.8	2.0
	2	2.0	2.5	2.2
	3	4.0	3.0	3.1
	4	5.3	2.9	3.4
	5	10.5	3.5	4.3
Panels A,B,C combined	1	0.6	–	1.6
	2	2.0	–	2.7
	3	4.0	–	3.2
	4	5.3	–	3.4
	5	10.0	–	4.3

[a] 1–7 Scale; 1= No oxidized flavor detectable; 7= Extremely strong oxidized flavor detectable.

Table 2—Correlation between TBA numbers and sensory scores for total and discriminating panel members

Panel	r value		r^2 value (%)	
	Total panel	Discriminating panel members	Total panel	Discriminating panel members
A	0.32*	0.53*	10	28
B	0.21*	0.45*	5	20
C	0.16*	0.44*	2	20
A,B,C	–	0.47*	–	23

*Sig. $P \leqslant 0.01$.

Table 3—Analyses of variance on scores given by total and discriminating panel populations—Summary of findings[a]

Panel population	Sample treatment	Sources of variation			
		Date	Judge	Date x Judge	Judge x Treatment
Total A	**	N.S.	**	N.S.	*
Total B	**	N.S.	**	*	**
Total C	N.S.	N.S.	**	*	**
Disc. A	**	N.S.	**	N.S.	N.S.
Disc. B	**	N.S.	**	*	**
Disc. C	**	N.S.	*	N.S.	N.S.

[a] N.S. = nonsignificant, $P > 0.05$
* $P \leqslant 0.05$
** $P \leqslant 0.01$

Table 4—Significant differences among sensory scores according to Duncan's multiple range test

Treatment variable	Total panel groups[a]			Discriminating panel groups[a]			A,B,C Combined
	A	B	C	A	B	C	
1	a	a	a	a	a	a	a
2	b	a	a	b	b	a	b
3	b	a	ab	c	b	b	c
4	b	a	ab	c	b	b	c
5	c	b	b	d	c	c	d
TBA numbers for initial range of flavor differentiation =	0.4–2.0	5.3–10.5	2.0–10.5	0.4–2.0	0.7–2.0	2.0–4.0	0.6–2.0

[a] See Table 1 for TBA numbers and sensory scores.

Table 5—Test for homogeneity of correlation coefficients to estimate effect of repeated tasting sessions on panelists' learning

Correlations Between Sensory Scores and TBA Numbers (r-values)			
Tasting session	Total panel A*	Total panel B	Total panel C
1	0.16	0.17	0.17
2	0.16	0.30	0.12
3	0.48	0.17	0.33
4	0.49	0.28	0.10
5	–	0.19	0.21
6	–	0.21	0.14
Tasting session	Discriminating panel A	Discriminating panel B	Discriminating panel C
1	0.45	0.41	0.56
2	0.44	0.49	0.42
3	0.69	0.50	0.58
4	0.57	0.43	0.34
5	–	0.51	0.71
6	–	0.37	0.44

*Sig. P = 0.01. All others nonsignificant.

date to date, but there was a significant date and judge interaction for some of the panel groups. Thus, the scores for the individual panelists showed the greater variability which probably accounted for the significant interactions. For the most part, panel groups that used the narrowest range of the sensory score sheet and whose scores gave the lower r-values with TBA numbers were those whose scores also had the greatest variability and interaction in these ANOVAs. Although a number of factors could have been responsible for the low correlation between panelists' scores and TBA numbers, it is suggested by the above data that panelist variability probably accounted for a large portion of the low r^2 values.

TBA number for oxidized flavor differentiation

To estimate the TBA number at which panelists first noted on oxidized flavor (detection threshold) Duncan's Multiple Range test was used. TBA numbers were grouped in ascending order with the corresponding mean sensory scores for each panel (see Table 1). The first two TBA number groups between which mean scores for a panel were significantly different was considered to be the initial range of flavor differentiation for the panel (Table 4). Since total panels A, B, and C were not homogenous, the initial ranges of oxidized flavor differentiation were considered separately for each panel. For panel A the range was between TBA 0.4 and 2.0. For panel B a significant difference in scores for oxidized flavor intensity occurred between TBA numbers 5.3 and 10.5. There is not a clear differentiation between scores for panel C. The only significant difference found was between TBAs of 2.0 and 10.5. The initial range of oxidized flavor differentiation for the total panel population tested in this study (A + B + C) would be from the TBA numbers of 0.4–10.5, or the entire range of treatment variables presented to the panelists. Thus, a range suggestive of a "threshold" value for all panelists was not evident. For the combined disc. panels, a TBA number of between 0.6 and 2.0 would represent the initial differentiation range. The latter figures are quite similar to those of Tarladgis et al. (1960) in which a TBA number of between 0.5 and 1.0 was stated as the threshold for rancid odor detection.

Scores from total panel A were significantly different among all treatment groups except treatments 2, 3 and 4. For the disc. sub-group of panel A, treatment variables 3 and 4 comprised the only samples between which scores did not differ significantly. A similar pattern was observed for panels B and C in that greater differentiation among treatment scores was found for the disc. sub-groups than for the total panel groups. When scores from disc. panels A, B and C were combined (Table 4), treatments 3 and 4 were the only treatment variables that were not scored significantly different from each other. It is suggested by these significant differences that panelists who were consistent in scoring also recognized differences in meat flavor relative to TBA numbers of the meat samples.

Effect of repeated trials on learning

Because the panelists rated each of the ground beef treatment variables four or six times, it was possible that some learning or "training" might have occurred from these repeated trials. In response to this issue, a test for homogeneity of correlation coefficients was performed (Steel and Torrie, 1960). The correlation coefficients for sensory vs TBA were compared for each panel population over each tasting session. These data are presented in Table 5. The only panel population in which a significant difference among r-values was found was total panel A (P=0.01). It is possible that some learning took place within this group. For all other panel groups, r-values were homogenous among the tasting sessions. It is suggested from these analyses that, on the whole, learning did not take place and these panelists were "untrained" throughout the study.

DISCUSSION & CONCLUSIONS

THESE DATA do not provide information about which meat samples the panelists liked. Generally, an affective sensory response, such as hedonic scoring (degree of liking) is recommended for use with inexperienced or consumer panelists. However, sensory characteristics other than an oxidized flavor might influence an individual's liking of a product. The goal of the present study was to relate human assessments of oxidized flavor to a chemical assessment of lipid oxidation. This could not have been accomplished with an affective test. This reasoning is supported by Trant et al. (1981). Further study will be needed to determine if

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Effects of Age, Breed, Sex and Muscle on Certain Mineral Concentrations in Cattle

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ABSTRACT

Concentrations of K, Na, Fe, Zn, Ca, Cu and Mg were determined in the longissimus dorsi, semimembranosus and diaphragm muscles. In 94 market weight animals the average concentrations of the elements in ppm of wet weight in the three muscles ranged as follows: Cu, 0.70–1.66; Fe, 18.0–38.1; Ca, 23.8–30.2; Zn, 26.8–40.5; Mg, 198–248; Na, 528–708; and K, 3060–3840. The concentrations of all seven elements in the diaphragm muscle were significantly different from those in the other two muscles. The Cu, Fe, Zn and Mg contents in the longissimus dorsi were significantly different from those in the semimembranosis. Sex and breed differences were less pronounced. In a group of 20 mature cows, Fe, Ca, Zn, Mg and Na were affected by age.

INTRODUCTION

KNOWLEDGE of trace mineral nutrition and the significance of mineral elements in metabolic processes of man and animals has increased rapidly during the last two decades. Several elements have been added to the list of those proven to be essential to sustain normal growth and function in mammals.

Because of agricultural and industrial practices, a redistribution of mineral elements in the environment has taken place, causing in certain areas the soils to become depleted in one or more essential elements. At the same time, other elements have been introduced into the environment of air, water and surface soil, which ultimately appear in the food chain as they are accumulated in plants and animals. Some of these elements are essential, while others are categorized as toxic metals (Ammerman et al., 1977; Doyle and Spaulding, 1978).

The inclusion of certain elements in feed to promote optimal growth and their retention in meat animals is of concern to agencies responsible for the regulation and recommendation of feed additives. In addition, it was pointed out by Mills (1974) that the criteria for animal nutrition may well be less rigorous than those for human nutrition. Acceptable levels of minerals for animals are directed towards optimal production, rate of growth and feed efficiency over a relatively short life span. In contrast, the intake of mineral elements for man has to be adequate and safe for optimal health throughout a lifetime.

Therefore, the evaluation of mineral concentrations in animal tissues becomes of major importance with respect to both safety and adequacy for human nutrition, particularly as minerals are more readily available to man in the form of animal products.

The objects of the present study are to evaluate the concentrations of several elements in three different bovine muscles and to determine the effects of age, breed and sex on their mineral content. The diaphragm muscle was included to determine if the mineral content of this readily accessible muscle is comparable to that of other muscles.

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MATERIALS & METHODS

MUSCLE SAMPLES were obtained from a total of 114 animals, 48 market weight heifers, 46 market weight steers and 20 mature cows. Slaughter weight of the market weight animals ranged from 388–576 kg, with an age range of 421–492 days. The following breeds of sire were represented by the market weight animals: Charolais–10 heifers and 14 steers; Simmental–10 heifers and 13 steers; Limousin–11 heifers and 9 steers; Chianina–17 heifers and 10 steers. All market weight animals were kept in a feedlot under the same feeding regime.

The effect of age on muscle mineral content was determined on the 20 mature, purebred Shorthorn cows, ranging in age from 3–12 years, (mean ± S.E. = 5.9 ± 0.6); the effects of muscle, breed of sire and sex on muscle mineral content were determined on the 94 market weight animals.

The different muscle samples were collected after the carcasses were aged for 6 days and frozen until processed for analysis. Moisture content was determined by drying samples in a forced air oven at 110°C overnight. Samples were dry ashed at 500°C. All elements in this study, K, Na, Fe, Zn, Ca, Mg and Cu, were analyzed by atomic absorption spectrophotometry (Varian AA-375 spectrophotometer) using the analytic methods outlined in the manual supplied by the Varian Company (1978). An attempt was also made to determine cadmium (Cd) and lead (Pb), but because of the extremely low levels present in these muscles, these two elements could not be evaluated with a satisfactory degree of accuracy and were therefore excluded from this study.

Mineral contents are expressed as ppm on a wet weight basis. Moisture contents of the muscles are presented to allow conversion to a dry weight basis. The data were analyzed using SAS general linear model (analysis of variance) and correlation programs, (SAS, 1979).

RESULTS

IN EVALUATING the effects of breed of sire (BOS), SEX and muslce (MUS) on the mineral concentrations, the data dealing with market weight cattle were subjected to analysis of variance (Table 1) to determine the extent of interactions between these variables. For MUS X BOS and MUS X SEX there were significant interactions involving the elements Ca, Zn, Na and possibly K.

Muscle effects

Differences in mineral content due to muscle effect are more obvious (Table 1) compared to other effects. Except for Ca, where sex effect is more important, muscle effect contributes tc the mean squares far in excess of any other source of variation. The least squares means for the different elements in each of the three muscles have been reported in Table 2. The means in this table were compared only when the analysis of variance (Table 1) indicated significant main effects and absence of interactions. The moisture contents reported in Table 2 were obtained on the samples used for the determination of the elements. In connection with other aspects of this work, fat content was also measured, but on another portion of the longissimus dorsi and semimembranosus; no fat determinations were obtained on the diaphragm. The moisture content in these two muscles was inversely related to the fat content and the variation in moisture content accounted for over ninety percent of the variation in fat content. Therefore, the differences in

moisture content reported in the data reflect to a large extent also the differences in fat content of these muscles.

The concentrations of all seven elements in the diaphragm were different from those in the other two muscles with higher concentrations of Cu, Fe, Zn and Na and lower concentrations of Ca, Mg and K. In addition, Cu, Fe and Mg contents differed significantly between the longissimus dorsi and the semimembranosus.

Breed of sire effects

Least squares means of mineral contents in each of the three muscles by breed of sire have been reported in Table 3. The means in this table have been compared only for those minerals for which the analysis of variance (Table 1) indicated the presence of significant ($P \leq 0.05$) muscle x breed of sire interactions. The Ca level in the longissimus dorsi was significantly higher in the Chianina than in the other three breeds of sire. In the longissimus dorsi, the Charolais had a significant lower Ca content. The Na content of the longissimus dorsi was significantly lower in the Limousin than that of the Charolais and the Chianina. There were no significant ($P \leq 0.01$) breed of sire differences for these two elements in the diaphragm muscle. Furthermore, breed of sire differences were much smaller in magnitude than those involving muscle.

Sex effects

As indicated in Table 1, Ca, Zn, Mg, Na and K were affected by sex. However, in all cases sex effects must be interpreted in light of existing muscle x sex and/or breed of sire x sex interactions. As indicated in Table 4, sex differences in mineral content are small and insignificant for the diaphragm muscle, but considerably larger for the longissimus dorsi and semimembranosus muscles. This may well be the main reason for the muscle x sex interaction. Steers were higher in Ca and Na and lower in Zn in both the longissimus dorsi and the semimembranosus muscles. In addition, the K level in the semimembranosus was higher in the steers than in the heifers. As in Tables 2 and 3, means were compared only for those minerals for which the analysis of variance (Table 1) indicated the presence of significant muscle x sex interactions.

Mature cows

Analysis of variance for moisture and mineral levels of the three muscles in the 20 mature cows is reported in

Table 1—Analysis of variance of moisture and mineral concentrations in muscles of market weight cattle (Table of Mean Squares)

Source of variation	Degrees of freedom	Compositional element							
		Moisture	Cu	Fe	Ca	Zn	Mg(X10)	Na(X1,000)	K(X10,000)
Muscle (MUS)	2	494***	23.48***	10,200***	1,280***	3,730***	5,870***	696***	1,560***
Breed of Sire (BOS)	3	20	0.04	11	280***	56**	10	14	53***
Sex	1	25	0.01	27	3,250***	800***	190**	398***	36*
MUS X BOS	6	2	0.03	12	140**	19	17	36**	16
MUS X SEX	2	2	0.07	18	490***	74**	45	203***	34*
BOS X SEX	3	6	0.02	10	10	22	13	2	30*
MUS X BOS X SEX	6	3	0.05	17	73	17	21	17	17
Error	223-233	3	0.03	13	42	14	18	10	8

*P<0.05; **P<0.01; ***P<0.001.

Table 2—Estimated least-squares means and standard errors for moisture and mineral concentrations in muscles of market weight cattle by muscle, by breed of sire and by sex

	Moisture content (%)			Mineral concentration (ppm wet weight)																			
				Cu			Fe			Ca			Zn			Mg			Na			K		
	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$
Muscle																							
Longissimus dorsi	90	72.9[a]	0.18	89	0.70[a]	0.019	90	18.0[a]	0.38	81	30.9	0.73	90	33.3	0.40	90	238[a]	1.43	83	573	11.0	80	3780	32.1
Semimembranosus	74	73.6[a]	0.20	74	0.82[b]	0.021	74	20.8[b]	0.43	72	30.2	0.80	74	26.8	0.45	74	248[b]	1.60	71	528	12.3	74	3840	34.1
Diaphragm	94	69.1[b]	0.18	92	1.66[c]	0.019	93	38.1[c]	0.38	93	23.8	0.69	93	40.5	0.40	93	198[c]	1.42	92	708	10.6	93	3060	30.3
Breed of sire																							
Charolais	62	71.3	0.22	61	1.08	0.024	61	25.1	0.48	55	26.4	0.94	61	32.4[a]	0.51	61	228	1.82	56	591	14.2	58	3450	39.3
Simmental	66	71.5	0.21	66	1.05	0.022	66	26.0	0.45	64	27.9	0.81	66	34.5[b]	0.46	66	226	1.66	66	596	12.3	65	3540	35.6
Limousin	60	72.1	0.22	59	1.03	0.023	60	25.5	0.47	57	27.5	0.87	60	33.1[ab]	0.48	60	229	1.73	60	599	12.9	60	3580	37.0
Chianina	70	72.6	0.20	69	1.08	0.022	70	26.1	0.44	70	31.4	0.79	70	34.2[b]	0.46	70	228	1.64	64	626	12.8	64	3680	36.7
Sex																							
Heifer	132	72.2	0.15	130	1.07	0.016	131	26.0	0.32	131	24.5	0.58	131	35.3	0.33	131	231[a]	1.19	129	561	8.9	125	3520	25.9
Steer	126	71.5	0.15	125	1.05	0.017	126	25.3	0.33	115	32.1	0.63	126	31.7	0.34	126	225[b]	1.23	117	645	9.6	122	3600	26.6

[a,b] Means within muscle, breed of sire, or sex with a different superscript are significantly different at $P \leq 0.01$.

Table 3—Estimated least-squares means and standard errors for mineral concentrations in muscles of market weight cattle by breed of sire within muscle

		Mineral concentration (ppm wet weight)																					
		Cu			Fe			Ca			Zn			Mg			Na			K		
Muscle	Breed of sire	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$	N	$\bar{X}$	$S_{\bar{X}}$
Longissimus dorsi	Charolais	23	0.70	0.038	23	17.1	0.77	18	29.3[a]	1.53	23	32.3	0.80	23	240	2.85	20	609[ab]	22.3	20	3700	64.1
	Simmental	23	0.68	0.038	23	18.7	0.75	21	29.3[a]	1.42	23	34.4	0.78	23	235	2.81	23	539[bc]	20.9	22	3690	61.1
	Limousin	19	0.64	0.041	20	18.0	0.81	18	29.0[a]	1.57	20	33.5	0.84	20	238	3.00	20	520[c]	22.3	20	3800	64.1
	Chianina	24	0.76	0.037	24	18.5	0.74	24	35.9[b]	1.34	24	33.2	0.77	24	239	2.77	20	624[a]	22.6	18	3940	67.7
Semimembranosus	Charolais	15	0.84	0.049	15	20.5	0.98	14	24.6[a]	1.92	15	24.8	1.02	15	249	3.66	14	481[a]	29.3	15	3610	78.1
	Simmental	20	0.80	0.040	20	21.3	0.80	20	31.2[b]	1.45	20	28.7	0.83	20	250	2.99	20	530[a]	22.3	20	3930	63.8
	Limousin	20	0.77	0.040	20	21.2	0.81	19	32.4[b]	1.51	20	26.2	0.84	20	250	3.00	20	541[a]	24.1	20	3824	64.1
	Chianina	19	0.86	0.041	19	20.3	0.82	19	32.7[b]	1.49	19	27.3	0.86	19	244	3.07	17	560[a]	21.5	19	3990	65.5
Diaphragm	Charolais	23	1.69	0.038	23	37.8	0.77	23	25.3[a]	1.38	23	40.0	0.80	23	197	2.85	22	681[a]	21.5	23	3030	60.9
	Simmental	23	1.66	0.038	23	37.9	0.75	23	23.2[a]	1.36	23	40.2	0.78	23	194	2.81	23	721[a]	20.9	23	2990	60.0
	Limousin	20	1.67	0.040	20	37.2	0.81	20	21.1[a]	1.46	20	39.7	0.84	20	199	3.00	20	737[a]	22.3	20	3120	64.1
	Chianina	26	1.62	0.037	27	39.5	0.72	27	25.6[a]	1.29	27	42.1	0.74	27	201	2.66	27	693[a]	19.8	27	3110	56.8

[a,b,c] Means for Ca and Na within muscle with a different superscript are significantly different at $P \leq 0.01$.

Table 5. In the analysis a linear model was used, with muscle as the main effect, age as a covariate, and a muscle x age term to determine if age effects are similar for all three muscles. The mean values for the minerals and moisture content of the three muscles are reported in Table 6, adjusted to the mean cow age of 5.9 years. As was the case with market weight cattle, the concentrations of minerals in the diaphragm muscle were significantly different from those in the other two muscles, with higher levels of Cu, Fe, Ca and Na and lower levels of Mg and K. The Zn level in the diaphragm muscle was significantly higher than that in the semimembranosus, but similar to that of the longissimus dorsi. Differences in mineral levels between the longissimus dorsi and the semimembranosus consisted of higher levels of Zn and Na and lower levels of Cu, Mg and K in the longissimus dorsi. Age effects were important for several minerals (Table 5). As the regression coefficients indicate, Fe, Ca and Na increased with age, while Zn decreased with age; Cu and K contents in the muscles were not affected by age.

Relationships of mineral content among the three muscles was determined by calculating correlation coefficients. Although several correlations in mineral content between muscles were significant ($P \leq 0.05$), their relationships were, in general, low. The variation in mineral content of any one muscle accounted for less than 27% of the variation in any other muscle. Similarly, correlations between minerals within the longissimus dorsi and within the semimembranosus were low. Within the diaphragm muscle, the relationships between mineral levels were somewhat higher, with those involving Ca showing a negative and the other minerals showing positive relationships. Because most correlation coefficients were low and of little predictive value, details have not been reported.

DISCUSSION

THE MINERAL LEVELS presented for the three muscles listed in Tables 2 and 6 are within the range of those reported by Schulz et al. (1974), Vavak (1975), Stabel-Taucher et al. (1975) and Kühne (1976). Differences in mineral content between muscles as indicated in Tables 2 and 6 are similar to those reported by Kühne (1976) and Kotula and Lusby (1975). It has been suggested that the different demands made on muscles is the reason for a variable distribution of certain elements. Kühne found this to be particularly true for Zn, which is found in higher concentrations in those muscles involved in movement.

Wagner et al. (1976), found that in swine the diaphragm muscle was higher in concentrations of Cu, Fe, Zn and Na, lower in Ca and similar in concentrations of Mg and K when compared to the longissimus dorsi muscle. The results reported in Table 2 show the same trend, except for

Table 4—Estimated least-squares means and standard errors for mineral concentrations in muscles of market weight cattle by sex within muscle

		Mineral concentrations (ppm wet weight)																			
		Cu			Fe			Ca			Zn			Mg			Na			K		
Muscle	Sex	N	$\bar{X}$	$S_{\bar{x}}$	N	$\bar{X}$	$S_{\bar{x}}$	N	$\bar{X}$	$S_{\bar{x}}$	N	$\bar{X}$	$S_{\bar{x}}$	N	$\bar{X}$	$S_{\bar{x}}$	N	$\bar{X}$	$S_{\bar{x}}$	N	$\bar{X}$	$S_{\bar{x}}$
Longissimus dorsi	Heifer	43	0.71	0.028	44	17.9	0.55	44	25.1[a]	0.99	44	36.1[a]	0.57	44	238	2.04	42	494[a]	15.4	38	3750[a]	46.6
	Steer	46	0.68	0.027	46	18.2	0.54	37	36.7[b]	1.08	46	30.6[b]	0.56	46	238	2.00	41	652[b]	15.8	42	3810[a]	44.3
Semimembranosus	Heifer	40	0.85	0.028	40	21.7	0.57	40	25.8[a]	1.03	40	28.6[a]	0.59	40	253	2.12	40	469[a]	15.7	40	3730[a]	45.2
	Steer	34	0.79	0.032	34	20.0	0.64	32	34.7[b]	1.23	34	24.9[b]	0.67	34	243	2.39	31	587[b]	19.0	34	3950[b]	51.0
Diaphragm	Heifer	47	1.64	0.027	47	38.4	0.54	47	22.7[a]	0.97	47	41.4[a]	0.56	47	201	2.01	47	722[a]	14.9	47	3090[a]	42.8
	Steer	45	1.68	0.027	46	37.8	0.54	46	24.9[a]	0.97	46	39.6[a]	0.56	46	195	2.00	45	694[a]	15.0	46	3040[a]	42.8

[a,b] Means within muscle with different superscripts are significantly different at $P \leq 0.01$.

Table 5—Analysis of variance of moisture and mineral concentrations in muscles of mature cows (Table of Mean Squares)

Source of variation	Degrees of freedom	Compositional element							
		Moisture	Cu	Fe	Ca	Zn	Mg	Na	K
Muscle	2	44.9***	4.70***	883***	505***	232***	2960***	63900***	2684000***
Age	1	3.1	0.05	68*	163*	168*	245	25100*	78800
Muscle X Age	2	a	a	a	a	a	550*	a	a
Error	56[b]	5.4	0.02	12	37	27	146	3820	50600

[a] Muscle X Age interaction was deleted from the model if its mean square was not significantly ($P < 0.05$) greater than the error mean square.
[b] Error degrees of freedom equals 54 for Mg since Muscle X Age is also in the model.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 6—Least-squares means and standard errors of moisture and mineral concentrations by muscle at the mean age of 5.9 years and regression coefficients defining relationships of moisture and mineral concentrations with age of 20 mature cows

	Moisture content (%)		Mineral concentration (ppm wet weight)													
			Cu		Fe		Ca		Zn		Mg		Na		K	
	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$
Means																
Longissimus dorsi	72.4[a]	0.52	0.70[a]	0.032	27.4[a]	0.78	30.0[a]	1.35	40.0[a]	1.17	216[a]	2.70	525[a]	13.8	3440[a]	50.6
Semimembranosus	73.8[a]	0.52	0.86[b]	0.032	28.7[a]	0.78	29.2[a]	1.35	35.8[b]	1.17	230[b]	2.70	475[b]	13.8	3617[b]	50.6
Diaphragm	70.8[b]	0.52	1.61[c]	0.032	39.5[b]	0.78	38.3[b]	1.35	42.5[a]	1.17	189[c]	2.70	587[c]	13.8	2910[c]	50.6
Regression coefficient (± SE)	—	—	—	—	0.39 ± 0.17		0.60 ± 0.29		−0.61 ± 0.25		[d]		7.5 ± 2.9		—	—

[a,b,c] Means within each element with different superscripts are significantly different at $P \leq 0.01$.
[d] Regression coefficients were not homogenous with respect to muscle (see Table 5). They were −2.6, −0.9 and 1.3 for the LD, SM and diaphragm muscles, respectively, with a standard error of 1.0.

Mg and K, which had a lower concentration in the diaphragm than in the longissimus dorsi muscle.

Breed of sire differences in mineral concentrations involved only Ca and Na (Table 3). In most instances these differences were small and not significant (diaphragm) and lacked consistency with respect to breed of sire and muscle. As the differences involved mainly the Charolais and Chianina breeds of sire (the larger and later maturing breeds), these differences may well be caused by variability in stage of maturity of the animals at the time of slaughter.

In comparing the mineral levels in the three different muscles, it is apparent that the diaphragm muscle is not significantly affected by breed of sire or sex, in contrast to the other two muscles. As the different breeds, sexes and muscles mature at different rates, it may well be that breed of sire, sex and muscle differences in mineral content at least partially arise from differences in maturity of the market weight cattle at time of slaughter.

Although the diaphragm muscle is readily accessible in the carcass, it is clear from the results that the mineral contents of that muscle have little or no value in predicting mineral levels in other muscles.

Comparisons of mineral contents in the muscles of the market weight cattle with those of the mature cows reveal several significant differences. Since the two groups differ not only in age and breed of sire, but also in their management, nutrition and physiological stress due to lactation, these differences should not be interpreted. Within this group of mature cows (age 3–12 years) concentrations of Fe, Ca, Zn, Mg and Na were affected by age. These results are similar to those reported by Kotula and Lusby (1975) who reported that Fe, Zn, Mg, Na and K were affected by age of steers in the range 1–6 years. With respect to Ca, these authors reported a decrease in muscle tissue with age, while in the present data, Table 6, the regression coefficient indicates an increase in Ca level with age.

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an oxidized flavor is objectionable to inexperienced or consumer panelists.

The overall objective of the study was to obtain insight into the relation between TBA numbers and consumers' perceptions of oxidized flavor in cooked ground beef. The r^2 values for sensory versus TBA were low. Variability in individual panelist scoring accounted partly for these low values. Although the score sheet used in this study was simple, identification of intensity of oxidized flavor probably was not an easy task for these inexperienced panelists. In addition, inconsistencies in the TBA test may have contributed to the low r^2 values. Nevertheless, of the 52 trained panelists who evaluated the beef samples, 28 (54%) were found to be consistent in their scoring. The TBA number range in which this sub-population, as a group, first noted a difference in intensity of oxidized flavor was from 0.6–2.0–surprisingly close to the 0.5–1.0 range for rancid odor detection determine by Tarladgis et al. (1960) with trained panelists. It might be expected that some portion of the general public would also note an oxidized flavor within this TBA number range. If the scores are indicative of the ability of these panelists to recognize an oxidized flavor in beef, then the TBA test was an indicator of the number of individuals who were sensitive to this flavor. To interpret TBA numbers in terms of consumer response to oxidized flavor, an estimation of the proportion of consumers expected to recognize an oxidized flavor at a given TBA number might be a more useful approach. Additional studies would be needed to test this.

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Studies in Lipid Composition and Physical State of Liver and Heart Mitochondria and Microsomes in Pigs Fed on Diets Containing Dried Biomass of Candida lipolytica (Toprina)

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ABSTRACT

The lipid composition and certain structural and functional features of liver and heart mitochondria and microsomes have been investigated in pigs fed diets containing 0%, 15%, 30% "Toprina" (dried biomass of *Candida lipolytica* grown on n-alkanes). The presence of "Toprina" in the diets and its odd-chain higher fatty acids (HFA) (especially 17:1 and 17:2) in total lipids, phospholipids, and neutral lipids of pig liver and heart mitochondria and microsomes does not result in any variations of membrane fluidity (evaluated by the unsaturation index, the unsaturation index/% of total saturated HFA, and by fluorescence polarization of perylene), nor does it result in changes of mitochondrial ATPase activity, sensitive to membrane lipid composition.

INTRODUCTION

INVESTIGATION of new protein sources for animal and human nutrition, having at the same time the characteristics of high biological value, of independence from agricultural and environmental limitations, and of relatively low costs, have stimulated industrial production of microorganisms. The zoo-technical use has been proposed for dried biomass of *Candida lipolytica* cultured, according to the industrial process of BP (British Petroleum) on n-alkanes extracted from petrol at a high purity level. The commercial product has been named "Toprina."

In a research program on the lipid components of Toprina, high levels of odd-chain fatty acids, mainly 17 carbon atoms and unsaturated (17:1 (n-8) and 17:2 (n-5) have been found (Borgatti and Trigari, 1979). This confirmed the tendency of yeasts grown on n-alkanes to enrich their cell lipids in fatty acids derived from incorporation from the medium of n-alkanes having chain with an odd number of carbon atoms (Klug and Markovetz, 1967; Rattray et al., 1975; Thorpe and Ratlege, 1972). Investigations on pigs (Borgatti et al., 1976a, b, c) and birds (Borgatti et al., 1979a, b, c; Leclercq, 1976) fed on diets containing different concentrations of Toprina, and on rats (Serlupi Crescenzi et al., 1978) receiving diets where the lipid contents consisted of lipids extracted from Toprina, demonstrated that odd-chain fatty acids were present in all tissues of experimental animals, with the exception of the brain. The exception could be due to a slower fatty acid turnover rather than to a lack of permeability of the blood-brain barrier. Only in rats the brain contained odd-chain fatty acids at a level five-fold lower than the carcass (Serlupi Crescenzi et al., 1978). Odd-chain fatty acids are rather unusual in diets and tissues of both man and higher animals of zootechnical interest; they are generally present in the animal kingdom in small amounts, about 1–3%. Only in ruminants higher contents, about 3–7%, are found (Viviani, 1970) and even higher in certain fishes [mullet and smelt (10–30%) (Addison and Ackman, 1970; Iyemgar and Schlenk, 1967; Schlenk, 1970). Even in such cases the source is from the diet, from ruminal bacterial flora for ruminants (Viviani, 1970) and from the type of plankton preferred as food for fishes (Addison and Ackman, 1970; Iyemgar and Schlenk, 1967; Schlenk, 1970).

The use of Toprina for zootechnical and, consequently, human nutrition has attracted research on possible toxic effects of odd-chain fatty acids, particularly unsaturated fatty acids and of their metabolism. Some studies on the rat (Schlenk, 1972) appeared to show that C17 fatty acids may substitute the corresponding C18 acids; in particular 17:1 (n-8), 17:2 (n-5) and 17:3 (n-2) could have the same physiological role of 18:1 (n-9), 18:2 (n-6) and 18:3 (n-3), respectively. To this purpose, it has been shown that 17:1 and 17:2 present in Toprina are (n-8) and (n-5) respectively (Borgatti and Trigari, 1979). The finding in our investigations of small amounts of 19:1 and traces of 19:2 only in diets containing Toprina confirms the capability of heart and liver tissues to metabolize dietary 17:1. On the other hand it is known that the activity of membrane-bound enzymes may change with changes of dietary fatty acids (Lenaz, 1977), mainly as a result of changes in fluidity of the membrane (Farias et al., 1975). It has been thought of interest therefore to perform functional and structural studies on mitochondrial and microsomal membranes from livers and hearts of pigs fed diets containing different amounts of Toprina.

EXPERIMENTAL

Animals and diets

For this study we have used dried biomass of *Candida lipolytica*, cultured on n-alkanes extracted from highly pure petrol with an industrial method (BP), and commercially known as Toprina. The product was furnished by the Ministry of Health (Roma, Italy). The zootechnical aspects of this study have been published elsewhere (Mordenti et al., 1977). We report only some details related to diets, animals, and mode of breeding.

Three groups of 10-day old intact male pigs, Large White x Lambrace crossbreeds, weighing 2.8 kg on the average were used. They were homogeneous in age, weight, and breed. The following diets were used: (a) control (T0%) containing 21.0% proteins (N x 6.25), 10% lipids, and 4,000 Kcal/kg; (b) and (c) containing 15% and 30% respectively of integral Toprina, and designated as T15% and T30%. The diets had the same protein, lipid, and caloric contents. The animals, bred in the same climatized environment in the dark, received food ad libitum. The studies were done in triplicate; the first two experiments consisted of 36 pigs each and the third one of 15, equally subdivided into the three experimental groups. The animals were sacrificed as soon as they had reached the established weight of 8–10 kg. The experimental time was 30, 37, and 42 days for the three experiments, respectively.

Lactating pigs were selected because of the zootechnical importance of swine. Animals in their periods of fastest post-natal growth are most sensitive and may best reveal metabolic alterations or pathological states as a result of the presence in food of lipids or lipid-soluble materials. Results obtained were believed to be of potential use in statements of Admissible Daily Intake (ADI) for man of specific lipid compounds.

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The diet composition was such that Toprina was present in amounts about 1.5 to threefold those normally advised in zootechnical use. The lipid content was lower than normal, so that the relative amount of lipids coming from Toprina would not be too low in comparison with total lipids. A further precaution was to keep constant the unsaturation level in the three diets, also keeping the sums of 17:1 + 18:1 and 17:2 + 18:2 constant by balancing the lipid sources in the diet on the basis of their fatty acid composition (Table 2).

MATERIALS

Standards for fatty acid analyses were obtained from Polyscience Corporation Chemical Division and from Applied Science Laboratories Inc., State College, PA. Solvents were obtained form Carlo Erba (Milano) and from Fisher Scientific Co. Other reagents: ATP and perylene from Sigma; purified sucrose, Tris (hydroxymethyl)-aminomethane (Tris buffer) and silver nitrate from Merck; silicic acid, 100 mesh, from Mallinkrodt; 14% boron trifluoride in anhydrous methanol (BDH, Laboratory Chemical Division); acid washed Chromosorb W 60–80 mesh and diethylene glycol succinate (DEGS) from Carlo Erba.

Preparation of mitochondria and microsomes

Hearts and livers were withdrawn from slaughtered animals, quickly washed, weighed and put in a 0.25M sucrose solution buffered to pH 7.2 by appropriate additions of 1M Tris base. In each experiment aliquots of equal weight from minced fat-free tissue and from two to three animals each were grouped in pools of 40–50g. Each pool was homogenized mildly with an Ultra-turrax homogenizer and mitochondrial and microsomal fractions were prepared according to Johnson and Lardy (1967). All steps were carried out at 0–4°C. Protein in the different fractions was assayed with a biuret method (Gornall et al., 1949). All samples of mitochondrial and microsomal fractions were transferred to 2 ml sealed glass vials and kept until the time of assay in liquid nitrogen. According to Fleischer and Kervina (1974) this treatment is suitable to prevent structural damage to the membranes.

Determination of mitochondrial ATPase

Mitochondrial ATPase activity was assayed according to Griffiths and Hought (1974), in liver and heart mitochondrial samples from each pool of the 2nd and 3rd experiments. All assays were done in triplicate. Specific activity was expressed as μmoles of phosphate hydrolyzed per minute per mg of mitochondrial protein.

Fluorescence polarization of perylene

Measures of fluorescence polarization (Azzi, 1974) were effected in mitochondrial and microsomal preparations from livers and hearts of pools from the 2nd and 3rd experiments, using perylene as a fluorescent probe. Determinations were made with a Perkin Elmer MPF III Spectrofluorimeter with a polarization accessory (018-0054 Hitachi Ltd.) in a thermostated cuvette chamber at 20–22°C. Each sample was excited at 410 nm and emission spectra were monitored between 430 and 460 nm, recording the fluorescence maximum. Polarization was calculated from duplicate experiments according to Shinitsky et al. (1971).

Lipid analysis

Total lipids (TL) were extracted from aliquots of mitochondrial and microsomal preparations with chloroform-methanol 2:1 (v/v) and purified according to Folch et al. (1957). The same method was employed for lipid extraction from Toprina and from the different diets, with a few modification. The extraction time was prolonged to 60 hr at a temperature of 40°C with continuous stirring.

Polar lipids (Pol) were separated from neutral lipids (NL) on silicic acid (Marks et al., 1960) obtaining a complete separation. Phospholipids were quantitatively evaluated by P determination according to the method of Bartlett (1959) as modified by Marinetti (1962). Total long chain fatty acids were obtained from aliquots of TL, Pol (containing phospholipids) and NL of all pools by saponification with methanolic KOH, removal of unsaponifiable materials, acidification and extraction with petroleum ether (40–60°C) of the saponified material (Viviani et al., 1964). Individual long chain fatty acids were methylated with 14% BF_3 in methanol (Morrison and Smith, 1964) and analyzed by gas-liquid chromatography associated with chromatography on silicic acid columns impregnated with 25% $AgNO_3$ and with catalytic hydrogenation of double bonds (Ahrens et al., 1959) in order to evaluate the degree of unsaturation (Viviani et al., 1968). Further identification was possible by the graphic evaluations of "carbon numbers" and by comparison with chromatograms of methyl esters of standard fatty acids.

Gas chromatography was accomplished on a Fractovap Line G from Carlo Erba equipped with a flame detector, using a 180 x 4 mm glass column filled with acid-washed Chromosorb W 60–80 mesh and 20% diethylene glycol succinate; pure N_2 was the carrier gas with a flow rate of 30 ml/min. The column temperature was kept isothermic at 190°C.

Degree of unsaturation

The unsaturation index was calculated from the fatty acid spectra according to the following formula (Bloj et al., 1973)

$$I = \frac{\text{n double bonds X \% fatty acid}}{100}$$

This value divided by the percentage of saturated fatty acids in the same sample yields the unsaturation index/% saturated fatty acid ratio.

RESULTS

TABLE 1 reports the content of total lipids, phospholipids and neutral lipids of "Toprina", in the diets used, and in the mitochondria and microsomes from hearts and livers of pigs in the different experimental conditions. Toprina contains 67% of its total lipids as phospholipids; therefore, the phospholipid increase and the neutral fat decrease in diets from T0% to T30% are consequences of the increase of Toprina content. It is clear that the presence of Toprina in the diet does not induce any significant change of TL, PL and NL of heart and liver mitochondria and microsomes of experimental animals.

Table 2 reports long chain fatty acid composition of Toprina and of the experimental diets. The presence in Toprina of high levels of odd-chain fatty acids is quite evident (53% of total fatty acids). The highest levels were found for 17:1 and 17:2 (26% and 14% of total fatty acids). In diets the content of odd-chain fatty acid is proportional to that in Toprina. Note that all diets have constant unsaturation index and unsaturation index/% saturated fatty

Table 1—Lipid content of Toprina, of experimental diets and of pig heart and liver mitochondria and microsomes

		%TL/fm[a]			%PL/TL[a]			%NL/TL[a]		
Toprina		9.05			66.82			33.18		
		T0% ± s.e.[b]	T15% ± s.e.	T30% ± s.e.	T0% ± s.e.	T15% ± s.e.	T30% ± s.e.	T0% ± s.e.	T15% ± s.e.	T30% ± s.e.
Average value of diet		8.2±0.3	8.9±0.1	9.6±0.1	1.1±0.1	14.8±0.9**	25.4±0.3**	98.9±0.1	84.8±0.4**	74.7±0.3**
Liver	Mitochondria	–	–	–	65.9±3.7	63.4±7.9 n.s.[c]	68.4±0.7 n.s.	34.2±3.7	36.7±7.9 n.s.	31.6±0.7 n.s.
	Microsomes	–	–	–	63.7±5.4	71.5±2.5 n.s.	72.4±4.6 n.s.	36.4±5.4	28.6±2.5 n.s.	27.6±4.6 n.s.
Heart	Mitochondria	–	–	–	63.0±3.8	73.0±3.1 n.s.	59.2±9.5 n.s.	37.0±3.8	27.0±3.1 n.s.	40.8±9.5 n.s.
	Microsomes	–	–	–	54.6±7.5	53.6±8.3 n.s.	49.3±9.8 n.s.	45.4±7.5	47.4±8.3 n.s.	50.7±9.8 n.s.

[a] TL = Total lipids; PL = Phospholipids; NL = Neutral lipids; %TL/fm = percentage of TL on fresh material; %PL/TL = percentage of PL on TL; %NL/TL = percentage of NL on TL
[b] s.e. = standard error
[c] n.s. = Comparison with T0% not significant $p < 0.05$
* = Comparison with T0% significant $p < 0.05$
** = Comparison with T0% significant $p < 0.01$

acids ratio. Care was taken to exclude the possibility that any observed effects on membrane structure could be due to unsaturation changes; for this reason the contents of 18:1 + 17:1 and 18:2 + 17:2 in T15% and T30% were the same as the contents of 18:1 and 18:2 in T0%.

Tables 3, 4, 5, 6 report the percent composition of fatty acids in TL, Pol, and NL of liver and heart mitochondria and microsomes in the three experimental conditions. In the same tables also total odd-chain fatty acids, the unsaturation index, and the unsaturation index/% saturated fatty acids ratio are reported. A general examination of the tables shows that, although total odd-chain fatty acids are present in all conditions, there is an increase in tissues of animals fed T15% and T30% proportional to the Toprina content. The increase of 17:0, 17:1, 17:2 is typical in T15% and T30%; in certain instances increase of 19:1 in heart and trace of 19:2 in liver have been found. On the other hand, even chain fatty acids 18:1 and 18:2 decrease slightly in T15% and T30%. Such changes could be the result of their decreased content in the diet.

Both the unsaturation index and the unsaturation index/% saturated fatty acids ratio do not undergo any significant change.

Tables 7 and 8 show data respectively on the fluorescence polarization of perylene as an index of membrane viscosity for the same organelles and on the activity of mitochondrial ATPase as a functional parameter. These parameters can be compared with odd-chain fatty acids content, unsaturation index, and unsaturation index/% saturated fatty acids ratio in the same samples. It is clear that, although odd-chain fatty acids increase as a result of diets containing from T0% to T15% and T30%, fluorescence polarization and ATPase activity, as well as the unsaturation of membrane lipids, do not undergo any significant changes.

DISCUSSION & CONCLUSIONS

DIETARY FATTY ACIDS may induce large changes in fatty acid composition of animals, since they are incorporated into their tissues either as such or after elongation or desaturation. A similar behavior has been demonstrated in several studies for odd-chain fatty acids of Toprina when administered to different experimental animals.

In agreement with those results, the present research demonstrates that odd-chain saturated and unsaturated fatty acids of Toprina are incorporated as structural components of all lipid fractions of mitochondria and microsomes of pig livers and hearts.

It has been known for a long time that activity of several membrane-bound enzymes is affected by membrane lipid composition (Lenaz, 1977). Among such enzymes, mitochondrial ATPase, plasma membrane (Na^+, K^+)-ATPase and erythrocyte acetyl-cholinesterase (Bloj et al., 1973; Farias et al., 1975) have been found to be affected in their allosteric regulation by changes in membrane lipids induced by lipid-free diets, or by diets containing lipids having different unsaturation. The above studies showed that the effects were mainly related to changes of membrane fluidity, as revealed by evaluation of the unsaturation index. Our investigations demonstrate that feeding pigs a Toprina content double that recommended for zootechnical use produces lipid changes consisting only in the presence of both saturated and unsaturated odd-chain fatty acids. On the other hand, presence in heart and liver membranes of these fatty acids or of their possible derivatives from elongation or desaturation, and of other lipid-soluble structures of Toprina such as residual n-paraffines, do not appear to alter the physical characteristics of the membranes studied. In fact investigation of the physical state of lipids by fluorescence polarization of the probe perylene and indirectly by the unsaturation index and the unsaturation index/% saturated fatty acids ratio did not reveal any fluidity change of lipids in all membranes studied. Moreover heart and liver mitochondrial Mg^{++}-ATPase did not show any significant change.

It appears possible to conclude that neither the ascer-

—Text continued on page 64

Table 2—Percent long chain fatty acids composition of Toprina and diets

Higher fatty acids	Toprina	Diets		
		T0% ± s.e.	T15% ± s.e.	T30% ± s.e.
12:0	0.5	18.1 ± 0.1	18.6 ± 1.0	17.1 ± 1.6
13:0	0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
14:0	1.7	11.5 ± 0.5	9.3 ± 0.4	8.8 ± 0.5
14:1	–	0.2 ± 0.0	0.1 ± 0.0	–
15:0	9.8	0.2 ± 0.0	1.4 ± 0.1	2.7 ± 0.2
15:1	0.6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:0	10.3	12.9 ± 0.6	11.1 ± 0.1	11.2 ± 0.9
16:1+17:0br[a]	7.2	0.3 ± 0.1	1.2 ± 0.1	2.0 ± 0.3
17:0	3.1	0.2 ± 0.0	0.5 ± 0.0	0.8 ± 0.1
17:1+18:0br	26.2	0.3 ± 0.1	4.6 ± 0.3	8.2 ± 0.4
18:0	1.2	4.1 ± 0.2	3.0 ± 0.1	2.9 ± 0.2
17:2	13.6	–	2.2 ± 0.1	5.3 ± 0.3
18:1	9.1	21.0 ± 0.8	18.2 ± 0.9	14.6 ± 3.2
19:0	0.1	0.1 ± 0.0	0.1 ± 0.0	Traces
18:2	15.4	29.2 ± 2.1	28.1 ± 0.5	25.2 ± 1.5
19:1+20:0br	0.6	–	–	–
20:0	0.3	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
18:3+20:1+19:2	0.1	0.6 ± 0.3	0.4 ± 0.2	0.4 ± 0.1
21:0	0.2	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
22:0	–	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
24:0	–	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Total odd chain	53.5	0.8 ± 0.1	9.0 ± 0.4**	17.7 ± 0.5**
Unsaturation index	0.9	0.8 ± 0.2	0.8 ± 0.1 n.s.	0.8 ± 0.1 n.s.
Unsaturation index/% saturated	3.3	1.6 ± 0.1	1.8 ± 0.2 n.s.	1.8 ± 0.1 n.s.

[a] br = branched fatty acids
Other abbreviations are explained in the text and in Table 1.

Table 3—Percent long chain fatty acids composition of total lipids, polar lipids and neutral lipids in liver mitochondria of pigs fed diets containing Toprina

Higher fatty acids	TL			Pol			NL		
	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.
A[a]	2.1±0.4	1.4±0.2	1.6±0.4	1.8±0.5	1.9±0.4	1.7±0.3	3.2±0.8	2.8±0.7	2.9±1.0
15:0	0.3±0.0	0.4±0.0	0.5±0.1	0.3±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.5±0.1	0.7±0.2
15:1+16:0br	0.2±0.0	0.2±0.0	0.2±0.0	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1
16:0	16.0±1.1	13.8±0.7	12.9±0.4	15.8±0.6	13.1±0.9	13.8±0.9	17.6±1.3	15.8±1.7	15.5±1.3
16:1+17:0br	1.5±0.2	1.3±0.2	1.1±0.1	1.5±0.2	1.2±0.2	1.2±0.1	2.2±0.5	2.1±0.4	2.2±0.2
17:0	0.8±0.1	1.6±0.3	2.0±0.3	0.9±0.2	1.7±0.1	1.9±0.3	0.9±0.1	1.9±0.1	2.0±0.3
17:1+18:0br	0.6±0.2	1.2±0.1	1.8±0.2	0.9±0.2	1.3±0.1	1.7±0.1	0.5±0.1	1.4±0.2	2.2±0.2
18:0	24.1±1.0	23.0±0.9	24.8±0.7	24.6±1.0	23.6±1.1	25.8±1.9	20.1±2.9	23.7±1.0	20.6±0.9
17:2	–	1.2±0.0	1.2±0.1	–	0.8±0.0	0.9±0.0	–	0.8±0.1	1.8±0.1
18:1	14.5±0.8	11.3±0.6	11.1±0.8	12.4±0.6	11.6±0.8	10.3±0.6	16.9±3.6	13.0±1.1	15.7±1.1
18:2	16.4±0.8	17.4±0.5	16.2±0.8	15.6±1.1	17.2±0.6	14.7±1.1	15.5±1.1	16.0±1.1	16.2±1.0
19:1+20:0br	0.4±0.1	0.4±0.1	0.6±0.2	0.3±0.0	0.3±0.1	0.7±0.1	0.6±0.2	0.4±0.1	0.7±0.2
18:3+19:2+20:1	0.3±0.1	0.4±0.1	0.7±0.1	0.2±0.0	0.4±0.1	0.4±0.1	0.7±0.4	0.4±0.1	0.9±0.2
18:3br	0.5±0.1	0.5±0.1	0.6±0.1	0.3±0.1	0.9±0.2	0.8±0.3	0.6±0.2	Traces	0.2±0.1
21:0	0.4±0.1	0.3±0.0	0.2±0.0	0.5±0.1	0.3±0.1	0.7±0.3	0.4±0.0	1.0±0.5	0.7±0.3
21:1+22:0br	1.0±0.3	0.6±0.1	0.9±0.1	0.6±0.1	1.1±0.2	1.4±0.4	0.7±0.2	0.8±0.3	0.8±0.3
20:2	0.7±0.1	0.8±0.1	0.8±0.1	0.9±0.1	0.8±0.3	0.9±0.2	0.8±0.2	0.4±0.0	0.6±0.1
20:4	16.4±1.0	19.3±1.6	18.5±1.0	16.4±1.1	18.1±0.9	17.8±0.9	13.8±1.4	15.3±1.1	13.4±1.6
22:1+23:0br	0.5±0.2	0.4±0.1	0.3±0.0	0.5±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.3±0.1	0.4±0.1
23:0	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.1	0.3±0.1	0.3±0.0	0.5±0.3	0.4±0.1	0.4±0.2
24:0	0.7±0.2	0.5±0.2	0.6±0.1	1.3±0.3	0.7±0.2	0.9±0.2	1.1±0.3	0.6±0.2	0.6±0.2
22:4	1.5±0.2	2.0±0.1	1.4±0.1	2.1±0.4	2.2±0.2	1.5±0.3	1.1±0.2	1.3±0.2	0.7±0.2
22:5	0.9±0.2	1.5±0.3	1.2±0.1	1.1±0.1	1.4±0.4	1.1±0.2	0.9±0.3	1.7±0.2	0.8±0.2
22:6	1.0±0.1	1.1±0.2	0.7±0.1	1.2±0.2	1.2±0.2	1.6±0.4	1.1±0.1	1.3±0.3	1.1±0.4
20:0+22:0	1.0±0.3	0.6±0.1	0.5±0.1	0.9±0.3	0.8±0.2	0.5±0.2	0.5±0.1	0.5±0.1	0.7±0.2
Total odd chain	4.5±1.0	8.2±0.6**	11.5±0.9**	5.1±0.8	8.3±0.7**	12.5±1.5**	6.7±1.5	8.9±1.2 n.s.	11.0±0.7*
Unsaturation index	1.3±0.1	1.5±0.1 n.s.	1.4±0.1 n.s.	1.3±0.1	1.5±0.1 n.s.	1.4±0.1 n.s.	1.2±0.1	1.2±0.1 n.s.	1.2±0.1 n.s.
Unsaturation index/ % saturated	2.9±0.2	3.7±0.2 n.s.	3.2±0.1 n.s.	2.9±0.2	3.5±0.2 n.s.	3.0±0.3 n.s.	2.6±0.2	2.8±0.4 n.s.	2.8±0.3 n.s.

[a] A = 12:0+13:0br+13:0+14:0br+14:0+15:0br
Other abbreviations are explained in the text and in the Tables 1 and 2.

Table 4—Percent long chain fatty acids composition of total lipids, polar lipids and neutral lipids in liver microsomes of pigs fed diets containing Toprina

Higher fatty acids	TL			Pol			NL		
	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.
A	2.0±0.4	1.7±0.4	1.5±0.2	1.8±0.5	2.7±0.8	1.6±0.5	3.2±0.5	3.5±0.8	2.6±0.5
15:0	0.3±0.1	0.4±0.0	0.5±0.2	0.2±0.1	0.6±0.1	0.4±0.1	0.3±0.1	1.1±0.3	0.6±0.1
15:1+16:0br	0.3±0.1	0.2±0.0	0.2±0.0	0.4±0.1	0.2±0.0	0.4±0.2	0.3±0.0	0.6±0.2	0.3±0.1
16:0	17.6±1.5	16.0±0.3	13.0±0.3	13.8±0.9	15.0±0.5	14.2±0.2	17.3±0.8	20.1±0.8	16.6±1.0
16:1+17:0br	1.5±0.1	1.1±0.2	1.0±0.1	1.4±0.3	1.2±0.2	1.2±0.3	2.4±0.8	2.6±0.4	2.2±0.3
17:0	0.7±0.1	1.8±0.2	2.1±0.2	0.7±0.1	1.4±0.2	2.7±0.4	0.7±0.1	1.9±0.3	2.0±0.2
17:1+18:0br	0.6±0.1	1.2±0.0	1.9±0.1	0.6±0.1	1.3±0.1	2.0±0.3	0.5±0.1	1.9±0.1	2.6±0.1
18:0	24.0±1.0	24.7±1.5	26.5±1.2	24.2±1.9	26.0±1.1	28.2±0.8	22.4±1.7	21.0±0.8	22.3±1.8
17:2	–	0.4±0.0	0.7±0.0	–	1.6±0.2	1.1±0.0	–	0.2±0.0	0.3±0.0
18:1	14.3±0.8	12.7±0.8	12.0±0.5	12.2±1.3	11.1±0.7	10.6±0.3	15.9±2.6	13.4±1.7	14.9±1.3
18:2	14.0±1.2	14.1±0.5	14.0±0.4	18.1±2.4	14.6±0.5	13.9±1.1	15.0±0.7	11.7±0.9	12.5±0.8
19:1+20:0br	0.2±0.0	0.2±0.0	0.4±0.1	0.3±0.1	0.3±0.1	0.7±0.2	0.7±0.4	1.0±0.3	0.9±0.3
18:3+19:2+20:1	0.3±0.1	0.3±0.1	0.4±0.1	0.5±0.2	0.3±0.1	0.5±0.1	0.3±0.1	0.7±0.2	0.6±0.1
18:3br	0.4±0.1	0.4±0.1	0.6±0.1	0.9±0.4	1.1±0.2	1.0±0.3	0.8±0.3	Traces	0.5±0.3
21:0	0.6±0.2	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.0	0.3±0.1	0.5±0.1	0.4±0.0	0.2±0.0
21:1+22:0br	0.7±0.1	0.6±0.1	0.6±0.1	0.7±0.1	0.9±0.1	1.0±0.3	0.5±0.1	0.8±0.2	0.4±0.1
20:2	0.8±0.1	0.5±0.1	0.8±0.1	1.1±0.3	1.1±0.3	1.0±0.2	0.6±0.2	0.3±0.1	0.7±0.3
20:4	15.7±1.3	17.0±0.9	18.3±0.8	17.4±1.0	15.7±0.9	15.5±1.2	14.4±1.8	10.6±1.1	13.1±1.2
22:1+23:0br	0.3±0.2	0.3±0.0	0.4±0.1	0.7±0.2	0.6±0.2	0.7±0.3	0.3±0.1	0.5±0.1	0.4±0.1
23:0	0.3±0.1	0.2±0.1	0.2±0.0	0.3±0.1	0.4±0.1	0.3±0.0	0.4±0.1	0.7±0.2	0.5±0.2
24:0	0.6±0.3	0.5±0.2	0.6±0.2	Traces	0.7±0.4	1.1±0.3	1.1±0.3	0.9±0.3	0.5±0.2
22:4	1.7±0.3	1.9±0.1	1.5±0.2	1.4±0.3	2.0±0.2	1.3±0.2	1.1±0.2	1.0±0.4	1.0±0.1
22:5	0.9±0.2	1.9±0.4	1.2±0.1	0.9±0.2	1.5±0.3	1.2±0.2	1.0±0.2	0.7±0.3	0.7±0.2
22:6	1.3±0.1	1.3±0.2	0.8±0.1	1.2±0.2	1.1±0.2	1.1±0.2	1.6±0.4	1.3±0.3	1.0±0.2
20:0+22:0	1.1±0.3	0.9±0.2	0.9±0.2	0.6±0.1	0.6±0.1	0.9±0.3	1.0±0.3	0.6±0.2	1.6±0.5
Total odd chain	3.3±0.6	7.5±0.5**	9.1±0.5**	3.8±0.5	9.5±1.3**	11.5±0.7**	5.8±1.3	9.4±1.2*	11.1±1.5*
Unsaturation index	1.3±0.1	1.4±0.1 n.s.	1.4±0.0 n.s.	1.4±0.1	1.3±0.0 n.s.	1.3±0.1 n.s.	1.2±0.1	1.0±0.1 n.s.	1.2±0.1 n.s.
Unsaturation index/ saturated	2.8±0.2	3.0±0.2 n.s.	3.0±0.1 n.s.	3.1±0.3	2.7±0.1 n.s.	2.5±0.2 n.s.	2.5±0.2	2.0±0.2 n.s.	2.4±0.3 n.s.

Abbreviations are explained in the text and in the Tables 1, 2 and 3.

Table 5—Percent long chain fatty acids composition of total lipids, polar lipids and neutral lipids in heart mitochondria of pigs fed diets containing Toprina

Higher fatty acids	TL			Pol			NL		
	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.
A	3.2±0.7	2.6±0.9	2.6±0.8	3.2±1.0	4.0±1.2	4.9±2.1	4.8±1.6	6.1±1.2	5.3±1.3
15:0	0.8±0.3	0.5±0.1	1.2±0.3	0.6±0.2	0.8±0.2	0.8±0.3	0.8±0.3	0.9±0.3	0.9±0.2
15:1+16:0br	2.6±0.5	2.6±0.3	2.3±0.5	2.8±0.7	2.2±0.7	2.1±0.6	2.3±0.6	2.5±0.5	1.8±0.4
16:0	15.6±0.6	14.2±0.9	14.4±0.9	15.0±0.6	15.7±1.8	14.0±1.3	17.3±1.4	16.5±1.6	16.6±1.6
16:1+17:0br	1.1±0.4	1.1±0.4	1.0±0.2	1.7±0.5	2.5±0.6	2.3±1.1	3.9±1.0	2.9±0.7	3.2±0.5
17:0	0.6±0.2	0.7±0.1	1.3±0.1	0.6±0.2	0.7±0.1	1.1±0.2	0.6±0.2	0.8±0.1	1.2±0.1
17:1+18:0br	1.4±0.4	1.7±0.5	2.3±0.2	1.7±0.3	2.2±0.3	2.5±0.5	2.0±0.3	2.4±0.2	2.6±0.4
18:0	16.0±0.6	12.3±1.0	14.3±0.9	15.2±0.8	13.7±0.4	14.5±1.6	17.2±2.0	15.9±1.6	15.5±0.8
17:2	–	1.3±0.0	1.5±0.0	–	0.7±0.0	1.2±0.0	–	0.8±0.0	0.5±0.0
18:1	14.1±0.8	14.2±0.5	13.2±0.9	13.5±0.4	14.7±1.4	14.1±1.1	16.6±0.4	16.0±0.8	14.2±0.9
18:2	24.9±4.3	29.4±2.8	23.2±4.4	24.4±2.9	23.1±2.7	20.0±3.8	15.6±3.4	20.4±1.9	19.9±3.4
19:1+20:0br	0.7±0.2	1.2±0.6	Traces	0.5±0.1	0.9±0.2	1.2±0.5	0.7±0.2	1.9±0.5	1.5±0.3
18:3+19:2+20:1	0.5±0.2	0.6±0.2	1.0±0.0	0.3±0.1	0.4±0.1	1.4±0.2	0.5±0.2	0.5±0.1	0.7±0.1
18:3br	0.2±0.0	Traces	Traces	Traces	0.3±0.1	Traces	0.5±0.1	0.4±0.1	Traces
21:0	1.2±0.1	1.1±0.4	1.1±0.3	0.4±0.1	0.9±0.3	0.9±0.3	0.7±0.2	0.9±0.2	0.9±0.2
21:1+22:0br	1.0±0.2	0.7±0.1	0.7±0.2	1.0±0.2	1.6±0.3	1.5±0.7	1.0±0.5	1.0±0.4	0.7±0.2
20:2	0.8±0.2	0.3±0.1	0.4±0.1	0.5±0.2	0.3±0.1	0.3±0.0	0.5±0.1	0.5±0.2	0.4±0.1
20:4	10.0±1.2	11.4±2.2	9.6±1.7	8.8±2.0	10.1±1.8	8.7±2.4	6.4±1.2	5.5±1.2	6.7±1.4
22:1+23:0br	0.5±0.1	0.4±0.2	0.7±0.3	Traces	0.3±0.1	0.3±0.1	0.5±0.1	0.3±0.1	0.4±0.0
23:0	1.1±0.3	1.0±0.4	0.9±0.4	0.6±0.1	0.4±0.1	0.8±0.3	1.0±0.4	0.7±0.3	0.8±0.3
24:0	1.8±0.3	1.5±0.5	0.9±0.2	1.0±0.2	0.9±0.3	1.3±0.4	1.0±0.4	1.2±0.3	0.9±0.3
22:4	0.7±0.2	0.8±0.2	1.1±0.6	0.8±0.2	0.7±0.1	1.1±0.3	0.8±0.3	0.6±0.2	Traces
22:5	0.3±0.0	1.1±0.5	0.5±0.1	0.5±0.1	0.7±0.1	0.4±0.1	0.3±0.0	0.5±0.2	1.3±0.4
22:6	1.1±0.3	0.8±0.4	0.4±0.1	1.4±0.5	1.1±0.4	2.3±0.9	1.3±0.5	1.4±0.4	1.8±0.4
20:0+22:0	1.1±0.5	0.6±0.1	0.8±0.3	0.4±0.1	0.8±0.2	1.3±0.6	1.1±0.4	1.6±0.3	0.6±0.1
Total odd chain	3.8±0.9	7.8±1.0*	9.6±0.8**	5.2±1.1	9.0±0.6*	9.9±0.5**	5.4±1.2	8.5±0.7*	10.0±0.4**
Unsaturation index	1.2±0.1	1.4±0.1 n.s.	1.2±0.1 n.s.	1.2±0.1	1.2±0.1 n.s.	1.2±0.1 n.s.	1.0±0.1	0.9±0.1 n.s.	1.0±0.1 n.s.
Unsaturated index/ % saturated	2.9±0.5	3.7±0.5 n.s.	3.0±0.5 n.s.	3.2±0.3	2.9±0.5 n.s.	2.8±0.4 n.s.	2.2±0.4	2.0±0.3 n.s.	2.4±0.4 n.s.

Abbreviations are explained in the text and in the Tables 1, 2 and 3.

Table 6—Percent long chain fatty acids composition of total lipids, polar lipids and neutral lipids in heart microsomes of pigs fed diets containing Toprina

Higher fatty acids	TL			Pol			NL		
	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.	T0%±s.e.	T15%±s.e.	T30%±s.e.
A	3.7±0.8	4.1±1.0	5.3±1.2	3.6±1.0	5.5±2.0	3.4±1.5	7.1±1.1	7.1±1.2	8.7±1.7
15:0	0.6±0.1	0.9±0.1	1.3±0.4	0.9±0.4	0.9±0.3	0.8±0.2	1.2±0.3	0.7±0.1	1.4±0.2
15:1+16:0br	2.6±0.4	2.9±0.5	2.1±0.1	2.9±0.5	2.3±0.3	2.0±0.6	1.7±0.5	1.2±0.2	1.2±0.2
16:0	19.1±2.3	18.0±1.1	17.2±1.2	16.3±1.4	17.1±1.2	14.9±1.8	20.8±0.5	20.9±1.4	18.2±1.3
16:1+17:0br	2.3±0.6	2.6±0.5	1.6±0.1	1.6±0.4	2.6±0.9	1.3±0.4	4.6±0.3	4.2±1.2	3.0±0.2
17:0	0.5±0.1	1.0±0.1	1.2±0.2	0.4±0.1	0.9±0.2	0.8±0.2	0.7±0.1	0.8±0.2	1.0±0.2
17:1+18:0br	1.9±0.2	2.0±0.3	2.5±0.4	2.2±0.1	2.0±0.6	2.6±0.5	1.9±0.4	1.9±0.3	2.9±0.3
18:0	16.4±0.7	14.7±1.5	16.9±0.4	14.5±1.4	13.7±1.2	16.2±0.8	15.1±1.3	15.6±1.3	15.3±1.2
17:2	–	0.5±0.0	1.1±0.0	–	0.4±0.0	1.2±0.0	–	0.4±0.0	0.4±0.0
18:1	15.6±0.9	12.5±0.6	12.7±1.0	14.8±1.7	14.2±1.0	14.9±0.4	19.0±1.8	18.8±3.7	16.6±1.8
18:2	17.4±2.9	16.4±2.3	15.3±2.6	18.6±2.2	15.7±3.4	19.8±3.1	10.3±1.6	12.0±1.7	14.0±1.3
19:1+20:0br	1.0±0.3	1.8±0.4	2.2±0.7	1.5±0.2	1.1±0.3	0.9±0.4	0.7±0.2	1.1±0.4	2.4±0.5
18:3+19:2+20:1	0.5±0.0	0.7±0.2	0.8±0.1	0.3±0.1	1.0±0.3	0.7±0.2	0.4±0.2	0.6±0.2	0.6±0.2
18:3br	Traces	Traces	0.2±0.0	0.6±0.2	0.5±0.3	0.2±0.1	0.7±0.2	0.2±0.1	0.6±0.1
21:0	0.7±0.1	0.7±0.2	0.6±0.2	0.2±0.1	0.8±0.4	0.9±0.1	1.0±0.2	1.1±0.4	0.8±0.3
21:1+22:0br	1.2±0.4	1.1±0.5	0.7±0.2	0.7±0.2	0.8±0.3	2.2±0.5	0.4±0.1	1.0±0.4	1.5±0.6
20:2	0.3±0.1	1.0±0.2	0.5±0.1	0.4±0.2	0.5±0.2	0.5±0.2	0.6±0.2	0.6±0.2	0.6±0.1
20:4	10.3±1.8	6.9±1.4	7.7±1.0	8.2±1.2	6.8±1.4	8.9±1.7	2.7±0.3	3.3±0.6	3.6±0.4
22:1+23:0br	0.4±0.1	0.2±0.1	0.3±0.1	0.6±0.2	Traces	0.3±0.0	0.4±0.1	0.5±0.1	0.6±0.1
23:0	0.7±0.2	0.5±0.2	0.7±0.3	0.8±0.2	1.2±0.5	0.7±0.2	1.1±0.3	0.8±0.2	1.2±0.3
24:0	1.3±0.1	1.5±0.3	1.1±0.2	2.0±0.2	1.5±0.6	1.2±0.3	2.4±0.7	1.2±0.8	1.4±0.6
22:4	0.6±0.1	1.5±0.1	Traces	0.2±0.1	1.3±0.6	0.9±0.4	Traces	Traces	Traces
22:5	0.5±0.1	1.8±1.0	0.8±0.2	0.7±0.4	1.4±0.6	0.6±0.1	Traces	0.7±0.3	3.2±1.9
22:6	1.4±0.4	1.5±0.2	2.7±0.5	0.9±0.2	0.9±0.3	1.3±0.3	2.0±0.5	2.6±0.5	1.9±0.3
20:0+22:0	0.7±0.1	2.5±1.0	0.6±0.1	0.7±0.3	1.1±0.6	0.9±0.3	0.9±0.4	0.8±0.2	1.3±0.4
Total odd chain	5.3±1.0	7.3±0.7 n.s.	12.3±1.5**	4.6±0.2	6.2±0.3**	10.8±0.8**	7.2±0.5	8.9±0.6*	11.5±0.6**
Unsaturation index	1.1±0.1	1.0±0.2 n.s.	1.0±0.1 n.s.	1.0±0.1	1.0±0.1 n.s.	1.1±0.1 n.s.	0.7±0.1	0.8±0.1 n.s.	0.8±0.1 n.s.
Unsaturated index/ % saturated	2.4±0.4	2.1±0.2 n.s.	2.0±0.2 n.s.	2.1±0.2	2.1±0.2 n.s.	2.4±0.4 n.s.	1.3±0.1	1.6±0.1 n.s.	1.7±0.2 n.s.

Abbreviations are expalined in the text and in the tables 1, 2 and 3.

Table 7—Fluorescence polarization of perylene in liver and heart mitochondria and microsomes of pigs fed T0%, T15%, and T30%

Liver	T0%	T15%	T30%
mitochondria	0.061 ± 0.002	0.067 ± 0.031 n.s.[a]	0.070 ± 0.002 n.s.
microsomes	0.074 ± 0.004	0.072 ± 0.002 n.s.	0.070 ± 0.002 n.s.
Heart			
mitochondria	0.079 ± 0.009	0.089 ± 0.006 n.s.	0.084 ± 0.009 n.s.
microsomes	0.100 ± 0.006	0.094 ± 0.004 n.s.	0.094 ± 0.004 n.s.

[a] n.s. = comparison with T0% not significant $p < 0.01$
Other abbreviations are explained in the text and in Table 1.

Table 8—Mg^{++} ATPase activity in liver and heart mitochondria of pigs fed T0%, T15%, and T30%

	T0%	T15%	T30%
	ATPase activity (μmoles $Pi \cdot min^{-1} \cdot mg^{-1}$)		
Liver	0.579±0.099	0.393±0.040n.s.	0.549±0.122n.s.
Heart	0.162±0.032	0.164±0.029n.s.	0.150±0.030n.s.

Abbreviations are explained in the text and in Tables 1 and 7.

tained content of odd-chain fatty acids from Toprina, nor the supposed presence of n-paraffines in mitochondrial and microsomal membranes, induce significant changes of enzyme tests (mitochondrial ATPase) and physical state of lipids.

Such conclusions must, however, be referred to our experimental conditions where the Toprina contents of diets did not largely exceed those recommended for zootechnical practice.

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Chemical and Physical Changes in Red Hake Blocks During Frozen Storage

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ABSTRACT

Storage stability of frozen red hake blocks over a 6-month period was studied under commercial conditions with processing modifications. The rate of DMA formation was significantly greater in samples dipped in polyphosphate and erythorbate compared to a reference (−90°C) or to a 5-day aged sample. There were no significant differences in the rate of decrease of extractable protein nitrogen over the 24-week period. A trained sensory panel found samples from aged fish to be generally more acceptable from a flavor and texture stand-point than the other treated samples. Mechanical shear forces tended to be higher for samples dipped in polyphosphate-erythorbate and lowest for 5-day samples among treated fillets. With proper handling red hake fillets may make a satisfactory commercial frozen block.

INTRODUCTION

IN 1979, the estimated sustainable maximum yield of red hake (*Urophycis chuss*) was 70,400,000 lb/year while the actual catch for that year was only 7,040,000 lb. The average catch for the previous five years, 1974-78, was even lower being 3,873,000 lb/year (Anon, 1980). This species, which has a mild flavor and slightly soft texture, represents a minor component of the U.S. fresh fish market. A possibility for the expansion of the red hake fishery would be the development of frozen fish blocks prepared from this species. The major impediment to the development of this industry is the occurrence upon storage of a tough rubbery texture in the flesh of the red hake (Gendron, 1980). It is generally recognized that the development of these changes in texture is due to the conversion of trimethylamine oxide (TMAO) in the frozen flesh to dimethylamine (DMA) and formaldehyde, with the latter serving as a cross-linking agent for the protein (Amano and Yamada, 1964; Yamada et al., 1969; Castell et al., 1971; Tokunaga, 1974; Dyer and Hiltz, 1974; Dingle and Hines, 1975; Castell et al., 1973a, b; Dingle et al., 1977; Gill et al., 1979; Crawford et al., 1979). This conversion with its subsequent textural changes was typical of gadoid fishes; this reaction was most pronounced in red hake (Castell et al., 1971). Destruction of the tissue by mincing leads to increased production of DMA and formaldehyde (Hiltz et al., 1976; Crawford et al., 1979). The maximal rate of conversion of TMAO to DMA and formaldehyde has been reported to occur at approximately −5 to −10°C. The extent of the reaction has been followed by measurement of DMA, formaldehyde, or loss in extractable protein nitrogen (Dingle et al., 1977); Gill and associates (1979) have reported that DMA was a better indicator of toughness than extractable protein nitrogen. Red muscle has been reported to contain higher amounts of enzymes converting TMAO to DMA and formaldehyde (Tokunaga, 1970; Dyer and Hiltz, 1974; Castell et al., 1973a) although Dyer and Hiltz did not find that removing red muscle improved the storage life of frozen stored fillets significantly.

Although the reaction of formaldehyde with proteins is most likely not the only factor involved in the toughening of gadoid fish texture with storage (Gill et al., 1979), nevertheless with certain species it appears to be of primary importance. The purpose of this study was to prepare frozen fish blocks from red hake fillets under commercial conditions varying the processing procedures with the hope of extending storage life in the frozen state. The changes in the frozen state were followed by chemical, physical and sensory evaluations.

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MATERIALS & METHODS

Preparation of samples

Red hake, caught the same day was purchased as whole fish at the docks in April 1979 and transported on ice to the National Marine Fisheries Services Gloucester Laboratory. A portion of the whole fish was processed the next day, and the rest was aged on ice for an additional 4 days in a refrigerator operating at 2–4°C. Processing was carried out as follows.

The fish were hand filleted, and fillets were skinned on a Jensen Model B skinning machine set by judgment at a setting which would maximize yield. One portion of the fillets from day-old fish was saberized or deep skinned to remove red muscle tissue. A portion of regular skinned fillets from day-old fish was dipped in a solution of 3% sodium erythorbate and 3% sodium tripolyphosphate prior to freezing.

Skinned fillets were packed into waxed boxes and frozen in 2.25 kg blocks in a Maerio #12 plate freezer exerting 600 PSI pressure and operating at −40°C. Boxes of frozen fillets were then placed in polyethylene bags and stored either at −90°C or −18°C.

Whole fish held an additional 4 days on ice were treated in a similar manner except they were all given the regular skinning and were not dipped. Table 1 summarizes the treatment of the various samples and indicates the terms used to identify each of the treatments throughout this paper.

Production of fish sticks

Beginning on day one of the study and at monthly intervals thereafter for 6 months, frozen blocks were taken from storage and

Table 1—Treatment of samples tested and terms used to identify each

Sample #	Days on ice	Skinning	Storage temp[a]	Identification
1	1	Regular	−90°C	Reference
2	1	Regular	−18°C	1-day
3	1	Regular	−18°C	Dipped
4	1	Saberized	−18°C	Saberized
5	5	Regular	−18°C	5-day

[a] Note: Samples 2, 3 and 4 were held at −90°C for 4 days following preparation. They were then transferred to storage at −18°C.

processed into fish sticks using standard commercial methods. Frozen blocks were cut on a bandsaw into sticks measuring 7.6 cm x 1.3 cm x 2.2 cm. The sticks were immediately dipped into a batter prepared by mixing 0.45 kg of commercial batter mix with 550 ml of deionized distilled water. They were then rolled in a quick browning commercial breading mix and deep fat fried for 45 sec in soybean oil held at 205°C. The browned fish sticks were then put on metal trays and placed in the plate freezer (without pressure) for 2 hr at −40°C, after which they were sealed in polyethylene bags and stored at −90°C. The following day they were placed in an ice chest and transported to the Amherst campus of the University of Massachusetts for shear measurements and sensory analysis.

The breading and battering of the sticks were periodically monitored so as to produce a high quality fish stick consisting of approximately 65% fish and 35% batter and breading.

Sensory evaluation and objective shear measurements

Fish sticks for sensory evaluation and shear measurement were placed frozen on an aluminum cookie sheet and cooked for 18 min at 205°C in a conventional air oven. They were kept warm in preheated, covered, ceramic dishes held under warming lights.

The sensory panel consisted of eight graduate students who participated in several training sessions prior to the initiation of the experiment. All testing was done in a specially designed room equipped with individual booths, each with controllable lighting and temperature, and air filtration.

Each panelist was presented with five warm fish sticks; one identified as a reference stick (stored at −90°C) and four treated sticks assigned numbers from a table of random digits. After peeling away the batter and breading from a portion of each stick, they were required to compare differences in intensity of texture and flavor between each treated stick and the reference stick. Differences for each treated stick were recorded on a 9-point scale which ranged from 1 (none) to 9 (extreme). Since general consumer acceptability of the coated stick was of interest, panelists were also asked to sample a portion of each intack stick and indicate its acceptability from a flavor and texture standpoint.

Shear measurements were made on a Warner-Bratzler shearing device fitted with 500 kg dynomometer marked in 25g units. Each stick was sheared in half, perpendicular to its long axis; then each half thus produced was itself sheared in half. Three sticks per treatment, including reference sticks, were sheared.

The entire sensory and shearing regimen was conducted mid-morning and mid-afternoon on each test day to provide a replicate.

Chemical analyses

Preparation of TCA extract. In preparation for the dimethylamine (DMA), trimethylamine (TMA), trimethylamine oxide (TMAO) and formaldehyde analyses, 50g of each fish sample, after thawing, was homogenized for 90 sec in 50 ml of deionized, distilled water and 100 ml of 10% trichloroacetic acid (TCA). This extract was filtered and aliquots were used for the aforementioned analyses.

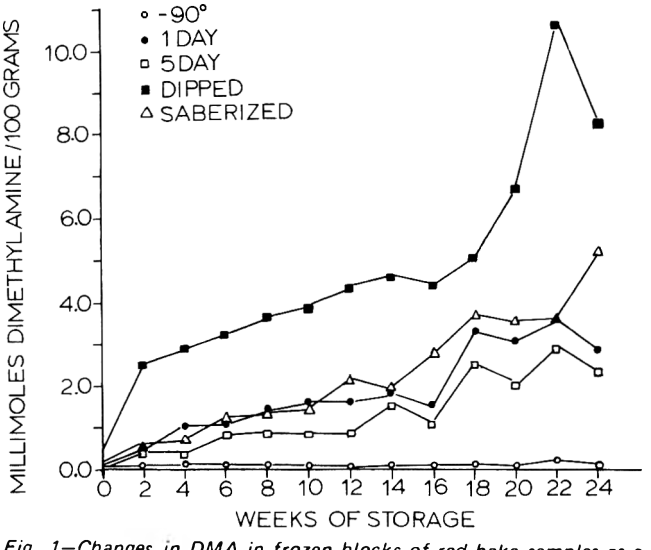


Fig. 1—Changes in DMA in frozen blocks of red hake samples as a function of time at −18° C. The manner of treatment of each sample is discussed in the text.

Dimethylamine. DMA was analyzed according to the method of Dyer and Mounsey (1945) with the exception that the aqueous layer was partitioned with 12 ml of 5% carbon disulfide in benzene. Shaking was accomplished with a Cole-Palmer Roto-Torque Model 7637 using the highest speed setting available.

Trimethylamine. TMA in the TCA extracts was analyzed according to the procedure of Dyer (1945), replacing 50% K_2CO_3 with 45% KOH as suggested by Shewan et al. (1971).

Trimethylamine oxide. TMAO in the TCA extract was determined by the method of Yamagata et al. (1969), using a 60°C water bath to achieve reduction of TMAO to TMA, rather than 80°C. The reduced extract was then subjected to the TMA procedure outlined above.

Free formaldehyde. Free HCHO in the TCA extract was determined by the procedure of Nash (1953).

Bound formaldehyde. Bound formaldehyde was calculated as the difference between values for DMA and free formaldehyde, based on the assumption that DMA and HCHO are produced in a 1:1 stoichiometry from TMAO.

% extractable protein nitrogen (expressed as % of reference). A protein extract was prepared according to the method of Dyer et al. (1950) and protein content in the extract was determined by the biuret procedure of Gornall et al. (1949).

2-thiobarbituric acid (TBA)-reactive substances. TBA-reactive substances was determined according to the procedure of Vyncke (1970).

Tissue pH. Tissue pH measurements were taken from a blend of fish: distilled water (1:5), homogenized for 45 sec.

Lipid. Fifty grams of finely chopped fish were homogenized for 2 min in a Waring Blendor with 50g anhydrous sodium sulfate and 100 ml chloroform (Bligh and Dyer, 1959). A 20 ml aliquot of chloroform was transferred to a tared beaker and evaporated to dryness to determine the weight of the lipid.

Peroxide number. Peroxide number was determined on the isolated lipid by the procedure of Riemenschneider et al. (1943).

Other analyses (AOAC). The protein content of the fillets was determined by Kjeldahl. Moisture content of fillets was determined by drying overnight in an air oven at 100°C. Ash was determined on a 10g sample after treatment in a muffled furnace at 525°C for 5 hr. (All analyses were performed in duplicate.)

A factorial experimental design was used and data were analyzed by analysis of variance. Comaprisons among treatment means were made using Duncan's multiple-range test (Cochran and Cox, 1957).

RESULTS

Chemical changes

Red hake fillets contained 82.2% water, 16.5% protein, 0.73% lipid and 0.86% ash. Fillet yields for 1-day fish vs 5-day fish were essentially the same with percentage values of 38.7 and 40.0, respectively. Deep skinning yielded 77.5% meat expressed as percent of fillet before skinning, while regular skinning yielded 88.7% meat.

Changes observed among the five samples of frozen red hake blocks in some selected chemical parameters as a function of storage time are presented in Fig. 1–5. The rate of accumulation of DMA (Fig. 1) is significantly different at the $P < 0.01$ level for the reference versus the sample dipped in polyphosphate and erythorbate, and significantly different at the $P < 0.05$ level for the 5-day sample versus the sample that was dipped in the polyphosphate-erythorbate mixture. With the exception of the reference sample stored at −90° C, all samples showed a steady increase throughout most of the storage period.

The change in extractable protein nitrogen with time is given in Fig. 2. Since the amount of EPN extracted from the reference samples varied somewhat, values were normalized. Therefore, the reference samples were omitted from the statistical analyses. There were no significant differences at either the 0.01 or 0.05 level over the 24 wk period; however, there was a significant difference at the $P < 0.05$ level between the 5-day old sample and the dipped sample when they were compared over the first 16 wk of storage. The 5-day old sample was not significantly different from the regular 1-day sample or the saberized sample.

As with DMA, the levels of free formaldehyde showed a general increase with storage time for all the samples with the exception of the reference at −90°C (Fig. 3). The reference and the sample dipped in erythorbate and sodium tripolyphosphate were significantly different at the $P < 0.05$ level of significance over the course of the experiment, and they were significantly different at the $P < 0.01$ level over the period of 16–24 wk. The reference and the 5-day sample developed significantly less free formaldehyde at the $P < 0.05$ level compared to the dipped sample over the first 16 wk. Fig. 4 represents bound formaldehyde which is the difference between DMA and free formaldehyde. Again there is a significant difference at the $P < 0.05$ level for the reference versus the sample that was dipped in the sodium tripolyphosphate-erthorbate mixture.

The results with TMA are shown in Fig. 5. There was a significant difference (at the $P < 0.01$ level) only between the reference and the sample dipped in the polyphosphate-erythorbate solution. There was no significant difference between either the reference or dipped samples and the others (1-day, 5-day, or saberized). The data indicated that there was a small conversion of TMAO to TMA even in samples in the frozen state (except for the reference). Whether this conversion was brought about by nonenzymic processes, by native tissue enzymes, or by enzymes which were elicited by microorganisms before the freezing process, is not known.

Sensory evaluation

Differences in intensity of flavor perceived by the sensory panel between treated samples and the reference sample stored at −90°C are shown in Fig. 6. With the exception of one testing period (16 wk) panelists found the greatest flavor differences to exist between the dipped samples and the reference. Comparisons were made among treatment means using Duncan's multiple-range test, and the differences observed by the panel between dipped and reference samples proved to be statistically significant ($P < 0.05$). Although there were not statistically significant differences between the other samples tested and the reference, 5-day samples tended to have low flavor difference scores. Several panelists described dipped samples as having a salty taste and occasionally commented that the flesh in the fish sticks had a yellowish color associated with it. In terms of overall acceptability of flavor, the largest number of rejections was associated with the dipped

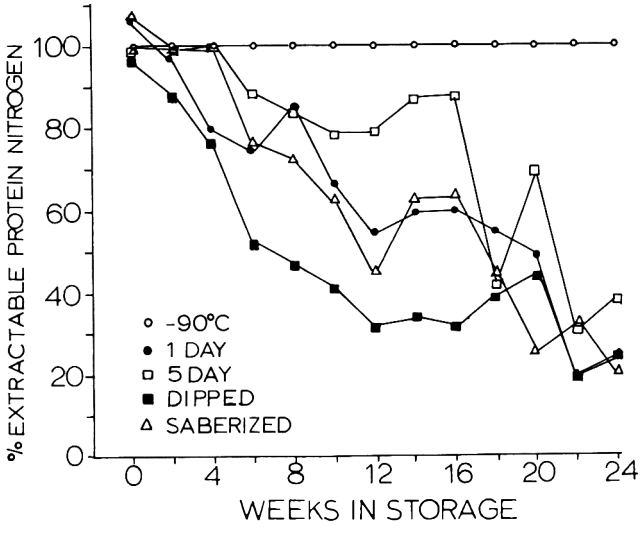


Fig. 2—Changes in extractable protein nitrogen in frozen blocks of red hake samples as a function of time at −18°C. The manner of treatment of each sample is discussed in the text.

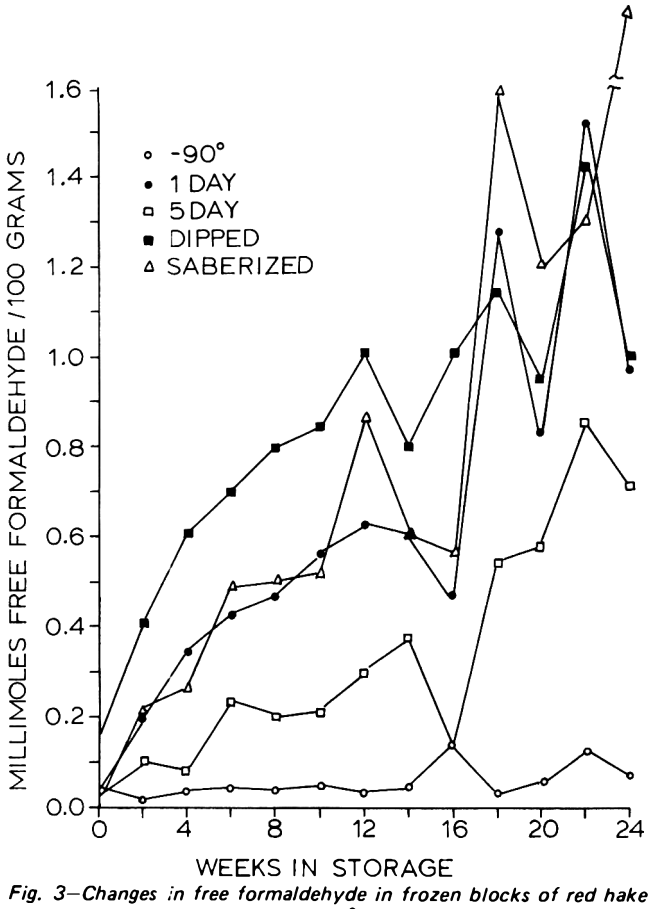


Fig. 3—Changes in free formaldehyde in frozen blocks of red hake samples as a function of time at −18°C. The manner of treatment of each samples is discussed in the text.

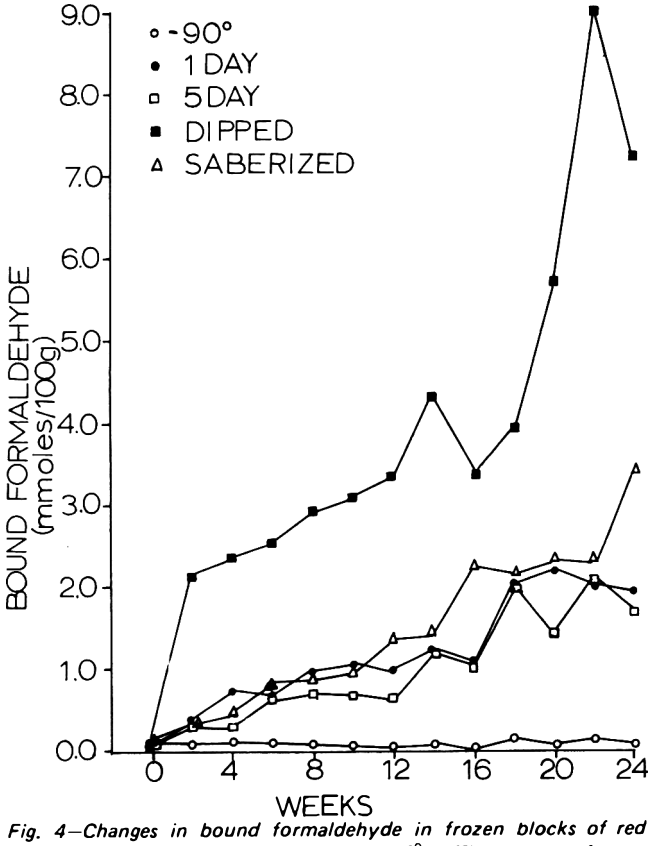


Fig. 4—Changes in bound formaldehyde in frozen blocks of red hake samples as a function of time at −18°C. The manner of treatment of each sample is discussed in the text. Bound formaldehyde was calculated as the difference in molar concentrations of DMA and free formaldehyde.

samples while the smallest number was associated with the 5-day samples. While one cannot predict general consumer acceptance from a small number of observations, the data suggests that the 5-day samples were quite similar to reference samples and both were considered to be of consistently high quality throughout the study by the trained panel.

The intensity of textural differences observed by sensory panelists between four treated samples and the reference sample are shown in Fig. 7. Panelists found less difference in texture between 5-day samples and the reference sample at all storage times except 8 wk. Overall, the greater similarity of 5-day samples to reference samples compared to the other three samples and the reference proved to be statistically significant ($P < 0.05$). Saberized, 1-day and dipped samples were approximately similar in texture throughout the study. The vast majority of panelists found the texture of 5-day sticks to be acceptable, and there were relatively few rejections compared to the other three treated samples.

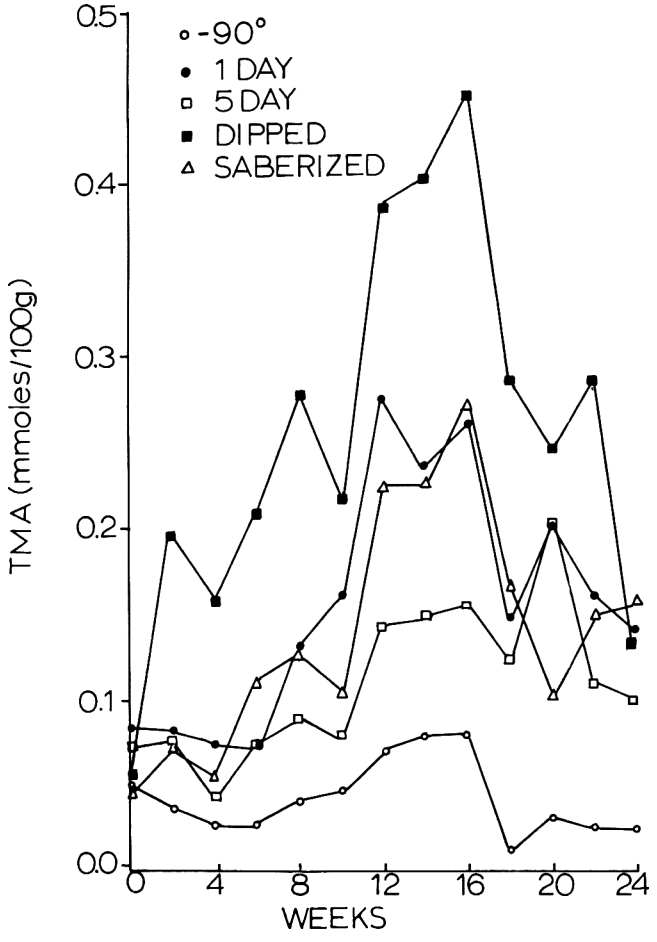


Fig. 5—Changes in trimethylamine in frozen blocks of red hake samples as a function of time at −18° C. The manner of treatment of each sample is discussed in the text.

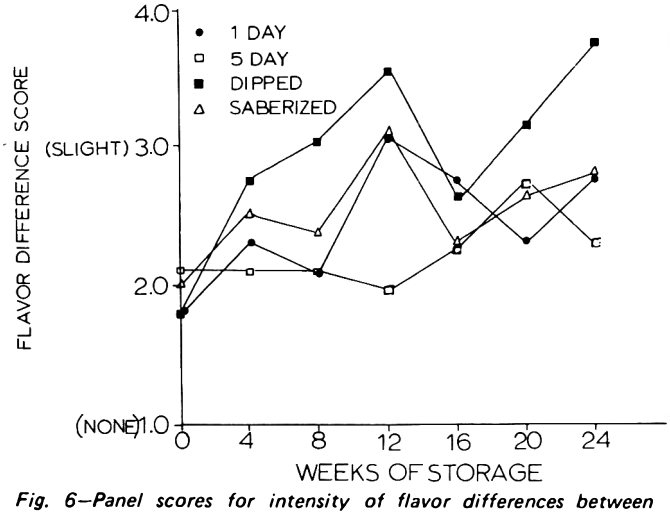


Fig. 6—Panel scores for intensity of flavor differences between treated and reference samples. The manner of treatment of each sample is discussed in the text.

Sensory evaluation by consumer-type panel

During the course of the sensory evaluation of the fish sticks prepared from the red hake samples, the general impression obtained from the expert panel was that the quality of the red hake fish sticks was high. Therefore, we carried out a consumer-type study on fish sticks prepared from red hake which were comparable in handling to the 1-day samples. The panelsist were obtained from the general population of students and staff at the University of Massachusetts in Amherst and had no previous experience in testing of fish sticks. They were simply asked to respond as to how they liked the fish sticks (including the batter) and whether they found the texture and flavor acceptable or unacceptable. Results of evaluations after storage at 0°F for 6½ months and 8½ months are shown in Table 2. After the storage period of 6½ months, 91% of the panelists liked the fish sticks moderately or better. This percentage decreased only slightly to 87% over the next 2 months of storage. Six percent of the panelists found the texture unacceptable and 5% found the flavor unacceptable after 6½ month storage; these figures remained essentially constant at 5% and 4%, respectively, after 8½ months storage.

Shear measurements

Mean shear values by treatments and time of storage are shown in Fig. 8. It is interesting to note that the sharpest increase in shear values for all samples occurred after 1

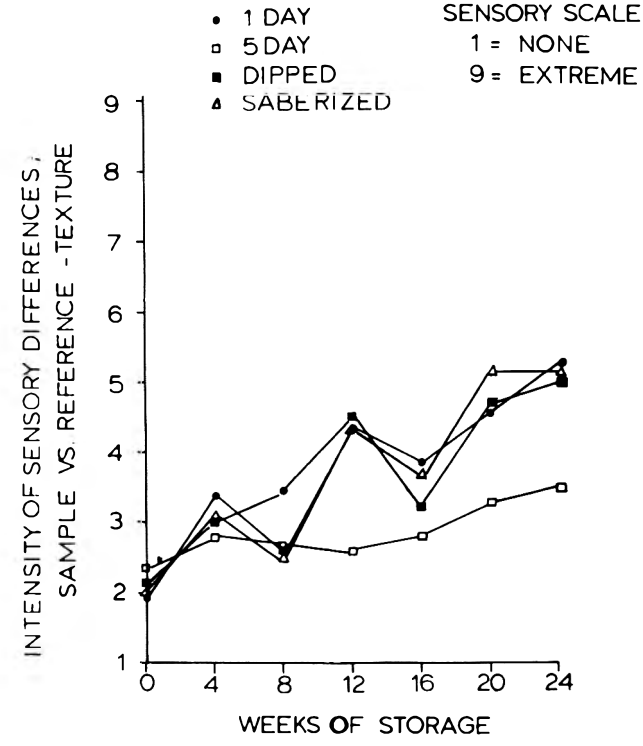


Fig. 7—Textural differences between treated and reference samples as determined by sensory panel. The manner of treatment of each samples is discussed in the text.

month's storage. Shear values for the reference samples held at −90°C were consistently low throughout the study indicating the effectiveness of extremely low temperature for preserving red hake in a condition most closely resembling the fresh state. At the end of the storage period, 5-day samples had the lowest shear values of treated samples, 0.76 kg, while the dipped samples had the highest values at 1.2 kg. Treatment means were compared by means of Duncan's multiple-range test. There were no significant differences among 1-day, 5-day, and saberized samples; however, dipped and reference samples differed significantly from all other samples and each other ($P < 0.05$). Obviously, from the standpoint of shear strength, the dipping process produced a tougher product than all other treatments.

DISCUSSION

CORRELATIONS between several of the variables examined in this study are shown in Table 3. A large number of variables were shown to be related. This, most likely, is indicative of the fact that changes in variables like DMA, EPN, and formaldehyde reflect chemical deterioration with time and that with time, flavor deteriorates and the texture becomes tougher. No cause-effect relationship should be assumed based on the correlations. These relationships may be useful in prediciting certain changes occurring in stored, frozen red hake.

In theory, the most important chemical parameter should be bound formaldehyde. This is determined as the difference between the DMA, which is assumed to be equivalent to the total amount of formaldehyde formed, and the free formaldehyde. The percentage of bound formaldehyde does not appear to vary greatly among the various samples examined. In actual fact, the amount of DMA appeared to be somewhat more useful as a measure of deterioration, at least from the point of view that the 5-day old sample was significantly different from the dipped samples in DMA content which did not show up in content of bound formaldehyde. This was probably due to the greater sensitivity of the tests for DMA than for formaldehyde and the fact that there was some variation among samples (about ± 20%) in percentage of bound formaldehyde. This would introduce greater variability into the bound formaldehyde results and make it less predictive as a measure of tissue deterioration.

Red hake is a lean fish with a total lipid content less than 1%. There was no evidence of rancidity as indicated by sensory response, nor were there any detectable values of peroxide numbers or thiobarbituric acid-reactive substances over the time period of storage. Textural changes appeared to be far more important with stored blocks of red hake than flavor changes.

Saberization was done to remove dark muscle tissue from the fillets. This process is often carried out to minimize lipid oxidation in tissue. However, there seemed to be no problem of lipid oxidation with normally skinned fish. It has been reported that the dark muscle tissue of fish also contains more active systems for the conversion of trimethylamine oxide to dimethylamine and formaldehyde

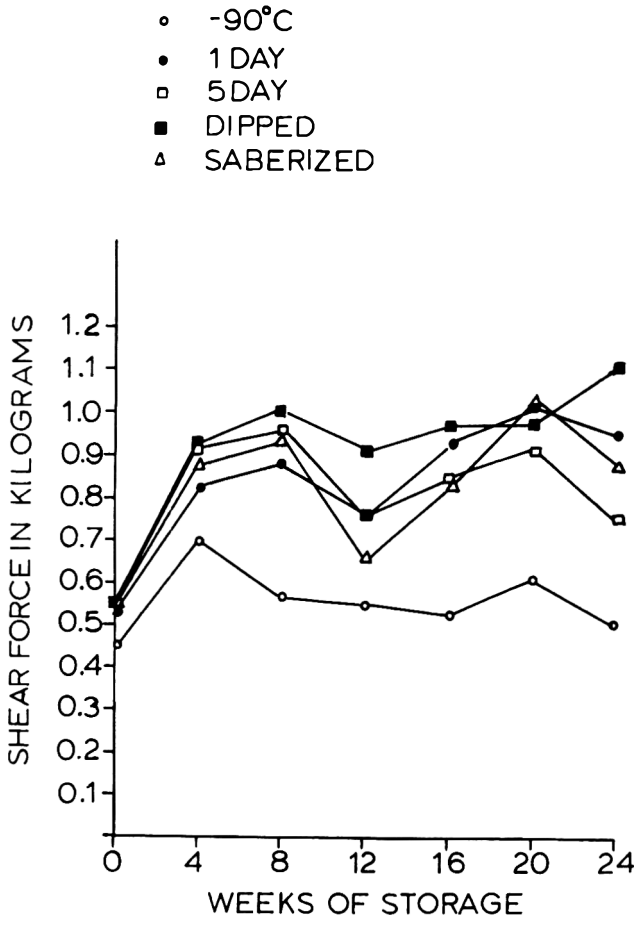


Fig. 8—Force in kilograms required to shear treated and reference samples. The manner of treatment of each sample is discussed in the text.

Table 2—Evaluation of fish sticks prepared from red hake blocks stored at −18°C (0°F)

	6½ month storage[a]		8½ month storage[b]	
	No. of Respondents	%	No. of Respondents	%
Like extremely	8	7	14	15
Like very much	49	43	43	45
Like moderately	46	41	26	27
Like slightly	7	6	8	8
Neither like nor dislike	0	0	2	2
Dislike slightly	2	2	1	1
Dislike moderately	1	1	0	0
Dislike very much	0	0	1	1
Dislike extremely	0	0	0	0

[a] In the samples prepared from blocks stored for 6½ months, 106 found the texture acceptable, 7 found it unacceptable; 106 found the flavor acceptable, 6 found it unacceptable.
[b] In the samples prepared from blocks stored for 8½ months, 89 found the texture acceptable, 5 found it unacceptable; 91 found the flavor acceptable, 4 found it unacceptable.

Table 3—Correlations between various chemical, physical, and sensory characteristics of red hake[a]

Characteristic measured	Correlation coefficient
TMA vs Flavor score	0.98*
DMA vs Flavor score	0.99*
DMA vs Shear value	0.98*
EPN vs Shear value	0.98*
Texture score vs Free HCHO	0.96**
Bound HCHO vs Flavor score	0.98**
EPN vs Flavor score	0.98**
TMA vs Shear value	0.96**
Bound HCHO vs Shear value	0.96**
Bound HCHO vs TMA	0.97**
DMA vs TMA	0.98**
EPN vs TMA	0.95**
DMA vs EPN	0.94**
Bound HCHO vs EPN	0.90**
Free HCHO vs EPN	0.92**

[a] Values for reference samples were not included in the calculations.
* $P < 0.01$
** $P < 0.05$

(Tokunaga, 1970; Dyer and Hiltz, 1974; Castell et al., 1973). However, no improvement was observed by any of our criteria for the saberized sample compared to the sample treated the same in all other respects. i.e., the 1-day old sample. Since approxiamtely 10% of the fillet is lost by the saberization procedure, there is no good reason to follow this procedure in the case of red hake used in frozen blocks.

Aging of samples for 4 extra days on ice prior to processing into frozen blocks produced a superior product as judged by chemical tests, i.e., DMA and free formaldehyde, and sensory textural properties. In several other parameters, the changes observed in the 5-day old sample, although not statistically significant, showed a trend towards being superior to the other treated samples (with the exception of the control stored at −90°C). The reason for this is not known. The level of TMAO at the beginning of the experiment had not yet decreased significantly, nor was the initial level of TMA increased above that of the other samples so one cannot ascribe this difference to a depletion of substrate. There could be, of course, other factors involved. The concentrations of the cofactors required for the breakdown of TMAO could change during the storage period. Another possibility could be that proteolytic enzymes would have time to act. The hydrolysis of the proteins could prevent the toughening which occurs in frozen storage. This seems the most likely explanation at the present time. The 5-day old sample was treated differently from the other four in one other respect; all of the other samples were held for an extra 4 days at −90°C after having been processed into frozen blocks. The aged sample did not undergo this process. This seems, however, a much less likely explanation of the difference.

Dipping the skinned fillets into a 3% solution of sodium tripolyphosphate and sodium erythorbate led to a significant deterioration of the sample with respect to chemical, physical, and sensory deterioration. The pH of the flesh that had been dipped in this solution was significantly higher compared to the other samples, approximately 7.9 vs 7.0. We have shown, however, that pH 7.0 is the optimum pH for the in situ production of dimethylamine and formaldehyde in frozen red hake flesh (unpublished observation). Sodium tripolyphosphate when added to tissue has shown only a negligible effect on the production of DMA. The most likely explanation for the effect of the dipping process is the presence of the erythorbate. Wh have shown more recently (unpublished data) that when erythorbate is added to the fish flesh the production of DMA is increased. Thus the most likely explanation for the observed results in these experiments was the presence of the erythorbate. The erythorbate and sodium tripolyphosphate were used as a dip in these experiments to reduce the potential for lipid oxidation. It is now clear that lipid oxidation is not a significant problem in the storage of this fish tissue; thus, there is no reason to use this dip process in the preparation of frozen blocks of red hake.

Earlier work (Dyer and Hiltz, 1974; Dingle et al., 1977) predicted the shelf-life of red hake to be much shorter than what we found in this study. The reason for this appears to be due to a large and nonlinear increase in the rate of TMAO breakdown with increasing temperature of frozen storage which is particularly marked at temperatures above −12°C (10°F) (Licciardello et al., 1981). Gill et al. (1979) did examine the breakdown of TMAO in frozen red hake at −17°C (+1.4°F) but carried out their studies for a relatively short time (40 days). The rate of deterioration of frozen blocks under good commercial storage conditions with proper temperature control is not as rapid as was once feared and with proper handling, red hake fillets may be satisfactory for commercial block production.

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Water-Thawing of Fish Using Low Frequency Acoustics

A. D. KISSAM, R. W. NELSON, J. NGAO, and P. HUNTER

ABSTRACT

Blocks of Pacific cod (plate frozen; 91-mm thickness and 12.7 kg) were thawed in an 18°C circulated water bath and simultaneously exposed to 1500 Hz acoustic energy not exceeding 60 watts. A ceramic transducer was positioned for light contact with a frozen block. A motorized belt moved the block in order to distribute the 239 cm^2 transducer over the 1500 cm^2 block surface. At 60 watts continuous input to the transducer, the block thawed in 71% less time than water-only controls. Quality analyses indicated that the flesh was not adversely affected by the acoustic waves.

INTRODUCTION

THE DEVELOPMENT of rapid freezing and thawing technology has progressed at remarkably different rates of innovation. Work on fish freezing systems has been underway since 1842, while thawing-rate improvement methods received little attention until the 1950's (Burgess et al., 1975). For the seafood industry, the development of large vessels having on board bulk freezing and storage capability has prompted increased interest in bulk thawing methods. To process large quantities of frozen fish, the shore-based industry must have the capability to thaw at rates matching the plant throughput. While quality is a major concern, the method used for thawing must also be conservative in size and operating cost.

High frequency (HF) or ultrasonic wave energy may be utilized to generate the required heat inside of the material through energy absorption. The negative aspects of poor penetration, localized heating, and high power requirements result in rejection of HF or ultrasonic absorption methods for thawing of bulk frozen fish (Brody and Antonevich, 1959; Waterman, 1963).

Early studies of ultrasonic absorption mechanisms showed inconsistencies in the classical mechanism. Absorption rates several times greater than predicted were observed at frequencies specific to a given material. Such absorption peaks were termed relaxation mechanisms and were associated with the physical properties of the material. Work on relaxation mechanisms showed that the physical properties of materials can be studied using ultrasonic measurements. The results of such measurements of the physical properties of the polycrystalline ice are the basis for this application of acoustic absorption to thawing of fish.

Theoretical aspects

Ice responds to frequencies in the low kilohertz range through a relaxational process. The period of relaxation is determined by the time lag between applied stress and resulting strain; hence the frequency of relaxation is determined by the crystal mechanical properties. In a polycrystalline sample, the crystals will be oriented randomly and exhibit differing rates of response to the stress/strain excitations of wave energy. The response will also be affected by impurities in the crystal resulting from natural frozen fluids in the flesh. Only a few electron-volts of energy need be applied to activate the relaxation mechanism in an ice crystal.

The relaxation response for polycrystalline ice varies with the temperature (Fig. 1). The relaxation frequency at food storage temperatures (above −30°C) would be greater than 790 Hz. This relaxation response has been described as the development of a quasi-viscous flow leading to melting at sub-normal temperatures (Fletcher, 1970).

The response to the wave energy, through molecular reorientation and stress/strain in the crystal, results in an upset in thermal equilibrium. Thermal currents between crystals develop, which increases the energy absorption. This is termed thermoelastic relaxation. The frequency has been approximated for polycrystalline material (Zener, 1940) using:

$$f_o = \frac{3\pi \bar{D}}{2d^2} \quad (1)$$

where f_o = thermoelastic relaxation frequency, Hz; D = thermal diffusity constant, m^2/s; and d = crystal grain size, m.

The approximate size of the extracellular ice crystals in post-rigor and slow-frozen cod and pollock ranges from 30μ to 60μ (μ, micron). This was determined by inspection of photographs (Love and Haraldsson, 1961; Tanaka, 1969).

The range of frequencies for the polycrystalline ice structure in frozen fish flesh is estimated using Eq. (1). The calculation is done for a cod muscle using a thermal diffusity at −10°C of 0.43 $(10^{-6})m^2/s$ (Jason, 1974). This calculation results in the range,

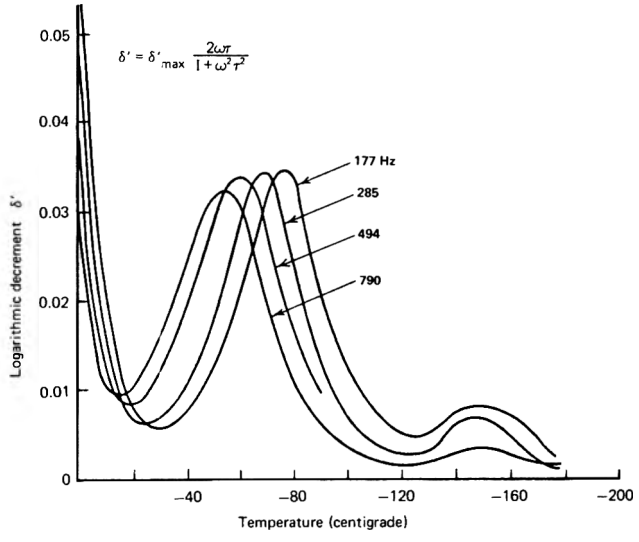


Fig. 1—The internal damping of sodium-chloride-doped polycrystalline ice samples for different resonant frequencies (Kuriowa, 1964).

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$$f_{30\mu} = 2251 \text{ Hz}$$
$$f_{60\mu} = 563 \text{ Hz}$$

Therefore excitation of the ice crystals at 1500 Hz would result in a variety of response levels. Mammalian flesh, vegetables, and fruits would all have a characteristic ice crystal size and possibly highly different frequency ranges. The range may extend from the low sonic to the ultrasonic.

Relaxation and thawing rate.

In thawing, the change in density requires a liquid mass transfer toward the solid region of the material. The liquid flow carries heat and the transfer rate will therefore affect the thawing rate. The effect of density change on the solidification rate of alloys has been mathematically modeled (Tien and Koump, 1970). A modification of the model for application to freezing foods (Bakal and Hayakawa, 1973) is shown in Eq (2):

$$\nu = \frac{\rho_2 - \rho_1}{\rho_2}\left[\frac{d\epsilon}{dt} + \frac{\chi - \epsilon}{2\Delta\epsilon}\frac{d(\Delta\epsilon)}{dt}\right] \quad (2)$$

where ν is the liquid flow velocity; ρ is density of the frozen (1) and unfrozen (2) regions; ϵ is the location of the frozen front from the surface; $\Delta\epsilon$ is the size of the phase change region; and χ is the variable distance from the surface.

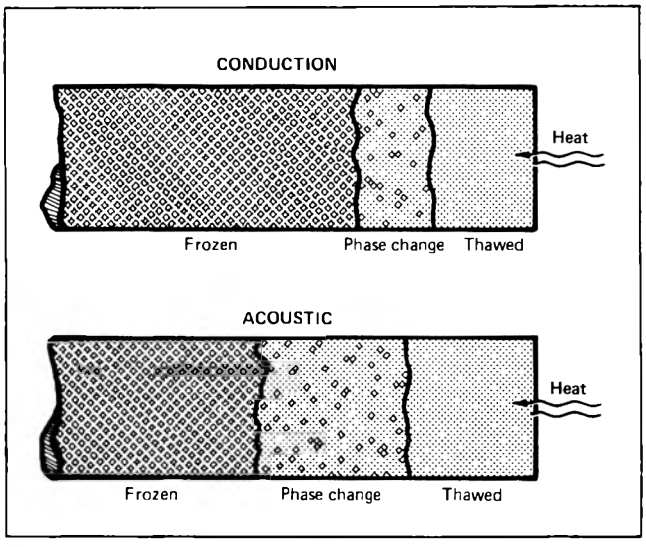


Fig. 2—Effect of ice relaxation frequency acoustic waves on the phase change region as compared to passive conduction heating.

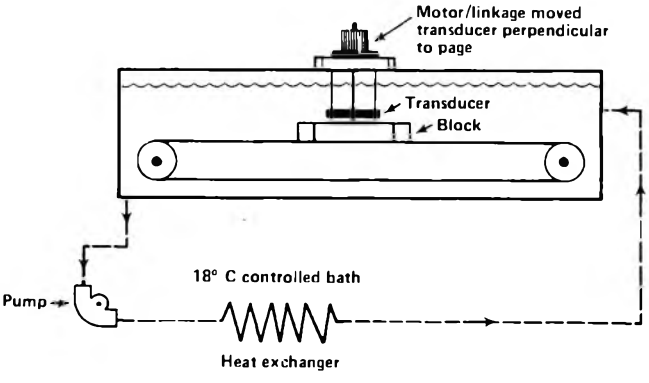


Fig. 3—Schematic of the experimental acoustic water-thawing system.

In Eq (2), the parameters affecting the liquid velocity are applicable to the reverse case of thawing. If derived for a thawing application, the mathematics deriving ϵ and $\Delta\epsilon$ would change. The basic relationships to liquid velocity driven by changing density would remain relevant.

The ice crystals at the frozen front (ϵ) rapidly approach phase transition when continuously exposed to relaxation frequency wave energy. Due to the large latent heat requirement, the phase change region ($\Delta\epsilon$) extends (Fig. 2). In comparison to a thawing process using only passive conduction heating, an acoustic-assisted process would have greater magnitudes for $d\epsilon/dt$ and $d(\Delta\epsilon)/dt$.

Acoustic extension of the phase change region requires that a greater mass of material simultaneously experience latent heating. Logically, such a situation increases the system demand for increased density and heat-energy laden moisture flow. If the magnitude of demand as a result of acoustic stimulation exceeds the hydrodynamic capacity of the material, the porosity would become a limiting factor in the thawing rate.

A one-dimensional Fourier heat conduction equation written for a multi-state system includes both heat flow by conduction and heat flow due to the flow of liquid (Tien and Koump, 1970). Conventionally, thawing rate increases with larger thermal gradients acting on the thermal conductivity of the material. Thermal gradient methods are limited by food quality considerations. They also ignore the heating contribution available by increasing the liquid transfer rate. The previously described process of ice crystal relaxation is a method available to influence the parameters controlling the liquid transfer rate.

EXPERIMENTAL SYSTEM

TESTS WERE CONDUCTED using blocks of headed-and-gutted Pacific cod. The blocks were formed in a plate freezer and weighed 12.7 ± 0.7 kg with a thickness of 91 ± 9 mm. Following freezing, a type T (c.c.) thermocouple was placed at the block center by drilling a small hole in the side of the block.

The thawing tank was 471 liters and continously mixed by discharge from the tank heat exchanger (Fig. 3). Temperature of the bath was 18°C. Water velocity over the submerged block was measured at 3.8 cm/s.

The transducer (Fig. 4) had a ceramic disc area of 239 cm^2 and its efficiency was estimated by the manufacturer at 50%. It was resonant at 1500 Hz and calibration data were provided to allow calculation of input power from the applied voltage. The transducer was driven by a function generator, audio amplifier, and a matching transformer. Input voltage was measured at the transducer leads.

The thawing tests were conducted with the block secured to the wire belt using two lengths of surgical tubing and the transducer positioned for light contact with the block. Motors moved the belt and transducer to distribute the 239 cm^2 transducer over the 1500 cm^2 block surface. The thawing time was measured at the center for the range of −29°C to −1°C. Power was applied to the transducer at 20 cW, 40 cW, and 60 cW (cW is continuous watts). Reduction in thawing time was referenced to control blocks thawed using only water. During the thawing test, a second thermocouple was inserted under the skin of a fish on the transducer contacted side. This second thermocouple measured any temperature difference in the surface flesh exposed to acoustics versus the control.

Immediately after thawing, fish from the transducer side of the 40 watt and 60 watt thawing tests were compared to the control for quality. Tests included salt-soluble protein extraction (Dyer et al., 1950), moisture and fat (AOAC, 1980), and water-holding capacity (Miyauchi, 1962). Sensory evaluation included triangle tests and quality attributes such as flavor, texture, and rancidity. The sensory test panelists were experienced in fish quality evaluations.

RESULTS & DISCUSSION

FIG. 5 AND 6 ILLUSTRATE the thawing rate improvements obtained using the 1500 Hz acoustic energy. These

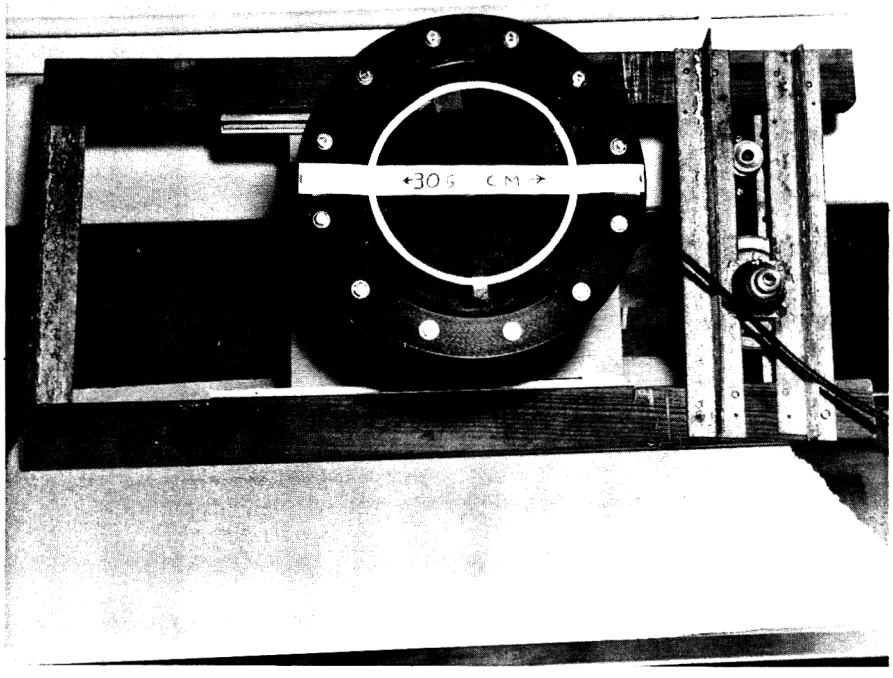


Fig. 4—The transducer measured 30.5 cm across and used a 239 cm^2 ceramic disc as indicated by the white circled region.

results represent the average of duplicate tests at each of the indicated transducer input power settings. The thawing times were consistent (± 2 min) with the average time.

The thermocouple placed under the skin of a fish contacted by the transducer did not measure any abnormal temperature in comparison to the controls. In no instance did the surface flesh reach the bath temperature of 18°C. The surface flesh temperature data indicates that the wave energy is not dispersed in the thawed flesh resions. Surface dispersion is a major drawback of HF absorption heating methods.

The impedance of the phase change region is distinctly different due to the nonuniform density and random location of ice crystals. Scattering and energy dispersion would result from such an interface between the frozen and thawed flesh regions. The internal friction resulting from thermal flow is not a function of the vibrational amplitude (Zener, 1940). Hence, the increases in transducer power required to maintain the relaxational process at the frozen front are a function of the size of the phase change region.

Using a 50% transducer efficiency, the output at the transducer face was 0.126 w/cm^2 for the 60 watt tests. If complete absorption is assumed, 38 watt-hours were delivered by the transducer to one surface of the fish block. Whole cod at −29°C requires 310 kJ/kg for thawing (Christie and Jason, 1975). An energy input requirement of 559 watt-hours for the half-block may be computed. Under optimum absorption conditions, the transducer has delivered 7% of the required thawing energy.

In a complete study of water-thawing cod blocks, circulation velocities over the block surface from 0.4 to 4.0 cm/s resulted in identical thawing rates (MacCallum and Ellis, 1964). The cyclic wave pressure of the acoustics in this experiment would not significantly improve surface heat transfer given the 3.8 cm/s flow over the block. The wave pressure is not contained by a rigid cell and would not force water between the carcasses. The carcasses were densely packed and were not separated during thawing.

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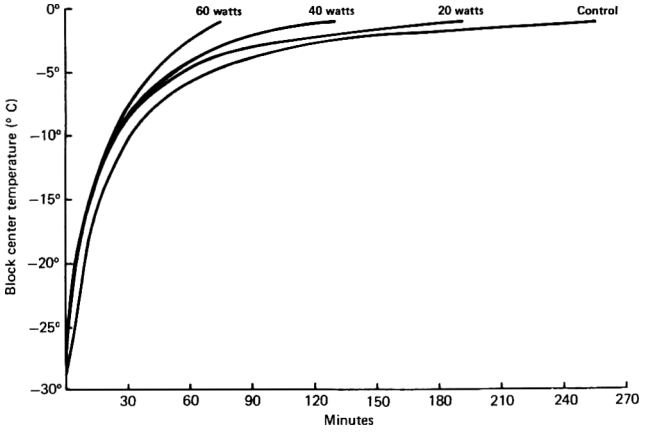


Fig. 5—Thawing rates obtained using acoustics at the indicated power levels versus no acoustic power (control).

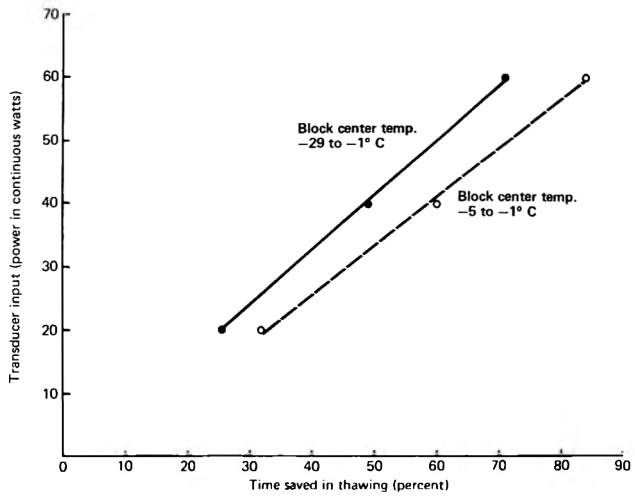


Fig. 6—Relationship of acoustic power to thawing time reduction.

Conduction heating processes will not deliver the energy required in the observed time unless the effects of relaxation and liquid transfer are considered. The control blocks thawed in this experiment required 255 min (−29°C to −1°C) using an 18°C water bath. A vacuum condensation process thawed 100 mm thick cod blocks in 290 min (−30°C to −1°C) using an 18°C vapor (Christie and Jason, 1975).

Sensory and quality analyses

The results of the quality analyses are presented in Tables 1–3. Fish obtained for this study were obtained from different locations over a 3-month period. Run 1 fish were larger with an average weight of 1600g as compared to runs 2 and 3 with an average weight of 980g. The fish were received headed and gutted, so it was difficult to tell if the fish had recently spawned.

In the triangle test, coded samples of two water-thawed and one acoustic-thawed, the correct response was not significant at the 5% level (Roessler et al., 1978). The panelists were not asked to provide an evaluation of sensory attributes for the triangle test samples.

The sensory attributes comparison of water-thawed, acoustic-thawed, and fresh cod samples were subjected to analysis of variance (Sokal and Rohlf, 1969). The objective of the variance analysis was to determine if there was any detectable difference based on a specific attribute. It was observed that there was no statistical difference at any level.

The chemical comparison of acoustic versus water-thawed flesh (Table 3) was consistent within a thawing test run. Variation between runs may be due to the fish size and spawning condition previously discussed. Several problems were encountered in the extraction of salt-soluble proteins and contributed to the generally low recoveries. The homogenization of flesh was not uniform due to bulking and froth formation. In centrifuge bottles, the proteins formed congealed pellets which were difficult to transfer to Kjeldahl flasks.

The analysis and sensory evaluation tests indicate that the flesh thawed using acoustics compares favorably with water-thawed. In comparison to fresh (nonfrozen) cod, the thawed samples for both control and acoustic compared favorably.

Table 1—Triangle taste panel results of acoustic versus water thawed cod

Transducer input power (continuous watts)	Comparisons (no.)	Correct responses (no.)	Significance (5% level)
40 (run 1)	8	3	Not significant
60 (run 2)	10	5	Not significant
60 (run 3)	10	6	Not significant

Industrial application

A higher throughput using a rapid thawing system may be attained by transferring frozen stock to a higher temperature storage in advance of complete thawing. This reduces only the sensible heating requirements, however.

The latent heating requirements are the most time-consuming thawing region. The control blocks in this experiment required 184 min to span the −5°C to −1°C phase

Table 2—Taste panel evaluation of acoustic and water thawed cod versus fresh cod

Thawing method	n	Sensory attributes: Flavor	Texture	Rancidity	Acceptability
Run 1					
Acoustic (40 watts)	8	4.0 ± 0.8	4.0 ± 1.1	4.8 ± 0.5	3.6 ± 1.1
Water	8	4.1 ± 0.9	4.0 ± 0.9	4.9 ± 0.4	3.9 ± 0.6
Fresh (non-frozen)	8	3.8 ± 1.2	4.3 ± 0.5	4.8 ± 0.5	3.8 ± 0.9
Run 3					
Acoustic (60 watts)	10	3.4 ± 0.8	4.0 ± 0.8	4.5 ± 0.8	3.4 ± 0.7
Water	10	3.5 ± 0.9	4.0 ± 1.1	4.8 ± 0.5	3.4 ± 0.9
Fresh (non-frozen)	10	3.6 ± 0.5	3.9 ± 0.8	4.8 ± 0.5	3.6 ± 0.5
Rating scales:		Flavor	Texture	Rancidity	Acceptability
		5 = very good	5 = normal	5 = none	5 = like very much
		4 = good	4 = slightly (soft, tough)	4 =	4 = like slightly
		3 = fair	3 = soft, tough	3 = slight	3 = neither like/dislike
		2 = borderline	2 = very (soft, tough)	2 =	2 = dislike slightly
		1 = poor	1 = mushy, rubbery	1 = strong	1 = dislike very much

Table 3—Chemical analyses of acoustic versus water-thawed cod

Thawing method	n	Moisture (%)	Fat (%)	Water holding capacity (%)	Salt soluble protein (%)
Run 1					
Acoustic (40 watts)	3	82.4 ± 0.0	1.6 ± 0.0	54.0 ± 1.4	58.6 ± 4.2
Water	3	82.5 ± 0.0	1.6 ± 0.0	49.9 ± 3.3	44.3 ± 3.2
Run 3					
Acoustic (60 watts)	3	79.9 ± 0.1	0.5 ± 0.0	70.1 ± 2.6	43.9 ± 1.3
Water	3	79.7 ± 0.3	0.6 ± 0.1	70.4 ± 3.5	41.7 ± 2.6

change region. The application of 1500 Hz acoustics at 60 watts input power to the transducer reduced this time to 34 min. The primary advantage of the acoustics was the reduction of the time held at phase change temperatures, an 82% reduction for this example.

In comparison to other heating methods, the low frequency and low power acoustics appear to stimulate heat transfer rather than aggressively apply energy. The major operating cost for an acoustic thawing system would be heating of the water bath.

Consider the requirements for a theoretical thawing unit of capacity 1300 kg/hr and using 150 dual-face transducers run in parallel at 60 cW each. This allows for 100 blocks per hour. The power input for the electronics, allowing for efficiency at the matching transformers and amplifiers, is approximately 18 kw-hr. The energy input required from the water bath is approximately 112 kw-hr. Additional energy is required for pumps and motors. Assume the total system consumes 150 kw-hr to thaw 1300 kg.

Operation of the acoustic system during a work shift to thaw 9100 kg would consume 1050 kw-hr: A system using only water would require 840 kw-hr. This assumes that the water process blocks were broken up mechanically or by hand to achieve the 9100 kg throughput.

The estimated 210 kw-hr additional energy must be balanced against the space devoted to one system versus several, reduced water demand, possibly reduced labor costs, and the rapidity of thawing. It is conceivable that several acoustic systems of 1300 kg/hr capacity could be installed in the space devoted to one conduction system with a total average capacity of 1300 kg/hr.

The system design for an acoustic/conduction thawing system incorporates the electronics into a simple circulated water bath. The transducers or the fish block must be moved to distribute the wave energy over the entire block surface. No insurmountable engineering task is apparent. Other design tasks include a parallel impedance matching transformer, specifications for the signal generator and amplification system, and a layout to minimize labor. The sound is audible and would require acoustic enclosures for the equipment in addition to ear protection for workers.

Transducers suitable for such a system are available with a quantity discount in effect at $300 per unit for 2000 units. Dual-face units are suggested such that one transducer could service two block surfaces simultaneously.

The acoustic/conduction process appears promising as a rapid thawing method, but construction of pilot units must be undertaken. Evaluation of its suitability as an investment to improve productivity is essential. A simple analysis of operation costs is insufficient in analyses comparing alternative thawing methods. A comprehensive study of a rapid thawing system would include the potential for improving the entire plant productivity and return on invested capital.

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Instrumental Analysis of Seafood Composition

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ROBERT M. GRODNER, JAMES A. COOK, ARTHUR F. NOVAK, and DON J. TOLODAY

ABSTRACT

Seafood products such as fish, crabs, shrimp and the aqueous solution from oysters, were analyzed for volatiles content by direct gas chromatography and mass spectrometry. Compounds were detected and identified which appear to be associated with freshness quality of the products. The data obtained suggests that the changing composition attending storage of seafood products can be effectively and rapidly analyzed by objective instrumental means.

INTRODUCTION

SEAFOOD has long been recognized as a highly perishable product which must be refrigerated immediately upon harvest from the water to retard deterioration. Even with reasonable handling and storage, bacterial growth in time, tends to impart odors and flavors to the product that reflect on freshness and quality. Thus, sensory evaluation has long been recognized as a most important factor for assessing the wholesomeness of seafoods. Unfortunately, establishing effective taste panels for such purposes is time consuming, costly (Martin, 1973; Larmond 1973), and ultimately is limited by the tasters' subjectivity. Many objective chemical tests have been proposed to measure decomposition of seafood (Farber, 1965), but such tests are not always applicable to detect the various types of spoilage which occur in different products. With the advent of direct gas chromatography, it has become feasible to detect many of the volatile components that contribute to the flavor quality of certain food products (Dupuy et al., 1971). Further, when direct gas chromatography (Dupuy et al., 1973) is coupled with mass spectrometry (Dupuy et al., 1976; Rayner et al., 1978; Legendre et al., 1979) it becomes possible to identify the specific volatile components that characterize flavor and indicate the sensory qualities of food products. Such techniques are readily adaptable to seafoods (Lerke and Huck, 1977). Mathematical models have been developed to effectively predict the flavor scores of canned tuna (Khayat 1978).

This paper examines the application of direct, objective, instrumental methods for determining the changing composition of volatile components attending storage of such seafood products as fish, crabs, shrimp, and the aqueous solution from oysters.

MATERIALS & METHODS

Materials

Silicone O-rings obtained from TekLab, Inc. (Baton Rouge, LA) were conditioned for 2 hr at 200°C. Sandwich-type silicone septa from Hamilton Co. (Reno, NV) were immersed in chloroform for 15 min, rinsed with chloroform, air-dried, and then conditioned for 2 hr at 200°C. Pyrex brand glass wool, manufactured by Corning Glass Works (Corning, NY) was heated at 200°C for approximately 16 hr to remove volatiles. Liners, 10 X 84 mm were cut from borosilicate glass tubing. Certified A.C.S. potassium carbonate (anhydrous) was obtained from Fisher Scientific Company. Porapak Q, 80/100 mesh, was obtained from Waters Associates (Framington, MA). Commercial and experimental seafood products were used.

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Analytical samples

Seafood samples used were of the following type: Fish products were fresh trout filets packed in serum cap containers and stored at −10°C for 2, 11, and 25 months. Crab samples were commercially purchased frozen lump meat, canned white crab meat, and the liquor from the canned product. Shrimp products were commercially obtained frozen samples analyzed immediately; after 14 and 21 days storage in polyethylene containers at 4°C. The liquor from freshly opened oysters was placed in a screw-capped glass jar and analyzed after 6 hr and 16 days storage at 4°C. A second sample of the same oyster liquor was treated similarly, but stored at 25°C for 16 hr.

Sample preparation

Approximately 200 mg of glass wool was placed at one end of a gas chromatographic injection-port glass liner. Between 100 and 300 mg of potassium carbonate was added on the glass wool and this was capped with about 50 mg additional glass wool. A quantity of 100–300 mg of seafood tissue or aqueous solution was added to the tube contents, and this was capped with about 50 mg additional glass wool.

Gas chromatography procedure

A Tracor 220 gas chromatograph (GC) equipped with dual flame ionization detectors, a Hewlett-Packard 3380A integrator and a Westonics MT 22 recorder were used. The construction of the GC has been described previously (Dupuy et al., 1977). The column was a 1/8 inch x 6 ft stainless steel U tube packed with Porapak Q, 80/100 mesh. Nitrogen was used as a carrier gas, with a pressure of 60 psi on the regulator. Rotometer settings were maintained at 40 ml per min. The pressures and rotameter settings for hydrogen and air were 30 psi, 45 ml per min and 50 psi, 560 ml per min, respectively.

The glass liner containing the sample was placed in the heated injection port of the GC at 140°C for 15 min to steam distill the volatiles and transfer them onto the head of the GC column which was maintained at ambient temperature during distillation. The liner with the spent sample was removed and the column temperature was quickly raised to 70°C, then programmed to rise 5°C per min to a final holding temperature of 215°C. This was maintained until elution of volatiles was complete.

Mass spectrometry procedure

Combined GC and mass spectrometry (MS) was performed with a Tracor Model 222 GC interfaced with a Hewlett-Packard Model 5930A mass spectrometer. Ionization potential was 70 eV, and the scan range was 33–450 Au. Data processing and scanning were accomplished with an INCOS 2000 mass spectrometer data system. In conjunction with the above mentioned equipment, a novel external inlet device was used, as previously described (Legendre et al., 1979), for making the MS determinations.

RESULTS & DISCUSSION

THE PROFILES of volatiles obtained from various seafood products in this study appear generally similar. Some specific compounds, however, seem to be associated with the decomposition that would be anticipated to occur

during certain storage conditions. Further, it is evident that in all instances, the quantity of overall volatiles tends to increase as the storage time or storage temperature increases. Fig. 1 demonstrates this effect quite well. It represents the profile of volatiles obtained from trout samples held in storage at $-10^{\circ}C$ from 2–25 months. It is clear that as the period of storage increases, the general quantity and intensity of volatile peaks increase. Additionally, certain specific volatiles appear to be associated with the changes that attend storage. For fish, the ethanol, dimethylsulfide, and trimethylamine peaks appear to be indicators of these changes. The ascending peak immediately associated with trimethylamine (determined recently to be n-propanol) might also be included among the freshness peak indicators since this peak occurs only in the 11 and 25-month samples. Additional observations and analysis of volatiles development during storage of fish would provide a more meaningful "profile print" of the decomposition process.

In applying the direct GC procedure to various seafood products to assess volatiles composition, it is essential to note that the same type of product may produce slightly differing profiles of volatiles, depending upon its source or prior preparation. Fig. 2, for example, shows the chromatograms from frozen lump crab meat, canned crab meat, and the fluid from the canned crab. While the chromatograms are essentially similar, the intensity of most peaks is increased in the canned product. This may reflect accelerated chemical changes associated with the significant heating required for canning. Fig. 2 also indicates that the fluid associated with the canned product (chromatogram 3) contains and releases the volatile components of the canned meat (chromatogram 2) quite well. The factor of solution homogeneity may function in this instance to provide a more representative picture of the volatiles present. The intensity of ethanol and trimethylamine, two important indices that seem associated with seafood composition, appear somewhat enhanced in the crab solution profile.

A comparison of frozen headless shrimp in various stages of decomposition are represented in the three chromatograms of Fig. 3. Chromatogram 1 indicates a shrimp product that would be judged in the market place as acceptable for consumption. It is evident from the profile

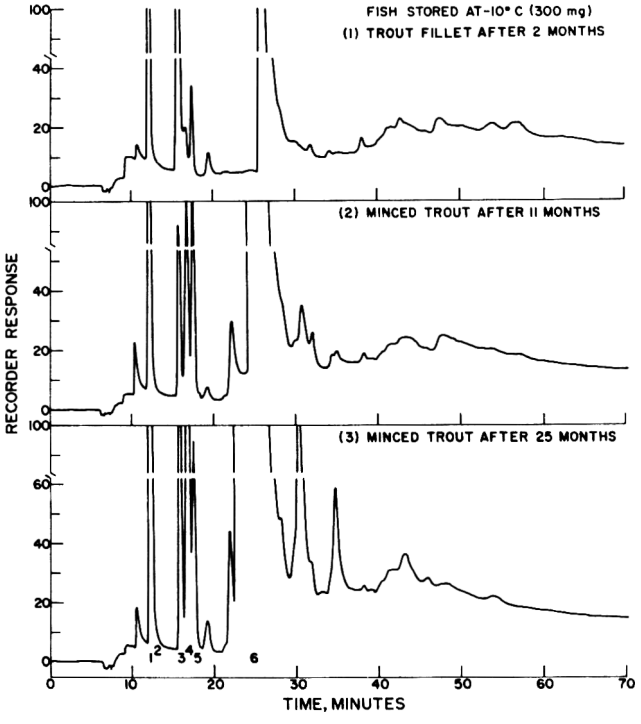


Fig. 1—Profile of volatiles obtained from trout samples after storage under refrigeration for varying periods of time: 1 and 2, Methanol and Methanethiol, (unresolved); 3, Ethanol; 4, Dimethylsulfide; 5, Acetone; 6, Trimethylamine.

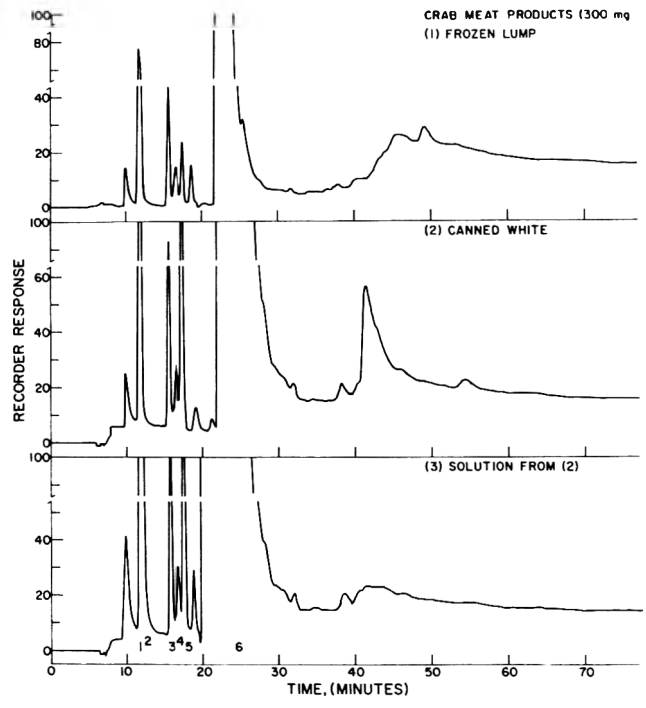


Fig. 2—Profile of volatiles obtained from crab meat samples and the aqueous solution from canned crab: 1 and 2, Methanol and Methanethiol, (unresolved); 3, Ethanol; 4, Dimethylsulfide; 5, Acetone; 6, Trimethylamine.

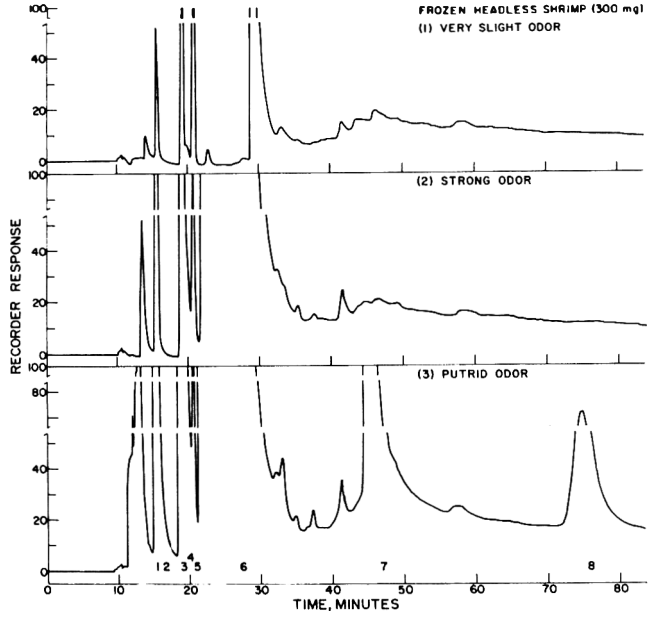


Fig. 3—Profile of volatiles obtained from shrimp at various stages of decomposition: 1 and 2, Methanol and Methanethiol, (unresolved); 3, Ethanol; 4, Dimethylsulfide; 5, Acetone; 6, Trimethylamine; 7, Methylpyridine (?); 8, Indole.

of volatiles that the number and intensity of volatile components are relatively low. Essentially no trimethylamine is present, and other odor producing products appear in subdued concentrations. In chromatogram 2, however, a dramatic elevation of trimethylamine is noted, and other volatile peaks are also increased. This chromatogram properly reflects the "strong odor" which characterizes such a material stored without the benefit of refrigeration for 24 hr. Chromatogram 3 obtained from a shrimp evincing the "putrid odor" characteristic of two days at ambient storage produces the greatest number of volatile components and these are present in high concentrations. Indole, which is not seen in the previous specimens, appears in this chromatogram and confirms the presence of a putrefactive process. It is important to recognize that while the quantity of volatiles present in a particular sample is highly indicative of its freshness quality, the type of specific peaks present play an equally essential role in assessing quality related factors. This is shown quite well in Table 1, where four components found in frozen shrimp are considered for their effect on the shrimp quality. It is obvious in this instance, that methanol, ethanol and trimethylamine are excellent indicators of the progressive deterioration that modify product odor from very slight to putrid. Acetone, however, does not appear to reflect these deteriorative changes. Thus, it is essential to determine by experimentation, the specific types of peaks that appear most sensitive to change with time for the specific product being analyzed. By monitoring such "indicator" peaks, subtle changes in product quality may be determined at an early stage.

Table 1 — Analysis of volatile components in frozen shrimp

Odor	Volatile concentration — integrator counts x 10^{-3}			
	Methanol[a]	Ethanol	Acetone	Trimethylamine
Very slight	7.5	11.6	9.3	130
Strong	9.0	81.0	4.0	2000
Putrid[b]	51.0	206.0	8.9	6500

[a] Contaminated with methanethiol.
[b] Indole count of 112,000 and methylpyridine (?) count of 350,000.

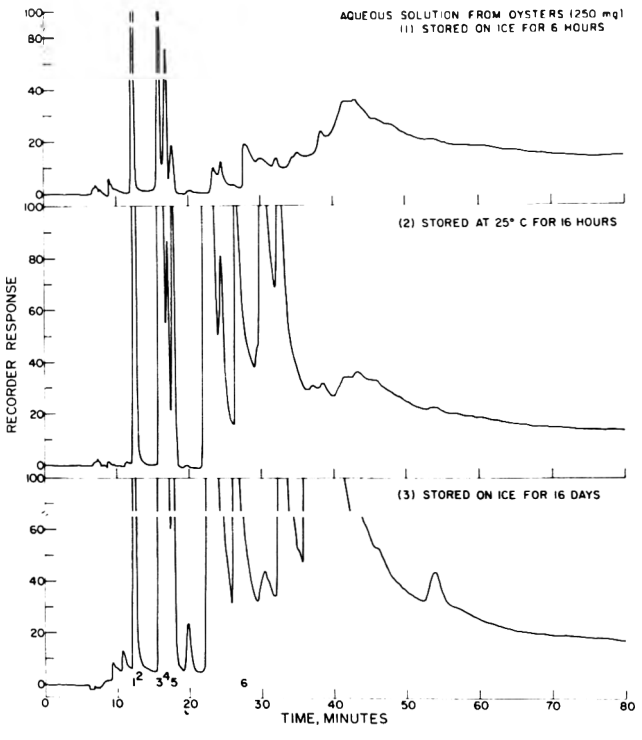


Fig. 4—Profile of volatiles obtained from the aqueous solution from oysters, stored at different temperatures for varying periods: 1 and 2, Methanol and Methanethiol, (unresolved); 3, Ethanol; 4, Dimethylsulfide; 5, Acetone; 6, Trimethylamine.

As indicated earlier, the solution associated with a seafood product provides an excellent medium to determine the volatile constituents of the particular product. Fig. 4 demonstrates this effect with the aqueous solution from oysters stored under varying conditions. Chromatogram 1, which represents a relatively fresh solution, reveals few volatiles in low concentrations. However, as the temperature and time of storage is increased, as shown in chromatogram 2, the intensity of the volatile peaks increases dramatically, and new volatile decomposition products become evident. When storage is continued for days, even below ambient temperatures, the volatiles and decomposition products become intense and indicative of the progressive deterioration of this seafood product.

It might well be reasoned that as a result of distillation heating the direct gas chromatographic technique may produce artifacts or spurious volatile peaks not present in the test sample. Although this is possible, it does not preclude effective use of the method to monitor on-going changes in profiles of volatiles, with time, because similar products are subject to identical analyses. Chromatograms 1 and 3 of Fig. 4 demonstrate the significant changes that occur in a homogeneous liquid specimen where the only variable is storage time. Thus it would appear that the change in the number and magnitude of specific volatile components can be reliably compared to assess alterations in product composition and quality.

Although much work remains to be done in this field to further refine the techniques and procedures, the basis for rapidly measuring and documenting the quality composition of seafood by objective instrumental means has been effectively demonstrated.

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Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply endorsement by the U.S. Dept. of Agriculture over others not mentioned.

Effect of Diamines, Polyamines and Tuna Fish Extracts on the Binding of Histamine to Mucin In Vitro

CHEU-HSIA CHU and LEONARD F. BJELDANES

ABSTRACT

Binding of [ring-2-^{14}C]-histamine to mucin was studied. Maximum binding of 2.5 μmol histamine/μmol mucin was observed at a histamine concentration of 0.5 mg/ml. Histamine binding was decreased by 46.5% by spermine, 25.3% by spermidine, 9.5% by cadaverine, and 11.1% by putrescine. These maximum effects were observed at molar ratios of histamine to polyamine of 1:1 for spermine and spermidine, 1:2 for cadaverine, and 1:2.3 for putrescine. The relative effects on histamine binding to mucin of extracts from wholesome canned tuna fish and a mixture of spermine, spermidine, cadaverine and putrescine were also examined. Although the total neutral aqueous extract of tuna had little effect on histamine-mucin binding (5.4% inhibition), the extract of bases from tuna and the prepared base mixture showed maximum inhibition of histamine binding to mucin of 23.1% and 21.4%, respectively. Certain other components of tuna extracts (NaCl, aspartic acid and glutamic acid) did not decrease histamine-mucin binding under the conditions used.

INTRODUCTION

SCOMBROID POISONING usually involves consumption of fish normally containing large amounts of free histidine in muscle tissue. The symptoms of poisoning are those of histamine poisoning. However, histamine does not appear to be the sole causative agent since histamine by itself is not toxic when taken orally (Douglas, 1980; Granerus, 1968; Weiss et al., 1932). Nevertheless, histamine taken in combination with wholesome tuna is reported to yield toxic effects in people even at moderate doses (100 – 180 mg histamine/100g tuna) (Motil and Scrimshaw, 1979). A possible interaction of histamine with other substances (potentiators) in food may explain these apparently inconsistent results.

Diamines are established potentiators of histamine's biological effects. Cadaverine and putrescine potentiate peroral toxicity of histamine in the guinea pig (Parrot et al., 1947; Bjeldanes et al., 1978). Spermine and cadaverine increase histamine transport across the gastrointestinal wall (Jung and Bjeldanes, 1979; Parrot, 1948; Parrot and Nicot, 1965) with little change in the relative amounts of histamine metabolites produced. In an effort to understand the mechanism by which certain diamines, polyamines and wholesome tuna fish alter the rates of histamine transport across the gut wall and/or potentiate the toxicity of histamine, the influence of these substances on the binding of histamine to gastric mucin was examined.

EXPERIMENTAL

Preparation of test solutions

All amine solutions including histamine dihydrochloride (Aldrich Chemical Co., Milwaukee, Wisconsin), cadaverine dihydrochloride, putrescine dihydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride (Calbiochem, San Diego, CA) were made in potassium hydrogen phthalate buffer of pH 3. Indicated concentrations (mg/ml) were computed based on weight of amine salt in solution.

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Binding of histamine to mucin

Two milliliters of each of the 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/ml histamine solutions were spiked with 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 μCi [ring-2-^{14}C]-histamine (Amersham Corp., Clearbrook, IL), respectively. Pig gastric mucin (15 mg) (Amend Drug & Chemical Co., Inc., Arlington Heights, IL) was added to the histamine solution and the mixture was stirred at 4°C overnight (about 18 hr). Mucin was precipitated from solution with 4 ml of 95% ethanol (pH 3) and collected by aspirating off the supernatant liquid. The collected mucin was dissolved with 2 ml of pH 3 buffer and precipitated once again with 4 ml of 95% ethanol. The supernatant was removed by aspiration. The histamine bound to mucin was then released by addition of 1 ml of pH 12 NaOH solution. Two milliliters of absolute methanol, 2 ml of 95% ethanol, and 3 drops of 6.25N NaOH were added consecutively to separate mucin from solution. Mixtures were centrifuged at 500 x *g* for 5 min and the supernatant concentrated down to 2 ml under a stream of nitrogen. Eight to ten drops of concentrated HCl were added to avoid precipitation when scintillation liquid was added. The final solution was poured into scintillation vials along with 14 ml of scintillation liquid, shaken until the solution was clear, and counted in a scintillation counter (Beckman Co., Irvine, CA). The separated mucin was treated with 2 or 3 drops of concentrated HCl and 2 ml of buffer. The mixture was transferred to a vial for radioactivity determination. The radioactivity remaining on mucin was negligible in all cases. All counts were corrected for counting efficiency and quenching efficiency and converted into μmol of mucin.

Effect of polyamines on the binding of histamine to mucin

Two milliliters of pH 3 buffer containing 0. 5 mg/ml histamine and various amounts of polyamine were spiked with 0.5 μCi [ring-2-^{14}C]-histamine and mixed with 15 mg of mucin. Each polyamine was examined at four different concentrations.

(1) Spermine group: 0.25, 0.7, 1.0, 2.0 mg/ml
(2) Spermidine group: 0.25, 0.5, 1.0, 2.0 mg/ml
(3) Cadaverine group: 0.5, 1.0, 2.0, 3.0 mg/ml
(4) Putrescine group: 0.5, 1.0, 2.0, 3.0 mg/ml.

Reaction conditions and extraction procedures are the same as described for the method for binding of histamine to mucin. Each point of Fig. 1 and 2 is a mean of at least triplicate determinations.

Extraction of canned tuna fish

The oil-drained tuna fish (600g, histamine content 2.7 mg/100 g) was blended with 520 ml of distilled water in a Waring Blendor for 2 min and centrifuged at 121,000 x *g* for 10 min. Half of the supernatant was evaporated to dryness *in vacuo* (Fraction 1). The other half was adjusted to pH 13 with 10N NaOH and extracted with two 100-ml portions of butanol. The combined organic phases were treated with 100-ml portions of 0.2N HCl. The aqueous phase was evaporated to dryness in vacuo (Fraction 2).

Effect of tuna fish extracts on histamine-mucin binding

The two tuna fish extracts were diluted with buffer to final volume of 16.2 ml and pH 3. Two milliliters of each fraction was spiked with 0.5 μCi radioactive histamine and treated as described in the method of histamine-mucin binding.

Effect of amine mixture on histamine-mucin binding

Two-milliliter portions of buffer containing 0.23 mg/ml spermine, 0.60 mg/ml spermidine, 0.19 mg/ml cadaverine, and 0.065 mg/ml putrescine were prepared for comparison with

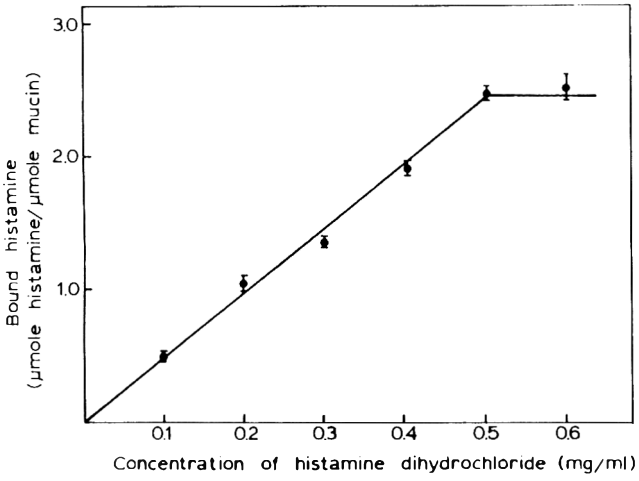


Fig. 1 — The binding of histamine to gastric mucin. Binding data were obtained after 2 ml of histamine di-HCl (0.1 — 0.6 mg/ml) and a tracer amount of [ring-2-^{14}C]-histamine di-HCl in pH 3 potassium hydrogen phthalate buffer were incubated with 15 mg of mucin overnight at 4°C. Bars indicate the range of standard error.

Fraction 1 and Fraction 2. The amine composition was similar to the determined value in Fraction 1 and Fraction 2 of extracts from wholesome canned tuna fish (Kim and Bjeldanes, 1979).

Effect of sodium ion and acidic amino acids on histamine-mucin binding

Procedures are the same as the method for histamine-mucin binding, only polyamine is substituted with 0.24N NaCl or 0.48N NaCl, or 0.36 mg/ml aspartic acid, or 0.40 mg/ml glutamic acid.

RESULTS & DISCUSSION

FIG. 1 SHOWS that the amount of histamine bound to mucin increased proportionally as a function of histamine concentration until a maximum was reached at a histamine concentration of 0.5 mg/ml (2.5 μmol mucin). A histamine concentration of 0.5 mg/ml was thus used in subsequent experiments. Fig. 2 shows that the inhibition of histamine binding by polyamines increased as the concentration of these amines increased until the maximum inhibition was obtained. The maximum inhibitions were 46.5% for spermine, 9.5% for cadaverine, and 11.1% for putrescine at a concentration of amine of 1 mg/ml. Spermidine induced a 25.3% reduction in histamine binding at a spermidine concentration of 0.7 mg/ml. All the inhibitions were significant except at 0.5 mg/ml for cadaverine or putrescine ($P < 0.05$). The molar ratio of polyamine to histamine at the point of maximum inhibition was 1:1 for spermine and spermidine, 2:1 for cadaverine, and 2.3:1 for putrescine.

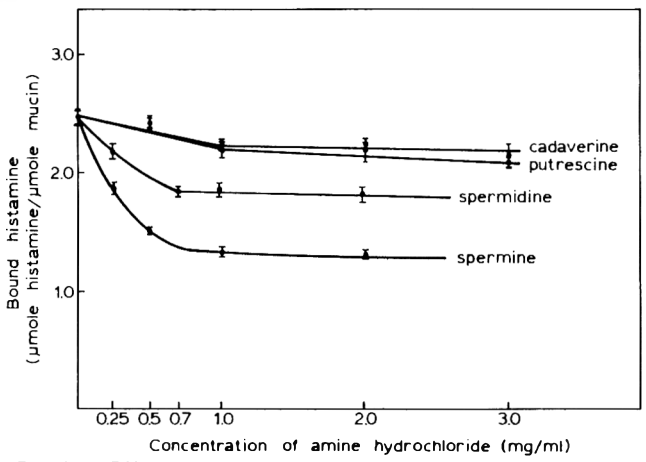


Fig. 2 — Effect of amines on histamine binding to gastric mucin. Bars indicate range of standard error.

Previous studies have shown that amines from tuna were present in Fraction 2, from wholesome canned fish (Kim and Bjeldanes, 1979). The results of inhibition studies (Table 1) show Fraction 1 had only 5.3% inhibition which was insignificant ($P < 0.2$), consistent with the poor extractability of amines from tuna fish into neutral water. Fraction 2, however, produced 23.1% inhibition. This value was very close to the inhibition level (21.4%) exerted by an amine mixture prepared to be comparable in amine composition to Fraction 2. To test the possible influence of salts on histamine-mucin binding, the effects of sodium chloride and acidic amino acids were examined. Sodium chloride showed no influence on binding at concentrations of 0.24N and 0.48N, nor did glutamic acid and aspartic acid at equimolar concentration with histamine.

The amines present in Fraction 2 appear to account for the inhibition effects of this fraction on histamine binding to mucin. Very similar results were obtained from inhibition studies with the amine extract (Fraction 2) and the prepared mixture of amines. However, the effects of diamines and polyamines on the binding of histamine to mucin do not appear to be additive. The expected inhibition level based on data presented in Fig. 2 should be greater than 46% — 23.4% from spermine and 22.8% from spermidine. The effect of cadaverine and putrescine would be negligible due to the comparatively weak effects of these substances on histamine-mucin binding and the small amounts of these substances present in samples. Half of the expected inhibition was obtained for both Fraction 2 and the prepared amine mixture.

Since oral histamine when taken by itself is not toxic even at doses which would be lethal if administered by the

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Table 1 — Effect of canned tuna fish extracts, sodium ion, and acidic amino acids on the binding of histamine to gastric mucin

Sample	(No.)	dpm ($\bar{X}$ ± S.E.)	μmol Histamine / μmol Mucin ($\bar{X}$ ± S.E.)	% Inhibition
Fraction 1	(3)[a]	3570.49 ± 84.03	2.333 ± 0.056	5.3[d]
Fraction 2	(4)	2879.45 ± 56.72	1.894 ± 0.037	23.1[b] (4:1)[c]
4-Amine mixture	(3)	2963.84 ± 31.75	1.937 ± 0.024	21.4[b] (4:1)
Na^+ (0.48 N)	(2)	3677.87 ± 69.39	2.404 ± 0.046	None
Na^+ (0.24 N)	(2)	3709.96 ± 3.91	2.425 ± 0.003	None
Glutamic acid (0.4 mg/ml)	(3)	3692.84 ± 86.63	2.414 ± 0.057	None
Aspartic acid (0.36 mg/ml)	(3)	3757.34 ± 113.88	2.456 ± 0.075	None

[a]Numbers in parentheses in this column indicate the number of samples in each group.
[b]P is calculated vs 0.5 mg/ml histamine group, *t*-test ($P < 0.001$).
[c]Figures in parentheses in this column represent levels of inhibition relative to Fraction 1.
[d]Not significantly different from zero

Nutritional Evaluation of Cereal–Cheese Whey Mixtures

M. HERNÁNDEZ, A. VEGA, and A. SOTELO

ABSTRACT

The chemical composition, amino acid content, PER, and digestibility of dehydrated sweet cheese whey, wheat flour, wheat bran, and lime-treated corn flour (*nixtamal,* used for making tortillas), as well as of 21 cereal-cheese whey mixtures were determined. The cheese whey was composed of 12% protein and 74% lactose. Diets containing a total of 8% protein were formulated with the cereal-cheese whey mixtures. The 50:50 (% protein) cereal-whey mixtures had the highest adjusted modified PERs, representing an increase over the cereals alone of 324% for the corn flour, 215% for wheat flour, and 177% for wheat flour, and 177% for wheat bran. The 50:50 (% protein) wheat bran mixture had the largest adjusted modified PER at 2.33 ± 0.13 (mean ± SEM) and the 50:50 wheat flour mixture, the smallest at 1.48 ± 0.21. Although the apparent digestibility was reduced from 10–14% for the latter two 50:50 cereal-whey mixtures compared to the cereals alone, this was not reflected in the PER. It was concluded that the nutritive value of all the cereals tested significantly increased with the addition of 15–25% or more (by weight) of dehydrated cheese whey.

INTRODUCTION

WHEY, a by-product of cheese manufacture, contains approximately 20% of the total protein, 50% of the solids, and all of the lactose of the milk used in the fabricating process (Weisberg, 1976; Jelen, 1979). Nine liters of whey are produced for each kilogram of elaborated cheese, but it is not used in liquid form because of transportation and contamination problems (Holsinger et al., 1974; Peterson et al., 1979). The dehydrated form of whey, prepared by the same process used to obtain milk powder (Forsum and Hambraeus, 1977), contains 74% lactose (Mavropoulou and Kosikowski, 1973), a concentration not easily tolerated by certain sectors of the population (Duncan, 1955).

It has been established that whey protein is of an excellent quality, even superior to casein, and can be used to adequately supplement cereals (Womack and Vaughan, 1972; Ashur et al., 1973; Forsum, 1975; Delaney, 1976; De Rham et al., 1977). The majority of these studies have been carried out with whey concentrates in which most of the lactose has been removed, and not with dehydrated cheese whey.

Since whey is most readily available in its dehydrated form, this study was designed to investigate its possible use as a supplement for the cereals most commonly used in the Mexican diet. The nutritional value of dehydrated cheese whey obtained from a factory located in Mexico City, of wheat flour and bran, of corn flour mixed with lime used for making tortillas (*nixtamal*), and of mixtures of the cereals and cheese whey was determined.

MATERIALS & METHODS

Materials

Spray-dried whey resulting from the manufacture of cottage cheese (sweet whey) was obtained from the Compañia Kraft de México, S.A. (Kraft Company of Mexico). Wheat bran was purchased from the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT-México) (International Center for the Improvement of Corn and Wheat), and wheat flour and dehydrated *nixtamal* (lime-treated corn four, MINSA brand) were purchased locally.

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Chemical analysis of raw materials

The raw products were analyzed according to the techniques described by the AOAC (1970). Crude fat was measured in the dried whey by the Mojonnier method, and lactose was quantified according to Teles et al. (1978). The amino acid composition of the cereals and dried whey was determined using a Perkin Elmer KLA-5 amino acid analyzer, following hydrolysis with 6N HCl for 18 hr at 110–115°C (Moore and Stein, 1963). Tryptophan was measured using the fluorometric technique described by Udenfriend (1962). A chemical score was calculated for each product with these data, using whole egg as the reference protein.

Cereal-cheese whey mixtures

Cereal-cheese whey mixtures were prepared in the following proportions (% weight): 5:95, 10:90, 15:85, 20:80, 25:75, and 30:70. A 50:50 (% protein) mixture was also made for each cereal-whey combination. The chemical scores of these mixtures were calculated and diets containing a total of 8% protein were prepared with each of the blends (Table 1). This concentration of protein was chosen due to the low levels of protein in the cereals.

Biological studies

A modified protein efficiency ratio (PER) for each diet was determined with Sprague Dawley male rats, 21–23 days old, individually caged and housed in a temperature-controlled (21°C) room. The animals were fed an experimental diet ad libitum over a period of 21 days. They were weighed twice a week and their food consumption was measured daily. The feces were collected for the last 7 days of the experiment, dehydrated and ground, and their nitrogen content was determined as a measure of apparent digestibility (WHO, 1965). Since numerous diets were tested, the study was divided into four parts. Each set of diets was compared against a control diet of 8% casein, and in two cases, against 10% casein.

Statistical analyses

The PER data were subjected to an analysis of variance as well as to the Scheffé multiple test. Correlation coefficients were also calculated for the PER and chemical score data (Steel and Torrie, 1960).

RESULTS & DISCUSSION

THE RESULTS of the chemical analysis of the raw materials are listed in Table 2. The values determined for the dried cheese whey were very similar to those reported by Glass and Hedrick (1977) and Jelen (1979). The amount of ash (7.05%) present in this product has been proposed by Riggs et al. (1955) to be responsible for the low nutritive value of whey powder protein. However, the large variation in the PER values that were obtained with whey (range: 0.63–2.47) suggests not only that the high level of lactose could be a major factor in the low nutritive value of this product, but also that rats might be particularly susceptible to this substance. The values obtained for the cereals were within the normal range (Leung, 1966; Sambucetti et al., 1976). The high protein content of wheat bran (17.88%) suggests that this cereal should be utilized to a much greater extent in the human diet.

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Amino acid analysis (Table 3) showed that the wheat flour was low in lysine; corn flour, in lysine and tryptophan; and wheat bran, in sulfur amino acids. These data are in accord with the values published by the FAO (1970). The proportion of lysine in the cheese whey was lower than was reported by Glass and Hedrick (1977), and phenylalanine was the limiting amino acid.

The chemical scores calculated for the cheese whey and cereals, alone as well as mixed, are listed in Table 4. It can be observed that the higher the proportion of whey, the greater is the chemical score, reaching 70.7% for a 50:50 (% protein) mixture of wheat flour-whey. In the case of the 50:50 (% protein) mixtures of corn- and wheat bran-whey, the limiting amino acids are different from the mixtures with less whey.

The modified PER values for each mixture are shown in Table 5, adjusted for a PER value of 2.5 for casein. No statistical differences were found between the PERs of the 8% and 10% casein diets (Riggs et al., 1955; Womack and Vaughan, 1972). However, the PERs obtained with 8% casein in the four sets of experiments were quite varied, possibly due to differences in the initial body weights of the rats. These variations decreased when the rats all weighed between 40–50g at the beginning of the experiment.

In general, the cereals had lower PERs than have been reported by other authors (Chandrasekharappa, 1979; Valencia et al., 1979). Wheat bran had the highest adjusted modified PER (0.84 ± 0.13), while whey had a value (1.45 ± 0.39) similar to that published by Riggs et al. (1955), although it was considerably less than the figure of 3.0 or more determined by McDonough et al. (1976), Schoppet et al. (1976), and Racotta et al. (1979). The difference in the quality of the protein in the cheese wheys measured by these investigators and ourselves could be due to the fact that the former used protein concentrates in which the lactose had been removed. It is known that lactose reacts with lysine during the drying process, reduc-

Table 1—Composition of experimental diets[a] using cereal-dehydrated cheese whey mixtures as a source of protein[b]

Cereal-cheese whey mixture (wt %)	Ingredients (g/100 g diet)					
	Cheese whey	Cereal	Dextrose	Corn oil	Salt mix[c]	Lactose[d]
Corn flour-whey						
0:100	64.5		16.4	11.0		47.7
100: 0		91.2		4.1	2.7	
95: 5	4.5	84.9		6.0	2.6	3.2
90: 10	8.9	78.6		8.2	2.3	6.6
85: 15	12.9	73.0		10.1	2.0	9.5
80: 20	16.9	67.3	1.6	10.4	1.8	12.5
75: 25	20.6	62.1	2.9	10.6	1.8	15.3
70: 30	24.6	56.4	3.7	10.6	1.5	18.2
59: 41[e]	32.2	45.6	6.3	10.7	1.1	23.8
Wheat flour-whey						
100: 0		79.4	0.5	13.0	3.6	
95: 5	4.0	74.8	1.2	12.9	3.3	3.0
90: 10	7.6	70.0	2.4	12.8	3.1	5.6
85: 15	11.3	65.5	3.3	12.6	2.8	8.4
80: 20	15.3	60.6	4.3	12.5	2.6	11.3
75: 25	18.9	56.1	5.2	12.4	2.4	14.0
70: 30	22.2	52.1	6.0	12.3	2.2	16.4
55: 45[e]	32.2	39.7	8.4	12.0	1.5	23.8
Wheat bran-whey						
100: 0		44.7	36.4	12.3	2.5	
95: 5	2.3	43.2	36.0	12.3	2.3	1.7
90: 10	4.6	41.5	35.0	12.2	2.3	3.4
85: 15	7.0	39.9	34.2	12.2	2.1	5.2
80: 20	9.5	38.1	33.4	12.2	2.0	7.0
75: 25	12.1	36.3	32.6	12.0	2.0	9.0
70: 30	14.8	34.5	31.8	12.0	1.8	11.0
41: 59[e]	32.2	22.4	26.4	12.0	1.0	23.8

[a] 2 g of vitamin mix (Nutritional Biochemicals, Cleveland, OH) were added to every diet.
[b] All diets contained a total of 8 % protein and were adjusted to 100g with cellulose fiber (Teklad Test Diets, Madison, WI).
[c] Roger-Harper mix (Teklad Test Diets, Madison, WI).
[d] The lactose was not added to the diet but a component of the dehydrated cheese whey.
[e] These prercent weights represented equal amounts of protein (50:50 o/o protein, cereal:whey).

Table 2—Chemical analysis of cereals and dehydrated cheese whey used as protein sources in experimental diets

Raw material	Constituents (g/100 g sample)					
	Moisture	Ether extract	Crude fiber	Protein[a]	Ash	Carbohydrate
Cheese whey	1.96	4.60		12.41	7.05	73.98[b]
Corn flour	8.83	4.07	2.47	8.77	1.46	74.40
Wheat flour	8.04	1.26		10.07	0.55	80.08
Wheat bran	5.03	3.78	7.96	17.88	3.42	61.93

[a] Factors used to calculate protein: cheese whey, N X 6.38; corn flour, N X 6.25; wheat flour, N X 5.70; wheat bran, N X 6.31.
[b] In the case of cheese whey, the carbohydrate is lactose.

Table 3—Amino acid composition of the raw materials used as protein sources in the experimental diets[a]

Amino acids	Dehydrated cheese whey	Corn flour	Wheat flour	Wheat bran
Tyrosine	0.884	1.846	1.362	1.019
Lysine	5.125	2.753	1.933	2.832
Phenylalanine	2.382	4.484	3.688	2.380
Histidine	0.561	2.438	1.290	2.384
Arginine	1.671	4.427	1.908	3.847
Aspartic acid	8.620	7.170	4.404	5.542
Glutamic acid	15.889	18.476	23.297	21.673
Threonine	7.310	3.170	2.523	1.938
Serine	5.671	4.686	4.096	5.823
Proline	7.215	6.168	11.282	3.819
Alanine	5.075	4.419	6.637	4.028
Glycine	2.598	4.062	3.634	3.005
Valine	5.836	4.888	4.361	3.483
Cysteine	1.414	1.426	1.896	1.009
Methionine	1.869	1.614	1.732	0.928
Isoleucine	5.930	3.134	3.387	3.620
Leucine	9.380	14.832	6.791	7.363
Tryptophan	1.753	0.685	1.290	0.920

[a] Values are expressed as g/16 g N.

Table 4—Chemical score of cereals, dehydrated cheese whey, and cereal-cheese whey mixtures

Composition of mixture (cereal; whey, wt %)	Corn flour-whey	Wheat flour-whey	Wheat bran-whey
100: 0	Lys, 50.0	Lys, 47.2	Met + Cys_2, 64.3
95: 5	Lys, 52.5	Lys, 50.3	Met + Cys_2, 64.3
90: 10	Lys, 55.0	Lys, 53.6	Met + Cys_2, 64.3
85: 15	Lys, 57.4	Lys, 56.3	Met + Cys_2, 64.4
80: 20	Lys, 59.6	Lys, 58.6	Met + Cys_2, 64.4
75: 25	Lys, 61.5	Lys, 61.3	Met + Cys_2, 64.5
70: 30	Lys, 63.7	Lys, 64.7	Met + Cys_2, 64.5
59: 41[a]	Met + Cys_2, 65.7		
55: 45[a]		Lys, 70.7	
41: 59[a]			Phe, 59.7
0:100	Phe, 47.8		

[a] The cheese whey and cereal provided equal amounts of protein (50:50, % protein) in these mixtures.

ing the availability of this amino acid, although spray drying produces less damage than the drum method (Rigg et al., 1955; Forsum and Hambraeus, 1977). Nevertheless, this is not the only factor, since cheese whey contains a high level of mineral salts which according to Womack and Vaughan (1974) and Weisberg (1976) provoke diarrhea in rats. The same effect has been observed in diets with a high lactose content (Womack and Vaughan, 1972). A diet in which cheese whey contributed all of the protein produced mild diarrhea in our rats, but this did not occur with any of the cereal-whey mixtures, including the one with the highest proportion of cheese whey (41% wheat bran, 59% cheese whey, 23.8% lactose). This mixture had the highest nutritional value of all those tested, with an adjusted modified PER of 2.33 ± 0.13 (Table 5).

It has been found that 21- to 23-day-old rats fed with diets containing 60% lactose do not survive for more than a week, and that they lose weight on diets containing 46–60% lactose (Duncan, 1955; Schinogoethe, 1976). Rats are particularly intolerant to this carbohydrate, which could provide a partial explanation for the wide variability in the data obtained from rats fed with a diet prepared with cheese whey alone. The high SEM (Table 5) suggests that each rat had a different tolerance level to lactose. However, in the case of human beings, it should be remembered that a small amount of lactose in the diet aids calcium absorption (Duncan, 1955), and that only certain groups in the general population demonstrate intolerance to this carbohydrate.

Table 6 shows the apparent digestibility of the different cereal-cheese whey mixtures. Cheese whey has the lowest value, and its addition to the cereals reduces their digestibility. This is especially apparent in the 50:50 (% protein) mixture of wheat flour with whey (14% decrease), followed by the 50:50 (% protein) wheat bran-whey mixture (10% decrease). However, the adjusted modified PERs of these mixtures are far superior to the cereals alone (Table 5), and this is true of the 50:50 corn four-whey mixture as well (Ashur et al., 1973).

Table 5—Adjusted modified PER of cereals, dehydrated cheese whey, and cereal-cheese whey mixtures[a]

Composition of mixture (cereal-whey, wt %)	Protein efficiency ratio (mean ± SEM)		
	Corn flour	Wheat flour	Wheat bran
100: 0	0.46 ± 0.13 (5)[b]	0.47 ± 0.14 (5)	0.84 ± 0.13 (6)
95: 5	1.27 ± 0.11 (6)*	0.72 ± 0.14 (5)	1.53 ± 0.17 (5)*
90: 10	1.34 ± 0.08 (6)*	0.97 ± 0.13 (6)	1.26 ± 0.29 (6)
85: 15	1.30 ± 0.13 (5)*	1.10 ± 0.16 (6)	1.97 ± 0.15 (6)*
80: 20	1.38 ± 0.20 (6)*	1.21 ± 0.10 (6)*	1.43 ± 0.21 (5)
75: 25	1.45 ± 0.18 (6)*	1.37 ± 0.12 (6)*	1.60 ± 0.28 (5)*
70: 30	1.71 ± 0.14 (6)*	1.31 ± 0.07 (5)*	1.57 ± 0.27 (5)*
59: 41[c]	1.95 ± 0.12 (5)* (324 %)[d]		
55: 45[c]		1.48 ± 0.21 (5)* (215 %)[d]	
41: 59[c]			2.33 ± 0.13 (5)* (177 %)[d]
Cheese whey	1.45 ± 0.39 (5)		

[a] Values were adjusted with respect to a PER of 2.50 for casein.
[b] Number of parentheses refers to number of animals used in each experimental group.
[c] The cheese whey and cereal provided equal amounts of protein (50:50, % protein) in these mixtures.
[d] Percentage increase in adjusted modified PER over the value of the cereal alone.
* $P < 0.05$, Scheffé multiple test.

Table 6—Apparent digestibility of cereals, dehydrated cheese whey, and cereal-cheese whey mixtures

Composition of mixture (cereal-whey, wt %)	% protein digestibility (mean ± SEM)		
	Corn flour	Wheat flour	Wheat bran
100: 0	75.5 ± 0.8 (5)	85.1 ± 0.7 (5)	78.9 ± 0.6 (5)
95: 5	76.8 ± 0.5 (5)	84.1 ± 0.9 (5)	76.6 ± 1.0 (5)
90: 10	79.1 ± 1.4 (6)	82.4 ± 1.0 (6)	75.9 ± 0.9 (6)
85: 15	68.2 ± 2.1 (5)	82.8 ± 1.1 (6)	76.5 ± 1.5 (6)
80: 20	78.2 ± 2.2 (6)	80.5 ± 1.4 (6)	71.1 ± 0.9 (5)
75: 25	77.7 ± 1.6 (6)	80.0 ± 0.3 (6)	74.6 ± 2.1 (5)
70: 30	70.9 ± 1.1 (6)	80.1 ± 0.9 (5)	73.9 ± 1.8 (5)
59: 41[a]	75.2 ± 1.2 (5)		
55: 45[a]		73.5 ± 1.1 (5)	
41: 59[a]			71.1 ± 2.3 (5)
Cheese whey	65.9 ± 1.4 (5)		
Casein	82.3 ± 0.8 (6)		

[a] The cheese whey and cereal provided equal amounts of protein (50:50, % protein) in these mixtures.

CONCLUSIONS

THE ADDITION of dehydrated cheese whey to the cereals significantly increased their nutritional value, especially in the case of the corn and wheat flours, although the highest adjusted modified PER value was obtained with the 50:50 (% protein) wheat bran-whey mixture. The best blend tested for the three cereals was one in which the cereal and whey each provided 50% of the protein in the diet. From the statistical analyses, it can be concluded that the addition of 15–25% dehydrated cheese whey markedly increases the nutritional value of a cereal diet, but is most effective when the protein is equally derived from both products. Wheat bran responded the least to supplementation, while corn flour showed the greatest increase in nutritional value.

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Modeling of Isothermal Diffusion of Whey Components from Small Curd Cottage Cheese During Washing

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ABSTRACT

The batchwise washing of whey components from small curd cottage cheese is modeled as an isothermal diffusion process in a porous medium with several refinements to account for the whey on curd surfaces that is carried into the washing system. Three geometric approximations are considered. Apparent diffusion coefficients for total solids and lactose at 25°C are determined. Results show that diffusion from a spherical particle with consideration of whey entrained in curd interstices by capillary forces is an acceptable basis for a mass transfer model.

INTRODUCTION

THE WASHING of cottage cheese curd accomplishes removal of whey components and enhancement of keeping quality of curd (Emmons and Tuckey, 1967). The mathematical modeling of the mass transfer of whey components from curd is critical to the eventual engineering optimization of the washing process. Based upon the electronmicroscopic work of Glaser et al. (1980), mass transfer of whey components from curd can be modeled as a diffusive flux through a porous medium. This paper reports results of such modeling. Three geometrical approximations to the shape of small curd cottage cheese particles are examined. Refinements were made to account for the initial charge of whey components carried into the washing system by entrainment on curd surfaces. The diffusion of whey components from curd, which limits the rate of the washing process, is the focus of the modeling.

The physical system considered is that of batch washing in which drained curd is combined with washing water (Emmons and Tuckey, 1967). Since the heat transfer in such a batch system is much more rapid than the diffusive mass transfer so that the curd reaches thermal equilibrium quickly, the washing is approximated as an isothermal (25°C) process. Two diffusing species considered are total solids and lactose.

THEORY

THE FOLLOWING PRESENTATION concerns the isothermal diffusion of solute (i.e., whey solids) from a porous network (i.e., cottage cheese curd). Several geometries are considered. Symbols are identified in the nomenclature section. The equations describe a situation in which a definite amount of drained curd, which still contains some entrained whey, is combined batchwise with a fixed volume of fresh wash water in an agitated tank. It is assumed that there is no chemical reaction in the system and that there is no convective mass transfer in the pores but only diffusion. Consider the two-phase system in Fig. 1 where the K-phase is the solid casein matrix, the γ-phase is the whey trapped, and the μ-phase is the wash water.

In the γ-phase the diffusion equation for the "i" species can be expressed as:

$$\frac{\partial \langle C_i \rangle^\gamma}{\partial t} = D^i_{eff} (\nabla^2 \langle C_i \rangle^\gamma) \quad (1)$$

where $\langle C_i \rangle^\gamma$, the intrinsic phase average concentration is (Ryan et al. 1981):

$$\langle C_i \rangle^\gamma = \frac{1}{V_\gamma} \int_{V_\gamma} C_i dV \quad (2)$$

Assuming the curd particle to be approximated by a sphere, Eq. (1) becomes:

$$\frac{\partial \langle C_i \rangle^\gamma}{\partial t} = D^i_{eff} \left(\frac{\partial^2 \langle C_i \rangle^\gamma}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial \langle C_i \rangle^\gamma}{\partial r} \right) \quad (3)$$

with initial and boundary conditions:

$$\text{at } t = 0, \text{ at any } r, \langle C_i \rangle^\gamma = \langle C^\circ_i \rangle^\gamma \quad (4)$$

$$\text{at } r = 0, \text{ for any time}, \frac{\partial \langle C_i \rangle^\gamma}{\partial r} = 0 \quad (5)$$

$$\text{at } r = a, \text{ for any time}, V_\mu \frac{\partial \langle C_i \rangle^\mu}{\partial t} = -\beta_\gamma A D^i_{eff} \frac{\partial \langle C_i \rangle^\gamma}{\partial r} \Big|_{r=a} \quad (6)$$

with the assumption that K, the partition coefficient equals one (i.e., $\langle C_i \rangle^\beta = \langle C_i \rangle^\gamma$, at r = a).

The solution of Eq (3) through (6) is given by Crank (1975) as:

$$\langle C_i(t) \rangle^\gamma = \langle C^\infty_i \rangle^\gamma \left\{ 1 + \sum_{n=1}^{\infty} \frac{6(1+\alpha)}{9+9\alpha+\alpha^2 q_n^2} \frac{a}{r} \frac{\sin q_n r/a}{\sin q_n} \cdot \exp(-D^i_{eff} q_n^2 t/a^2) \right\} \quad (7)$$

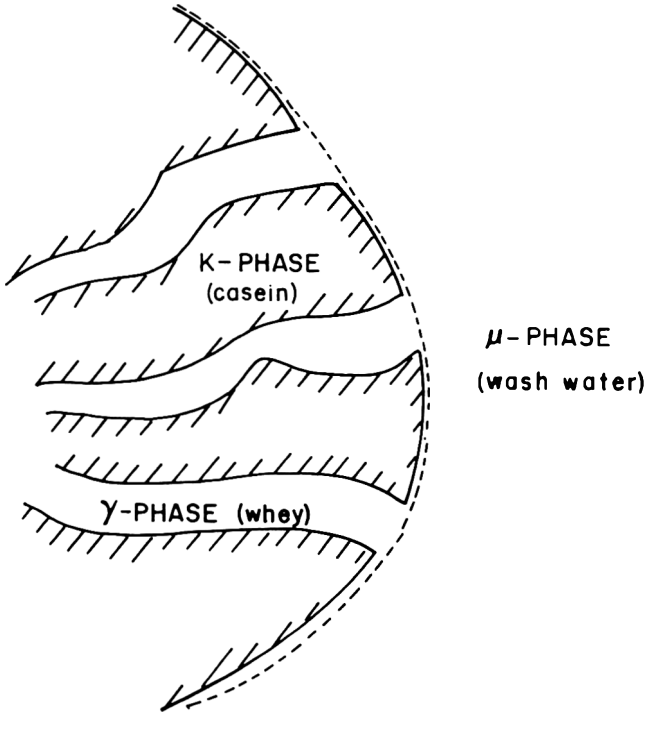


Fig. 1—Porous structure of the curd particle.

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where the q_n's are the nonzero roots of:

$$\tan q_n = \frac{3q_n}{3+\alpha q_n^2} \tag{8}$$

and α is defined as:

$$\alpha = \frac{V_\mu}{V_\gamma} \tag{9}$$

and $V_\gamma = \beta_\gamma V_{curd}$

Whey solids are comprised of numerous components from low molecular salts to whey protein. One solution to the problem of representing all solids by a single pseudocomponent is to utilize a lumped parameter model (Bailey, 1975). The diffusivity of the single component, which is experimentally denoted as "total solids," is defined as:

$$D_{TS} = \frac{\int_0^\infty \langle C^\infty_i \rangle^\gamma (D_{eff}) \cdot D_{eff} \cdot d\, D_{eff}}{\int_0^\infty \langle C^\infty_i \rangle^\gamma (D_{eff}) \cdot d\, D_{eff}} \tag{10}$$

To calculate the total solids concentration in the wash water as diffusion progresses, expressed as a ratio to the concentration at equilibrium, the lumped parameter approach can be incorporated into Eq (7). Integration over the total volume of spheres yields the solution for the total solids concentration in the wash water (Bressan, 1980):

$$\left(\frac{M_{TS}(t)}{M^\infty_{TS}}\right)_\mu^{\text{for spheres}} = 1 - \sum_{n=1}^{\infty} \frac{6\alpha(1+\alpha)}{9+9\alpha+\alpha^2 q_n^2} \cdot \exp\left(-D_{TS}\, q_n^2\, t/a^2\right) \tag{11}$$

For the case of an infinite slab of thickness 2L, Eq (1) becomes:

$$\frac{\partial \langle C_i \rangle^\gamma}{\partial t} = D^i_{eff} \cdot \frac{\partial^2 \langle C_i \rangle^\gamma}{\partial x^2} \tag{12}$$

with initial and boundary conditions:

$$\text{at } t = 0, \text{ at any } x \ \langle C_i \rangle^\gamma = \langle C^\circ_i \rangle^\gamma \tag{13}$$

$$\text{at } x = 0, \text{ for any time} > 0, = \frac{\partial \langle C_i \rangle^\gamma}{\partial x} = 0 \tag{14}$$

$$\text{at } x = \pm L, \text{ for any time} > 0, V_\mu \frac{\partial \langle C_i \rangle^\mu}{\partial t} = \pm \beta_\gamma D^i_{eff} \frac{\partial \langle C_i \rangle^\gamma}{\partial x}\Bigg)_{x=\pm L} \tag{15}$$

The sheet occupies the space $-L \leqslant x \leqslant L$, while the solution is of limited extent and occupies the spaces $-L-b \leqslant x \leqslant -L$, $L \leqslant x \leqslant L+B$. Eq (15) expresses a condition such that the rate at which solute leaves the sheet equals that at which it enters solution.

The solution for the infinite slab is (Crank, 1975):

$$\left(\frac{M_{TS}(t)}{M^\infty_{TS}}\right)_\mu^{\text{for inf. slab}} = 1 - \sum_{n=1}^{\infty} \left[\frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-q_n^2\, D_{TS}\, t/L^2\right)\right] \tag{16}$$

where $\alpha = \frac{b}{\beta_\gamma L}$ and the q_n values are the nonzero roots of $\tan q_n = -\alpha q_n$.

The solution of Eq (1) for a finite slab (cube) arises from the product of three solutions for infinite slabs according to Newman's Rule (Carslaw and Jaeger, 1959). The solution for the cube is:

$$\left(\frac{M_{TS}(t)}{M^\infty_{TS}}\right)_\mu^{\text{for cube}} = 1 - \left[\sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \cdot \exp\left(-q_n^2 D_{TS} t/L^2\right)\right]^3 \tag{17}$$

The solution for a finite cylinder begins with the solution of Eq (1) for an infinite cylinder:

$$\frac{\partial \langle C_i \rangle^\gamma}{\partial t} = D^i_{eff} \cdot \frac{1}{r} \cdot \frac{\partial}{\partial r}\left(\frac{r \partial \langle C_i \rangle^\gamma}{\partial r}\right) \tag{18}$$

with initial and boundary conditions:

$$\text{at } t = 0, \text{ at any } r, \langle C_i \rangle^\gamma = \langle C^\circ_i \rangle^\gamma \tag{19}$$

$$\text{at } r = 0, \text{ for any time}, \frac{\partial \langle C_i \rangle^\gamma}{\partial r} = 0 \tag{20}$$

$$\text{at } r = a, \text{ for any time}. A \frac{\partial \langle C_i \rangle^\mu}{\partial t} = -\beta_\gamma a\, D^i_{eff} \frac{\partial \langle C_i \rangle^\gamma}{\partial r}\Bigg)_{r=a} \tag{21}$$

The solution for the infinite cylinder (Crank, 1975) is:

$$\left(\frac{M_{TS}(t)}{M^\infty_{TS}}\right)_\mu^{\text{for inf cylinder}} = 1 - \sum_{n=1}^{\infty} \frac{4\alpha(1+\alpha)}{4+4\alpha+\alpha^2 q_n^2} \cdot \exp\left(-q_n^2\, D_{TS} t/a^2\right) \tag{22}$$

where $\alpha = A/\Pi a^2 \beta_\gamma$ and q_n's are the nonzero roots of $\alpha q_n J_0(q_n) + 2J_1(q_n) = 0$.

The solution for finite cylinder geometry is obtained as the product of infinite slab and infinite cylinder solutions:

$$\left(\frac{M_{TS}(t)}{M^\infty_{TS}}\right)_\mu^{\text{for finite cylinder}} = 1 - \left[\left(\sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-q_n^2\ D_{TS}\ t/L^2\right)\right) \cdot \left(\sum_{n=1}^{\infty} \frac{4\alpha(1+\alpha)}{4+4\alpha+\alpha^2 q_n^2} \cdot \exp\left(-q_n^2\ D_{TS}\ t/a^2\right)\right)\right] \tag{23}$$

The solution for the cube and finite cylinder were obtained assuming that Newman's rule is valid for fixed finite values of α (Schwartzberg, 1980).

The drained curd that is added to the wash water carries with it a mass of whey solids which comes from whey adhering to the surface of the drained curd. Three refinements to the previously described equations were considered to account for the change in concentration of whey solids arising from entrained material, in addition to the most simple case which neglected the phenomenon and which considered only the diffusion of solids from curd pores (Case 1).

The first refinement (Case 2) assumes that the solids added to the wash water in the first 30 sec are due to the entrained whey, and subtracts this value from subsequent points as a correction. The second refinement (Case 3) is based upon an extrapolation of the initial points of increasing concentration over time for the first 90–120 sec back to zero time and subtraction of this intercept value from subsequent points.

The third refinement (Case 4) is based upon the conventional Prandtl boundary layer equations for flow past a flat vertical plate (Bird et al., 1960) as an estimation of the thickness of the whey layer after draining By solving these equations under the specific conditions of the problem:

$$h = \sqrt{\frac{2\mu D_p}{g\rho_w t_d}} \tag{24}$$

The Prandtl boundary layer correction is combined in Case 4 with another correction. The final correction involves estimation of the amount of whey trapped between curd particles as a result of capillary forces, based upon the assumption that the volume of liquid remaining between curd particles is 4% of the volume of the curd bed (Bear, 1972). The amount of whey solids contributed by such entrained liquid is subtracted from measured points to yield data sets applicable to the general diffusion model.

EXPERIMENTAL

Cheese samples

Samples of small curd cottage cheese were taken from a commercial creamery after cooking and before draining and were transported to the pilot plant. Curd was sieved in whey as described by Perry and Carroad (1980) between No. 4 ASTM mesh and No. 7 ASTM mesh screens to yield particles of average diameter 0.378 cm and average volume 0.0282 cm^3. Curd was cooled in whey to the experimental washing temperature (25°C) before washing. Curd was drained in No. 7 ASTM mesh screens for 30 min at 25°C to minimize whey entrainment.

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Washing

A 400-g sample was combined with 3000 ml of water (demineralized and then adjusted to pH 4.5 with H_3PO_4) at 25°C. Washing occurred in a 4000 ml baffled beaker situated in a thermostatically controlled water bath. Agitation was provided by a turbine impeller at 250 rpm. Seventeen samples were taken at specified times over a 3-hr period, filtered free of curd and fines through cotton, and assayed for total solids and lactose. Washing was repeated four times.

Assays

Total solids concentration of wash water was determined gravimetrically in duplicate using microwave drying (CEM Corporation, NC: CEM Moisture/Solids Analyzer AVC Model MP). Average curd density was determined by dividing a mass of curd by its volume as determined by water displacement. Lactose was determined spectrophotometrically (Acton, 1977).

Computation

Model equations for each geometry were fitted to experimental data by varying the value of the diffusivity to minimize least-squared differences. The eigenvalues q_n were obtained by using a modified Newton-Raphson method.

The first ten (10) terms of the series were evaluated when fitting the experimental data to the model equations. The β_γ value was calculated by using the following expression:

$$\beta_\gamma = \frac{<C_i^\infty>^\beta \cdot \frac{V_u}{V_{curd}}}{<C_i^\infty>^\gamma - <C_i^\infty>^\mu} \qquad (25)$$

A nonlinear least-squares program (Burroughs B-6700 computer, program BMD-07R) was employed to obtain the diffusion coefficients and their standard deviation. Model equations were also compared to the data by means of percent deviation (Heldman, 1974 according to:

$$\% \text{ Deviation} = \sqrt{\frac{\sum_{n=1}^{N} \frac{(VT - VE)^2}{VE}_n}{N - 1}} \times 100 \qquad (26)$$

where VT is the theoretical value of M_t/M_∞, VE is the experimental value, and N is the number of points considered.

RESULTS & DISCUSSION

THE INCREASE in concentration of total solids and lactose as washing of small curd cottage cheese proceeds is shown in Fig. 2. It is seen that the mass transfer is almost complete after 50 min, with most of the washing occurring in the first 20 min. After 20 min, 87% of the equilibrium concentration of total solids in the washing water has been achieved. At the same time, 93% of the quilibrium concentration of lactose has been reached, so that 78% of the concentration of components other than lactose is reached based on a mass balance. Constituents other than lactose include whey proteins which are expected to diffuse slowly, as well as salts and lactic acid.

The various model equations previously presented were fitted to the experimental data to verify the applicability of diffusion in accounting for the washing of whey from the curd structure. The three geometries with the several refinements (Cases 1–4) were tested. The fitting of equations to data yielded a diffusion coefficient which gave closest agreement between theory and experiment. Then, the diffusivity so determined was used to predict a curve of increasing concentration of total solids or lactose in the water with time.

All geometries were based on curd particles of volume 0.0282 cm^3. For the sphere, the radius was 0.189 cm. For the cube the side length was 0.304 cm. For the cylinder the radius was 0.189 cm and height was 0.252 cm. Density of unwashed curd was measured as 1.062 g/ml. Case 1 was a pure diffusion model. In Case 2, the ratios of total solids (or lactose) concentration at 30 sec in the water to that at equilibrium were subtracted from all subsequent data points. In Case 3, the concentration values obtained by extrapolation back to initial time for total solids and for lactose were subtracted from the data before empirical fitting of the equations.

For Case 4, the equivalent particle diameters were 0.378 cm for the sphere, 0.324 cm for the cylinder, and 0.304 for the cube. With viscosity of whey equal to μ_w = 1.18 × 10^{-2} g/cm-sec and density, ρ_w = 1.0273 g/cm^3 (Lim, 1970) and at a time of 900 sec, the thickness of the whey layer covering a curd particle, based on Prandtl boundary layer analysis, was found to be 9.92 × 10^{-5} cm for the sphere, 9.18 × 10^{-5} cm for the cylinder, and 8.89 × 10^{-5} cm for the cube. The total volume of whey assumed to be introduced into the washing system from surface covering was 0.593 cm^3 for the sphere, 0.641 cm^3 for the cylinder, and 0.660 cm^3 for the cube. The total volume of the curd bed (470.8 cm^3) was assumed to entrain 4% of its volume as whey based on capillary forces, or 18.8 cm^3. This value was added to the surface layer volumes for an average volume for all geometries of 19.43 cm^3 of surface whey carried into the washing system. This represents 0.77g of lactose and 1.32g of total solids.

Typical results are shown in Fig. 3 and 4 where the predicted curves for the pure diffusion case of spherical geometry are compared to the data of Fig. 2. Even for this simple case (Case 1), the model fits the data well. The diffusion coefficients at 25°C for all models tested are listed in Table 1. All values are of reasonable order of magnitude and the lactose values are uniformly larger than the associated total solids values, indicating that the whey proteins are more significantly influencing the diffusion of total solids than are the salts and low molecular weight components. Percent deviations and the theoretical and experimental values of the coefficient of the first term of the series are listed in Table 2. The sphere and cylinder models show the best fit to the data. In particular, the Case 4 sphere and cylinder models show the best fit to the data. In particular, the Case 4 sphere model shows highly acceptable agreement between experiment and theory. Associated with this model would be diffusion coefficients of 0.34 × 10^{-5} cm^2/sec for total solids and 0.38 × 10^{-5} for lactose, both at 25°C.

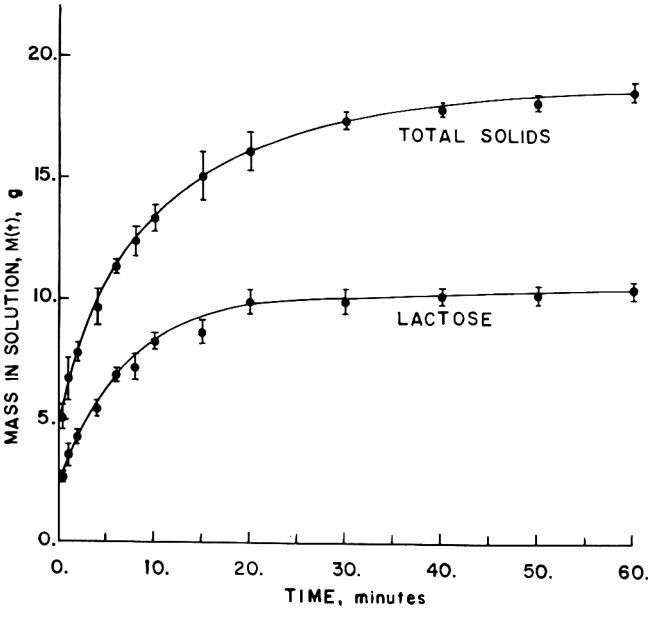


Fig. 2—Increasing concentration of total solids and lactose in washing water for first 60 min of washing small curd cottage cheese. Points represent average values of four experiments. Bars represent standard deviations.

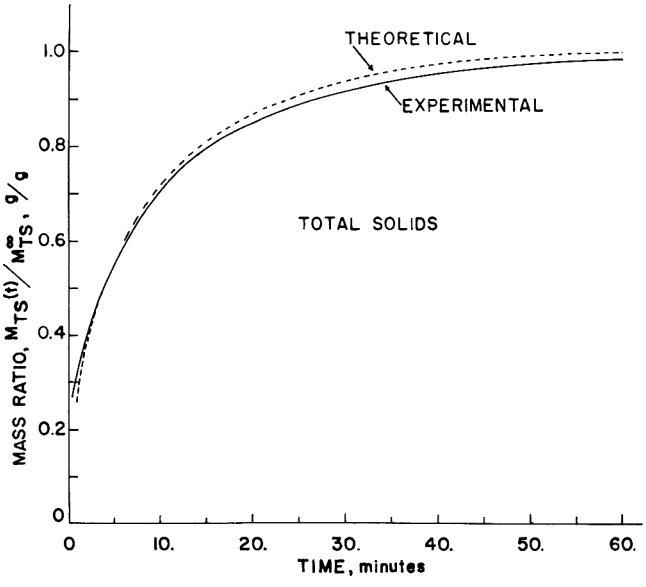


Fig. 3—Comparison of experimental and theoretical results of diffusion of total solids from small curd cottage cheese. Theoretical curve is for pure diffusion (Case 1) from a spherical particle. Data points (shown previously in Fig. 2) are omitted for clarity.

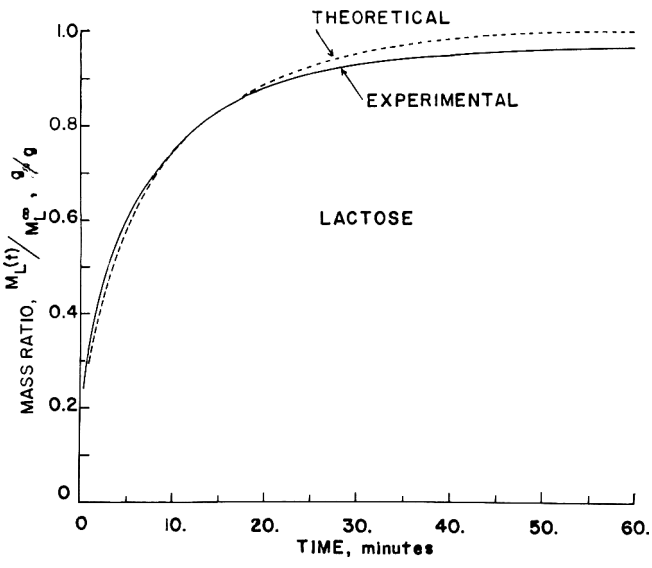


Fig. 4—Comparison of experimental and theoretical results of diffusion of lactose from small curd cottage cheese. Theoretical curve is for pure diffusion (Case 1) from a spherical particle. Data points (shown previously in Fig. 2) are omitted for clarity.

Table 1—Comparison of diffusivity values obtained from various models of washing of small curd cottage cheese[a]

	Diffusivity of total solids			Diffusivity of lactose		
	Cube	Cylinder	Sphere	Cube	Cylinder	Sphere
Case 1	0.28(0.061)[b]	0.51(0.047)	0.40(0.020)	0.34(0.050)	0.53(0.041)	0.44(0.022)
Case 2	0.22(0.066)	0.32(0.055)	0.24(0.029)	0.25(0.072)	0.35(0.068)	0.29(0.035)
Case 3	0.19(0.071)	0.29(0.070)	0.23(0.039)	0.27(0.088)	0.39(0.077)	0.32(0.029)
Case 4	0.26(0.050)	0.42(0.038)	0.34(0.009)	0.32(0.045)	0.40(0.036)	0.38(0.010)

[a] All values are times 10^{-5} cm^2/sec
[b] Numbers in parentheses represent standard deviation based upon four experimental determinations

Table 2—Percent deviation between experimental data and predicted increases in concentration based on models. Experimental and theoretical values of the coefficient of the first of the series for various models

	Total solids			Lactose		
	Cube	Cylinder	Sphere	Cube	Cylinder	Sphere
Case 1	3.62	3.18	2.30	4.02	4.10	3.88
Case 2	6.36	6.53	5.56	5.86	6.39	5.72
Case 3	8.81	9.12	8.14	6.22	6.40	5.88
Case 4	2.20	2.71	1.82	3.52	3.46	3.20

B_1 (coefficient of the first term of the series)	Cube	Cylinder	Sphere	Experimental
	0.498	0.532	0.581	0.571

The results show that a diffusive mass transfer model describes adequately the washing of whey components from small curd cottage cheese, and that such a model yields a diffusion coefficient of expected magnitude (i.e., 10^{-5} cm^2/sec). Such results can be applied to optimization of the washing process to remove preferentially the unwanted whey species and can be extended to remove the isothermal assumption.

NOMENCLATURE

a = radius of the curd particle, cm
A = cross-sectional area of curd particle, sq cm
b = solution extent, cm
$\langle C_i \rangle^{\gamma}$ = concentration of component 'i' in the liquid inside the particles (γ-phase), g/cu cm
$\langle C_i^o \rangle^{\gamma}$ = initial concentration of component 'i' in the liquid inside the particles (γ-phase), g/cu cm
$\langle C_i^{\infty} \rangle^{\mu} = \langle C_i^{\infty} \rangle^{\gamma}$ = concentration of component 'i' at infinite time, g/cu cm
D^i_{eff} = effective diffusion coefficient of component 'i', sq cm/sec
D_{TS} = effective diffusion coefficient of total solids, sq cm/sec
D_p = particle diameter, cm
g = gravity acceleration, sq cm /sec
h = whey film thickness, cm
J_0 = Bessel function of order zero
J_1 = Bessel function of order one
K = partition coefficient
L = thickness of the infinite slab, cm
$M_{TS}(t)$ = mass of total solids in the liquid phase at time 't', g
M^{∞}_{TS} = mass of total solids in the liquid phase at infinite time, g
N = number of data points
q_n = nonzero roots of the transcendental equations
r = radius of the particles, cm
t = time, min
t_d = drainage time (min)

V_γ = volume of whey trapped (γ-phase) sq cm
V_μ = volume of the solution, sq cm
V_{curd} = volume of curd sq cm
VE = experimental value of M_{TS}/M_∞
VT = predicted value of M_{TS}/M_∞
x = main coordinate in the slab case
α = volume of liquid/volume of liquid occluded into the particles
β_γ = fraction of solid volume occupied by the occluded liquid
μ_w = whey viscosity, g/cm sec
μ = wash water phase
ρ_w = whey density, g/cu cm
γ = whey phase
κ = casein phase

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intravenous route, a barrier to histamine absorption must be present in the gut. A proposed barrier is the relatively large amount of mucin to retard histamine transport out of the gut combined with a series of metabolic processes which detoxify histamine as it is freed from the mucin (Parrot and Nicot, 1965). Results of studies with two amines present in fish (cadaverine and putrescine) indicate that histamine metabolism is affected little by these substances when administered orally (Jung and Bjeldanes, 1979; Parrot and Nicot, 1965).

The inhibition of histamine binding to mucin by diamines and polyamines observed in the present study is consistent with the hypothesis that these substances can liberate histamine from intestinal mucin and increase the amount of histamine absorbed in the intestinal lumen (Jung and Bjeldanes, 1979; Parrot, 1948; Parrot and Nicot, 1965; Parrot, 1949), which results in an increase in histamine toxicity. Of the amines studied, spermine and spermidine may play a major role in the potentiation of histamine toxicity from histamine-spiked wholesome tuna fish. The low levels of cadaverine and putrescine and the absence of apparent potentiation effects or interaction among the amines on histamine binding to mucin suggest that cadaverine and putrescine may play a lesser role in histamine toxicity from ingestion of spiked wholesome tuna. Further studies in vivo are required to substantiate the proposed role of gastric mucin as a barrier to absorption of histamine in the gut.

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Heat Transfer During the Washing of Small Curd Cottage Cheese

JUAN A. BRESSAN and PAUL A. CARROAD

ABSTRACT

The heat transfer which occurs during the cooling of small curd cottage cheese during washing is mathematically modeled as a conduction process. The thermal diffusivity for curd was determined experimentally and shown to agree with predictions of correlations in the literature. Heat transfer occurs rapidly in curd, and is a relatively less significant design basis for washing systems than mass transfer.

INTRODUCTION

THE WASHING of cottage cheese curd involves simultaneous heat and mass transfer. This is true both for batch washing systems as described by Emmons and Tuckey (1967) in which temperature is lowered stage-wise by using progressively colder wash waters as well as in continuous washers. Previous work (Bressan et al., 1981a, b) investigated the mass transfer phenomenon associated with curd washing and has yielded a mathematical model based on diffusive mass transfer which acceptably describes isothermal washing and an expression for variation in the effective mass transfer diffusion coefficient with temperature.

The previous work is extended in this paper to ascertain the relative importance of heat transfer during curd washing compared to the mass transfer phenomenon. Such analysis is important in establishing a methodology for designing curd washing systems. A mathematical model describing heat transfer in small curd cottage cheese particles is proposed and experimentally verified. The thermal diffusitivity of curd is determined and compared to values predicted by correlations in the literature, showing close agreement. Comparison is made between the rates of heat and mass transfer.

THEORY

SMALL CURD COTTAGE CHEESE particles are modeled as spheres of radius = a and uniform initial temperature T_i for heat transfer analysis. In the washing process, curd is contacted batch-wise with well-stirred water of mass = m_w, specific heat = c_w, and initial temperature = T_o. The system is assumed to be adiabatic (i.e., no loss of heat from the washing system to the surroundings).

The surface temperature of the spheres is assumed equal to the temperature of the bulk fluid for all times $t > o$. This assumption corresponds to assuming that the film heat transfer resistance is negligible, or equivalently that the film heat transfer coefficient is large. The coefficient may be estimated by using the correlation of Brian and Hales (1969) as presented by Loncin and Merson (1979) for particles in suspension:

$$\frac{Nu}{(Pr)^{1/3}} = \left(\frac{D_p^4 \cdot P/m}{\nu^3}\right)^{1/6}$$

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By calculating P/m from a power number correlation (Foust et al., 1960), the film coefficient is estimated to be approximately 9000 watt/$m^2 \cdot K$. The corresponding Biot number is 45. By inspecting the Heisler (1947) charts for spheres, one concludes that under the conditions of this study the film heat transfer resistance can be neglected.

Considering only radial conduction of heat, the governing equation is:

$$\frac{\partial T}{\partial t} = \alpha\left(\frac{\partial^2 T}{\partial_t 2} + \frac{2}{r}\frac{\partial T}{\partial r}\right) \quad (2)$$

The initial condition is: at $t = o$, $T = T_i'$ (3)

where T_i' is the constant value, uniform throughout the sphere, equal to $T_i - T_o$.

The boundary conditions are:

$$\text{at } r = o(t>0), \qquad \frac{\partial T}{\partial r} = o \quad (4)$$

$$\text{at } r = a(t>o), \qquad -4\pi a^2 K\frac{\partial T}{\partial r} = m_w \cdot C_w \cdot \frac{\partial T}{\partial t} \quad (5)$$

The solution to the system of Eq (2) to (5), which gives the temperature profile for a particle is (Carslaw and Jaeger, 1959):

$$T_c'(t) = \frac{T_i'}{K+1} - \frac{2KaT_i'}{3r}\sum_{n=1}^{\infty} e^{-\alpha q_n^2 t/a^2} \left(\frac{K^2 q_n + 3(2K+3)q_n^2 + 9}{K^2 q_n^4 + 9(K+1)q_n^2}\right) \cdot \sin\left(\frac{q_n \cdot r}{a}\right) \cdot \sin(q_n) \quad (6)$$

The temperature of the fluid (r = a) is given by:

$$T_w'(t) = \frac{T_i'}{K+1} - 6KT_i'\sum_{n=1}^{\infty} e^{-\alpha q_n^2 t/a^2} \cdot \left(\frac{1}{K^2 q_n^2 + 9(K+1)}\right) \quad (7)$$

In Eq (6) and (7), the q_n's are the roots of:

$$\tan q_n = \frac{3q_n}{3+Kq_n^2} \quad (8)$$

The ratio of the heat capacity of the fluid to the heat capacity of the curd is:

$$K = \frac{3m_w C_w}{4\pi a^3 \rho_c C_c} = \frac{m_w C_w}{m_c C_c} \quad (9)$$

In considering cooling of the curd, which is important for its keeping quality, the temperature profile of the slowest cooling point where r = o is of interest. The temperature profile at the center can be derived from Eq (6) by calculating the following limit:

$$\lim_{r\to o}\left[\frac{\sin(q_n r/a)}{r}\right] \quad (10)$$

Applying L'Hospital's rule:

$$\lim_{r\to o}\left[\frac{\sin(q_n r/a)}{r}\right] = \frac{q_n}{a} \quad (11)$$

Substituting Eq (11) into Eq (6) yields:

$$T'_{c \text{ at center}}(t) = \frac{T'_i}{K+1} - \frac{2}{3} KT'_i \sum_{n=1}^{\infty} e^{-\alpha q_n^2 t/a^2} \cdot \left[\frac{K^2 q_n^4 + 3(2K+3)q_n^2 + 9}{K^2 q_n^4 + 9(K+1)q_n^2}\right] \cdot q_n \cdot \sin(q_n) \quad (12)$$

Eq (6) and (7) may be rearranged to yield the dimensionless temperature profiles:

$$\theta_c = \frac{T_{center}(t) - T_o}{T_{\infty center} - T_o} = 1 - \frac{2}{3} K(K+1) \sum_{n=1}^{\infty} e^{-\alpha q_n^2 t/a^2} \cdot \left[\frac{K^2 q_n^4 + 3(2K+3)q_n^2 + 9}{K^2 q_n^4 + 9(K+1)q_n^2}\right] \cdot q_n \cdot \sin(q_n) \quad (13)$$

$$\theta_w = \frac{T_w(t) - T_o}{T_\infty - T_o} = 1 - 6K(K+1) \sum_{n=1}^{\infty} e^{-\alpha q_n^2 t/a^2} \cdot \left[\frac{1}{K^2 q_n^2 + 9(k+1)}\right] \quad (14)$$

EXPERIMENTAL

UNWASHED small curd cottage cheese was sampled at cooking temperature (58°C) from a commercial creamery vat after cooking. Curd was sieved in whey as described by Perry and Carroad (1980) to obtain a fraction of mean particle radius a = 1.89 mm. Curd was drained for 30 min at 58°C. Drained curd (400g) was added to tap water (3000 ml) at 23.9°C in a baffled and insulated beaker. The mixture was continuously agitated at 250 rpm by a turbine impeller (four blades, impeller diameter 5 cm) as necessary to maintain curd particles in suspension without breaking them. Liquid temperature was measured by a digital thermometer (Hewlett-Packard 2802A); readings were taken at 5 sec intervals. Experiments were repeated three times, with average values reported.

The mathematical model was fitted to experimental data using a nonlinear least-squares program (Burroughs 6700 computer) to yield a value of thermal diffusivity which minimized differences. In such a calculation, the roots of Eq (8) were determined from a computational algorithm using a modified Newton-Raphson method.

RESULTS & DISCUSSION

Specific heat

The specific heat of cottage cheese curd was estimated in two ways, using the equation proposed by Dickerson (1969):

$$C_p = 0.4 + 0.006 \cdot (\text{water content, \%}) \quad (15)$$

and that proposed by Heldman (1975):

$$C_p = 0.34 \cdot X_c + 0.37 \cdot X_p + 0.4 \cdot X_f + 0.2 X_a + X_m \quad (16)$$

where X is mass fraction and the subscripts denote c = carbohydrate, p = protein, f = fat, a = ash, and m = moisture.

Both equations resulted in the same estimate of $C_{p\ curd}$ = 0.87. This value of specific heat of curd was combined with the stated mass ratio of curd to water used experimentally to yield K = 8.62.

Thermal diffusivity and temperature profiles

The experimentally determined dimensionless temperature profile for the liquid surrounding the curd particles is shown in Fig. 1, along with the theoretical curve [Eq (14)] which best fitted the data (r^2 = 0.94). The associated value of thermal diffusivity is $\alpha = 1.13 \times 10^{-3}$ cm²/sec. Using this value of thermal diffusivity in Eq (13) yielded the temperature profile at the center of a curd particle, also shown in Fig. 1.

Thermal diffusivity values for cottage cheese curd are calculable from correlations available in the literature. According to Riedel (1969), for products containing at least 40% water and at temperatures between 0°C and 100°C:

$$\alpha_{Riedel} = 0.0885 \times 10^{-6} + [(\alpha_{water} T - 0.0885 \times 10^{-6}) \cdot X_{water}\ (\text{cm}^2/\text{sec})] \quad (17)$$

For the experimental conditions of this study, $\alpha_{Riedel}^{20°C} = 1.32 \times 10^{-3}$ cm²/sec.

Sweat and Parmelee (1978) proposed the following correlation for thermal conductivity of dairy products:

$$k\ \frac{\text{watt}}{\text{m·C}} = 0.141 + [0.00412 \cdot (X_{water}, \%)] \quad (18)$$

For this study, k = 1.11×10^{-3} cal/cm·sec·C (= 0.465 watt/m·C). Combining this value of thermal conductivity with the previously stated specific heat value and an experimentally determined mass average density of 1.062 g/cm³, yields a value of $\alpha_{Sweat\ \&\ Parmlee} = 1.20 \times 10^{-3}$ cm²/sec.

Theoretical temperature profiles for the liquid were recalculated using these values of thermal diffusivity and selected points are illustrated in Fig. 1. There is very little difference between the profile based on the thermal diffusivity value obtained by best fitting the model to the data and those based on using the values of thermal diffusivity obtained from correlations. The mathematical model works well and the thermal diffusivity value of curd which was

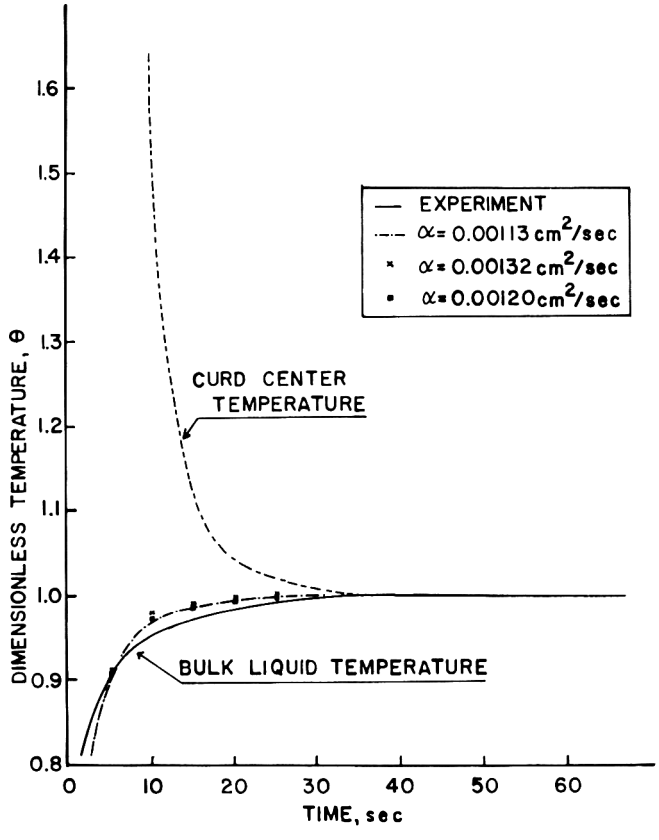


Fig. 1—Dimensionless temperature profiles for liquid surrounding curd and for center of curd particle during washing. Insert identifies different bulk liquid temperature profiles.

determined in this study agrees with the values predicted from existing correlations.

Effect of changes in K

The value of K is determined by the mass ratio of water to curd and is a variable in the design of curd washing process. Fig. 2 illustrates the result on the temperature profile of varying K between 2 and 10. It is evident that the time needed to reach thermal equilibrium is not appreciably affected by K.

CONCLUSION

RESULTS of this study indicate that the heat transfer process involved in batch washing of cottage cheese curd is quite rapid relative to the mass transfer process involved, the latter requiring approximately 20 min to reach equilibrium (Bressan et al., 1981a). For design purposes, heat transfer considerations may be neglected and mass transfer may be regarded as the limiting rate phenomenon which forms the basis of design for batch washing operations of cottage cheese curd.

NOMENCLATURE

a = radius of the curd particle, cm
B_i = Biot number
C_w = specific heat of water
C_c = heat capacity of curd, cal/g°C
D_p = particle diameter, cm
h = film heat transfer coefficient, watt/m^2°K
k = thermal conductivity, watt/m°C
K = ratio of the heat capacity of the fluid to that of the curd

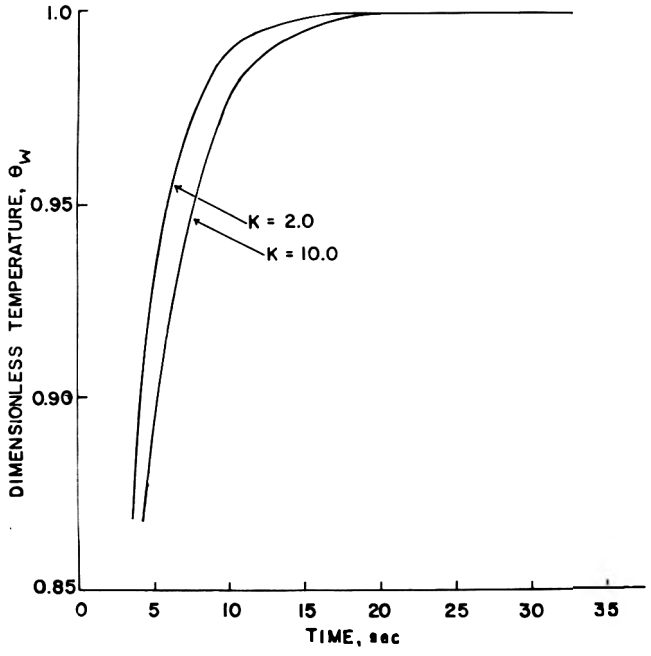


Fig. 2—Dimensionless temperature profile for liquid surrounding curd for various K values.

m_w = water mass, g
m_c = curd mass, g
Nu = Nusselt number
P/m = power of the agitator for kilogram of total mass, cm^2/sec^3
Pr = Prandtl number
q_n = roots of equation 7
r = particle radius, cm
t = time, sec
T = temperature (°C)
T_o = initial water temperature
T_i = initial curd temperature
$T_i' = T_i - T_o$ = initial curd temperature considering T_o as origin of temperature
$T_c'(t)$ = curd temperature at time t, considering T_o as origin of temperature
$T_w'(t)$ = water temperature at time t, considering T_o as origin of temperature
$T_{center}'(t)$ = particle center temperature at time (t) considering T_o as origin of temperature.
$T_w(t)$ = water temperature at time t
$T_{center}(t)$ = particle center temperature at time t
T_∞ = water equilibrium temperature
$T_{\infty\ (center)}$ = particle center temperature at equilibrium
x_{water} = mass fraction of water
α = thermal diffusivity, cm^2/sec
ν = kinematic viscosity, cm^2/sec
θ_c = dimensionless temperature ratio for curd center
θ_w = dimensionless temperature ratio for liquid

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Kinetics of Browning and Protein Quality Loss in Whey Powders During Steady State and Nonsteady State Storage Conditions

T. P. LABUZA and M. SALTMARCH

ABSTRACT

Comparison was made between the amount of browning and protein quality loss during storage of whey powder under steady state conditions (25, 35 and 45°C and a_w's of 0.33, 0.44 and 0.65 at each temperature) and a fluctuating temperature condition of 25/45°C with alternating 5 day periods at each temperature. The results showed that the maximum rate of browning and loss of protein quality (ϵ-DNP-lysine and RNV by Tetrahymena assay) occured at a_w 0.44 rather than at a higher a_w as is found for many dried foods. The Q_{10}'s for the reactions were about 5 and 3.7 respectively, for browning and protein quality loss. Of major importance was that storage losses at constant a_w, but under the fluctuating temperature condition, were significantly greater than at the mean temperature of 35°C. Using steady state data from the 25, 35 and 45°C conditions, the prediction of browning and protein quality losses for the fluctuating condition compared favorably (± 10%) to actual losses, based on the simple Arrhenius relationship which indicates that temperature history does not significantly change reaction mechanisms.

INTRODUCTION

WHEY POWDERS have received increased attention during the last 10 years as inexpensive and nutritious alternative ingredients in many food products. With this increased use, there has arisen a practical need to predict the shelf life of both whey powders and the food products into which they are incorporated as a principal ingredient.

Nonenzymatic browning via the Maillard reaction is one important mode of deterioration in whey powders which limits shelf life (Saltmarch et al., 1981). Whey powders contain relatively high concentrations of lactose and protein high in lysine content. In the presence of moisture these components readily participate in the Maillard reaction. This interaction may result in a decrease in protein quality which is accompanied or followed by undesirable color changes.

Both temperature and water activity storage conditions have an effect on the Maillard reaction. Studies by Henry et al., 1948; Coulter et al., 1949; Ben-Gara and Zimmerman, 1972; Huss, 1970, 1947a, b; Holsinger et al., 1973 have indicated that the Maillard reaction increases with increasing temperature in dehydrated milk products. Studies on a number of dry foods humidified over a wide range of water activities indicate a maximum for the rate of the Maillard reaction in the intermediate moisture food water activity range (Lea and Hannan, 1947; Hendel et al., 1955; Sharp, 1957; Sharp and Rolfe, 1958; Labuza, 1970; Wolfrom et al., 1974; Potthast et al., 1976). Specifically in dried milk powder stored at 40°C, Loncin et al. (1968) reported a maximum for both loss of lysine and brown pigment formation in the 0.6–0.7 water activity (a_w) range. A study conducted by Ben-Gara and Zimmerman (1972) indicated greater losses of chemically available lysine in nonfat dried milk stored at a_w's of 0.6 than 0.4 in the 20–40°C range. Burvall et al. (1978) found that in both lactose hydrolysed and convential dried milk powders, loss of biologically available lysine was greatest at an a_w of 0.62. The rate of the reaction is related to the state of the water. An increase in the water content (and thus the a_w) above the monolayer value acts to dissolve and mobilize reactant species for the Maillard reaction, thereby increasing the reaction rate. As a_w and water content continue to increase further, however, the amount of water present eventually begins to decrease the reaction rate as a result of dilution (Labuza, 1970; Eichner, 1975) and product inhibition (Eichner and Karel, 1972). The result is a maximum in rate at some intermediate a_w, usually around 0.6–0.8 (Labuza and Saltmarch, 1981).

The first objective of this study was to gain better understanding of the effect of both temperature and a_w on browning and protein quality loss in whey powders. The second objective was to determine if kinetic data from steady state storage conditions could be used to predict actual browning and protein quality losses that occur under fluctuating storage temperature conditions, based on the equations developed by Labuza (1979).

MATERIALS & METHODS

Materials

Two types of sweet whey powder were utilized in the present study. The first was a non-hygroscopic spray-dried sweet whey powder (Product No. 0425, Lot No. 0662, Mid-America Farms, Springfield, MO) and the second was a hygroscopic spray-dried sweet whey powder (Product No. 0400, Lot No. 5751, Mid-America Farms). Sweet whey powders were chosen for this study because they are commonly used by the food industry. The powders were stored under dry conditions ($a_w < 0.1$) at 4°C until utilized for the accelerated shelf life studies.

Methods

Whey powder samples for both the steady and non-steady state storage studies were prepared for storage by being equilibrated to the desired a_w using the methods of Saltmarch and Labuza (1980a, b). Samples in open containers (about ¼ in. thick) were placed in desiccators over the appropriate saturated salt solution ($MgCl_2$, 0.33 a_w; K_2CO_3, 0.44 a_w; $NaNO_2$, 0.65 a_w). The samples were equilibrated under vacuum for 2 wk at room temperature which was enough to affect the maximum degree of crystallization at each a_w. The a_w of the samples was measured at the end of this period as well as during storage by a vapor pressure manometric technique modified by Lewicki et al. (1978) to insure proper equilibration. There were no changes in a_w from the humidification value at any time, although as noted by Saltmarch and Labuza (1980a), the moisture content dropped very slightly at the 0.44 value as recrystallization takes place. No change occurred in moisture content of a_w 0.33 since crystallization is limited. At a_w 0.66, adsorption of water by the protein masks any loss that may occur due to recrystallization (Saltmarch and Labuza, 1980a).

At the start of the storage studies, the samples were then transferred to desiccators at a_w's of 0.33, 0.44 and 0.65 and the desired storage temperature (25°, 35°, or 45°C) and stored open to the atmosphere. For the nonsteady state storage study, the open samples were transferred between chambers maintained at 25°C and 45°C

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every 5 days. Come-up time to temperature was less than 35 min. Samples were stored at least 210 days at 25°C, 95 days at 35°C, 35 days at 45°C and 80 days for the 25/45°C fluctuating storage. Sampling was spread out to include seven or eight sampling times during this period of storage. Under these conditions then, both a_w and temperature were controlled although moisture content could vary if equilibration were slow. However, since the moisture difference was small, this was not felt to be a problem.

Evaluation of brown pigment formation was done by a new three-enzyme modification of the Choi et al. (1949) method for milk powders. This method is based on the principle that the proteolytic enzymes trypsin, chymotrypsin and peptidase will more easily solubilize the brown pigments which can then be measured spectrophotometrically. This digest can also be used in the biological assay for protein quality. The major modification was incubating 1.5g in 50 ml of deionized, distilled water with 1.5 ml of the enzyme solution. This enzyme solution was prepared with 5.3 mg/ml trypsin (Type IX Crystallized Sigma No. T-1034), 10.3 mg/ml chymotrypsin (Type II, Sigma No. C-4129 and 4.3 mg/ml peptidase (Grade I, Sigma, No. P-7625) in 50 ml water.

At least duplicate measurements of three replicates were analyzed for the initial value and duplicate measurements of single samples at each time during storage.

Protein quality losses defined in this study were measured as loss of chemically available lysine (ϵ-DNP-lysine) using a 1, fluoro-2,4 dinitrobenzene tagging reagent in the Booth (1971) modification of the Carpenter (1960) method. The second method used was the *Tetrahymena thermophilia* bio-assay developed by Sutton (1978) where the amount of protozoan growth is presumed to correlate to the relative nutritional value (RNV) of a food. However, instead of visual counting, a Coulter counter was used. Sampling for the lysine analysis was done using seven or eight replicates for the initial value at each test condition and one analysis at each of the eight time periods during storage. For the RNV assay, triplicate counts of six replicates were used for the initial value and triplicate counts of two duplicate samples were analyzed at each of the eight time periods during storage.

Rate constants were obtained for brown pigment formation assuming a zero order rate relationship based on the work of Mizrahi et al. (1970a, b) on dried cabbage and as found for most dry foods (Labuza and Saltmarch, 1981). This rate relationship is defined as follows:

$$B = B_o + k_z t \quad (1)$$

where: B = brown pigment concentration at t; B_o = brown pigment concentration at t = 0; k_z = zero order rate constant; t = time. The early stages of protein quality loss via the Maillard reaction have been shown by Warmbier et al. (1976) to obey a first order reaction relationship in intermediate moisture model food systems. Based on this, a first order rate relationship was assumed for losses of ϵ-DNP-lysine and RNV in the whey powders as in Eq (2):

$$A = A_o \exp(-k_f t) \quad (2)$$

where: A = remaining ϵ-DNP-lysine or RNV at time t; A_o = ϵ-DNP-lysine or RNV at t = 0; k_f = first order rate constant; t = time.

The temperature dependence of browning and protein quality loss was determined by use of the typical Arrhenius relationship to obtain activation energies (E_A) and Q_{10} values.

Actual brown pigment formation and protein quality losses in the whey powders stored under fluctuating temperature conditions were compared to predicted losses using the kinetic model solutions derived by Hicks (1944), Schwimmer et al. (1955), and Labuza (1979). These kinetics are based on the simple Arrhenius relation, that the rate increases logarithmically as an inverse function of absolute temperature and that a history effect is not important (i.e., storage at one temperature does not influence the rate constant at another temperature). Labuza's (1979) solutions simplify the mathematics by using Q_{10}'s rather than activation energies (E_A). Kamman and Labuza (1981) showed this to be an acceptable model for prediction of thiamin loss in pasta during fluctuating temperature storage of pasta held at constant moisture content (sealed bags). For brown pigment formation, Eq (3) for a zero order reaction undergoing a square-wave storage temperature sequence was utilized:

$$B = B_o + (\tfrac{1}{2} k_{T_m} t)\left[Q_{10}^{\left[\frac{a}{10}\,\frac{T_m+10}{T_m+a}\right]} + Q_{10}^{-\left[\frac{a}{10}\,\frac{T_m+10}{T_m-a}\right]}\right] \quad (3)$$

where: B = brown pigment concentration at total time t (measured at end of each period); B_o = brown pigment concentration at time zero; k_{T_m} = rate at constant mean temperature T_m (°K); a = amplitude of square-wave fluctuation = 10°C; Q_{10} = (Rate at T + 10)/Rate at T where: $\log_{10} Q_{10} = 2.2\, E_A/(298.15)(308.15)$ and T = temperature in °Kelvin.

For the fluctuating condition, the predicted effective rate constant where a = 10 is:

$$k_{eff} = \frac{k_{T_m}}{2}\left[Q_{10} + Q_{10}^{-\left[\frac{T_m+10}{T_m-10}\right]}\right] \quad (4)$$

For the loss of ϵ-DNP-lysine and relative nutritional value, a first order reaction solution gives Eq (5):

$$A = A_o e^{-k_{eff}\, t} \quad (5)$$

where: A = remaining ϵ-DNP-lysine or RNV at t; A_o = ϵ-DNP-lysine or RNV at time zero; k_{eff} = fluctuating rate constant as in Eq. (4).

RESULTS & DISCUSSION

Rate constants (O.D./g solid per day) for brown pigment formation listed in Table 1 were obtained from the slopes of linear plots of measured brown pigment concentration (O.D. units/g solids whey powder) versus time for each temperature and a_w treatment condition. Fig. 1 shows a typical plot for the fluctuating condition where the average value is plotted at each time. All other treatments showed similar straight lines with r^2 values of 0.99 or greater except for a few cases as noted in Table 1. The coefficient of variation for the browning measurement was 6%. Table 1 also indicates the 95% confidence limits for each rate constant. First order rate constants listed in Table 2 for loss of ϵ-DNP-lysine were derived from the slopes of semi-log plots of fraction remaining ϵ-DNP-lysine versus time for each temperature and a_w treatment condition. Fig. 2 shows a plot of these data at 25°C. In all cases the r^2 value for a first order reaction was larger than for zero order; however, at some conditions the data showed poorer correlation than in browning because of the greater variability of the lysine

Table 1—Brown pigment formation rate constants [(OD/g solids/day) x 10^4 [a]

Whey powder	a_w	25°C	35°C	45°C	Fluctuating temperature[b]	Activation energy kcal/mole	Q_{10}
Hygroscopic	0.33	3.67 ± 0.26	18.25 ± 2.2(B)	137.2 ± 10.2	64.7 ± 2.6	34.03	6.52
	0.44	8.46 ± 0.40	34.54 ± 1.7	236.2 ± 19.2	109.5 ± 3.8	32.25	5.92
	0.65	5.98 ± 0.36	21.4 ± 2.4(B)	152.3 ± 20.3(B)	74.9 ± 5.84	30.32(B)	5.32
Non-Hygroscopic	0.33	2.80 ± 0.15	12.86 ± 0.76	85.04 ± 4.8	42.3 ± 1.9	32.08	5.87
	0.44	6.13 ± 0.40	28.77 ± 1.7	143.9 ± 23.0(A)	69.4 ± 2.3	29.6	5.12
	0.65	4.84 ± 0.27	16.5 ± 1.4	104.1 ± 3.8	48.5 ± 2.1	28.8	4.90

[a] ± 95% Confidence limits; For all cases $r^2 > 0.99$ except (A) $r^2 = 0.98$; (B) $r^2 = 0.86$.
[b] Alternating 5 days (25°C/45°C)

measurement (coefficient of variation 15%). It can also be seen that there are larger 95% confidence limits for each condition (generally greater than 20%) as contrasted with about 5% for the browning. Table 3 lists the calculated rate constants for loss in relative nutritional value (RNV) along with the relevant statistics. Because of the large coefficient of variation (10%) as measured on the initial values, the poorer fit as with free ϵ-DNP-lysine was expected. In a few cases the r^2 is less than 0.6 and the confidence range is ± 50% of the mean value. Even with a low correlation to first order, using a polynomial to fit the data would preclude testing the Arrhenius approach for prediction of unsteady state data from steady state conditions. Thus, the values were calculated and used as is, to thus test the predictability of Eq (5). Fig. 3 shows a plot of the RNV for hygroscopic whey stored at 45°C which showed the best correlations and the greatest losses. These results suggest that the RNV method may not be as useful as would be desirable to measure protein quality losses. This is unfortunate since animal studies would be far to expensive to use for determination of kinetic data and would probably show less correlation. It also should be noted that the RNV half life in Table 3 was greater than the lysine half life at 25 and 35°C, suggesting that lysine was not the limiting growth factor.

Several observations may be made about the effect of temperature and a_w on browning and protein quality losses in whey powders from the results presented. As has been noted in other studies on milk powders, the rates of browning and protein quality loss increase with storage temperature (Coulter et al., 1949; Henry et al., 1948. Ben-Gara and Zimmerman, 1972; Huss, 1970, 1974a, b; Holsinger et al., 1973). Secondly, the highest rate of browning and protein quality loss generally occurs at the 0.44 a_w condition studied. This value of course could be different if other a_w's were available near this range. There is some overlap of the 95% confidence limits with the higher a_w, which was not noted by Saltmarch et al. (1981). This is significant since Loncin et al. (1968) has found the maximum rate to be in the 0.6–0.7 a_w range in milk powders stored open at

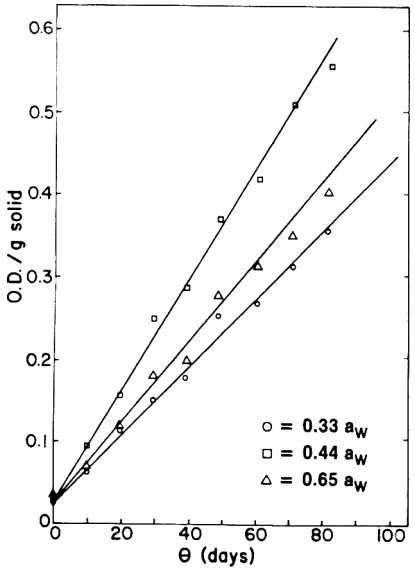


Fig. 1—Brown pigment formation in nonhygroscopic whey powder stored for five day alternating periods at 25 and 45° C.

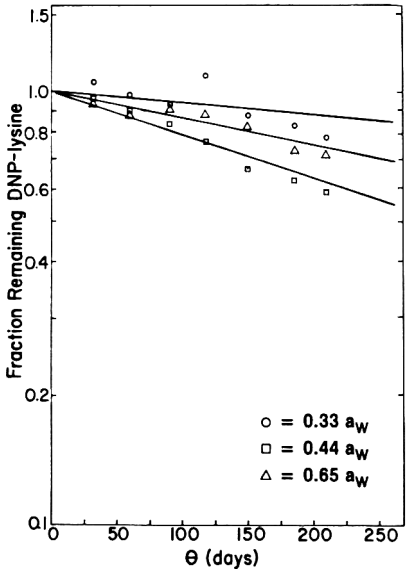


Fig. 2—Loss of DNP-lysine in nonhygroscopic whey powder (25° C).

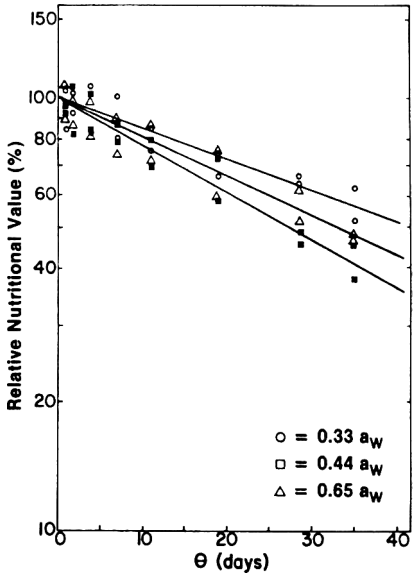


Fig. 3—Loss of relative nutritional value in hygroscopic whey powder (45° C).

Table 2—Rate constant and half-life for lysine loss (DNP-Method)[k_L (days)$^{-1}$ x 10^3][a]

Whey powder	a_w	25°C	35°C	45°C	Fluctuating temperature[b]	Activation energy kcal/mole	(r^2)	Q_{10}
Hygroscopic	0.33	1.15 ± 0.31	6.91 ± 1.26	18.5 ± 9.6	17.1 ± 2.7	25.11	(0.86)	3.99
	0.44	2.67 ± 0.17	13.94 ± 1.69	30.0 ± 11.0	18.0 ± 1.2	22.20	(0.90)	3.40
	0.65	1.81 ± 0.6	9.61 ± 1.34	20.9 ± 7.4	15.7 ± 0.9	23.04	(0.89)	3.56
Nonhygroscopic	0.33	1.12 ± 0.5	7.88 ± 0.8	13.3 ± 4.6	13.7 ± 1.6	23.89	(0.81)	3.73
	0.44	2.53 ± 0.2	13.3 ± 1.0	21.6 ± 3.7	17.9 ± 3.7	20.19	(0.90)	3.05
	0.65	1.59 ± 0.4	10.2 ± 2.2	20.8 ± 3.9	14.6 ± 2.3	24.45	(0.91)	3.85
			Half-life in days[c]					
Hygroscopic	0.33	602 (0.86)	100 (0.91)	38 (0.66)	41 (0.93)			
	0.44	260 (0.99)	50 (0.96)	23 (0.72)	38 (0.99)			
	0.65	383 (0.80)	72 (0.94)	33 (0.74)	44 (0.99)			
Nonhygroscopic	0.33	619 (0.73)	88 (0.97)	52 (0.75)	51 (0.96)			
	0.44	274 (0.86)	68 (0.89)	32 (0.91)	47 (0.94)			
	0.65	436 (0.86)	68 (0.89)	33 (0.91)	47 (0.94)			

[a] ± 95% Confidence limit (r^2 in bracket next to half-life)
[b] Five day alternating period 25/45°C
[c] r^2 in parentheses is for log ϵ-DNP-lysine vs days

40°C. Unfortunately, they did not furnish information about the range of a_w's tested. Burvall et al. (1978) found greatest losses of biologically available lysine at 0.62 water activity, but they did not measure losses at a_w's between 0.33 and 0.62. Saltmarch and Labuza (1980a) recently presented data which indicate that the maximum in rate at low a_w for whey powder is related to the transition of lactose from the amorphous to crystalline form. The lactose crystallization could result in a locally high water content region during the collapse of the amorphous lactose just prior to crystallization, which could act to increase the reaction rate. However, since most of the crystallization takes place during the early preincubation, this cannot explain the constant rate of browning throughout storage except that the remaining uncrystallized lactose is imbedded in the protein matrix in such a way that it is readily available for reaction, but not crystal formation. Recently, Kim et al. (1981) compared the extent of browning in whey stored open as in this study versus in closed containers after similar prehumidification. It was found that the sealed samples at a_w 0.44 showed a faster browning rate than the open ones because the water lost from the lactose during recrystallization remains in the pouch and increases the overall a_w of the system. This does not occur with open storage. It should be noted that despite the fact that the nonhygroscopic whey was 95% crystallized (see Saltmarch and Labuza, 1980b), the loss of RNV and lysine was within the 95% confidence limits for the hygroscopic whey and the browning rate was only slightly less for each condition. This suggests that the low level of amorphous lactose in both systems is still in excess, relative to the concentration of reactive amines. Thus, lactose is not rate limiting which may not be the case in other foods where diffusion and concentration limitations cause the maximum rate to occur at a higher a_w value.

The major finding in this study is that the rates of browning and protein quality loss under square-wave fluctuating temperature conditions exceed those found at the calculated mean temperature of 35°C. Hicks (1944), Schwimmer et al. (1955) and Labuza (1979) predicted that the reaction rate under fluctuating conditions would be greater than under steady state conditions at the calculated mean temperature. Wu et al. (1974, 1975a, b) showed this in a series of studies on enzyme and protein denaturation under sine-wave conditions. However, their studies were limited because of using zero order kinetics for a first order reaction in addition to problems of come-up and come-down times. Kamman and Labuza (1981) also found a faster rate for the simple loss of thiamin in pasta stored under a square wave condition and showed Eq (3) and (5) to be good for prediction of losses in a product stored under fluctuating temperature conditions. It should be noted in Fig. 1 that a straight line was obtained by plotting the results of analyses made at the end of each fluctuating period. This was also found for RNV and ϵ-DNP results as noted by the high r^2 values, and thus, suggest that moisture changes during the temperature fluctuation do not affect the reaction rate.

Tables 1 and 3 list the activation energies calculated from a plot of ℓn k vs 1/T. The values were obtained by calculating the best regression line through the extremes of the 95% confidence limits for each test condition. Because of the high degree of confidence for the browning data, good correlations exist for the activation energies. Values in the 30–34 kcal/mole range should be expected based on surveys of the literature for browning (Labuza and Saltmarch, 1981; Warmbier et al., 1976). For loss of ϵ-lysine, a poorer correlation was found (Table 3), however, the best straight line through all three temperatures gives and E_A of 20–25 kcal/mole which is what is usually found for lysine loss. It can also be seen that a_w has little effect on the activation energy if the 95% confidence limits are used in comparing the systems.

Table 3 lists the activation energies for loss of RNV. Except at a_w 0.33 for both whey powders, the activation energy is close to that for lysine loss. Although the overall correlations were poorer for the actual RNV data as compared to lysine loss, in every case a much better correlation in the Arrhenius plot was found.

Actual brown pigment formation, loss of ϵ-DNP-lysine and loss of relative nutritional value data for the hygroscopic whey stored under nonsteady state temperature conditions were compared in Table 4 to predicted values obtained by using Eq (3) and (5). In almost all cases the predictions were quite good despite the poorer fit for the steady state protein quality data. These results would then justify the procedure of using steady state temperature data for prediction of changes in fluctuating temperature conditions, even when there is a possible break in the activation energy plot. It would also seem that the prior temperature history a product goes through is not very important in the browning reaction and does not change the reaction rate constant at other temperatures. Thus, the Arrhenius approach is applicable in prediction of unsteady state changes from constant condition data for browning and protein quality loss.

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Table 3—Rate constant and half-life for loss of relative nutritional value (RNV) [rate constant[a] $(days)^{-1} \times 10^3$]

Whey powders	a_w	25°C	35°C	45°C	Fluctuating temperature[b]	Activation energy kcal/mole	(r^2)	Q_{10}
Hygroscopic	0.33	0.53 ± 0.23	3.62 ± 1.63	15.6 ± 3.6	9.86 ± 1.31	32.6	(0.92)	6.04
	0.44	2.16 ± 0.48	8.10 ± 2.91	25.5 ± 3.8	12.32 ± 0.4	23.2	(0.94)	3.59
	0.65	1.56 ± 0.12	5.49 ± 1.22	20.8 ± 3.3	8.69 ± 1.39	24.3	(0.98)	3.82
Nonhygroscopic	0.33	0.73 ± 0.33	3.64 ± 1.48	15.5 ± 4.2	8.23 ± 1.25	29.5	(0.91)	5.09
	0.44	1.95 ± 0.36	7.67 ± 1.20	24.6 ± 4.8	11.56 ± 1.06	23.9	(0.97)	3.74
	0.65	1.40 ± 0.31	5.14 ± 1.39	18.5 ± 3.5	9.64 ± 1.12	24.4	(0.95)	3.84
			Mean half-life in days[c]					
Hygroscopic	0.33	1307 (0.57)	191 (0.52)	44 (0.81)	70 (0.93)			
	0.44	321 (0.83)	86 (0.63)	27 (0.91)	56 (0.91)			
	0.65	444 (0.84)	126 (0.82)	33 (0.90)	80 (0.89)			
Nonhygroscopic	0.33	949 (0.54)	190 (0.57)	45 (0.75)	84 (0.90)			
	0.44	356 (0.88)	90 (0.90)	28 (0.85)	60 (0.96)			
	0.65	495 (0.83)	135 (0.75)	37 (0.86)	72 (0.94)			

[a] ± 95% Confidence limits
[b] Five day alternating period 25/45°C
[c] r^2 In parentheses is for regression of log RNV vs time

A further comparison is made in Table 5 where the actual and predicted rate constants for the fluctuating condition are compared between themselves as well as to the constant 35°C values. Again, it is quite clear that the constant 35°C temperature rates would underpredict the rate in fluctuating storage conditions. For browning, the predicted rate constants are generally smaller than the actual value but are (except for one case) close to the 95% confidence limits. The predicted protein loss rate constants are higher (except for one case) possibly due to the poorer correlation of the Arrhenius plot. The values, however, are close to the 95% confidence limits of the actual value. Because only three temperatures are used, the 95% confidence limits for E_A, Q_{10}, and therefore, the k_{eff} are so large that it was not meaningful to use them in the comparison to the actual losses in the fluctuating condition. Overall, however, these results could be interpreted to suggest that the kinetic model for square-wave predictions is quite good for both zero order and first order reactions, even when a phase change of one of the reactants (lactose) may be taking place. In most cases, the difference between predicted and actual amount of change is ± 10% which would seem to be quite acceptable given the analytical limitations. Since most food products during storage do not undergo as drastic a temperature shift, i.e., they would usually follow a sine wave, it is left to be tested whether a similar equation for sine wave storage will give as good a prediction. Finally, the results suggest that significant protein quality loss can occur in whey powder during normal plant storage with little difference between hygroscopic and non-hygroscopic whey powders if left in an unprotected condition or if sufficient moisture gain occurs through the bags to raise the a_w close to the crystallization point.

Table 4—Experimental vs predicted values during storage of hygroscopic whey powder in a fluctuating temperature sequence [Browning (OD/100 g solids)]

Day	0.33 a_w		0.44 a_w		0.65 a_w	
	BP_e[a]	BP_p[b]	BP_e	BP_p	BP_e	BP_p
0	1.9	1.9	1.9	1.9	2.1	2.1
20	16.4	14.1	21.4	22.8	19.4	13.9
40	29.4	26.2	43.9	43.8	30.0	25.6
60	41.9	38.4	69.6	64.8	47.2	37.4
80	54.7	50.5	89.1	85.8	63.0	49.1

Fraction remaining lysine

Day	0.33 a_w		0.44 a_w		0.65 a_w	
	DNP_p[a]	DNP_e[b]	DNP_p	DNP_e	DNP_p	DNP_e
0	1.00	1.00	1.00	1.00	1.00	1.00
20	0.53	0.74	0.64	0.60	0.66	0.69
40	0.44	0.56	0.48	0.36	0.52	0.48
60	0.33	0.42	0.35	0.22	0.38	0.33
80	0.30	0.31	0.32	0.13	0.27	0.23

Relative nutritional value

Day	0.33 a_w		0.44 a_w		0.65 a_w	
	RNV_e[a]	RNV_p[b]	RNV_e	RNV_p	RNV_e	RNV_p
0	100.0	100.0	100.0	100.0	100.0	100.0
20	82.4	80.0	75.3	73.2	83.9	80.0
40	65.8	63.9	59.9	53.6	71.9	64.0
60	51.1	51..1	47.3	39.2	56.8	51.3
80	46.4	40.9	38.7	28.7	51.7	41.0

[a] Experimental value for storage at 25/45° C alternating 5 days
[b] Predicted from either Eq (3) or (5)

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Table 5—Comparison of rate constants for browning and protein quality loss under fluctuating and 35° C storage conditions

		Brown pigment mean formation rate (OD/g solid/day) x 10^4			Mean loss rate constant for ε-DNP-Lysine ($days^{-1}$) x 10^3			Mean loss rate constant for relative nutritional Value ($days^{-1}$) x 10^3		
		Fluctuating		35°C	Fluctuating		35°C	Fluctuating		35°C
	a_w	Exp.	Predicted	Exp.	Exp.	Predicted	Exp.	Exp.	Predicted	Exp.
Hygroscopic	0.33	64.7 ± 2.6	60.7	18.3	17.1 ± 2.7	14.6	6.9	9.9 ± 1.3	11.2	3.6
	0.44	109.5 ± 3.8	104.8	34.5	18.0 ± 1.2	25.6	13.9	12.3 ± 0.4	15.6	8.1
	0.65	74.9 ± 5.8	58.8	21.4	15.7 ± 0.9	18.4	9.6	8.7 ± 1.4	11.1	5.5
Nonhygroscopic	0.33	42.3 ± 1.9	38.7	12.9	13.7 ± 1.6	15.7	7.8	8.2 ± 1.3	9.6	3.6
	0.44	69.4 ± 2.3	76.2	28.8	17.9 ± 3.7	22.3	13.3	11.6 ± 1.1	15.3	7.7
	0.65	48.5 ± 2.1	41.9	16.5	14.6 ± 2.3	20.8	10.2	9.6 ± 1.1	10.5	5.1

Shape Characterization for a Simple Diffusion Analysis of Air Drying of Grains

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ABSTRACT

This work proposes a simple method to characterize which of the elemental geometries (slab, cylinder or sphere) would be a better approximation for the diffusion analysis of air drying of grains having "nonconventional shapes." The method is tested with experimental drying data obtained for soybeans, rough rice, wheat and peanuts.

INTRODUCTION

SEVERAL STUDIES have been made on the rate of moisture diffusion through cereal grains and oilseeds, mainly in connection with problems of drying. Diffusion models have been applied for the drying of wheat (Becker and Sallans, 1955), sorghum (Suárez et al., 1980b), soybeans (Suárez et al. 1980a), among many others.

Various grains and oilseeds do not have "conventional" shapes, that is those which may be assimilated to one of the most simple geometries such as a slab, a sphere or a cylinder. Although the diffusion equation may be solved for nonconventional shapes (Rotstein et al., 1974), it is more usual to assimilate grain shape to that of a sphere, slab or cylinder for which solutions of Fick's equation are readily available. However, and depending on the particular grain shape it is not always easy to decide "a priori" which of the above simple geometries will best represent reality.

The objective of this work is to propose a simple method to characterize which of the elemental geometries (slab, cylinder or sphere) would be the most adequate for a grain having a "non-conventional" shape. This is useful to facilitate the analysis of drying data through a moisture diffusion model.

EXPERIMENTAL

Laboratory drier

The drier consists of a centrifugal fan which blows the air over electric bar elements into a chamber at the base and then upwards through a vertical duct. A single layer of grain was used for each drying experiment the progress of which was followed by weighing the sample periodically in a precision balance. The equipment and experimental procedure have been described in detail in a previous publication (Suárez et al., 1980b).

It is to be noted that the air velocity employed in this work produces fluidization of the layer of grains. However, this did not cause any damage or loss of materials.

Materials

Local varieties of soybeans, wheat, whole peanuts and rough rice were used as model grains for the drying experiments. It was necessary to add water to all grains for the drying experiments. This was done by humidification at 90% RH following the technique described by Suárez et al. (1980b). Moisture content of grains at equilibrium was found to be 29%, 26%, 24% and 20% (dry basis) for soybean, wheat, rice, and peanuts, respectively. These values were used as initial (and uniform) moisture content of the grains in the drying runs.

The grains were screened prior to humidification and drying to obtain samples of uniform size. In order to describe the geometry of the grains used, the following measurements were made. A dial micrometer was used to determine the major axis and minor axis of rice, wheat, and peanuts as well as the diameter of soybean. Forty grains for each product were measured and the results (average) are shown in Table 1.

Volumetric tests were also conducted to obtain radius estimates of different grains. The total volume of a large quantity of grain was measured using a picnometer (with toluene as the fluid). The average grain volume was calculated by dividing the total sample volume by the total number of grains. The equivalent spherical radius was then computed from the formula for the volume of a sphere using the average grain volume. As one may expect, some degree of shrinkage occurred during grain drying; therefore, the measurements were made on both, "wet" (i.e. grains rehumidified for drying) and fully dehydrated samples. The results are shown in Table 2 which shows the equivalent spherical radius at the initial moisture content (indicated and at dryness. It can be seen that the average shrinkage is only between 6–8%.

Moisture content of the grains was determined gravimetrically using a vacuum oven method at 70°C over magnesium perchlorate, and expressed on a dry basis (g water/g solids).

Equilibrium moisture content

Selected values of equilibrium moisture content at the various drying temperatures used were determined by equilibration against saturated salt solutions which provided known constant relative humidities (Iglesias et al., 1975). The measurements correspond to the desorption branch of the isotherm. Table 3 shows the values obtained and used for the drying plots.

RESULTS & DISCUSSION

ALL DRYING EXPERIMENTS were performed using an

Table 1—Geometric characterization of the grains used[a]

Grain	Minor axis (cm)	Major axis (cm)
Rough Rice	0.219	0.881
Wheat	0.281	0.558
Peanuts (whole)	0.786	1.32
Soybean	diameter (cm): 0.740	

[a] Values reported are the average of over forty samples of each different grain.

Table 2—Equivalent spherical radius of the different grains

Grains	ESR[a] of dry grain (cm)	Initial moisture content (% d.b.)	ESR[a] of "wet" grain (cm)	% Reduction in ESR
Soybean	0.715	29–31	0.772	8
Peanuts (whole)	0.801	18–20	0.870	7.9
Wheat	0.366	24–26	0.388	6
Rough Rice	0.356	24	0.378	6.2

[a] ESR = equivalent spherical radius

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air velocity of 13 m/sec, which as shown in previous publications (Suárez et al., 1980a, b) is enough to ensure that internal mass transfer is the main resistance to the rate of loss of moisture.

It is also noteworthy that the drying analysis of grains in the moisture range here considered, may be done by neglecting heat transfer effects and the process treated as a purely mass transfer one (Suárez et al., 1980a, b). In these conditions Fick's second law of diffusion may be used to fit the experimental data using the appropriate geometry.

Solutions of Fick's law for the most simple geometries

Infinite slab

$$m^* = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left\{-(2n+1)^2 \pi^2 \frac{Fo}{4}\right\} \quad (1)$$

Infinite cylinder

$$m^* = \sum_{n=1}^{\infty} \frac{4}{\mu_n^2} \left\{\exp -\mu_n^2 Fo\right\} \quad (2)$$

where μ_n are the roots of the Bessel's function.

Sphere

$$m^* = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left\{-n^2 \pi^2 Fo\right\} \quad (3)$$

Table 3–Equilibrium moisture content of the grains for corresponding drying conditions

Grain	Air temp (°C)	R.H. (%)	m_e (d.b.)
Soybean	71.0	4–9	0.0
Peanuts (whole)	50.0	11	0.02
Rough Rice	40.0	19	0.05
Wheat	40.0	11	0.04
	50.0	17	0.05

In all cases Fo is the Fourier number (Luikow, 1968) for mass transport and $m^* = (m-m_e)/(m_o-m_e)$, where m_o and m_e, are mean moisture content at any time, initial moisture content, and equilibrium moisture content, respectively. The lengths used in the Fo numbers were the equivalent spherical radius, corresponding to the fully dehydrated grains.

Among grains studied in this work, soybean is the one which has the more regular shape, i.e. geometrically it may be considered as a sphere with very little doubt. For this reason it was studied first. Eight replicate drying runs were performed at constant air temperature (71.0°C) and air velocity (13 m/sec) and the data were fitted using Fick's law solution in spherical coordinates [Eq. (3)]. Fig. 1 shows the predicted and measured drying plots for soybean (only for five replicates for the sake of clarity). For m^* approximately less than or equal to 0.3 Fick's law for spheres reduces to a straight line (in semilogarithmic coordinates) (with an intercept of $6/\pi^2$ or 0.608). The intercept of the various experimental lines representing log m^* vs Fo (in the straight line region) were calculated using linear regression analysis and the results are shown in Table 4. It can be seen that the average value of the intercepts for the eight replicates is 0.594 ± 0.016 a value which is in very good agreement with 0.608 as predicted by Eq (3). This agreement supports the assumption that geometrically the soybean may be considered as a sphere. Although in this case this was expected because of the regular shape of soybean this procedure may be used as a criterion for cases where the grain has a less conventional shape.

In this connection it is interesting to show what happens when the drying data are fitted with Fick's law solution corresponding to a geometry very different from reality. For this purpose the drying data of soybean were also analyzed using the solution for an infinite cylinder [Eq (2)]. The results are presented in Fig. 1 which shows that the fit is not as good as in the case shown in Fig. 1; nevertheless, one may accept that the data are still relatively fitted by

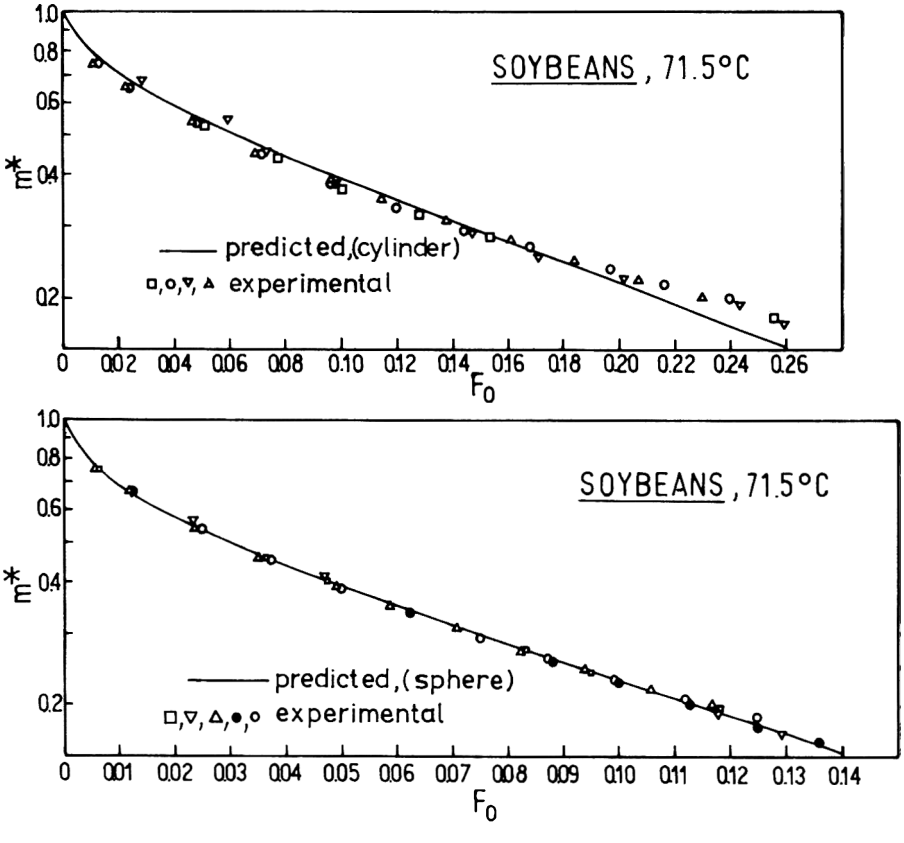


Fig. 1–Comparison of predicted and experimental drying behavior for soybean.

Fick's law for cylindrical coordinates. In order to make a quantitative comparison the data were processed through a nonlinear regression method in a computer and the deviation between experimental data and predictions [for both Eq (2) and (3)] was expressed as standard deviation,

$$S.D. = (\Sigma \frac{(Obs\text{-}Pred.)^2}{n-1})^{1/2}$$

The average S.D. for all drying runs fitted with solution for spherical coordinates was found to be $0.733.10^{-2}$, while for cylindrical coordinates it was $0.845.10^{-2}$. It may be concluded that both criteria, value of intercept and S.D. of the fit may be used to characterize the most adequate simple geometry for characterizing a particular grain shape.

The above criteria may now be used to characterize the most adequate geometry for grains having less conventional shapes, i.e. wheat, rough rice, and peanuts, in this study.

Fig. 2, 3 and 4 show predicted (using different coordinates) and measured drying plots for rough rice, peanuts, and wheat. The experimental data in the straight line region were processed through a linear regression program in order to calculate the intercepts. The results are shown in Table 5. It can be seen that the intercept for rough rice is relatively close to the value predicted by Fick's law solution for a sphere [Eq. (3)], thus supporting the usual drying literature assumption that geometrically rice may be considered as a sphere (Steffe and Singh, 1980). For peanuts the intercept was found to be 0.735 which is close to the value of 0.701 expected for an infinite cylinder, while for wheat the intercept is again close to the value expected for a spherical geometry. In this case this supports again, the usual literature assumption that wheat may be considered as a sphere for diffusional analysis of drying data (Becker and Sallans, 1955).

The results obtained by analysis of the intercepts were now compared with the S.D. criterium, as it was applied for soybeans. Table 6 compares the S.D. (average for various runs) for drying runs fitted with different solutions of Fick's law. It can be seen that the results agree with those obtained through the intercept analysis. Rough rice is better fitted considering spherical coordinates than cylindrical ones, and the same applies for wheat. For peanuts, cylindrical geometry appears closer to reality than rectangular one, in agreement with the conclusion obtained through calculation of intercepts.

Table 7 shows the diffusion coefficients for the various grains (at a given gemperature), calculated using the most appropriate geometry in each case.

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Table 4—Intercept of the drying plots for soybeans dried at 71.0°C

Run	Intercept
1	0.618
2	0.604
3	0.594
4	0.589
5	0.568
6	0.599
7	0.605
8	0.583
average = 0.594 ± 0.016	

Table 5—Average values of intercepts of the drying plots for various grains having less conventional shapes

Grain	Intercept (average)	Drying temp (°C)
Rough rice	0.620	40.5
Peanuts (whole)	0.735	50.0
Wheat	0.621	40.0

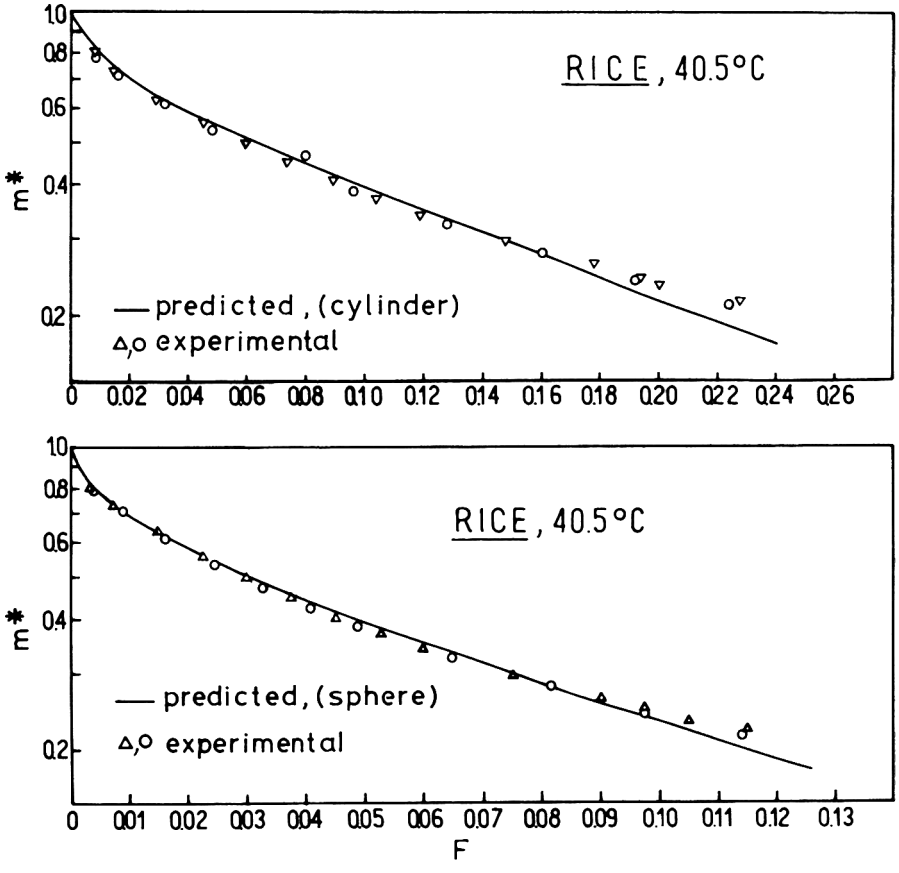


Fig. 2—Comparison of predicted and experimental drying behavior for rice.

Table 6–Comparison of the S.D. between experimental data and various predictions for different grains

Grain	S.D.
Rough Rice	0.425×10^{-2} (Eq. 2) – 0.27×10^{-2} (Eq. 3)
Wheat	0.33×10^{-2} (Eq. 2) – 0.46×10^{-3} (Eq. 3)
Peanut (whole)	0.135×10^{-2} (Eq. 2) – 0.29×10^{-2} (Eq. 1)

Table 7–Calculated values of diffusion coefficients

Grain	Geometry adopted	Temperature (°C)	Diffusion coefficient (cm^2/sec)
Soybean	sphere	71.5	9.3×10^{-7}
Peanuts (whole)	cylinder	50.0	4.0×10^{-7}
Rough Rice	sphere	40.5	1.3×10^{-7}
Wheat	sphere	40.0	1.8×10^{-7}

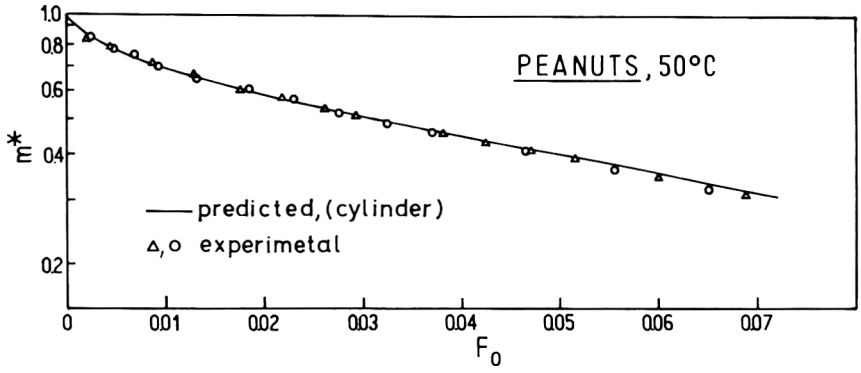
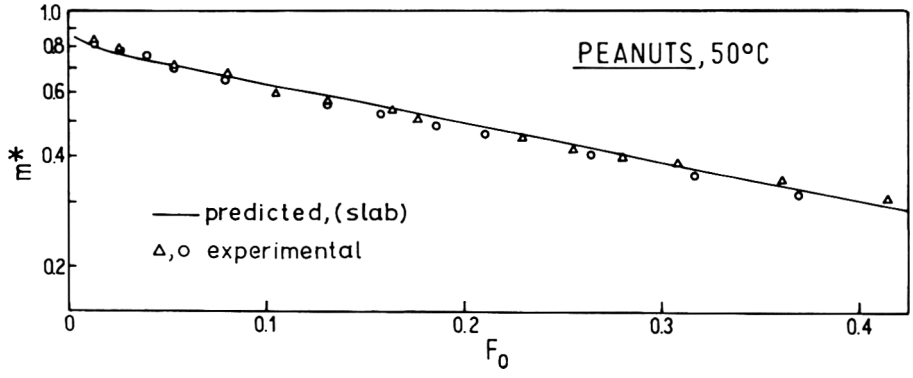


Fig. 3–Comparison of predicted and experimental drying behavior for peanuts.



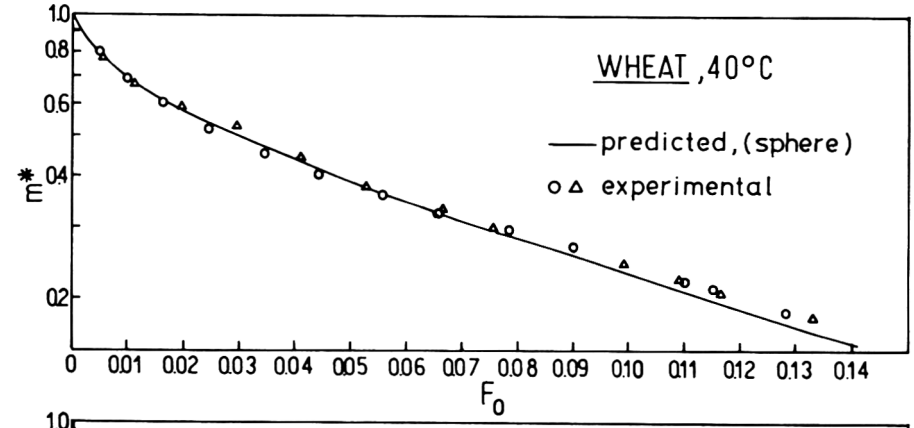
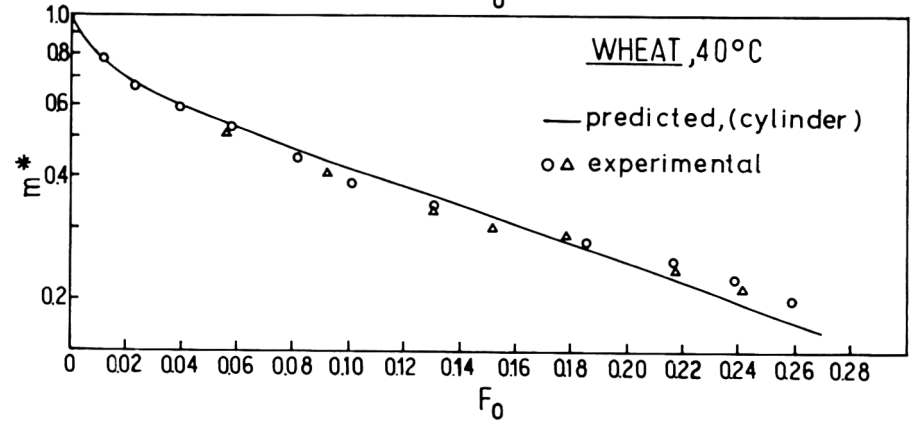


Fig. 4–Comparison of predicted and experimental drying behavior for wheat.



Changes in Volatile Sulfur Compounds of Whisky During Aging

MASAHIRO MASUDA and KI-I-CHI NISHIMURA

ABSTRACT

Volatile sulfur compounds of malt whiskies were analyzed using a gas chromatograph equipped with a flame photometric detector by direct injection and solvent extraction methods. Sulfur compounds identified by coupled gas chromatography-mass spectrometry and by matching GC retention times were dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, 3-(methylthio)propanal, 3-(methylthio)propanol, 3-(methylthio)propyl acetate, ethyl 3-(methylthio)-propanoate, dihydro-2-methyl-3(2H)-thiophenone, 2-thiophene-carboxyaldehyde, 5-methyl-2-thiophenecarboxaldehyde, benzothiophene and benzothiazole. Quantitative changes in these volatile sulfur compounds were studied by analyzing 48 aged and unaged malt whiskies by these methods. Dimethyl sulfide, 3-(methylthio) propyl acetate, dihydro-2-methyl-3(2H)-thiophenone and ethyl 3-(methylthio)propanoate decreased rapidly during aging and disappeared within three years. Dimethyl disulfide decreased gradually during aging. Dimethyl disulfide proved to be formed during distillation by the action of copper.

INTRODUCTION

MANY VOLATILE sulfur compounds have been found in alcoholic beverages such as beers (Garga-Ulloa, 1980), wines (Schreier et al., 1974b; Leppänen et al., 1980) and brandies (Dellweg et al., 1969). Ronkainen et al. (1973) reported the presence of hydrogen sulfide, methanethiol, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and other compounds in grain spirits. DMDS, dimethyl trisulfide (DMTS) and diethyl sulfite in whiskies and some alcoholic beverages were quantitatively determined by Leppänen et al. (1979). These sulfur compounds are said to play an important role in the flavor of alcoholic beverages because of their characteristic odor of low threshold (Sato and Takahashi, 1977).

Some sulfur compounds have been reported to decrease during aging. Hydrogen sulfide, methanethiol, carbon disulfide and DMS that were present in a fresh raw grain spirit were not found in an aged sample (Ronkainen et al., 1973). DMDS and DMTS in spirits also decreased during aging Leppänen et al., 1979). However, the change in volatile sulfur compounds during aging of malt whiskies has not yet been studied.

We were interested in the role of sulfur compounds in the flavor of malt whiskies and adopted a direct injection method for the analysis of low-boiling sulfur compounds, such as DMS and DMDS, and a solvent extraction method for the analysis of higher-boiling sulfur compounds using a gas chromatograph with a flame photometric detector. This report concerns the identification of 12 sulfur compounds in malt whiskies and new results on the quantitative changes in some volatile sulfur compounds during aging and on the formation of DMDS during distillation.

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EXPERIMENTAL

Malt whiskies

Seven unaged and 41 aged malt whisky samples were obtained from our distillery for this study. Among them 16 aged whiskies were produced by storing the same lot of a new make (unaged malt whisky). Malt whiskies analyzed by direct injection and solvent extraction methods were not always the same.

Reference compounds

DMS, DMDS, benzothiophene, benzothiazole, methyl ethyl sulfide and dinonyl sulfide were commercial products (Tokyo Kasei Co., Japan). DMTS and 3-(methylthio)propanol were gifts from Dr. T.L. Peppard and Dr. K. Yamaguchi, respectively. These sulfur compounds were purified by preparative gas chromatography. 3-(Methylthio)propanal, dihydro-2-methyl-3(2H)-thiophenone and 2-thiophenecarboxaldehyde were synthesized according to Kaneko and Mitsui (1938), Karrer and Schmid (1944), and King and Nord (1948), respectively. 5-Methyl-2-thiophenecarboxaldehyde were synthesized after Wolff-Kishner reduction of 2-thiophenecarboxaldehyde (King and Nord, 1949) by formulation of the 2-methylthiophene formed with N-methylformanilide as in the synthesis of 2-thiophenecarboxaldehyde: b.p. 91–93°C/10 mm; m/z (%): 125 (100), 126 (80), 97 (34), 53 (30), 45 (16), 127 (12), 39 (9), 69 (8). 3-(Methylthio)propanol was also prepared by reduction of methyl 3-(methylthio)propanoate (Merck) with lithium aluminum hydride: b.p. 80–83°C/10 mm. 3-(Methylthio)propyl acetate was prepared by acetylation of 3-(methylthio)propanol with an acetic anhydride-pyridine mixture: b.p. 91–93°C/15 mm. Ethyl 3-(methylthio)propanoate was prepared by alkali hydrolysis, followed by esterification of methyl 3-(methylthio)propanoate: b.p. 95–97°C/20 mm.

Separation and identification

Two liters of unaged malt whisky (diluted with the same amount of water) was mixed with 100g of sodium bicarbonate, then extracted three times with 1L of ether-pentane mixture (1:1). The extracts were washed with 500 mL of water, dried over sodium sulfate, and then concentrated by the same method described for the solvent extraction method.

The extracts were chromatographed on 10g of silica gel 60 (Merck) eluted with ether. The eluate was separated into 30 fractions by high pressure liquid chromatography (HPLC) using a column (250 mm x 10 mm i.d.) of Licrospher Si 100 5μ (Merck) eluted with a gradient of 0–100% of dichloromethane in hexane at 3 mL/min. A Waters ALC/GPC 202/401 liquid chromatograph fitted with a UV detector (254 nm) was used.

Fractions containing sulfur compounds (checked by a gas chromatograph equipped with FPD and FID) were analyzed by gas chromatography-mass spectrometry (GC-MS). Column and operation conditions were the same as those used in the solvent extraction method. A Finnigan 4000 mass spectrometer directly connected with the capillary column effluent was used.

Sulfur compounds identified by GC-MS analysis were confirmed by co-injection of authentic compounds with whisky extracts using two different columns.

Direct injection method

Fifty microliters of an internal standard solution consisting of 10 mg/L methyl ethyl sulfide in ethanol was added to 1 mL of malt whisky, and after shaking several times, 3 μL portions of the sample were analyzed by gas chromatography.

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Solvent extraction method

Fifty milliliters of malt whisky (diluted with 100 mL of water) was mixed with 75 μL of an internal standard solution consisting of 100 mg/L dinonyl sulfide in ethanol and 5g of sodium bicarbonate, then extracted twice with 75 mL of pentane. The pentane layers were washed with 40 mL of water and dried over anhydrous sodium sulfate. The extracts were concentrated in a KD concentrator with a Schneider column and then carefully in a rotary vacuum evaporator until most of the solvents were gone. Two-microliter portions of the extracts were analyzed by gas chromatography. Commercial grade pentane (Wako Chemicals, Japan) was carefully purified by redistilling on a rectifying column, 2m x 2.5 cm i.d., packed with stainless steel helices prior to use for extraction.

Gas chromatography

A Hitachi 163 gas chromatograph fitted with a flame photometric detector was connected to a Hitachi 834S chromatoprocessor.

For the direct injection method, a glass column, 2m x 3 mm i.d., packed with 3% PEG 20M on Gas Chrom Q (80-100 mesh) was used. The operating conditions were as follows: column temperature, 70°C; injection temperature, 150°C; carrier gas, 30 mL/min nitrogen.

For the solvent extraction method, a glass capillary column, 100m x 0.5 mm i.d., coated with PEG 20M was used. The operating conditions were as follows: column temperature, 80–210°C (3°C/min); injection temperature, 250°C; carrier gas, ca 4 mL/min nitrogen; make-up gas, 30 mL/min of nitrogen.

For identification of the sulfur compounds, the following columns were also used: a glass column, 2m x 3 mm i.d., packed with 5% DC-11 on Chromosorb G, AW-DMCS (60–80 mech) and a glass capillary column, 50m x 0.25 mm i.d., coated with OV 101.

For purification of the sulfur compounds, a Varian 90-P gas chromatograph equipped with a column (10 ft x 1/4 in) packed with 10% PEG 20M on Celite (60–80 mesh) was used.

Table 1—Sulfur compounds identified in malt whiskies

Compounds	Method of identification[a]	Previously reported in[b]
Dimethyl sulfide (DMS)	MS RT-1,2	Beer (1,10,16), Wine (14,16) Sake (9), Rum (14) Grain spirit (5)
Dimethyl disulfide (DMDS)	MS RT-1,2,3,4	Beer (2,16), Wine (14, 16) Sake (9), Whisky (14) Brandy (14), Rum (14) Grain spirit (5)
Dimethyl trisulfide (DMTS)	MS RT-3,4	Whisky (14), Brandy (14) Rum (14), Hops (11)
3-(Methylthio)-propanal	RT-3,4	Beer (3,6)
3-(Methylthio)-propanol	RT-3,4	Beer (6,10), Wine (4,7) Brandy (15)
3-(Methylthio)-propyl acetate	MS RT-3,4	Beer (6,10), Wine (7) Apple brandy (12)
Ethyl 3-(methylthio)-propanoate	MS RT-3,4	Beer (6), Wine (7) Brandy (15)
Dihydro-2-methyl-3(2H)-thiophenone	MS RT-3,4	Beer (6)
2-Thiophenecarboxaldehyde	MS RT-3,4	Beer (8), Brandy (13,15)
5-Methyl-2-thiophenecarboxaldehyde	MS RT-3,4	Beer (8), Brandy (13)
Benzothiophene	MS RT-3,4	
Benzothiazole	MS RT-3,4	

[a] The following four columns at optimum isothermal ocnditions were used in determining agreement of known and unknown retention times: RT-1, PEG 20M packed column; RT-2, DC-11 packed column; RT-3, PEG 20M capillary column; RT-4, OV 101 capillary column.

[b] Numbers correspond to the references reported by the following authors: (1) Kepner et al. (1963); (2) Drews et al. (1969); (3) Visser and Lindsay (1971); (4) Muller et al. (1971); (5) Ronkainen et al. (1973); (6) Schreier et al. (1974a); (7) Schreier et al. (1974b); (8) Pickett et al. (1976); (9) Sato et al. (1977); (10) Nakajima et al. (1978); (11) Peppard (1978); (12) Schreier et al. (1978); (13) Heide et al. (1978); (14) Leppänen et al. (1979); (15) Schreier et al. (1979); (16) Leppänen et al. (1980).

Calibration

Calibration curves were obtained with 60% alcoholic solutions containing 10–800 μg/L DMS and DMDS for the direct injection method and containing 10–800 μg/L DMDS, 2.5–40 μg/L DMTS, 5–100 μg/L dihydro-2-methyl-3(2H)-thiophenone, 2.5–40 μg/L ethyl 3-(methylthio)propanoate, 10–400 μg/L 3-(methylthio) propyl acetate, 5–100 μg/L 2-thiophenecarboxaldehyde, 2.5–40 μg/L 5-methyl-2-thiophenecarboxaldehyde, 5–60 μg/L benzothiophene and 2.5–40 μg/L benzothiazole for the solvent extraction method.

RESULTS & DISCUSSION

TABLE 1 lists sulfur compounds identified in malt whiskies in this study. These sulfur compounds were identified by gas chromatography-mass spectrometry (GC-MS) and by matching GC retention times using polar and nonpolar columns. 3-(Methylthio)propanal and 3-(methylthio)propanol were identified only by the correspondence of GC retention times with those of authentic compounds, because of their low concentrations in malt whiskies. These sulfur compounds, except for dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS), were identified in whisky for the first time. DMS and DMDS have been found in many alcoholic beverages such as beer, wine and sake. DMTS has been found in hops

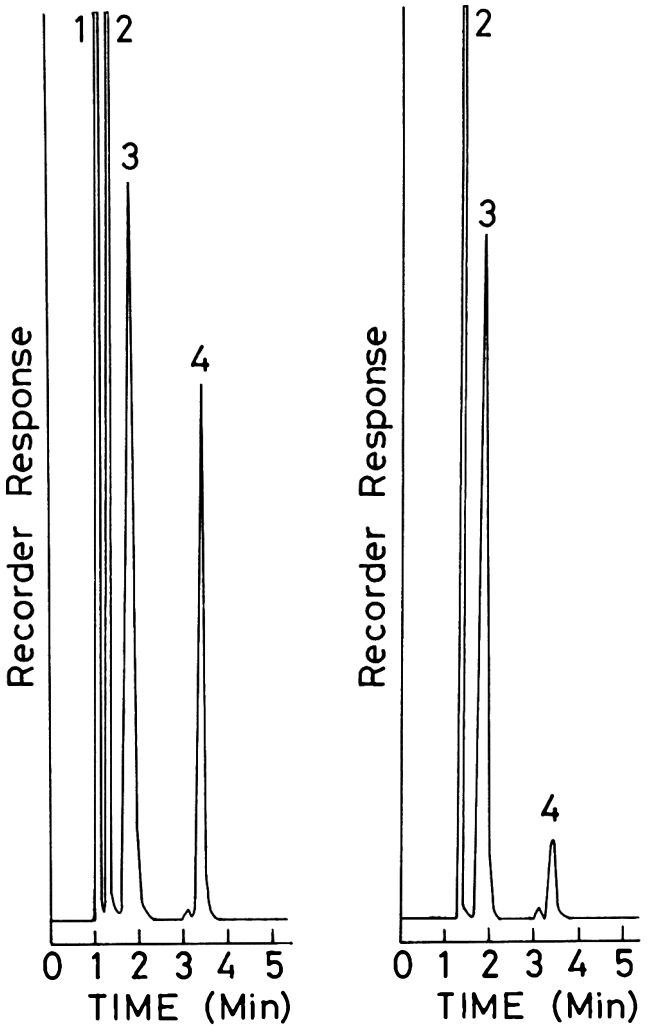


Fig. 1 — Gas chromatograms of an unaged (left) and a 5-yr-aged (right) malt whisky by FPD: (1) dimethyl sulfide (DMS); (2) methyl ethyl sulfide (internal standard); (3) ethanol; (4) dimethyl disulfide (DMDS).

(Peppard, 1978) and distilled beverages (Leppänen et al., 1979). 3-(Methylthio)propanal, 3-(methylthio)propanol, 3-(methylthio)propyl acetate, ethyl 3-(methylthio)propanoate and dihydro-2-methyl-3(2H)-thiophenone have been identified in beer (Schreier et al., 1974a) and wine (Schreier et al., 1974b). 2-Thiophenecarboxaldehyde and 5-methyl-2-thiophenecarboxaldehyde have been identified in beer (Pickett et al., 1976) and brandy (Heide et al., 1978). Benzothiophene and benzothiazole have not previously been found in alcoholic beverages, but together with 2-thiophenecarboxaldehyde and 5-methyl-2-thiophenecarboxaldehyde, these compounds have been said to be formed by Maillard reactions in heat-processed foods (Schutte, 1974).

Gas chromatograms of an unaged and a 5-yr-aged malt whisky analyzed by the direct injection method are shown in Fig. 1. DMS and DMDS were quantitatively determined using methyl ethyl sulfide as the internal standard. Methyl ethyl sulfide was reported to be present in bourbon whiskies by Kahn et al. (1968), but we found no peak in that region of gas chromatograms of malt whiskies. The results were summarized in Table 2, which lists the DMS and DMDS contents in malt whiskies of various ages. In this report, the concentrations of sulfur compounds are all expressed on a 100% alcohol basis.

Table 2—DMS and DMDS concentrations (μg/L at 100% alcohol) in malt whiskies of various ages

Malt whisky age (yr)	0	1	2–3	4–8
No. of samples	6	6	7	6
DMS	470	0	0	0
DMDS	370	330	180	120

Table 3—DMDS concentrations (μg/l at 100% alcohol) during the course of whisky production and in a low wine distilled in a glass still

	Wash	Low wine	New make	Low wine (glass)[a]
DMDS	trace	330	300	30

[a] A low wine made by distilling a wash in a glass still.

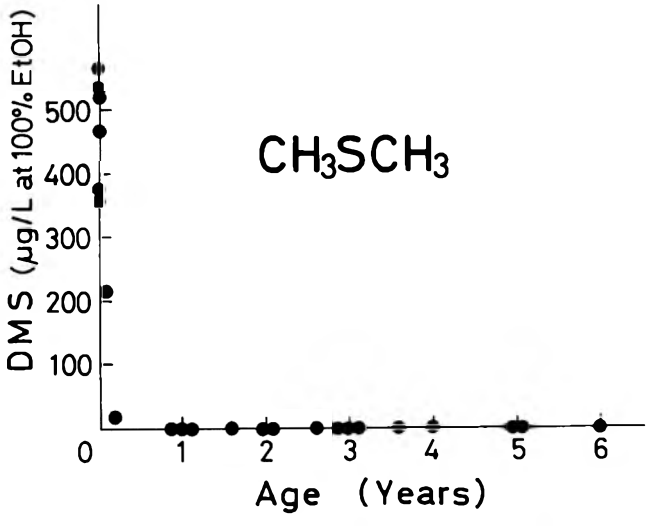


Fig. 2 — Changes in DMS concentrations during aging of malt whiskies.

DMS was present at 350–560 μg/L in unaged whiskies, but decreased rapidly during aging and disappeared within a year (Fig. 2). DMDS was present at 250–620 μg/L in unaged whiskies and decreased gradually during aging (Fig. 3). The phenomenon of DMDS decreasing during aging was confirmed by analyzing aged whiskies produced by storing the same lot of a new make (Fig. 4). DMDS decreased more rapidly in hogshead barrels (about 230L) than in puncheon barrels (about 500L), presumably because of the comparatively larger surface area and contact area between barrel and whisky in the former. The decrease of DMDS in whiskies and brandies during aging has been reported by Leppänen et al. (1979). As the DMDS content of malt whiskies of various ages varied very widely depending on the original content and the type of barrel used, estimation of the age from the DMDS content seems very difficult.

DMS was also found in washes (fermented worts) where very little DMDS was present. The formation of DMS in beer and wort has been reported by many authors (Nakajima and Narziβ, 1978, Dikenson, 1979).

Table 3 lists the DMDS content in a wash, a low wine (first distillate), a new make (second distillate) and a low wine distilled in a glass still and offers evidence that DMDS was formed during distillation. When a low wine was made

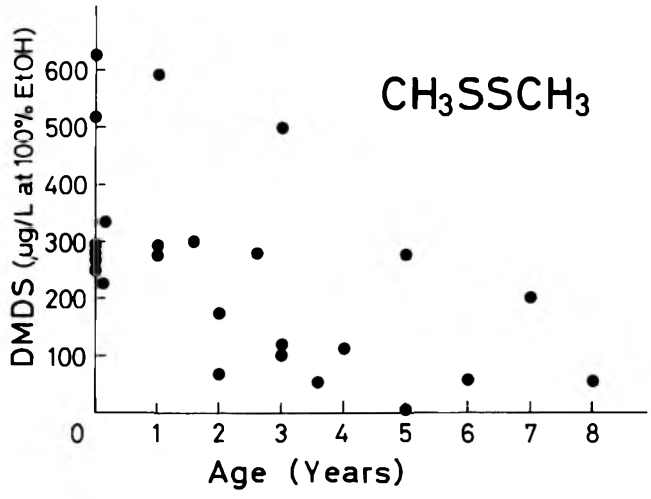


Fig. 3 — Changes in DMDS concentrations during aging of malt whiskies.

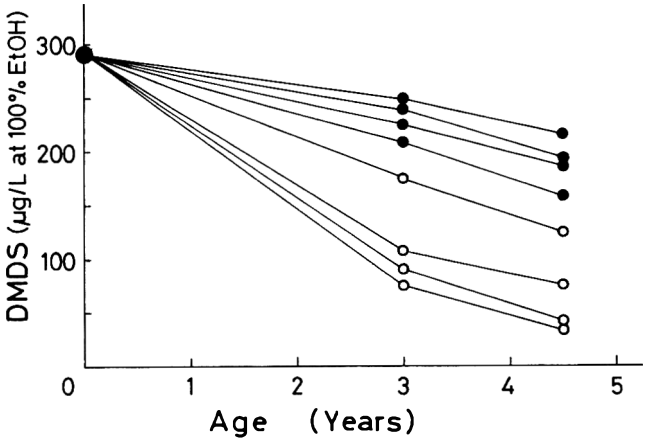


Fig. 4 — Changes in DMDS concentrations during aging of the same lot of new make in puncheons (•) and hogshead barrels (○).

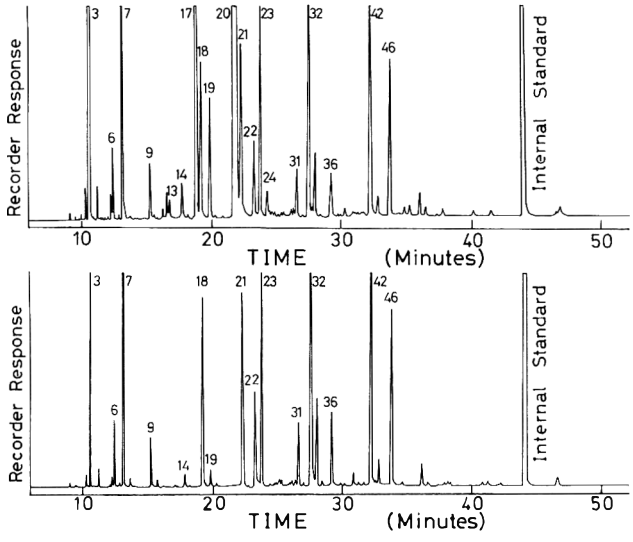


Fig. 5 — Gas chromatograms of pentane extracts of an unaged (top) and a 8-yr-aged (bottom) malt whisky by FPD. The peak numbers correspond to the numbers in Table 3.

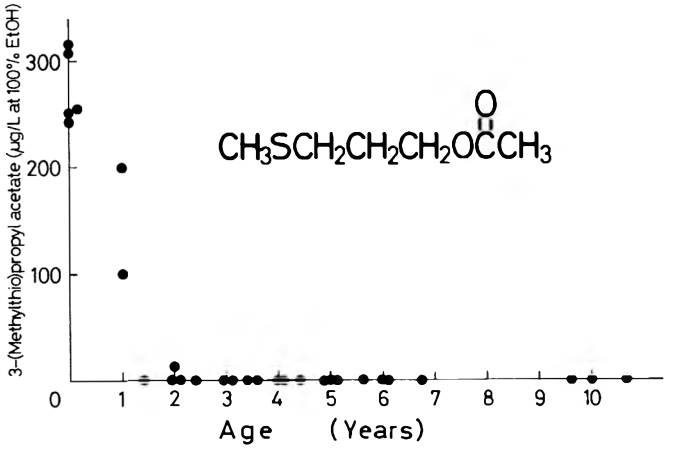


Fig. 6 — Changes in 3-(methylthio)propyl acetate concentrations during aging of malt whiskies.

by distilling a wash in a glass still, the amount of DMDS formed was about one-tenth of the amount formed in a copper still. These results were confirmed by distilling a wash in glass and copper stills in the same shape under the same conditions. Therefore, copper is involved in the formation of DMDS, which is very interesting as copper metal has been said to eliminate the unpleasant odor caused by volatile sulfur compounds (Tanner, 1969).

Gas chromatograms of pentane extracts of an unaged and an 8-yr-aged malt whisky are shown in Fig. 5. Many peaks that seem to be attributable to sulfur compounds were detected by chromatography on a glass capillary column. Table 4 lists the concentrations of sulfur compounds which were ascertained to be present in malt whiskies.

Quantitative changes in 3-(methylthio)propyl acetate and dihydro-2-methyl-3(2H)-thiophenone during aging are shown in Fig. 6 and Fig. 7 respectively. These compounds were present in relatively large amounts in unaged whiskies, but decreased rapidly with aging and disappeared within three years. Quantitative changes in ethyl 3-(methylthio) propanoate during aging are shown in Fig. 8. This compound also decreased rapidly with aging. 3-(Methylthio) propanal and 3-(methylthio)propanol were present in very small amounts in unaged whiskies and disappeared during aging. DMDS was also measured, but the reproducibility of this solvent extraction method was poorer than that of the direct injection method because the high volatility of this compound. Besides these sulfur compounds, some unidentified peaks also decreased with aging. On the other hand, the amounts of DMTS, 2-thiophenecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, benzothiophene and benzothiazole did not change. Leppänen et al. (1979) reported that DMTS also decreased slightly with aging, but we found no quantitative change in this study. Further investigation is necessary, but even if a decrease does occur, it is probably very slight.

Table 4—Concentrations (μg/L at 100% alcohol) of sulfur compounds in malt whiskies of various ages

Peak[a] No.	Compound	0 Yr[b] (5)[c]	1 Yr (4)	2 Yr (6)	3–5 Yr (8)	6–10 Yr (7)
3	DMDS	359	345	272	149	91
9	DMTS	12	16	15	19	14
13	3-(Methylthio)-propanal	7	t[d]	0	0	0
17	Dihydro-2-methyl-3(2H)-thiophenone	77	53	3	0	0
19	Ethyl 3-(methylthio) propanoate	21	11	t	0	0
20	3-(Methylthio)-propyl acetate	274	139	3	0	0
23	2-Thiophenecarboxaldehyde	66	83	49	60	54
24	3-(Methylthio)-propanol	8	0	0	0	0
31	5-Methyl-2-thiophenecarboxaldehyde	15	15	10	16	16
32	Benzothiophene	39	27	27	31	38
42	Benzothiazole	18	16	14	15	32

[a] The peak numbers correspond to the numbers in Fig. 5.
[b] Malt whisky age.
[c] Number of samples.
[d] Trace.

In summary, twelve sulfur compounds were identified in malt whiskies, nine of which had not been reported previously in whiskies. This study has also shown for the first time that many sulfur compounds decrease during aging of malt whiskies and that among them, DMS, 3-(methylthio)propyl acetate, dihydro-2-methyl-3(2H)-thiophenone and ethyl 3-(methylthio)propanoate decrease very rapidly and disappear within three years. Although many aliphatic sulfur compounds such as sulfides and disulfides decreased during aging, the amounts of aromatic sulfur compounds such as thiophene and thiazole derivatives did not change. Considering their volatility, the decrease of these sulfur compounds is presumed to be due to interactions with oak ingredients and not to simple evaporation through oak barrels. We are now investigating the mechanism of the decrease of surfur compounds during aging of malt whisky and the roles of these compounds in whisky flavor.

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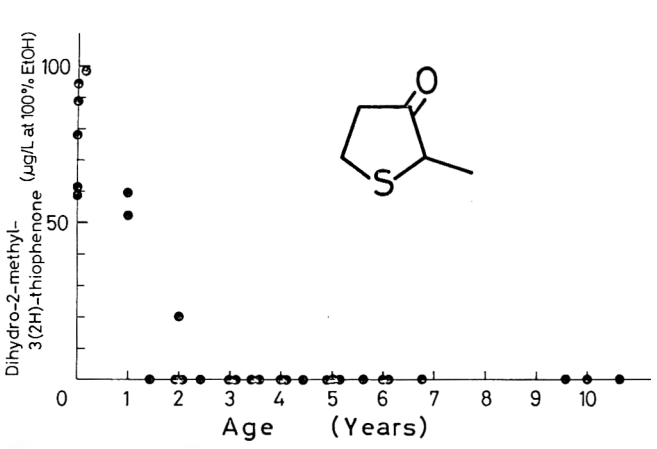


Fig. 7 — Changes in dihydro-2-methyl-3(2H)-thiophenone concentrations during aging of malt whiskies.

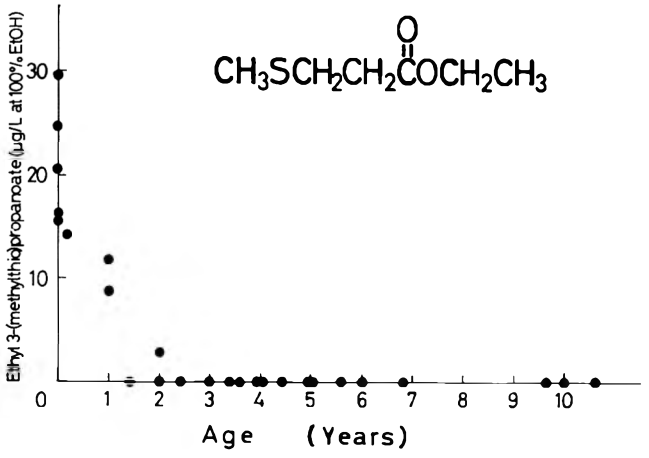


Fig. 8 — Changes in ethyl 3-(methylthio)propanoate concentrations during aging of malt whiskies.

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Antibacterial Effect of Tertiary Butylhydroquinone Against Two Genera of Gram Positive Cocci

M. RACCACH and E. C. HENNINGSEN

ABSTRACT

The minimum inhibitory concentration (MIC) of tertiary butylhydroquinone (TBHQ) against *Pediococcus pentosaceus* in a bacteriological medium was 15 μg/ml for 10^3 to 10^5 cells/ml and 20 μg/ml for 10^6 cells/ml. Heat stress and sodium chloride were each synergistic to the inhibitory effect of TBHQ. Production of both L and D lactic acid was inhibited by TBHQ. Fermentation of sucrose was totally inhibited by 5 μg/ml TBHQ as compared to 10 μg/ml TBHQ for L-arabinose, D-galactose and maltose and to 15 μg/ml TBHQ for glucose. TBHQ (50 μg/ml) did not inhibit growth of 1.2×10^3 cells of *S. aureus* z-88, but 30 μg/ml totally inhibited growth of 3.0×10^3 cells of *S. aureus* 100 for 36 hr. TBHQ (100 μg/ml) was bactericidal against the latter strain. TBHQ (10–50 μg/ml) slightly inhibited production of nonheat treated nuclease but had little or no effect on heat treated nuclease of *S. aureus* z-88.

INTRODUCTION

TERTIARY BUTYLHYDROQUINONE (TBHQ) is commonly used as an antioxidant to prevent the autoxidation of lipids. It also possesses antimicrobial properties (Davidson et al., 1981; Ericksen and Tompkin, 1977; Raccach, 1980; Robach and Stateler, 1980). Raccach (1980) while comparing the effects of BHA, BHT and TBHQ on the fermentative activity of the meat starter culture *Pediococcus pentosaceus* found that TBHQ was the most inhibitory (98%). The reports on the effect of TBHQ on *Staphylococcus aureus* (Davidson et al., 1981; Robach and Stateler, 1980) were limited to growth of the pathogen. The effect of TBHQ on two strains of *S. aureus* was reported (Robach and Stateler, 1980) but did not include a comparison based on equal cell concentrations. These findings stimulated us to conduct the present study. The purpose of this study was to evaluate more extensively the effect of TBHQ on the fermentative activity of *P. pentosaceus* and on the growth and nuclease production by *S. aureus* in defined systems.

MATERIALS & METHODS

Stock cultures

Pediococcus pentosaceus (ATCC 10791) was obtained from the American Type Culture Collection. *S. aureus* 100, an enterotoxin A producer, was obtained from the Food Research Institute at the University of Wisconsin. *S. aureus* z-88 which produces enterotoxin A and D was also used. The staphylococci were transferred daily for three consecutive days in brain heart infusion (BHI) broth (Difco) and *P. pentosaceus* in lactobacilli MRS broth (Difco). All cultures were incubated statically at 35°C. The last transfer was used as the inoculum (1%, v/v) for the experimental work.

Preparation of cultures

The cells of a 16–18 hr culture were harvested (16,300 × *g*, 10 min, 4°C) and washed three times with cold sterile 0.1% (w/v) peptone water and resuspended in the same diluent. Whenever desired, *P. pentosaceus* was diluted to 10^3 cells/ml and heat stressed in a water bath at 52°C for 20 min. *P. pentosaceus* either stressed or non-stressed was inoculated into 50 ml quarter strength lactobacilli MRS broth (QSMRS) to provide 10^3–10^6 cells/ml. QSMRS broth was found adequate for these experiments, providing 0.5% (w/v) glucose comparable to the levels of carbohydrates used in meat systems. Staphylococci were always inoculated into BHI broth to provide a concentration of 10^3 cells/ml.

TBHQ

Tenox TBHQ (food grade) was obtained from Eastman Chemical Products (Kingsport, TN).

A stock solution of TBHQ (20 mg/ml) was prepared with 95% (v/v) ethanol. The stock solution was kept refrigerated in the dark. Every 4–5 wk a fresh stock solution was prepared. The stock solution was diluted with 95% ethanol and added to either BHI or QSMRS to provide the desired concentration (0.5–100 μg/ml). In all instances the volume of the TBHQ solution did not exceed 1% (v/v) of the total volume to which it was added.

Enumeration of cultures

P. pentosaceus was enumerated in lactobacilli MRS agar by the pour plate technique and the staphylococci were surface spread on Baird-Parker medium. Both media were incubated at 35°C for 24–48 hr. In some instances growth of the cultures in either BHI or QSMRS broth was measured spectrophotometrically (560 nm). Uninoculated broth was used as blank.

Measurement of lactic acid and pH

Lactic acid was titrated with 0.1N NaOH to pH 8.3, using an Altex digital pH meter (model 3560, Beckman Instruments, Inc.) with a combination electrode. L and D-lactic acid isomers were determined spectrophotometrically (Double beam, Beckman model 24 spectrophotometer) using L and D-lactic acid dehydrogenase, respectively, and NAD^+. The increase in NADH was determined at 340 nm (Boehringer Manheim, 1979).

Minimal inhibitory concentration (MIC)

MIC for the purpose of this study was defined as the concentration of TBHQ which inhibited ≥90% of lactic acid production by *P. pentosaceus* for at least 24 hr.

Inhibition of lactic acid production

Inhibition of lactic acid production was calculated according to the following equation:

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{Lactic acid produced in treatment}}{\text{Lactic acid produced in control}} \times 100 \right]$$

The control did not contain the antioxidant.

Fermentation of carbohydrates

L and D-arabinose, D-galactose, D-lactose, maltose, sucrose and L and D-xylose were used. Quarter strength lactobacilli MRS broth was prepared by omitting the glucose and adding the appropriate carbohydrate (0.5% w/v).

Staphylococcal nuclease

The turbidimetric assay of Erickson and Deibel (1973) was used. The sources of the enzyme were supernatants from 24 and 48 hr cultures of *S. aureus* z-88. The enzymatic activity was tested before ("total" nuclease) and after (heat stable nuclease) a heat treat-

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ment of 15 min in boiling water. The reaction mixture (9 ml) contained 1000 μg denatured DNA (Sigma Herring Sperm Type VIII sodium salt), 0.5–1.0 ml culture supernatant and 10^{-3}M $CaCl_2$ in boric acid-borax buffer (0.2M in terms of borate) pH 8.6. After an incubation period of 15 min at 37°C the reaction was stopped with 1.0 ml of 4N HCl. The undepolymerized DNA and the hydrochloric acid formed turbidity which was read spectrophotometrically (600 nm) after 10 min. The absorbance values were translated into μg/ml undepolymerized DNA using a standard curve (y = −0.055 + 0.006x; where y = absorbance, and x = undepolymerized DNA). The depolymerized DNA was obtained by difference (100 μg/ml − μg/ml undepolymerized DNA). Nuclease units per ml supernatant were calculated according to the following equation:

Nuclease units per ml = DNA depolymerized per ml/incubation period (in minutes).

The results of each experiment are the arithmetic averages of three independent trials.

RESULTS & DISCUSSION

Effect of TBHQ on *P. pentosaceus*

The MIC of TBHQ against *P. pentosaceus* in QSMRS was 15 μg/ml for 10^3–10^5 cells/ml and 20 μl/ml for 10^6 cells/ml. These inhibitory effects were effective for only 36–48 hr. TBHQ at concentrations below the MIC for 10^3 cells/ml of *P. pentosaceus* inhibited also the production of lactic acid. Inhibitions of 11.3 and 41.0% of lactic acid production were obtained with 5 and 10 μg/ml TBHQ, respectively (Table 1). Doubling the concentration of TBHQ from 5.0–10.0 μg/ml increased the inhibition of lactic acid production almost four times. TBHQ (5 μg/ml) inhibited lactic acid production less than it did the growth of 10^3 pediococci/ml (11% vs 20%, respectively).

Most commercial lactic acid cultures are either frozen (Raccach, 1980) or lyophilized (Genigeorgis, 1976). These preservation methods stress bacterial cells (Israeli et al., 1974; Raccach and Juven, 1976) to different extents depending on the nature of the process. Heat stressing was used in this study because it was the fastest way to obtain stressed cells of *P. pentosaceus*. The stressed cells produced about 21% less lactic acid than nonstressed cells (Table 1). TBHQ (5 μg/ml) inhibited (45.5%) lactic acid production by stressed cells while 10 μg/ml TBHQ increased this value to 76.5%. Stress was found synergistic to the inhibitory effect of TBHQ. TBHQ (10 μg/ml) alone inhibited 41% of lactic acid production and stress alone inhibited 20.8% (Table 1). When stress and TBHQ were combined a 76.5% inhibition was obtained. This value was higher than the sum (61.8%) of TBHQ and stress.

Meat lactic acid cultures are added to meat systems containing, in some instances, up to 3.6% sodium chloride (Raccach, 1980). Sodium chloride (3.5% w/v) alone inhibited 21.9% of lactic acid production by *P. pentosaceus* (Table 2) and 5 μg/ml TBHQ alone inhibited 11.3% (Table 1). TBHQ combined with sodium chloride inhibited 85.4% of lactic acid production, a value higher than the sum (33.2%) of each (Table 2). This result indicates that synergism existed between TBHQ and sodium chloride. This was not the case when BHA, BHT and sodium chloride were tested against *P. pentosaceus* (Raccach, 1980). This finding has practical implications concerning the fermentation of meat systems. TBHQ and sodium chloride may delay the fermentation period to attain the desired final acidity enabling the growth of undesirable microorganisms such as *S. aureus*, thus affecting the safety of the product. In meat, the inhibitory effect of phenolic type antioxidants including TBHQ may be smaller than in QSMRS broth. Raccach (1980) found that BHA and BHT increased the fermentation time (to pH 5.0) of a meat system of the Genoa type by only 9.1–16.7% compared to 65% inhibition in APT broth. It is conceivable that TBHQ added to meat will partition between the fat of the meat and the bacterial cell lipids, leaving a smaller portion to affect the lactic acid culture.

P. pentosaceus produces a racemic mixture of lactic acid (Doelle, 1971). The production of both isomers was inhibited by 5 μg/ml TBHQ (87.1 and 93.8% of L and D-lactic acid, respectively) (Table 3). This inhibition increased with the increase in TBHQ concentration. The differences in lactic acid inhibition shown in Tables 1 and 3 may be due to the different method used to determine lactic acid.

So far, glucose was the only carbohydrate used in QSMRS. The following experiments were conducted in order to examine the effect of TBHQ on the fermentation of other carbohydrates by *P. pentosaceus*. *P. pentosaceus* did not produce acid from D arabinose, D-lactose and from both L and D-xylose. TBHQ inhibited the lactic acid fermentation of the monosaccharides L-arabinose and D-galactose (Fig. 1). This inhibition increased with an increase in concentration of TBHQ. In the case of L arabinose, growth (measured spectrophotometrically) was slightly more inhibited than lactic acid production. TBHQ (10 μg/ml)

Table 1—Effect of TBHQ on lactic acid production by Pediococcus pentosaceus[a]

Treatment	pH	Lactic acid (%)	Inhibition of lactic acid (%)
Nonstressed			
Control	3.8	0.396	
TBHQ (μg/ml)			
5.0	4.0	0.350	11.3
10.0	4.4	0.230	41.0
Stressed			
Control (nonstressed)	3.8	0.380	
No TBHQ, stressed	4.0	0.300	20.8
TBHQ (μg/ml)			
5.0	4.9	0.160	45.5
10.0	5.7	0.090	76.5

[a] 10^3 cells/ml

Table 2—Combined effect of NaCl (3.5% W/V) and TBHQ (5 μg/ml) on lactic acid production by Pediococcus pentosaceus[a]

Treatment	pH	Lactic acid (%)	Inhibition of lactic acid (%)
Control (No TBHQ)	3.8	0.68	
NaCl	3.6	0.53	21.9
TBHQ + NaCl	6.2	0.10	85.4

[a] 10^3 cells/ml

Table 3—Effect of TBHQ on the production of L and D-lactic acid by Pediococcus pentosaceus[a]

	Lactic acid isomer			
	L		D	
Treatment TBHQ (μg/ml)	Amt produced (g/l)	Inhibition (%)	Amt produced (g/l)	Inhibition (%)
0	4.27	0.0	0.80	0.0
5.0	0.55	87.1	0.05	93.8
7.5	0.53	87.6	0.03	96.3
10.0	0.26	93.9	0.01	98.8

[a] 10^3 cells/ml

inhibited 80% of lactic acid production from L arabinose with >90% inhibition of growth. The same trend was found with D-galactose with the exception of 7.5 μg/ml TBHQ. TBHQ (10 μg/ml) inhibited >90% of lactic acid production from D-galactose with a slightly higher inhibition of growth (Fig. 1). The effect of TBHQ on the lactic acid fermentation of the disaccharides maltose and sucrose was also examined (Fig. 2). *P. pentosaceus* in the presence of 5.0–7.5 μg/ml TBHQ produced more lactic acid from maltose than from sucrose. Evidently, 5.0 μg/ml TBHQ completely inhibited both the lactic acid fermentation of sucrose and the growth of the culture while 10 μg/ml TBHQ were required for the complete inhibition of maltose fermentation. This may be due to the different glycosidic linkages of the two disaccharides and/or to the inhibition of production or activity of the appropriate hydrolysing enzymes. This result may have extremely important practical implications since sucrose can be substituted for glucose in fermented meat systems (Acton et al., 1977). The inhibition of lactic acid fermentation of sucrose by TBHQ was much larger than that of glucose. This finding should be tested in meat systems before a final conclusion is drawn.

The fermentation of sucrose and the growth of *P. pentosaceus* in the presence of TBHQ (0.5–7.5 μg/ml) is shown in Fig. 3. Both lactic acid production and growth of *P. pentosaceus* were inhibited with the increase in concentration of TBHQ. The inhibition of growth of *P. pentosaceus* was slightly higher than lactic acid production at 1 and 3 μg/ml TBHQ and about twice as high at a concentration of 2 μg/ml TBHQ. The increase in inhibition of lactic acid production was linear with the increase in concentration of TBHQ up to 4 μg/ml (Fig. 3). Higher concentrations of TBHQ did not increase the inhibition. The hydrophobic nature of the TBHQ molecule like other phenolic type antioxidants may promote strong interactions with the hydrocarbon zones of cell membrane structures and may cause perturbing effects on membrane associated functions such as a decrease in permeability to essential metabolites (Snipes et al., 1975; Singer and Wan, 1977). One such perturbing effect may be on the phosphoenol pyruvate (PEP): sugar phosphotransferase system (PTS). Any degree of inactivation of this sytem will hinder the transport of the carbohydrate, resulting in a slower and diminishing fermentative activity. Surak (1977) found that the primary effect of TBHQ on *Tetrahymena* was the inhibition of DNA, RNA, protein synthesis and the metabolism of ^{14}C acetate.

Effect of TBHQ on staphylococci

TBHQ at concentrations between 10 and 50 μg/ml did not inhibit the growth of S. aureus z-88 (1.2×10^3 cells in BHI broth) (Table 4). The amount of "total nuclease" (non heat treated) produced by *S. aureus* z-88 slightly decreased with the increase in concentration of TBHQ up to 50 μg/ml. At 50 μg/ml TBHQ, "total nuclease" production was 85% of the control (no TBHQ) (Table 4). After an incubation of 48 hr, 50 μg/ml TBHQ was slightly inhibitory decreasing "total nuclease" production to 90% of the control. Heat stable nuclease (supernatant boiled for 15 min) was hardly affected by TBHQ (Table 4). These findings are extremely important because it is generally accepted that the detection of heat stable staphylococcal nuclease or thermonuclease in foods indicates that the food contained at one time high *S. aureus* counts (1.0×10^6 cells/ml or more)

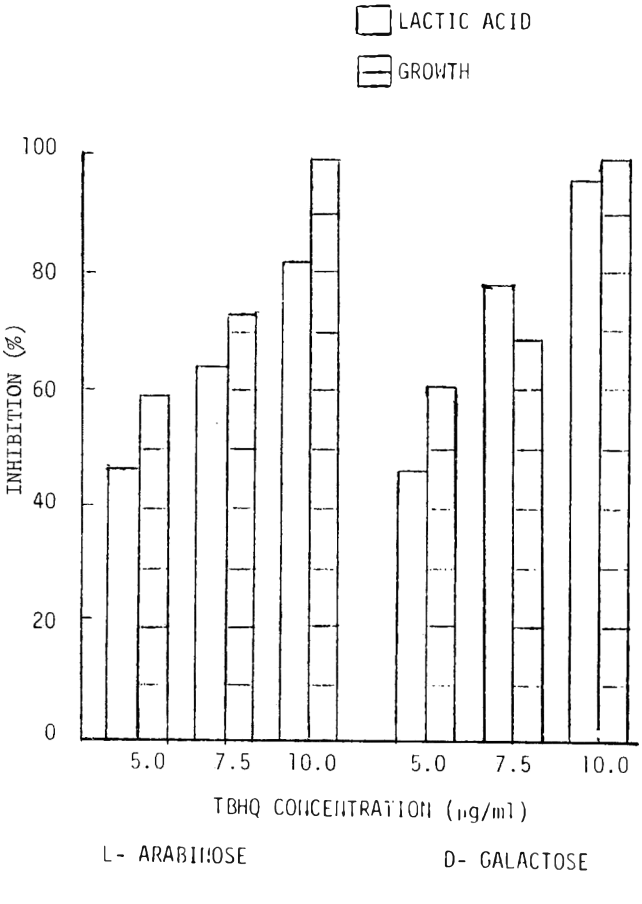


Fig. 1—Effect of TBHQ on growth and fermentation of L-arabinose and D-galactose by P. pentosaceus.

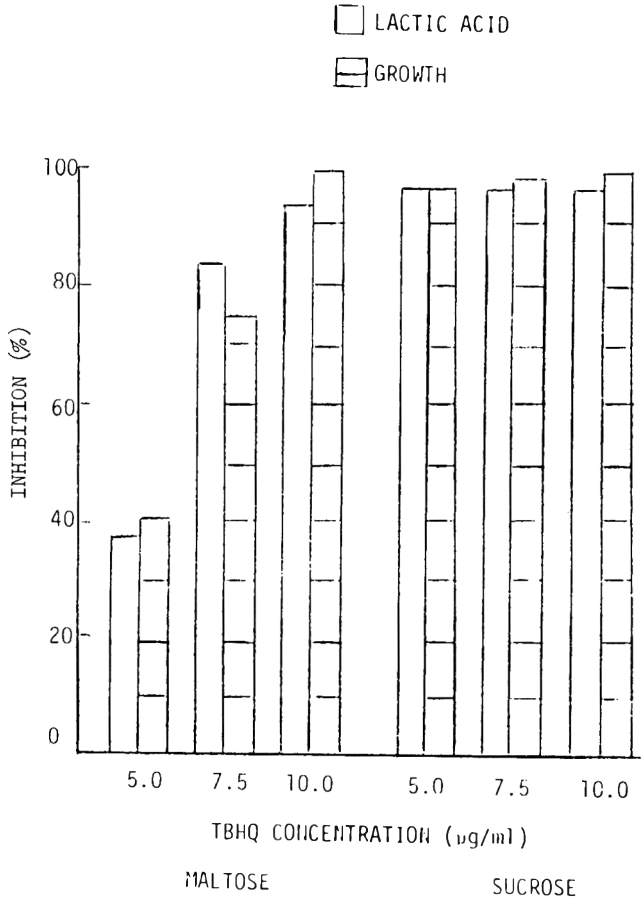


Fig. 2—Effect of TBHQ on growth and fermentation of maltose and sucrose by P. pentosaceus.

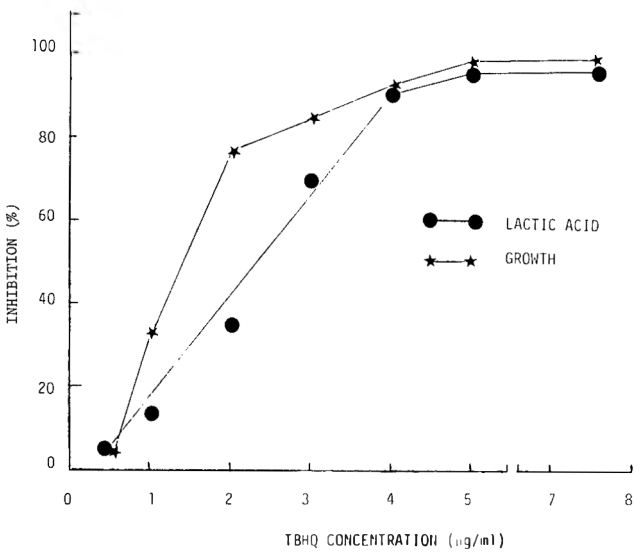


Fig. 3—Effect of TBHQ on growth and fermentation of sucrose by P. pentosaceus.

(Tatini et al., 1976; Koupal and Deibel, 1978). The food would then be hazardous to eat because of the possible presence of enterotoxin(s). Any food additive or food component which may adversely affect the detection of heat stable staphylococcal nuclease may lead to false negative results affecting the safety of the product. For example, heat stable nuclease of *S. aureus* was not detected in boiled eggs or in foods containing eggs even though *S. aureus* was present in the product ($0.3–3.5 \times 10^8$ cells/g) and produced enterotoxin(s) (Park et al., 1978).

A concentration of 30 µg/ml TBHQ was bacteriostatic against 10^3 cells/ml of *S. aureus* 100 for 36 hr, but 50 µg/ml TBHQ extended this period to 48 hr (Table 5). A bactericidal effect was obtained with 100 µg/ml TBHQ. On the other hand, a concentration of 50 µg/ml TBHQ did not inhibit the growth of 10^3 cells/ml of *S. aureus* z-88 (Table 4). It appears that the effect of TBHQ against *S. aureus* is strain dependent. There is no published comparison on the effect of TBHQ on the same cell concentration of different strains of *S. aureus*. The reports in the literature show that 25 µg/ml TBHQ were bactericidal against 10^4 cells/ml of *S. aureus* S-6 but only bacteriostatic (for 36 hr) against 10^3 cells/ml of *S. aureus* 12600 in tryptic soy broth (TSB) (Robach and Stateler, 1980). The same concentration of TBHQ (25 µg/ml) was inhibitory (for 72 hr) against 2.4×10^5 cells/ml of an unspecified strain of *S. aureus* in TSB (Davidson et al., 1981).

In summary, this study showed that TBHQ inhibited the growth of both *P. pentosaceus* and *S. aureus* 100 but was not inhibitory against the growth of *S. aureus* z-88. TBHQ inhibited lactic acid production by *P. pentosaceus* but had little or no effect on the production of staphylococcal nuclease by *S. aureus* z-88. In a fat-containing product, such as meat, the effect of TBHQ and other phenolic type antioxidants on microorganisms will be smaller than in a laboratory medium. This was shown with pediococci (Raccach, 1980) and *S. aureus* (Raccach, 1981) in fermented meat systems. Robach et al. (1977) have shown the same phenomenon with BHA used against *Vibrio parahaemolyticus* in a crab meat homogenate. Thus, one should be very careful when interpreting these and other results from studies with laboratory media. It is important, however, to conduct studies in laboratory media in order to elucidate the mode of action and characterize the optimal conditions for the antimicrobial activity of TBHQ and other food additives.

Table 4—Effect of TBHQ on growth and nuclease production by Staphylococcus aureus Z-88

	Cell count[a]		Nuclease "Total"[b]		Nuclease Heat stable[c]	
Treatment	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
(TBHQ µg/ml)	(CFU/ml)		(Nuclease Unit/ml)			
0	2.0×10^8	1.7×10^8	5.9	5.9	5.8	5.9
10	1.0×10^8	1.6×10^8	5.5	5.6	5.7	5.8
20	1.0×10^8	1.5×10^8	5.3	5.6	5.6	5.7
30	3.4×10^8	1.2×10^8	5.4	5.5	5.6	5.7
40	3.5×10^8	1.2×10^8	5.2	5.4	5.5	5.7
50	1.8×10^8	1.0×10^8	5.0	5.3	5.4	5.5

[a] Initial count of 1.2×10^3 cells/ml
[b] Untreated supernatant
[c] Supernatant boiled for 15 min

Table 5—Effect of TBHQ on Staphylococcus aureus 100

	Incubation period (hr)			
Treatment	0	24	36	48
(TBHQ µg/ml)	(CFU/ml)			
0	3.0×10^3	2.0×10^9	1.7×10^9	1.6×10^9
30	3.2×10^3	2.8×10^3	3.1×10^3	4.7×10^6
50	3.0×10^3	3.1×10^3	2.9×10^3	3.1×10^3
100	3.3×10^3	$<1.0 \times 10^1$		

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Mass Balance Model for Enthalpy of Water Binding by a Mixture

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ABSTRACT

A thermodynamic approach was based on sorption data over a wide range of water activity (a_w) at each of three temperatures. The hypothesis tested was the total partial enthalpy change for the water of a mixture is equal to the sum of the partial enthalpy changes for the water of the individual ingredients at the same a_w. This was tested with sorption data for starch, casein, sucrose, starch:casein (1:1) and starch:sucrose (9:1) at 7.2, 20 and 30°C. Experimental values for the starch:casein mixture were in agreement (±5%) with those calculated from data on the individual ingredients, validating the hypothesis for a mixture of polymers. The starch:sucrose indicated a greater variation as the sucrose concentration increased to saturation.

INTRODUCTION

THERMODYNAMIC PARAMETERS of moisture sorption by foods has become increasingly important with the rising interest in intermediate moisture foods (IMF). It is believed that the thermodynamics of equilibrium sorption data provides a basis for explaining how water associates with foods at different energy levels (Palnitkar and Heldman, 1970).

Presently there is no generally agreed upon term that associates energy with bound water in a food system. Among the many terms which have been used are (1) heat of vaporization (Brooker et al., 1974); (2) heat of adsorption (Brunauer et al., 1938; Brunauer, 1943); (3) partial enthalpy (Fish, 1957); (4) force of water binding (Leung et al., 1976); (5) integral heats of sorption (Othmer and Sawyer, 1943); and (6) isosteric heat of sorption (Dole and McLaren, 1947; Smialek, 1980). Compounding the problems, some authors reported the energy as the total heat associated with the adsorption of water vapor by the solid, including the heat of liquefaction of water, while others reported the difference between these values. Brunauer et al. (1938) reported as the energy of binding only the most tightly bound water, i.e., the monolayer. Thus, the binding energy of the remaining large portion of the water, the water of interest in IMF, is ignored. Rockland and Nishi (1980) pointed out that the total binding energy of individual ingredients will be reflected in the equilibrium water activity of the mixture.

Calorimetric measurements of binding enthalpy are based on the heat released when water is added to a dry solid. A major drawback of this method lies in accurately measuring the extremely small quantities of heat evolved (Iglesias and Chirife, 1976).

The most common method for determination of water binding enthalpy is through the use of the Clausius-Clapeyron equation. This method is based on sorption data at three temperature and applies to the whole range of water sorption. It has been widely used for nonfood (Zettlemoyer et al., 1975) as well as for food substances (Almasi, 1978).

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In order to relate the enthalpy change of binding to sorption characteristics of IMF, a great amount of experimental data would be required to determine the enthalpy change for each and every food. However, the laboratory work could be greatly reduced if the binding enthalpy change of a known mixture could be calculated from the enthalpy change for each ingredient. We have explored this possibility with the aid of a mass balance enthalpy equation and the calculated partial enthalpy change of water of the ingredients.

Derivation of mass balance enthalpy equation

Based on the mass balance approach shown valid (Lang and Steinberg, 1980, 1981a) for the quantity of water bound at a given water activity (a_w), we hypothesized that, at a given a_w, the ingredients of a mixture behave independently of each other with their own associated water. If this is valid, the total partial enthalpy change for the water of mixture is equal to the sum of the partial enthalpy changes for the water of the individual ingredients at the same a_w. This can be stated as follows:

$$\Delta\bar{h} = \sum_i \frac{\Delta\bar{H}_i}{M} m_i w_i \qquad (1)$$

where $\Delta\bar{h}$ = total partial enthalpy change of water (cal/g dry mixture); $\Delta\bar{H}_i$ = partial molal enthalpy change of water for the ith ingredient at a given a_w (cal/mole water); M = molecular weight of water; m_i = moisture content of ith ingredient (g H_2O/g dry ingredient); and w_i = weight of ith ingredient (g dry ingredient/g dry mixture).

The individual partial molal enthalpy changes of water for the various ingredients and the mixture can be calculated from the relationship between the activity of water and the temperature at constant composition and moisture content,

$$\frac{\partial \ln a_w}{\partial T} = \frac{-\Delta\bar{H}_i}{RT^2} \qquad (2)$$

Assuming that the partial molal heat capacities of water in the bound and standard states are constant over the range of temperatures employed, $\Delta\bar{H}_i$ can be expressed as a linear function of the temperature,

$$\Delta\bar{H}_i = \Delta_o\bar{H}_i + (\bar{c}_p - c_p^\circ)\,T \qquad (3)$$

where $\Delta_o\bar{H}_i$ = the intercept of the linear relationship; $\bar{c}_p$ = the partial molal heat capacity of the bound water; and c_p° = the partial molal heat capacity of pure water.

Substituting (3) into (2) and integrating we obtain,

$$\ln a_w = \frac{\Delta_o\bar{H}_i}{RT} - \frac{(\bar{c}_p - c_p^\circ)}{R} \ln T + C \qquad (4)$$

Isotherm data are obtained for each ingredient at each of at least three temperatures. Accuracy is increased by expressing the data in a linear form (see below). The temperature of interest, in this case 20°C, is chosen and the moisture content calculated at any a_w level of interest. This moisture content is entered into the isotherms at the

other temperatures to calculate the a_w values at the same moisture content for all three temperatures. These a_w values when substituted into Eq (4), will yield simultaneous equations, the solutions of which will define the values $\Delta_o\bar{H}_i$ and $(\bar{c}_p^{\circ} - \bar{c}_p)$ and C.

Substitution of $\Delta_o\bar{H}_i$ and $(\bar{c}_p^{\circ} - \bar{c}_p)$ into (3) and using the temperature of interest will yield $\Delta\bar{H}_i$, the partial molal enthalpy change of the water for the ingredients or the mixture at the temperature.

MATERIALS & METHODS

Materials

Corn starch was Argo brand (Best Foods, Inc., Englewood Cliffs, NJ) at 9% moisture, casein was Fisher purified (Fisher Scientific Co., Fairlawn, NJ) at 4% moisture and sucrose was Domino brand (Amstar Corp., New York, NY) at 5% moisture. Mixtures were prepared by hand mixing until uniform in the proportions given in the tables.

Saturated salts

The saturated salts used and their corresponding water activities at the three temperatures used are shown in Table 1. In each case, the a_w values for the salts were obtained either directly from those accepted by the National Bureau of Standards (Greenspan, 1977) or were determined in this laboratory by calibration against these accepted values (McCune et al., 1981).

Equalibration at constant a_w

To determine the isotherms of starch and casein, duplicate one gram aliquots were equilibrated against the saturated salt solutions shown in Table 1, as described by Lang et al., (1981).

The isotherms for the sucrose were determined using the isopiestic technique described by McCune et al. (1981). By this method, a secondary standard, cellulose, was equilibrated to solutions of known moisture content and the water activity read from a calibration curve of weight gain vs a_w. For each material, isotherms were experimentally determined in duplicate at 7.2, 20 and 30°C, over a range of 0.33–0.95 a_w.

Moisture determination

Moisture content was determined in triplicate by the vacuum oven method (AOAC, 1970) using 60°C and 29.8 in Hg vacuum for 36 hours.

Linear isotherms

To avoid the large errors inherent in graphical interpolation of the conventional isotherm, the equilibrium sorption data for each material and temperature were fitted to the Smith (1947) equation. Lang and Steinberg (1981a, b) showed that the isotherms for many homogeneous food materials can be linearized over an a_w of 0.30–0.95 by this equation. Thus, by using the regression equation of the linearized isotherm for each material, the moisture content at any a_w, between 0.30 to 0.95, could be found.

Table 1—Equilibrium relative humidities of the saturated salt solutions at the temperatures used for moisture equilibration

Salt	a_w 7.22°C	20°C	30°C
KNO_3	0.961[a]	0.946[a]	0.923[a]
$BaCl_2$	0.918[b]	0.910[b]	0.896[b]
$ZnSO_4$	0.912[b]	0.890[b]	0.865[b]
K_2CrO_4	0.868[b]	0.859[b]	0.860[b]
Li_2SO_4	0.847[b]	0.842[b]	0.841[b]
$(NH_4)_2SO_4$	0.822[a]	0.813[a]	0.806[a]
NaCl	0.757[a]	0.755[a]	0.751[a]
NaBr	0.628[a]	0.591[a]	0.560[a]
$MgCl_2$	0.335[a]	0.331[a]	0.324[a]

[a] Greenspan (1977)
[b] Calibration against Greenspan values (McCune et al., 1981).

RESULTS & DISCUSSION

Isotherm data

The linear equation parameters obtained by regression analysis of the isotherm data for starch, casein and sucrose and two binary mixtures of these ingredients at 7.2, 20 and 30°C are given in Table 2.

Calculation of partial molal enthalpy changes of water

Selecting 20°C as the temperature of interest, the moisture content of the ingredients and mixtures were calculated from the linear form of the appropriate isotherms at various water activities and are reported in Table 3. From these moisture content values, the corresponding a_w values required to obtain the same moisture content at 7.2°C and 30°C were calculated from the respective isotherms. Substituting these a_w values into Eq (4), three simultaneous equations were formed which yielded the appropriate values of $\Delta_o\bar{H}_i$ and $(\bar{c}_p - \bar{c}_p^{\circ})$. Substituting

Table 2—Smith equation parameters of water sorption data at three temperatures for three ingredients and two mixtures

[Equation: Moisture content (dry basis) = $a_i + b_i \log (1 - a_w)$]

	Parameters		
	Intercept, a_i	Slope, b_i	Correlation coefficient
Starch			
7.2°C	0.09708	−0.1705	−0.9999
20 °C	0.09890	−0.1485	−0.9999
30 °C	0.09127	−0.1450	−0.9999
Casein			
7.2°C	0.03993	−0.1696	−0.9999
20 °C	0.04698	−0.1427	−0.9999
30 °C	0.04154	−0.1390	−0.9999
Sucrose			
7.2°C	−0.7238	−1.4406	−0.9999
20 °C	−0.6052	−1.2683	−0.9999
30 °C	−0.5106	−1.1378	−0.9999
Starch:Sucrose (9:1)			
7.2°C	0.00567	−0.3182	−0.9923
20 °C	0.01143	−0.2780	−0.9923
30 °C	0.01923	−0.2568	−0.9924
Starch:Casein (1:1)			
7.2°C	0.06017	−0.1745	−0.9999
20 °C	0.06112	−0.1602	−0.9999
30 °C	0.05795	−0.1459	−0.9999

Table 3—Calculated moisture contents of three ingredients and two mixtures at various water activities at 20° C

Water activity (a_w)	Moisture Content (g/g) dry basis				
	Starch	Casein	Sucrose	Starch:Casein (1:1)	Starch:Sucrose (9:1)
0.95	0.2921	0.2326	1.0449	0.2696	0.3731
0.93	0.2704	0.2118	0.8595	0.2462	0.3325
0.90	0.2474	0.1897	0.6631	0.2214	0.2894
0.87	0.2305	0.1734	0.5186	0.2031	0.2578
0.85	0.2212	0.1645	0.4397	0.1932	0.2405
0.83	0.2132	0.1568	–	0.1844	–
0.80	0.2027	0.1467	–	0.1731	–
0.75	0.1883	0.1329	–	0.1576	–
0.70	0.1765	0.1216	–	0.1449	–

these quantities into Eq (3), the values of the partial molal enthalpy changes of water for the various ingredients and mixtures at selected water activities were calculated and are reported in Table 4.

The Mass Balance Model, Eq (1)

Sample calculation: a macromolecule and a sugar

Consider the mixture, starch: sucrose, 9:1 (as in basis), 1g of dry sample will contain 0.8892g starch and 0.1108g sucrose. Assuming that we wish to calculate the $\Delta\bar{h}$ of the mixture at an a_w of 0.90 and 20°C, we first calculate the moisture content of the starch alone under these conditions from the appropriate starch isotherm:

$$m_D = 0.9890 - 0.1485 \log (1 - 0.90) = 0.24740\text{g } H_2O/\text{g starch}$$

Then from the isotherm at 7.2°C, we can calculate the a_w at this moisture content:

$$a_w = 1 - 10^{[(0.24740 - 0.09708)/-0.1705]} = 0.86866 \quad \text{at } 7.2^\circ C$$

From a similar calculation based on the isotherm at 30°C, the a_w at this moisture content and temperature is 0.91620. Substituting these a_w values into Eq (4) at the three temperatures, we obtain the following simultaneous equations:

Table 4—Partial molal enthalpy changes of water for three ingredients and two mixtures at various water activities at 20°C

Water activity (a_w)	Partial molal enthalpy changes of water, $\Delta\bar{H}_i$ (cal/mole)				
	Starch	Casein	Sucrose	Starch:Casein (1:1)	Starch:Sucrose (9:1)
0.95	−220.77	−218.01	−127.90	−228.69	−203.42
0.93	−285.19	−274.32	−137.99	−294.63	−248.89
0.90	−374.81	−348.64	−133.06	−383.76	−302.42
0.87	−459.22	−415.04	−108.64	−465.06	−343.01
0.85	−514.02	−456.21	− 82.81	−516.16	−364.29
0.83	−568.10	−495.52	–	−565.37	–
0.80	−648.66	−551.69	–	−636.42	–
0.75	−783.73	−640.03	–	−749.70	–
0.70	−923.07	−724.24	–	−859.32	–

Table 5—Comparison of partial enthalpy changes of water for binary mixtures with those calculated from the partial molal enthalpy changes of the individual ingredients at several water activities at 20°C

Mixture	Water activity (a_w)	$\Delta\bar{h}$ (cal/g)		Deviation
		Experimental[a]	Calculated[b]	
Starch:Casein (1:1)	0.95	−3.422	−3.189	+6.82
	0.93	−4.026	−3.741	+7.09
	0.90	−4.716	−4.393	+6.85
	0.87	−5.243	−4.914	+6.27
	0.85	−5.534	−5.216	+5.75
	0.83	−5.788	−5.491	+5.14
	0.80	−6.116	−5.864	+4.11
	0.75	−6.558	−6.417	+2.14
	0.70	−6.912	−6.921	−0.13
Starch:Sucrose (9:1)	0.95	−4.213	−4.005	+4.94
	0.93	−4.593	−4.536	+1.25
	0.90	−4.858	−5.119	−5.37
	0.87	−4.908	−5.571	−13.51
	0.85	−4.863	−5.837	−20.04

[a] Application of Eq (4) to isotherm data at three temperatures for the mixture.
[b] Application of Mass Balance Eq (1) to isotherm data at three temperatures for the components.

$$\ln 0.86866 = \frac{\Delta_o\bar{H}_i}{1.9869}\left(\frac{1}{273.15 + 7.22}\right) - \frac{(\bar{c}_p - c_p^\circ)}{1.9869}\ln(273.15 + 7.22) + C$$

$$\ln 0.90000 = \frac{\Delta_o\bar{H}_i}{1.9869}\left(\frac{1}{273.15 + 20.00}\right) - \frac{(\bar{c}_p - c_p^\circ)}{1.9869}\ln(273.15 + 20.00) + C$$

$$\ln 0.91620 = \frac{\Delta_o\bar{H}_i}{1.9869}\left(\frac{1}{273.15 + 30.00}\right) - \frac{(\bar{c}_p - c_p^\circ)}{1.9869}\ln(273.15 + 30.00) + C$$

Solving these equations, we obtain

$$\Delta_o\bar{H}_i = -3{,}909.7 \text{ cal/mole } H_2O$$
$$(\bar{c}_p - c_p^\circ) = +12.059 \text{ cal/deg/mole } H_2O$$
$$C = 41.084$$

Substituting these values in Eq (3),

$$\Delta\bar{H}_i = -3{,}909.7 + 12.059\,(273.15 + 20.00) = -374.6$$

However, these calculations include rounding errors; when all the number involved in this calculation were carried to eight digits, the answer was

$$\Delta\bar{H}_i = -374.81 \text{ cal/mole } H_2O \text{ at } a_w = 0.90 \text{ and } 20^\circ C.$$

A similar calculation for sucrose yields the values:

$$m_D = 0.66308\text{g } H_2O/\text{g sucrose}$$

and

$$\Delta\bar{H}_i = -133.06 \text{ cal/mole } H_2O \text{ at } a_w = 0.90 \text{ and } 20^\circ C.$$

Substituting the above values in Eq (1):

$$\Delta\bar{h} = \frac{-374.81}{18.015} \times 0.24740 \times 0.8892_{\text{ starch}} + \frac{-133.06}{18.015} \times 0.66308 \times 0.1108_{\text{ sucrose}}$$

or

$$\Delta\bar{h} = -5.119 \text{ cal/g sample (dry basis).}$$

The experimental value was −4.858 cal/g sample (Table 5), so the deviation is −5.37%.

The values obtained for the partial enthalpy changes of water of the binary mixtures and their comparison with the calculated values obtained from the individual ingredients (Table 5) are in agreement (small deviation) for the starch:casein mixture over the range of water activities considered since the precision in the calculation of $\Delta\bar{h}$ appears to be of the order of ±5%. However, the results for the starch:sucrose system indicate a greater variation. While this variation may also be due to the propagation of experimental error, one cannot overlook the possibility that the presence of sucrose in the system influences the enthalpy of water binding by the starch since the sucrose is present as a highly concentrated aqueous solution. In contrast, the water present in the starch:casein mixture is primarily

associated with macromolecules so no apparent liquid is present. Further work on this phenomenon is needed to sharpen the precision of the measurements and to clarify the behavior of water here.

CONCLUSIONS

THE HYPOTHESIS that the ingredients of a mixture behave independently of each other with their own associated water and that, therefore, the partial enthalpy changes of their associated water are additive appears to apply to the starch:casein system. However, for systems containing sucrose, the water binding phenomenon needs to be further elucidated.

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Further Studies on the Roles of the Head and Tail Regions of the Myosin Molecule in Heat-induced Gelation

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ABSTRACT

Heat induced gelation properties of the two proteolytic fragments of myosin, heavy (HMM) and light meromyosin (LMM), were studied by rigidity measurement in a band type viscometer and by a direct examination using a scanning electron microscope. A heat induced network forming ability for both LMM and HMM was found in 0.6M KCl at a pH 6.0. LMM produced gels corresponding to a reversible helix-coil transition at temperatures ranging from 40–70°C, with little evidence of aggregation as assessed from a turbidity change of the system. Contrary, HMM associated irreversibly producing a gel with increased rigidity at pH 5.0 and a salt concentration of 0.1M. Oxidation of SH-groups appeared to be involved only in HMM and not in LMM gelation process.

INTRODUCTION

IT IS NOW BECOMING widely accepted that binding properties of sectioned and formed or comminuted cured meat products originate from the heat-induced gelation of myosin in vertebrate mescles (Fukazawa et al., 1961a, b; Samejima et al., 1969, 1981; Nakayama and Sato, 1971; Macfarlane et al., 1977; Ford et al., 1978; Ishioroshi et al., 1979, 1980; Siegel and Schmidt, 1979a, b; Yasui et al., 1979, 1980). The proteins show a great tendency to bind to and cross-link each other upon heating.

Studies of the heat-induced interactions of myosin molecules have been complicated by the gelation of solutions and production of insoluble aggregates. To simplify the problem, we (Samejima et al., 1981) digested skeletal muscle myosin with chymotrypsin in the presence of EDTA and obtained two fragments, the low ionic strength soluble (soluble at 0.1M KCl and pH 6–7) fragment of myosin, subfragment-1 (S-1, M_w = 120,000), which is a single headed fragment without tail and the low ionic strength insoluble (insoluble at 0.1M KCl and pH 6–7, but soluble at 0.6M KCl and pH 6–7) fragment, rod (M_w = 220,000), which is an entirely coiled coil helical fragment without the head [Fig. 1 (I)]. In our preceding paper (Samejima et al., 1981), it has been reported that the heat-induced gelation of myosin consists of two reactions, i.e., aggregation and network formation of the head and tail regions of the molecules which respectively show independent transition temperatures at about 43°C and 53–55°C.

As is generally known from biochemical studies on myosin, other types of fragments [Fig. 1 (I)] are obtainable by chymotryptic treatment in the presence of divalent metals (Weeds and Pope, 1977). The low ionic strength soluble fragment is heavy-meromyosin (HMM, M_w = 340,000), a double-headed fragment with a part of the tail connected through a "hinge region" and the low ionic strength insoluble fragment is light-meromyosin (LMM, M_w = 140,000), apparently another part of the helical tail region. Although Samejima et al. (1969) first studied the heat-gelling properties of HMM and LMM, they recognized later that proteolytic artifacts were affecting their results (Samejima et al., 1972). Thus the heat-gelling properties of these subfragments of myosin are not yet well understood. It is, however, these properties which are most important in understanding the cross-linked state in the heat-induced gelation of myosin molecules because HMM and LMM are similar to myosin in having two globular heads and comparable solubility properties, respectively.

The present study is a continuation of our attempts to distinguish the contribution of various regions of the myosin molecule to the heat-induced gelation of this protein. The experiments were, therefore, undertaken to compare the association properties of skeletal muscle myosin, HMM and LMM.

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EXPERIMENTAL

Materials

Chymotrypsin, phenylmethyl sulphonyl fluoride (PMSF), ATP and dithiothreitol (DTT) were purchased from Sigma Chemical Co. Ltd. All other chemicals used were of the highest purity obtainable commercially and were supplied by Kanto Chemical Co. Ltd., Nakarai Chemical Co. Ltd. and Wako Pure Chemical Co. Ltd.

Myosin and myosin subfragments preparation. Skeletal muscle myosin was prepared from rabbit M. longissimus thoracis essentially according to Perry (1955).

HMM was prepared by chymotryptic digestion of myosin in 0.6M KCl and 20 mM K-phosphate buffer (pH 7.0). Skeletal myosin (about 10 mg/ml) was digested with 0.03 mg/ml of chymotrypsin at 25°C for 10 min in the presence of 1mM $MgCl_2$ and 1mM DTT. Digestion was terminated by the addition of 0.1M PMSF to a final concentration of 0.5 mM, and then the protein solution was dialyzed overnight against 50 mM KCl and 10 mM K-phosphate (pH 7.0) at 4°C with three changes of outer solution. Solubilized HMM thus obtained was further purified by ammonium sulfate fractionation and subsequent DEAE column chromatography (Weeds and Pope, 1977). From the insoluble residue of the HMM preparation, LMM was made using the ethanol fractionation described in our preceding paper (Samejima et al., 1976, 1981).

Chymotryptic subfragment-1 (S-1) and myosin rod (rod) were prepared from insoluble myosin filaments (in 0.12M KCl) essentially by the method of Weeds and Pope (1977).

Methods

SDS-Polyacrylamide gel electrophoresis. All protein samples were boiled for 3 min in the presence of 1% sodium dodecylsulfate (SDS) and 1% β-mercaptoethanol to assure proper dissociation and to prevent degradation by proteolytic enzymes. SDS-polyacrylamide gel electrophoresis was performed according to Weber and Osborne (1969) using 7.5% polyacrylamide gels.

The molecular weight of the subfragments was estimated by co-electrophoresing each fragment with standard marker proteins (Weber and Osborne, 1969).

Optical rotation (OR) and circular dichroism (CD) measurements. The measurements of OR and CD were carried out with a JASCO ORD/UV-5 recording spectropolarimeter equipped with a CD attachment as described previously (Samejima et al., 1974). OR and CD measurements were made at the cotton trough minimum (λ = 233 nm) and θ_{222}, respectively, using protein solutions (0.5 mg/ml) in 0.6M KCl and 20 mM phosphate buffer (pH 6.0). The protein solution at about 20°C was placed into a jacketed 0.2 cm cell and the temperatue raised stepwise by up to 70°C. The

solution was held at each temperature for 20 min to ensure thermal equilibration before measuring OR or CD. Calculation for helicity have been reported in detail elsewhere (Samejima et al., 1974).

Difference spectrum. Ultraviolet absorbance changes accompanying melting of LMM was measured with a Hitachi recording spectrophotometer type 323. The experiments have been carried out at different wave lengths of incident light over the spectral range of 340–250 nm and a differential spectrum was obtained (Samejima et al., 1976).

Turbidity measurements. Turbidity measurements were made with the same spectrophotometer as in the case of difference spectrum experiments at the fixed wave length of 370 nm (Samejima et al., 1981), The reference cell was maintained at constant temperature (20°C) and the sample cell was adjusted to varying temperatures (20–70°C) by means of circulating water through separate jackets.

Gelation. To monitor the occurrence of heat-induced gelation of myosin subfragments, we measured changes in the rigidity (shear modulus) of the protein sample under various conditions by means of the band-type viscometer reported earlier (Yasui et al., 1979). A sample solution or suspension in a cuvette (1 cm) was placed in the thermostatically controlled cell holder and incubated at each temperature for 20 min prior to measurement.

Determination of sulfhydryl groups. The Ellman procedure (1959) was employed for the determination of sulfhydryl groups in myosin subfragments. The assay with 5.5′-dithiobis-(2-nitro benzoic acid) (DTNB) was carried out in saturated urea and borate (0.2M, pH 8.1. The absorbance readings were measured against appropriate blanks at 412 nm and calculations were made using the extinction coefficient of 1.36×10^4 as given by Ellman (1959).

Determination of protein concentrations. Protein concentrations of the myosin subfragments were estimated from their respective extinction coefficients (Godfrey and Harrington, 1970; Young et al., 1964) or by Biuret measurements standardized against bovine serum albumin.

RESULTS & DISCUSSION

SDS-Polyacrylamide gel electrophoresis

Typical SDS-polyacrylamide gel electrophoretograms of HMM and LMM are shown in Fig. 1(II) (a) and (b), respectively. The purity of each protein was similar to that reported by others (Tonomura, 1972; Samejima et al., 1976; Goodno et al., 1976; Weeds and Pope, 1977), and the estimated subunit molecular weight of each heavy chain

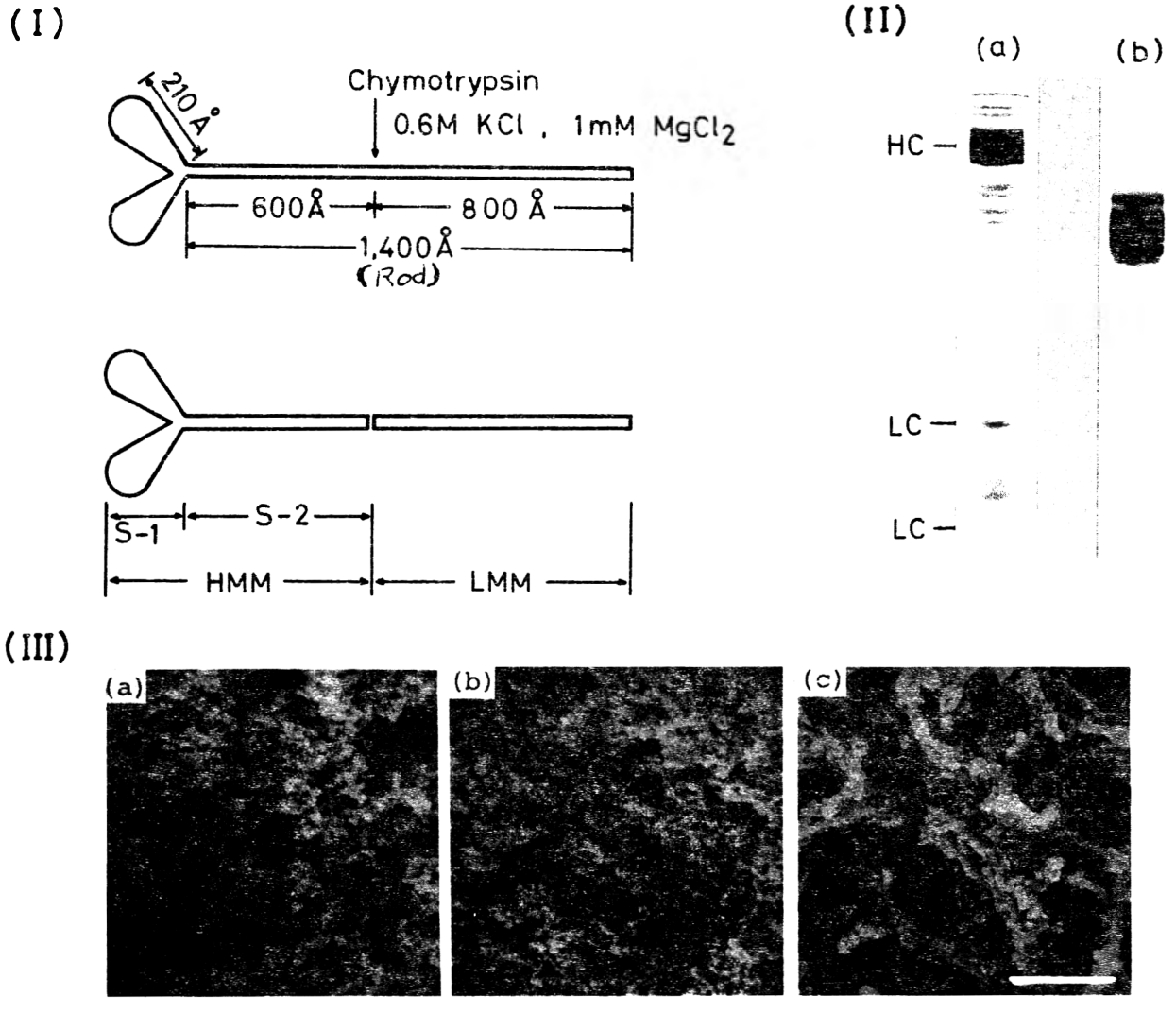


Fig. 1—Schematic representation of myosin molecule and its chymotryptic cleavage into HMM and LMM (I), SDS-polyacrylamide gel electrophoretograms of HMM and LMM preparations (II) and scanning electron micrographs of heat-set gels from HMM and LMM (III). (I) A myosin molecule and its fragmentation into HMM and LMM by chymotryptic digestion in the presence of $MgCl_2$. Skeletal myosin (about 10 mg/ml) in 0.6M KCl, 20 mM phosphate (pH 7.0), 1 mM $MgCl_2$ and 1 mM DTT was digested with α-chymotrypsin (0.03 mg/ml) at 25°C for 10 min. The reaction was stopped with 0.5 mM PMSF. (II) SDS-polyacrylamide gel electrophoretograms of HMM (a) and LMM (b). 30 μg of HMM and LMM were loaded on each gel (7.5%), respectively. HC:HMM heavy chain; LC:light chains of HMM. (III) Scanning electron micrographs of heat-set gels of HMM [(a) and (b)] and LMM(c). (a) 0.6M KCl, pH 6.0 and 60°C; (b) 0.6M KCl pH 5.0–5.5 and 60°C; (c) LMM, 0.6M KCl, pH 6.0 and 65°C. Bar length is 5 μm.

from its mobility was 200,000 for myosin, 130,000 for HMM, and 70,000 for LMM. Molecular weight and helical content of our HMM and LMM preparations were 340,000 and 55% for HMM and 140,000 and 100% for LMM, respectively.

To check a possiblity as to whether proteolytic artifacts were involved due to survival of chymotrypsin, HMM and LMM preparations thermally treated at 65°C and pH 6.0 for 20 min were examined by sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis. No further fragmentation of subfragment molecules into smaller polypeptides was observed, indicating complete inactivation of chymotryptic activity. Molecular weights of the proteins used were taken as 480,000 for myosin, 340,000 for HMM, 120,000 for S-1 (Tonomura, 1972) and 140,000 for LMM (Weeds and Pope, 1977).

HMM and LMM, and ultrastructures of their heat-set gels

Fig. 1 (I) shows a schematic representation of the limited chymotryptic digestion of myosin into HMM and LMM in the presence of 1 mM $MgCl_2$ at pH 7.0. The myosin and its subfragments are all soluble in 0.6M KCl at pH 6.0. Since the heat-induced gelation of myosin is maximum at high salt concentrations (0.4–1.0 M) (Ishioroshi et al., 1979), these were adopted as standard condition for the gelation versus temperature experiments reported in this paper.

Transparent solutions of these proteins containing 4.5 mg protein/ml become more of less turbid upon heating and at the same time, they form gels with different values of rigidity depending on the conditions used as will be seen in Fig. 2–7. Ultrastructures of gels formed from HMM and LMM are illustrated in Fig. 1(III)(a), (b) and (c). Scanning electron micrographs clearly show that lacy [Fig. 1(III)(c)] and sponge-like [Fig. 1(III)(a) and (b)] network structures are produced by LMM and HMM, respectively. Both types of gels were reported previously (Yasui et al., 1979) to be formed on the heat-induced gelation of myosin. The results imply that the very poor gel formability of the globular head region (S-1) of myosin (Samejima et al., 1981) is improved by being joined to the low ionic strength soluble tail region (S-2) through a hinge, the S-1/S-2 linkage, and that elimination of the S-2 region from the myosin total rod at a second hinge of the HMM/LMM junction does not affect substantially the heat-induced gel formability of the tail region of the molecule. This may confirm the critical role of the coiled coil α-helical tail in the heat-induced gelation of myosin.

To investigate thermo-gelling properties of HMM and LMM in comparison with those of S-1 (single bed) and the rod (tail) reported by Samejima et al. (1981), rigidity measurements were conducted under various conditions.

Gelation characteristics of HMM and LMM.

Fig. 2 and 3 compare the gelation curves of the frag-

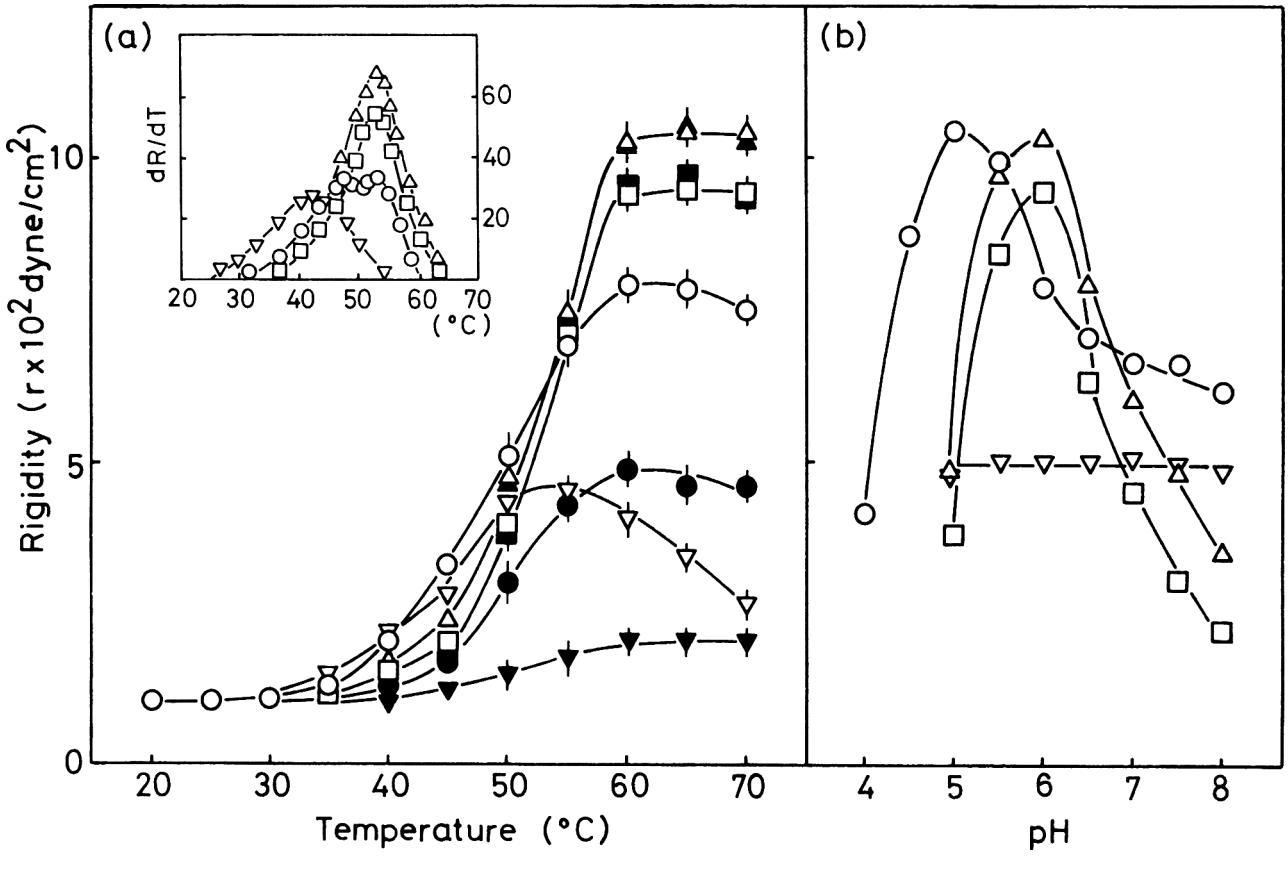


Fig. 2–Rigidity versus temperature (a) and pH (b) profiles of S-1, HMM, rod and LMM in the presence and absence of DTT. (a) Changes in rigidity of each protein system [4.5 mg/ml protein, 0.6M KCl, 20 mM phosphate (pH 6.0) with or without 1 mM DTT] which was plotted as a function of temperature. (▽), S-1; (▼), S-1 with DTT; (○) HMM; (●), HMM with DTT; (△), rod; (▲), rod with DTT; (□), LMM; (■), LMM with DTT. The error bars indicate the standard errors of measurements (n = 3–5).

Insert: Derivative plots as a function of temperature. Differential rigidity (dR/dT) of S-1, HMM, rod and LMM was plotted against temperature. (b) Changes in rigidity of each protein solution [4.5 mg/ml protein, 0.6M KCl, 20mM citrate 9pH 4.0–5.5), phosphate (pH 6.0–7.0) or Tris-HCl (pH 7.5–8.0) buffer which was plotted as a functin of pH. Temperature for gelation were 65°C for rod and LMM, 60°C for HMM and 55°C for S-1. (▽), S-1; (○), HMM; (△), rod; (□), LMM.

ments of myosin. LMM has been found to yield qualitatively similar curves to those of the myosin rod in the rigidity versus temperature, pH [Fig. 2(a) and (b)] and salt concentration [Fig. 3(a)] profiles. That is, the temperature profile revealed a single transition temperature (Tm) of about 53°C which is almost identical with that of the rod under the same conditions [Fig. 2(a), insert] and the maximum rigidity at about 65°C [Fig. 2(a)], pH profile gave the maximum at about 5.8–6.0 [Fig. 2(b)], and the gelation was developed maximally at the salt concentration range higher than 0.5M. However, the gel formability as measured by the rigidity versus protein concentration profile [Fig. 3(b)] indicates that the heat-induced gel strength increases proportionally to 1.2 power of LMM concentration. This value is considerably lower than those obtained with myosin and the rod, 1.8 (Ishioroshi et al., 1979) and 1.6 [Fig. 3(b)].

The maximum rigidity value obtained with the LMM system standardized at 4.5 mg/ml was between 940 ± 30 dyne/cm^2, and it seems slightly lower than that of the rod, 1050 ± 20 dyne/cm^2, reported by Samejima et al. (1981). This weight concentration roughly corresponds to 1.0 × 10^{-5}M of myosin whose rigidity value has been reported to be between 1800 ± 50 dyne/cm^2 (Ishioroshi et al., 1979). The rigidity value obtained with the LMM, the rod and the myosin standardized at 1.0 × 10^{-5}M (1.4 mg/ml for the LMM and 2.2 mg/ml for the rod) are 120, 480, and 1800 dyne/cm^2, respectively [Fig. 3(b) and Ishioroshi et al., 1979]. Therefore, in terms of molar basis, the gel forming ability of LMM is one-fifteenth of myosin and one-fourth of the myosin rod.

Under the same experimental conditions as LMM, HMM behaved similarly to myosin. The rigidity versus temperature profile is biphasic with two cooperative transition temperatures, Tm_1 at 45°C and Tm_2 at about 54°C [Fig. 2(a), insert] which are almost identical with those of myosin (Ishioroshi et al., 1979). Addition of 1 mM DTT to the HMM system gives rise to reduction of rigidity development as a whole and, at the same time, to disappearance of Tm_1 [Fig. 2(a)], whereas it exerted no influence over the rigidity profile of LMM [Fig. 2(a)]. We (Samejima et al., 1981) have reported that the heat-induced gelation properties of S-1 were characterized by very poor gel forming ability upon heating (450 ± 35 dyne/cm^2 in terms of rigidity), by no pH- or salt concentration-dependency of gelation and furthermore by a low value (0.6) obtained from the slope of log rigidity versus log protein concentration plot. The rigidity value from the heat-set gel at 60°C of HMM was found to increase from 450 ± 35 dyne/cm^2 for the S-1 to about 790 ± 50 dyne/cm^2 using the same protein concentration of 4.5 mg/ml. As described earlier for LMM, the gel forming ability of HMM on the molar basis is about 5 times greater than that of S-1.

Unlike the case with the heat-induced gelation of S-1, the gelation of HMM revealed an appreciable pH-dependence between pH 4.0 and 7.0 in 0.6M KCl, and the maximum rigidity (1040 ± 30 dyne/cm^2) was reached at pH 5.0, though the values in the acidic region were somewhat scattered and dependent on the time elapsed after adjustment of the pH with buffer [Fig. 2(b)].

As expected from its similarity in solubility to S-1, the heat-induced gelation of HMM showed little salt concentra-

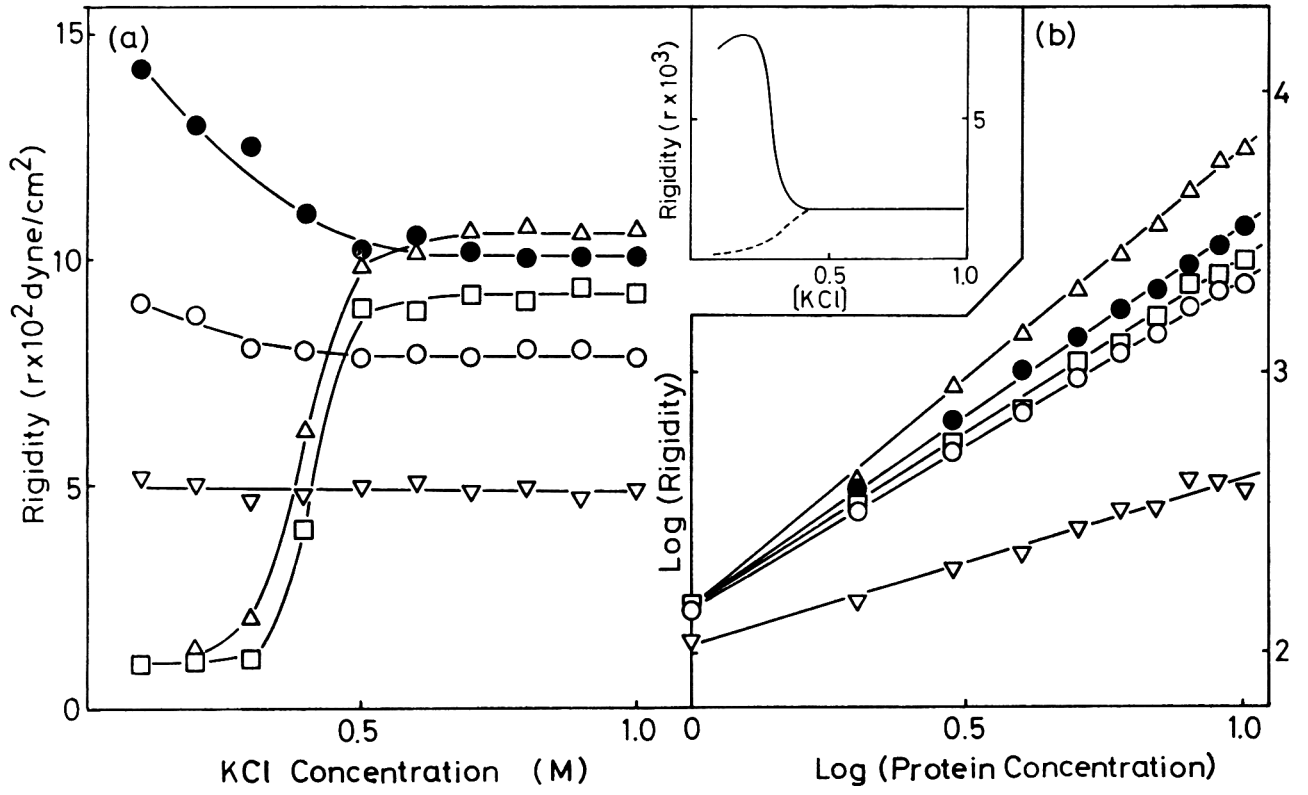


Fig. 3–Rigidity versus ionic strength (KCl concentration) profiles (a) and effects of protein concentrations (b) on the heat-induced gelation of S-1, HMM, rod and LMM. (a) Insert: Rigidity versus ionic strength profiles of freshly prepared (solid line) and 15 day-aged (dotted line) myosin quoted from the paper by Ishioroshi et al. (1979). Measurements were performed at pH 6.0 and 60°C for HMM (○), at pH 6.0 and 65°C for rod (△) and LMM (□), at pH 6.0 and 55°C for S-1 (▽) and pH 5.0 and 60°C for HMM (●). 4.5 mg/ml protein were used throughout. (b) Conditions and symbols used were the same as in (a) except that various concentrations of proteins in 0.6M KCl were used.

tion dependence, if the experiments were carried out at pH 6.0 [Fig. 3(a)]. However, the rigidity versus salt concentration profile of HMM depended remarkably on salt concentrations lower than 0.3M [Fig. 3(a)], at pH 5.0. The maximum rigidity attained was about 1400 ± 40 dyne/cm^2 at 0.1M. It is, therefore, considered that the reaction proceeds optimally at pH 5.0 and 60–65°C and in 0.1M KCl rather than in our standard condition, at pH 6.0 and 65°C and in 0.6M neutral salt. The effect of varying protein concentration from 0.1 to 1.0% was also examined on quantitative gel formability of HMM and the results are shown in Fig. 3(b). The rigidity of the heat-induced HMM gel increases proportionally to the 1.15 power of HMM concentration at pH 6.0 and in 0.6M KCl and to the 1.3 power of HMM concentration at pH 5.0 and 0.1M KCl, respectively.

Myosin shows two transition temperatures of Tm_1 at 43°C and Tm_2 at 55°C, whereas the rod and LMM reveal only one Tm at 53°C. The heat-induced gelation profiles of LMM expressed quantitatively by rigidity have indicated that the relation characteristics of LMM are quantitatively almost the same as those of myosin and the rod, i.e., the gel being formed optimally at pH 6.0 and 65°C in 0.6M KCl, though absolute values of rigidity of LMM are far lower than those of myosin and the rod. Under these conditions, the gelation of myosin has been reported to consist of two reactions (Samejima et al., 1981). The one originates from thermal aggregation of the head region of the molecule (Tm_1 at 43°C) and the other from helix-coil transition of the tail (Tm_2 at 53–55°C). The former is selectively abolished by the addition of 1 mM DTT, but it does not affect the latter, thus suggesting that the three dimensional network structure essential for the gelation is provided by the LMM segment of the tail without oxidation of SH groups.

Some physicochemical changes underlying the gelation

As shown in Fig. 4, no change in SH contents in LMM was observed in the course of the heat-induced gelation illustrated in Fig. 2, while melting of the secondary structure with a single Tm at 50–55°C took place (Fig. 5) together with an exposure of aromatic side chains from the hydrophobic core of the molecule to the more hydrophilic surface environment as manifested by the difference spectrum measurements (Fig. 6). It should be mentioned that formation of the network structure by LMM occurs almost independently of change in turbidity (Fig. 7) which is regarded as an index of thermal aggregation of both S-1 and myosin (Samejima et al., 1981). These findings may offer enough evidence to conclude that the potentiality for the heat-induced gelation which is developed at high ionic strength and pH 6.0 is intrinsically preserved in the LMM segment of the myosin rod.

A decrease of 8.6 mole of SH groups per mole of HMM took place during the course of the heat-induced gelation of HMM at pH 6.0 in 0.6M KCl (Fig. 4). This finding is consistent with the effect of DTT on the gelation of this fragment [Fig. 2(a)] and also with the results on the heat-induced gelation of the S-1 and myosin reported by Samejima et al. (1981). Thus it leads to the conclusion that oxidation of SH groups on and/or in the head region of HMM is involved in the heat-induced gelation of the fragment as far as the gelation under this particular condition is concerned.

The behavior in the heat-induced gelation of HMM observed in our standard condition has given us the impression that the globular head region of myosin is modulated to act like myosin by being linked with S-2 segment of the rod. Careful examination of the results so far obtained suggests peculiarities of this fragment distinct from myosin and other fragments. For instance, due to the resemblance to

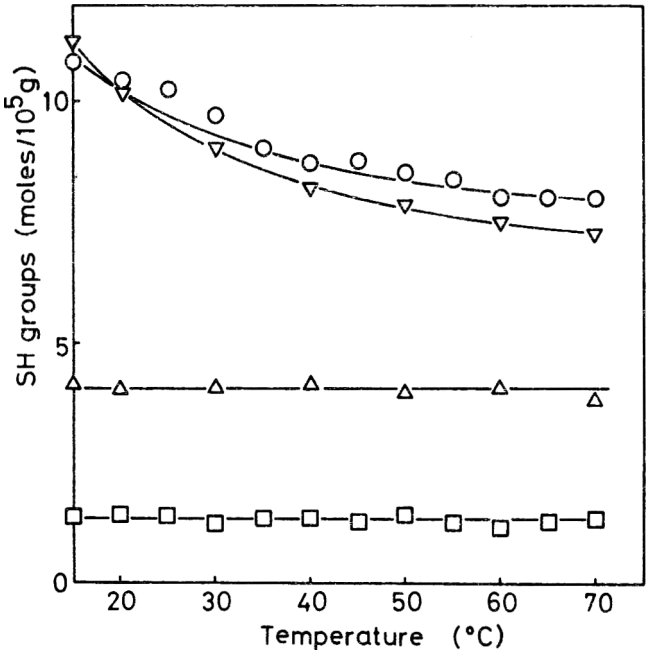


Fig. 4—Changes in the sulfhydryl contents of thermally treated S-1 (▽), HMM (○), rod (△) and LMM (□). Proteins (2 mg/ml) in 0.6M KCl and 20 mM phosphate buffer (pH 6.0) were incubated for 20 min at temperatures 20–70°C. Aliquots were withdrawn at the temperatures incubated and served for SH determination. Points were the mean of measurements on 2–3 different samples.

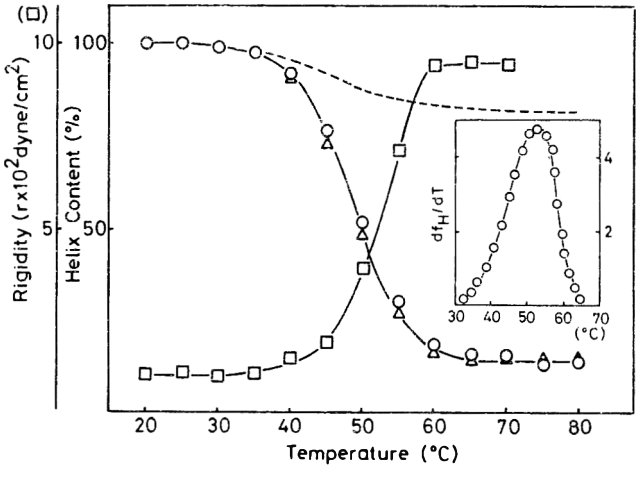


Fig. 5—Changes in helical content of LMM during the course of stepwise heating from 20–70°C. Helical contents were derived from the measured OR and CD parameters during thermal treatment. The temperature of the protein solutions, which contained 0.5 mg/ml LMM in 0.6M KCl with 20 mM phosphate buffer (pH 6.0) was raised stepwise. OR (○) and CD (△) measurements were performed with 2 mm quartz cell after 20 min incubation at each temperature. (□) represents changes in rigidity in Fig. 2 (a) of the LMM as a function of temperature. Dotted line: reversibility of the helix content of the LMM obtained from OD and CD measurements.

Insert: Derivative plot of fraction of helix (f_H) of the LMM ($\Delta f_H/\Delta T$) as a function of temperature.

the gelation of myosin, the two transition temperatures in the rigidity versus temperature profile of HMM might be considered as Tm_1 and Tm_2 for the S-1 and for the S-2, respectively. According to Tsong et al. (1979), however, of the two transition temperatures in the thermal melting curve of the myosin rod, the lower one which is not reflected in the gelation profiles (Samejima et al., 1981) is the melting transition of the S-2 portion of this fragment (~46°C), whereas the higher transition (~55°C) of the rod probably reflects melting of the LMM segment of this structure.

Since HMM possesses no LMM segment in its tail, it seems to be contradictory to presume from the result in Fig. 2(a) that the S-2 segment is responsible for the higher Tm in the heat-induced gelation of HMM. Goodno et al. (1976), however, observed that the melting temperature of the S-2 in 0.6M KCl rose from 43°C to about 52°C with decreasing pH from 7.0 to 6.0. This stabilizing effect of the pH on the structure of the S-2 segment may give us an explanation of the discrepancy described above and support our view that in the heat-induced gelation of HMM, the lower Tm stands for thermal aggregation of the S-1 region, whereas the higher Tm reflects unfolding of the S-2 segment attached to the S-1 in the HMM molecule.

Because of their instability against various denaturing conditions, myosin and its globular subfragments are readily aggregated by the thermal treatment (Yasui et al., 1960; Kawakami et al., 1971) and, in advanced stages of aggregation, the reaction can be measured as increase in turbidity of the system (Samejima et al., 1981). On the basis of their studies of the effect of DTT on both the heat-induced aggregation and gelation of myosin and the S-1, Samejima et al. (1981) recognized that the aggregation as measured by turbidity at 370 nm was associated with the early stage of the gelation of myosin and the S-1 in 0.6M KCl and at pH 6.0 whose Tm is about 43°C. Accordingly, the loss of transparency of the system appears concomitantly with the onset of gelation.

Turbidity measurements on the HMM system (Fig. 7) showed, on the other hand, an appreciable increase in turbidity which depends strongly on the pH of the system, i.e., at pH 5.0, HMM being already in the state of suspension even at 10°C. At pH 6.0, however, the subfragment solution turned turbid with completion of the gelation at 60°C. Cursory analysis of turbidity curves suggests that midpoints of turbidity changes during the course of stepwise heating at pH 5.0, 5.5, and 6.0 are < 10°, 36° and 56°C, respectively. Comparison of turbidity changes in Fig. 7 to those in rigidity in Fig. 2(a) indicated that the changes are not parallel. Hence, turbidity changes of the HMM system are probably a manifestation of changes in the self-association property, as well as the structural stability of the S-2 region of the molecule, both of which are highly pH-dependent. In fact, the diminished pH-dependence of the turbidity changes as shown by the S-1 (Fig. 7) under the same conditions suggests an alteration of the associating property of this fragment by linking the S-2 segment of the rod. The self-associating property and relative stability of the S-2 were found to increase remarkably as the pH is decreased (Goodno et al., 1976; Sutoh et al., 1978).

Notwithstanding its inhibitory effect on the early phase of the heat-induced gelation of HMM in 0.6M KCl and at pH 6.0 [Fig. 2(a)], DTT (1 mM) fails to affect the change in turbidity of HMM under the same conditions (Fig. 7). This may indicate that the extensive aggregation of HMM molecules is not involved in the SH-dependent phase of the heat-induced gelation under these experimental conditions, and the results may also support the view described in our earlier paper (Samejima et al., 1981) in which two types of

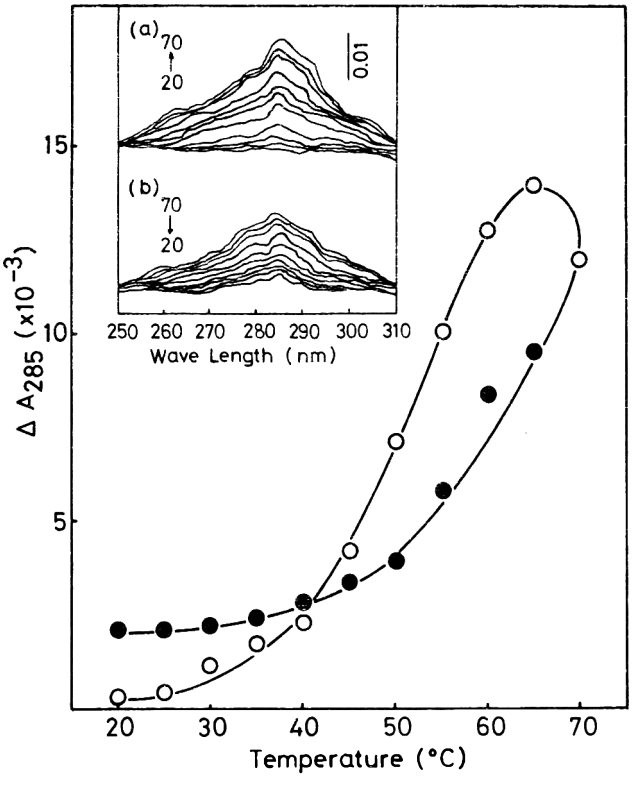


Fig. 6—Effect of temperature on $-A_{285}$ of the LMM. The temperature of the protein solution [0.6 mg/ml of LMM, 0.6M KCl and 20 mM phosphate buffer (pH 7.0)] was raised (○) or lowered (●) between 20 and 70°C.

Insert: Difference spectra of the LMM induced by heating (a) and cooling (b) system.

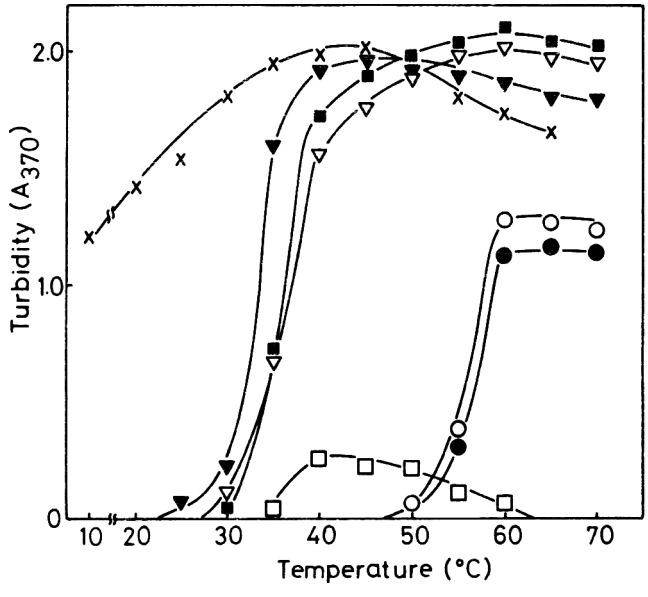


Fig. 7—Changes in turbidity of S-1, HMM and LMM during the course of stepwise heating. Different aliquots were held at just one temperature for a specified time and measured. Conditions were the same as in Fig. 2(a) and (b) except that 0.6 mg/ml protein was used in place of 4.5 mg/ml protein. (○), HMM at pH 6.0 without DTT; (●), HMM at pH 6.0 with DTT; (■), HMM at pH 5.5; (x), HMM at pH 5.0; (□), LMM at pH 6.0; (▽), S-1 at pH 6.0; (▼), S-1 at pH 5.5.

thermal aggregation of the head region of myosin molecule, the SH-dependent and the SH independent ones, have been postulated.

Since the turbidity measurements only reflect the extensively aggregated state of particles regardless of the nature of association or sorts of bonding between particles, it is deduced that a sort of oligomerization which is so small as to escape turbidity measurements may possibly occur at the early stage of gelation. Actually, as is presumed from viscosity-rigidity analogy (Nakagawa, 1978), in this type of experiment made by means of a band-type viscometer, Kawakami et al. (1971) reported that the reduced viscosity of the HMM solution measured by the use of Ostwald type viscometer demonstrated a clearly increasing trend at the early stage of the thermal denaturation. Judging from our earlier work on myosin and the S-1 (Kawakami et al., 1971; Samejima et al., 1981), the reaction corresponds to the heat-induced gelation at the lower Tm which originates from thermal aggregation of head region and is SH-dependent.

In context with the results from our earlier work (Samejima et al., 1981) the present findings indicate that the LMM plays primary roles in the heat-induced gelation of myosin at pH 6.0 and high ionic strength. Under these conditions, the LMM molecule tends to exist mostly as monomers or dimers (Burke et al., 1973) and is able to associate each other to yield a three dimensional network system [Fig. 1(III)(c), 2 and 3], as it undergoes a partially irreversible helix-coil transition upon heating (Fig. 5 and 6). This gelling property is more pronounced by an increase in length of the double stranded helical tail portion. Namely, the myosin rod whose length increases from about 80 nm of LMM to 140 nm (Takahashi, 1978) by covalent connection of the S-2 to the LMM exhibited quantitatively greater gel forming ability (Fig. 2 and 3, and Samejima et al., 1981). Furthermore, the gel forming ability is improved to a great extent with linking of head regions to the tail (Samejima et al., 1981).

It is intriguing that the S-2, the low ionic strength soluble helical segment of the rod, can not substitute for the LMM under our standard conditions, for the HMM which has two heads linked with the S-2 reveals quite different gelation profiles as a function of pH and ionic strength [Fig. 2(b) and 3(a)] than those of the rod and the LMM. However, when it comes to the gelation at low ionic strength, e.g., at 0.1M KCl, the heat-induced gelation profile of HMM qualitatively mimics that of freshly prepared myosin [Fig. 3(a) and Ishioroshi et al., 1979].

In the range of physiological ionic strength, the LMM and the rod show a strong tendency to self-associate and precipitate, and it is clear [see Fig. 3(a)] that these segments of the myosin molecule do not undergo heat-induced gelation under these conditions. In addition, the heat-induced gelation of the isolated S-1 has been found to be independent of pH and ionic strength (Samejima et al., 1981).

The S-2, the segment which connects the LMM and the S-1 portions of the intact myosin molecule is quite soluble in its isolated state over a broad range of ionic strength (Lowey et al., 1967, 1969), despite the fact that the secondary structure of this fragment is similar to that of LMM. The self-association and thermal stability of this fragment have been reported to be unexpectedly strengthened on lowering the pH below 6.0 (Sutoh et al., 1978; Goodno et al., 1976). Thus it is conceivable that the heat-induced gelation of HMM may possibly be responsible for that of freshly prepared myosin (Ishioroshi et al., 1979) occurring at low ionic strength where the LMM region of the myosin tail exerts little influence over the reaction and the masked effect of the S-2 portion can apparently emerge. Our preliminary studies on the heat-induced gelation of HMM at pH around 5.0 and ionic strength of 0.1 indicated (data not shown here) that the gelation is entirely different from that occurring under the present standard conditions. To explore the possible significance of this S-2 region of myosin in the heat-induced gelation, investigations on the changes in the physicochemical properties of the isolated S-2 fragment are now under way in our laboratory and the results will be published elsewhere.

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Relationships of Chewing Sounds to Judgments of Crispness, Crunchiness and Hardness

Z. M. VICKERS

ABSTRACT

The relationships between oral and auditory judgments of crispness, crunchiness and hardness were examined. Twenty subjects used magnitude estimation to judge the crispness, crunchiness and hardness of sixteen food items and sixteen tape recorded biting and chewing sounds. Correlations between oral and auditory judgments of each attribute were generally large. In addition, all three attributes appear to be very closely related.

INTRODUCTION

IN 1976 Vickers and Bourne hypothesized that crispness in foods was an auditory sensation. Christensen and Vickers (1981), in an effort to test this hypothesis, clearly demonstrated that auditory sensations were not essential for judgments of crispness. They, in turn, suggested that the stimulus for crispness may be vibratory. If the Vickers and Bourne hypothesis is true, auditory sensations alone should be sufficient stimuli for making judgments of food crispness. The results of Vickers and Wasserman (1980) show that food crushing sounds can be judged for crispness. One objective of this study was to further test the Vickers and Bourne hypothesis by determining if crispness judgments can be made solely on the basis of auditory sensations.

A second objective of this research was to examine the relationships between crispness and crunchiness. Several scientists have produced evidence to indicate that crispness and crunchiness are closely related sensations. Moskowitz and Kapsalis (1974) studied the use of 15 texture descriptors for 71 different foods. The regression equations they derived showed crispness to be most closely related to the quality of crunchiness. Crunchiness was most closely related to crispness and to hardness. Vickers (1979) suggested that crispness and crunchiness are both auditory sensations and differ in pitch. Vickers and Wasserman (1980) used multidimensional scaling to arrange 15 food sound descriptors in two-dimensional space. The descriptors crisp and crunchy were very close to each other in this space, indicating that they have similar meanings when used to describe sounds. Vickers and Wasserman also regressed scores for each of these descriptors on a two-dimensional multidimensional scaling solution of 18 different food sounds. The crispness and the crunchiness axes crossed the two-dimensional space at roughly the same angle, again indicating that the two attributes are similar. The present study examines the relationships between oral and auditory judgments of crispness and crunchiness.

Subjects participating in this study were also asked to judge the hardness of both the food samples and the sounds. Hardness (or firmness) has been shown to be related to judgments of crispness (Brennan et al., 1970; Jeon et al., 1975; Vickers and Christensen, 1981). Although subjects can associate the quality of hardness with food crushing sounds (Vickers and Wasserman, 1980), hardness is generally regarded as an oral-tactile sensation. Judgments of hardness were included in this study for comparison with judgments of crispness and crunchiness because judgments of food sample hardness were expected to show a relatively small correlation with judgments of sound hardness.

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EXPERIMENTAL

Subjects

Twenty University of Minnesota students, 16 females and 4 males, served as subjects. They were solicited by an advertisement in the campus newspaper. Subjects were chosen in order of inquiry and were paid for their participation.

Preparation of samples

Nine different food samples, purchased in local supermarkets were used in this study. From these foods sixteen different samples, some with altered crispness, were prepared for testing (Table 1). This series of wet crisp and dry crisp foods was chosen to represent a broad range of crispness levels and textures. The foods were cut into rectangular shapes. (Celery was an exception. Each rib was cut in half longitudinally. Measures of height and width thus correspond to the nonrectangular half-rib sections.)

The level of crispness of the dry crisp samples was adjusted by altering their water activities to approximately those shown in Table 1. The samples were placed in desiccators containing one of the following saturated solutions: LiCl (a_w = 0.11), MgCl (a_w = 0.32), K_2CO_3 (a_w = 0.44), and NaCl (a_w = 0.75), The samples remained in the desiccators for 2 wk. They were then wrapped individually in Saran and stored in airtight containers. The crispness of the wet crisp foods was adjusted by blanching them in boiling water for the length of time indicated in Table 1. Wet crisp foods were refrigerated in water until the day of testing.

Preparation of tape

The tape recording contained sounds produced by biting and chewing the foods listed in Table 1. The sounds were produced by the author and consisted of a bite and two subsequent chews. During both the biting and chewing, a microphone (Bruel and Kjaer 4133) was held against the outer ear immediately above the opening

Table 1

Product	Dimensions in mm	
	Height	Width
1. Celery, fresh	9.7	16.7
2. Celery, blanched 45 sec	8.25	15.0
3. Celery, blanched 3 min	7.9	14.8
4. Turnips, fresh	11.7	12.1
5. Turnips, blanched 30 sec	10.6	11.1
6. White radish, fresh	5.9	12.5
7. White radish, blanched 60 sec	5.2	10.6
8. Gingersnap (Nabisco), a_w = 0.11	6.6	21.0
9. Gingersnap (Nabisco), a_w = 0.32	6.6	21.0
10. Triscuit® (Nabisco), a_w = 0.32	5.0	19.4
11. Triscuit® (Nabisco), a_w = 0.44	5.0	19.4
12. Triscuit® (Nabisco), a_w = 0.75	5.0	19.4
13. Waverly Wafer® (Nabisco), a_w - 0.11	4.8	36.0
14. Ry Krisp® (Ralston Purina), a_w = 0.32	5.7	30.7
15. Lorna Doone® (Nabisco), a_w = 0.75	9.5	20.6
16. Saltine (Nabisco), a_w - 0.44	4.0	25.0

of the ear canal. This recording method produced very realistic sounds. Sounds were recorded on Scotch Brand 207 tape using a Nagra IV-SJ tape recorder (tape speed 19.05 cm/sec). A Bruel and Kjaer 2619 pre-amp and a QSJA-BK microphone amplifier were connected between the microphone and the tape recorder. Ten "bite and two chew" samples of each food were recorded. The tape segment containing the best of these ten sounds ("best" defined as the segment that sounded to the author most characteristic of the food product) was removed. These 16 "best" segments were then spliced in random order into a single tape. Three copies of these tapes were cut and spliced into three more tapes each containing the 16 sounds in a different random order. Individual sounds on all the tapes were separated by segments of nonmagnetic tape.

EXPERIMENTAL

SUBJECTS PARTICIPATED in a single test session lasting approximately 35 minutes. Each subject was tested separately by an experimenter who was present throughout the test. The experimenter read the task instructions, answered procedural questions, distributed samples and operated the tape recorder. Subjects used magnitude estimation (modulus-free) to judge the crispness, crunchiness and hardness of the food samples and of the sounds. Subjects were asked to make their judgments of the food samples by biting the food with their incisors (keeping the lips clear) and chewing it twice with their molars.

During the test session the subjects judged three separate and sequential sets of the 16 food samples (one set for crispness, one set for crunchiness, and one set for hardness) and three separate and sequential sets of the 16 biting and chewing sounds. Half of the subjects listened to the sounds first and then judged the foods. The other half judged the food samples before listening to the sounds. The order in which the subjects judged the qualities of crispness, hardness and crunchiness was randomized. The order of the food samples within a set was also randomized. Before the testing of both the foods and the sounds began, the subjects practiced using magnitude estimation (modulus-free) to assign numbers to five food or sound samples for the quality they would be judging in the first of the three sets. During these practice trials the experimenter would encourage and reinforce ratio thinking.

After judging each sample set, subjects assigned numbers to verbal concepts using the same numerical scales they had used to evaluate the foods or sounds. This scoring was intended to anchor the magnitude estimation results to the following five terms arranged in order of intensity: least crisp (crunchy, hard) food imaginable, slightly, moderatley and extremely crisp (crunchy, hard) and most crisp (crunchy, hard) food imaginable.

Analysis

The magnitude estimation data were first normalized by setting the geometric mean of each judge's nonzero scores to 10.0. Normalization procedures were performed separately for the three sensory attributes and the oral and auditory judgments (a total of six separate normalizations per judge). Regression lines and correlation coefficients for relationships between the different sensory parameters were computed using median normalized data.

RESULTS

THE RELATIONSHIP between oral and auditory judgments of crispness is shown in Fig. 1. The correlation coefficient for all data points on this plot is 0.82 (slope = 0.77). Points 2, 3 and 12 on this plot were clearly outliers. If these points are omitted and the correlation coefficient is recalculated, r = 0.96 (slope = 0.89). The outlying foods (celery blanched 45 sec, celery blanched 3 min and Triscuits a_w = 0.75) are scored crisper when judged by auditory cues alone than when judged by biting and chewing the food. Correlations (including samples 2, 3 and 12) between bite and chew judgments of crispness and auditory judgments of crispness appeared to be somewhat larger (r = 0.76) for the ten subjects who judged the sounds first than for those who judged by biting and chewing first (r = 0.66). In both groups samples 2, 3 and 12 were scored significantly lower for crispness when bitten and chewed than when judged by sound alone (paired t-test $p < 0.05$). Subjects making oral judgments first tended to score samples 2, 3 and 12 in the same manner as subjects who scored the sounds first. There was no difference in the variability of the crispness scores assigned to the foods or sounds between the two groups of subjects (paired t-test).

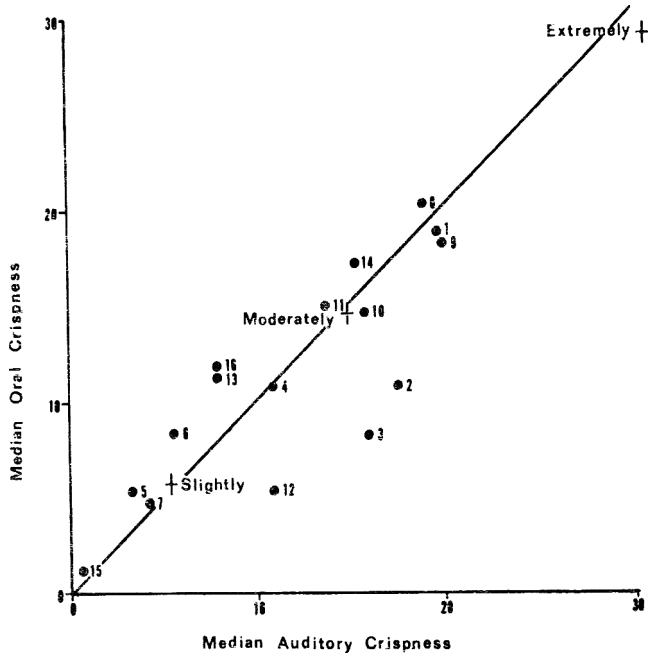


Fig. 1—Relationship between oral crispness and auditory crispness. Each point is the median judgment for 20 subjects. The identifying numbers refer to the list of foods in Table 1. The solid black line represents the function X = Y. Also included are the median values assigned to the concept scale: slightly, moderately and extremely crisp.

There is a large correlation (r = 0.95) and close to one-to-one relationship between crunchiness judged by biting and chewing the food and crunchiness judgments made on the sound alone (Fig. 2). Samples 2, 3 and 12 are not outliers on this plot although they occupy positions similar to their location in Fig. 1.

There is also a fairly large correlation (r = 0.89) and a one-to-one relationship between judgments of hardness made by biting and chewing the foods and hardness judged from the sounds alone (Fig. 3). If foods 2 and 3 are considered outliers and omitted, the recalculated correlation coefficient is 0.97.

The outlying foods (celery blanched 45 sec and celery blanched 3 min) are found to be harder when judged by auditory cues alone than when judged by biting and chewing the food. They fall in a similar position on Fig. 3 (the hardness plot) as on the crispness plot (Fig. 1). Correlations (including samples 2 and 3) betwen bite and chew judgments of hardness and auditory judgments of hardness appeared to be slightly larger (r = 0.90) for the ten subjects who judged the sounds first than for those who judged by biting and chewing first (r = 0.84). In both groups, samples 2 and 3 were scored significantly lower for hardness when bitten and chewed than when judged by sound alone (paired t-test $p < 0.05$). Subjects judging the texture first tended to score samples 2 and 3 slightly but no significantly lower than subjects who scored the sounds first (t-test). There was no difference in the variability of the hardness scores assigned to the foods or the sounds between the two groups of subjects (paired t-test).

The variability of crispness, hardness and crunchiness judgments was similar between judgments made by biting

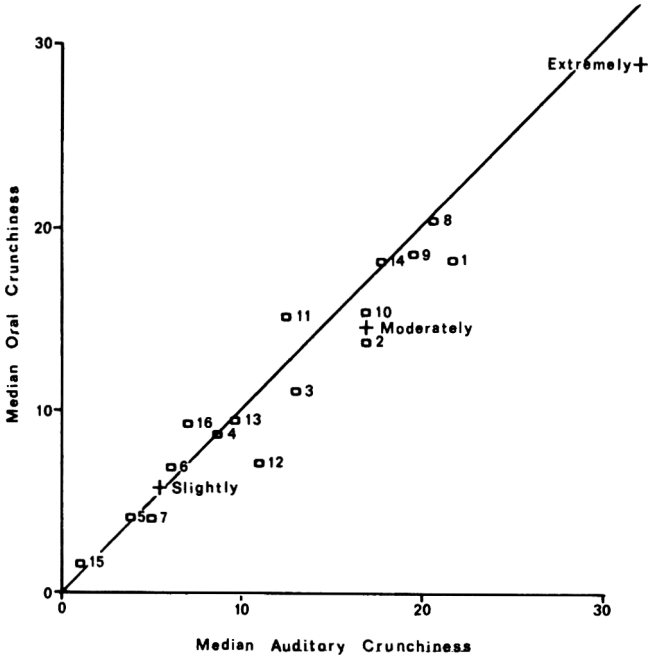


Fig. 2—Relationship between oral crunchiness and auditory crunchiness. Details as for Fig. 1.

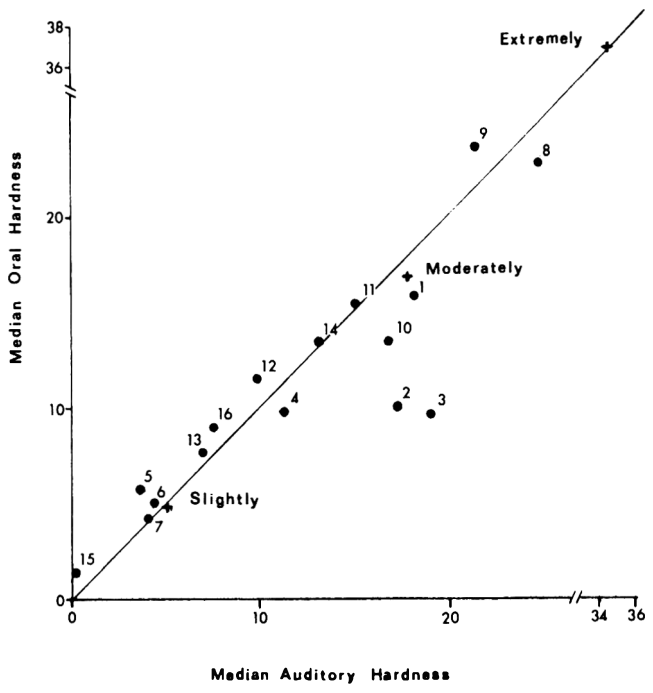


Fig. 3—Relationship between oral and auditory hardness. Details as for Fig. 1.

and chewing the foods and judgments made on the sound alone. Mean standard errors for each attribute for either test method fell between 5.5 and 8.2

By using the concept scale the subject's judgments could be anchored to adjectives describing the intensity of the attributes. Fig. 1–3 illustrate that very similar numerical values were assigned to the same levels of sensory intensity whether the test procedure was biting and chewing the food or listening to the sound. Fig. 4 and 5 indicate that this similarlity extends between attributes as well.

Crispness and crunchiness are clearly very closely related (Fig. 4 and 5). The very large correlations ($r = 0.97$ and $r = 0.96$) and one-to-one relationships (slopes = 0.94 and 0.98) exist whether judgments are made on the basis of biting and chewing the foods or by only listening to the sounds.

Correlations between hardness and crispness or cunchiness were as follows: oral hardness vs oral crunchiness ($r = 0.90$), auditory hardness vs auditory crunchiness ($r = 0.93$), oral crispness vs oral hardness ($r = 0.88$), auditory crispness vs. auditory hardness ($r = 0.97$).

DISCUSSION

THE BULK OF THE DATA in Fig. 1 show that crispness judgments can be made in the absence of oral-tactile cues or on the basis of sound alone. This inference, viewed together with the results of Christensen and Vickers (1981) which showed that crispness judgments could be made in the absence of auditory cues, would lead to the conclusion that information reaching the brain by either the auditory or oral route produces very similar crispness judgments.

The outliers on the crispness plot (Fig. 1) provide an interesting perspective to judgments of crispness. They were judged more crisp when only the sound was evaluated than when the food was actually bitten and chewed. These three products (celery blanched 45 sec, celery blanched 3 min, and Triscuits $a_w = 0.75$) are familiar foods that have had their crispness lowered. The other foods with adjusted crispness were less common and/or the altered level was not dramatically different from that normally present. Whether these three outliers should be considered an unfortunate confusion or a real part of the concept of crispness is unclear.

In the process of heating or adjusting the water activity of these foods several mechanical properties (e.g., rigidity, hardness, and toughness) that may contribute to the concept of crispness also change. These changes in physical characteristics may be reflected in oral cues to a far greater extent than in auditory cues. In such cases the oral crispness sensation would differ from the auditory crispness sensation. Whether the oral sensation predominates or the mouth and auditory sensations are integrated in such a situation cannot be resolved from this study.

The large correlation between judgments of crunchiness made with and without oral-tactile cues indicates that crunchiness judgments can be made on the basis of sound alone. The fact that points 2, 3 and 12 are not outliers on the crunchiness plot (Fig. 2) may indicate that the oral-tactile qualities that detract from the crispness of these foods are not as important for judgments of crunchiness.

An initially surprising result of this study was the very high correlation between hardness judged by biting and chewing the foods and hardness judged by only listening to the sounds. If hardness is an oral-tactile sensation as the traditional literature on food texture clearly suggests, why can the subjects judge sounds for hardness nearly as accurately as they can judge the foods? In other words, to what acoustical quality are they attributing the sensation of hardness?

One possibility is that subjects could be judging the sounds for loudness and equating loudness to hardness. This possibility would be reasonable because Vickers and Wasserman (1980) considered loudness to be one of two basic criteria for distinguishing among food crushing sounds. However, earlier research has indicated that loudness is not so closely related to hardness (firmness) when both judgments are made by biting and chewing the food ($r = 0.84$, Vickers and Christensen, 1981) or when both judgments are made on sound alone ($r = 0.66$, Vickers and Wasserman, 1980). Thus if subjects had judged the sounds for loudness instead of hardness, one would not have expected such a

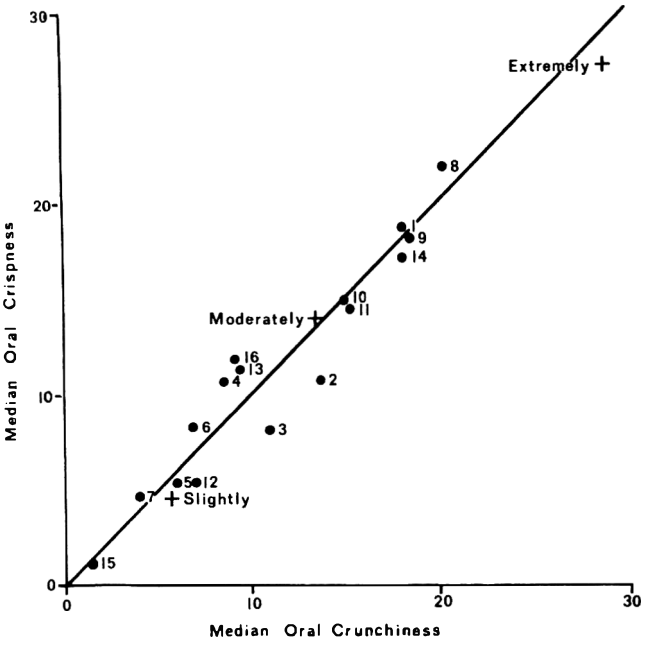


Fig. 4—Relationship between oral crispness and oral crunchiness. Details as for Fig. 1.

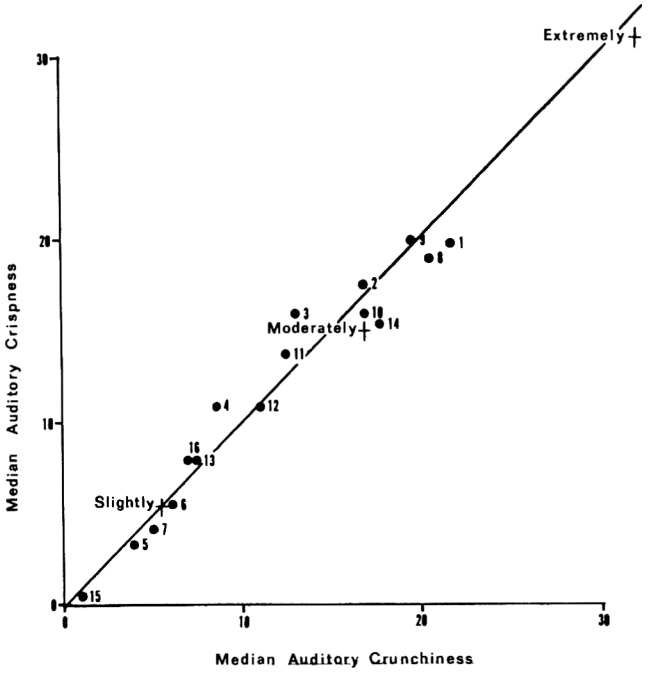


Fig. 5—Relationship between auditory crispness and auditory crunchiness. Details as for Fig. 1.

high correlation between the bite-and-chew and the sound-only procedures unless the subjects judged loudness during both test procedures.

The data seem to suggest that the food sounds possess information that may be used to distinguish among degrees of hardness. This suggestion is supported by evidence from the Vickers and Wasserman (1980) study where hardness was included as a descriptor because several people had insisted on using it to describe and contrast biting and chewing sounds.

Evidence from several earlier studies (Vickers and Christensen, 1981; Moskowitz and Kapsalis, 1975; and Vickers and Wasserman, 1980) shows that hardness and crispness do not vary independently. Vickers and Christensen (1981) actually provide evidence for hardness being a component of the crispness sensation. The large correlation (r = 0.97) between auditory crispness and auditory hardness in this study also supports this idea. If hardness is a component of crispness and crispness can be an auditory sensation, then hardness, in the context of crisp foods, could also be an auditory sensation.

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Copper and Zinc Binding to Dietary Fiber Sources: An Ion Exchange Column Method

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ABSTRACT

The ion exchange capacities of dietary fiber sources for copper and zinc were studied via a factorial design: 4 fiber sources X 2 treatments X 3 solutions. The treatments were: (1) neutral detergent and (2) enzymatic. The treated fiber residues were used as the stable matrix in columns and mineral-containing solutions were pumped through the columns. The amount of minerals bound varied with fiber type and treatment type. All fiber residues bound more copper than zinc. Binding of both minerals was reduced when they were applied in combination. The treatment of the fibers produced residues with dissimilar physical properties. These results indicate the variability introduced by methodology.

INTRODUCTION

DIETARY FIBER may influence mineral availability from foodstuffs. Reinhold et al. (1975) examined the binding of calcium, zinc and iron to various wheat breads and wheat bran. They found that even white bread and dephytinized bran bound zinc. They concluded from the data that fiber interfered with mineral metabolism. Reinhold et al. (1976) found that high fiber diets containing Bazari bread made from high extraction wheat flour, produced negative calcium and zinc balances.

One mechanism by which dietary fiber may influence mineral availability is cation exchange (Stiles, 1976). Dietary fibers contain acidic groups, such as uronic acids and acidic side chains on closely associated protein. Uronic acid content has been strongly correlated to calcium binding in dietary fiber residues (James et al., 1978). McConnell et al. (1974) determined cation exchange capacity (CEC) values for residues from fruit and vegetables, while Rasper (1979) determined CEC values for various cereal sources of dietary fiber.

Saccharides may also influence CEC and mineral binding. Glucopyranosides have been shown to complex zinc chloride by Richards and Williams (1970). Evans et al. (1979) used Sephadex to isolate zinc-binding ligands from biological fluids. They determined that the Sephadex interfered with recovery by acting as a cation exchanger. Dietary fiber is a heterogeneous material and its constituent polysaccharides could act similarly to Spehadex in the binding of cations.

The purpose of this experiment was to investigate the ability of isolated fiber residues to bind copper and/or zinc.

EXPERIMENTAL

Preparations

The fiber sources used were wheat bran (Gooch Milling & Elevator Co.), soy bran (ADM, Co,), oat hulls (National Oat Co.), and cellulose (Alphacel, ICN Pharmaceuticals). Two preparations from each fiber source were made to obtain the different dietary fiber residues. One batch was subjected to a detergent (NDF) treatment (without amylase) (Van Soest and Wine, 1967) and the other was subjected to pepsin-pancreatin enzymatic (EIR) treatment (Hellendoorn et al., 1975). Residues were ground to pass through a 60 mesh screen (US Series). Particle size of the residues was controlled by sieving. It was necessary to remove the particles smaller than 150 mesh size (except for soy bran which passed through 150 mesh but not 200 mesh) in order to achieve passage of liquid through the material.

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Analyses

The treated fiber residues were analyzed for protein via microkjeldahl. Copper and zinc concentrations were determined via dry ashing (450°C) and flame atomic absorption spectrophotometry. The cation exchange capacity (CEC) was determined as described by Helfferich (1962).

Column procedure

The column experiment was designed as a factorial experiment as follows: 4 dietary fiber residues by 2 treatments (NDF, EIR) by 3 mineral-containing solutions (Cu alone, Zn alone, Cu-Zn in combination). The columns were run in duplicate in random order. A sample of 0.25g of treated fiber residue was used as the column bed for each run.

The sample was poured as a slurry into a glass column (Bio Rad Labs, Econo columns, 0.7 cm X 15 cm). Flow rate was controlled at 1 ml/min by a peristalic pump (Gilson Medical Electronisc, Inc.). Ten ml fractions were collected in graduated test tubes.

Each column was subjected to a series of eluting solutions as follows: (1) H_2O; (2) 0.1N HCl; (3) 25 mM $NaHCO_3$, pH 6.8; (4) 10 ppm mineral (nitrate form) in 25 mM $NaHCO_3$, pH 6.8; (5) H_2O; and (6) 0.1N HCl. The approximate volume of mineral containing solution required to achieve saturation of the fiber residue was determined in preliminary experiments and varied with both fiber and treatment. Fractions were collected continuously throughout the run and their exact volumes were recorded immediately after collection. They were transferred to snap-cap vials and acidified with 50 ul 6N HCl to prevent precipitation. The fractions were directly aspirated from the vials for atomic absorption spectrophotometry.

Statistical analysis

The protein analyses and mineral determinations were performed in duplicate, while there were four replicates in the CEC determinations. The column data were subjected to a multi-way analysis of variance using the Statistical Package for Social Sciences program (Nie et al., 1975). Using Pearson's correlation, the millimoles of mineral bound per gram fiber were related to the protein content of the treated fiber residues.

RESULTS & DISCUSSION

TABLE 1 contains the values for the analyses of the fiber sources and the residues from the neutral detergent (NDF) and enzymatic (EIR) treatments. The treatments, NDF and EIR, resulted in reduced endogenous mineral contents in the residues compared to the original material. The treatments affected the mineral concentrations dissimilarly. There was less zinc and copper in the residues after the NDF treatment than after the EIR treatment (except for the oat hulls). Protein content in the treated residues was also reduced from the original levels. The soy bran and oat hulls had similar protein contents after either treatment. The wheat bran showed a large difference in protein content between the two treatments, which indicated dissimilarity introduced by the treatments. The CEC values for cellulose and wheat bran were not affected by the two treatments.

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This may indicate that although the treatments resulted in different mineral and total protein contents, the specific chemical constituents which determine CEC characteristics may have been affected similarly. Soy bran's CEC was much higher after the EIR treatments, while oat hulls' CEC was much higher after the NDF treatment. Thus, in contrast to the wheat bran and cellulose, the chemical constituents influencing the CEC of soy bran and oat hulls appeared to be greatly affected by treatment.

Fig. 1 shows the mineral concentration profile for the fractions from both treatments, NDF and EIR, of cellulose, when copper was applied alone. All trials produced similar profiles to Fig. 1. The lag in the curve after the mineral-containing buffer was begun, indicated binding of mineral to the matrix. There were large differences between fiber types in the volumes needed to reach the breakthrough region. Saturation of the column was defined as the area where concentration of mineral in the effluent equaled concentrations in the eluent. The large peak indicated that the bound mineral was released from the matrix to the solution, upon addition of HCl.

Table 1—Results of chemical analyses on the untreated fiber sources and treated fiber residues

Treatment	Fiber source	Copper (ppm)	Zinc (ppm)	Protein[a] (%)	CEC[b] (meq H^+/ g fiber)
Untreated	Wheat bran	15.3	106.3	19.3	–[c]
	Soy bran	9.0	46.6	18.5	–
	Oat hulls	17.0	31.8	4.6	–
	Cellulose	2.2	1.8	0.0	–
NDF	Wheat bran	5.7	4.3	9.2	0.091±0.007[d]
	Soy bran	1.1	T	3.6	0.109±0.008
	Oat hulls	13.9	10.5	0.6	0.114±0.006
	Cellulose	T	T	0.0	0.018±0.003
EIR	Wheat bran	8.4	21.0	4.2	0.095±0.008
	Soy bran	5.8	18.8	3.6	0.184±0.013
	Oat hulls	29.7	6.4	0.6	0.036±0.003
	Cellulose	1.9	1.6	0.0	0.019±0.001

[a] Protein = N X 6.25
[b] CEC = cation exchange capacity
[c] CEC values from untreated were not determined
[d] Mean ± standard deviation

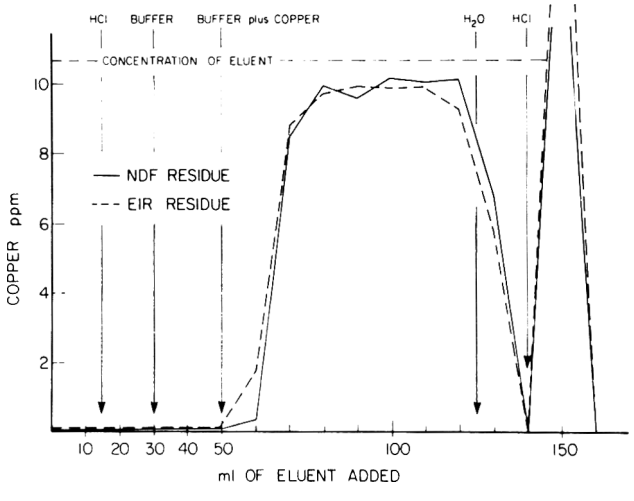


Fig. 1—Copper concentration profile for NDF- and EIR-treated cellulose residues.

The millimoles of minerals bound to the column material are shown in Table 2. The fibers differed in the amounts of both copper and zinc bound. The EIR-treated residues from soy bran and oat hulls bound copper at a higher percentage of their CEC's than did the NDF-treated residues. Less zinc bound to the residues than did copper. When presented in combination, in equimolar concentrations, the binding of both minerals was reduced from single mineral application levels. This reduction may have resulted from increased ionic strength, as Evans et al. (1979) found that zinc binding capacity of Sephadex decreased as ionic strength increased. Further experiments should be done with varying types and concentrations of trace minerals.

The analysis of variance confirmed the significant influences of fiber type, treatment type and solution type on the amount of minerals bound to the fiber residues. The Pearson's correlation coefficients indicated that the amount of copper and zinc bound to the fiber residues was related to protein content for the NDF-treated residues when the minerals were presented singly (0.997 and 0.998 respectively). However, that was not the case when the minerals were presented in combination (0.360 and 0.438 for Cu and Zn, respectively). The correlations of protein content to amount of mineral bound were not significant for any of the EIR-treated residues. Since not all the mineral binding was correlated to protein, it was possible that the fibers, i.e. various polysaccharide constituents, including uronic acids, were influencing the binding. Further research should identify the amounts of these polysaccharide constituents and examine possible relations to mineral binding.

This experiment led to two conclusions. First, the residues produced by these two treatments, neutral detergent and enzyme, did not have equivalent physical properties. In further investigations the physiochemical differences caused by methodology must be acknowledged. Second, dietary fiber sources vary dramatically in their abilities to bind minerals. This must be remembered when fiber sources are included in the diet.

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Table 2—Millimoles Of Minerals Bound To Column Material

		Minerals alone				Minerals combined			
		WB	SB	OH	Cellulose	WB	SB	OH	Cellulose
Copper	NDF	0.089±0.0016[a]	0.054±0.0003	0.039±0.0019	0.011±0.0001	0.074±0.0001	0.045±0.0003	0.068±0.0004	0.001±0.0001
	EIR	0.087±0.0021	0.112±0.0011	0.026±0.0006	0.012±0.0013	0.068±0.0046	0.087±0.0011	0.029±0.0026	0.001±0.0002
Zinc	NDF	0.064±0.0025	0.046±0.0004	0.029±0.0021	0.005±0.0001	0.040±0.0034	0.025±0.0003	0.036±0.0001	0.003±0.0004
	EIR	0.056±0.0035	0.095±0.0045	0.019±0.0020	0.005±0.0007	0.032±0.0013	0.053±0.0019	0.012±0.0022	0.003±0.0007

[a] Mean ± standard deviation

Isolation and Identification of Volatile Compounds from Roasted Peanuts

CHI-TANG HO, MIN-HSIUNG LEE, and STEPHEN S. CHANG

ABSTRACT

Volatile flavor compounds were isolated from 70 kg of freshly roasted Florida Runner peanuts by a specially designed apparatus. The isolated volatile flavor compounds were subjected to extensive gas chromatographic fractionation and the pure fractions obtained were identified by infrared and mass spectrometry. A total of 131 compounds was identified. The following compounds are reported for the first time as flavor components of roasted peanuts: five lactones, incluing γ-crotolactone, 3-methyl-γ-crotolactone and 5-hydroxy-4-nonenoic acid, lactone; four pyrazines, three pyrroles; three pyridines, 13 sulfides, including three monosulfides, six disulfides and four trisulfides; seven thiazoles, two thiophenes, five furanoids, including 2-methyl-3(2H)furanone; five oxazoles, three oxazolines, including 2-methyl-3-oxazoline; 12 hydrocarbons, one alcohol, one ketone, two acids, two esters, 2-acetoxy-2-butene, and maltol.

INTRODUCTION

PEANUTS are one of the most important agricultural products in the United States. The volatile flavor compounds of roasted peanuts have been studied by many researchers. Pattee et al. (1965) first reported the identification of fourteen compounds from high-temperature-cured peanuts. Most of the compounds identified were carbonyl compounds resulting from the autoxidation of the unsaturated fatty acids at elevated temperatures.

Mason et al. (1966) made the first positive identification in roasted peanuts of low molecular weight pyrazines, followed by identification of monocarbonyls (Mason et al., 1967). Johnson et al. (1971a, b) reported spectral evidence for 47 new constituents of peanuts, including 22 pyrazines.

An important work in the identification of the steam volatile components from roasted Spanish peanuts were reported by Walradt et al. (1971). A total of 187 compounds was identified. One hundred forty-two of them were reported for the first time from roasted peanuts. In a most recent review, Van Straten (1977) stated that a total of 279 compounds had been identified in roasted peanut flavor.

Recently, a method has been employed for the collection, characterization, and quantitation of headspace volatiles from roasted peanuts, using Tenax GC adsorption polymer (Buckholz, 1979; Buckholz et al., 1980a). Statistical analysis was used to correlate sensory and instrumental analysis. Stepwise regression showed good correlation between sensory properties and selected GC peaks (Buckholz et al., 1980b).

In the present paper, we wish to report a systematic chemical analysis of the volatile flavor components isolated from freshly roasted peanuts.

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EXPERIMENTAL

Material used

Freshly roasted peanuts (kept at −20°C) were used for isolation of the volatile compounds. Florunner peanuts were roasted in a commercial roaster to meet the Neotec color reflection of 25.0 ± 0.3 (Green). Experienced expert tasters confirmed that the roasted peanuts had a typical roasted peanut aroma and flavor.

Isolation of volatile flavor compounds (VFC)

The VFC were isolated from 70 kg of roasted peanuts using the apparatus described by Chang et al. (1977). The principle of the apparatus is the removal and subsequent condensation of the volatile compounds in the headspace of the roasted peanuts. Nitrogen gas was used to remove the volatile compounds from roasted peanuts. Seven kg of roasted peanuts were used for each isolation which lasted 48 hr at 70°C. A total of ten isolations was run. The total condensate collected in the traps cooled with dry ice and acetone was treated in a manner similar to that described by Herz and Chang (1966). The condensate (a total of 200 ml) was washed out and extracted with analytical grade ethyl ether (Mallinckrodt). The ethyl ether extract (2200 ml) of the condensate was dried with anhydrous sodium sulfate and then concentrated to a volume of 50 ml with the use of a 30-plate Oldershaw column. It was finally concentrated to a volume of 5 ml with a spinning band still.

Fractionation of the flavor isolate

The initial preparative gas chromatography of the isolated volatile roasted peanut flavor compounds was performed on a Perkin-Elmer Sigma 3 gas chromatograph equipped with a flame ionization detector, fitted with a 1/8 in. O.D. x 12 ft stainless steel column, packed with 10% OV-17 on 80/100 mesh Chromosorb W. The flow rate was 30 ml/min with a column temperature programmed from 40–240°C at a rate of 3°C/min. The chromatogram was divided into 24 broad fractions (Fig. 1). Each broad fraction was accumulatively collected according to the method of Thompson et al. (1978).

Each of the 24 broad fractions was subjected to a second fractionation using a 1/8 in. O.D. x 12 ft stainless steel column packed with 10% SP-1000 liquid stationary phase on 80/100 mesh Chromosorb W. Some selected subfractions were chromatographed for a third time using a 1/8 in. O.D. x 12 ft stainless steel column packed with 10% OV-101 liquid stationary phase on 80/100 mesh Chromosorb W.

Identication of the gas chromatographic fractions

The gas chromatographic fractions were identified by the combination of infrared and mass spectrometry, according to the procedure reported previously (Smouse and Chang, 1967). Mass spectrometry was performed on a Du Pont 21-490 Mass Spectrometer with a jet separator interfaced to a Varian Moduline 2700 Gas Chromatograph fitted with an FID detector, and a 1/8 in. O.D. x 12 ft stainless steel column packed with 10% OV-101 on 80/100 mesh Chromosorb W. The flow rate was 30 ml/min and the column temperature was programmed from 40–240°C at a rate of 3°C/min.

RESULTS & DISCUSSION

A TOTAL of 131 compounds was identified in the volatile flavor isolated from 70 kg of roasted peanuts. Table 1 lists the compounds identified which consisted of 12 aliphatic hydrocarbons, seven aromatic hydrocarbons, seven alcohols, seven aldehydes and ketones, five acids, nine esters and lactones, 21 pyrazines, seven pyrroles, six pyridines,

15 sulfides, eight thiazoles, three thiophenes, 11 furanoids, 10 oxazoles and oxazolines, and two miscellaneous compounds. Seventy compounds identified are being reported for the first time in roasted peanut flavor. The identifications were primarily based on mass spectral data; the infrared data were used to aid the identifications whenever samples were large enough to obtain the infrared spectra. Tables 2 and 3 list the mass spectral data and infrared spectral data of some volatile compounds in roasted peanuts which were not published previously.

Hydrocarbons, alcohols, aldehydes, ketones, and acids

The aliphatic hydrocarbons may not play a significant role in the roasted peanut flavor, since they possess a relatively weak aroma.

A relatively small number of alcohols, aldehydes and ketones were identified in this study.

Five lower acids were identified. Isobutyric acid, which is the main compound in the biggest fraction, No. 4, is a major component in roasted peanut flavor and has a strong butter-like aroma.

Esters and lactones

Three esters and six lactones were identified in this study. The unsaturated lactones might play an important role in roasted peanut flavor. In our opinion, 2-crotolactone and 3-methyl-2-crotolactone, had a good nutty odor. 5-Hydroxy-4-nonenoic acid, lactone was identified for the first time in a food system and contributed a nutty, fried aroma. The synthesis and sensory property of 5-hydroxy-4-nonenoic acid, lactone has been reported recently (May et al., 1978). Lactones are thermal decomposition products of fats. A number of saturated and unsaturated lactones were identified in the volatile decomposition products produced by fats under simulated commercial frying conditions (Chang et al., 1978).

Pyrazines

With the identification of 2,5-dimethylpyrazine in the volatiles of potato chips (Deck and Chang, 1965), pyrazines were uncovered as an extremely important class of flavor compounds in foods. As early as 1966, Mason et al. (1966), identified five pyrazine compounds in the volatiles isolated from roasted peanuts and found that they made a substantial contribution to the "nutty" character of roasted peanut aroma. Johnson et al. (1971a, b) identified 22 pyrazines and Walradt et al. (1971) identified 21 pyrazines in roasted peanut aroma. A total of 21 alkylpyrazines has been identified in this study. The pyrazine compounds had a pleasant roasted nut-like note. The mechanisms by which pyrazines form in food have been reviewed previously (Maga and Sizer, 1973).

Pyrroles and pyridines

Seven pyrroles and seven pyridines were identified in the volatiles of roasted peanuts. N-methylpyrrole was described as having a sweet, woody odor. 2-Acetylpyrrole was previously identified in roasted peanuts (Walradt et al., 1971), beef stew (Peterson et al., 1975) and 14 other natural products (Ohloff and Flament, 1979). Peterson et al., (1975) described 2-acetylpyrrole as having a rather unpleasant plastic, antiseptic odor.

The importance of pyridine derivatives as aroma constituents has remained quite limited so far (Ohloff and Flament, 1979), although they widely occur in various breakdown flavors such as those of beef stew (Chang and Peterson, 1977) and baked potato (Coleman et al., 1981). 2-Acetylpyridine had already been identified earlier in

—Text continued on page 132

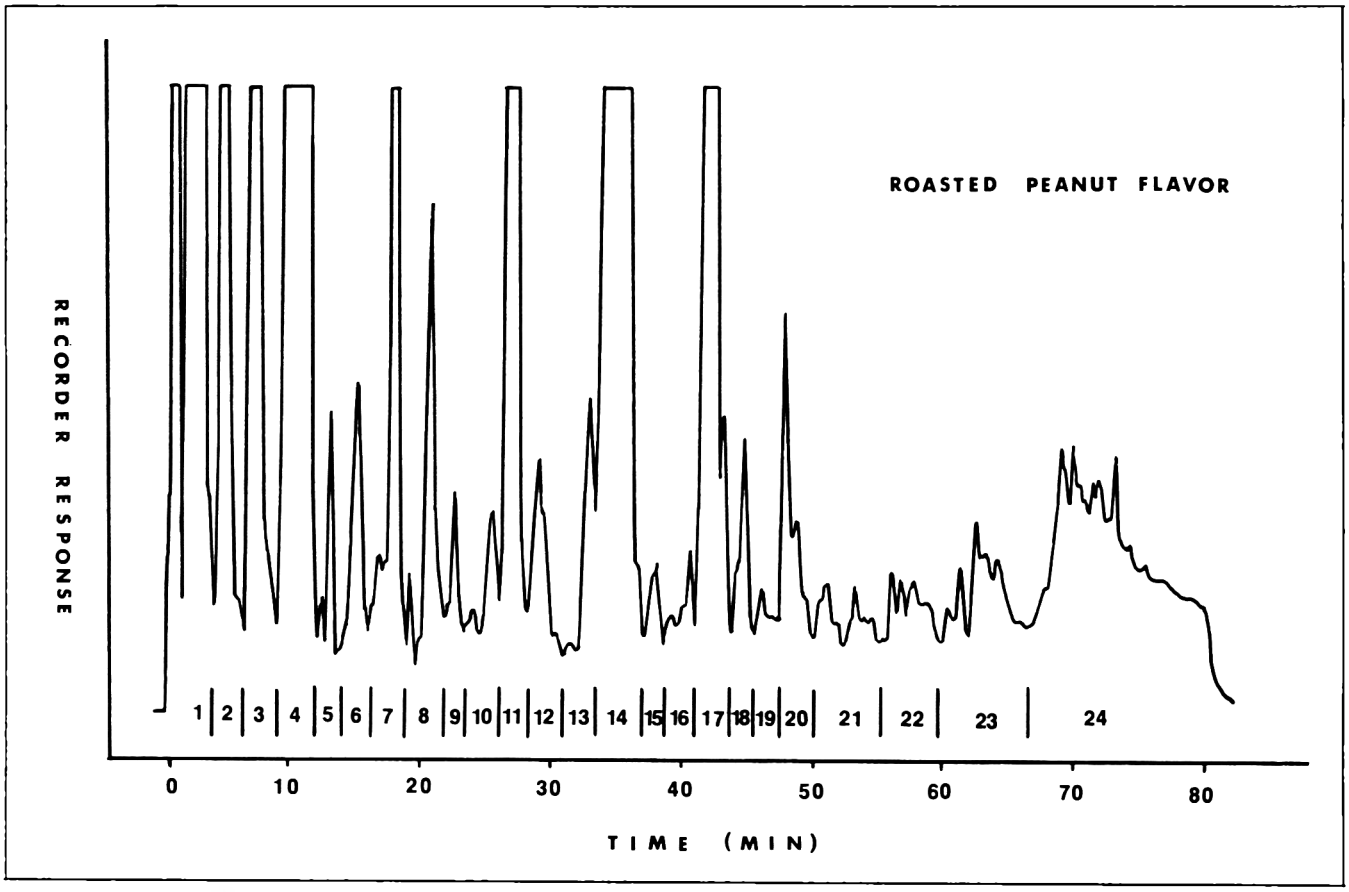


Fig. 1—Preparative gas chromatogram of the roasted peanut volatiles by using a 10% OV-17 column.

Table 1—Volatile compounds identified in roasted peanut flavor

Identification	New to roasted peanut flavor	Mass spectrum reference	Infrared spectrum reference
A. Aliphatic hydrocarbons			
n-pentane		ten Noever de Brauw et al. (1979)	
isopentane	+	MSDC (1974)	
n-nonane	+	ten Noever de Brauw et al. (1979)	
3-methylnonane	+	MSDC (1974)	
4-methylnonane	+	MSDC (1974)	
undecane		ten Noever de Brauw et al. (1979)	
tridecane		ten Noever de Brauw et al. (1979)	
2-methyltetradecane	+	MSDC (1974)	
4-ethyltetradecane	+	MSDC (1974)	
2-methylhexadecane	+	MSDC (1974)	
isobutylcyclopentane	+	MSDC (1974)	
n-hexylcyclopentane	+	MSDC (1974)	
B. Aromatic hydrocarbons			
toluene		Min et al. (1977)	
ethylbenzene		Min et al. (1977)	
p-xylene		Min et al. (1977)	
n-butylbenzene	+	Min et al. (1977)	
n-hexylbenzene	+	Min et al. (1977)	
n-heptylbenzene	+	Min et al. (1977)	
l-methylnaphthalene		MSDC (1974)	
C. Alcohols			
ethanol		MSDC (1974)	Pouchert (1978)
n-butanol		Stoll et al. (1967)	Pouchert (1978)
n-pentanol		Stoll et al. (1967)	Pouchert (1978)
2,4-dimethyl-3-pentanol	+	MSDC (1974)	
2-phenylethanol		Kinlin et al. (1972)	
benzyl alcohol		MSDC (1974)	
4-vinylphenol		MSDC (1974)	Table 3
D. Aldehydes and Ketones			
isobutylaldehyde		MSDC (1974)	Pouchert (1978)
benzaldehyde		Stoll et al. (1967)	Pouchert (1978)
2-hexanone		ten Noever de Brawu et al. (1979)	
4-methyl-3-penten-2-one		Cornu and Massot (1979)	
cyclopentanone		MSDC (1974)	
2-methylcyclopentanone	+	ten Noever de Brauw et al. (1979)	
acetophenone		MSDC (1974)	Pouchert (1978)
E. Acids			
isobutanoic acid		ten Noever de Brauw et al. (1979)	Pouchert (1978)
propionic acid		MSDC (1974)	Pouchert (1978)
pentanoic acid		MSDC (1974)	Pouchert (1978)
2-methylbutanoic acid	+	MSDC (1974)	
2,2-dimethyl-3-butenoic acid	+	MSDC (1974)	
F. Esters and Lactones			
methyl acetate	+	Stoll et al. (1967)	Sadtler (1961)
ethyl acetate		Cornu and Massot (1979)	Pouchert (1978)
trimethyl orthoacetate	+	MSDC (1974)	
γ-butyrolactone		Stoll et al. (1967)	Pouchert (1978)
γ-crotolactone	+	MSDC (1974)	Table 3
3-methyl-γ-butyrolactone	+	Table 2	Table 3
3-methyl-γ-crotolactone	+	Table 2	Table 3
5-hydroxypentanoic acid, lactone	+	MSDC (1974)	
5-hydroxy-4-nonenic acid, lactone	+	May et al. (1978)	
G. Pyrazines			
pyrazine		Bondarovich et al. (1967)	
methylpyrazine		Kinlin et al. (1972)	Bondarovich et al (1967)
2,3-dimethylpyrazine		Kinlin et al. (1972)	
2,5-dimethylpyrazine		Deck and Chang (1965)	Deck et al. (1973)
2,6-dimethylpyrazine		Kinlin et al. (1972)	Deck et al. (1973)
2-acetylpyrazine		Walradt et al (1970)	
trimethylpyrazine		Kinlin et al. (1972)	Deck et al. (1973)
2-methyl-5-ethylpyrazine		Kinlin et al. (1972)	Deck et al. (1973)
isopropylpyrazine		Bondarovich et al. (1967)	
2-methyl-5-vinylpyrazine		Bondarovich et al. (1967)	
2,3-dimethyl-5-ethylpyrazine		Friekel et al. (1971)	Freikel et al. (1971)
2,5-dimethyl-3-ethylpyrazine		Kinlin et al. (1972)	Bondarovich et al. (1967)
2,5-dimethyl-3-vinylpyrazine	+	Buttery et al. (1971)	

(Continued)

Table 1—Continued

Identification	New to roasted peanut flavor	Mass spectrum reference	Infrared spectrum reference
2,5-dimethyl-3-propylpyrazine	+	ten Noever de Brauw et al. (1979)	
2,5-dimethyl-3-isopropylpyrazine	+	Buttery et al. (1971)	
2-methyl-5,6-diethylpyrazine		Kinlin et al. (1972)	Bondarovich et al. (1967)
2-methyl-6-propenylpyrazine		ten Noever de Brauw et al. (1979)	
2-methyl-6,7-dihydro-5H-cyclopentapyrazine		Pittet et al. (1974)	Pittet et al. (1974)
5-methyl-6,7-dihydro-5H-cyclopentapyrazine		Pittet et al. (1974)	
3,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine	+	Pittet et al. (1974)	
5,6,7,8-tetrahydroquinoxaline		Pittet et al. (1974)	
H. Pyrroles			
pyrrole		Cornu and Massot (1979)	
N-methylpyrrole		Ledl and Severin (1974)	Pouchert (1978)
N-ethylpyrrole		Ledl and Severin (1974)	
2-acetylpyrrole		Kinlin et al (1972)	Table 3
N-methyl-2-formylpyrrole		Stoll et al. (1967)	
1,2,5-trimethylpyrrole	+	MSDC (1974)	
3-methyl-4-ethylpyrrole	+	MSDC (1974)	
I. Pyridines			
pyridine		MSDC (1974)	
2-methylpyridine	+	ten Noever de Brauw et al. (1979)	
3-methoxypyridine	+	MSDC (1974)	
2-acetylpyridine		Ferretti and Flanagan (1971)	
2-(n-pentyl)pyridine		ten Noever de Brauw et al. (1979)	
methyl nicotinate		MSDC (1974)	
nicotinylacetone	+	MSDC (1974)	
J. Sulfides			
2-thia-3-hexene	+	MSDC (1974)	
5-methyl-2-thiahexane	+	MSDC (1974)	
3,3-dimethyl-2-thiapentane		MSDC (1974)	
propylbutylsulfide	+	Table 2	
dimethyldisulfide		Kinlin et al. (1972)	
methylpropyldisulfide	+	Table 2	
1.1-di-(methylthio)ethane	+	MSDC (1974)	
methylbutyldisulfide	+	Table 2	
dipropyldisulfide		Table 2	
propylbutyldisulfide	+	Table 2	
di-sec-butyldisulfide	+	Dubs and Stussi (1978)	
dimethyltrisulfide	+	ten Noever de Brauw et al. (1979)	
methylethyltrisulfide	+	Table 2	
dipropyltrisulfide	+	Table 2	
dibutyltrisulfide	+	Table 2	
K. Thiazoles			
2-methylthiazole	+	Vitzthum and Werkhoff (1974a)	
4-methylthiazole		Vitzthum and Werkhoff (1974a)	
5-methylthiazole	+	Vitzthum and Werkhoff (1974a)	
5-butylthiazole	+	Table 2	
2-isopropyl-4,5-dimethylthiazole	+	Buttery and Ling (1974)	
4-butyl-2,5-dimethylthiazole	+	Buttery et al. (1973)	
2-propyl-4,5-diethylthiazole	+	Table 2	
2,4-dimethyl-5-ethylthiazole	+	Pittet and Hruza (1974)	
L. Thiophenes			
2-ethylthiophene	+	ten Noever de Brauw et al. (1979)	
2-acetylthiophene		Stoll et al. (1967)	
2-acetyltetrahydrothiophene	+	Table 2	
M. Furans			
furfural		Heyns et al. (1966)	
furfuryl alcohol		Stoll et al. (1967)	Pouchert (1978)
2-methyl-3(2H) furanone	+	Mulders (1973)	
5-methyl-3(2H)furanone	+	Seydel et al. (1967)	Table 3
2-methyltetrahydrofuran-3-one		Stoll et al. (1967)	
3-hydroxy-2-methyltetrahydrofuran	+	van den Ouweland and Peer (1975)	
2-acetyl-5-methylfuran		Heyns et al. (1966)	
5-methylfurfuryl alcohol	3	Vitzthum and Werkhoff (1974b)	
2-acetylfuran		Heyns et al. (1966)	

(Continued)

Table 1—Continued

Identification	New to roasted peanut flavor	Mass spectrum reference	Infrared spectrum reference
2-(n-butyl)furan	+	Heyns et al. (1966)	
2-(n-pentyl)furan		Chang et al. (1966)	
N. Oxazoles and Oxazolines			
4,5-dimethyloxazole		Vitzthum and Werkhoff (1974a)	
2,4,5-trimethyloxazole		Vitzthum and Werkhoff (1974a)	
2-acetyloxazole	+	Table 2	
2-methyl-5-propyloxazole	+	Table 2	
2-pentyloxazole	+	Table 2	
2-ethyl-5-butyloxazole	+	Table 2	
2,4-diethyl-5-propyloxazole	+	Table 2	
2-methyl-3-oxazoline	+	Table 2	
2,4-dimethyl-3-oxazoline	+	Mussinan et al. (1976)	
2,4,5-trimethyl-3-oxazoline	+	Chang et al. (1968)	Chang et al. (1968)
O. Miscellaneous			
2-acetoxy-2-butene	+	MSDC (1974)	Table 3
maltol	+	Stoll et al. (1967)	

Table 2—Mass spectral data of some volatile compounds identified in roasted peanut flavor

Compound	Characteristic MS data m/e (relative intensity)						
3-methyl-γ-butyrolactone	M^+ 100,	42 (100),	41 (67),	56 (43),	39 (21),	27 (18),	43 (11),
		29 (9),	40 (8),	100 (7),	55 (5).		
3-methyl-γ-crotolactone	M^+ 98,	98 (100),	43 (29)	56 (24),	71 (24),	91 (21),	21 (17),
		39 (10),	29 (6).				
propylbutylsulfide	M^+ 132,	43 (100),	89 (65),	56 (60),	47 (53),	55 (48),	132 (45),
		57 (44),	44 (40).				
methylpropyldisulfide	M^+ 122,	122 (100),	43 (73),	41 (71),	80 (62),	39 (38),	82 (34),
		45 (33),	64 (27),	59 (26).			
methylbutyldisulfide	M^+ 136,	58 (100),	136 (90),	43 (86),	41 (84),	80 (78),	39 (29),
		55 (25),	45 (22),	71 (21),	59 (20).		
dipropyldisulfide	M^+ 150,	150 (100),	43 (78),	75 (70),	108 (65),	107 (54),	42 (52),
		39 (48),	41 (30).				
propylbutyldisulfide	M^+ 164,	57 (100),	43 (93),	108 (92),	164 (59),	41 (39),	29 (25),
		66 (14),	122 (10).				
methlethyltrisulfide	M^+ 140,	61 (100),	45 (22),	94 (10),	46 (9),	140 (8),	79 (9),
dipropyltrisulfide	M^+ 182,	43 (100),	45 (64),	44 (55),	73 (52),	41 (50),	72 (37),
		61 (32),	76 (27),	108 (24).			
dibutyltrisulfide	M^+ 210,	57 (100),	41 (37),	29 (33),	89 (20),	210 (16),	122 (16),
		178 (10),	55 (9),	154 (8).			
5-butylthiazole	M^+ 141	98 (100),	71 (60),	126 (38),	83 (36),	43 (34),	59 (32),
		57 (21),	84 (20),	141 (9).			
2-propyl-4,5-diethylthiazole	M^+ 183,	154 (100),	155 (88),	59 (35),	168 (34),	41 (33),	43 (30),
		85 (25),	112 (24),	141 (23),	183 (21).		
2-acetyltetrahydrothiophene	M^+ 130,	43 (100),	87 (16),	45 (4),	44 (3),	42 (1),	86 (1),
		130 (1).					
2-acetyloxazole	M^+ 111,	111 (100)	43 (82)	68 (79),	69 (78),	41 (44),	39 (25),
		42 (15),	112 (11).				
2-methyl-5-propyloxazole	M^+ 125,	96 (100),	110 (98),	55 (87),	42 (80),	82 (58),	43 (55),
		125 (55).					
2-pentyloxazole	M^+ 139	42 (100)	97 (42),	110 (41),	111 (40),	41 (35),	59 (26),
		139 (25),	124 (23).				
2-ethyl-5-butyloxazole	M^+ 153,	110 (100),	153 (34),	43 (31),	124 (30),	82 (29),	39 (19),
		98 (18),	42 (16),	55 (15).			
2,4-diethyl-5-propyloxazole	M^+ 167	138 (100),	43 (47),	139 (42),	84 (28),	152 (27),	41 (27),
		167 (23),	112 (22).				
2-methyl-3-oxazoline	M^+ 85	43 (100),	31 (62),	55 (41),	29 (32),	85 (30),	42 (28),
		58 (24),	70 (22).				

Table 3—Infrared spectral data of some volatile compounds identified in roasted peanut flavor

Compound	Characteristic IR data cm^{-1}							
4-vinylphenol	3580,	3340,	1605,	1508,	1262,	1170		
γ-crotolactone	1780,	1152,	1100,	1045				
3-methyl-γ-butyrolactone	1705,	1235,	1170,	1102				
3-methyl-γ-crotolactone	1755,	1665,	1378,	1225,	1116			
2-acetylpyrrole	3445,	3280,	1640,	1428,	1402,	1316,	1140,	1045
dimethyltrisulfide	2990,	2970,	1488,	1465,	1392,	1360,	1170,	1040
dipropyltrisulfide	2985,	2965,	1455,	1390,	1370,	1242,	1160	
5-methyl-3(2H) furanone	2960,	1745,	1660,	1068				
2-acetoxy-2-butene	2990,	1722,	1455,	1368,	1266,	1134		

roasted peanut aroma (Walradt et al., 1971) and had an odor of popcorn (Pittet and Hruza, 1974). 2-Pentylpyridine was reported to possess a fatty or tallow-like odor and to have an extremely low threshold concentration of 0.6 ppb (Buttery et al., 1977). Pyridines might play an important role in the flavor of roasted peanuts.

Thiazoles

Numerous thiazoles have been identified in flavors of a wide variety of food such as meat (Chang and Peterson, 1977), coffee (Vitzthum and Werkhoff, 1974a) and potato products (Buttery and Ling, 1974; Coleman et al., 1981). However, in the flavor of roasted peanuts only three thiazoles, namely thiazole, 4-methylthiazole and benzothiazole, were reported (Walradt et al., 1971). The present study identified eight thiazoles. Among them, 2-isopropyl-4,5-dimethylthiazole and 2-propyl-4,5-diethylthiazole had a pleasant nutty aroma and could be important to the flavor of roasted peanuts.

Oxazoles and oxazolines

The occurrence of oxazoles has only been reported in five food flavors, namely coffee (Stoffelsma et al., 1968); Vitzthum and Werkhoff, 1974b), cocoa (Vitzthum et al., 1975), barley (Harding et al., 1978), potato (Coleman et al., 1981), and meat products (Peterson et al., 1975; Chang and Peterson, 1977). No oxazole had been reported previously in peanut flavor. In the present study, seven oxazoles were identified in the flavor of roasted peanuts. Most oxazoles identified were characterized as having a green nutty aroma.

The first oxazoline, 2,4,5-trimethyl-3-oxazoline, was reported by Chang et al. (1968) in the flavor of boiled beef. So far, oxazolines have been found to occur only in meat aroma (Hirai et al., 1973; Mussinan et al., 1976). Among the three oxazolines identified in the flavor of roasted peanuts, 2-methyl-3-oxazoline has never been reported in food flavor.

Other heterocyclic compounds

Due to their wide distribution and the diversity of their structures, furanoid compounds play an important role in food flavor. They are usually formed by degradation of carbohydrates. Eleven furanoid compounds were identified in the flavor of roasted peanuts. 2-Methyl-3(2H)-furanone and 5-methyl-3(2H)-furanone were identified for the first time in roasted peanut flavor. The former compound was previously isolated from the aroma of white bread and was claimed to be very reminiscent of bread aroma (Mulder et al., 1973). 2-Pentylfuran, presumably formed by autoxidation of linoleate, imparted a beany and grassy flavor impression when added to vegetable oil at a concentration up to 10 ppm. (Smouse and Change, 1967).

The pyrone derivative, maltol, found for the first time in roasted peanut flavor, was already discovered in 1894 as one of the character impact compounds in malt (Brand, 1894). Since then maltol has been identified as an important flavor constituent in various thermally processed foodstuffs (Ohloff and Flament, 1979).

Thiophenes have been identified in various cooked and roasted products like roast beef (Min et al., 1977), roasted coffee (Stoffelsma et al., 1968), and roasted peanuts (Walradt et al., 1971). Three thiophenes were identified in this study.

Sulfides

Fifteen symmetrical or mixed sulfides, disulfides and trisulfides were identified in the roasted peanut flavor in this study. Among them, only dimethyldisulfide and dipropyldisulfide had been previously identified in roasted peanut flavor (Johnson et al., 1971a, b; Walradt et al., 1971).

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Effects of Acids on Potassium Sorbate Inhibition of Food-Related Microorganisms in Culture Media

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ABSTRACT

Effects of potassium sorbate in combination with citric, lactic, phosphoric, and hydrochloric acids on the growth of six food-related microorganisms were studied. Trypticase soy broth with 0.1 or 0.2% sorbate adjusted to pH 5.5 with any of the acids was bacteriostatic to *Yersinia enterocolitica* 0:17. These combinations at pH 5.5 were also bacteriostatic to *Salmonella* group D, and extended the lag phase of *Pseudomonas fluorescens* for 24 hr. Combinations of either organic acid with 0.2% sorbate at pH 5.5, reduced the growth rate of *Lactobacillus plantarum* and an unidentified lactobacillus isolated from frankfurters. *Pseudomonas aeruginosa* was unaffected by any sorbate-acid combination. Organic acids, specifically citric and lactic, potentiate the antimicrobial action of potassium sorbate.

INTRODUCTION

THE INCLUSION of potassium sorbate as an antimicrobial agent in a variety of meat products has been extensively investigated. With the addition of at least 0.1% sorbate with and without 40 ppm of nitrite, the growth and/or subsequent toxin production of *Clostridium botulinum* was retarded in chicken emulsions (Sofos et al., 1980), skinless precooked, uncured sausage links (Tompkin et al., 1974), bacon (Ivey et al., 1978) and frankfurter-type mixtures formulated with deboned chicken meat, beef, or pork in conjunction with soy products (Sofos et al., 1979). In addition, potassium sorbate ranging from 0.1–0.26% delayed the growth of several food-related organisms e.g., *Staphylococcus aureus* in both bacon (Pierson et al., 1979) and skinless precooked, uncured sausage links (Tompkin et al., 1974), *Salmonella* and *Escherichia coli* in vacuum-packaged oven roasted turkey breasts (To and Robach, 1980a), and *Vibrio parahaemolyticus* in crab meat and flounder homogenates (Robach and Hickey, 1978).

Fresh poultry products dipped in a solution containing at least 5% potassium sorbate have been shown to possess an extended refrigerated shelf life as compared to untreated samples. Cunningham (1979) showed that fresh poultry (drumsticks and breasts) dipped for 30 sec in a 10% potassium sorbate solution had a 20-day shelf life at 4°C which was approximately twice as long as the untreated samples, based on off-odor development and time for total plate counts to reach 10^7 cells/cm^2. In a similar study, Robach (1979a) demonstrated that freshly chilled broilers had an extended refrigerated shelf life (19 days) when dipped in a 5% sorbate solution for 30 sec. However, when poultry products were dipped in a 15% sorbate solution for 1 min, these commodities acquired unacceptable sensory characteristics (Cunningham, 1979). When fresh poultry breasts and whole broilers were inoculated with *Salmonella* or *S. aureus* prior to a 1 min immersion in a 5% sorbate dip, the growth of *Salmonella* (Robach and Ivey, 1978; To and Robach, 1980b) was significantly reduced after at least 7 days of refrigerated storage.

In culture media, 0.2% potassium sorbate inhibited growth of three *V. parahaemolyticus* strains (Robach and Hickey, 1978) and *Pseudomonas putrefaciens* (Robach, 1979b) in trypticase soy broth (TSB) plus 2.5% NaCl and TSB, respectively, at pH 6.0. However, lactic acid bacteria, the common spoilage organism of vacuum packaged meat commodities, appears to be resistant to potassium sorbate when incorporated into conventional media at 0.1 and 0.2% (Emard and Vaughn, 1952).

Potassium sorbate in combination with other chemicals displays a synergistic inhibition for several foodborne pathogens. Using TSB (pH 6.0) incubated at 37°C, Robach and Stateler (1980) showed that certain combinations of sorbate with NaCl, tertiary butylhydroquinone (TBHQ) or butylated hydroxyanisole (BHA) potentiated sorbate's inhibitory activity against the growth of either *S. aureus* S–6 or 12600 strains, whereas BHA-sorbate combinations had synergistic antimicrobial activity against the growth of *Salmonella typhimurium* (Davidson et al., 1981).

The antimicrobial effectiveness of potassium sorbate is pH dependent. Huhtanen and Feinberg (1980) showed that poultry frankfurter emulsions inoculated with *C. botulinum* and acidified with H_3PO_4 or glucono-δ-lactone to a pH of at least 5.4 did not increase the mean swell rates. Further, the inhibition of *C. botulinum* spore germination, growth, and toxic production by sorbic acid (0.2%) alone or in combination with nitrite was pH dependent (Sofos et al., 1980). For pH values above 6.20, this effect was not observed.

The antimicrobial action of various organic acids has been reported by several investigators. Chung and Goepfert (1970) showed that the minimum pH for growth of salmonellae was dependent on the type of organic acid used to adjust the pH. Lactic acid, citric acid, and HCl required a pH of 4.40, 4.05 and 4.05, respectively, to inhibit the growth of salmonellae indicating antimicrobial activity independent of pH reduction. By dipping poultry carcasses into several organic acids or hydrochloric acid adjusted to pH 2.5, Mountney and O'Malley (1965) showed that the amount of inhibition on growth of poultry carcass microflora did not depend on the pH, but rather on the organic acid used in the immersion solution, i.e., citric and lactic acid were more inhibitory than HCl. The objective of this investigation was to determine if a synergistic antimicrobial effect exists between potassium sorbate and citric, lactic, phosphoric, or hydrochloric acids on the growth of *Yersinia enterocolitica*, *Salmonella*, *Pseudomonas*, and lactic acid bacteria in TSB and APT broth.

MATERIALS & METHODS

Bacterial strains

The six microorganisms used in this study were *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas fluorescens* ATCC 10796, *Salmonella* group D serotype isolated from minced chicken, *Yersinia enterocoliticia* serotype 0:17 isolated form a patient at Mt. Sinai Hospital, New York, by Dr. Edward J. Bottone, *Lactobacillus plantarum* (obtained from Duplo), and a lactic acid bacterium isolated

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from hot dogs. Lactic acid bacteria and the other cultures were maintained on APT and trypticase soy agar (TSA) slants, respectively, and stored at 4°C. Stock cultures were transferred monthly.

Media and inoculum preparation

The test medium for the lactic acid bacteria was APT broth; TSB was used for the others. The media were prepared according to the manufacturer's specifications with the exception that only 90% of the required amount of distilled water was used for rehydration in order to accommodate the proper volume of sorbate solution. One hundred fifty milliliters of media were dispensed into 250 ml Erlenmeyer flasks and autoclaved for 10 min at 121°C. Potassium sorbate granules were dissolved in distilled water to give 5 and 10% (wt/vol) concentrations on the day of use. These solutions were filter sterilized through a 0.2 μm membrane and 3.3 ml volumes were pipetted into the broths to yield final sorbate concentrations of either 0.1 or 0.2%. An equal volume of distilled water was added to flasks used as controls.

Stock solutions of lactic (88.2%), citric (50.0%), phosphoric (85.0%), and 1N hydrochloric acids were filter-sterilized through a 0.2 μm membrane. Half of the flasks at each sorbate level were adjusted to pH 5.5 and the other half to pH 6.0 using the sterilized acids in the amounts shown in Table 1. Ten milliliter volumes of the adjusted media were pipetted into sterile 16 x 150 mm screw-cap test tubes and incubated for 24 hr at 20°C to check for contamination.

The six bacterial cultures were transferred from slants to their respective broths and incubated for 24 hr at 25°C. The cultures were diluted in sterile distilled water to a concentration of approximately 10^4–10^5 cells/ml.

Growth and enumeration procedures

Duplicate tubes containing either 0.0, 0.1, or 0.2% sorbate adjusted to pH 5.5 or 6.0 with each acid were inoculated with 0.1 ml of the desired culture. Initial plate counts were taken from 10% of the tubes inoculated with each organism. The tubes were incubated at 25°C and samples were taken at 24-hr intervals for 4 days or until counts reached 10^8 cells/ml.

Counts were obtained using the pour-plate technique. Lactic acid bacteria were plated on APT agar and *P. aeruginosa*, *P. fluorescens*, *Salmonella*, and *Y. enterocolitica* were plated on TSA. All plates were incubated for 72 hr at 25°C. Counts were made using a Fisher Bacterial Colony Counter (Model 480) on plates containing 30–300 colonies. The data for each species were transformed into log bacterial counts/ml and the geometric mean calculated.

Chemicals

APT broth and agar were purchased from Difco. TSA and TSB were purchased from BBL Laboratories. Concentrated hydrochloric acid and citric acid (crystals) were obtained from Mallinckrodt, Inc., St. Louis, MO, lactic acid (88.2% aqueous solution) from J.T. Baker Chemical Co., Phillipsburg, NJ., and phosphoric acid (85% aqueous solution, reagent grade) from Eastman Kodak Co., Rochester, NY. Potassium sorbate (granules, lot #16922-A) was bought from ICN-K & K Laboratories, Inc., Plainview, NY.

RESULTS

Salmonella group D and *Y. enterocolitica* 0:17

The effects of potassium sorbate and acid in TSB on the growth of *Salmonella* and *Y. enterocolitica* cells are presented in Fig. 1, 2, and 3. Both microorganisms were more sensitive to the antimicrobial effects of potassium sorbate than to the antimicrobial effects of HCl, citric, lactic, or phosphoric acids. The antibacterial activity of potassium sorbate increased with a corresponding decrease in pH (Huhtanen and Feinburg, 1980; Sofos, et al., 1980). At pH 6.5, independent of the acid utilized, the growth of *Y.*

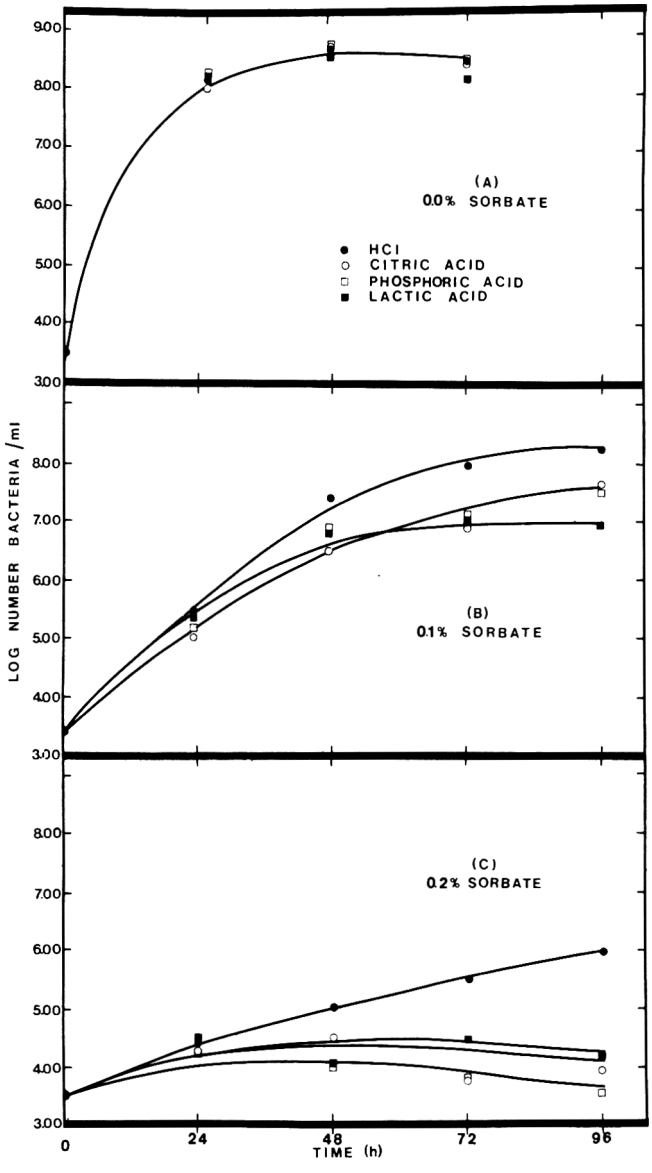


Fig. 1—Effect of sorbate on the growth of Y. enterocolitica 0:17 at pH 6.0. Cells from a 24-hr culture were inoculated into trypticase soy broth containing (A) 0.0% sorbate, (B) 0.1% sorbate, and (C) 0.2% sorbate. The pH of the broth was adjusted to 6.0 with HCl, citric acid, phosphoric acid or lactic acid. The media were incubated at 25°C.

Table 1—The molarities and amount of acid added to adjust the pH of TSB and APT broth[a]

Media	pH	Citric		Phosphoric		Lactic		Hydrochloric	
		%	mM	%	mM	%	mM	%	mM
TSB[b]	6.0	0.134	6.38	0.102	10.41	0.169	18.76	0.065	17,83
	5.5	0.195	9.30	0.127	12.96	0.238	26.42	0.086	23.59
APT	6.0	0.060	2.86	0.038	3.88	0.105	11.66	0.032	8.78
	5.5	0.150	7.19	0.102	10.41	0.185	20.54	0.060	16.46

[a] For a particular acid and pH level, the same amount of acid was required in media containing 0.0, 0.1 or 0.2% potassium sorbate.
[b] Trypticase soy broth

enterocolitica and *Salmonella* was not inhibited at 0.0 and 0.1% levels of sorbate. However, the growth of both microorganisms decreased by approximately 1 log cycle after 24 hr incubation in TSB containing 0.2% sorbate (data not presented).

At pH 6.0, in combination with each acid, 0.1% sorbate decreased the growth of *Y. enterocolitica* cells approximately 3 log cycles after 24 hr incubation at 25°C (Fig. 1A and 1B). However, after 96 hr, the lactic acid-0.1% sorbate combination decreased the total number of cells by more than 1 log cycle compared to the HCl-0.1% sorbate combination. By increasing the sorbate level to 0.2% and adjusting the pH to 6.0 with citric, lactic, or phosphoric acids, growth of *Y. enterocolitica* was decreased to a 1 log cycle increase at 24 hr; a net increase of less than 1 log cycle was observed at 96 hr (Fig. 1C). In TSB adjusted to pH 5.5, the growth rate of *Y. enterocolitica* without sorbate (Fig. 2A), was slower than the growth rate without sorbate at pH 6.0. With the addition of 0.1 or 0.2% sorbate, the growth of *Y. enterocolitica* cells was essentially zero (Fig. 2B and 2C).

The growth rates of *Salmonella* in TSB adjusted to pH 5.5 (Fig. 3A) and 6.0 (data not presented) without sorbate were similar. *Salmonella* was slightly inhibited by 0.1% sorbate at pH 5.5 (Fig. 3B). The growth rate was dependent on the type of acid used to adjust the pH. Citric acid appeared to be the most effective in increasing the antimicrobial action of 0.1% sorbate at pH 5.5, (lag phase extension and reduction in the total number of cells) when compared to HCl or phosphoric acid adjusted media. At the 0.2% sorbate concentration, however, all acids were equally effective in suppressing the growth of *Salmonella* at pH 5.5 (Fig. 3C).

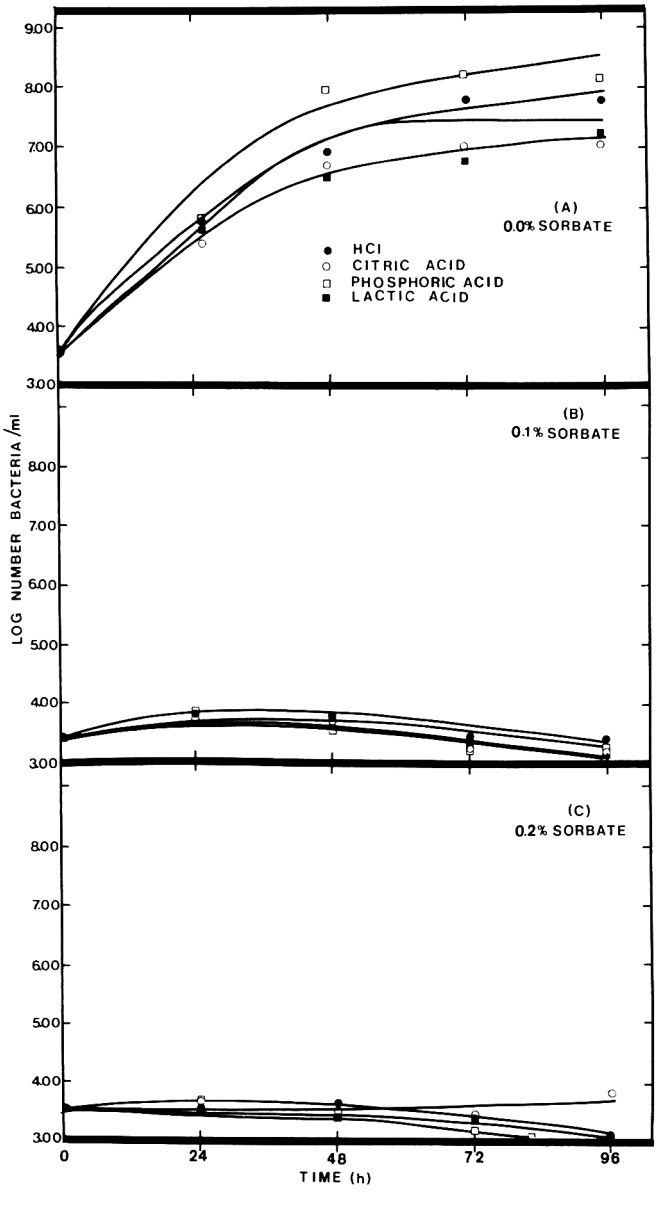


Fig. 2—Effect of sorbate on the growth of Y. enterocolitica 0:17 at pH 5.5. Cells from a 24-hr culture were inoculated into trypticase soy broth containing (A) 0.0% sorbate, (B) 0.1% sorbate and (C) 0.2% sorbate. The pH of the broth was adjusted to 5.5 with HCl, citric acid, phosphoric acid or lactic acid. The media was incubated at 25° C.

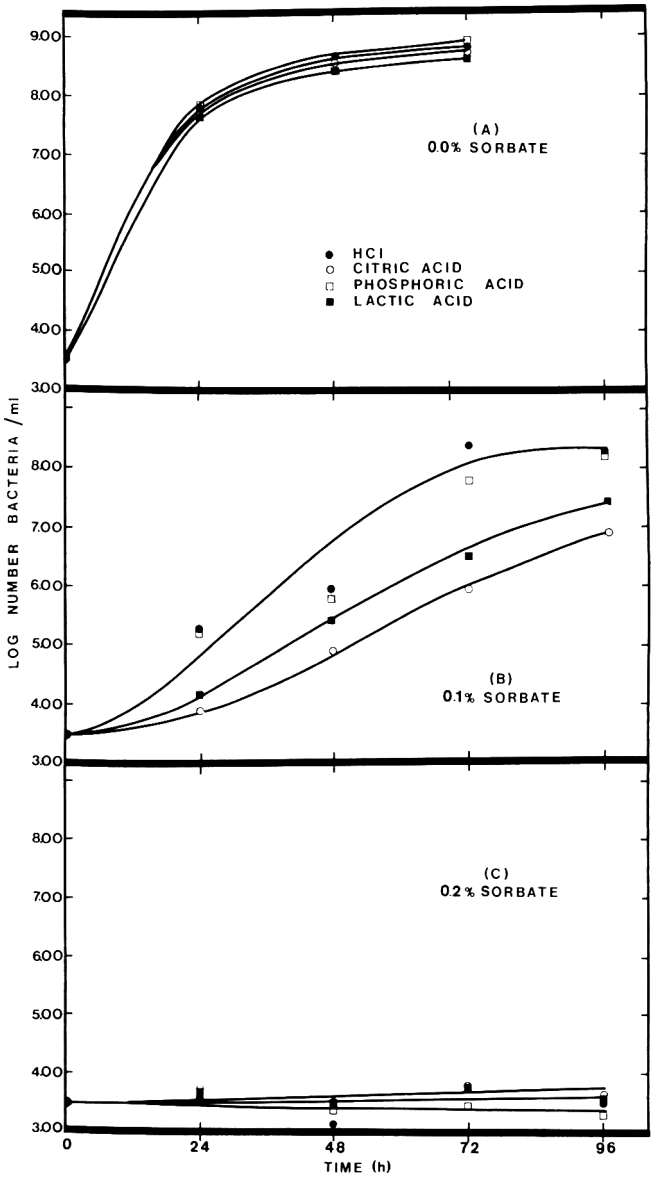


Fig. 3—Effect of sorbate on the growth of Salmonella group D serotype cells at pH 5.5. Cells from a 24-hr culture were inoculated into trypticase soy broth containing (A) 0.0% sorbate, (B) 0.1% sorbate, and (C) 0.2% sorbate. The pH of thd broth was adjusted to 5.5 with HCl, citric acid, phosphoric acid or lactic acid. The media were incubated at 25° C.

P. aeruginosa and *P. fluorescens*

The growth of *P. aeruginosa* was unaffected by any combination of 0.2% sorbate and acid at pH 5.5 or 6.0 (data not presented). The effects of sorbate on the growth of *P. fluorescens* in TSB at pH 5.5 are presented in Fig. 4. The *P. fluorescens* cells showed some inhibition by the sorbate-acid combinations at pH 5.5. Phosphoric or lactic acid suppressed growth approximately 1 log cycle more than HCl after 24 h (Fig. 4A). Citric acid and lactic acid with 0.1% sorbate extended the *P. fluorescens* lag phase with respect to HCl with 0.1% sorbate by 27 and 34 hr, respectively (Fig. 4B).

The lag phase of *P. fluorescens* in media with 0.2% sorbate in combination with lactic or citric acid was prolonged almost 24 hr more than the lag phase with HCl (Fig. 4C). The log phase of growth of *P. fluorescens* was similar in all acids at pH 5.5. At this sorbate level (0.2%), the cell populations of *P. fluorencens* increased to 10^7 cells/ml in lactic or citric acid in 89 and 82 hr, respectively, whereas in HCl adjusted TSB, only 56 hr were required.

L. plantarum and *Lactobacillus* sp. isolated from frankfurters

Citric, lactic, or phosphoric acids did not potentiate the antimicrobial effectiveness of 0.0 or 0.1% sorbate on the growth of the *Lactobacillus* isolate in APT broth at pH 5.5 (Fig. 5A and 5B). However, at the 0.2% sorbate level, the rate of growth and maximum cell population of the *Lactobacillus* isolate were less when APT was adjusted with these three acids than with HCl (Fig. 5C).

The effects of sorbate on the growth of *L. plantarum* in APT broth adjusted to pH 5.5 are presented in Figure 6. The increase in concentration of sorbate caused a decrease in the rate of growth of *L. plantarum*. Little, if any, inhibitory effect on the growth of *L. plantarum* was observed for the acids at the 0.0 and 0.1% sorbate levels. Without sorbate, *L. plantarum* counts reached 10^8 bacterial cells/ml in less than 24 hr. When 0.2% sorbate was incorporated into the APT broth, the slope of the log phase was about half that without sorbate. However, citric, lactic, or phosphoric acids potentiated the antimicrobial effectiveness of sorbate at pH 5.5.

DISCUSSION

IN ACCORDANCE with previously published data (Robach, 1978; Robach, 1979b; Robach and Ivey, 1978; Robach and Hickey, 1978), the increase in the concentration (from 0.0 to 0.2%) of potassium sorbate caused an increase in the antimicrobial effect of this compound. The effects were manifested by either an increase in the lag phase, a decrease in the growth rate, and/or a reduced total population yield. Sensitivity to the antimicrobial effects of potassium sorbate was species dependent. For example, at pH 5.5, a 0.1% sorbate concentration was bacteriostatic to *Y. enterocolitica* serotype 0:17 (atypical), but did not inhibit *Salmonella* group D. With *P. fluorescens* an increased concentration of sorbate at pH 5.5 extended the lag phase, but did not affect the growth rate. These results contrast with those of Robach (1978) who showed that 0.1% sorbate at pH 5.5 was bacteristatic for 2 strains of *P. fluorescens* for 5 days in TSB at 24°C. This discrepancy could be attributed to the different methods of incubation. They utilized a shaker culture system whereas we incubated under static conditions. Sorbate (0.2%) at pH 5.5 had no effect on the growth rate of *P. aeruginosa*. Lactic acid bacteria and other catalase-negative microorganisms are somewhat resistant to the antimicrobial action of sorbate (0.1–0.2%) at a pH of approximately 6.0 (Emard and Vaughn, 1952). Two-tenths percent sorbate slightly decreased the growth rate of 2 strains of lactic acid bacteria in APT broth at a pH of 5.5 adjusted with HCl. Lactic acid bacteria and various psychrophilic strains of *Streptococcus* are the major spoilage microorganisms of processed, anaerobically packaged meat products (Ayres et al., 1980). The data obtained in this investigation indicate that 0.2% sorbate alone would not be practical for use in extending the refrigerated shelf lives of these foods.

As observed by other investigators (Sofos et al., 1980; Huhtanen and Feinberg, 1980; Robach, 1978), the antimicrobial effectiveness of potassium sorbate is pH dependent. Our results show that with a decrease in pH from 6.5 to 5.5 using HCl, the antimicrobial effect of sorbate increased with all bacterial strains examined, except the lactic acid bacterium isolate from frankfurters and *P. aeruginosa*. These results further demonstrate that it is the un-

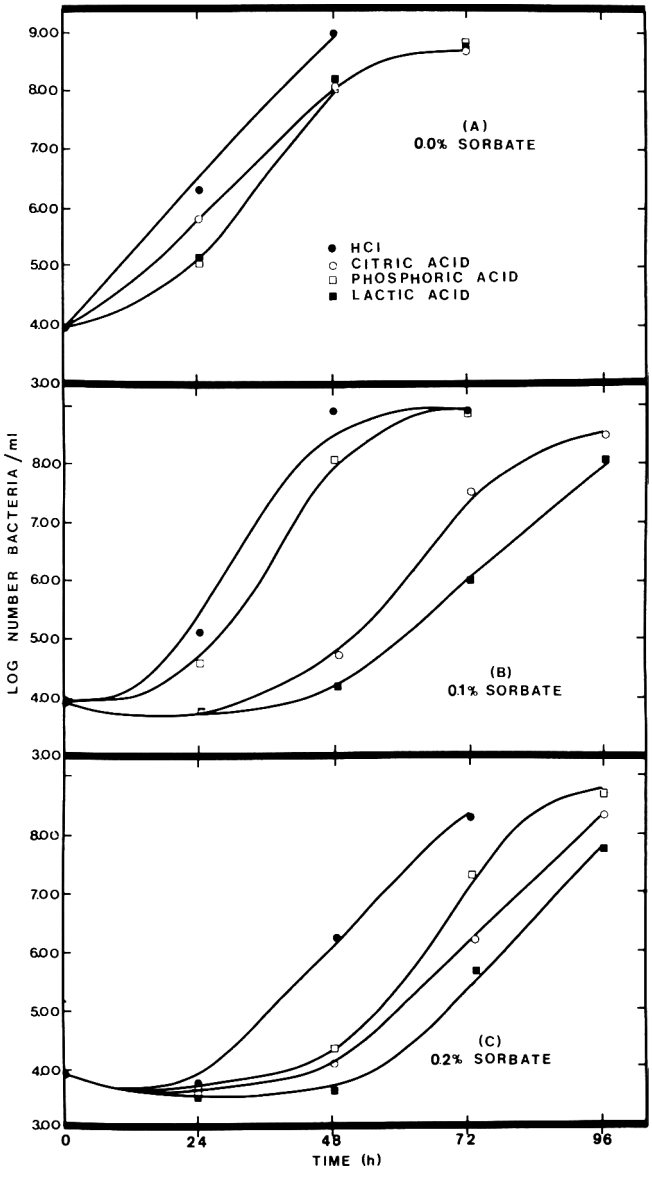


Fig. 4—Effect of sorbate on the growth of P. flourescens ATCC 10796 at pH 5.5. Cells from a 24-hr culture were inoculated into trypticase soy broth containing (A) 0.0% sorbate, (B) 0.1% sorbate, and (C) 0.2% sorbate. The pH of the broth was adjusted to 5.5 with HCl, citric acid, phosphoric acid or lactic acid. The media were incubated at 25° C.

dissociated sorbic acid molecule which posseses antimicrobial capabilities. The magnitude of the antimicrobial effect of the sorbate-acid interaction depended on the species of microorganism.

In our investigation, phosphoric, citric, or lactic acids potentiated the antimicrobial effectiveness of 0.1 or 0.2% sorbate. Each of the organic acids affected the growth of a particular bacterial strain differently. For *Y. enterocolitica* and *Salmonella*, citric or phosphoric acid in combination with sorbate demonstrated an obvious inhibition of growth (Fig. 1 and 3). Since *Salmonella* and *Y. enterocolitica* species have been routinely isolated from fresh poultry and pork products (Jensen, 1954; Nilehn, 1969; Bottone, 1977; Stern and Pierson, 1979), the application of a citric or phosphoric acid-sorbate spray or dip on pork or poultry carcasses may decrease the numbers of these microorganisms. Citric, lactic, or phosphoric acids in APT (pH 5.5) with sorbate had synergistic antimicrobial activity against lactic acid bacteria (Fig. 5 and 6). Therefore, the application of citric, phosphoric, or lactic acid-sorbate mixture to reduce the surface pH (5.6–5.8) of anaerobically packaged, fresh and/or processed meat products might increase the microbiological shelf lives of these food commodities.

The results of this investigation demonstrate that combinations of either citric or lactic acids and potassium sorbate synergistically delayed the initiation of growth and/or restrained the total growth of some bacterial strains associated with fresh meats. An investigation of the antimicrobial activities of citric acid-sorbate combinations in artificially and naturally contaminated food systems is now in progress.

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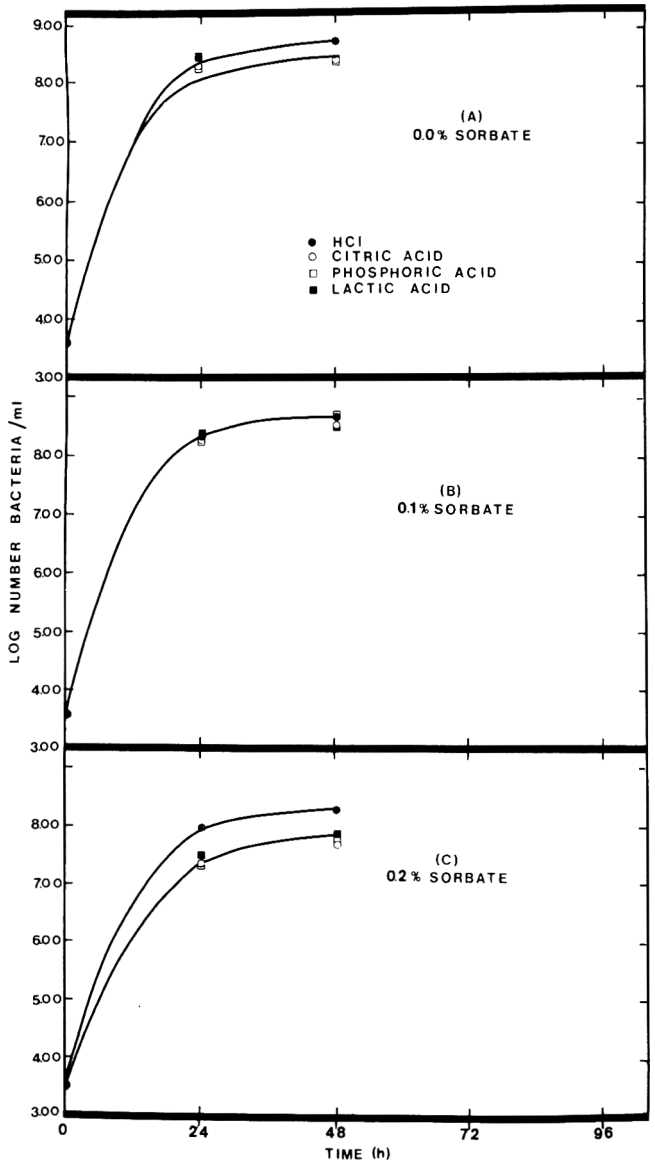


Fig. 5–Effect of sorbate on the growth of a frankfurter isolated lactic acid bacteria at pH 5.5. Cells from a 24-hr culture were inoculated into APT broth containing (A) 0.0% sorbate, (B) 0.1% sorbate and (C) 0.2% sorbate. The pH of the broth was adjusted to 5.5 with HCl, citric acid, phosphoric acid or lactic acid. The media was incubated at 25° C.

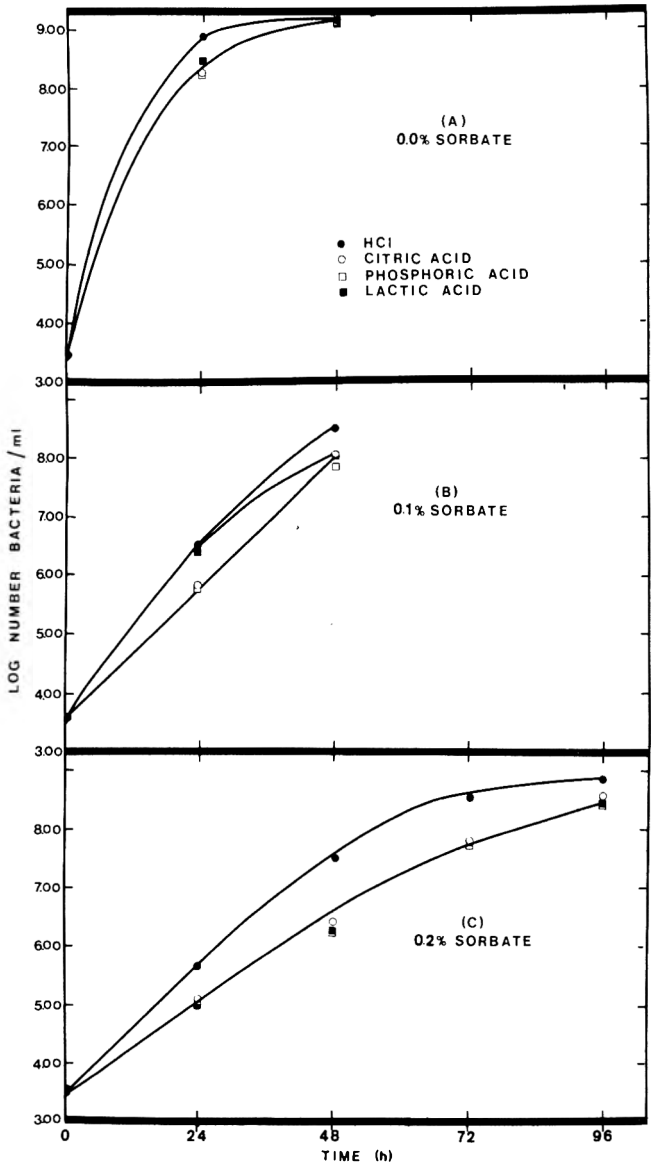


Fig. 6–Effect of sorbate on the growth of L. plantarum at pH 5.5. Cells from a 24 h culture were inoculated into APT broth containing (A) 0.0% sorbate, (B) 0.1% sorbate and (C) 0.2% sorbate. The pH of thd broth was adjusted to 5.5 with HCl, citric acid, phosphoric acid or lactic acid. The media were incubated at 25° C.

Expanded Tables for Conversion of a Proportion of Correct Responses (P_c) to the Measure of Sensory Difference (d') for the Triangular Method and the 3-Alternative Forced Choice Procedure

J. E. R. FRIJTERS

ABSTRACT

The Triangular Method differs from the 3-Alternative Forced Choice Procedure with respect to the type of instructions and the design of the experimental procedure involved. Therefore the functional relationship between a proportion of correct responses (P_c) and the sensory discriminability between stimuli (expressed as d') is not identical for the two procedures. This paper provides tables to enable easy and precise conversion of values of P_c into values of d' for each of the procedures.

TRIANGLE TEST

TRADITIONALLY the Triangle Test or Triangular Method is used for testing of statistical hypotheses stating the indiscriminability of sensory stimuli of a similar kind (e.g. Peryam, 1958). However, the method is also often used to study sensory discriminating abilities of human observers.

Basically, the aim of the experimental procedure involved is to obtain a proportion of correct responses under forced choice instructions. When the experiment is completed, the proportion of correct responses observed is tested against the chance proportion of 0.3333. Theoretically, the latter proportion must be obtained if the triangle stimuli are sensorially perfectly indiscriminable, or differently stated, if the observer has no ability to discriminate at all.

An experimentally observed proportion of correct responses (P_c) can be statistically tested using the binomial test, although a χ^2-1 sample test can equally well be carried out on raw frequencies of correct and incorrect responses (Amerine et al., 1965). In order to permit rapid analysis of data, statistical tables have been produced (Roessler et al., 1948; 1956; 1978). These tables contain the number of correct responses out of a particular number of observations required in order to exceed a specified level of significance.

Although the statistical Triangle Test is widely known as one of the "sensory difference tests," application of the procedure does not result in a *measure* of sensory difference if a statistically significant result has been obtained. The Thurstone-Ura Model for the Triangular Method does provide such a measure (Frijters, 1979).

The aim of this paper is twofold. It discusses the differences between the Triangular Method and the 3-Alternative Forced Choice (3-AFC) task and it shows that a sensory difference can be measured equally well with each of these procedures. Tables of d', the metric for sensory difference, are given and their use is illustrated with an example. First, however, attention is paid to a model related to those prevalent in bioassay, and which can be used assess differential effects on response behaviour of sensory dissimilar stimuli. This model has been called the Triangular Constant Method Model (Frijters, 1979).

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TRIANGULAR CONSTANT METHOD MODEL

A DOSE-EFFECT MODEL for the Triangular Method was developed by Bock and Jones (1968). The aim of this model is the development of a relation between a particular difference between stimuli (mostly a concentration difference) on the one hand and the difference between responses at these stimuli on the other.

Assume that a number of stimuli are available, denoted as S_s and S_j ($j = 1,2, \ldots ,n$). Let the respective stimuli have concentration t_s and t_j ($j = 1,2, \ldots ,$), and let $t_j > t_s$.

Assume further that in a particular experiment "triangles" are prepared from stimuli S_s and stimuli S_j, so that a series of triangles is presented to each of the participating subjects. After completion of the experiment, a number of j proportions of correct responses can be determined.

In the Triangular Constant Method Model each P_c is corrected first for the effect of guessing using the formula:

$$P_{corrected} = (3P_c - 1)/2 \qquad (1)$$

Next, each $P_{corrected}$ is linearly converted into a standard normal deviate denoted as z_{js}, using the cumulative distribution function of the standard normal distribution (Bock and Jones, 1968; Finney, 1971).

Now a simple functional relation can be developed of the following general form:

$$z_{js} = a + b\,(t_j/t_s) \quad (t_j > t_s) \qquad (2)$$

where z_{js} represents the "behavioral effect" resulting from the difference t_j and t_s, which are the concentrations of stimuli S_j and S_s; t_j and t_s are the concentrations of S_j and S_s; a is a constant; and b is the regression coefficient. The latter two parameters can be estimated by simple linear regression.

Bock and Jones (1968) also describe a Minimum Normit χ^2 solution and a Maximum Likelihood solution which is particularly useful for an optimal estimation of a sensory threshold. In order to facilitate the Minimum χ^2 solution Gacula and Kubala (1978) produced a table of Weights and Weighted z_{js} values.

For an example of an application of the Triangular Constant Method Model the reader is referred to Bock and Jones (1968, p. 100–103). It is noted, however, that a modification of this model has been proposed (Frijters, 1981a) since the original version appeared invalid.

THURSTONE-URA MODEL

THE ABOVE MODEL relates the stimulus continuum to the response continuum. Such a model is therefore called a Stimulus-Response Model (S-R Model, or Dose-Effect Model). The Thurstone-Ura Model is a psychometrical model for the Triangular Method (Frijters, 1979). It relates the sensory continuum (mediating between stimulus and response continuum) to the response continuum (see Frijters, 1980a or 1981b). It deals with the O-R part of a Stimulus-Organism-Response Model (S-O-R Model) (Fig. 1.).

In this model the three stimuli of a triangle, say S_s, S'_s and S_j (S_s and S'_s are physically identical, i.e. they both have concentrations t_s) give rise to the corresponding internal sensations $\underline{x}_s$, $\underline{x}'_s$ and $\underline{x}_j$. It is further assumed that

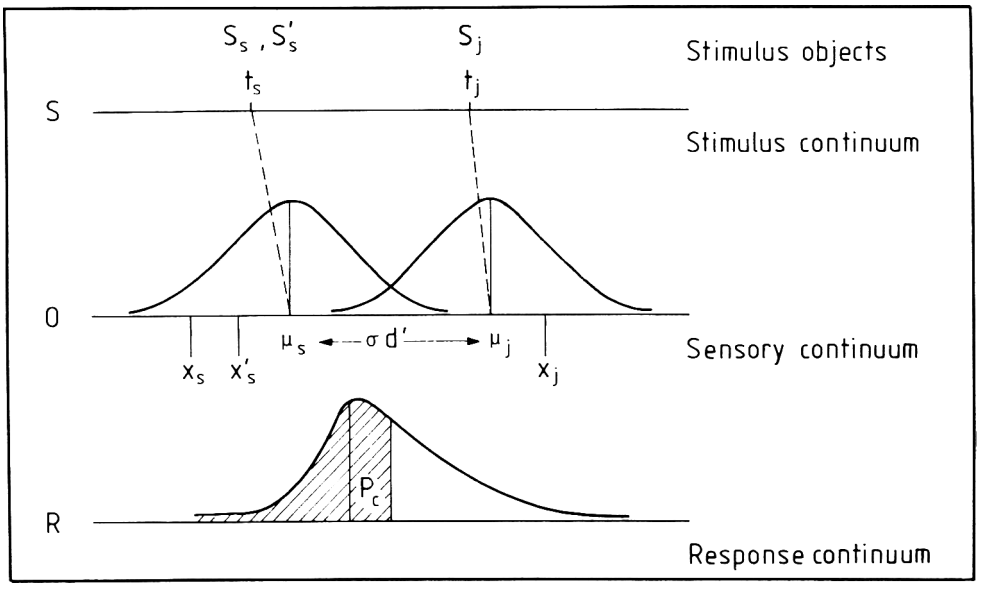


Fig. 1—S-O-R Model for the Triangular Method and 3-AFC procedure. The stimuli S_s, S'_s and S_j taken here as an example (without loss of generality) have physicochemical values t_s, t'_s and t_j respectively. On a particular trial these stimuli give rise to the sensations x_s, x'_s and x_j. In fact the sensations are random values drawn from the respective distributions $f(\mu_s)$ and $f(\mu_j)$. These distributions have equal variance σ^2, and their means μ_s and μ_j are located at $\sigma d'$ from each other. Given either the Thurstone-Ura model for the Triangular Method or the 3-AFC Model for the 3-AFC task the value of d′ can be obtained from a proportion of correct responses resulting from a number of trials. The parameter d′ represents a measure for sensory discriminability or sensory difference.

these sensory responses are mutually independently distributed with equal variances σ^2, having respective means μ_s, μ_s and μ_j (See Fig. 1). In the Normal variant of the Thurstone-Ura model the sensory distributions are assumed to be Gaussian (Frijters, 1979).

On the basis of these and some other assumptions (Ura, 1960) an equation has been developed relating P_c to the parameter of sensory discriminability which in the Theory of Signal Detection (Green and Swets, 1966) is denoted as d′. This parameter represents the standardized distance between the means of the sensory distributions resulting from the stimuli S_s and S_j respectively. The equation is given by:

$$P_c = 2\int_0^\infty \{\Phi(-u\sqrt{3}+d'\sqrt{(2/3)} + \Phi(-u\sqrt{3}-d'\sqrt{(2/3)}\} \times \exp(-\tfrac{1}{2}u^2)/\sqrt{(2\pi)}\, du \quad (3)$$

in which:

$$d' = \frac{\mu_j - \mu_s}{\sigma} \quad (\mu_j > \mu_s) \quad (4)$$

and Φ is the cumulative distribition function of the standard Normal distribution. Eq. (3) has been numerically solved by various authors who produced Tables of d′ (Ura, 1960; David and Trivedi, 1962; Bradley, 1963; Frijters et al., 1980). The present Table 1 is the most expanded Table of d′ since it contains the values of P_c corresponding to the values of d′ ranging between 0.00 and 4.89 increasing with steps of 0.01. This table allows a precise conversion of P_c to its corresponding value of d′. Thus, using an experimentally obtained proportion of correct responses as an unbiased estimate of P_c in (3), Table 1 provides the corresponding value of d′.

The transformation of an experimentally obtained P_c into d′ opens the possibility to establish a psychophysical relation between the ratio of the physicochemical stimulus parameters t_j and t_s on the hand and d′ on the other (S-O part of an S-O-R Model, see Fig. 1).

If an experiment has been carried out using triangles composed of a reference stimulus level S_s and stimuli S_j (j = 1,2, . . . , n) a series of j values of P_c has been obtained. These are then converted into the corresponding values of d′. After this conversion the following psychophysical function can be established (Frijters, 1980b):

$$d' = b \log (t_j/t_s)\ (t_j > t_s) \quad (5) \text{ where}$$

t_i and t_s are the concentrations of the stimuli S_j and S_s respectively. Note that this function is fitted through the origin, because if $t_j \rightarrow t_s$, and thus $\log (t_j/t_s) \rightarrow 0$, then $d' \rightarrow 0$ (Frijters, 1980b; 1981c).

3-ALTERNATIVE FORCED CHOICE MODEL

SENSORY ADAPTATION within triangles may exert some influence on the discriminative ability of the observer, i.e. it lowers sensory sensitivity. Adaptation results in a higher P_c for triangles containing two physicochemically weak stimuli plus one stronger, than for triangles composed of two strong plus one weaker stimulus (Hopkins, 1954; Filipello, 1956; Frijters, 1977). This observation led the ASTM (1968) to recommend the use of triangles only of the combination with two physicochemically weak stimuli plus one stronger, and to avoid the use of triangles of both combinations. (Thus if S_j is stronger than S_s, only the permutations $S_jS_sS'_s$, $S_sS_jS'_s$ and $S_sS'_sS_j$ should be used, and not all six permutations $S_jS_sS'_s$, $S_sS_jS'_s$, $S_jS_sS'_s$, $S_jS'_sS_s$, $S_jS_sS'_j$ and $S_sS_jS'_j$.) As pointed out (Frijters, 1981c) this recommendation leads to a hybrid psychophysical procedure, which is a mixture of the 3-Alternative Forced Choice Procedure (3-AFC) and the traditional Triangular Method. In the 3-AFC procedure, which is a particular case of the family of multiple-AFC procedures (Green and Swets, 1966), only tri-stimulus sets are used composed of two physicochemically weak plus one stronger stimulus, or the sets contain two strong plus one weaker stimulus. In the first version the instructions are given to select the *strongest* stimulus, whereas in the symmetrical version the instructions state to select the *weakest* stimulus. This is different from the Triangular Method in which the subject is asked to select the *odd* stimulus from each triangle presented.

The difference in design and instructions between the 3-AFC procedure and the Triangular Method may seem of

marginal relevance only. However, theoretically it has been shown (Frijters, 1979) that these differences can have drastic influences on the proportion of correct responses experimentally obtained without affecting the corresponding value of $\underline{d}'$, and this was experimentally confirmed (Frijters, 1980b).

As for the Triangular Method under the Thurstone-Ura Model, for the 3-AFC task the relationship between P_c and $\underline{d}'$ can be specified under similar assumptions. The Normal variant of the 3-AFC Model is given by (Frijters, 1979):

$$P_c = \int_0^\infty \left\{\Phi^2 (u+d') + \Phi^2(-u+d')\right\}\exp(-\tfrac{1}{2}u^2)/\sqrt{(2\pi)}\, du \quad (6)$$

where Φ is the cumulative distribution function of the standard Normal distribution, and $\underline{d}'$ is identical to (4).

Table 2 contains the values of P_c corresponding to the values of $\underline{d}'$ ranging between 0.00 and 3.99 increasing with steps of 0.01. If an experimentally obtained P_c is used as an unbiased estimate of P_c in (6). Table 2 can be used to obtain the corresponding values of $\underline{d}'$.

If in a particular experiment triangles are presented composed of stimuli of a reference level S_s and stimuli S_j

Table 1—The Thurstone-Ura Model (Normal variant)

Function: $P_c = 2\int_0^\infty \left\{\Phi(-u\sqrt{3}+\underline{d}'\sqrt{(2/3)} + \Phi(-u\sqrt{3}-\underline{d}'\sqrt{(2/3)}\right\} \exp(-\tfrac{1}{2}u^2)/\sqrt{(2\pi)}\, du$

Decimal Places: 4 Decimal point omitted.

Examples:

P_c	$\underline{d}'$
0.4494	1.19
0.8576	3.59

$\underline{d}'$	0	1	2	3	4	5	6	7	8	9
0.0	3333	3333	3333	3334	3334	3335	3336	3337	3339	3340
0.1	3342	3344	3346	3348	3351	3353	3356	3359	3363	3366
0.2	3369	3373	3377	3381	3386	3390	3395	3399	3404	3410
0.3	3415	3420	3426	3432	3438	3444	3451	3457	3464	3471
0.4	3478	3485	3493	3500	3508	3516	3524	3532	3541	3549
0.5	3558	3567	3576	3585	3594	3604	3614	3623	3633	3644
0.6	3654	3664	3675	3686	3697	3708	3719	3730	3742	3753
0.7	3765	3777	3789	3802	3814	3826	3839	3852	3865	3878
0.8	3891	3904	3918	3931	3945	3959	3973	3987	4001	4015
0.9	4029	4044	4059	4073	4088	4103	4118	4134	4149	4164
1.0	4180	4196	4211	4227	4243	4259	4275	4292	4308	4324
1.1	4341	4358	4374	4391	4408	4425	4442	4459	4477	4494
1.2	4511	4529	4546	4564	4582	4599	4617	4635	4653	4671
1.3	4689	4708	4726	4744	4763	4781	4800	4818	4837	4855
1.4	4874	4893	4912	4931	4950	4969	4988	5007	5026	5045
1.5	5064	5083	5103	5122	5141	5161	5180	5199	5219	5238
1.6	5258	5277	5297	5317	5336	5356	5375	5395	5415	5435
1.7	5454	5474	5494	5513	5533	5553	5573	5593	5612	5632
1.8	5652	5672	5692	5712	5731	5751	5771	5791	5811	5830
1.9	5850	5870	5890	5910	5929	5949	5969	5988	6008	6028
2.0	6048	6067	6087	6106	6126	6146	6165	6185	6204	6224
2.1	6243	6263	6282	6301	6321	6340	6359	6379	6398	6417
2.2	6436	6455	6475	6494	6513	6532	6551	6570	6588	6607
2.3	6626	6645	6664	6682	6701	6720	6738	6757	6775	6793
2.4	6812	6830	6848	6867	6885	6903	6921	6939	6957	6975
2.5	6993	7011	7028	7046	7064	7082	7099	7177	7134	7151
2.6	7169	7186	7203	7221	7238	7255	7272	7289	7306	7322
2.7	7339	7356	7373	7389	7406	7422	7439	7455	7471	7487
2.8	7504	7520	7536	7552	7568	7584	7599	7615	7631	7646
2.9	7662	7677	7693	7708	7723	7739	7754	7769	7784	7799
3.0	7814	7829	7843	7858	7873	7887	7902	7916	7931	7945
3.1	7959	7973	7987	8001	8015	8029	8043	8057	8071	8084
3.2	8098	8111	8125	8138	8152	8165	8178	8191	8204	8217
3.3	8230	8243	8256	8268	8281	8294	8306	8319	8331	8343
3.4	8356	8368	8380	8392	8404	8416	8428	8440	8451	8463
3.5	8475	8486	8498	8509	8521	8532	8543	8554	8565	8576
3.6	8587	8598	8609	8620	8631	8641	8652	8662	8673	8683
3.7	8694	8704	8714	8724	8734	8744	8754	8764	8774	8784
3.8	8794	8803	8813	8823	8832	8841	8851	8860	8869	8879
3.9	8888	8897	8906	8915	8924	8933	8941	8950	5959	5968
4.0	8976	8985	8993	9001	9010	9018	9026	9035	9043	9051
4.1	9059	9067	9075	9083	9090	9098	9106	9113	9121	9129
4.2	9136	9144	9151	9158	9166	9173	9180	9187	9194	9201
4.3	9208	9215	9222	9229	9236	9242	9249	9256	9262	9269
4.4	9275	9282	9288	9295	9301	9307	9313	9320	9326	9332
4.5	9338	9344	9350	9356	9362	9367	9373	9379	9385	9390
4.6	9396	9401	9407	9412	9418	9423	9429	9434	9439	9444
4.7	9449	9455	9460	9465	9670	9475	9480	9885	9490	9494
4.8	9499	9504	9509	9513	9518	9523	9527	9532	9536	9541

Table 2—The 3-Alternative Forced Choice Model (Normal variant)

Function: $P_c = \int_0^\infty \{\Phi^2(u+d') + \Phi^2(-u+d')\} \exp(-\frac{1}{2}u^2)/\sqrt{(2\pi)}\, du$

Decimal Places: 4, Decimal points omitted.

Examples P_c 0.6870, 0.9894 — d' 1.99, 3.59

d'	0	1	2	3	4	5	6	7	8	9
0.0	3333	3361	3389	3418	3446	3475	3504	3532	3561	3590
0.1	3619	3648	3678	3707	3736	3765	3795	3824	3854	3884
0.2	3913	3943	3973	4003	4033	4063	4093	4123	4153	4183
0.3	4214	4244	4274	4305	4335	4366	4396	4427	4457	4488
0.4	4518	4549	4580	4610	4641	4672	4702	4733	4764	4795
0.5	4825	4856	4887	4918	4949	4979	5010	5041	5072	5103
0.6	5133	5164	5195	5226	5256	5287	5218	5348	5379	5410
0.7	5440	5471	5501	5532	5562	5593	5623	5653	5684	5714
0.8	5744	5774	5804	5835	5865	5895	5924	5954	5984	6014
0.9	6044	6073	6103	6132	6162	6191	6220	6249	6279	6308
1.0	6337	6365	6394	6423	6452	6480	6509	6537	6565	6594
1.1	6622	6650	6678	6705	6733	6761	6788	6816	6843	6870
1.2	6898	6925	6951	6978	7005	7032	7058	7084	7111	7137
1.3	7163	7189	7215	7240	7266	7291	7316	7342	7367	7392
1.4	7416	7441	7466	7490	7514	7539	7563	7587	7610	7634
1.5	7658	7681	7704	7727	7750	7773	7796	7819	7841	7863
1.6	7886	7906	7929	7951	7973	7994	8016	8037	8058	8079
1.7	8100	8120	8141	8161	8181	8201	8221	8241	8261	8280
1.8	8300	8319	8338	8357	8376	8394	8413	8431	8450	8468
1.9	8486	8503	8521	8539	8556	8573	8590	9607	9624	8641
2.0	8657	8674	8690	8706	8722	8738	8753	8769	8784	8800
2.1	8815	8830	8845	8859	8874	8888	8903	8917	8931	8945
2.2	8959	8972	8986	8999	9012	9026	9039	9051	9064	9077
2.3	9089	9102	9114	9126	9138	9150	9162	9173	9185	9196
2.4	9207	9218	9229	9240	9251	9262	9272	9283	9293	9303
2.5	9313	9323	9333	9343	9352	9362	9371	9380	9390	9399
2.6	9408	9417	9425	9434	9442	9451	9459	9467	9476	9484
2.7	9492	9499	9507	9515	9522	9530	9537	9544	9552	9559
2.8	9566	9573	9597	9586	9593	9599	9606	9612	9618	9625
2.9	9631	9637	9643	9649	9654	9660	9666	9671	9677	9682
3.0	9687	9693	9698	9703	9708	9713	9718	9723	9727	9732
3.1	9737	9741	9746	9750	9755	9757	9763	9767	9771	9775
3.2	9779	9783	9787	9791	9795	9798	9802	9805	9809	9812
3.3	9816	9819	9823	9826	9829	9832	9835	9838	9841	9844
3.4	9847	9850	9853	9856	9858	9861	9864	9866	9869	9871
3.5	9874	9876	9878	9881	9883	9885	9887	9890	9892	9894
3.6	9896	9898	9900	9902	9904	9906	9908	9910	9911	9913
3.7	9915	9917	9918	9920	9921	9923	9925	9926	9928	9929
3.8	9931	9932	9933	9935	9936	9937	9939	9940	9941	9942
3.9	9944	9945	9946	9947	9948	9949	9950	9951	9952	9953

(j = 1,2, . . . , n) a series of j values of P_c is obtained. Conversions of these proportions into values of d' makes it possible to establish a psychophysical function in a similar way as described in the previous section for the Triangular Method under the Thurstone-Ura Model. Because of the similarity further elaboration of this issue is omitted.

A WORKED EXAMPLE

IN ORDER TO ILLUSTRATE the use of the present Tables of d' a brief example is given. The data are taken from a previous olfactory study (Frijters, 1981c) to which the reader is referred for complete experimental details.

To each of 12 subjects a random series of 18 triangles was presented twice. A triangle contained two stimuli S_s from a reference level and one stimulus S_j (j = 1, . . . , 6). Position of the stimulus S_j within a triangle was at random (thus the three permutations of one combination were used). A stimulus was a particular concentration of linalylacetate diluted in diethyphtalate. The molarities of the respective stimuli were: 0.127 (S_s), 0.202 (S_1), 0.282 (S_2), 0.400 (S_3), 0.555 (S_4), 0.741 (S_5) and 1.177 (S_6). The subjects were instructed to select the *strongest* stimulus from each triangle presented, and to guess if in doubt (3-AFC procedure).

Table 3—Number and proportion of correct responses and corresponding value of d' for each of the six concentration levels

Triangle combination	Correct responses	P_c	d'
$S_sS_sS_1$	42/72	0.583	0.83
$S_sS_sS_2$	46/72	0.639	1.02
$S_sS_sS_3$	63/72	0.875	2.06
$S_sS_sS_4$	66/72	0.917	2.36
$S_sS_sS_5$	67/72	0.931	2.49
$S_sS_sS_6$	69/72	0.958	2.83

$d' = 3.29 \log (S_j/S_s)$, $r = 0.99$, $s_{(b)} = 0.29$.

The number and proportion of correct responses was

determined for each concentration level after completion of the experiment. Using Table 2 the values of $\underline{d}'$ corresponding to the proportions were determined. Using simple linear regression a psychophysical function represented by Eq (5) was fitted. For the results which are self-explanatory, see Table 3.

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Comparison of Optimization Techniques for Application to Food Product and Process Development

S. NAKAI

ABSTRACT

The super-simplex optimization was modified by incorporating a quadratic regression subroutine. The new algorithm was found to be more efficient in converging at the optimum than the following algorithms: fractional factorial designs, sequential single-factor search, pattern-search method, Morgan-Deming simplex and super-simplex. The efficiency of the modified super-simplex optimization was dependent on the fitness of the regression equation to the model equation. Response surface analysis was accurate only when the model equation was quadratic factorial and the boundary covered the optimum. When a constraint was imposed to restrict the search within the boundary, unlike other simplex algorithms including the weighted centroid method, the modified super-simplex algorithm circumvented a problem of the search stalling at the boundary. Since it is not unusual for optimization to be restricted within boundaries, the modified super-simplex algorithm could be useful in optimizing food processing and analysis. When the modified super-simplex technique is applied to food research, even higher optimization efficiency could be expected by incorporating backward stepwise multiple regression analysis instead of the quadratic regression subroutine with regression equations of set formulae. To obtain a regression equation fit better to the true response surface, all information, including graphics, should be utilized.

INTRODUCTION

AN ULTIMATE GOAL of most scientific research and investigation is to find the optimum conditions for achieving the objectives. Food processing is not an exception and the search for the most efficient process is of utmost importance to many food processors. The improved productivity should be maintained in research, in pilot plant studies and on production line; however, the human brain can usually think of only one factor at a time. For more than two factors, especially when they are interacting, the computer approach can do a better job in optimization. The use of computers for optimizing research and development in manufacturing was advocated by Frazer (1981).

Finding an optimum solution for a processing problem by selecting from a large set of potential influencing factors at various steps in a process is a difficult task since it requires a large number of experimental runs. To improve the efficiency of finding optimum solutions for such problems, different statistical techniques have been proposed. Multi-factorial designs have been used over the past 40 years; however, the methods are not very efficient when employed for optimization. Since the choice of the factor levels depends entirely on the experimenter's judgement, there is always a risk of missing the true optimum when the levels are set too far apart, or of being inefficient when the levels are too close together.

Linear programming is an efficient and versatile method for optimization which has been used for product formulation, menu preparation and process control (Harper and Wanninger, 1970). However, in order to utilize this technique, linear equations which correlate the variables involved with the parameters of product quality, have to be previously known. Linear regression analysis can be applied to available processing data to derive the required linear regression equations. However, when the correlation is quadratic, quadratic programming is preferred (Boot, 1964).

Since its introduction by Box (1957), the evolutionary operation (EVOP) quickly became popular in food processing (Box, 1975). This technique is advantageous since the method can be applied directly to the processing line and is therefore suitable for quality control. However, the Box EVOP is restrictive and unsuitable for applying to research and development (Kramer and Twigg, 1970). To improve the versatility of the original two-factor EVOP, a variety of EVOP's have been developed (Lowe, 1964). One of the most advanced techniques is simplex EVOP (Spendley et al., 1962), which was later modified by Nelder and Mead (1965). Simplex optimization owes most of its success to its ability to handle multifactors including mutual interactions; because of this ability the probability of missing the true optimum is diminished even when the response surface contains a ridge (Morgan and Deming, 1974). The number of applications of Morgan and Deming's version of the simplex EVOP have been increasing in analytical chemistry (Deming and Parker, 1978; Dols and Armbrecht, 1976) and in chemical engineering (Winicov et al., 1978). Dols and Armbrecht (1976) stated "in the course of method development for analysis of agricultural products, an optimization procedure should be performed before critical performance characteristics are measured," and also "other things being equal, the simplex method is more effective in terms of the number of experiments required." A computer-assisted, self-directed chemical synthesis system has been designed using the simplex algorithm (Winicov et al., 1978).

More recently, the super modified simplex optimization was introduced by Routh et al. (1977). The method is essentially a simplex optimization expedited by incorporating a quadratic curve fitting to determine the vertex immediately following the reflection vertex to replace the worst vertex in the preceding simplex.

For mechanical and economic reasons, it is not unusual for one to have to constrain the conditions in food processing or analysis. Examples of constraints are oven temperature, sample pH, and reactant concentration, etcetera. Nelder and Mead (1965) suggested that for a nonnegative constraint, the function should be modified to take a larger positive value, and any trespassing over the boundary should be kept inside by a contraction move. However, this contraction usually decreases the speed of convergence. Routh et al. (1977) used the intersection (V_r') between the boundary and the vector joining V_w and V_r as an effective V_r, where V_w and V_r are the worst vertex and the reflection vertex, respectively. This adherence to the boundary constraint did not seem to cause any serious problem when the gradient was large near the boundary. However, we found that the sequential search for the optimum sometimes stalled at the boundaries when we used the simplex optimization of Morgan and Deming (1974) or of Routh et al. (1977).

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To overcome this drawback in the algorithms, we introduced a subprogram of "quadratic, factorial regression analysis" into the computer program of the super-simplex optimization of Routh et al. (1977). This modified super-simplex optimization (MSS) has been successful in converging quickly onto the optimum without stalling at the boundaries. Optimization experiments in food research are usually slow and difficult to reproduce, therefore, the comparison of different optimization techniques is very time-consuming. Furthermore, the optimized data would not be accurate when the measurement of the quality parameters is unreliable. To avoid these problems, especially to obtain objective and reliable comparison results, many researchers are using mathematical models (Hillstrom, 1977). Simply assigning some food processing parameters as the independent variables in the models would create model problems in food processing.

This paper deals with a comparison of the modified super-simplex optimization with the following: the sequential single-factor method (Friedman and Savage, 1947); the three level fractional factorial designs of Taguchi (1957); the patternsearch method of Hooke and Jeeves (1961); the simplex optimization of Morgan and Deming (1974); the super modified simplex optimization of Routh et al. (1977) the weighted centroid method of simplex optimization (Ryan et al, 1980); and the central composite rotatable designs of response surfaces (Cochran and Cox, 1957). Mathematical models were used for an objective evaluation of the comparison.

EXPERIMENTAL

Optimization models and computation

Model equations in three and five variables were formulated according to the method of Brown and Gerard (1967):

$$y = 20-5x_1-2x_2-4x_3-6x_1x_2-x_1x_3+3x_2x_3+4x_1^2+4x_2^2+x_3^2 \quad \text{(minimization)} \quad (1)$$

and

$$y = 1.34+33.6x_1+25x_2+34.4x_3+22x_4+7x_5-4x_1x_2-12x_1x_3-10x_1x_4-4x_1x_5-16x_2x_3-12x_2x_4-6x_2x_5-15x_1^2-18x_2^2-20x_3^2-26x_4^2-10x_5^2 \quad \text{(maximization)} \quad (2)$$

The response surfaces of Eq (1) are shown in Fig. 1. The response value calculations, factorial analyses, simplex optimizations and multiple regression analyses were performed using a Monroe 1880 programmable calculator and an Amdahl 470 V8 computer.

Fractional factorial designs for selection of influential factors

Two-level designs, $L_8(2^7)$ and $L_{16}(2^{15})$, of Taguchi (1957) were used for three- and five-factor models to select the possible significant factors and interactions between them.

Fractional factorial designs for optimization

The significant factors and interactions, as determined above, were used for analysis of variance in the $L_{27}(3^{13})$ factorial designs of Taguchi (1957). On the basis of the response curve, the levels of the factors for the subsequent factorial design were chosen and the computation was continued.

Sequential single-factor method

This method is probably the most common for optimization. It requires all factors but one to be held constant while a univariate search is carried out on the chosen factor. The optimized factor is then held constant, and a different factor is searched. The optimum levels of each factor thus found would generate the optimum response.

Patternsearch method

The method of Hooke and Jeeves (1961) is one of the most

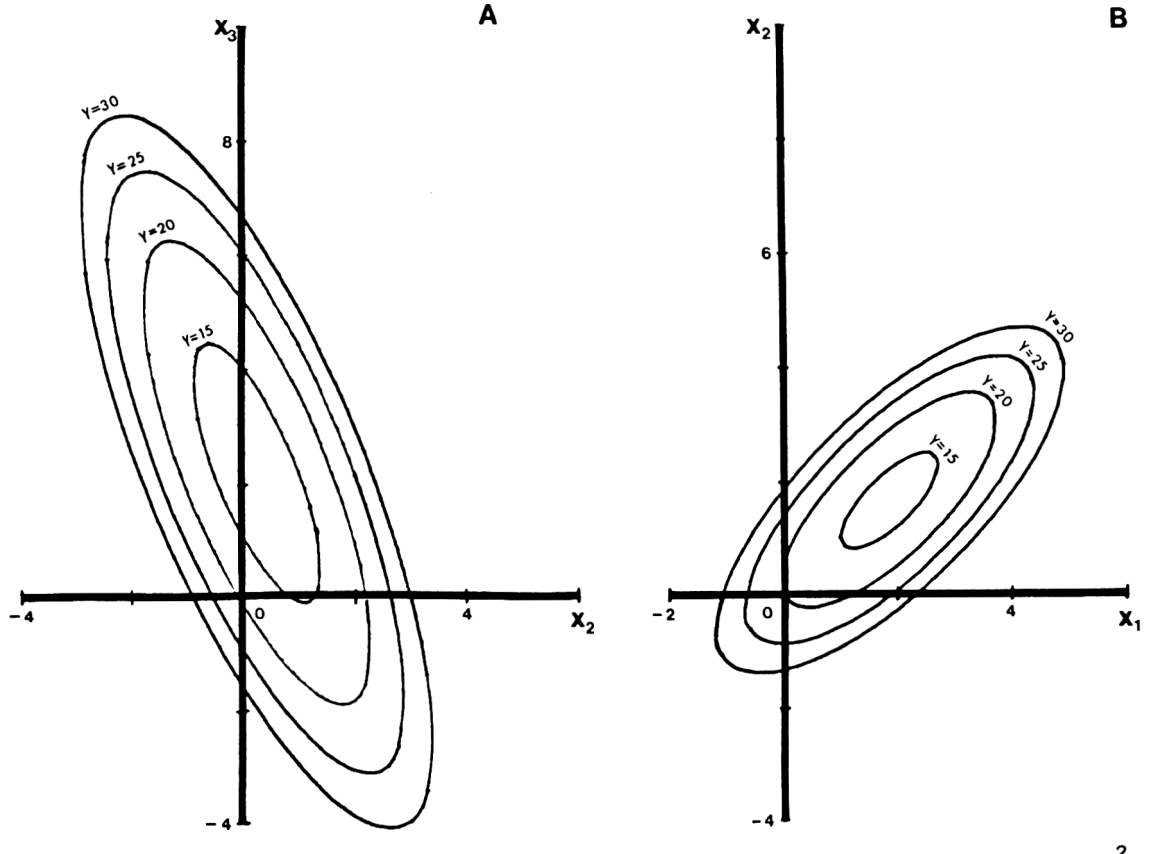


Fig. 1—The response surfaces of the three variable model for minimization: $Y = 20-5x_1-2x_2-4x_3-6x_1x_2-x_1x_3+3x_2x_3+4x_1^2+4x_2^2+x_3^2$; A: $x_1 = 1.08$; B: $x_3 = 0$.

widely used direct search methods. The method is iterative, by changing alternately one variable at a time with a decreasing step length for the changes, thus the search narrows down to focus on the optimum.

Simplex optimization

The original simplex algorithm developed by Morgan and Deming (1974) was slightly modified as shown in Fig. 2. Simplex iteration was terminated when three successive response values did not differ when rounded off to the requisite decimal places.

Factorial-simplex-quadratic regression coordinated technique

The data of the fractional factorial design, $L_8(2^7)$ or $L_{16}(2^{15})$, and of the initial simplex were combined and used for quadratic factorial regression analysis. The factors and their interactions, which were significant in the factorial analysis of variance, were used as the independent variables for the regression analysis. The partial derivatives of the regression equation were equated to zero and the resultant simultaneous equations solved for each variable. The response value obtained replaced the worst vertex of the starting simplex. The regular simplex optimization was subsequently continued.

Super-simplex optimization

Super-simplex optimization programs were written for both the Monroe calculator and the Amdahl computer using the algorithm of Routh et al. (1977). To use a computer program of multiple regression analysis or polynomial regression analysis currently available as the subroutine subprogram for calculating the quadratic regression coefficients, a_1 and a_2, the part of the program used to calculate the variance of estimate and F-ratio was erased or skipped since it introduced a mathematical error.

Modified super-simplex optimization

The above super-simplex optimization program was modified by incorporating a subprogram of quadratic, factorial regression analysis, and the subsequent vertex was calculated from data of 10–14 vertices: 4 data from the starting simplex in the case of three-variable optimization, and an additional 6–10 data, one from each subsequent vertex (Fig. 3). The calculated vertex replaced the worst vertex of the preceding simplex only when it was better than the worst.

Weighted centroid method of simplex optimization

The centroid was calculated by adding one extra best data in the equation of Spendley et al. (1962).

Central composite rotatable designs of response surfaces

A computer program for calculating the minimum of the three-variable model from data at different levels of variables was written for the Monroe calculator using the algorithm developed by Cochran and Cox (1957).

Comparison of various simplex optimizations and response surface analysis

As Hillstrom (1977) recommended, at least 20 random starts were chosen for comparison. To randomize the starting point and initial simplex size simultaneously, the lower boundary of each factor was uniformly fixed at 0 or −0.2 and the upper boundary was chosen by using a uniform random-number generator of the Monroe calculator. Mathematical models for minimization were selected from the papers of Nelder and Mead (1965) and Ryan et al. (1980). The equations were slightly adjusted before use to simulate conditions of food processing and analysis, i.e. accuracy of the y values within two digits after the decimal point which can be obtained within an iteration limit of 150, hopefully 50–100 iterations.

For multiple regression analysis in the modified simplex optimization for 4 and 5 factor models $y = \Sigma x_i + \sum_{i \neq j} x_i x_j$ excluding Σx_i^2 was used for more frequent checking of possible stalling since a large number of vertices have to run prior to regression analysis when too many independent variables are included in the regression equation. Also the multiple regression analysis was modified to include all previous regression vertices, even if they did not replace the worst, to maintain good deviation among vertices which is necessary for a better y estimate. Similarity among vertex values for regression analysis immediately restricts the search capacity. Because of accumulating regression vertices, multiple regression analysis is saturated with vertex values, thus multiple regression analysis is applied only to the earlier stage of iteration. This is reasonable since the variability of vertex values decreases at the later stage of iteration thus losing effectiveness of multiple regression analysis.

RESULTS & DISCUSSION

Comparison of fractional factorial designs, single-factor method, patternsearch method and simplex optimization

The fractional factorial design was not efficient for the optimization of the three-factor model since it required 108 experiments to approach within a reasonable range of the minimum (Fig. 4 and 6). As observed by Morgan and Deming (1974), the results of the factorial designs are highly

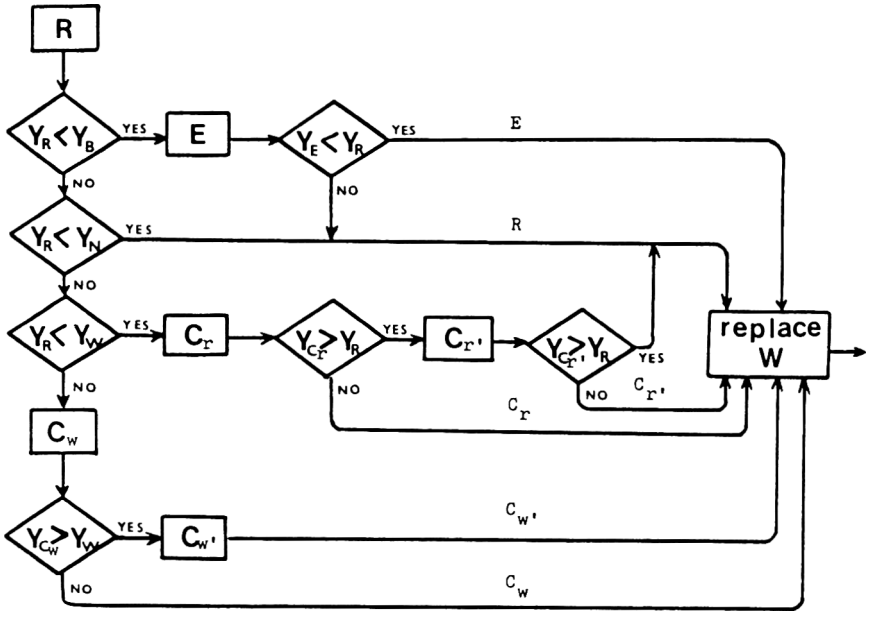
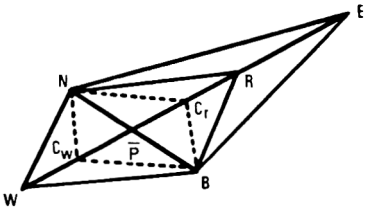


Fig. 2—Flow chart of the simplex minimization. Calculation of the new vertex, $V = \bar{P} + \kappa(\bar{P} - W)$.



W: Worst vertex
B: Best vertex
N: Next-to-the-worst vertex
$\bar{P}$: Centroid of N-B vertices
R: Reflection, $\kappa = 1.0$
E: Expansion, $\kappa = 2.0$
C_r: Contraction of R, $\kappa = 0.5$
C_W: Contraction of W, $\kappa = -0.5$
$C_{r'}$: Massive contraction of R, $\kappa = 0.25$
$C_{W'}$: Massive contraction of W, $\kappa = -0.25$
Y stands for the response

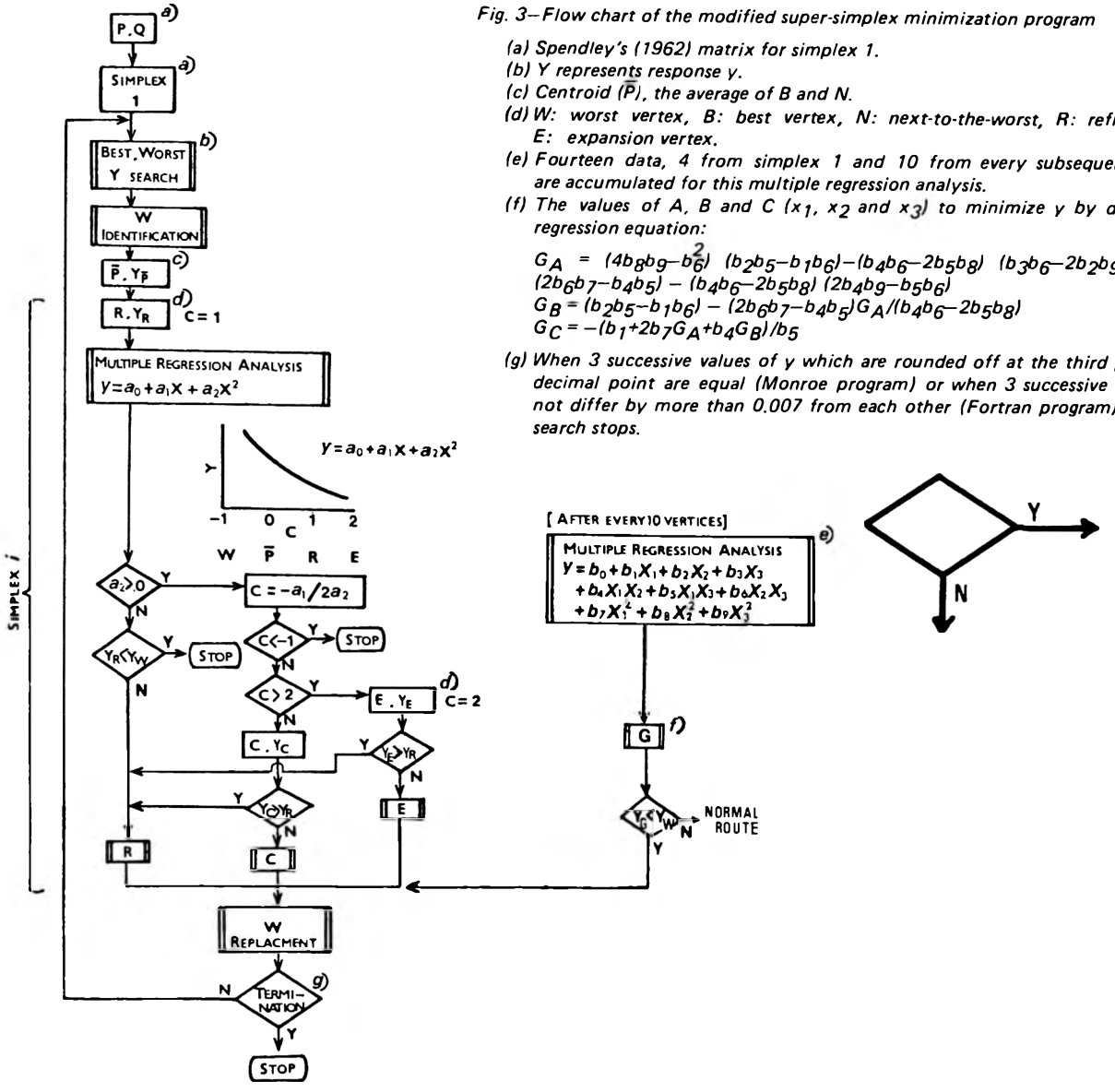


Fig. 3—Flow chart of the modified super-simplex minimization program

(a) Spendley's (1962) matrix for simplex 1.

(b) Y represents response y.

(c) Centroid ($\bar{P}$), the average of B and N.

(d) W: worst vertex, B: best vertex, N: next-to-the-worst, R: reflection vertex, E: expansion vertex.

(e) Fourteen data, 4 from simplex 1 and 10 from every subsequent 10 vertices, are accumulated for this multiple regression analysis.

(f) The values of A, B and C (x_1, x_2 and x_3) to minimize y by derivatizing the regression equation:

$$G_A = (4b_8b_9-b_6^2)(b_2b_5-b_1b_6)-(b_4b_6-2b_5b_8)(b_3b_6-2b_2b_9)/(4b_8b_9-b_6^2)(2b_6b_7-b_4b_5)-(b_4b_6-2b_5b_8)(2b_4b_9-b_5b_6)$$

$$G_B = (b_2b_5-b_1b_6)-(2b_6b_7-b_4b_5)G_A/(b_4b_6-2b_5b_8)$$

$$G_C = -(b_1+2b_7G_A+b_4G_B)/b_5$$

(g) When 3 successive values of y which are rounded off at the third place from the decimal point are equal (Monroe program) or when 3 successive values of y do not differ by more than 0.007 from each other (Fortran program), the iteration search stops.

dependent upon the choice of levels tested whether a small region is explored comprehensively or a large region superficially.

After attaining a minimum of 12.94 from only 16 iterations for the three-factor model (Fig. 5), the sequential single factor method was incapable of approaching closer to the true optimum, 12.71, calculated by direct differentiation [$\delta f(x)/\delta x = 0$] of the model. Further iterations did not improve the minimization. According to Morgan and Deming (1974), the single-factor approach is unsatisfactory if the chosen factors are interdependent.

The patternsearch method needed 100 to 115 iterations prior to reaching a minimum of 12.75 for the three-factor model (Fig. 5). The method was again inefficient for optimization.

The simplex optimization required 50 (Fig. 6) and 65 iterations with optimum responses of 12.71 and 29.66 for three- and five-factor models, respectively. The direct differentiation yielded the maximum response of 29.68 for the five factor model.

The most effective method was a combination of factorial designs, multiple regression analysis, and simplex optimization. A minimum of 12.71 after only 7 iterations (Fig. 6) and a maximum of 29.68 after only 10 iterations were obtained for three- and five-factor models, respectively. However, these numbers do not include the number of experiments used in the factorial designs. The optimum response values obtained by the factorial-simplex-quadratic regression coordinated method were in good agreement with those calculated by the direct differentiation technique. The reliability of this method was further assessed by including random errors of 2.5% and 5% to all of the response data obtained for the five-factor model to simulate real food processing conditions. It was found that this manipulation necessitated only a couple of additional iterations for convergence, demonstrating high precision of simplex optimization. Factorial designs for selection of significant factors and iteractions are frequently a prerequisite for simplex optimizations as shown by Morgan and Deming (1974) since the vertex number for convergence quickly increases as the number of factors increases (Nelder and Mead, 1965). Whenever feasible during an optimization procedure, utilizing all available data from the past experiments in quadratic, factorial regression analysis could be useful in accelerating optimization.

Comparison of response surface analysis and various simplex optimizations using computing models with the boundary constraint

Comparison using a quadratic factorial model. Equation

1 was used for the comparison. As seen in Table 1, the speed of convergence for the Morgan-Deming simplex algorithm and the super-simplex algorithm was about the same. However, occasionally the latter reached the minimum after 30% fewer vertices than the former.

When boundaries were set for the variables, and all trespassing values for variables were replaced by the nearest boundary values, the search for the minimum was sometimes stalled at the boundaries (Table 1, examples 1, 2 and 3). Modification of the super-simplex optimization program by incorporating the quadratic, factorial regression analysis circumvented this problem with no stalling (Table 1). It was found that this modified program was fastest in convergence for most of the examples with different boundaries.

The rotatable designs of response surfaces yielded, for the three-variable model, optimum values very close to the true optimum when the boundaries were set broad enough to include, within their ranges, the variable values corresponding to the true minimum. The number of experiments needed in this design was only 20. Furthermore, this method does not require a complicated program for computation.

However, when the set boundaries of each variable did not cover the variable values for he true minimum, or when the level ranges were too narrow, the optimization became inaccurate (Table 2). Cochran and Cox (1957) warned of the potential risk of missing the true optimum and recommended a further confirmatory test of the calculated optimum values. Extrapolation of a regression equation for prediction of values beyond the ranges of data used for regression analysis is risky since the predicted values are frequently unreliable. This situation is similar to the relations between factorial designs and simplex optimization as described by Morgan and Deming (1974). Selection of the proper levels or boundaries of variables is extremely important for the factorial designs as well as for the response surface analysis for optimization.

Usually at the beginning of food processing or analytical experiments, the location of the optimum is unknown. Therefore, the proper boundary settings which include the true optimum inside their ranges, are entirely dependent upon the experimenter's intuition. Setting of too broad boundary ranges is risky and may overlook the local detail surrounding the true optimum. Above all, the same technical and economic constraints that forced the boundary setting may prevent proper selection of sufficiently broad ranges. In cases such as this, it is preferable to use iterative searching methods, e.g., the modified super-simplex optimization.

One potential drawback of the modified super-simplex optimization is the increasing complexity in computer programming as the number of variables increases. Pre-screening of the most significant factors by preliminary factorial designs is helpful. The data from this preliminary analysis can be used in quadratic, factorial regression analysis to expedite the optimization. Sequential optimizations including various simplex techniques are sometimes difficult to carry out especially when even one experiment is quite time-consuming. Conducting needed experiments all at once is preferable. Response surface designs may be most efficient in this case.

In some optimization algorithms, a difficulty is encountered in searching for an optimum when the model response surfaces contain a ridge (Cochran and Cox, 1959). The response surfaces of the three-variable model used in this study, shown in Fig. 1, indicate the presence of moderately sinking ridges. Therefore, this model may be a suitable example for the comparison of different optimization techniques.

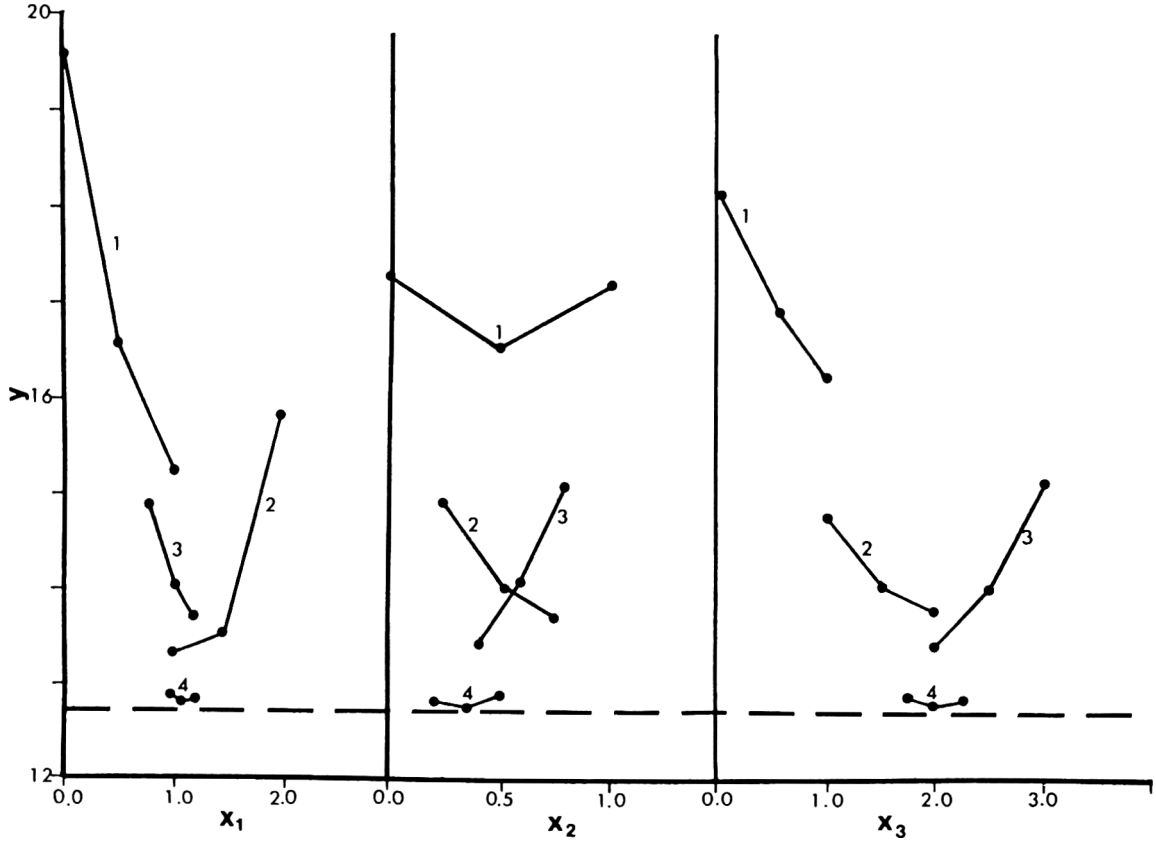


Fig. 4—Response curves of a three factor factorial analysis 1: 1st factorial, 2: 2nd factorial, 3: 3rd factorial, 4: 4th factorial, – – – True minimum.

More extensive comparison using different models. In Table 3, the super-simplex optimization is not different from the Morgan-Deming simplex algorithm in terms of efficiency and stalling. The modified super-simplex optimization considerably improved the efficiency as the number of vertices to reach a minimum is 27.5 compared to 43.2 for the Morgan-Deming simplex optimization. In addition, the modified super-simplex optimization has no stalling problem compared to 14 stallings for the Morgan-Deming simplex algorithm.

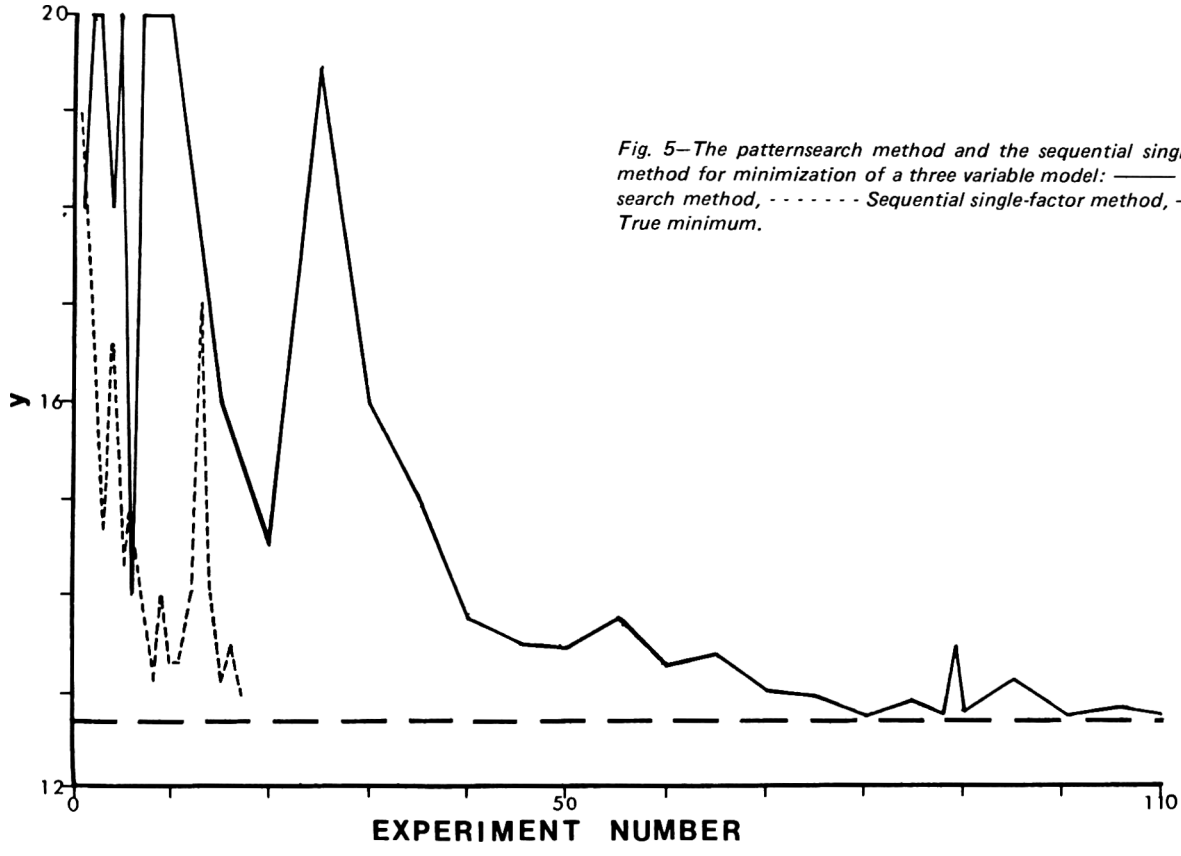


Fig. 5—The patternsearch method and the sequential single-factor method for minimization of a three variable model: ——— Patternsearch method, - - - - - - - Sequential single-factor method, — — — — True minimum.

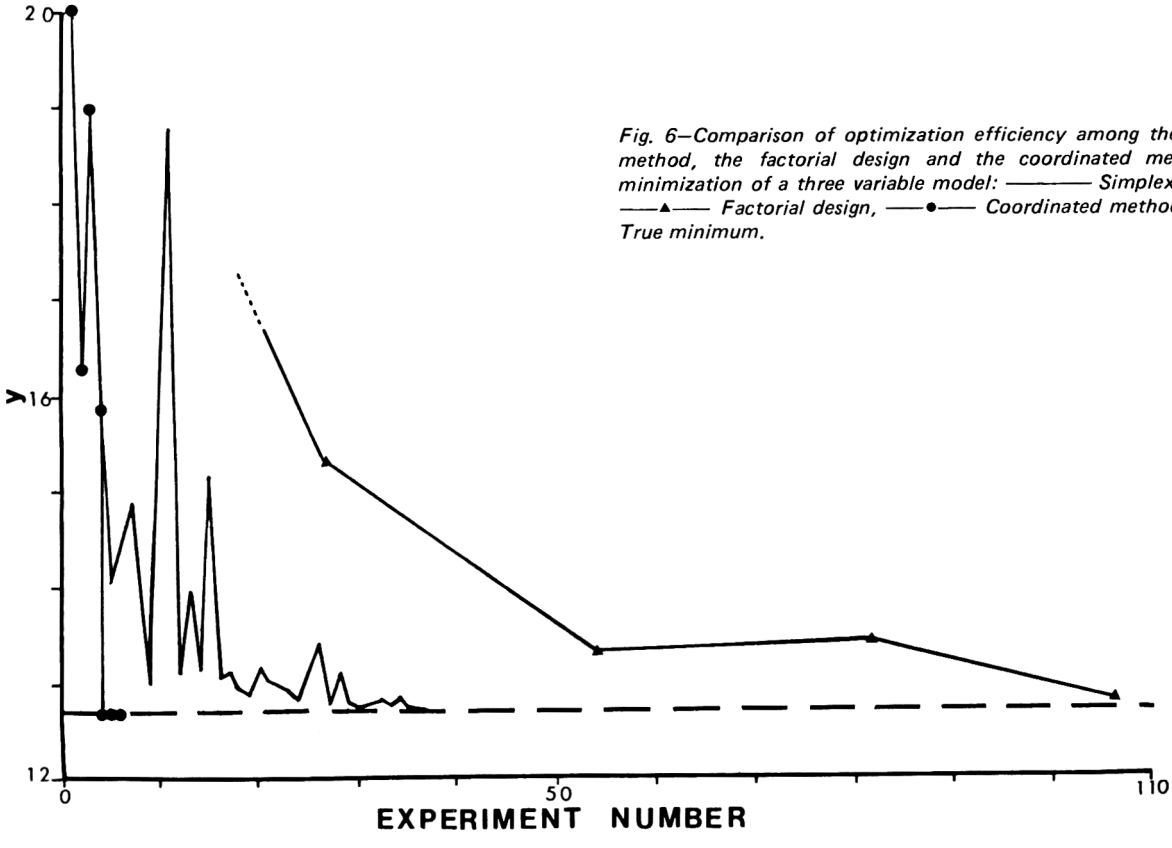


Fig. 6—Comparison of optimization efficiency among the simplex method, the factorial design and the coordinated method for minimization of a three variable model: ——— Simplex method, ——▲—— Factorial design, ——●—— Coordinated method, — — — True minimum.

The weighted centroid method of Ryan et al. (1980) is shown to be of about the same efficiency as the modified super-simplex optimization, however, stalling occurred for nine cases.

Response surface analysis shows excellent results for this model when the ranges of search include the expected minimum. However, when the ranges are outside of the minimum, the accuracy of the prediction of minimum rapidly deteriorated. In one case, the predicted minimum was 107.68 compared to the theoretical value of 12.71. This problem was already shown in Table 2 for arbitrary boundaries.

A more complicated model, Fletcher and Powell's helical valley (Nelder and Mead, 1965), was used in Table 4. The Morgan-Deming simplex optimization had four cases of stalling. The modified super-simplex optimization had five cases of stalling when the multiple regression analysis was carried out with data of 12 vertices. However, by changing this number of data to 10 or 14 the stalling could be completely avoided. This manipulation also avoided degeneration which was observed with the Morgan-Deming simplex optimization that gave the minimum value of 32.06 in one case compared to the theoretical value of 10.00. However, the efficiency of modified super-simplex optimization is not significantly different from that of the Morgan-Deming algorithm. When a t-test was carried out for nonstalling, nondegenerated cases, there was no significant difference ($p > 0.05$) between numbers of vertices. It appears that the multiple regression analysis incorporated into the optimization procedure accelerates the optimization only when the regression equation fits the model equation.

This is exactly the case when response surface analysis was applied to this model. Due to lack of fitness, the deviation of the calculated minimum value from the theoretical value is outrageous, 4308.13 compared to theoretical 10.00. Since there is no promise that all food processings or analyses follow quadratic factorial forms, e.g., Eq (1) and (2) indiscriminate application of response surface analysis without a follow-up check of the computed optimum value should be cautioned against.

A four-factor model, Powell's quartic function used by Nelder and Mead (1965), was slightly modified in Table 5. The modified super-simplex optimization had no stalling

Table 1—Comparison of three different simplex optimization programs

Example no.	Boundaries x_1	Boundaries x_2	Boundaries x_3	Computed minimum[f] Vertex	x_1	x_2	x_3	Y_{min}	Program
1. MDS stalled									
LL[d]	0.1	0.1	0.1	24	0.71	0.10	0.10	17.45	MDS[a]
UL[e]	5.0	5.0	5.0	50	0.92	0.14	2.14	12.76	SSO[b]
				27	1.07	0.23	2.16	12.71	MSS[c]
2. SSO stalled									
LL	0.1	0.2	0.1	47	1.14	0.33	2.04	12.72	MDS
UL	4.0	2.0	6.0	44	0.10	0.20	1.77	16.12	SSO
				29	1.07	0.23	2.19	12.71	MSS
3. Broader boundaries									
LL	0.0	0.0	0.0	42	0.92	0.00	2.43	12.75	MDS
UL	5.0	5.0	5.0	42	0.99	0.07	2.48	12.73	SSO
				29	1.08	0.26	2.16	12.71	MSS
4. Boundaries set outside of optimum variable values									
LL	0.1	0.1	0.1	18	1.00	0.43	1.50	12.98	MDS
UL	1.0	0.5	1.5	18	1.00	0.50	1.50	13.00	SSO
				25	1.00	0.44	1.50	12.98	MSS
True optimum					1.08	0.24	2.20	12.71	

[a] Morgan-Deming simplex optimization
[b] Super-simplex optimization
[c] Modified super-simplex optimization
[d] Lower limit
[e] Upper limit
[f] Variable values underlined were stalled at the boundaries

Table 2—Comparison of modified super-simplex optimization and response surface analysis

	Boundaries x_1	Boundaries x_2	Boundaries x_3	Computed minimum Vertex	x_1	x_2	x_3	Y_{min}	Program
Boundaries outside of optimum variable values									
LL[a]	0.1	0.1	0.1	20	1.00	0.21	1.50	13.32	RSF[c]
UL[b]	1.0	0.5	1.5	25	1.00	0.44	1.50	12.98	MSS[d]
Too narrow boundaries									
LL	0.8	0.1	2.0	20	0.80	0.10	2.50	12.85	RSF
UL	1.2	0.3	2.5	12	1.04	0.17	2.29	12.71	MSS
True optimum					1.08	0.24	2.20	12.71	

[a] Lower limit
[b] Upper limit
[c] Response surface analysis
[d] Modified super-simplex optimization

compared to 7 for the Morgan-Deming simplex optimization. The efficiency of the modified super-simplex optimization was not significantly diffrent from that of the Morgan-Deming optimization.

A five-dimensional paraboloid used by Ryan et al. (1980) was slightly modified in Table 6 to obtain more practical y values. The difference observed in the number of vertices was significant ($p < 0.01$) according to t-test. Unstalled data only were used in this t-test for efficiency comparison since the number of vertices for stalled cases is not really the number of vertices of convergence. The number of vertex data used in multiple regression was 18, but when the number of vertices to converge was too large, 20 data were used to expedite the optimization process. Sometimes 16 data were also used to improve the proximity of computed minimum values. In no cases did the computation encounter stalling.

Incorporation of multiple regression analysis into the Morgan-Deming algorithm was also tried in an attempt to avoid stalling. The work was quickly suspended after finding that the Morgan-Deming simplex is less flexible in search than the super-simplex algorithm. This is an advantage of the Morgan-Deming algorithm when the search does not have a boundary constraint since a better proximity of the coverage value than that for super-simplex optimization can be expected as seen in Table 3. The values are 12.72 and 12.77, respectively, compared to a theoretical value of 12.71. However, for an effective use of the incorporated multiple regression analysis in the modified super-simplex algorithm, somewhat scattered data are required for preventing the calculated y value from going astray or stalling.

Application of simplex optimization techniques to food research

Simplex optimization and its modified techniques have been used in several experiments of food analysis, and product and process development. The best fit component molecular weights and relative concentrations of three proteins in their mixture were calculated from its equilibrium ultracentrifugation data (van de Voort et al., 1979). The Scholte equation was used for computation:

$$\sum^{n} c(\xi_i)/c_0 = f_1 \sum^{n} K_{1i} + f_2 \sum^{n} K_{2i} + f_c \sum^{n} K_{3i} + \sum^{n} \xi_i$$

where $c(\xi_i)$ is the protein concentration at ξ_i in the centrifuge cell; ξ_i, a reduced expression of the radial distances at n different positions in the cell; c_o is the initial protein concentration; f_{1-3} are the weight fractions of proteins 1–3; $K_{1\text{-}3i}$, molecular weight related parameters. Multiple regression analysis was used to solve this equation. By setting boundaries for the molecular weight of each protein molecule, simplex optimization searched for the best fit mol wt to maximize the F-value of regression. A prohibit-trespassing subroutine was incorporated into the optimization program to restrain the search within reasonable mol

Table 3—Comparison of various simplex optimizations[a]

Model: $y = 20 - 5x_1 - 2x_2 - 4x_3 - 6x_1x_2 - x_1x_3 + 3x_2x_3 + 4x_1^2 + 4x_2^2 + x_3^2$ (1)

	MDS	SSO	MSS	MWS	RSF
Unstalled					
No. of cases	34	31	48	39	30
Vertices no.	43.2±9.6[b]	41.2±7.6	27.5±2.7	26.2±3.2	20
Min. value	12.72±0.01	12.77±0.08	12.71±0.003	12.72±0.04	12.71±0.000
Largest min.	12.76	12.95	12.72	12.95	12.71
Stalled					
No. of cases	14	17	0	9	na[c]
Vertices no.	31.6±8.2	40.9±9.7	na	36.9±16.2	na
Min value	15.97±2.60	13.60±1.27	na	14.33±2.21	na
Largest min.	19.76	16.00	na	19.02	na
Ranges outside of min.					
No. of cases	10	10	10	10	10
Vertices no.	39.9±6.9	45.6±9.8	41.5±8.3	31.5±4.2	20
Min. value	13.48±0.76	13.50±0.70	13.48±0.94	13.51±0.93	30.41±33.1
Largest min.	15.26	14.98	15.79	15.79	107.68

[a] Range: 0 (fixed) ~ 10, Theoretical minimum: 12.71, MDS: Morgan-Deming, SSO: super-simplex, MSS: modified super-simplex, MWS: weighted centroid method, RSF: response surface analysis.
[b] mean ± standard deviation
[c] not applicable

Table 4—Comparison of various simplex optimizations[a]

Model: $y = \left\{100[x_3 - 10\theta(x_1,x_2)]^2 + [\sqrt{x_1^2 + x_2^2} - 1]^2 + x_3^2\right\} x2 + 10.00$ (3)

where $2\pi\theta(x_1,x_2) = \arctan(x_2/x_1)$ when $x_1 > 0 = \pi + \arctan(x_2/x_1)$ when $x_1 < 0$

	MDS	MSS	RSF
Unstalled			
No. of cases	26	30	30
Vertices no.	73.7±25.5	66.4±26.0	20
Min. value	11.51±4.75	10.16±0.21	4804.13±24353.25
Largest min.	32.06	10.80	133705.08
Stalled			
No. of cases	4	0	na
Vertices no.	53.8±11.9	na	na
Min. value	11.68±1.69	na	na
Largest min.	13.46	na	na

[a] Range: −0.2 (fixed) ~ 2.0, Theoretical minimum: 10.00

Table 5—Comparison of various simplex optimizations[a]

Model: $y = \{(x_1 + 10x_2)^2 + 5(x_3 - x_4)^2 + (x_2 - 2x_3)^4 + 10(x_1 - x_4)^4\} \times 2 + 10.00$ (4)

	MDS	MSS
Unstalled		
No. of cases	23	30
Vertices no.	44.5±8.6	44.0±15.2
Min. value	10.04±0.06	10.3±0.04
Largest min.	10.24	10.16
Stalled		
No. of cases	7	0
Vertices no.	51.4±8.0	na
Min. value	13.45±2.52	na
Largest min.	16.95	na

[a] Range: —0.2(fixed) ~ 2.0, Theoretical minimum: 10.00

Table 6—Comparison of various simplex optimizations[a]

Model: $y = (x_1^2 + x_2^2 + x_3^2 + x_4^2 + x_5^2) \times 100 + 10.00$ (5)

	MDS	MSS
Unstalled		
No. of cases	23	34
Vertices no.	79.5±13.7	61.9±18.9
Min. value	10.04±0.03	10.12±0.14
Largest min.	10.09	10.55
Stalled		
No. of cases	11[b]	0
Vertices no.	96.1±10.5	na
Min. value	11.97±1.87	na
Largest min.	14.09	na

[a] Range: —0.2(fixed) ~ 2.0, theoretical minimum: 10.00
[b] Eight cases degenerated with a largest min. value of 507.38. The table contains only the minimum value found during computation.

wt ranges, otherwise the search frequently went astray during the optimization process while an optimum was simply being searched mechanically. The obtained values of the molecular weights and relative concentrations of three model proteins in their mixtures were in good agreement with the theoretical values.

Data transformation for linearization of food research data was optimized (Fujii and Nakai, 1980). A and B in a transformation equation, $t = (y + A)^B$ to fit to a linear equation $t = a + bx$, were optimized for the largest R^2 values. The boundary setting was again useful in this case since mathematical error should be avoided for small B value. A good agreement was obtained between the calculated curves and the model curves.

The amount of lysine covalently bound to wheat gluten was maximized (Li-Chan et al., 1979). Carbodiimide mediated condensation reaction was used for the coupling. ϵ-Amino group of lysine was protected by benzylidene derivatization to avoid isopeptide formation. N^{ϵ}-Benzylidenelysine amount, carbodiimide amount and pH were used as factors in this optimization. The best condition was obtained after 15 vertices, pretty quick since this was only for confirmation of the optimum found by other method.

The modified super-simplex optimization was also applied to ion exchange amino acid analysis in the search for quicker analytical conditions. Five factors (pH, ionic strength, temperature, temperature programming and n-propanol concentration) were optimized for the best resolution of the separated amino acid peaks. A practical chromatographic condition was established after 34 vertices. The condition found was considerably different from the recommended procedure of the manufacturer.

Other applications of the modified super-simplex optimization include the searches for the largest yield of extracted protein from defatted soy flours using pH, temperaure and NaCl concentration as factors and the largest loaf volume of bread containing whey powder using heating conditions of whey (pH, temperature, time and solids concentration) as factors. In both cases, the best values were obtianed before completing 20 experiments. Selective precipitation of β-lactoglobulin from cheese whey was also optimized using pH, temperature, Fe concentration and extent of desalting as factors. After 34 experiments, the best precipitation condition was obtained.

For application to research, three programs were written for the Monroe calculator involving calculations of the vertices in the initial simplex, the reflection vertex and the contraction vertex using a quadratic regression subroutine. After repeating a certain number of experiments, the response values were plotted vs each factor. These plots assisted in the determination of the regression formula: whether cubic terms should be introduced, and which product terms should be included. The formula of the regression equation affected the required number of experiments to be performed prior to backward stepwise regression analysis. The backward stepwise regression analysis was useful in selecting significant terms in the regression equation, thus expediting the optimization process. The best fit regression equation was used for computation of the next vertex. These additional manipulations, when applied to mathematical models, further reduced the number of vertices required for optimization.

As mentioned earlier, when starting a new research project and the knowledge of potential factors is lacking, two level fractional factorial analysis is recommended prior to simplex optimization for selecting significantly influencing factors for use in the optimization process (Morgan and Deming, 1974). The results of the factorial analysis could be used in determining the regression formula, especially for determining the need for product terms in it. The entire data of factorial analysis could be used in the stepwise regression analysis to improve the accuracy of the obtained regression equation.

When simultaneous runs of several experiments are more desirable than the iterative run, i.e., one experiment at a time to save time rather than saving the number of experiments, the application of response surface analysis for approximation is advocated if it is followed by simplex optimization for confirming the validity of the obtained optimum.

Boundary setting should be flexible, whenever possible, as the trespassing vertex frequently suggests the direction of move towards the optimum. Often, restarting a new simplex with different ranges of factors before the completion of the search for the optimum in the preceding simplex process is useful in terms of the overall efficiency. This is because the speed of approaching the optimum is fast in the early stage of the optimization process followed by a long period of slow access. Frequent use of graphics during optimization process to plot data or best fit curves from the regression equation is helpful not only for more efficient optimization (Frazer, 1981) but also to avoid reaching local optima rather than the global optimum.

All of these strategies are certainly directing us towards the future computerization of research planning and, possibly, some parts of research performances as well.

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—Continued on page 176

Regulation of Aflatoxin Biosynthesis: Effect of Exogenously Supplied Cyclic Nucleotides

G. TICE and R. L. BUCHANAN

ABSTRACT

The effects of cAMP, cGMP, AMP, and GMP on aflatoxin accumulation were studied using flask and test tube cultures of *Aspergillus parasiticus*. The response to cGMP supplementation was variable, depending on the type of culture system employed. Cyclic AMP produced a dose-related increase in aflatoxin production, with 5.0 mM supplementation resulting in as much as an 18-fold increase in toxin accumulation. Stimulation of aflatoxin production by cAMP was not attributable to nutritional effects, and the results suggest that cAMP may be a key regulatory control point governing aflatoxin biosynthesis.

INTRODUCTION

AFLATOXINS are a group of closely related polyketide secondary metabolites synthesized by strains of *Aspergillus parasiticus* and *Aspergillus flavus*. This group of compounds is noted for its toxicity and carcinogenicity in test animals, and there is evidence linking aflatoxin contamination of foods and feeds with the etiology of liver cancer in domestic animals, and possibly man. Since their initial discovery, significant advances have been made in elucidating the chemistry and toxicology of aflatoxins, as well as identifying the environmental parameters that influence aflatoxin production. However, significantly less information is available concerning the biochemistry of aflatoxin synthesis, including both the mechanism of biosynthesis and the metabolic controls that regulate the process. Potentially, this information could be useful in developing methods for controlling aflatoxin contamination of foods and feeds.

Recently, Applebaum and Buchanan (1979) reported that the intracellular levels of cyclic adenosine-3′,5′-monophosphate (cAMP) and cyclic guanosine-3′,5′-monophosphate (cGMP) fluctuated during the growth of *A. parasiticus*. Specifically, cAMP levels increased during exponential growth, reaching a maximum during the late exponential growth phase, and then decreased sharply during the stationary growth phase. Conversely, the intracellular levels of cGMP were low during the exponential growth phase, but rose sharply during the stationary growth phase. Maximal aflatoxin production occurred during the period when the cyclic nucleotides were fluctuating. Zonneveld (1976) noted a similar pattern of cAMP accumulation in *Aspergillus nidulans*.

The above observations provide circumstantial evidence that cAMP and/or cGMP may play a role in the regulatory mechanisms that control aflatoxin biosynthesis. The objective of the present study was to determine which, if either, of the cyclic nucleotides influence aflatoxin production. This was achieved by examining the effect of exogenously-supplied cAMP and cGMP on aflatoxin production by *A. parasiticus*.

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EXPERIMENTAL

Microorganism

Aspergillus parasiticus NRRL 2999 was used throughout the study. Stock cultures were maintained on potato dextrose agar (Difco) slants, and stored at 4°C.

Medium

Modified AM medium (Applebaum and Buchanan, 1979) containing 60g glucose/L was used throughout the study. The pH of the medium was adjusted to 4.5 using 10N HCl.

Spore suspensions

Spore suspensions were prepared by transferring 5 ml of sterile distilled water plus Tween 80 (20 μl/100 ml) to a slant culture of *A. parasiticus*, agitating vigorously, and then transferring the harvested conidia to a sterile bottle. This procedure was repeated a total of three times. The spore suspension was enumerated with a hemocytometer, and diluted to a final concentration of 10^6 conidia/mL.

Culture techniques

Flask cultures were prepared by transferring 25 ml of medium to 125-ml Erlenmeyer flasks. The flasks were stoppered with foam plugs and autoclaved for 15 min at 15 psi. The flasks were then supplemented with either cAMP, cGMP, AMP, or GMP (Sigma Chem. Co.). Each flask was inoculated with 1.0 ml of spore suspension to produce an inoculum of 4×10^4 conidia/ml. All flasks were incubated without agitation at 28°C. After 3, 4, 5, 7, and 10 days of incubation, triplicate cultures of each nucleotide concentration were examined for aflatoxin production, mycelial dry weight, and pH.

Subsequent experimentation was conducted using 3-ml test tube cultures (Buchanan and Ayres, 1976). These cultures were prepared by transferring 3 ml portions of sterile medium supplemented with AMP, cAMP, GMP, or cGMP (0.00, 0.05, 0.50, and 5.00 mM) to sterile test tubes (12.5 x 1.4 cm). Each tube was then inoculated with 0.1 ml of spore suspension (final concentration: 3×10^4 conidia/ml), and incubated without agitation at 28°C. After 5, 6, and 7 days of incubation, triplicate cultures of each nucleotide concentration were examined for mycelial dry weight and aflatoxin production.

Aflatoxin analyses

Aflatoxins were extracted from the flask cultures by adding 25 ml of chloroform to each flask, agitating vigorously, and then transferring the contents to a 125-ml separatory funnel. The chloroform layer was drawn off through a Whatman #1 filter paper into a round bottom flask. This procedure was repreated a total of three times, and then the filter paper was rinsed with chloroform and the washings added to the round bottom flask. The volume of the extracts was reduced to 10 ml using a flask evaporator, and the samples were stored at −20°C while awaiting further analysis.

The test tube cultures were extracted by adding 3 ml of chloroform, vortexing, and then allowing the two layers to separate. The chloroform layer was then drawn off with a Pasteur pipette and transferred to a 15 ml vial. The procedure was repeated a total of three times. The chloroform extracts were then evaporated to dryness under N_2, and subsequently redissolved in 2.0 ml of chloroform. The samples were then stored at −20°C while awaiting further analysis.

Aflatoxins were separated by thin-layer chromatography using Absorbosil-1 plates (20 x 20 cm) developed with chloroform/acetone (9:1). All samples were assayed in duplicate and compared against an authentic aflatoxin $B_1 + B_2 + G_1 + G_2$ standard (Hor-

witz, 1975). Aflatoxins were quantitated fluorodensitometrically using a fluorometer equipped with a TLC scanner (model 111, Turner Associates, Inc.). The sum of aflatoxins B_1, B_2, G_1, and G_2 was used as a measure of total aflatoxin production.

Mycelial dry weights

After chloroform extraction, the mycelia were collected on the preweighed filter papers, rinsed with distilled water, and allowed to air dry. The mycelia were then placed in a 100°C oven for 24 hr, and subsequently cooled in a desiccator. Mycelial dry weights were then determined gravimetrically.

RESULTS & DISCUSSION

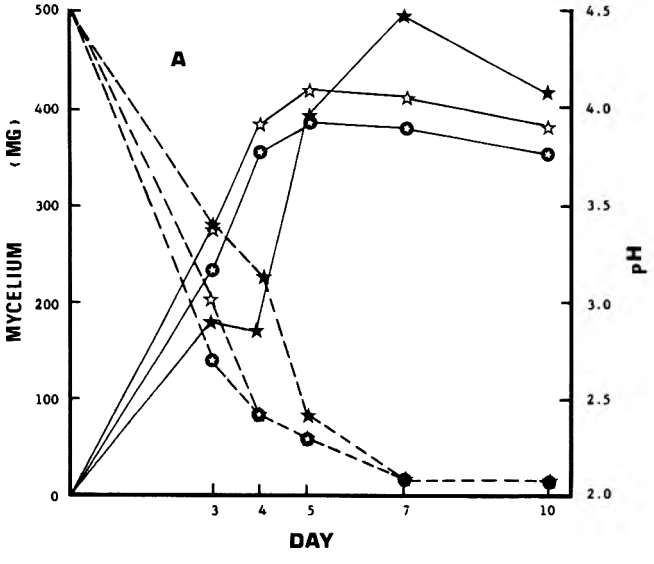
THE EFFECTS of cAMP (0, 1, and 5 mM) on growth, pH, and aflatoxin production by flask cultures of *A. parasiticus* are depicted in Fig. 1. The 1.0 mM cAMP-supplemented cultures displayed a normal growth pattern, though there was a slight increase in mycelial mass during the latter part of the growth curve. The 5.0 mM cAMP cultures displayed an atypical growth pattern, with an apparent lag period occurring between days 3 and 4. This was followed by a sharp increase in mycelial mass between the 4th and 5th day. The cultures appeared to have an extended exponential growth phase; however, the significance of this is unclear due to the earlier lag period. Supplementation with cAMP appeared to produce a dose-related retardation of the pH decrease associated with the growth of *A. parasiticus*; however, by the 5th day the pH's of supplemented and control cultures were equivalent.

Cyclic AMP strongly stimulated aflatoxin production. Supplementation with 1.0 mM cAMP resulted in an approximate five-fold increase in aflatoxin production, while the 5.0 mM level yielded an 18-fold increase. The stimulation was in large part transitory since the cAMP-supplemented cultures degraded the aflatoxin to a greater extent than did the control cultures; however, the aflatoxin levels in the 5.0 mM cultures remained elevated during the stationary phase. The cyclic nucleotide appeared to prolong the period of aflatoxin production, with maximum aflatoxin accumulation occurring on day 4 in the control cultures and day 5 in the supplemented cultures.

The effect of cGMP on growth, pH, and aflatoxin production in flask cultures is depicted in Fig. 2. There was little difference in the growth of the control and 1.0 mM cGMP-supplemented cultures. The 5.0 mM cultures appeared to have increased mycelial mass during the latter stages of the microorganism's growth cycle. In addition, there was a lag period similar to that noted for cAMP; however, in this instance the lag period may be an artifact since it was largely due to a low mycelial dry weight in a single sample. Cyclic GMP supplementation produced pH changes similar to those noted with cAMP. The 1.0 mM and 5.0 mM cGMP levels increased aflatoxin production by two- and threefold, respectively. The 5.0 mM supplementation level prolonged the period of aflatoxin production, and increased the amount of toxin remaining during the stationary growth phase.

In order to help distinguish between potential regultory and nutritional responses produced by the cyclic nucleotides, the effects of 5.0 mM AMP and GMP on growth, pH and aflatoxin production were determined using flask cultures. These results are summarized in Fig. 3. AMP appeared to slightly inhibit growth between days 3 and 7. The nucleotide produced an approximate twofold increase in aflatoxin production. GMP inhibited the growth of *A. parasiticus*, and caused an approximate 75% decrease in aflatoxin accumulation. Neither AMP nor GMP affected the pH of the cultures.

This initial experimentation indicated that cAMP strongly stimulated aflatoxin production. AMP and cGMP also increased aflatoxin production to a lesser degree, while GMP inhibited accumulation of the mycotoxins. Previous investigations (Wold and Suzuki, 1973) have suggested that high concentrations of one cyclic nucleotide can cause it to mimic the effect of the other. In order to determine if the stimulatory effect of cGMP may have been due to it mimic-



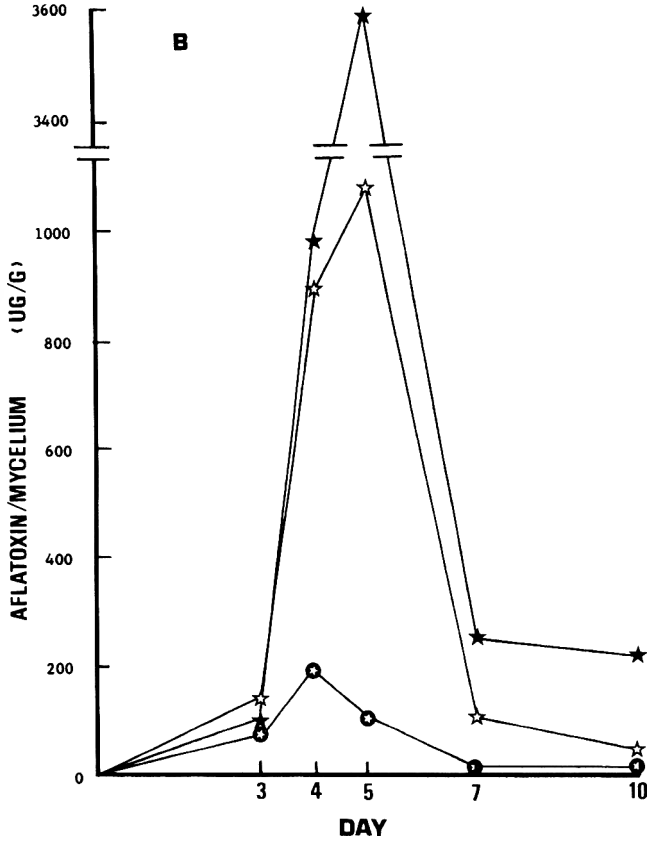
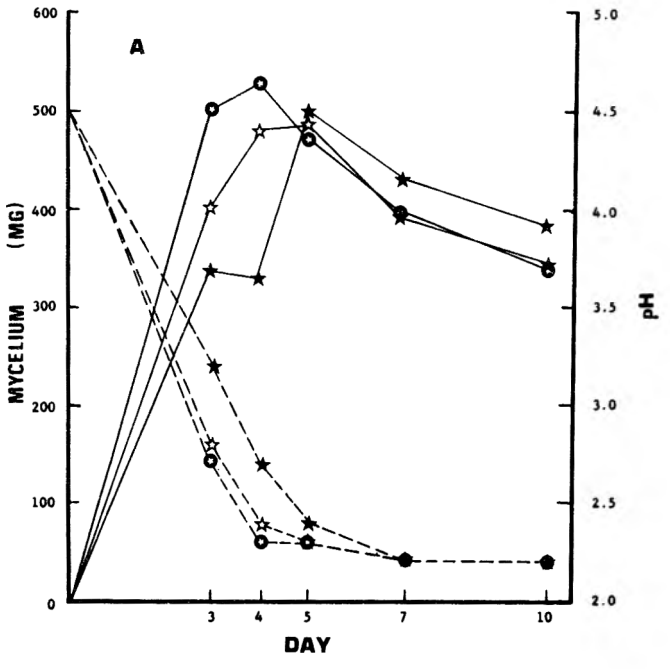


Fig. 1—Effect of 0 (☺), 1 (☆), and 5 (★) mM cAMP on growth and pH (A), and aflatoxin production (B) in A. parasiticus flask cultures.

ing cAMP, and to further characterize the effect of the noncyclic nucleotides, the effects of exogenously supplied cAMP, AMP, cGMP, and GMP were reexamined using a wider range of concentrations (0.00, 0.05, 0.50, and 5.0 mM). To facilitate the large number of samples, 3-ml test tube cultures were employed. These results are depicted in Fig. 4 and Table 1. It should be noted that maximal growth and the initiation of aflatoxin accumulation typically occurs between 60 and 72 hr, while toxin production was not evident until the sixth day in the tube cultures employed in the present study. The delay appeared to be due to an extension of the time needed for germination. The reason for this extended germination time was not readily apparent, and once growth was initiated, the cultures appeared normal.

The relatively small amount of growth and its inherent variability in test tube cultures precluded detecting small differences in mycelial mass; however, the technique was sufficient for evaluating the nucleotide effects on aflatoxin production. Cyclic AMP caused a dose-related stimulation of aflatoxin accumulation, with the highest supplementation level producing a fivefold increase. The highest levels



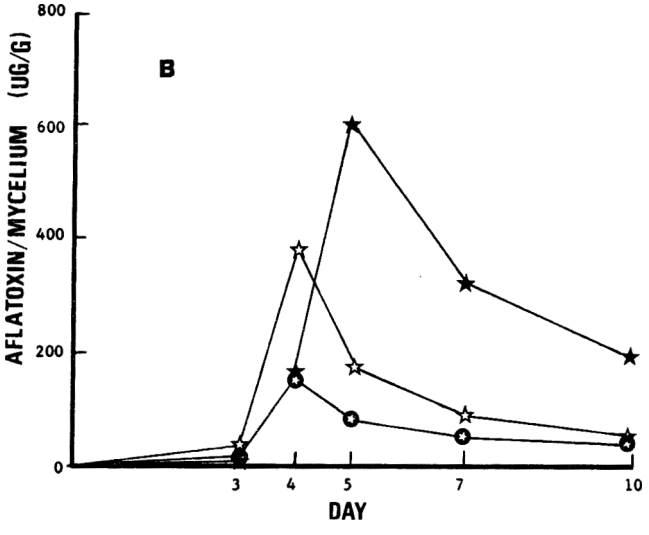


Fig. 2—Effect of 0 (⊛), 1 (☆), and 5 (★) mM cGMP on growth and pH (A), and aflatoxin production (B) in A. parasiticus flask cultures.

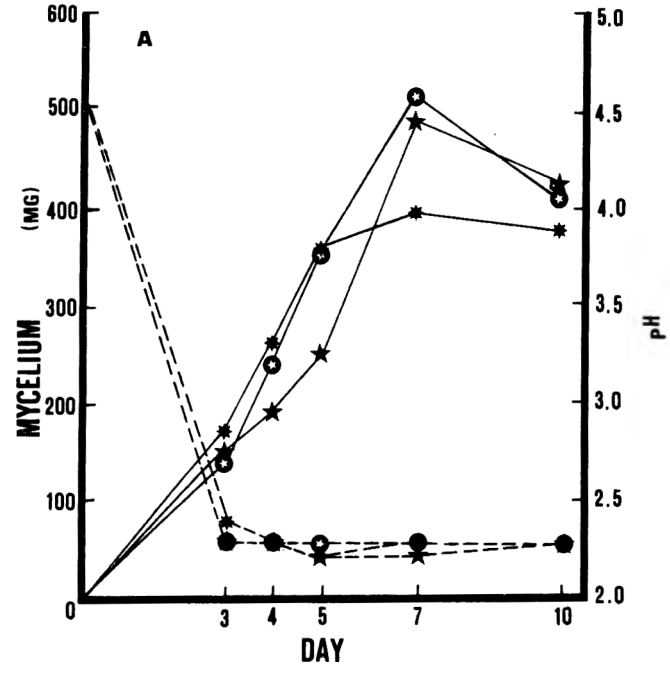
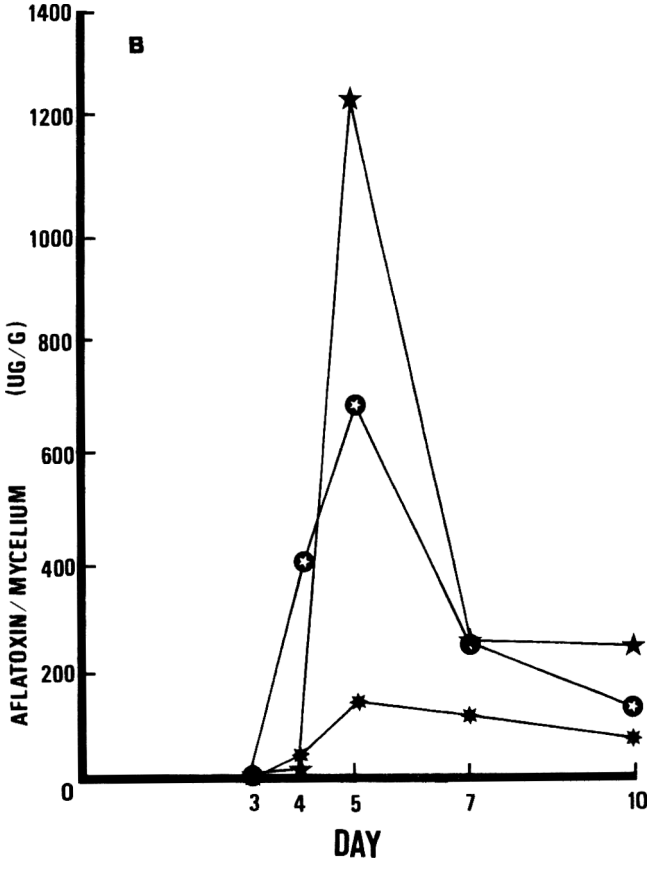


*Fig. 3—Effect of AMP and GMP on growth and pH (A), and aflatoxin production (B) in A. parasiticus flask cultures. (★ = 5 mM AMP, * = 5 mM GMP, ⊛ = control).*



of cGMP appeared to stimulate the initial (day 6) production of aflatoxin, but later samples had less aflatoxin than did the control cultures. Supplementation with 5.0 mM AMP produced an approximate 2-fold increase in aflatoxin accumulation while lower concentrations of the nucleotide appeared to be slightly inhibitory. GMP supplementation had little effect on aflatoxin production in the test tube system.

The culture data strongly support the previous observations that cAMP stimulates aflatoxin production. Comparison of the effects of cAMP and AMP indicates that little of cAMP's activity can be attributed to nutritional effects, strengthening the supposition that cAMP plays a regulatory role in the synthesis of aflatoxins. The stimulation of aflatoxin production (threefold) by cGMP noted in the flask cultures was not evident in the test tube cultures. The reason for this difference requires further investigation. One possibility is that the differences may be due to the decreased surface area associated with the tube cultures. However, the lack of stimulation supports the hypothesis that cGMP was acting as a cAMP mimic in the earlier experimentation. The inhibitory activity noted for GMP in the flask cultures was also not evident in the tube cultures, possibly for the same reasons for the differential response to cGMP. Wold and Suzuki (1973) that GMP inhibited citric acid accumulation during secondary metabolism by *Aspergillus niger*. Additional research is warranted to more fully characterize the inhibitory activity of GMP, particularly since such studies may help to explain the inhibitory activity of caffeine (Buchanan and Fletcher, 1978; Nartowicz et al., 1979; Lenovich, 1981). Buchanan and Fletcher

Table 1—Effect of cAMP, AMP, cGMP, and GMP on the Growth of A. parasiticus in 3.0-ml Test Tube cultures

Supplement	Conc (mM)	Incubation time (Day) 5	6	7
Control	0.00	15.0±4.2[a]	25.9±7.5	31.9±1.3
cAMP	0.05	16.1±5.3	29.1±0.9	30.8±1.7
	0.50	21.4±6.2	28.5±0.8	27.2±5.7
	5.00	13.0±2.7	21.5±3.0	35.4±3.6
AMP	0.05	16.5±3.7	26.9±6.9	27.0±1.0
	0.50	15.4±9.0	26.8±1.4	33.8±2.2
	5.00	7.1±3.4	23.7±3.8	31.0±7.5
cGMP	0.05	18.5±4.7	24.3±2.5	30.7±3.7
	0.50	26.1±7.8	25.8±2.4	33.7±3.6
	5.00	14.5±0.9	27.4±5.4	35.3±1.9
GMP	0.05	11.1±10.7	29.5±5.2	32.6±4.8
	0.50	15.3±2.0	31.4±1.5	31.2±1.7
	5.00	13.8±3.6	27.3±7.9	31.7±1.1

[a] mg dry weight; mean ± standard deviation

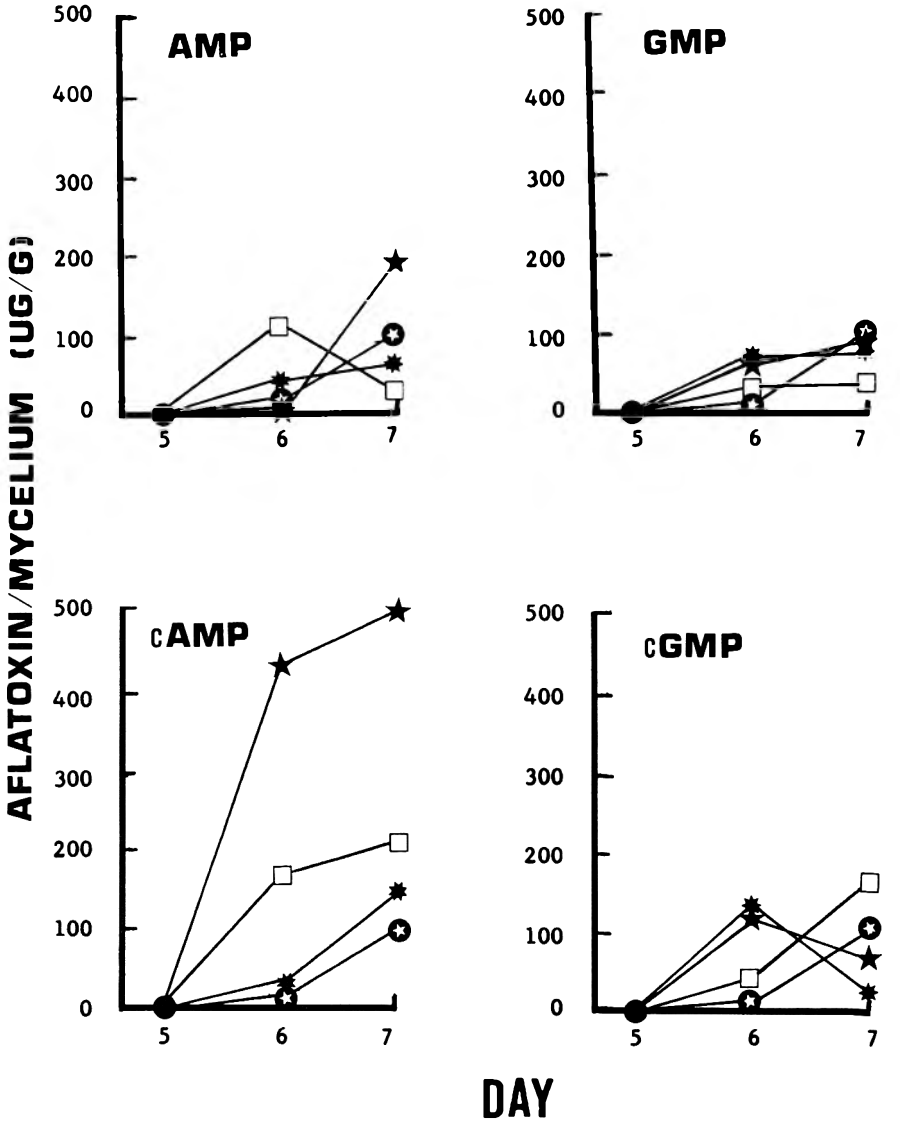


Fig. 4—Aflatoxin production by A. parasiticus in 3.0-ml test tube cultures containing 0.00 (⊛), 0.05 (*), 0.50 (□), and 5.00 (★) mM cAMP, AMP, cGMP, or GMP.

(1978) suggested that caffeine inhibition of aflatoxin production may be due to an accumulation of cAMP; however, the presen results do not support that hypothesis.

Other investigations have indicated that cAMP can influence the accumulation of secondary metabolites by fungal species. Wold and Suzuki (1976) reported that the accumulation of citric acid by *A. niger* was regulated by the intracellular concentrations of cAMP and zinc. Wolf and Mirocha (1977) found that exogenous cAMP stimulated zearalenone production, and concluded that the intracellular concentration of the nucleotide is one of the regulatory parameters controlling the synthesis of the mycotoxin. Jones and Bu'Lock (1977) reported that dibutyryl-cAMP increased carotenoid pigment accumulation by *Mucor hiemalis* and *Mucor mucedo*.

The mechanism by which cAMP influences the production of aflatoxins and other secondary metabolites awaits further elucidation. One possiblity is that the nucleotide affects the uptake or utilization of glucose and other carbohydrates. Shih and Marth (1974) demonstrated that there was accelerated glucose utilization during the period of aflatoxin production. They correlated this with decreased respiratory activity during the late exponential growth phase. Applebaum and Buchanan (1979) demonstrated that the intracellular concentration of cAMP tended to be highest just prior to the period of accelerated glucose utilization. Abdollahi and Buchanan (1981a, b) reported that the metabolism of a carbohydrate source is necessary for the induction of the aflatoxin biosynthetic pathway, but did not find that cAMP was directly involved in the induction process. It has been reported that cAMP stimulates phosphofructokinase (Mansour, 1969) and pyruvate kinase (Milman and Yurowitzki, 1967), and inhibits glyceraldehyde phosphate dehydrogenase (Yang and Deal, 1969) in various eukaryotic systems. If similar regulatory controls exist in *A. parasiticus*, cAMP may be stimulating carbohydrate catabolism, which results in increased aflatoxin production. Additional research is currently underway to evaluate possible relationships among cAMP, carbohydrate metabolism, and aflatoxin production.

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AIR–DRIED GRAINS – DIFFUSION ANALYSIS . . . From page 100

CONCLUSIONS

THE ABOVE ANALYSIS may be used to characterize the most convenient geometry to simplify the diffusion analysis of drying of grains having nonconventional shapes. It is noteworthy that the application of the "intercept values" analysis requires that the data must correspond to the straight line region, which for spheres begins below $m^* = 0.35$ and for cylinder below $m^* = 0.50$. This implies that one should use drying data corresponding to low moisture contents (i.e. 6 $\sim$ 7%) where the experimental error must be significant unless a sound experimental device is used. A correct determination of equilibrium moisture contents is also essential.

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Changes in Soluble and Bound Peroxidase–IAA Oxidase During Tomato Fruit Development

RONNIE L. THOMAS, JOSEPH J. JEN, and CHARLES V. MORR

ABSTRACT

Soluble peroxidase activity increased dramatically during the early stages of tomato fruit development, reaching a maximum at the mature-green stage. Ionically and covalently bound peroxidases were also observed, and the activities of these fractions increased steadily throughout fruit development. IAA (indoleacetic acid) oxidase activity was observed in both soluble and bound fractions and paralleled the peroxidase activity. Tissue homogenates from juvenile fruit caused an extended induction period in IAA oxidation reactions catalyzed by the purified tomato fruit peroxidase, suggesting the occurence of a high concentration of phenolic-type auxin protectors in this tissue. As the fruit developed, tissue homogenates showed a reduction in the amount of auxin protectors as the IAA oxidizing capacity of the fruit increased.

INTRODUCTION

PREVIOUS STUDIES have shown that tomato peroxidase is capable of oxidizing IAA (Frenkel, 1972; Huang and Haard, 1977: Thomas and Jen, 1980). It has been postulated that the presence of IAA represses fruit ripening (Haard, 1977), and Vendrell (1969) demonstrated that the application of exogenous IAA to fruit causes a delay in the ripening process. Thus, it appears that fruit are subject to modifications in hormonal balance during senescence as are other plant parts. Nagle and Haard (1975) reported that IAA decreases in tomato fruit during ripening, and Frenkel (1972) observed an increase in the IAA oxidase activity of tomato peroxidase during the ripening of tomatoes. However, Kokkinakis and Brooks (1979) reported that the yield of peroxidase per gram of tissue decreased with fruit ripening in tomatoes. Although there have been some conflicting results, it does appear that a capacity for IAA oxidation is developed in tomato fruit during ripening (Frenkel, 1972).

Cytochemical studies of tomato fruit peroxidase showed that the enzyme is localized on the inner-membrane surface of the tonoplast and the cell walls (Thomas and Jen, 1981). Other investigations involving the subcellular fractionation of peroxidase from various sources have concluded that part of the cellular peroxidase is bound to the cell wall (Ridge and Osborne, 1970; Haard, 1973; Gordon and Allridge, 1971; Twohing et al. 1973; Yung and Northcote, 1975).

Since numerous peroxidase isoenzymes typically exist often with different properties and substrate specificities, it has been difficult to assign physiological roles to peroxidase. An analysis of the changes in both soluble and bound peroxidase was performed to determine the true IAA oxidizing capacity of developing tomato fruit and the contribution of each fraction to IAA oxidation.

Previous studies have also shown that the IAA oxidase activity of tomato peroxidase is influenced by phenolic compounds (Thomas et al., 1981). Monohydroxyphenols serve as cofactors in the reaction, but o-dihydroxyphenols are inhibitory. Haard (1977) has suggested that IAA oxidase activity may be modulated by changes in the phenolic pool. In this report the effect of tissue homogenates from tomatoes at various stages of development on the IAA oxidase activity of a purified, tomato fruit peroxidase was measured to determine if such a regulatory mechanism might exist.

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MATERIALS & METHODS

Fruit

Tomato plants (*Lycopersicon esculentum* Mill var. Walters) were grown in the greenhouse at Clemson University during the period of December, 1979 through March, 1980. Two flowers (first and second) per cluster were pollinated and tagged at anthesis, and fruit load was limited to 10–15 fruit per plant. Fruits were harvested at 2-wk intervals at the beginning and at 1-wk intervals near the end of the growing period. Also, the following stages of development were characterized: (I) cell division from 0–21 days; (II) cell expansion from 21–42 days; (III) mature-green from 44–56 days; (V) breaker from 56–63 days; (V) pink from 63–70 days; and (VI) red ripe from 70–77 days (Martin et al., 1979).

Sampling

Pericarp tissue of three to five fruit from each harvest date was chopped into small pieces. Two 50-g samples were randomly chosen and were treated separately in subsequent operations. Peroxidase extracts were prepared according to the procedure of Gkinis and Fennema (1978) as described below.

Soluble peroxidase

Tissue samples were homogenized in a Waring Blendor in the presence of 0.05M phosphate buffer (pH 6.0) containing 1% polycar AT for 1 min. The homogenate was centrifuged at 30,000 × *g* for 30 min at 4°C, and the supernatant was referred to as the soluble fraction.

Ionically bound peroxidase

The residue from the previous step was mixed with 20 ml of cold water (0°C), homogenized for 5 sec, and then centrifuged at 30,000 × *g* for 15 min at 4°C. The supernatant was discarded, and the wash was repeated. The resulting residue was homogenized for 15 sec in the presence of 20 ml of cold (0°C) 1M NaCl. The homogenate was centrifuged at 30,000 × *g* for 20 min at 4°C, and the supernatant was referred to as the ionically bound fraction.

Covalently bound peroxidase

The residue from the previous step was washed twice with cold water (0°C) as described above. The resulting residue was suspended in 20 ml of 0.2M acetate buffer (pH 5.5), containing 0.4g pectinase and 0.2g cellulase (Ridge and Osborne, 1970; Haard and Marshall, 1976; Liu and Lamport, 1974). The samples were incubated with gentle horizontal shaking overnight at 35°C and centrifuged at 30,000 × *g* for 20 min. The supernatant was referred to as the covalently bound fraction.

Enzyme assays

Peroxidase activity of the fractions was determined by adding 50 μl of the enzyme extract to 2.5 ml of 0.5% guaiacol and 0.25%

hydrogen peroxide in 0.1M potassium phosphate buffer (pH 6.0) and following the increase in absorbance at 470 nm. One unit of peroxidase activity was defined as a change of 0.1 absorbance unit per minute. The amount of peroxidase in the fractions is reported as units of activity per gram of dry weight tissue.

Each fraction was dialyzed vs distilled water for 5 hr, concentrated to a small volume, and IAA oxidase activity was determined by O_2 uptake using a Clark-type oxygen electrode as described by Huang and Haard (1977). The standard reaction mixture contained 0.5 mM IAA, 0.1 mM $MnCl_2$, and 0.1 mM 2,4-dichlorolphenol, and 0.5 ml of the enzyme extract in a total volume of 3 ml. One unit of IAA oxidase activity was defined as a 0.1 μM change in O_2 concentration per minute. The amount of IAA oxidase in the fractions is reported as units of activity per gram of dry weight tissue. The moisture content of the fruit was determined by drying a 5-g sample of homogenized tissue in vacuo at 70°C for 24 hr.

Tissue homogenates

Twenty grams of tomato pericarp tissue were placed in 50 ml of distilled water and boiled for 15 min. The tissue was homogenized in a Waring Blendor for 2 min, and the homogenate was centrifuged at 2,000 x *g* for 20 min. The supernatant was recovered, and the volume readjusted to 50 ml with distilled water. A 1:50 dilution of each homogenate was prepared, and 0.1 ml of the diluted homogenate was added to the standard IAA oxidase assay mixture described above to determine the effect of the homogenate on the IAA oxidase activity of a purified, tomato fruit peroxidase. The purified, tomato fruit peroxidase was prepared according to the method of Thomas and Jen (1980). IAA oxidase activity was determined as described above, except that 50 μl of the purified enzyme preparation was used, and 0.1 ml of tissue homogenate was added to the reaction mixture.

Electrophoresis

Disc gel electrophoresis was performed according to the method of Davis (1964). Peroxidase activity was located by incubation of the gels in a solution of 0.05% guaiacol and 0.005% H_2O_2 in 0.1M potassium phosphate buffer (pH 6.0).

RESULTS & DISCUSSION

FIG. 1 shows that soluble peroxidase activity increased dramatically during the early stages of fruit development, reaching a maximum at the mature-green stage (III). Soluble peroxidase activity remained high throughout the breaker (IV) and pink (V) stages before decreasing in the later stage of development. IAA oxidase activity in the soluble fraction paralleled the peroxidase activity of this fraction (Fig. 1), consistent with the observation of Frenkel (1972) that an enhancement of the major isozyme component of peroxidase and IAA oxidase occurs as the tomato fruit ripens. It is significant that IAA oxidase activity increases prior to the respiration climaceteric, which typically occurs at the beaker (IV) to pink (V) stages of development (Martin et al., 1979) and is accompanied by a dramatic evolution of ethylene (Frenkel, 1972). Ethylene has often been considered as the regulatory agent influencing fruit ripening, or the ripening hormone (Burg, 1962; Burg and Burg, 1965; Pratt and Goeschl, 1969). However, since fruits at maturity usually contain sufficient ethylene to trigger ripening, it seems unlikely that the gas is the sole hormonal factor controlling fruit ripening. Mapson (1970) proposed that the onset of ripening is attributed to the ability of the tissue to respond to ethylene due to the disappearance of a restraining factor rather than to an increase per se in ethylene concentration. After ripening has commenced, ethylene evolution is considered no more than an adjunct process and only indicates the ability of some fruit to accomplish an autocatalytic synthesis of the gas (Frenkel, 1972; Mapson, 1970). Such a restraining factor, or resistance to ripening (Hanson, 1966), implies that the presence of ethylene alone is not enough to account for the triggering of fruit ripening (Frenkel, 1972). It has been suggested that other growth-regulatory systems in fruits undergo changes prior to ripening, and that such modifications could account for the changes in the resistance to ripening and to ethylene action (Pratt and Goeschl, 1969; Crane, 1969; Dilley, 1969). Since IAA is considered to be a naturally occurring retardant of fruit ripening (Frenkel, 1972), it is conceivable that IAA must be inactivated before fruit ripening can occur. The appearance of high IAA oxidase activity prior to the onset of ripening (Fig. 1) supports this viewpoint.

Although most of the peroxidase activity was observed in the soluble fraction, activity was also observed in the ionically and covalently bound fractions (Fig. 2 and 3). The bound activity was minimal during the early stages of development but steadily increased throughout fruit development. Since proteins attached to cell walls may be ionically or covalently bound, these results are in agreement with previous cytochemical localization studies, which showed an increase in peroxidase activity in the cell walls

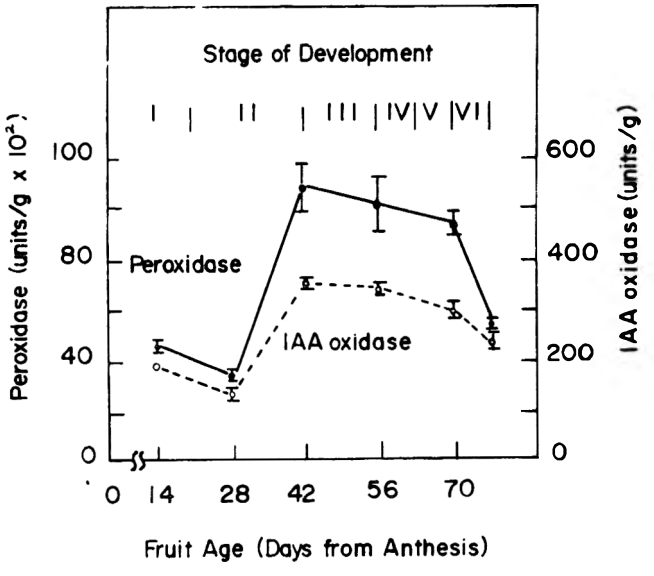


Fig. 1—Changes in the amount of soluble peroxidase and IAA oxidase in tomato fruit during development. Each value is the mean of four determinations derived from two samples, with two determinations per sample. Error bars represent the standard error. Stages of development are described in "Materials and Methods."

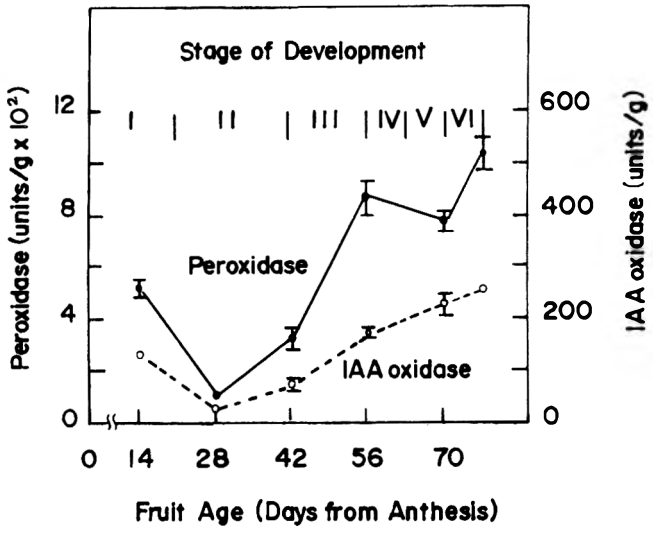


Fig. 2—Changes in the amount of ionically bound peroxidase and IAA oxidase in tomato fruit during development. For details see legend of Fig. 1.

of tomato fruit cells during fruit development. Two possible functions of cell wall peroxidase are to destroy IAA and to catalyze the polymerization of lignin (Fielding and Hall, 1978). Since tomato fruit cells do not lignify, it is likely that the primary role of the wall-bound peroxidase is to oxidize IAA. Fig. 2 and 3 show that IAA oxidase activity in the ionically and covalently bound fractions parallels peroxidase activity. Thus, the wall-bound peroxidase may function in oxidizing IAA as the hormone enters the cell. Such a role for wall-bound peroxidase has physiological significance since IAA entering fruit cells from other parts of the plant might otherwise delay the ripening process. Furthermore, this is spatially possible since binding of IAA occurs at the plasmalemma (Poovaiah and Leopold, 1976).

It was interesting that the IAA oxidase to peroxidase ratios in the bound fractions were on the average about 10 times greater than in the soluble fraction (Table 1), indicating that bound peroxidase has a greater capacity for oxidizing IAA than the peroxidase found in the soluble fraction. Furthermore, the IAA oxidase to peroxidase ratios in the covalently bound fractions were approximately twice that in the ionically bound fractions. These results suggested that different isozymes were possibly involved. Fig. 4 shows the isozyme patterns of each fraction at different stages of fruit development. In the soluble fractions one major and one minor isozyme was observed throughout fruit development. In the covalent fractions a similar isozyme to the major soluble isozyme was observed along with the appearance of two new isozymes during fruit development. Whether or not the covalent isozyme pattern represents *de novo* synthesis of new molecular forms could not be ascertained from this study, but it appears that at least some of the covalently bound peroxidase deposited in the cell wall is identical to the isozyme predominately found in the soluble fraction. The isozyme patterns of the ionically bound fractions show the appearance of two cationic peroxidases during fruit development, indicating that totally different isozyme forms are found in the cell wall. Thus, the occurence of different isozyme forms in the ionically and covalently bound fractions may explain the greater capacity of the peroxidase in these fractions to oxidize IAA. The bound peroxidases may be more important in auxin degradation than the peroxidase in the soluble fraction. Although the IAA oxidase activity of peroxidase in the soluble fraction is highest just prior to the onset of ripening, the activity in this fraction gradually declines during ripening (Fig. 1) whereas the IAA oxidase activity of the bound fractions continues to increase (Fig. 2 and 3). Furthermore, the intensification of the IAA oxidase activity of the peroxidase in the bound fractions indicates a greater specificity for IAA.

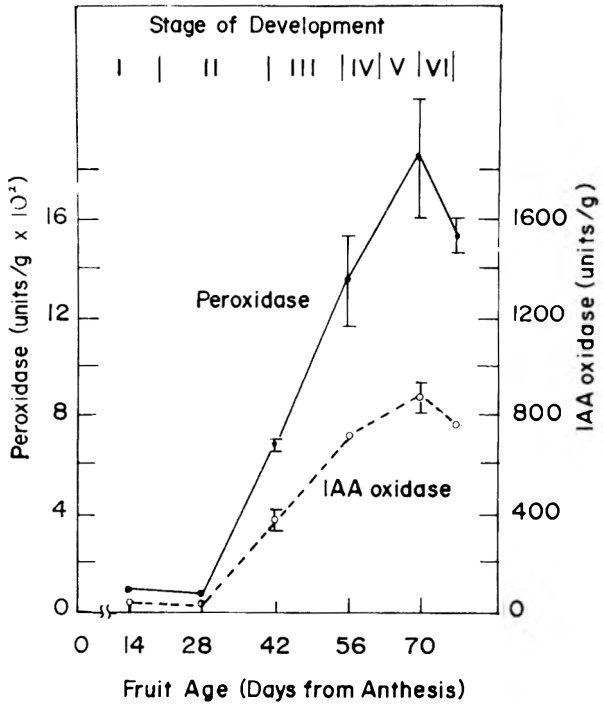


Fig. 3—Changes in the amount of covalently bound peroxidase and IAA oxidase in tomato fruit during development. For details see legend of Fig. 1.

Since previous studies have shown that IAA oxidation by tomato peroxidase is influenced by phenolic compounds (Thomas et al., 1981) and data reported here show an increased capacity of IAA oxidation in tomato fruit during their development, it might be expected that changes in the phenolic pool of tomato fruit would occur during fruit development. Fig. 5 shows the effect of tomato tissue homogenates from different stages of development on the length of the induction period typically observed in IAA oxidation by peroxidase when inhibitory phenolics are present. The length of the induction period has been shown to be proportional to the concentration of the phenolic (Thomas et al., 1981). The tissue homogenate from juvenile tissue (Stage I) caused an extremely long induction period. However, as fruit development continued the length of the induction period produced by tissue homogenates drastically decreased and remained at a low level throughout the later stages of development. These results agree with the observation that auxin protectors with available o-dihydroxyphenolic groups are abundant in juvenile tissue (Stonier and Yang, 1973). Furthermore, as the inhibitory effects of the naturally occurring auxin protectors decrease, the capacity of IAA oxidation in the soluble and bound fractions increases. At the mature-green stage of development (Stage III) tomato fruit have a high capacity for destroying IAA, since IAA oxidase activity in the soluble fraction is at a maximum and naturally occurring auxin protectors are at a minimum. This capacity for IAA destruction is maintained throughout ripening by the increased IAA oxidase activity of the bound peroxidases.

Cytochemical localization studies (Thomas and Jen, 1981) have shown that peroxidase is not found in the cytoplasm of tomato fruit cells but only in the cell walls and on the inner-membrane surface of the tonoplast. It is assumed that the soluble peroxidase obtained from tissue extraction is derived from the tonoplast, the enzyme being only loosely bound to the membrane (possibly as a peripheral protein). Further verification of the cellular location of the soluble peroxidase obtained by tissue extractions is needed. However, it is likely that the auxin protectors are localized in the vacuole, and the tonoplast location of peroxidase is spatially very significant from the viewpoint of regulation of IAA oxidation. This also suggests that IAA destruction can occur at the tonoplast or in the vacuole, as well as in the cell wall.

In the study of the physiology of fruit ripening, the most perplexing problem has been the understanding of the events which initiate the ripening process. Ethylene has

Table 1—Ratios of IAA oxidase activity to peroxidase activity in the soluble and bound fractions from tomato fruit at various stages of development

	IAA Oxidase/Peroxidase					
	Stage					
	I	II	III	IV	V	VI
Soluble	0.04	0.06	0.03	0.03	0.03	0.05
Ionically bound	0.25	0.25	0.22	0.20	0.29	0.24
Covalently bound	0.50	0.48	0.54	0.52	0.47	0.50

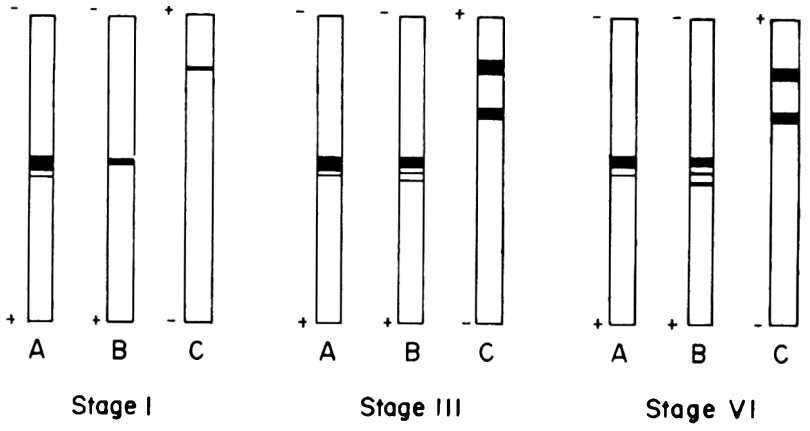


Fig. 4—Isozyme patterns of tomato fruit peroxidase from the soluble, ionically bound, and covalently bound fractions at different stages of development. (A) soluble (B) covalently bound, (C) ionically bound. Samples from the ionically bound fraction were run at pH 4.3 with polarity reversed.

often been considered to be the ripening hormone because of its promotive effects (Burg and Burg, 1965), but there is evidence that other factors are involved in determining the ability of fruit tissue to respond to ethylene, such as modifications in hormonal balance (Pratt and Goeschl, 1969). It would be logical to consider that a fruit should be subject to the same modifications in hormonal balance during senescence as are other plant parts, namely a general decline of the plant growth regulators, gibberellins, auxins, and cytokinins (Frenkel, 1972). Such hormonal changes have been observed in fruit tissue (Crane, 1969; Dilley, 1969). Since peroxidase is capable of oxidizing IAA and the appearance of high IAA oxidase activity is commensurate with the schedule of fruit ripening, the physiological function of peroxidase in fruit ripening seems to be auxin degradation. This would be a very significant role, since the presence of such growth substances appears to be the cause of resistance to ripening.

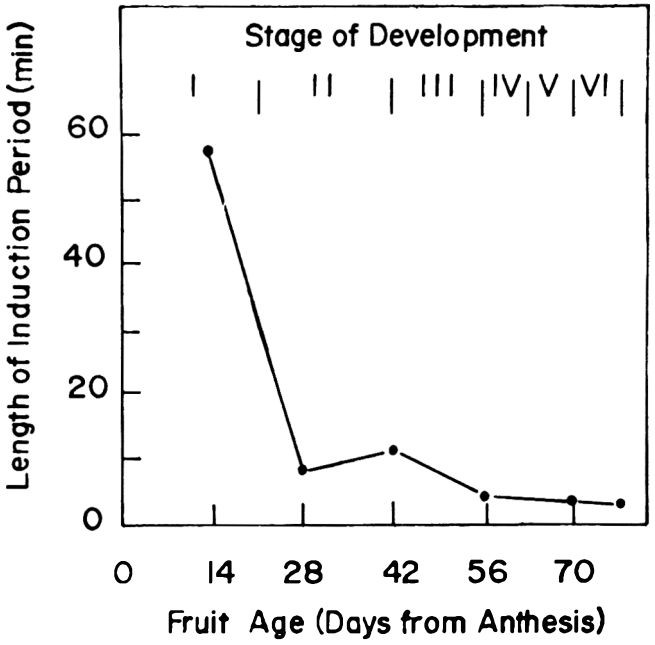


Fig. 5—The effect of tomato tissue homogenates from different stages of development on the length of the induction period in IAA oxidation by purified tomato peroxidase. Stages of development are described in "Materials and Methods."

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Betalains in Beet Root Tissue Culture

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ABSTRACT

The goals of this study were to culture red beet cells via plant tissue culture techniques and to compare the pigment levels produced in callus culture with pigment levels of natural beet root. A modified Murashige and Skoog medium was used to maintain red, yellow and nonpigmented cell lines. It was found that the red pigment, betanin, content of the red cell line has to be increased 2–6 times to match levels found in natural beet root.

INTRODUCTION

BETANIN, the red pigment produced by beet root, is important to the food industry as a color additive because the FDA has banned the use of red pigments, FD&C Red No. 2 and 4 in food products (Riboh, 1977). In addition, the use of nitrates and nitrites which imparts red color to meat products has been questioned because of evidence that these compounds produce carcinogenic nitrosamines in the stomach (Fiddler et al., 1972). Betanin, being a natural pigment, can be used in food products (von Elbe and Maing, 1973) such as sausage (von Elbe et al., 1974), yogurt, ice cream, sherbet (Pasch et al., 1975), and maraschino cherries (Anonymous, 1979).

Betalains, the pigments produced by *Beta vulgaris*, are comprised of two main groups: the red-violet betacyanin group of which betainin is principal pigment and the yellow betaxanthin group of which vulgaxathin I is the principal pigment.

Commercial beet powder is obtained from beet roots. An alternative source of betanin could be beet tissue culture which consists of a number of techniques for growing plant organs or cells on agar or in suspension culture. The possibility of using plant tissue culture techniques to produce secondary products has been discussed by Ingle and Lasure (1978) and Dougall (1977). The accumulation of betalains in beet callus culture was reported by Constabel and Nassif-Makki (1971). Saguy et al. (1978) described a quick quantitative betalains assay method.

The goals of this study were to culture a betanin producing beet cell line via plant callus culture and to compare pigment levels produced in callus culture with pigment levels of beet root.

EXPERIMENTAL

Cell line selection

The beet variety Detroit dark red (Burpee Seed Co.) was the source of the cell lines used in this study.

The first step in the cell selection procedure was to disinfest 12g of seeds by immersing them in a solution of 0.25M phosphoric acid and containing 5.25% sodium hypochlorite in a Corning 1372 milk dilution bottle for 15 min. The seeds were then washed with sterile water and placed on Difco plate count agar and allowed to germinate at 25°C. Callus initiation was performed by placing sterile excised root tips on a modified Murashige and Skoog (1962) medium (MMS) at 25°C. In addition to the normal constitutents, MMS contained naphthaleneacetic acid (NAA, 1 mg/L) and kinetin (0.3 mg/L). Callus initiation occurred after 1–3 wk. Friable red callus were subdivided and transferred to fresh MMS agar. Friability, the quality of easily crumbling into small pieces, is a desirable characteristic of callus since it facilitates subculturing. The callus cultures were photographed before and after each transfer to document color development. The cultures used in the study were obtained from a single seed.

The callus lines were maintained by incubation at 25°C on MMS agar, 20 ml. of medium per 100 x 15 mm plastic petri plate (Lab Tek Products), and were subcultured every 3 wk.

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Betalains assay

Betanin from raw beets was isolated according to the methods of von Elbe et al. (1972) and used as a standard for assay. The assay method developed by Saguy et al. (1978) was used to determine betanin, vulgaxathin I, betalamic acid and browning substances. Betalamic acid is an intermediate in the biogenesis of the betalains, while browning substances are suspected of being oxidized pigment (Kimler, 1971). The spectrophotometric assay is based on a nonlinear curve-fitting of the spectrum with the predicted absorbance for the individual pigments and eliminates the need for time consuming separations.

Samples were first ground with a small amount of McIlvaines buffer, (Anonymous, 1968) pH 5.0 and then transferred quantitatively to graduated centrifuge tubes. The samples were diluted with buffer so that the absorbances at 537 and 478 nanometers were 1.0 or less in a 1 cm light path. Centrifugation in a Sorval RC2B refrigerated centrifuge (5°C) at 15,000 rpm for 10 min removed the cellular debris. A spectrophotometric scan (360–600 nm) of the supernate was performed with a Varian Cary 219 spectrophotometer. Assays were performed on the betanin standard, the unknowns and the unknowns supplemented with the betanin standard. Eighteen wavelengths were then selected to provide the data for the calculations. The wave lengths selected were 360, 400, 410, 426, 430, 440, 450, 460, 476, 478, 490, 500, 510, 520, 537, 550, 560, and 600 nanometers. Results were calculated as mg of pigment per liter. It was necessary to correct for dilution and moisture content of the samples. Moisture was determined by drying a known amount of sample in a 47°C vacuum oven for 24 hr and expressing the moisture as a percentage of the sample.

RESULTS & DISCUSSION

THREE CELL LINES were selected from a single seed of Detroit dark red beet. These cell lines consisted of red, yellow and nonpigmented callus and were selected by subculturing callus portions with the desired characteristics of friability and pigmentation. They have been maintained over a period of 3 yr and 52 transfers on MMS agar.

The cell lines are not stable in an exact sense since spontaneous color changes in subcultured callus were observed photographically over a number of transfers. However, enough of the population retains the initial color so that the original pigment is present at all times. Callus cultures which appear to be totally of one color appear under 7X magnification to be composed of the other colors also, i.e., red, yellow, brown and unpigmented. A pattern of color development was observed and is diagrammed in Fig. 1. Beet callus may be initiated containing any of the colors in Fig 1 but color change of the whole callus proceeds only in the direction of the arrows or remains unchanged. This variation is an example of one of the major problems associated with commercialization of products from plant tissue culture.

Table 1—Comparison of standard deviations[a]

Method	No. of wavelengths	Betanine	Vulgaxathin	Betalamic Acid	Browning
Nilsson (1970)	3	6.59 ± 3.09	10.79 ± 10.33		
Saguy et al. (1978)	10	1.38 ± 0.66	5.36 ± 3.45	5.63 ± 3.64	3.96 ± 2.97
Saguy et al. (1978)	20	1.32 ± 0.64	4.99 ± 2.93	5.24 ± 3.09	3.38 ± 2.07
Weller-Lasure[b]	18	5.01 ± 0.48	2.81 ± 1.10	0.62 ± 0.30	0.19 ± 0.04

[a] Results are in mg pigment/L.
[b] The sample was lypholized beet powder.

Table 2—Comparative betalains assay[a]

Sample	% Moisture of callus	Betanin	Vulgaxathin	Betalamic acid	Browning
Red callus	97	1.34	3.40	0.75	0.05
Yellow callus	96	0	0.71	0.13	0.07
Nonpigmented callus	97	0	0	0	0.07
Raw beet	89	4.36	4.19	0.76	0.16
Reported range in raw beet[b]		2.61 – 8.70			

[a] mg pigment/g dry weight.
[b] Pasch et al. (1975) and Riboh (1977).

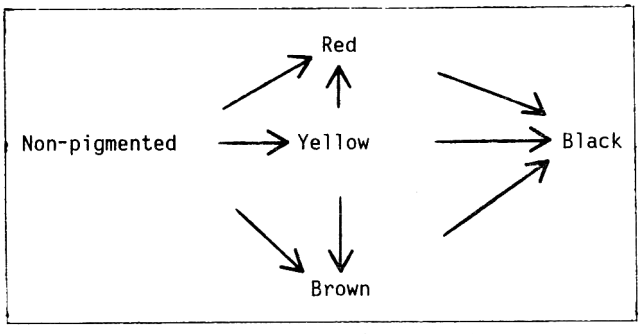


Fig. 1—Observed pattern of color development

The amounts of betanin, vulgaxathin I, betalamic acid and browning substances produced in callus culture were compared with those of beet root. The results of the assays performed in this study compare favorably with those of previously published values (Table 1).

According to Pasch et al. (1975), the betanin content of raw beet is in excess of 100 mg/100g of fresh weight while a review of natural colorants by Riboh (1977) stated that the betanin content of raw beets ranges from 30–100 mg/100g fresh weight. The moisture content of raw beets was determined experimentally to be 88.6%, thus these literature values correspond to 2.61–8.70 mg of betanin per gram of dry weight. Results of the assays comparing the pigment levels of the three callus lines and beet root (Table 2) showed that the betanin content of the red callus has to be increased 2–6 times to match reported levels in beet root.

Microscopic observations (100X) of the red callus revealed that approxiamtely 40% of the cells are red, 50% nonpigmented, 6% yellow and 4% brown. In addition the intensity of the pigment varies from cell to cell. Observation of the yellow callus showed that only 1–2% of the cells had yellow pigment while the rest were unpigmented with a few brown cells. These observations confirmed the assays in that the red callus contained more vulgaxathin than the yellow callus and that the yellow callus did not contain appreciable levels of betanin. The nonpigmented callus contained unpigmented cells with only a rare pigmented cell of red or yellow. It was interesting to note that the levels of vulgaxathin and betalamic acid were comparable in the beet root and red callus.

The goals of this study, namely to cultivate cells of red beet using plant tissue culture techniques, select red cell lines and compare the pigment levels in the whole beet root and the callus cultures were achieved. In the process, we have made several observations. First, the medium we selected as the best for pigment production contained NAA. This supports the data of Constabel and Nassif-Makki (1971). Secondly, we were able to physically select cell lines containing predominantly one color and maintain these lines over a period of 3 yr. Thirdly, we were able to use the procedure as described by Saguy to estimate betanin, vulgaxathin and betalamic acid concentrations.

The objectives of further work should be to increase the betanin concentration in the red cell line to the level found in beet root and to stabilize the cell line so that only red pigmented cells are produced. This might be achieved by establishing single-cell-derived cell lines and selecting for stable red clones.

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Betacyanine and Betaxanthine Decolorizing Enzymes in the Beet (*Beta vulgaris* L.) Root

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ABSTRACT

Both enzymatic betacyanine and betaxanthine decolorizing activity were found in subcellular tissue extracted from the beet root. The optimum pH for betaxanthine degradation was pH 3.4, similar to that for betacyanine. Enzyme preparations from different cultivars, i.e., red or yellow, vary in their activities toward betacyanine and betaxanthine. A major portion of the decolorizing activity was found in tissue separated from the epidermal portion of the beet root where most pigments were accumulated. Sodium chloride, cellulase, and pectinase were selected as agents to solubilize the tissue bound enzyme. Although there was little success in bringing the bound enzyme into a soluble form, it was found that NaCl showed inhibitory effect on the beet betacyanine decolorizing enzyme. Commercial enzyme preparations also contained betacyanine decolorizing activity.

INTRODUCTION

EARLIER RESEARCH has shown the existence of a betacyanine decolorizing enzyme in fresh table beet tissue (Soboleva et al., 1976; Lashley and Wiley, 1979). The enzyme systems involved in this action are closely bound to the subcellular components of the beet root tissue and most active at a temperature of 40°C and a pH of 3.4. This pH optimum does not seem to correspond to optimum pH's for the various oxygenases and hydrolases normally found in beet tissue. The literature to date has not revealed the presence of a betaxanthine decolorizing enzyme. Possible enzymatic decolorizing problems of both betacyanines and betaxanthines must be considered in the overall commercial development of natural beet pigments.

The objectives of this study were to develop more information about the betalaine decolorizing enzymes. Included were studies of cultivar relationships to pigment decolorizing activity, location of major enzyme activity anatomically in the beet root, attempts to solubilize the decolorizing enzymes, and further purification and characterization of the enzyme systems.

It was anticipated that these studies would suggest means of preventing enzymatic destruction of betalaine colorants during processing or as an ingredient in formulated foods. Enzymic degradation studies might also provide insights into the mechanism of betacyanine destruction by environmental factors such as pH, air, light, and heat.

EXPERIMENTAL

Raw product

The beet roots (*Beta vulgaris* L.) used in this experiment were commercial cultivars of Ruby Queen, Monoking Explorer, Burpee's Golden and Dark Red Detroit from New York State. The roots were stored at 2°C and 85% relative humidity for one to three months prior to the experiments.

Crude enzyme extraction

The enzyme was extracted from whole beet root except in the special study relating to distribution of enzyme within the beet root. The whole beet root was hand-chopped to small pieces. Two hundred grams of chopped beet were added to 200 ml of chilled 0.01M phosphate buffer (pH 7.0) and homogenized in a Waring Blendor for 5 min. Larger tissue fragments were removed by filtering through cheesecloth and centrifuging at 200 x *g* for 5 min using a Beckman Model J-21 B centrifuge. The supernatant was further centrifuged at 1200 x *g* for 12 min to separate tissue bound enzyme from beet juice. The enzyme-containing sediment was re-suspended in fresh buffer solution and centrifuged to remove pigments; this was repeated 6 times. The washed sediment was then suspended in 10 ml buffer solution for enzyme analyses. Protein content of the preparation was determined by the Lowry method according to Gernardt and Beevers (1968), and ranged from 9.69 mg/ml to 12.60 mg/ml.

Solubilizaton of tissue bound beet enzyme

Commercial enzyme preparations pectinase (Clarex-L, Miles Laboratories) and cellulase (cellulase-TAP, International Enzyme Co.) were used to solubilize the enzyme from subcellular tissue. One ml of beet enzyme was added to 2 ml 0.1M pH 4.8 buffer and 1 ml of Clarex-L or 10% cellulase-TAP, diluted to 10 ml and held at room temperature for 20 hr. The enzyme suspension was centrifuged at 5000 x *g* for 5 min. Decolorizing activity was determined in both supernatant and sediment.

Sectioning of beet root tissue

To study the distribution of decolorizing enzyme within the beet root, intact beet root was separated into epidermal and central portions. The epidermal portion was removed from the intact root to approximately a 3–4 mm depth from the outer surface; the remainder was regarded as central portion. The enzyme was extracted from each portion using the procedure mentioned earlier.

Preparation of substrates

Beet juice was prepared by continuous counter-current diffusion with preliminary inactivation of oxidase enzyme at 85°C (Wiley and Lee, 1978). The juice was treated with 0.05% pectinase solution (Clarex-L) for 24 hr at room temperature, filtered through 15 micron diatomaceous earth, and concentrated to 35°Brix at 40–45°C in a rotary vacuum evaporator. The concentrated beet juice was loaded in a SP-Sephadex C-25 cation exchange column (40–120 micron) and eluted with 1 mM Na_2HPO_4 to isolate the major betaxanthines and betacyanines (Solomos et al., 1981). Concentration of the pigments was determined by measuring optical absorbance at 476, 537, and 600 nm (Nilsson, 1970; Wiley and Lee, 1978).

Measuring enzyme activity

Enzyme decolorizing activity was determined by measuring decrease of pigment concentration in reaction mixture similar to the method of Lashley and Wiley (1979). The reaction mixture consisted of 0.2 ml to 1 ml of enzyme preparation and 8 ml to 15 ml of substrate in a centrifuge tube which was incubated at 40°C in the dark. The reaction proceeded for 20 min to 5 hr depending on the enzyme activity and the concentration of substrate and was ended by centrifuging out the enzyme sediment at 5000 x *g* for 5 min in a Beckman Model J-21 B centifuge. The supernatant was then used to determine the remaining pigment by measuring its optical absorbance. This was calculated as $\Delta A = A_o - A_t$, where $A = 1.095 (A_{537} - A_{600})$ for betacyanine and $A = A_{476} - 0.258 \times A_{537} - 0.742 \times A_{600}$ for betaxanthine. The control containing the same amount of substrate and with 0.01M phosphate buffer replacing the enzyme preparation accompanied each set of tests. A heat inactivated enzyme preparation was also checked for decolorizing activity.

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In examining the effect of NaCl on the decolorizing activity, a necessary amount of NaCl was incorporated into the substrate solution. The effect of low oxygen tension was examined by purging substrate mixture with nitrogen gas for 10 min prior to adding enzyme preparation and then continuously during the reaction.

RESULTS & DISCUSSION

Preliminary tests

To examine if betaxanthine and/or betacyanine decolorizing enzymes were present in extracts prepared from beet roots, three preliminary tests were conducted. Environmental or chemical loss and enzymatic loss of betaxanthine after 2.5 hr incubation with extracts from the Monoking Explorer cultivar were 63%. Loss of pigment in the control (without enzyme) was 18%; betaxanthine loss due to enzymatic reaction was 45%. After 5 hr in 40°C incubation, the enzymatic loss was 42%, and chemical reaction loss was 33%. These data suggested that after approximately 2.5 hr incubation, the enzymatic reaction was completed whereas the chemical reaction continued to destroy the pigment. The enzyme may be denatured or inhibited by the products generated by the chemical or enzymatic reaction. Further data showed that the extract from Monoking Explorer, a highly red pigmented cultivar, exhibits a very strong betacyanine decolorizing activity similar to that reported by Soboleva et al. (1976) and Lashley and Wiley (1979), i.e., 70% of the betacyanine color was lost in 60 min. Preliminary data also showed that the extract from Burpee's Golden, a yellow beet cultivar, exhibited stronger activity toward betaxanthine than betacyanine.

Influence of pH on betaxanthine decolorizing activity

The effect of pH on the betaxanthine decolorizing activity was examined over the range of 2.4–5.3 using an enzyme extract from Monoking Explorer. The reaction proceeded for three time intervals, 1, 1.5, and 3 hr (Fig. 1). The optimum pH for enzymatic betaxanthine decolorizing activity was 3.4 which was also the optimum pH for betacyanine decolorizing activity (Lashley and Wiley, 1979; Soboleva et al., 1976). Both higher and lower pH's can decrease the total loss of betaxanthine if the decolorizing enzyme was not properly inactivated; however, chemical destruction was intensified by lowering the pH.

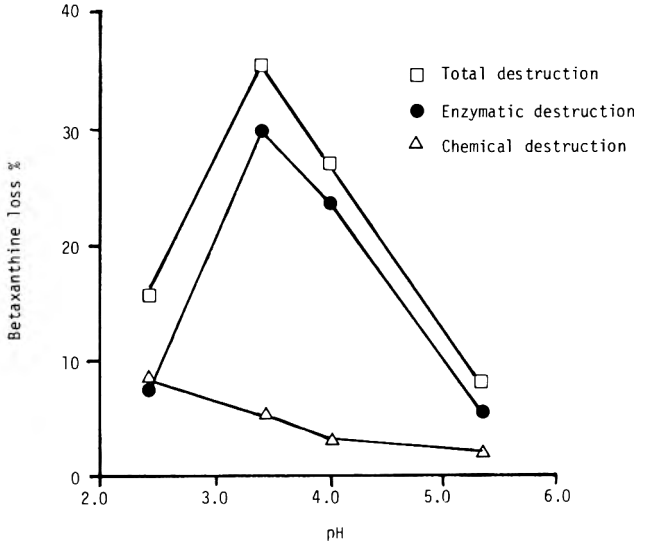


Fig. 1—Influence of pH on betaxanthine decolorizing activity of Monoking Explorer enzyme. Reaction mixtures consist of 10 ml betaxanthine and 0.3 ml enzyme, reacted at 40° C for 1 hr.

Distribution of decolorizing enzyme within the beet root tissue

The distribution of decolorizing enzymes in beet root was examined by using enzyme extracts prepared from epidermal and central portions of the Ruby Queen cultivar. The epidermal portion of the beet root contains more enzymatic decolorizing activity than the center portion (Table 1). It is suggested that the location of the betalaine decolorizing enzyme system may be associated with the highest pigment accumulation sites in the beet root which are generally found in the epidermal area.

Comparisons of betalaine decolorizing activity among various beet cultivars

Table beet cultivars, Monoking Explorer, Ruby Queen, and Burpee's Golden were used to compare relative betaxanthine and betacyanine decolorizing activity. The enzyme preparation from Burpee's Golden displays stronger betaxanthine decolorizing activity, but weaker betacyanine decolorizing activity than the two red beet cultivars, Monoking Explorer and Ruby Queen (Table 2). Data suggested that the specificity of the betalaine decolorizing enzyme in beet root may be related to the major pigment present in the root. Data also indicate that Monoking Explorer exhibited a stronger overall betalaine decolorizing activity

Table 1—Betalaine decolorizing activity of enzyme from the epidermal and the central portion of the Ruby Queen cultivar

Tube[a]	Substrate	A_o	$A_{2\ hr}$	Pigment loss %	Enzymatic pigment loss %
Control	Betaxanthine	0.258	0.202	22	
Epidermal portion	Betaxanthine		0.144	44	22
Central portion	Betaxanthine		0.169	35	13
Control	Betacyanine	0.519	0.454	13	
Epidermal portion	Betacyanine		0.238	54	41
Central portion	Betacyanine		0.379	27	14

[a] Reaction mixtures consist of 10 ml substrate and 0.3 ml enzyme, reacted at 40° C and pH 3.4 for 2 hr

Table 2—Comparison of betalaine decolorizing activity among various cultivars

Tube[a]	Substrate	A_o	A_t	Pigment loss %	Enzymatic pigment loss %
Control	Betaxanthine	0.176	0.160	9	
Monoking Explorer	Betaxanthine		0.090	49	40
Ruby Queen	Betaxanthine		0.099	44	35
Burpee's Golden	Betaxanthine		0.054	69	60
Control	Betacyanine	0.509	0.496	3	
Monoking Explorer	Betacyanine		0.140	73	70
Ruby Queen	Betacyanine		0.230	55	52
Burpee's Golden	Betacyanine		0.381	25	22

[a] Reaction mixtures consist of 10 ml substrate and 0.2 ml enzyme, reacted at 40° C for 1 hr for betaxanthine and 30 min for betacyanine

Table 3—Effect of NaCl on beet enzyme activity

Tube[a]	NaCl conc	A_o	$A_{30\ min}$	Betacyanine loss%	Enzymatic betacyanine loss %
Blank	0	0.845	0.803	5	
Reaction 1	0		0.416	51	46
Reaction 2	0.5M		0.613	27	22
Reaction 3	1.0M		0.784	7	2

[a] Reaction mixtures consist of 10 ml substrate and 0.2 ml enzyme, reacted at 40° C and pH 3.4 for 30 min.

Table 5—Effect of pH on betacyanine decolorizing activity of commercial pectinase and cellulase

Tube[a]	A_o	$A_{1\ hr}$	Betacyanine loss %	Enzymatic betacyanine loss %
pH 3.4				
Blank	0.747	0.673	10	
Cellulase		0.452	39	29
Pectinase		0.361	52	42
pH 4.8				
Blank	0.738	0.708	4	
Cellulase		0.377	49	45
Pectinase		0.333	55	51
pH 7.0				
Blank	0.742	0.706	5	
Cellulase		0.522	30	25
Pectinase		0.684	8	3

[a] Reaction mixtures consist of 0.1 ml 10% cellulase-TAP (protein content 14.70 mg/ml) or 0.1 ml Clarex-L_5 (protein content 33.18 mg/ml) and 10 ml substrate, reacted at 40° C for 1 hr.

than Ruby Queen. Since Monoking Explorer grown in New York and Maryland contained significantly more of the betaxanthines and betacyanines than Ruby Queen (Shannon, 1972; Ng and Lee, 1978), it is likely that the amount of betalaine decolorizing enzymes existing in beet root tissue relates to the amount of betalaines present in the tissue.

Effect of sodium chloride and low oxygen tension on the decolorizing activity

The effect of NaCl on beet enzyme activity was examined with an enzyme preparation from Dark Red Detroit and purified betacyanine as substrate. Data in Table 3 indicate that NaCl has an inhibitory effect on the beet enzyme activity; 0.5M and 1.0M NaCl inhibited approximately 50% and 95%, respectively, of the activity.

It has been reported that oxygen is necessary for enzymatic decolorization of betanin (Soboleva et al., 1976). To confirm this observation, betacyanine decolorizing activity of the beet enzyme was examined under anaerobic conditions (Table 4). A substantial amount of the activity was inhibited when the reaction mixture was bubbled with nitrogen.

Decolorization of betacyanine by other enzyme systems

The attempt to bring the tissue bound enzyme into a soluble forms was not successful since most enzyme activity still remained in the sediment. However, during the course of the experiment, it was found that the two commercial enzyme preparations, Clarex-L and cellulase-TAP, showed betacyanine decolorizing activity but no betaxanthine decolorizing activity. The two commercial enzyme preparations also had somewhat different characteristics in betacyanine decolorizing activity than the endogenous beet

Table 4—Effect of reduced oxygen level on beet enzyme activity

Tube[a]	A_o	$A_{30\ min}$	Betacyanine loss %	Enzymatic betacyanine loss %
Control blank	0.761	0.702	8	
Control reaction		0.287	62	54
N_2 bubbled blank	0.763	0.728	5	
N_2 bubbled reaction		0.589	23	18

[a] Reaction mixtures consist of 8 ml substrate and 0.2 ml enzyme, reacted at 40° C and pH 3.4 for 30 min.

Table 6—Effect of NaCl on betacyanine decolorizing activity of commercial pectinase and cellulase

Tube[a]	A_o	$A_{30\ min}$	Betacyanine loss %	Enzymatic betacyanine loss %
Blank	0.750	0.728	3	
Cellulase no NaCl		0.479	37	34
Cellulase + 0.5M NaCl		0.230	70	67
Cellulase + 1.0M NaCl		0.185	76	73
Pectinase No NaCl		0.411	45	42
Pectinase + 0.5M NaCl		0.172	77	74
Pectinase + 1.0M NaCl		0.140	81	78

[a] Reaction mixtures consist of 0.2 ml 10% cellulase-TAP or Clarex-L and 10 ml substrate, reacted at 40° C and pH 4.8 for 30 min.

root enzymes. First, they exhibited higher activity at pH 4.8 than at pH 3.4, the optimum pH for the beet root enzymes (Table 5). Second, NaCl greatly intensified the decolorizing activity of these two enzymes (Table 6).

SUMMARY

THE CRUDE ENZYME extracts from the beet root showed decolorizing activity to both betaxanthines and betacyanines. The attempt to clarify whether these two reactions were initiated by the same enzyme or by different enzymes was not achieved due to the difficulty of solubilizing the tightly bound macromolecules. In addition to the decolorizing enzyme found in the beet root, a betacyanine decolorizing enzyme was also found in several commercial enzyme preparations. The mechanism of enzymatic degradation of the betalaines, the existence and the nature of the responsible enzymes and factors that affect their activity, are open for further study. Such understanding will be utilized to improve betalaine recovery from the beet root during industrial operations and to increase their stability in food products during processing or storage. Perhaps, the elucidation of enzymatic destruction of betalaines will provide information for determining the environmental or chemical degradation mechanism of the pigments themselves.

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Processing of Potato Protein Concentrates and Their Properties

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ABSTRACT

Protein recoveries from potato juice by ultrafiltration, polyelectrolyte-coagulation and cryoconcentration were compared. The best yield was obtained by ultrafiltration. Depending on the method of potato juice concentration, differences were observed in: foaming and emulsifying properties, wettability, swelling, and buffer capacity of preparations. The dried preparations contained a high level of proteolytic enzymes inhibitors and glucoalkaloids. Thermal inactivation of preparations before drying led to 43–48% destruction of protease inhibitors and 81–89% glycoalkaloids. At the same time it was observed that thermal treatment led to distinct changes in the amino acid composition of the proteins and had an adverse effect on the properties of the dried preparations.

INTRODUCTION

THE PROCESSING of one metric ton of potatoes to starch, produces 5–12m^3 of potato juice which contains 30–41% of proteins in total solids. According to Bode (1974) high-quality starch can be obtained using recycled water. In that case only 300 liters of water per metric ton of processed potatoes was used.

Potato proteins contain about 71% tuberin, 7.6% glutein, 6.6% albumin, 3% globulin, 1.7% prolamin, and 8.8% other proteins (Kappor and Desborough, 1975. These proteins are rich in lysine; the sulfur amino acids are limiting (Rexen, 1976). Kaldy and Markakis (1972), Knorr (1978; 1980), on the basis of the amino acid composition, suggested that these proteins had high nutritional value, and Kappor and Desborough (1975) obtained promising results in nutritional experiments.

Different methods of protein recovery from potato juice have been reported (Eriksson and Sivik, 1976; Finley and Hautala, 1976; Knorr, 1977, 1978, 1980; Meister and Thompson, 1976; Rosenau et al., 1978).

Strolle et al. (1980) presented five alternatives for potato juice utilization: (1) ion exchange recovery process; (2) biological oxidation of waste; (3) protein recovery by the process developed by the above authors followed by biological oxidation of the remaining solids; (4) protein recovery as in (3) with additional concentration of the remaining solids to 60% t.s.; and (5) concentrating the entire effluent to 60% solids by multieffect evaporation.

According to further research by Strolle et al. (1980) concentration leads to considerable loss in available lysine with simultaneous preservation of trypsin inhibitors. The authors did not obtain favorable results using the concentrate in poultry feeding tests; additional drying enabled them to use dried soluble potato solids in poultry feeding but not more than 5–10% of the fodder.

In our work we present results on protein recovery from potato juice by ultrafiltration, coagulation with polyelectrolytes, and cryoconcentration, as well as physicochemical and nutritional properties of the resultant protein preparations.

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EXPERIMENTAL

FOR THE RECOVERY of proteins from potato juice the scheme illustrated in Figure 1. Potato juice was separated from starch using an Alfa-Laval pulp centrifuge. The potato juice (50 dm^3) containing about 5% of total solids was concnetrated in a Danish DDS Lab. Module (20–0.36) ultrafilter using the "600" membranes, which rejected substances of molecular weight above 6000 Daltons, at a pressure or 1.0 MPa. Concentration was carried out to three different volumes: 1:5; 1:10, 1:15 at an average permeate flux of 2–7 dm^3/m^2/hr. The permeate flux decreased when the solids content in the UF-concentrate increased.

The following polyelectrolytes were used: WF-1-Rokryzol (nonionic polyacrylamide coagulant, molecular weight 3 x 10^3); WF-2-Rokryzol (anion high-molecular acrylamide acid) and its methyl derivative, as well as CMC-methyl carboxycellulose and Magnoflocd (Allied Colloids Ltd., England) at a concentration of 0.15 g/dm^3 at pH 4.5, ensured an 85% recovery of nitrogen substances. All the polyelectrolytes were nontoxic.

The precipitate was separated by centrifugation at 2000 x *g* in a Sharples centrifuge (Type-1A-Laboratory).

Cryoconcentration of potato juice was carried out as follows: potato juice was frozen in an alcohol bath at −18°C, water crystals separated from the concentrated juice in a sieve with a 0.2 mesh diameter, and the process repeated three times. The concentrates were dried in an Anhydro-Laboratory S spray dryer (air temperature 150°C inlet and 60°C outlet, Fig. 1). All experiments were replicated five times.

In concentrates and dreid preparations the solids content, Nitrogen Solubility Index (NSI) (Puski, 1975) as well as the starch content and reducing sugars were determined. Nitrogen substances were separated on Sephadex gel G-100 (Sephadex, 1970). Buffer capacity (Coffman and Gracia, 1977) titration curves for 0.5% protein solutions were plotted against pH using 0.1N HCl or 0.1N

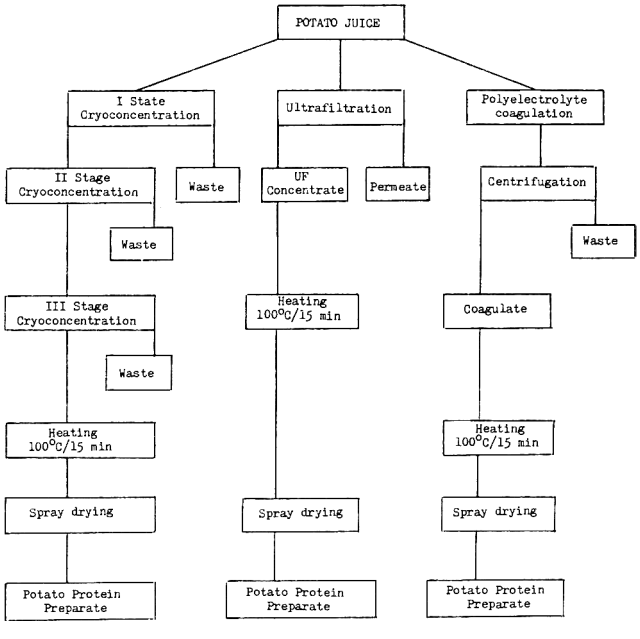


Fig. 1—Simplified flow diagram for the processing of potato protein concentrates.

NaOH (Kirchmeier, 1977). Water absorption (Hermansson, 1972), whipping properties (Puski, 1975), lipolytic properties (Webb et al., 1970), proteolytic enxyme inhibitor activity (Wojnowska et al., 1979) and the glycoalkaloides (Schwartz, 1962) were also investigated. The nutritional value of the proteins was calculated according to the Essential Amino Acids Index (EAAI), and in nutritional experiments on rats the Net Protein Utilization Index (NPU) was determined (Miller and Bender, 1965).

RESULTS & DISCUSSION

Recovery of nitrogen substances

The concentration of potato juice 1:5 by ultrafiltration (UF) led to an increase in the solids content from 5.64% to 6.25% and in nitrogen substances from 1.63% to 2.37%, respectively. After spray drying, this concentrate contained 13.47% water, 46.6% nitrogen substances, and 17.3% mineral substances (Table 1).

The tenfold UF-concentrated potato juice contained 13.06% total solids, including 8.99% nitrogen substances. After spray drying, the preparation contained 9.56% water, 58% nitrogen substances, and 15.66% mineral substances (Table 1). The 15-fold UF concentrated potato juice contained 18.9% solids, including 16.27% nitrogen substances. After spray drying, the preparation contained 6.1% water, 74.23% nitrogen substances, and 8.65% mineral substances. The UF-waste contained from 1.71% solids at a concentration of 1:5 to 1.91% at a concentration of 1:15, including 1;16% to 0.55% nitrogen substances nonprecipitable in 12% TCA. On the other hand, the waste, after potato protein coagulation at 95°C, still contained 2.42% solids, including 0.85% nitrogen substances (Table 1).

The results stimulated further research of ultrafiltration of potato juice, since there is a possibility of obtaining dry preparation of acceptable water content, high protein content and as low as possible mineral substances content. The conditions of drying our concentrates, milder than previously reported (Strolle et al., 1980; Holm and Eriksson, 1980), enabled us to obtain dried preparations with water content as low as 4%, particularly after previous ultrafiltration.

The highest recovery of nitrogen substances, 85% of those precipitable in 12% solution of TCA, was obtained with a Magnoflocd concentration of 0.15 g/dm^3 and pH 4.5. The precipitate contained 6.16% organic substances and 2.04% mineral substances. After spray drying, the preparation contained 4% water, 56.2% nitrogen substances, 26.37% starch and 1.2% reducing sugars (Table 2).

The post-coagulate waste contained 2.42% solids, including 0.13% nitrogen substances nonprecipitable in 12% TCA solution, 0.5% mineral substances, and 1.5% starch.

The least favorable results were obtained with cryoconcentration (Table 3). After procesisng, the concentrate contained 11.5% total solids and 5.19% nitrogen substances. After spray drying the preparation contained 17.7% water, 35.36% nitrogen substances, 27% starch, and 19.46% of mineral substances. The post-cryoconcentration waste contained 4.08% solids, 2.28% nitrogen substances and 0.8% mineral substances. During two-stage cryoconcentration the loss of total solids was as high as 41.05%, while during a three-stage concentration, 38.9% was lost. Taking into consideration power consumption, this method is not appropirate for concentrating potato juice.

Physicochemical proprrties

Fat emulsifying properties distinguish the potato preparations among the known vegetable protein preparations (Finley and Hautala, 1976). One hundred mg of spray dried preparation, UF-concentrated to 1:15, emulsified 139.42 cm^3 of oil; UF-concentrated to 1:10, 147.88 cm^3 oil; and UF-concentrated to 1:5, 81.3 cm^3 oil. The dried preparation previously concentrated by cryoconcentration emulsified 132 cm^3 oil, while the preparation after polyelectrolyte coagulation emulsified 120 cm^3 oil (Table 4). The

Table 1—Chemical composition of concentrated and dried protein preparation

Kind of sample	Conc degree	Total solids (%)	Organic substances (%)	Organic substances (%)$^{t.s.}$	Mineral substances (%)	Mineral substances (%)$^{t.s.}$	Nitrogen substances N_{total} x 6.25 (%)	Nitrogen substances N_{total} x 6.25 (%)$^{t.s.}$	Nitrogen substances $N_{prec.}$ in 12% TCA (%)	Nitrogen substances $N_{prec.}$ in 12% TCA (%)$^{t.s.}$
Potato juice		5.64	4.83	85.65	0.80	14.32	1.63	29.03	0.16	2.90
UF concentrate[a]	1:5	6.25	5.58	89.42	0.66	10.57	2.37	38.00	0.23	3.80
Post-UF waste	1:5	1.71	1.32	76.85	0.39	23.20	1.16	67.98	–	–
Post UF drying prep	1:5	86.53	69.23	80.00	17.30	19.99	46.66	53.92	5.18	5.95
Post UF concentrate	1:10	13.06	11.94	91.46	1.11	8.54	8.99	68.81	1.18	9.03
Post UF waste	1:10	1.87	1.27	67.87	0.60	32.15	0.73	39.50	–	–
Post UF drying prep	1:10	90.44	75.28	83.23	15.66	10.77	58.03	64.16	8.15	8.98
Post UF concentrate	1:15	18.90	18.91	96.37	0.68	3.63	16.27	86.11	2.38	12.64
Post UF waste	1:15	1.91	1.39	72.89	0.51	27.11	0.55	29.19	–	–
Post UF drying prep	1:15	93.90	85.25	90.78	8.65	9.21	74.23	79.04	8.60	9.16
Thermal coagulate[b]	–	6.03	5.65	93.66	0.38	6.34	4.69	77.70	0.46	9.16
Post coagulate waste		2.42	1.79	73.90	0.63	16.10	0.85	35.38	–	–

[a] UF-ultrafiltration
[b] 95°C; pH 4.5, 15 min

Table 2—Chemical composition of preparations after protein coagulation with Magnoflocd—dose of 0.15 g/dm^3

Kind of sample	Total solids (%)	Organic substances (%)	Organic substances (%)$^{t.s.}$	Mineral substances (%)	Mineral substances (%)$^{t.s.}$	Nitrogen substances N_{total} x 6.25 (%)	Nitrogen substances N_{total} x 6.25 (%)$^{t.s.}$	Nitrogen substances $N_{prec.}$ in 12% TCA (%)	Nitrogen substances $N_{prec.}$ in 12% TCA (%)$^{t.s.}$	Reducing sugars (%)	Starch (%)
Potato juice	5.64	4.83	85.6	0.81	14.20	1.64	29.00	0.164	2.90	5.6	–
Coagulate	8.20	6.16	75.1	2.04	24.90	5.38	65.50	0.480	5.86	1.9	6.20
Drying preparation	96.02	87.10	90.7	22.82	23.70	56.20	58.62	5.230	5.42	1.2	26.37
Post-coagulate waste	2.42	1.92	79.33	0.50	20.70	0,86	35.37	–	–	0.9	1.50

concentrates prior to drying displayed larger oil emulsifying capacities. Heating the emulsion for 30 min at 80°C did not lead to phase breaking. The results indicate the possibilities of utilizing potato protein preparations as natural emulsifiers of hydrophobic substances in the food processing industry. More detailed data concerning the emulsifying properties of concentrated and dried protein preparations were presented previously (Wojnowska et al., 1979). The emulsification values obtained correspond with results of other authors using undenatured potato protein concentrates (Holm and Eriksson, 1980).

The foaming properties of these preparations should also be mentioned. Probably the ability to form stable foam depends upon saponin concentrations. The preparations after UF concentration to 1:10 and 1:15 exhibited, after 10 min of foaming, a 1000 and 1100% foam volume increase, while the dried preparations showed 650 and 525%, respectively. The preparation after juice ultrafiltration to 1:5 gave a foam volume increase of 650% and dried concentrates presented similar properties (Table 5).

The whipping capacity of the Magnoflocd-coagulated preparation and of that concentrated by cryoconcentration depended upon their concentrations. The dried Magnoflocd-coagulated preparation dissolved in water at a level equal to 5, 10, 12.5, and 15% gave 150, 175, 200, and 225% foam volume; dried post-cryoconcentrated preparation yielded 200, 280, 320, and 400% foam volume, respectively. The post-cryoconcentrated preparation, however, formed foam of less stability (Table 5). The foaming properties are similar to those reported earlier (Knorr, 1980).

Water absorption capacity (wettability) is no less important. At 83% relative humidity and 20°C the dried preparations presented the following wettabilities: after UF, at a concentration of 1:5–0.39 cm^3 water/g; at 1:10–0.37; at 1:15–0.34; after cryoconcentration–0.25; after Magnoflocd-coagulation–0.4; and after thermal coagulation–0.17 cm^3 water/g (Wojnowska et al., 1979).

The heating of concentrates to 100°C for a period of 15 min to inactivate anti-nutritional substances and their subsequent drying led to a lowering of the wettability of the preparations to 0.20 cm^3 water/g, except for the preparation obtained by polyelectrolyte coagulation, whose wettability remained at the same level.

Strolle et al. (1980) observed that the application of a more drastic temperature of drying and the addition of $Ca(OH)_2$ to the concentrates before drying, to a 5% concentration in the dried preparation, reduced their wettability.

Essential differences were observed in the swelling capacity of individual preparations (Fig. 2). The dried preparations, after UF-concentration to 1:10 and 1:15, showed swelling capacity of 0.55 cm^3 water/g and 0.49 cm^3 water/g, respectively, but the preparation after UF-concentration to 1:5 was much lower–0.33 cm^3 water/g. Also, preparations after UF-concentration to 1:15 and 1:10 swelled more intensively during the first 10 min, while in

Table 3—Chemical composition of preparation after three-stage cryoconcentration

Kind of sample	Total solids (%)	Organic substances (%)	Organic substances (%)t.s.	Mineral substances (%)	Mineral substances (%)t.s.	Nitrogen substances N_{total} x 6.25 (%)	N_{total} x 6.25 (%)t.s.	$N_{prec.}$ in 12% TCA (%)	$N_{prec.}$ in 12% TCA (%)t.s.	Reducing sugars (%)	Starch (%)
Potato juice	5.64	4.83	85.6	0.81	14.20	1.64	29.00	0.164	2.90	5.60	–
Preparations:											
before drying	11.50	8.90	77.70	2.61	22.30	5.19	45.10	0.420	3.65	1.40	2.95
after drying	82.31	62.85	76.10	19.46	23.60	35.36	41.73	3.27	3.95	1.20	27.0
Post-cryoconc waste	4.08	32.40	79.62	0.80	20.50	2.28	55.9	2.03	5.03	1.80	–

Table 4—Emulsifying properties of potato protein preparation

Preparation	Emulsifying activity[a]	Emulsion stability[b]	Emulsifying capacity % emulsified oil phase	Emulsifying capacity V_{100}[c]
UF-concentration (1:5)	79.06	82.04	66.44	8.31
Dried product	69.94	95.08	76.47	139.42
UF-concentration (1:10)	45.14	44.70	78.23	3.99
Dried product	44.54	48.20	81.09	147.88
UF-concentration (1:15)	44.74	44.74	68.55	8.33
Dried product	40.02	92.48	75.00	81.30
Preparation after protein coagulation with Magnoflocd				
before drying	98.00	99.80	52.50	3.20
dried product	98.43	99.67	72.80	120.00
Post-cryoconc prep				
before drying	93.50	95.20	52.70	4.02
dried product	87.60	92.00	28.20	132.00

[a] Emulsifying activity = $\frac{\text{Volume of emulsified layer}}{\text{Volume of whole}} \times 100$

[b] Emulsion stability = $\frac{\text{Volume of emulsified layer}}{\text{Volume of whole}} \times 100$ after heating at 80°C/30 min.

[c] V_{100}—the amount of oil emulsified by 100 mg protein

Table 5—Whipping properties of potato protein preparations

Preparation		Whipping capacity % volume increase	A[a]
UF-concentration (1:5)		650	8.50
Dried product		650	7.94
UF-concentration (1:10)		1000	10.80
Dried product		650	8.14
UF-concentration (1:15)		1100	13.43
Dried product		525	25.18
Dried preparation after protein coagulation with Magnoflocd			
	5.0	150	15.36
Level in H_2O	10.0	175	17.92
(%)	12.5	200	15.97
	15.00	225	15.27
Dried post-cryoconc prep			
	5.00	200	12.3
Level in H_2O	10.0	280	14.2
(%)	12.5	320	15.3
	15.0	400	17.2

[a] A—Ratio of vlume after whipping in cm^3, to the whipped preparation weight after whipping in (g).

case of UF-concentration to 1:5 swelling occured between 25–30 min (Fig. 2). A similar swelling capacity and kinetics was shown by the polyelectrolyte-concentrated preparation–0.35 cm^3 water/g. The lowest results were obtained with the dried post-cryoconcentration preparation–0.25 cm^3 water/g, and measurable swelling capacity was observed within the first 15 min while during the next 15 min swelling was slight–0.03 cm^3 water/g.

The buffer capacity of dried preparations was different and depended upon the degree of concentration of potato juice (Fig. 3). The lowest values were observed in dried preparations after UF. Certain differences were also observed between buffer capacities of concentrates and dried preparations obtained from them.

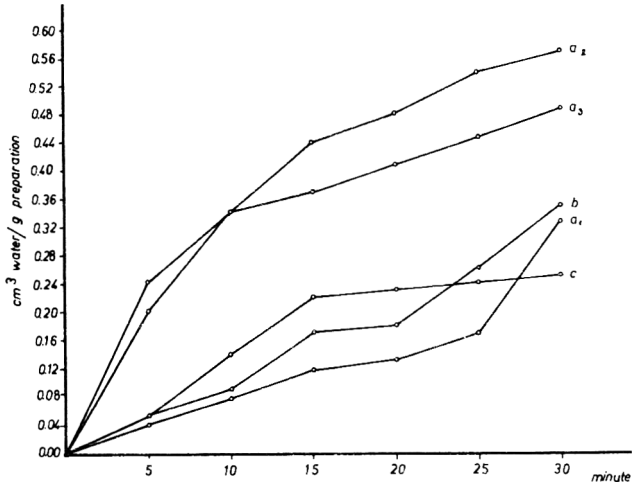


Fig. 2–Differences in the swelling capacity of individual preparations: UF-concentrated dried preparation of the concentration level –(a_1) 1:5; (a_2) 1:10; (a_3) 1:15; (b) dried polyelectrolyte-concentrated preparation; (c) dried cryoconcentration preparation.

The chromatographic separation of nitrogen substances on gel Sephadex G-100 shows that the fraction of nitrogen substances of the lowest molecular weight was considerably reduced in concentrates and dried preparations in comparison to the potato juice (Table 6). The results indicate that the applied methods of concentration did not ensure full recovery of the nitrogen fraction.

A detailed analysis of the data shows that, seemingly, during the concentration of potato juice there is an interaction of individual protein fractions and especially of molecular weight from 50 x 10^3 to 90 x 10^3 (Table 6). The drying of the concentrates probably leads to thermal degradation of proteins of high molecular weight of about 200 x 10^3. Some support of the observed changes in protein fractions of the concentrates and preparations is given by the hysteresis results, expressed by α-coefficeints calculated from the titration curves of 0.5% protein solution in the range pH 3–10 (Wojnowska et al., 1979). It is possible that the reduction of cation and anion concentrations in the concentrates is a results of the changes in the side chains of proteins and hence an increase in ionogenic groups and a rise in the value of the α-coefficient.

An index of changes in the proteins of dried preparations might also be their solubility (NSI) which depends both on the method of concentration of potato juice as well as on its degree of concentration. The NSI of dried preparation of UF concentrated to 1:5 is equal to 53.6%; 1:10, 72.4%; 1:15, 24.5%; after Magnoflocd polyelectrolyte coagulation, 55.8%; and after cryoconcentration, 60.2% (Wojnowska et al., 1979).

Table 6–Chromatographic separation of nitrogen substances on gel Sephadex G-100

Potato juice	a	200		175.8					79.5		63.1		52.4	9.4		12.3		
	b		2.8		7.6					12.0		5.2		9.4			63.0	100[c]
Proteins after UF																		
conc 1:5	a	200							79.4				56.2			11.8		
	b		12.5							13.8				38.0			36.8	101.1
conc 1:10	a	190				94.4							50.8		25.6	9.5		
	b		5.2				15.3							41.2	24.5		13.8	100.0
conc 1:15	a			178.2							61.6		51.8			8.9		
	b				39.9							7.0		20.3			38.7	95.9
Dried prep after UF																		
conc 1:5	a								70.8		59.6					7.1		
	b									22.3		38.2					39.5	100.0
conc 1:10	a			180.8					80.9				40.5			9.5		
	b				10.1					12.3				35.3			42.3	100.1
conc 1:15	a								79.7		59.5					12.6		
	b									14.6		44.0					41.1	100.0
Proteins after polyelectrolyte coagulation																		
coagulum	a	190.5				144.5			72.4							13.1		
	b		14.9				19.6			28.7							36.8	100.0
dried preparation	a			178.0					75.4				40.9					
	b				27.8					32.2				45.6				105.0
Proteins after cryoconcentration																		
concentrate	a			172.8				89.1					53.7		38.8	15.8		
	b				19.4				14.8					24.1	16.3		27.2	101.8
dried conc	a			180.0				89.1		75.8			43.6			10.4		
	b				20.6				15.2	26.5				4.8			32.8	

[a] Molecular weight of peak proteins x 10^3
[b] Nitrogen content at peak in percent
[c] Percentage recovery of nitrogen on the column

These data suggest considerable changes in the protein structure.

A certain dependency was observed between the oil emulsifying capacity and the solubility of dried preparations.

Substances lowering the nutritional value of potato proteins

A specific group of potato proteins are inhibitors of proteolytic enzymes. In potato juice there are also glycoalkaloids and their aglycones, cyclic aminoalcohols of steroidal structure. Three basic groups of glycoalkaloids were distinguished: solaidin, solasodin, and veratramin. The toxicity of glycoalkaloids occurring in potatoes is still doubtful (Sujkowski and Skrzeczkowski, 1977); therefore, one should take into account that during potato juice concentration a simultaneous concentration of both proteolytic enzymes and glycoalkaloids occurs. Obviously in the concentrated potato juice there was an increase in the concentration of these substances.

The lowest values of trypsin inhibitors and glycoalkaloids in the concentrate were obtained by thermal coagulation of potato juice (Table 2). In the dried preparations, no matter what the method of concentration used, no significant differences were observed in the glycoalkaloids content but there were considerable differences in the trypsin inhibitor contents (Table 7).

The method proposed by Omosiye and Cheryan (1979) for removing trypsin inhibitors from soya protein concen-

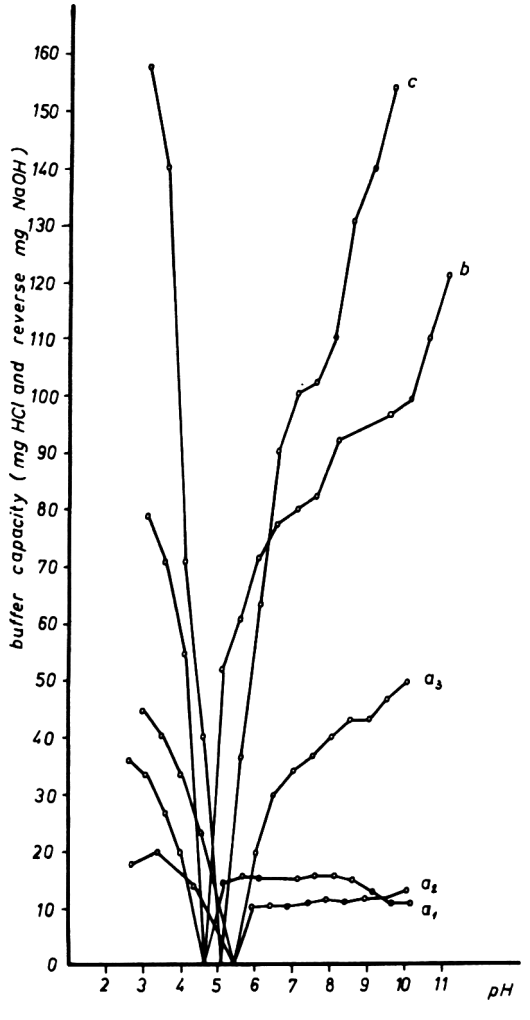


Fig. 3—Buffering capacity of protein preparations obtained: dried product after UF-concentration degree: (a_1) 1:5; (a_2) 1:10; (a_3) 1:15; (b) dried polyelectrolyte-concentrated preparation; (c) dried cryoconcentration preparation.

Table 7—Substances limiting the biological value of potato proteins

Method of obtaining preparations	Glycoalcoloids mg/100g preparate	Proteolytic enzyme inhibitors TUI[a] mg protein
Ultrafiltration	360.00	589.80
Cryoconcentration	369.70	1521.00
Polyelectrolitic	386.70	1461.00

[a] Trypsin Unit Inhibitors (TUI)

Table 8—Activity of proteolytic enzyme inhibitors and glycoalkoloid content in protein preparations heated to 100° C for 15 min

Method of obtaining preparates	After heating: Glycoalkaloid content (mg/100g preparate)	After heating: Activity of protease inhibitors TUI[a] mg protein	D[b]: Glycoalkaloids	D[b]: Protease inhibitors
Ultrafiltration	47.20	177.18	80.9	47.05
Cryoconcentration	58.20	863.75	83.8	43.22
Polyelectrolytic coagulation	43.30	758.60	88.8	48.08

[a] Trypsin Unit Inhibitors (TUI)
[b] D—Percentage decrease in content of anti-nutritional substances after heating at 100° C for 15 min in relation to the content of the following in concentrated preparates

Table 9—Amino acid composition of potato protein preparations before and after heating in 100° C for 15 min

Amino acids g/16g N	Potato juice	Method of protein recovery: Ultrafiltration A[a]	Ultrafiltration B[b]	Cryoconc A	Cryoconc B	Polyelectrolytic coagulation A	Polyelectrolytic coagulation B
Phenylalanine	3.96	5.80	4.64	5.72	4.02	5.59	4.08
Tyrosine	4.44	4.86	3.80	4.68	3.88	4.92	3.49
Leucine	5.50	8.70	5.60	7.79	5.88	7.04	5.58
Isoleucine	5.89	5.95	4.28	6.30	3.93	6.12	3.74
Methionine	1.71	2.93	1.20	2.20	0.96	2.29	1.00
Valine	6.59	6.93	4.20	6.70	3.18	6.24	5.19
Cystine	1.29	1.60	1.18	1.90	1.29	1.26	0.96
Alanine	4.35	4.72	3.52	4.01	3.11	4.19	3.19
Glycine	4.52	5.59	5.01	4.64	3.28	3.69	3.27
Proline	3.95	4.65	3.98	4.80	3.26	3.84	4.12
Glutamic acid	12.80	11.90	9.28	10.56	8.76	10.24	7.46
Aspartic acid	12.20	11.74	10.28	10.00	10.58	12.00	10.05
Arginine	5.29	5.81	4.20	5.58	4.75	5.12	4.57
Serine	4.52	4.75	3.28	5.26	1.81	5.45	2.20
Histidine	1.76	2.14	1.28	2.02	1.59	1.79	1.46
Treonine	3.84	5.62	3.57	5.02	2.28	5.26	3.32
Lysine	5.28	7.04	4.50	6.40	4.23	6.52	5.39
Total essential amino acids	40.22	51.57	35.25	47.73	29.43	47.21	34.21
CS/met. + cys.	52.63	79.40	44.0	71.92	41.00	62.20	34.00
EAAI[c]	79.00	98.30	60.10	95.00	48.00	91.40	64.21
NPU[d]	–	–	48.20	–	30.30	–	52.80

[a] A—preparation before heating
[b] B—preparation after heating
[c] Essential amino acids index
[d] Net protein utilization

trates by ultrafiltration seemed to be inefficient in the case of potato proteins. The potato protein preparations (without thermal processing) used as nutritional components (10% of proteins in the diet) caused death of rats during the eighth to tenth day of the nutritional experiment.

The low content of trypsin inhibitors in the thermal coagulated preparation encouraged us to heat individual concentrates at 100°C for a period of 15 min and then dry them. The thermal process led to a 43–48% reduction of the anti-proteolytic activity of potato protein preparations, and a 81–89% reduction in glycoalkaloids (Table 8).

It was observed that the thermal treatment led to considerable changes in the amino-acid composition of proteins, and also had an adverse effect upon the physicochemical properties of the dried preparations (Wojnowska, 1979).

The amino acids in the concentrates were reduced by 31, 38 and 28%, the chemical score from 43–45%, and EAAI by 39, 49 and 30% as a result of thermal processing. The potato concentrate after cryoconcentration proved to be the least resistant to heat, while the most resistant was the preparation after polyelectrolyte-coagulation.

Although thermal inactivation of anti-nutritional substances made it possible to carry out the nutritional experiment, the NPU values seem to be relatively low–48.2, 30.3, 52.8%; the differences reflected the level of amino acid degradation (Table 9).

From the results presented it seems that the utilization of protein concentrates from potato juice requires, apart from highly efficient methods of protein recovery, the development of an effective method of inactivation of anti-nutritional substances. The high content of essential amino acids in the preparation proteins, 51.57 g/16g N after UF, as well as 47.21 g/16g N after polyelectrolyte coagulation, and 47.73 g/16g N after cryoconcentration, prove the need of the utilization of these valuable proteins (Table 9).

Special attention must be paid to the determination of the "concentration limit" of anti-nutritional substances which limit the biological value of proteins in concentrates or dried preparations.

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Two Distinct Pectin Fractions from Senescent Idared Apples Extracted Using Nondegradative Methods

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ABSTRACT

After removal of water-soluble pectin from senescent Idared apples, an additional sizable pectin fraction could be extracted using Na_2EDTA under nondegradative conditions. This chelator-soluble fraction did not appear to owe its insolubility in the cell wall solely to molecular charge. The galacturonic acid-rich polymers of chelator-soluble pectin were notably lower in arabinose galactose and rhamnose residues than those of water-soluble pectin.

INTRODUCTION

MAJOR CHANGES occur in the pectic substances of apple cell walls during fruit senescence. Galactose residues are lost (Bartley, 1974; Tavoki and Wiley, 1968) and the amount of water extractable pectin increases (Doesburg, 1957; Knee, 1971).

This paper describes an additional sizable pectin fraction extractible after water-soluble pectin has been removed, using a chelator of divalent ions, Na_2EDTA. Like water-soluble pectin, this chelator-solubilized pectin is extracted without breaking covalent bonds. This specific fraction was observed by Joslyn and Deuel (1963), but regarded as insignificant and not studied further. Less specifically, pectic materials have been released from apples where the normal cell wall interactions have been interfered with by agents believed to bind calcium or by esterifiying the carboxyl groups of polyuronides (Knee, 1978; Doesburg, 1957).

MATERIALS & METHODS

Plant material

Apples (cultivar Idared) grown at Geneva, NY were stored in air at 0°C. After different storage periods, acetone insoluble solids (AIS) were prepared as described by Knee (1973) using 80% acetone. The AIS were refluxed for 10 min at 65–70°C in 80% acetone to inactivate endogenous enzymes (O'Beirne, 1980) and further washed with 80% acetone (500 ml per 125g fresh weight).

Extraction of pectic substances

The extractibility of pectic substances from AIS in a number of media was investigated promptly after enzyme inactivation. For a neutral aqueous medium, a 0.1M Tris solution adjusted to pH 7 with M HCl was used. To examine the effects of chelating divalent ions, a 0.1M Tris, 0.1M disodium ethylenediamine tetraacetic acid (Na_2EDTA) solution adjusted to pH 7 with M HCl was used. The extracting solutions were added to the AIS until the volume, in ml, was 2X the numerical value for fruit fresh weight (grams). The resulting suspensions were magnetically stirred for appropriate periods of time at 1°C.

After extraction, solids were separated by filtration using Whatman 541 filter paper with suction. The filtrate was centrifuged (27,000x *g*) to remove cell wall debris, then 4 vol of acetone at 1°C were added to precipitate the extracted pectic substances. The pectin was harvested by centrifugation (27,000x *g*). Where present Na_2EDTA was removed from filtrates by dialysis (Spectrapor 1) for 72 hr at 1°C against distilled water prior to precipitation of extracted pectin. The centrifuged pectic material was dissolved in 0.01M phosphate/0.001M Na_2EDTA to give about 500 μg/ml and precipitated again with 4 vol acetone. (The phosphate solutions used here and elsewhere were made up of equimolar amounts of KH_2PO_4 and Na_2HPO_4.) Finally, this centrifuged material was re-dissolved in 0.01M phosphate/0.001M Na_2EDTA. This material was either used immediately for further work or stored at −40°C.

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Ion exchange chromatography

DEAE Sephacel (Pharmacia Inc.) was used to separate ionizable pectic substances from those with little or no charge (Knee, 1970).

About 40 ml of preswollen DEAE Sephacel were equilibrated as directed by the manufacturer to provide a column 2 cm^2 x 11 cm. Samples for application to the column contained about 7 mg galacturonic acid residues and about 1 mg covalently bound residues of other sugars dissolved in 12–14 ml of buffer containing 0.01M phosphate, 0.001M Na_2EDTA, pH 6.9. Na_2EDTA, a chelator of divalent ions, was included in all buffers used in column chromatography to preclude possible cation-mediated interactions between pectic substances and chromatographic media (Knee, 1970). Material which did not bind to the DEAE Sephacel was eluted using the buffer solution. This was followed by a phosphate gradient formed by addition of a solution containing 1.0M phosphate, 0.001M Na_2EDTA to a solution of 0.01M phosphate and 0.001M Na_2EDTA. Operating pressure (gravity) was 25–30 cm H_2O; flow about 18 ml/hr.

For large scale preparation of pectic substances for gas chromatographic analysis of neutral sugars covalently bound to charged pectic substances, a column bed about 11 cm x 5.3 cm^2 was used. Samples containing 50–70 mg galacturonic acid residues in about 100 ml buffer were applied. 1,1,1-Trichloro-2-methyl-2-propanol (0.05%) was added to the buffers used between runs and the packing material was regularly replaced; 7.5 ml fractions were collected.

Colorimetric analyses

Galacturonic acid was quantified using the m-hydroxy diphenyl procedure of Blumenkrantz and Aboe-Hansen (1973). Total fruit pectin was determined by the method of McCready and McComb (1951); Klerzyme H.T. (G.B. Fermentations Industry) was used in place of pectinol 100D.

Nongalacturonic acid residues present were estimated in terms of glucose equivalents by the sulphonated alpha-napthol procedure of Barrett and Northcote (1965). A correction was made for the absorbance of any galacturonic acid present.

Gas chromatography (GC)

Neutral sugars covalently bound in pectic fractions were quantified after hydrolysis as alditol acetate derivatives by GC on cyanosilicone SP 2330 (Supelco Inc., Pa.) using inositol as an internal standard. The overall procedure is outlined in Fig. 1. The hydrolysis and reduction to alditols is essentially as described by Albersheim et al. (1967). Subsequent procedures are those of Supelco Inc. (Anon., 1977) with minor modifications. Without the ion exchange step, browning of the samples occurred during derivatization and recovery of the internal standard was poor.

The degree of esterification (DE) of pectin fractions with methanol was determined by measuring methanol by the gas chromatographic procedure of Lee et al. (1975) after alkaline hydrolysis.

Fruit firmness in kg was determined using a penetrometer with an 8 mm plunger (made by effe gi, Italy).

Fruit receiving a calcium chloride dip were immersed for 30 sec in a 4% $CaCl\ 2H_2O$ solution containing 0.125% Tween 20.

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RESULTS & DISCUSSION

ACETONE INSOLUBLE SOLIDS (AIS) of Idared apples were suspended in a succession of neutral aqueous extraction media, followed by neutral aqueous media containing Na_2EDTA. A distinct and sizable chelator-soluble pectin fraction was extractible after water-soluble pectin was removed (Table 1). This contrasts with the observations of Joslyn and Deuel (1963). However, these workers reported difficulties with their titrimetric estimation of pectin due to co-precipitation of EDTA.

A significant chelator-soluble pectin fraction was found to be extractible from stored apples in two seasons (Tables 2, 3). In the 1978 crop the magnitude of the water-soluble pectin and chelator-soluble pectin fractions remained relatively constant over the storage period sampled (4.5–11 months). Fig. 2 shows that fruit texture was relatively stable at this time also. In the 1979 crop (Table 3) samples were examined at an earlier stage of post-harvest physiology. Both water-soluble pectin and chelator-soluble pectin increased in magnitude, and fruit firmness decreased. Such an increasing level of chelator-soluble pectin has been reported by Massey et al. (1964).

Water-soluble pectin and chelator-soluble pectin were subjected to ion exchange chromatography on DEAE Sephacel (Fig. 3, 4). Water-soluble pectin consisted of an unbound, neutral sugar-rich fraction and a bound, galacturonic acid-rich fraction (Fig. 3), similar to the findings of Knee (1970). Chelator-soluble pectin, examined here for the first time (Fig. 4), consisted almost entirely of a bound galacturonic acid rich fraction. Colorimetric analyses indicated that about twice as much neutral sugar was associated with the bound polymers of water-soluble pectin as with the bound polymers of chelator-soluble pectin (Fig. 3, 4). With the same gradient, water-soluble pectin had a higher neutral sugar/galacturonic acid ratio throughout elution than the chelator-soluble pectin (Fig. 3, 4).

The amounts of specific neutral sugars found in DEAE cellulose-bound polymers of chelator-soluble pectin and water-soluble pectin were quantified (Table 4). As found in the colorimetric procedures, chelator-soluble pectin at both 5.5 and 11 months storage had about half the mole percent of neutral sugar found in water-soluble pectin. Chelator-soluble pectin contained less than one mole percent of each sugar except arabinose. The level of rhamnose in chelator-soluble pectin was half that of water-soluble pectin. Since rhamnose residues severely kink the normally ribbon-like helical conformation of polygalacturonic acid (Rees and Wight, 1971), this low rhamnose content suggests that molecules of chelator-soluble pectin may have a more linear conformation than those of water-soluble pectin. The existence of intact high MW rhamnogalacturonan chains with such low levels of rhamnose has not been reported in apples previously. Levels similar to, or lower than, those in chelator-soluble pectin have been reported only for fragments of pectic material separated after degradative treatments (Barrett and Northcote, 1965; Knee et al., 1975; Neukom et al., 1980).

In comparison with chelator-soluble pectin, water-soluble pectin is richer in those neutral sugar residues (arabinose, galactose) which normally make up pectin sidechains. The mole percent neutral sugar (13.9) is similar to that found in

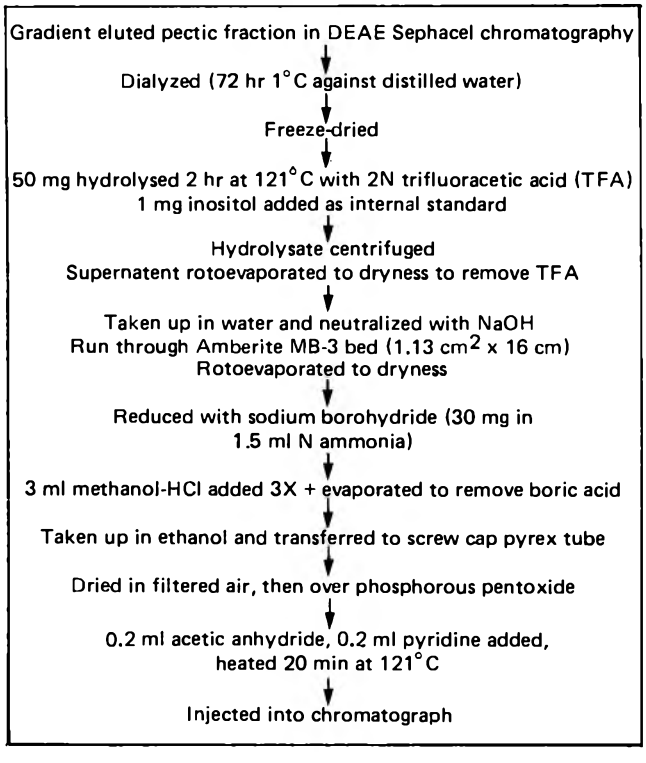


Fig. 1—Steps in hydrolyzing and derivatizing neutral sugars from pectic fractions for gas chromatographic analysis.

Table 1—Extractibility of pectic substances from AIS of Idared cortical tissue after 11 months storage

Sequence of extracting media	Time (hr)	μg galacturonic acid/g FW[a] (mean of 2 extractions)
neutral aqueous[b]	2	615
neutral aqueous	24	315
neutral aqueous	2	59
neutral aqueous	2	49
neutral chelating[c]	2	510
neutral chelating	24	77
neutral chelating	2	15
neutral chelating	2	10

[a] FW here and elsewhere denotes 'fresh weight'
[b] Neutral aqueous solution = 0.1M Trls-HCl pH7
[c] Neutral chelating solution = 0.1M Trls-0.1M Na_2 EDTA-HCl pH7

Table 2—Amounts of pectic substances extracted from Idared apples stored 4.5 – 11 months at 0° C (1978 season)

Storage period (months)	μg galacturonic acid/g FW[a]		
	Chelator soluble[b]	Water soluble[b]	Total
4.5	550±30	–	–
5.5	630	1200	–
6	650±25	825±40	6000±10
11	510±10	990±70	6500±0

[a] Values with a range represent means of two extractions.
[b] Based on data in Table 1 the extraction procedure was shortened to a 2 plus 24 hr extraction for water-soluble pectin followed by a 2 plus 24 hr extraction for chelator-soluble pectin.

Table 3—Amounts of pectic substances extracted from Idared apples stored 0.5, 1 and 5 months at 0° C and from those stored 5 months at 0° C after a calcium chloride dip (1979 season)

Storage period (months)	μg galacturonic acid/g FW[a]		Fruit firmness[b] (kg)
	Chelator soluble[c]	Water soluble[c]	
0.5	213± 7	102± 1	6.66±0.42
1	280±35	103±17	
5 undipped	570±20	335± 5	5.29±0.36
5 dipped	480± 0	265± 5	5.10±0.47

[a] Mean of two extractions with range
[b] Mean of 20 apples at room temperature
[c] These fractions are described in footnote b, Table 2.

the charged material of the water-soluble pectin fractions of Barrett and Northcote (1965), (13) and Knee (1978), (17.8).

Chelator-soluble pectin was solubilized in the presence of Na_2EDTA, suggesting that divalent cations such as calcium, may have been involved in insolubilizing these molecules. If this were so, a difference in molecular charge between water-soluble pectin and chelator-soluble pectin might account for the differences in their solubility. At 6 months storage, water-soluble pectin had a higher DE than chelator-soluble pectin but at 11 months storage both fractions had similar values (Table 5). Since pectic substances of DE 50–60 are readily precipitated by calcium in vitro (Doesburg, 1965), $CaCl_2$ was added to the neutral aqueous extraction media at 11 months storage (Table 6). Much less water-soluble pectin was solubilized and the chelator-soluble fraction became correspondingly greater. The data in Tables 5 and 6 suggest that the differences in solubility between the two fractions has no simple relationship to differences in molecular charge.

To examine the effects of exogenously applied calcium, a sample of apples from the 1979 crop was dipped in $CaCl_2$. At 5 months storage there was no difference in fruit firmness between control and treated apples but both water-soluble pectin and chelator-soluble pectin was extracted in lower amounts in treated apples (Table 3). There was no evidence that chelator-soluble pectin was more affected than water-soluble pectin. It is interesting, however, that the levels of both fractions were lower, since Doesburg (1965) has hypothesized that calcium tends to facilitate other processes of pectin insolubilization in the cell wall.

This paper describes diversity in the pectic substances of apple cell walls by (1) demonstrating two separate fractions on the basis of mode of extraction and (2) showing them to be different structurally. Neither molecular charge of the pectin molecule or the effects of exogenously applied calcium could be directly related to the diversity observed. Neukom et al. (1980) have made a similar suggestion based on observations on apple pectin fragments released after a degradative procedure. Such diversity may be explained by the presence of more than one structural type of pectin molecule in the cell wall or by the existence of a heterogenous molecular structure.

Differences in the relative amounts and types of neutral sugar residues both in the rhamnogalacturonan mainchain and present as sidechains can reflect the functionality of pectin in the cell wall (Rees, 1969; Rees and Wight, 1969). The low levels of arabinose galactose and rhamnose in chelator-soluble pectin allow for interaction between neighboring pectin molecules over relatively long chainlengths. Such interaction would be required, for example, for the chainstacking interaction with calcium proposed by Rees (1972) and for other noncovalent interactions. As is pointed out by Darvill et al. (1980), noncovalent interactions in cell walls are particularly important for pectic substances

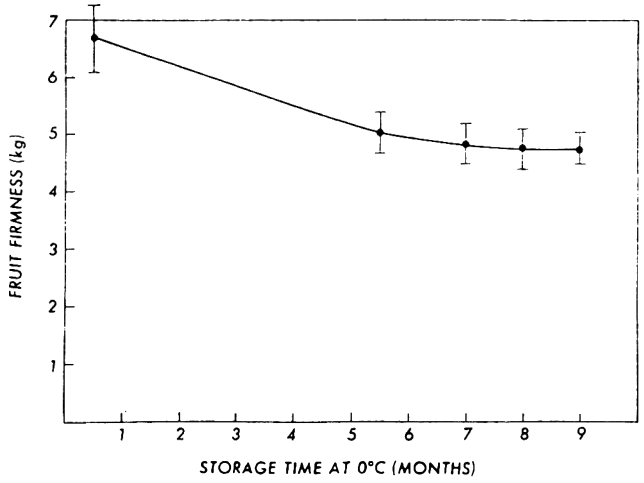


Fig. 2—Effect of storage time on firmness of Idared apples (1978 crop).

Table 4—Neutral sugars associated with the fraction of water-soluble pectin and chelator-soluble pectin which is eluted by a phosphate gradient from DEAE Sephacel

	Water-soluble pectin[a]	Chelator-soluble pectin[a]	
	5.5 Months storage[b]	5.5 Months storage[b]	11 Months storage[c]
	Moles/100 moles galacturonic acid		
β-L-Arabinose	9.4	4.4	4.2
β-D-Galactose	5.4	0.90	0.93
β-D-Glucose	0	0.15	0.34
β-L-Rhamnose	1.2	0.61	0.58
β-D-Xylose	0.14	0.19	0.33
Mole % neutral sugars	13.9	5.9	6.0

[a] These fractions are described in footnote b, Table 2.
[b] Values for 5.5 months storage based on a regular extraction (125g cortex tissue from 8—10 fruits)
[c] Values for 11 months storage based on a bulk extraction (375g cortex tissue from 10 fruits)

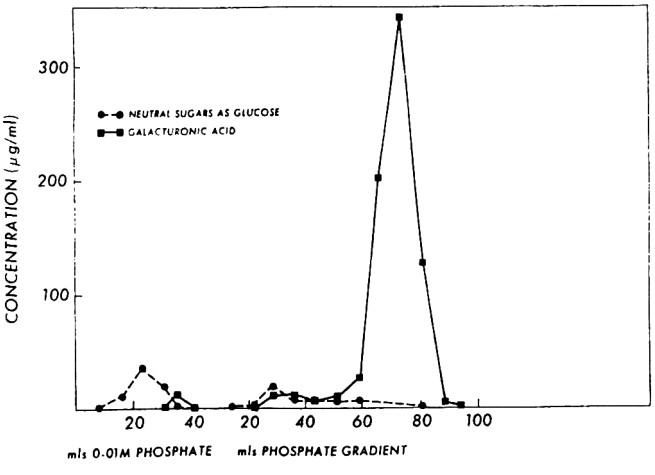


Fig. 3—Elution pattern of water-soluble pectin from Idared apples stored 11 months (DEAE Sephacel).

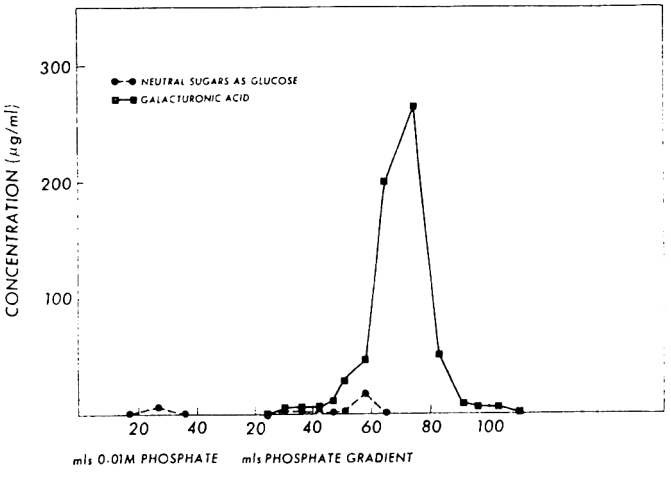


Fig. 4—Elution pattern of chelator-soluble pectin from Idared apples stored 11 months (DEAE Sephacel).

Table 5—Degree of esterification of water-soluble pectin and chelator-soluble pectin at 6 and 11 months

	6 months storage[a]	11 months storage[b]
	Moles methanol/100 moles galacturonic acid residues	
Water-soluble pectin[c]	54	15±4
Chelator-soluble pectin[c]	32	12±8

[a] Values for 6 months storage represent a single extraction (6—10 fruits used).
[b] Values for 11 months storage represent means of three separate extractions with standard deviations.
[c] Extraction sequence was as in Table 1; water-soluble pectin refers to material extracted in the first 2 hr extraction period while chelator-soluble pectin refers to material extracted in the first 2 hr extraction with Na_2EDTA.

Table 6—Effect of adding $CaCl_2$ to the neutral aqueous extraction media on the % distribution of extractible pectin between water-soluble pectin and chelator-soluble pectin (Idared apples stored 11 months)

	% Extractible pectin		
	Regular extraction	with $CaCl_2$[a]	Change
Water-soluble pectin[b]	65	41	−24
Chelator-soluble pectin[b]	35	59	

[a] Neutral aqueous solution: 0.1M Tris, 0.1M $CaCl_2$ adjusted to pH 7 with M HCl. Data based on replicated extractions.
[b] These fractions are described in footnote b of Table 2.

and this type of interaction is not reflected in the cell wall model proposed by Keegstra et al. (1973). The characterization of a distinct chelator-soluble fraction may be a step towards understanding some noncovalent interactions in apple cell walls, and in cell walls generally.

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Rupture Properties of Wheat Gluten in Simple Tension: The Role of Hydrogen Bonds

ARTURO E. INDA and CHOKYUN RHA

ABSTRACT

Gluten obtained from hard red winter wheat flour was freeze-dried and reconstituted wth deuterium oxide and aqueous solutions of urea to selectively alter the extent of hydrogen bonding. The rupture properties of the modified glutens were studied in simple tension at seven rates of strain covering three orders of magnitude (2.86×10^{-2} to 28.6 min^{-1}). Tensile strength and rupture strain decreased steadily with the extent of hydrogen bonding disruption, i.e., with increase in urea concentration. For a given type of modified gluten, the strain at rupture was independent of the rate at which the material was deformed and the tensile strength increased with increasing deformation rate.

INTRODUCTION

THE UNIQUE rheological properties of wheat doughs are determined primarily by the properties of gluten, the complex mixture of proteins, lipids and water that is formed during the dough-making process.

Although relatively little is known in detail about the relation between the molecular structure and the physical properties of gluten, some general aspects of its behavior can be explained in terms of its amino acid composition, which is summarized in Table 1. For example, the limited hydration of gluten at or near neutrality can be attributed to its low content of ionizable amino acids, and to the relatively high concentration of nonpolar residues (Wall and Beckwith, 1969). Cysteine or cystine residues, although present in small amounts, are very important because they can form intramolecular and intermolecular disulfide bridges (Wall and Beckwith, 1969). The most distinctive feature of the gluten proteins, however, is their very high content of glutamine, about 35% of all the amino acid residues (Wu and Dimler, 1963). The polar aliphatic side chains of this amino acid can participate in hydrogen bonding through their amide group. Intermolecular, rather than intramolecular, hydrogen bonding is favored due to the relatively high content of proline, which hinders α-helix formation. Indeed, X-ray diffraction studies have shown that the only ordered structures in gluten are those of the phospholipids present (Traub et al., 1957; Grosskreutz, 1960).

Disulfide bonds, primarily in the glutenin fraction, have been widely studied in regard to their ability to form a covalent network which, in turn, is considered to determine to a large extent the viscoelastic properties of dough and gluten (Beckwith and Wall, 1966; Bietz et al., 1973; Bloksma, 1975). There is, however, evidence suggesting that the gluten proteins do not form a covalent network. For example, Stanley et al. (1968) were able to disassociate gluten proteins using a mixture of phenol-acetic acid-water, and Kobrehel and Bushuk (1977) solubilized glutenin completely in water using sodium salts of fatty acids; both studies were done under conditions where disulfide bonds are stable. Rheologically, gluten behaves like an uncrosslinked, high molecular weight polymer. In stress relaxation, for example, the stress decays to zero at about 10^4 sec (Bar-David and Lerchenthal, 1975). Since polymeric networks cannot be dissolved or dispersed without breaking covalent bonds (Flory, 1953) and since they sustain an equilibrium stress in a stress relaxation experiment (Ferry, 1980), the three studies mentioned above as examples show that the rheological properties of gluten cannot be accounted for primarily in terms of intermolecular disulfide bridges.

On the other hand, the effects of hydrogen bond disruption on the rheological properties of dough and gluten have also been recognized. Jankiewicz and Pomeranz (1965), using a Brabender Farinograph, observed a rapid decrease in the consistency of dough containing 3M urea solution. Doguchi and Hlynka (1967), also with a Farinograph, found that the consistency of gluten decreased linearly with increasing concentration of urea.

This has led to the conclusion that the presence of noncovalent bonds throughout the structure of gluten must play a key role in its properties. The premise is that the overall behavior is the result of the cooperative effect of both types of bonds (Kasarda et al., 1978). The object of this study is to examine the rupture properties of gluten, whose secondary bonding characteristics have been selectively modified, when it is subjected to simple tension in a three-orders-of-magnitude range of strain rates.

Rupture properties are particularly useful because they describe the ultimate capacity of a material to sustain imposed stresses or strains. The failure envelope, which is the line joining the rupture points observed under different test conditions, depends only on the structural characteristics of the material, (Smith, 1963) and, as such, provides an effective means to analyze the effect of hydrogen bonding modification on the rheological properties of wheat gluten.

MATERIALS & METHODS

Wheat flour and gluten

The wheat flour was provided by the U.S. Grain Marketing Research Laboratory, Manhattan, KS, and consisted of a blend of hard red winter wheat, mainly the Newton variety, grown within 50 miles of Manhattan, KS. Gluten was separated from flour according to AACC method 38-10 (AACC, 1969). *—Continued on next page*

Table 1—Summary of the amino acid composition of gluten[a]

Amino Acids	% of Total
Ionizable	≃ 6%
Polar	≃ 45%
Apolar	≃ 49%
GLUTAMINE	≃ 35%
PROLINE	≃ 15%
CYSTINE/2	≃ 2%

[a] Adapted from Wu and Dimler (1963)

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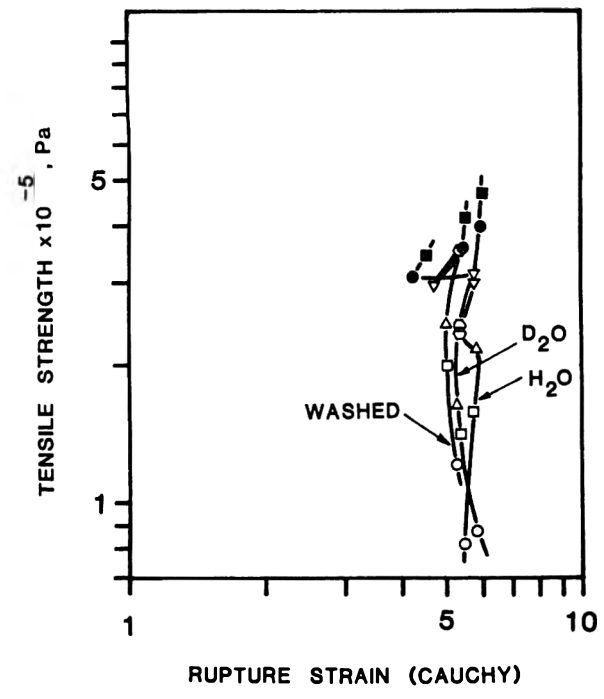


Fig. 1—Failure envelopes of washed gluten and freeze-dried gluten reconstituted with water and deuterium oxide. The rates of strain are, in min.$^{-1}$: — ○ — 2.86 x 10^{-2}; — □ — 1.43 x 10^{-1}; — △ — 2.86 x 10^{-1}; -◇- 1.43; — ▽ — 2.86; — ● — 14.3; — ■ — 28.6

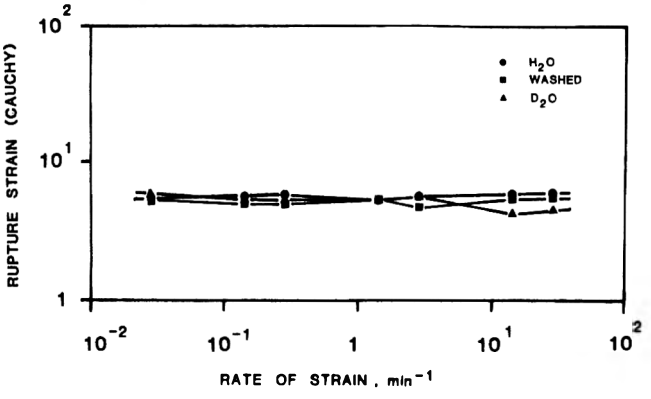


Fig. 2—Rate of strain dependency of the rupture strain for the same types of gluten shown in Fig. 1

Gluten moisture and protein determination

Moisture was determined by AACC method 44-40 (AACC, 1969). Nitrogen was determined by the micro Kjeldahl procedure, AOAC method 47.021 (AOAC, 1975). Protein was calculated multiplying Kjeldahl nitrogen by 5.7. The moisture and protein contents thus calculated were 57.2% and 35.6% (83.2% on dry basis), respectively.

Specimen preparation

Part of the gluten was frozen with liquid nitrogen and freeze-dried in a Virtis freeze-drier Mod. 10-MRTR (Virtis Research Equipment, Gardiner, NY). The dry material was ground in a Waring Blendor during 1 min and sieved through a U.S. Standard sieve No. 50. The resulting powder was kept at room temperature in a desiccator under nitrogen atmosphere before reconstitution. Gluten was reconstituted with water, deuterium oxide and aqueous urea solutions of various concentrations, maintaining the same water to protein ratio of the original gluten. After reconstitution, all gluten samples were shaped into slabs approximately 3.5 mm thick between 10 cm x 10 cm plexiglass plates lined with Teflon, and stored at -20°C. For rheological testing, gluten strips of 35 mm length were cut from the frozen slabs and allowed to thaw at room temperature during approximately 2 hr in a closed Petri dish lined with teflon. The dimensions of the strips were measured with a dial caliper. The cross sectional area of the specimens ranged from 10 mm^2 to 20 mm^2.

Rheological testing

The gluten strips were stretched to the point of rupture in an Instron Universal Testing Machine Mod. 1122 (Instron Corporation, Canton, MA) using an Instron tension load cell Mod. 2511-101 with capacity of 500g. The specimens were elongated at seven rates of strain covering a range of three orders of magnitude, from 2.86 x 10^{-2} min^{-1} to 28.6 min^{-1}. These are the rates of strain obtained when strips of 35 mm length are extended at crosshead speeds varying from 1 mm/min to 1000 mm/min. To avoid moisture losses during testing, the gluten specimens are surrounded by a flexible polyethylene cylinder of 6.5 cm. diameter whose inside surface was wetted before each run. The polyethylene cylinder was attached to the instrument so that its height increased along with the travel of the crosshead. All tests were conducted at 23 ± 1°C.

Data analysis

The reported values of tensile strength and rupture strain were calculated using the double exponential frequency distribution function (Kase, 1953), which is used to describe the rupture behavior of elastomeric materials (Kase, 1953; May, 1964). Each point on the failure envelopes is the mode, or most probable value, of this distribution function, obtained from eight experimental points using the method of Kase (1955). Previous unpublished results (Inda et al., 1979) showed that this number of replications was sufficient to describe well the rupture behavior of gluten using the double exponential distribution.

The ordinate in the failure envelopes is the true tensile strength, or force per unit cross sectional area of deformed material. The latter was calculated assuming the volume of the specimens remained constant. The abscissa is the Cauchy measure of strain, which describes the relative deformation of the material, also at the point of rupture.

RESULTS & DISCUSSION

DEUTERIUM OXIDE, which forms slightly stronger hydrogen bonds than water (Vinogradov and Linnell, 1971), has been reported to strengthen the structure of dough, gluten and bread. Kretovich and Vakar (1964) found that, in a creep experiment, gluten made with deuterium oxide had a lower tensile compliance than gluten made with water. Using an extensigraph, Tkachuk and Hlynka (1968) observed higher resistance to extension with deuterated gluten and lower loaf volume in bread prepared with deuterated dough. On the other hand, these same authors did not observe differences between both types of gluten when the resistance to mixing was measured using the farinograph.

Fig. 1 shows the failure envelopes obtained with washed gluten, i.e., gluten which was not freeze-dried, and with gluten reconstituted with water and deuterium oxide. A comparison of washed gluten, which is included as a control, and gluten reconstituted with water shows that freeze drying had only a minor effect. At four of the seven rates of strain, gluten reconstituted with deuterium oxide behaved similarly to gluten reconstituted with water. Deuterated gluten was less strong at 2.86 x 10^{-1} min^{-1}, and both less strong and less extensible at the two highest rates of strain. This suggests that the effect of deuterium oxide on the rheological properties of gluten is strain rate dependent. No direct comparison can be made, however, with the results obtained by Kretovich and Vakar (1964) and by Tkachuk and Hlynka (1968) due to differences in the materials and in the deformation patterns.

The unique feature of these results, as shown in Fig. 2 is that, although the strain rates cover three orders of magnitude, rupture strain is largely independent of strain rate. Most of the standard deviations of the distributions representing both rupture strain and tensile strength ranged from 10–20% of the values of the corresponding modes. For all three types of gluten, the modes of the distributions representing rupture strain fell within a very narrow range of values of strain, between 5 and 6. Similar behavior was observed by Bar-David and Lerchenthal (1975), with re-

constituted commercial vital gluten, in creep experiments. In that study, gluten ruptured at a Cauchy strain of about 4.5, independently of the value of the imposed constant stress. Rinde et al. (1970), however, found that the rupture strain of reconstituted commercial gluten increased from 1.4 to 3.5 when the rate of strain in simple tension increased from 1.3 x 10^{-1} min^{-1} to 12.6 min^{-1}. The moisture content of the gluten used in those experiments was 50%, or 7% lower than that of the gluten used in this study. Although it has been reported that the rheological properties of gluten are strongly dependent on moisture content (Cumming and Tung, 1975), there is no evidence to suggest that the shape of the rupture envelope obtained by Rinde et al. (1970) is related to the lower moisture content of the gluten used.

Tensile strength, on the other hand, increased steadily with rate of strain, as shown in Fig. 3. The same behavior was observed by Rinde et al. (1970) and by Bar-David and Lerchenthal (1975); this happens, as expected, because there is less time allowed for stress relaxation during the period of extension as the rate of strain increases.

Fig. 4 shows the failure envelopes of gluten reconstituted with urea aqueous solutions to examine the effect of increased disruption of hydrogen bonding on the tensile properties. Again, most standard deviations of the distributions representing both tensile strength and rupture strain ranged from 10% to 20% of the values of the corresponding modes. The failure envelope of gluten reconstituted with water is included as reference. Since complete disruption of hydrogen bonding in most proteins usually requires placing them in an excess amount of highly concentrated (8M–10M) urea solutions (Haschemeyer and Haschemeyer, 1973), it is evident that Fig. 4 shows the effects of partial disruption only. The rupture envelopes shift considerably toward lower values of tensile strength and rupture strain as the concentration of urea increases. For example, in the case of gluten reconstituted with 5M urea, both properties decreased by a factor of about 2.4 with respect to gluten reconstituted with water.

The effects of urea concentration and rate of strain on tensile strength and rupture strain are shown by separate for each property in Fig. 5 and 6.

Fig. 5 shows that rupture strain is independent of rate of strain, as in the case of gluten reconstituted with water and deuterium oxide, and that the effect of urea concentration can be described reasonably well (linear regression coefficient = - 0.95), in the range studied, by a straight line in double logarithmic coordinates. Fig. 6 shows that the strain rate dependency of tensile strength generally follows the same pattern observed in Fig. 3. No explanation was found for the atypical point obtained with gluten reconstituted with 5M urea and extended at the lowest strain rate.

When a viscoelastic solid is stretched at a constant rate of deformation, the stress sustained at any moment is the result of two competing processes: stretching of the high molecular weight chains, which increases the stress, and stress relaxation, which decreases it. The relative contribution of each process to the observed stress depends on the time scale of the experiment and on the viscoelastic characteristics of the material, but the stress always increases with increasing extension of the material. At some point, rupture begins with the formation or growth of a crack, which concentrates the stress, and continues with the propagation of the crack across the sample. Fig. 4 shows that these critical conditions in gluten are reached at lower values of stress and strain as the extent of hydrogen bonding disruption increases. Under these conditions, less hydrogen bonds are formed so that the polypeptide chains interact less with neighboring chains, at least through hydrogen bonding . Consequently, the tensile resistance of the material decreases because it depends now on a diminished number of interacting chains.

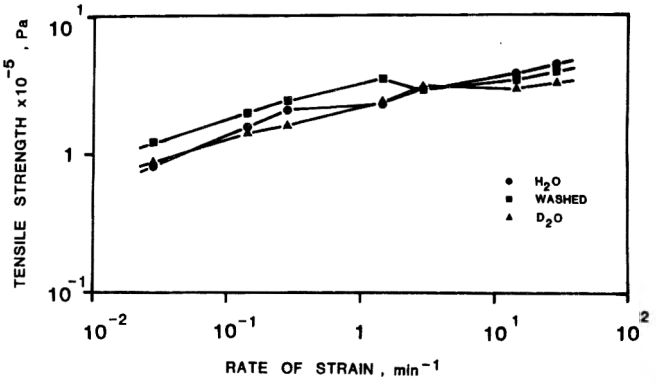


Fig. 3—Rate of strain dependency of the tensile strength for the same types of gluten shown in Fig. 1.

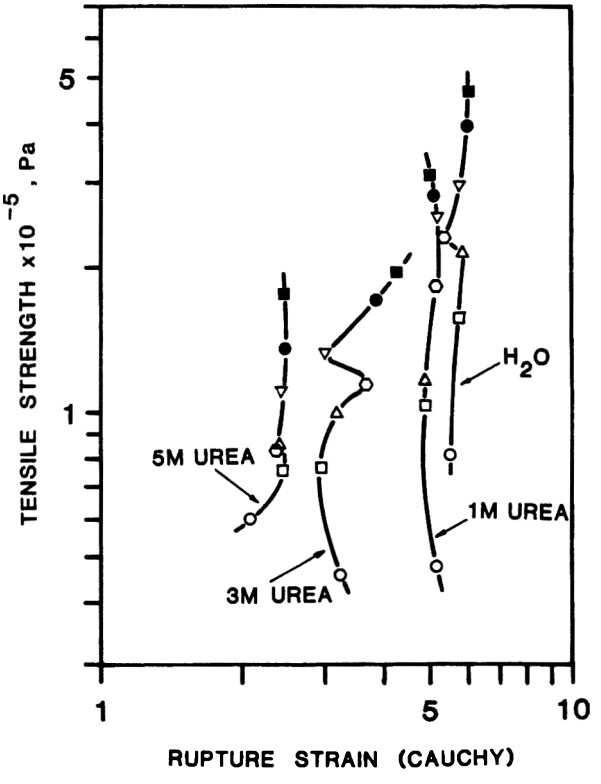


Fig 4—Failure envelopes of gluten reconstituted with water and with aqueous urea solutions. The symbols represent the same rates of strain described in Fig. 1.

The results reported in this study clearly show that even modest disruption of hydrogen bonding in gluten has considerable effects on its rheological properties. This confirms and extends the results obtained by Doguchi and Hylnka (1967) and by Jankiewicz and Pomeranz (1965), with a Brabender Farinograph, in providing further experimental evidence that hydrogen bonds play a key role in the structure and properties of wheat dough and gluten.

CONCLUSIONS

DEUTERIUM OXIDE did not affect the rupture properties of gluten under most of the conditions studied. At high rates of strain, deuterated gluten became less extensible and had lower tensile strength than gluten made with water. Partial disruption of hydrogen bonding in gluten affects significantly its rupture behavior. Rupture occurs at low values of stress and strain as the extent of disassociation increases, and this effect is related to the decrease in hydro-

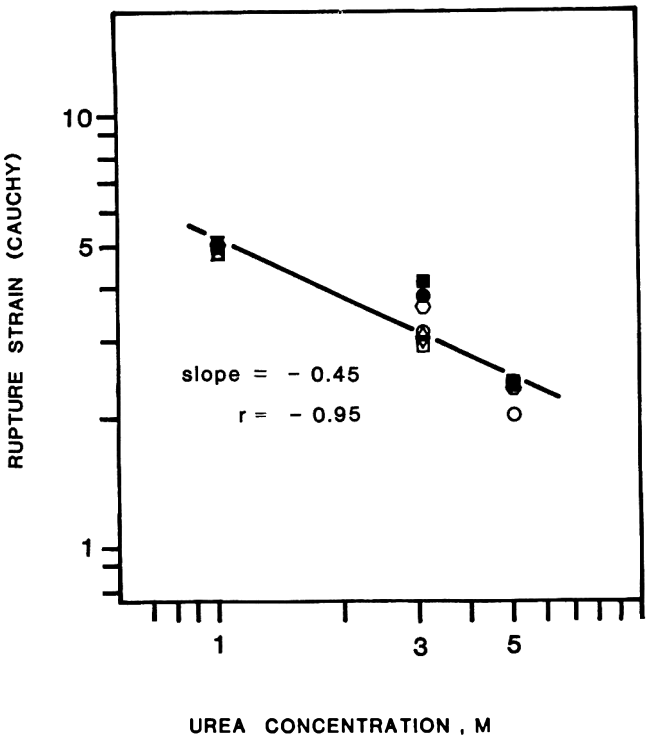


Fig. 5—Effects of urea concentration and rate of strain on the rupture strain of gluten. The symbols represent the same rates of strain described in Fig. 1

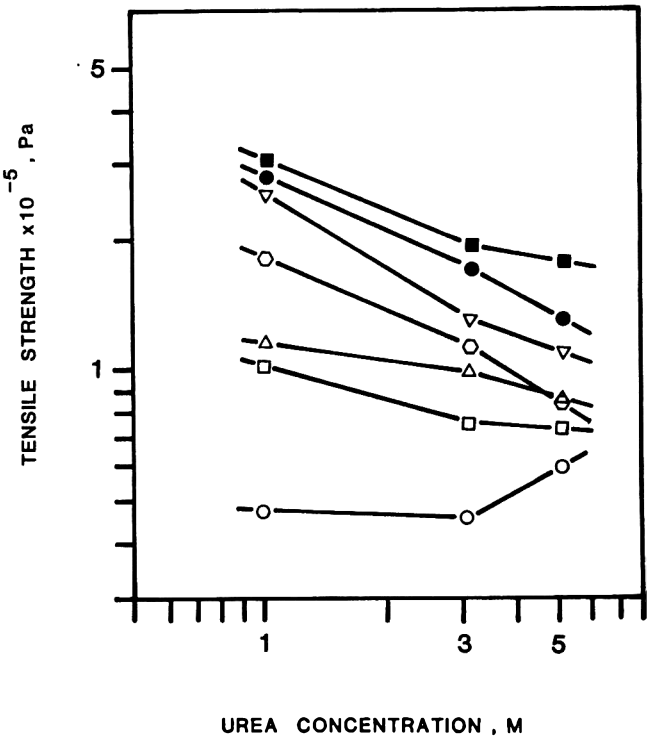


Fig. 6—Effects of urea concentration and rate of strain on the tensile strength of gluten. The symbols represent the same rates of strain described in Fig. 1.

gen bonding interaction between polypeptide chains. In all cases, tensile strength increased with strain rate, but rupture strain was virtually constant.

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Modified Atmosphere Storage of Rockfish Fillets

KIRK L. PARKIN, MARY JO WELLS, and W. DUANE BROWN

ABSTRACT

A modified atmosphere (MA) of 80% CO_2 and 20% air was effective in extending shelf life of fresh rockfish fillets. Aerobic plate counts and trimethylamine levels were significantly lower ($P < 0.1\%$) for the fillets held in MA compared to those held in air. A decline in surface pH was observed on fillets stored in MA, presumably due to the absorption of CO_2 and its hydration to carbonic acid. Oxidation-reduction potentials suggested a more aerobic environment on fillets stored in MA. There were no significant differences in weight loss between control and MA samples. Sensory evaluations indicated that fillets held under MA were of good quality for at least 13 days of storage.

INTRODUCTION

FRESH FISH and other seafood products are perhaps the most susceptible to postmortem deterioration of the muscle foods consumed in the world today. Thus, extension of the shelf life of fresh seafood products would be very advantageous to the industry. This could result in increasing the marketing range of fresh products and in enabling greater amounts of seasonal surplus to be marketed fresh.

Coyne (1933) observed that concentrations of CO_2 up to 100% were very effective in preserving fresh fish, with optimal conditions being observed with atmospheres containing 40–60% CO_2. Shewan (1950) suggested levels of 30–40% CO_2. Tarr (1954) called for at least 40–50% CO_2 concentrations to derive maximal benefits of modified atmosphere (MA) storage of fresh fish. Brown et al. (1980) observed rockfish fillets and salmon steaks held under 20% and 40% CO_2 atmospheres and found the 40% CO_2 atmosphere to be the more beneficial of the two. In the summer of 1978, over 2 million pounds of salmon were successfully shipped under a MA composed of 60% CO_2 (Veranth and Robe, 1979). Wolfe (1980) has provided a useful summary dealing with the use of CO_2-enriched atmospheres for fish and other products.

Prior to the present study, most of the research into the application of MA systems to the preservation of fresh fish had involved the use of CO_2 concentrations of 60% or below. It seemed likely that the preservative effect of CO_2 should increase with its concentration in the MA. Therefore, it was the intention of this study to evaluate the effectiveness of a MA highly enriched with CO_2 (80%) as a preservative medium for fresh rockfish fillets. Product quality was measured by common microbiological and chemical indices, accompanied by sensory panel evaluation, and comparisons were drawn between the MA held fillets and fillets held as air controls.

EXPERIMENTAL

Storage and handling

Fresh skin-on rockfish fillets (Sebastes spp) were obtained from a local distributor, and were sized to approximately 70–100g. The fillets were placed on racks and in containers as previously described (Brown et al., 1980). The MA was effected by evacuating the storage cylinder to 16, and then 17 in. of vacuum, with each of these evacuation steps being followed by the release of the vacuum to atmospheric pressure by the inflow of 100% CO_2. The initial MA charge was verified to be 80±5% CO_2 (balance air) as measured by a Carle model 8000 gas chromatograph using Porapak and molecular sieve columns. Control samples were prepared similarly except that the environment inside the storage cylinder consisted of a normal air atmosphere. All cylinders were stored at 35±2°F in the dark. At intervals of 0, 3, 7, 10 and 14 days, 6 fillets from each of the storage atmospheres were removed and each fillet was subjected to all the chemical and microbiological analyses employed. Samples for sensory analyses were taken on days 6 and 13. The MA cylinders were regassed to 80±5% CO_2 (balance air) at each of these intervals. All handling of the fish was done with disposable vinyl gloves to minimize contamination.

Aerobic plate count

A core sample of approximately 1g was obtained from the flesh side of each of the fillets with a twice flame sterilized cork borer, representing a 2.0 cm^2 surface area. After homogenization for 1 min in 99 ml of sterile 0.9% NaCl, the appropriate serial dilutions (all in sterile 0.9% NaCl) were pour plated in duplicate in Standard Methods Agar with 0.5% NaCl, as suggested by Liston and Matches (1976). The plates were incubated at 20°C for at least 4 days and plate counts were calculated and expressed on the basis of numbers of organisms per cm^2 surface area.

Surface pH

Measurements were made with a Beckman model 39507 surface pH combination electrode using a Radiometer pH meter, model 28.

Surface oxidation reduction (redox) potential

Measurements were made with Beckman platinum inlay and reference model 39402 electrodes, using a Corning model 12 pH meter. The electrodes, using a Corning model 12 pH meters. The electrodes were standardized with reference solutions of saturated hydroquinone at pHs 7.00 and 4.01, corresponding to standard potential of +286 and +463 millivolts, respectively.

Chemical analyses

Samples of fillets (25g) were extracted with 50 ml of 0.6N perchloric acid solution for 1 min in a Waring Blendor, and then filtered through Whatman #2 filter paper. Preliminary studies indicated that these acid extracts (pH about 1.0) were stable for at least 1 wk with respect to trimethylamine (TMA) and hypoxanthine (Hx) contents.

For convenience, analyses for these components were accomplished the day following the extraction, after overnight storage of the acid extract at 35°F in the dark. At time of analysis, the total volume of the extract was neutralized with 5N KOH until a pH of 6.5–7.0 was obtained. The neutralized extract was diluted to 75 ml and held in ice to facilitate the precipitation of perchlorate. At this point, the neutralized extract was subjected to TMA and Hx analyses as described below.

Trimethylamine

Trimethylamine content of the fillets was measured by the method of Chang et al. (1976) using a modified Orion ammonia specific ion electrode.

Further modifications were employed in accordance with the procedure used by Brown et al. (1980) in order to improve the sensitivity of the analysis. These alterations consisted of changing the internal filling solution to contain 0.01M TMA-HCl/0.05 M KCl and increasing the amount of formaldehyde in the incubation step to 0.37% (v/v). —Continued on next page

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Hypoxanthine

Hypoxanthine was analyzed by the method of Beuchat (1973) as modified by Brown et al. (1980).

Weight loss

Weight loss was calculated as the percent difference between the weights of each individual fillet at the beginning of the storage period and at the time of sampling.

Sensory analysis

Eleven judges were briefed on the scoresheets employed, and were given a training session using stored fish having expected low and med-range visual and odor differences.

Sensory panel evaluations were conducted at 6 and 13 days of storage for MA and air control treatment groups, both being compared to fresh rockfish fillets. Approximately 2 hr before the panel was conducted, the fillets were removed from storage. Random samples of cut and sized fillets from each treatment group were placed in small, marked cups. These cups were covered with a perforated aluminum lid to hide the samples and were capped with a second, removable aluminum lid. The filled cups (two fresh, one MA, and one air control) were placed on trays of ice along with a reference (fresh) sample. The judges ranked these samples according to odor strength in relation to the reference, in the order of least to most off-odor.

For color evaluation, fillets were placed in covered glass petri dishes to prevent odor from biasing the color evaluation. Dishes consisting of one fresh, two MA, and two air control samples were placed on an iced tray along with a reference (fresh) sample. Under standard fluorescent lighting, the judges were instructed to view the flesh and skin color at a 45° angle. Color was judged on 10 cm unstructured scale with the reference marked in the middle. Flesh color evaluations were scaled from 0 (light) to 10 (dark) and skin color evaluations were scaled from 0 (dark red) to 10 (no red).

Data for the skin and flesh color were subjected to analysis of variance and the calculated means tested with the least significant difference test (Amerine et al., 1965) to calculate significance. Odor data were calculated by the "R" index method (O'Mahony et al., 1979) and reported as pooled and mean results.

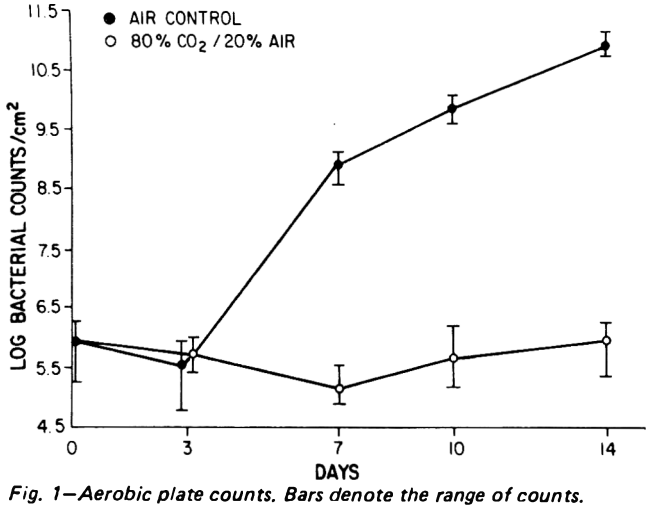


Fig. 1—Aerobic plate counts. Bars denote the range of counts.

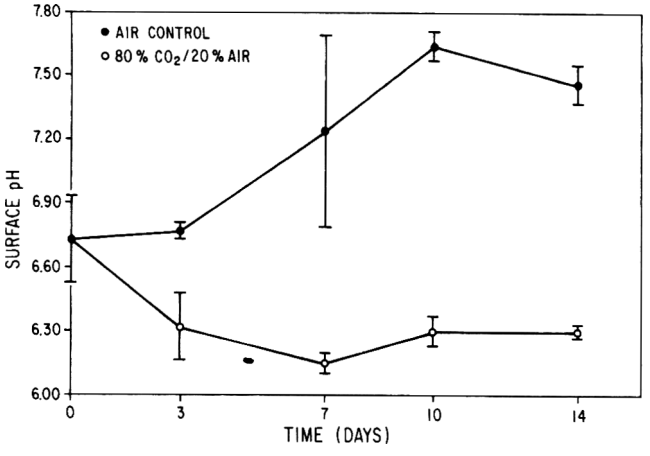


Fig. 2—Surface pH values. Bars denote standard deviations.

RESULTS & DISCUSSION

Aerobic plate counts

Surface plate counts are presented in Fig. 1. The fillets held as air controls showed a spoilage pattern such as is generally observed for a fresh product. By the 10th day, each of the fillets was thoroughly coated with a thick slime, indicative of extensive bacterial growth, and could be classified as being putrid.

In complete contrast to the air held fillets, fillets held in the MA showed negligible bacterial growth during the entire 14 day storage period. This trend reflects previous observations, verifying the bacteriostatic effect of CO_2. The differences in bacterial counts between fillets stored in air and those stored in MA were 6,000 and 950,000 fold at 7 and 14 days, respectively. Analysis of variance verified that these differences were statistically significant at the 0.1% level. Whether or not the bacteriostatic effect observed for the MA is manifest in decreasing the number of specific types of bacteria while permitting growth of other types was indiscernible, since differential counting was not included in the experimental protocol. It would be of interest to know patterns of differential microbial growth for this commodity stored under MA.

Surface pH

The results of the surface pH measurements are presented in Fig. 2. The surface pH of the air control fillets consistently rose from the onset of the study period, reaching the greatest value after about 10 days. This pH trend is generally observed during normal refrigerated storage of fresh rockfish fillets (Lauder et al., 1970). The pH on the surface of the fillets stored in the MA displayed an opposite trend. After a substantial decrease over the first 3 days, a pH of about 6.3 or below was maintained throughout the remainder of the storage period. These values are similar to those observed by Coyne (1933) in the storage of cod under 100% CO_2 atmospheres. Statistically, the difference in pH values between the fillets undergoing the separate treatments was significant at the 0.1% level, as confirmed by analysis of variance. These pH values represent a 10-fold difference in $[H^+]$ as early at 7 days storage. Also, the pH measurements indicate as early as 3 days a 2.5 fold difference in $[H^+]$ between fresh fillets and those subjected to the MA treatment. It is certainly possible that this induced pH decrease may be a force in the bacteriostatic effect imparted by CO_2.

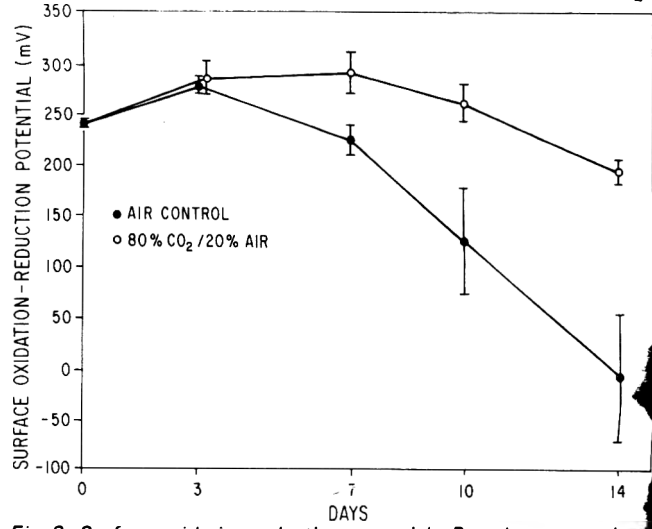


Fig. 3—Surface oxidation-reduction potentials. Bars denote standard deviations.

Presumably, the drop in pH on the surface of the fillets held in MA results from the dissolution of CO_2 (as carbonic acid) into the aqueous environment of the fish flesh.

Surface oxidation-reduction potential

The redox potential (E_h) on the surface of the air control fillets dropped rapidly (tending toward anaerobiosis), especially between 7 and 14 days of storage (Fig. 3). The lowest mean value of E_h was reached on the 14th day, being of anaerobic character at −4 mv. It is likely that the extensive bacterial slime formation hindered O_2 diffusion into the flesh, contributing to these anaerobic conditions. Also, it has been well documented that, in periods of logarithmic growth or stationary phases of bacteria, very low E_h values are found in inoculated media (Hewitt, 1950; Oblinger and Kraft, 1973). In any event, obvious spoilage had taken place on these fillets before the potential dropped to a mean value of +100 mv, indicating that spoilage was primarily due to the growth of aerobic bacteria. It was also noted that the trend of E_h closely paralleled bacterial growth, suggesting that E_h may provide an index of the quality of fresh fish fillets.

Based on E_h measurements, the MA appeared to promote a more aerobic environment on the rockfish fillets than did the air atmosphere. Analysis of variance indicated that the difference in E_h between the two treatments was statistically significant at the 0.1% level. By convention, the more positive potentials observed on the MA held fillets should indicate conditions that are inhibitory to the growth of strict anaerobes. Even after 14 days of storage the potentials on these fillets reflected a truly aerobic character, as a mean E_h of +198 was observed.

Trimethylamine

The results of TMA analysis are presented in Fig. 4. The initial TMA values indicated that the fillets were procured in very good condition. During the first 3 days of storage, low levels of TMA remained in all of the fillets. However, after 3 days of storage, the air control fillets showed a rapid accumulation of TMA, with very high levels being reached by 10–14 days. This trend of TMA production and accumulation appears to follow closely the bacterial growth pattern, as expected.

According to Connell (1978), the upper limits of TMA levels in acceptable chilled stored fish are 10–15 mg TMA-N/100g flesh (equivalent to 7.1–10.7 = μmoles TMA/g flesh). As was noted previously, the fillets held in air were characterized by a "fishy" odor by the 7th day, yet might still be considered acceptable. The rate of TMA production in the fillets held in the MA also seemed to correlate closely with bacterial growth. In this case, TMA levels remained low throughout the entire 14-day storage period. At the end of the study period, there was about 7 times more TMA in the flesh of the air control fillets than in those held in MA. The difference between treatments with respect to TMA accumulation was determined to be statistically significant at the 0.1% level.

From these results, it appears that TMA provides an adequate quality index for fillets stored under each of the two treatments. One must assume that the lower levels of TMA observed in the fillets held under the MA is a result of the bacteriostatic effect of CO_2. However, a secondary factor that may contribute is that TMA production has been shown to be dependent on the pH of the system, with more active TMA production taking place at higher pH values (Castell and Snow, 1949).

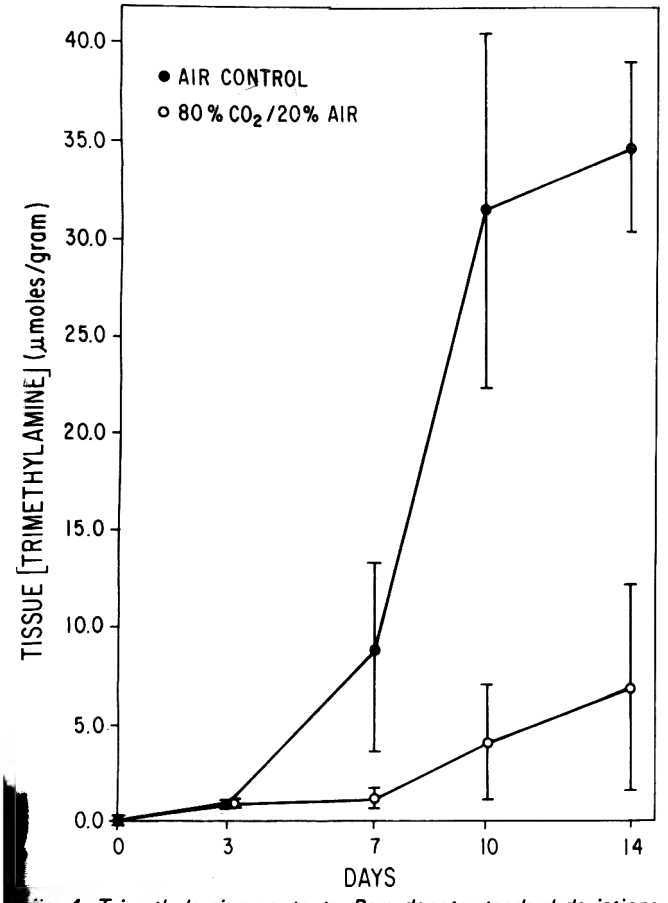


Fig. 4—Trimethylamine contents. Bars denote standard deviations.

Hypoxanthine

The results are presented in Fig. 5. For the air held fillets, maximum levels of this compound were reached and maintained between 7 and 14 days, paralleling closely the trends of bacterial growth and TMA production. Thus, it

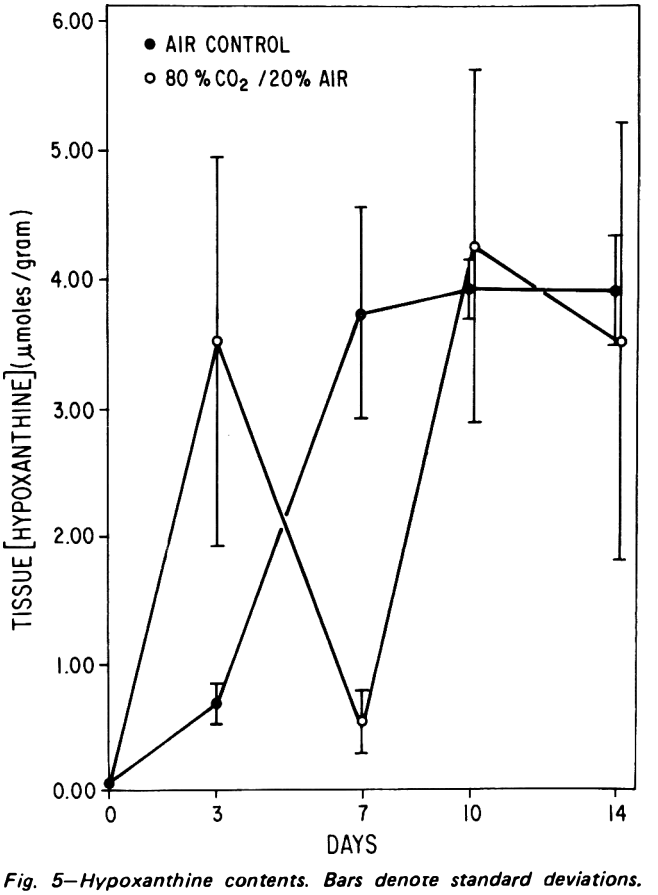


Fig. 5—Hypoxanthine contents. Bars denote standard deviations.

Table 1—Mean sensory scores for visual differences among storage treatment groups and fresh samples of rockfish, using unstructured category scaling (n=22)[a]

	Day	Fresh	Treatment group MA	Air
Skin	6	4.7a	4.8a	5.6a
	13	5.1a	5.8ab	7.0b
Flesh	6	6.1a	6.4a	8.5b
	13	4.1a	4.6a	6.6b

[a] Scores in the same row not followed by a common letter are significantly different at the 0.1% level. Sensory score based on a 10-point scale: Flesh (0=light, 10=dark), skin (0=dark red, 10=no red).

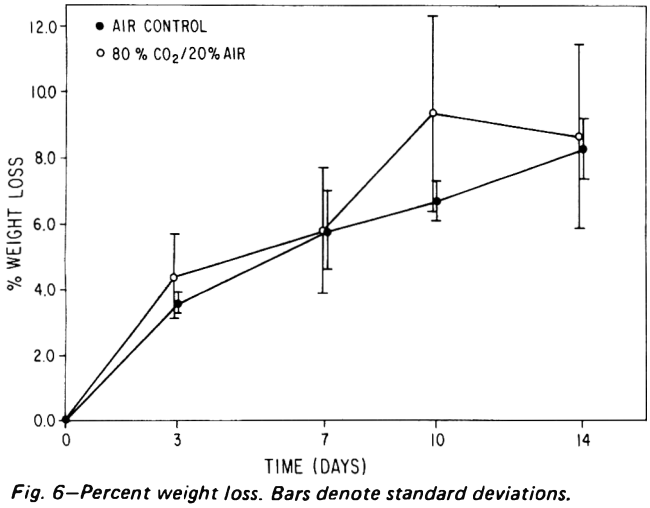


Fig. 6—Percent weight loss. Bars denote standard deviations.

appears that this test is valid in assessing the relative quality of rockfish fillets held under normal chill storage conditions. The pattern of Hx accumulation of these fillets correlates with those observed for other Pacific coast fish (Jones et al., 1964). In contrast, the pattern of Hx accumulation in the fillets held in MA was observed to be very erratic. While this assay may provide for a chemical index in assessing the quality of the air held fillets, it does not seem to be a test that is applicable to fillets or material held under MA.

Weight loss

There was little difference in weight loss with respect to the type of storage atmosphere, although the mean values for the fillets held in the MA seemed somewhat greater, particularly at 10 days (Fig. 6). However, there was no statistically significant difference between the two treatment groups.

Sensory

There were no significant visual differences in skin color among treatment groups on day 6, but on day 13, the fresh and air control samples were significantly different at the 0.1% level (Table 1). However, there was no significant difference between the fresh and MA samples with respect to skin color after 13 days of storage. Presumably, the MA delays the fading of the skin color that was observed with the air control samples.

With respect to flesh color, significant differences at the 0.1% level were found between the air control sample and the MA and fresh samples at 6 and 13 days. Again, no difference was detected between the fresh and MA samples, even after 13 days.

Mean "R"-indices of sensory scores for odor differences were calculated for the three treatments (Table 2). Significant differences were found between fresh and air control treatments on Day 6. However, there was no significant difference between the fresh and MA treatment groups at this point of storage. On day 13, there was a significant difference between the fresh and the MA and air control samples. Pooled results were also tabulated because of the smaller number of replications used for comparison.

Table 2—R-index sensory scores for odor differences among storage treatment groups and fresh samples of rockfish[a]

Data	Day	Fresh	Treatment group MA	Air
Mean	6	75.8a	87.9ab	100.0b
	13	71.2a	92.4b	100.0b
Pooled	6	75.6	88.0	100.0
	13	74.6	95.1	100.0

[a] Scores in the same row not followed by a common letter are significantly different at the 0.1% level.

CONCLUSION

MODIFIED ATMOSPHERE SYSTEMS have been shown to provide a technological avenue worth pursuing in their application to the handling of fresh seafood products. As a result of this and previous studies, dramatic extensions of shelf life have been observed for products held under such systems. It may be that modified atmosphere technology ultimately can be employed in the packaging steps of distribution as well as for bulk shipping or storage of fresh seafood products.

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The Fate of Hypochlorous Acid During Shrimp Processing: A Model System

H. A. GHANBARI, W. B. WHEELER and J. R. KIRK

ABSTRACT

After immersion of shrimp in a 100mg/L solution of $HO^{36}Cl$, the distribution and chemical forms of chlorine were determined. There was a 2–5% loss of Cl_2 into the atmosphere, but no significan photolysis of HOCl was measured during the 30 min of treatment. More than 80% of the HOCl reacted during the incubation period, forming Cl^- or incorporating into organic molecules. Only 1–3% ^{36}Cl was incorporated into the shrimp. Organic molecules in the immersion solution also showed ^{36}Cl incorporation; the 10,000 x *g* precipitable fraction contained 2–5% ^{36}Cl and the soluble organic fraction 1–2% ^{36}Cl.

INTRODUCTION

CHLORINE is listed as a "Generally Recognized As Safe" (GRAS) chemical for use in food processing. Chlorine is used in water for washing and cooling raw food products and for cleaning food-handling equipment. Because of its bactericidal properties, the use of chlorine is considered essential in food processing operations for esthetic and safety reasons. The use of chlorine in the food industry has been reviewed by Mercer and Somers (1955) and Kirk and Mitchell (1980).

Chlorine readily dissolves in water to predominantly form hypochlorous acid in the pH range 5–7, which is a powerful oxidizing agent and an effective chlorinating agent (White, 1972; Ghanbari et al., 1980, 1981a; Kirk and Mitchell, 1980). Cunningham and Lawrence (1979) have reported chlorine incorporation in chicken skin lipids and pork belly fat when these meats were exposed to chlorinated cooling water. Hypochlorous acid can react with organic molecules dissolved or suspended in water. Reactions and reaction products of chlorine in water were reviewed by National Research Council (1979).

Studies involving use of chlorine in the food industry generally concern the bactericidal property of HOCl, changes in the quality of food products, and to a very limited extent, incorporation of chlorine in the organic compouds found in foods. There is little or no information concerning the fate of chlorine used in food processing. This study concerns the fate of chlorine as hypochlorous acid and its distribution and chemical form in processed shrimp. The experimental protocol is presented as a model to be used in similar studies concerning the processing of foods. Information obtained on the fate of chlorine will permit the efficacious rather than indiscriminate use of chlorine in food processing.

EXPERIMENTAL

Materials

Fresh shrimp were purchased from a small shrimping company in Crystal River, FL. [^{36}Cl]-hydrochloric acid was purchased from New England Nuclear. All other chemicals used in this study were reagent or analytical grade.

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Chlorine generation

Chlorine gas was generated by the dropwise addition of 2 ml of 3N HCl solution to 1g potassium permanganate in a closed gas generation apparatus (Ghanbari et al., 1981b). Chlorine gas was trapped in 50 ml of 0.02M NaOH solution to yield a final pH of 6–7. Using this system, the gas is efficiently trapped predominately as HOCl.

To prepare $HO^{36}Cl$, $H^{36}Cl$ was first neutralized with NaOH and then added in microliter amounts to potassium permanganate in the reaction flask to generate Cl_2 (g). The amount of $Na^{36}Cl$ used was determined by the specific activity (cpm/mg/L) desired in the final solution.

Available chlorine

Available chlorine was measured by iodiometric titration (Franson, 1976; Masschelein, 1979).

Liquid scintillation counting

Radioactivity was determined using liquid scintillation counting in Aquasol (New England Nuclear) using a Searle Analytic 92 Liquid Scintillation System with windows set at 50-800. Counting efficiency was determined by quench curve, and counter reliability was monitored by including standard with each run.

Biological material pyrohydrolysis

Samples of shrimp homogenates were combusted in a "Biological Material Pyrohydrolizer" (BMP) in the presence of O_2 and H_2O, and the combustion products were passed through a 900°C zone. Any Cl in the sample was converted to HCl vapor and collected as the pyrohydrolysate by condensation (Ghanbari et al., 1981b). Pyrohydrolysis permitted larger samples to be used in chlorine incorporation studies because it minimized any color or chemical quenching or chemiluminescence in samples prepared for liquid scintillation counting.

Total organic carbon analysis

Total organic carbon (TOC) was determined using a Beckman Model 915 TOC analyzer equipped with model 865 IR analyzer.

Exposure of shrimp to HOCl solution

Fresh shrimp were "headed," cleaned and stored frozen at −10°C until use. Approximately 30g of shrimp were immersed in 100 mg/L (available chlorine) solution of $HO^{36}Cl$, pH 6 at a ratio of 5:1 (v/w HOCl solution:shrimp) to simulate washing and fluming of shrimp during commercial processing. This mixture was protected from light and incubated at room temperature (21–23°C) with constant stirring. Aliquots of the wash solution were sampled after 0, 1, 5, 15, and 30 min and analyzed for radioactivity and available chlorine. The chlorine exposure was terminated at the end of 30 min by adding 4 ml of 0.1N $Na_2S_2O_3$ and 1 ml of 1N HCl. Sodium thiosulfate reduced any remaining HOCl to Cl^- and HCl lowered the pH (to about 2) for a complete HOCl quenching and also provided unlabeled Cl^- ions which minimized nonspecific binding of $^{36}Cl^-$ to organic molecules and tissue. The quenched mixture was incubated for 10 min with constant agitation, the immersion solution was then poured off and saved for further analysis.

Determination of ^{36}Cl in shrimp and inorganic compounds in the immersion solution

Treated shrimp were rinsed three times with 0.1M NaCl, cut in small pieces and ground into a homogenous paste. Approximately 0.5g of the paste was weighed into a quartz boat and placed in the BMP for ^{36}Cl determination.

–Continued on next page

Aliquots of the wash water were counted for ^{36}Cl (0.5 ml, duplicate). Insoluble organic compounds and particulate matter were removed from the wash water by either centrifugation at 10,000 x *g* for 20 min or by filtration through a 0.45 micron filter (Millipore Corp.). Radioactivity was determined in the clear wash water (using duplicate 0.5 ml samples).

Incorporation of chlorine into soluble organic compounds in the clarified aqueous fraction was determined by sequential precipitation of $^{36}Cl^-$ by excess Ag^+ and precipitation of unreacted Ag^+ by excess Cl^- (Faith et al., 1980). A 4 ml aliquot of the clear immersion solution was mixed with 0.4 ml of 0.2M $AgNO_3$, centrifuged at low speed, and filtered through a 0.45 micron filter. A 2.2 ml aliquot of the filtrate was mixed with 0.2 ml of 0.2M NaCl, centrifuged and filtered. After each filtration step, more Ag^+ and Cl^- (respectively) were added to a 200 µl aliquot of the filtrates to test the completion of $^{36}Cl^-$ and Ag^+ precipitation. The final filtrate was free of $^{36}Cl^-$ and any radioactivity in this filtrate was attributed to chlorine incorporation into soluble organic compounds in the immersion solution.

Experimental controls

Experimental controls and blanks were important in the design of this model. One control (B) was identical to the experimental run (A) except Na thiosulfate and HCl were added before adding shrimp. This control tested for "nonspecific" incorporation of $^{36}Cl^-$. A second control (C) was identical with the experimental run (A) but did not contain shrimp. This control was used to determine the loss of $^{36}Cl_2$ to the atmosphere, loss of HOCl by photolysis and to test the completeness of $^{36}Cl^-$ precipitation by $AgNO_3$ and filtration. The third control (D) was identical to the experimental run except HOCl was nonradioactive. This control provided information on chemiluminescence and/or quenching in Liquid Scintillation Counting (LSC). Total organic carbon (TOC) was also monitored using aliquots from this control.

RESULTS & DISCUSSION

CHLORINE, when added to water, undergoes rapid and almost complete hydrolysis to form hypochlorous acid and a chloride ion, equilibrium being established within a few seconds in favor of HOCl in the pH range used in this study.

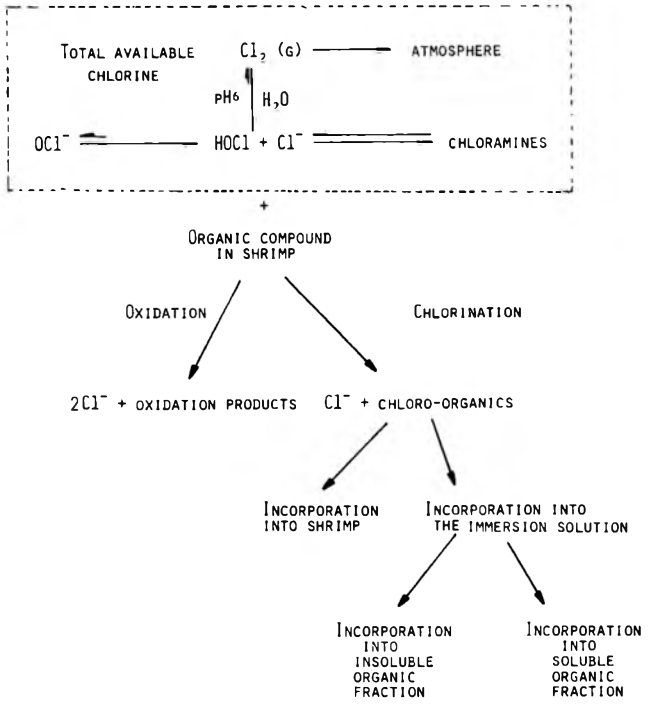


Fig. 1—Schematic presentation of fate of chlorine in shrimp processing.

Fig. 1 is a schematic presentation of the fate of Cl in a solution of HOCl containing shrimp tissue and organic matter. Using $HO^{36}Cl$ the fate of Cl and available chlorine in shrimp processing was quantitated.

Fate of available chlorine.

Data describing the residual chlorine in the $HO^{36}Cl$ immersion solution when shrimp were incubated in an open beaker at 24°C for various time periods are shown in Table 1. Changes in available chlorine in experimental samples designated A (with $HO^{36}Cl$) and D (with HOCl) were parallel throughout the incubation period because no isotopic effect was observed, as expected. After 30 min of incubation, only 17.1% of original available chlorine remained in the immersion solution with the greatest portion of available chlorine reacting in the first few minutes after immersion of the shrimp. In the control experiment containing $HO^{36}Cl$ and no shrimp, 95.8% of the original available chlorine remained in the solution at the end of 30 min. The 4.2% loss of available chlorine was due to either photolysis or escape into the atmosphere. Containing shrimp, $HO^{36}Cl$ and $Na_2S_2O_3$, experimental run B had no residual chlorine in the immersion solution as expected.

Data from the scintillation counting experiments corresponding to the experiments outlined in Table 1 are shown in Table 2. In experimental run C 95.7% of the labeled chlorine remained in solution after 30 min indicating that only 4.3% of ^{36}Cl had been lost. Radioactivity in experimental samples described in run D was zero and in run B radioactivity stayed constant. Radioactivity in run A decreased 5.2%. These data represent ^{36}Cl incorporated into the shrimp tissue and organic matter in the immersion solution as well as $^{36}Cl_2$ loss to the atmosphere.

Chlorine incorporated into shrimp

Because of the chemiluminescence of shrimp homogenates dissolved in NaOH or Protosol (New England Nuclear), ^{36}Cl incorporation into shrimp was determined by pyrohydrolysis of the shrimp homogenates. Applicability of pyrohydrolysis for quantitation of chlorine in shrimp samples was tested, and the results are shown in Fig. 2. After 10 min of combustion in the BMP the counts in the condensate were essentially at the initial background level. To insure against cross-contamination, each sample combustion was followed by a 10 min blank combustion to detect any carry-over of ^{36}Cl. ^{36}Chlorine incorporated into the shrimp was calculated using the following equation:

$$\text{Percent } ^{36}Cl \text{ incorporated into shrimp} = W(a_A - a_B)/a_T 0 \times 100$$

Table 1—Changes in the available chlorine during incubation of shrimp in HOCl solution. About 20g of shrimp (5 shrimp) were incubated for 30 min in 5X (W/V) solution containing 100 mg/L HOCl

Experimental and control runs	Residual chlorine after incubation period (mg/L) 0 min	1 min	5 min	15 min	30 min
Shrimp + $HO^{36}Cl$ (A)	100	60.1	48.6	32.1	17.1
Shrimp + 4 ml $Na_2S_2O_3$ + $HO^{36}Cl$ (B)	0	0	0	0	0
$HO^{36}Cl$ (C)	100	99.4	98.8	97.9	95.8
Shrimp + HOCl (D)	100	61.5	47.2	31.2	16.8

where W = weight of shrimp in grams; a_A = radioactivity in shrimp samples from run A, in dpm/g; a_B = radioactivity in shrimp samples from run B, in dpm/g; and a_T0 = Total radioactivity at time 0 in dpm. Also,

$$a_A \text{ or } a_B = a \times v/w$$

where: a = radioactivity per ml of pyrohydrolysate, dpm/ml; v = volume of pyrohydrolysate ml; and w = weight of shrimp homogenate sample used in pyrohydrolysis, g. Chlorine incorporation into shrimp during experiments outlined in Tables 1 and 2 was 2.82% of total ^{36}Cl present or 7.94 micromoles of chlorine per 20g of shrimp.

Loss of ^{36}Cl to the atmosphere

The loss of radioactivity from the immersion solution in experimental run D after 30 min of incubation was 5.2%. Of this loss, 2.82% was incorporated into shrimp and the balance, i.e. 2.38% of ^{36}Cl, was assumed to be lost to the atmosphere. This loss was greater than in experimental run C because $HO^{36}Cl$ concentration remained high, which would favor the loss of Cl_2 into the atmosphere, as compared to experimental run A where the concentration of $HO^{36}Cl$ decreased rapidly reaching a final concentration of 17 mg/L at the end of 30 min.

Incorporation of chlorine into organic molecules in the wash water

The difference in the level of ^{36}Cl in the wash water for the experimental run A before centrifugation (or filtration) and after centrifugation was assumed to be the amount of ^{36}Cl incorporated into insoluble organic molecules and particulate matter. Radioactivity in the immersion solution of the control run (B and C) remained essentially unchanged after centrifugation. Total organic carbon in the immersion solution of experimental run D decreased from 720 mg/L level before centrifugation to 468 mg/L after centrifugation (252 mg/L insoluble organics). Chlorine incorporation in the insoluble fraction of the immersion solution was 1.92%.

Chlorine incorporated into the soluble organic fraction of the immersion solution was calculated using the following formula:

$$\text{Percent } ^{36}Cl \text{ incorporated into soluble fraction} = V_T \times 5/6\ (a_{Af} - a_{Bf})/a_T0 \times 100$$

where: V_T = total volume of the immersion solution, ml; a_{Af} = radioactivity in the final filtrate of run A, dpm/ml; a_{Bf} = radioactivity in the final filtrate of run B, dpm/ml; a_T0 = total radioactivity in the wash water at 0 incubation time, dpm; and 5/6 = dilution factor for the 2 precipitation-filtrations.

Chlorine incorporation into soluble organic molecules in the immersion solution in this study was 1.41%. Total organic carbon did not change by filtration of the clear immersion solution from experimental run D indicating no loss of soluble organics as a result of repetitive filtration.

In summary, of the 100 mg/L HOCl in the experimental run A, experimental data indicate 2.5% was lost to the atmosphere as Cl_2 (g), 17.2% remained in the immersion solution as residual chlorine, and 80.5% was destroyed by oxidation/chlorination reactions after 30 min of incubation.

—Continued on page 197

Table 2—Changes in the ^{36}Cl level during incubation of shrimp in HO^{36} Cl solution. About 20g of shrimp (5 shrimp) were incubated for 30 min in 5X (W/V) solution containing 100 mg/L HOCl. Original radioactivity in the solution was about 10^5 dpm/ml

Experimental and control runs	Percent radioactivity left after incubation period (mg/L)				
	0 min	1 min	5 min	15 min	30 min
Shrimp + HO^{36} Cl (A)	100	96.2	95.0	95.3	94.8
Shrimp + 4 ml $Na_2S_2O_3$ + HO^{36} Cl (B)	100	100.1	100	99.9	100
HO^{36} Cl (C)	100	99.3	99.0	97.8	95.7

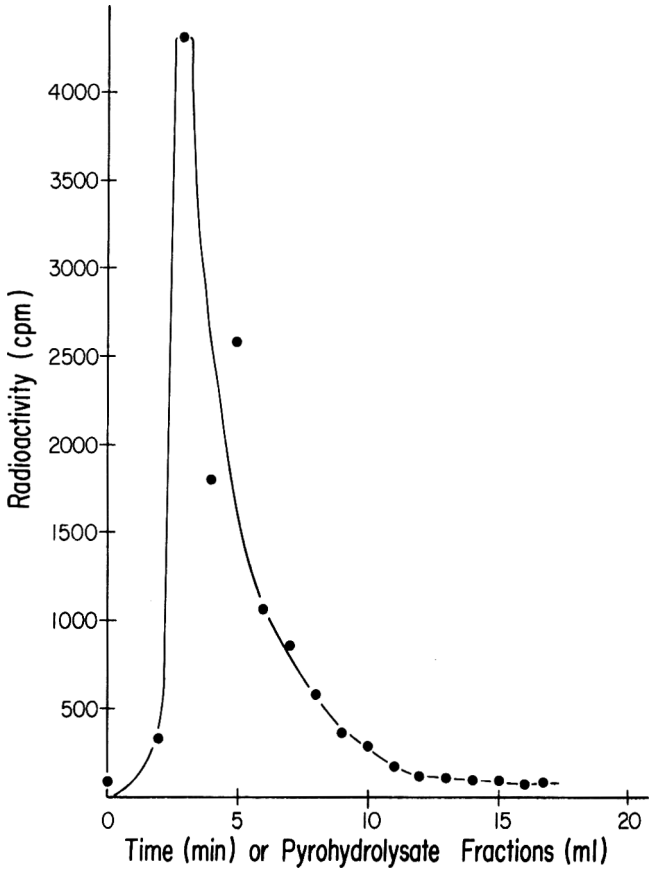


Fig. 2—Recovery of ^{36}Cl from shrimp samples using Biological Material Pyrohydrolyser. Weight of sample 440 mg, temperature 900° C, water 1 ml/min, O_2/CO_2 (95/5) flow 100 ml/min.

Table 3—Fate of $HO^{36}Cl$ and ^{36}Cl in immersion solution containing shrimp. This is a composite of data collected for 30 min incubation of shrimp in $HO^{36}Cl$ solution. Experimental conditions as in Tables 1 and 2.

Form of ^{36}Cl	No. of Expts.	% of original radioactivity	
		Average	Range
Photolysis	4	N.D.[a]	N.D.
Loss to the atmosphere as $^{36}Cl_2$	7	3.15	2–5
Residual chlorine as HOCl	11	18.2	0–26
Incorporation into shrimp	4	2.42	1–3
Incorporation into insoluble organic fraction	4	2.95	2–5
Incorporation into soluble organic fraction	4	1.52	1–2

[a] N.D. — Not detectable.

Chemical Composition and Shelf Life Study of Grayfish (*Squalus acanthias*)

SUDIP N. JHAVERI and SPIROS M. CONSTANTINIDES

ABSTRACT

Seasonal proximate composition of grayfish (*Squalus acanthias*) yielded 14.5% lipid, 1.3% urea, 12.6% protein, and 1% nonprotein nitrogen, which was higher than that observed in the teleosts. Mineral analysis showed that mercury was present below 1.0 ppm FDA limit. Total saturated fatty acids in the male grayfish amounted to 45.3% as opposed to 26.6% in the female fish. The total monoenes were 40% higher in the female than in the male fish. The cholesterol levels averaged 68 mg/100g tissue in both the sexes. The keeping quality of grayfish was indicative of rapid deterioration. Storage life of dressed grayfish in ice was observed to be between 6 and 8 days. The frozen storage stability of fillets pretreated with sodium tripolyphosphate and ascorbate solutions was measured by determining the TBA value, thaw drip and sensory analysis.

INTRODUCTION

THE AQUATIC RESOURCES of the Northwest Atlantic Ocean are being extremely exploited for use as food fish (Rathjen, 1974). However, this continual depletion of stocks has been salvaged by the establishment of the 200-mile fishing limit. Thus there are over 200 million metric tons of fish now available for consumption (Bardach and Parisier, 1978). In order to relieve pressure on the existing stocks of traditional species, a concerted effort must be made on an industrial scale to explore the possibilities of utilizing nontraditional or underutilized species for human consumption.

Development of underutilized fishery demands a thorough knowledge of each of these species in the areas of biology, chemistry, catching technique, maximum sustainable yield, product development, marketing and industry response. Thus, a new approach must be taken for our underexploited marine species in order to counteract the possible exhaustion and overexploitation of our highly consumed resources.

One such predominant underutilized species found in the Northwest Atlantic Ocean is grayfish (*Squalus acanthias*), commonly known as the spiny dogfish. With its slender body and a shark-like appearance, grayfish measures between 50–100 cm in length and weigh from 3.5–9.8 kg. Females are slightly larger than males. Grayfish are found in coastal waters from the Gulf of St. Lawrence to Cape Lookout, NC (Bigelow and Schroeder, 1948). Since it is a cartilaginous fish, it belongs to the sub-class elasmorbranchii, with depositions of calcium fluorophosphate forming the skeleton (Ronsivalli, 1978). A typical feature of the elasmobranchs is the high amounts of nonprotein nitrogen (NPN) including urea, trimethylamine oxide, betaine and sarcosine (Gordievskaya, 1971).

Until 1950, spiny dogfish were caught in the U.S. primarily as a source of vitamin A, extracted from their livers. More than 100 million pounds of dogfish were landed yearly, in the years preceding 1949. In 1957 the total production amounted to less than 2 million pounds. The development of methods of synthesizing vitamin A, around 1950, was probably the reason for this dramatic reduction.

In the years that followed, the stocks of grayfish grew to a point where they were beginning to impose severe economic costs on the fishing industry. Attempts were made in order to find commercial uses for this species (Holmsen, 1968). In England, the market for dressed grayfish was about 13.7 million pounds in 1974. Since then this number has grown. Similar markets for dressed carcasses exist in France, Germany, Belgium and other countries in Western Europe, to varying degrees (Anon, 1975).

In 1976, about 17 thousand pounds of grayfish were landed in the New England area. This figure has increased to more than 3400 metric tons in 1980 (Current Fisheries Statistics, 1980). A potential market for dogfish belly flaps exists in Germany and U.K. However, in the U.S., this species is considered a nuisance to the commercial fisherman because they damage fishing gear with their rough skin and spines.

In this study, seasonal proximate composition, fatty acid distribution, cholesterol level and mineral composition were conducted on the edible portions of the fish. The keeping quality of dressed grayfish in ice was measured by determining total bacterial load, nonprotein nitrogen and urea levels. Frozen storage stability of fillets was studied by determining rancidity, drip loss and organoleptic quality.

MATERIALS & METHODS

Handling of fish

Fresh grayfish were obtained from the R.I. Fisherman's Co-Op. The fish were caught in the southern New England waters either near Block Island or Nantucket Sound. On board the fish were kept covered in ice. Within 6–8 hr of the catch, the fish were landed and transferred to the laboratory where they were headed, gutted, bled, washed in cold water and iced. The fish obtained every month, weighed 3 kg on an average and measured between 70–100 cm. In order to obtain a homogeneous sample mass, muscle tissue from each fish, from a representative batch was removed from dorsal, ventral and along the sides. The flesh was homogenized in a blender and this was called the edible portion.

Proximate composition

An average of five, fresh grayfish were brought in monthly (1977), filleted and the flesh was homogenized to obtain edible portion. Protein, moisture, ash and urea were determined on the edible portion according to the AOAC (1975) procedure. Lipid was extracted by the Bligh and Dyer (1959) method. Nonprotein nitrogen (NPN) was measured according to the procedure of Shenouda et al. (1979). The protein nitrogen was calculated by subtracting NPN from total nitrogen and multiplying this value by the factor, 6.25.

Fatty acid analysis

Lipids from the edible portions of five male and five female fresh grayfish (June 1977) was extracted (Bligh and Dyer, 1959) and esterified according to Metcalfe et al. (1966). Aliquots of the methyl esters were injected into a Varian Aerograph, 1200 series, single column gas chromatograph, equipped with a flame ionization detector and operated isothermally at 175°C. A 15% DEGS (Diethylene glycol succinate) column on 180–200 mesh size chromosorb W-HP was used with a nitrogen flow rate of 40 ml/min, hydrogen of 75 ml/min and compressed air at 400 ml/min. Hewlett-Pack-

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ard electronic integrator Model 3380A was used to identify and quantify the individual fatty acids in the samples and the PUFA standards obtained from Supelco, Inc. (Supelco Park, Bellefonte, PA 16823).

Cholesterol determination

The colorimetric method of Tu et al. (1967) was modified, by using a single, stable reagent prepared according to Wybenga et al. (1970) as follows: tubes containing 2 ml of lipid extract (Bligh and Dyer, 1959) from the fatty acid analysis preparation, in chloroform were placed in a water bath at 60°C. After the chloroform was completely evaporated, 4 ml ethanol and 0.5 ml 50% KOH was added to the tubes. This mixture was saponified at 60°C for 90 min. After cooling, 3 ml 5% NaCl and 10 ml petroleum ether were added to each tube and the tubes were shaken vigorously. Two ml of the ether extract were evaporated in a water bath at 50°C and to 2 ml glacial acetic acid were added to this mixture. At this point, 5 ml cholesterol reagent were added and the mixture was shaken well. The tubes were transferred to a boiling water bath for 45 sec and immediately placed in an ice bath. Absorbance was read at 555 nm against a reagent blank.

Mineral analysis

Five male and five female fresh grayfish (Sept., 1977) were individually measured and weighed. The average length and weight of the male fish were 89.9 cm and 1.4 kg; and that of the female fish were 95.0 cm and 3.2 kg, respectively. The edible portion was freeze-dried and 10 mg of each sample was weighed into a Teflon beaker. Samples were digested at a low temperature for 24 hr. The ash from each sample was mixed with 300 ml of ultra-pure HNO_3 and allowed to set overnight. Each sample was made up to a final volume of 1 ml by the addition of doubly deionized H_2O. Five ml ultra-pure H_2SO_4 were next added to each sample to increase sensitivity during atomic absorption. These samples were analyzed on a Perkin-Elmer Model 603 spectrophotometer with a Deuterium Arc-Background corrector by comparing with standards in 3N HNO_3. All the minerals were analyzed by flameless absorption using a 2100 graphite furnace.

Mercury

Mercury was determined by using a Coleman Model MAS-50 Mercury Analyser System. Five male and five female individually measured and weighed grayfish were filleted, homogenized and freeze-dried. The length and weight of the fish are specified above in the mineral analysis section. Two hundred mg of freeze-dried sample were digested with 4 ml 0.5N H_2SO_4 and 1 ml conc HNO_3 at 58°C. This was followed by oxidizing the samples with 15 ml 5% $KMnO_4$ solution for 24 hr. Aliquots of this mixture were injected into Coleman MAS-50 analyzer to determine Hg concentrations.

Keeping quality in ice

Twenty-five fresh grayfish were headed, gutted and washed. All the traces of blood were removed before storing them under ice at 4°C. The water formed from ice was removed daily and replaced with fresh ice. At regular intervals during the 2 wk of storage, fish were analyzed for total bacterial counts, NPN and urea concentrations. Sensory observations were also made during this storage period.

Total bacterial counts

The total microbial populations were estimated, at regular time intervals, according to Wittfogel (1955) and Shenouda et al (1979).

Frozen storage stability

Fillets from 20 fresh grayfish were divided into two groups. Fillets in group one were dipped in a solution (1:2 w/v) containing 0.5% food grade ascorbic acid and 8% food grade sodium tripolyphosphate (TPP) for 90 sec, drained, sealed in polyethylene pouches and frozen at −15°C. The second group was washed and the fillets were packed in pouches and frozen without any treatment.

Thiobarbituric Acid Value (TBA) was measured, as a rancidity index on the pre-treated and the control group, according to the procedure of Yu and Sinnhuber (1957).

Percent drip loss was measured by slow thawing of frozen samples at 4°C (Shenouda et al., 1979). During each analysis the weights of triplicate samples were within 50g of each other.

Sensory evaluation

The sensory evaluation was carried out on the two groups of frozen fish fillets. At the time of evaluation, small portions of fillets were soaked in 5% lemon and 2% vinegar for 5 min, dipped in egg white, covered with corn starch and bread crumbs and stir fried at 250°F, till light brown. These fillets were presented to 11 panelists who had past experience in evaluating seafoods. The panelists were asked to evalute the product for appearance, odor, texture and flavor using a modified evaluation score sheet (Fig. 1; Sheehy et al., 1977). This score sheet consisted of various descriptive terms which were designated numerical ratings from +4 to −4, with the most acceptable and desirable properties rated 0, and the extreme characteristics on either side of the scale. Once the data were obtained, analysis of variance was performed in order to identify significant differences.

RESULTS & DISCUSSION

Proximate composition

The seasonal proximate composition of the edible portion was determined monthly for a period of 7 months due to the ease of availability of the fish (Table 1). A character-

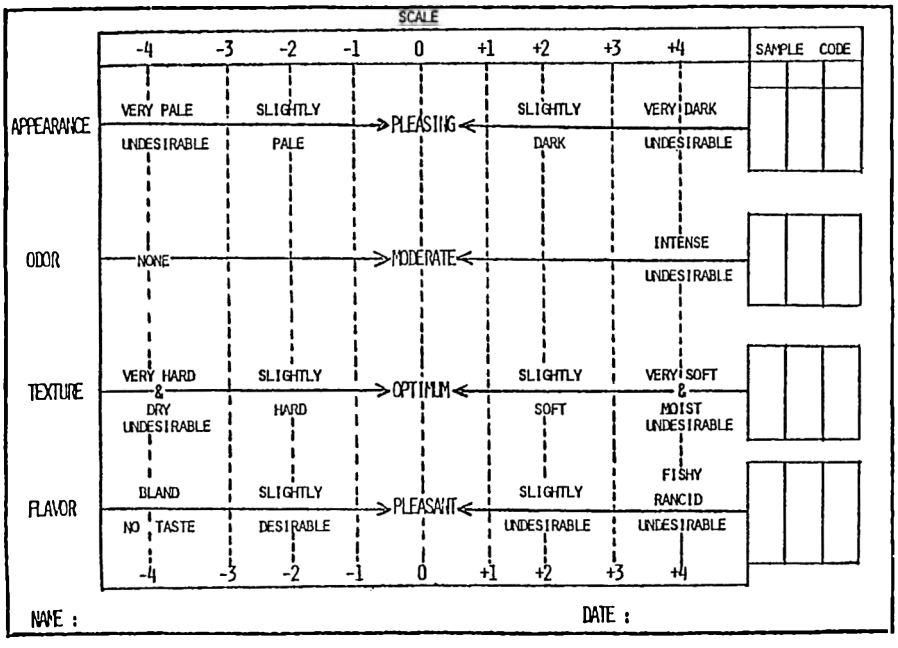


Fig. 1—Sensory evaluation score sheet.

Table 1—Seasonal proximate composition of grayfish (Squalus acanthias)[a]

Month (1977)	Moisture	Lipid	Protein	Ash	Urea	NPN
January	67.8	17.9	10.9	1.0	1.4	1.2
February	70.9	15.8	15.0	1.0	0.9	0.7
March	72.5	15.0	12.0	1.0	1.3	1.1
April	76.1	11.7	12.7	0.9	1.4	1.0
May	70.7	14.6	11.5	0.8	1.5	1.1
June	72.1	15.0	12.4	0.7	1.5	1.1
September	70.4	11.8	13.6	0.8	1.2	0.9
Mean ± S.D.	71.5 ± 2.5	14.5 ± 2.2	12.6 ± 1.4	0.9 ± 0.1	1.3 ± 0.2	1.0 ± 0.2

[a] Percent value from an average of triplicate analysis.

Table 2—Fatty acid and cholesterol levels in male and female grayfish (Squalus acanthias)[a,b]

Fatty Acid	Male (%)	Female (%)
14:0	1.0 ± 0.1	1.3 ± 0.1
16:0	32.6 ± 2.5	22.6 ± 0.1
18:0	11.7 ± 0.3	2.7 ± 0.1
Total Saturates	45.3	26.6
16:1	3.1 ± 0.2	6.3 ± 0.04
18:1	15.3 ± 0.5	26.3 ± 0.3
20:1	3.5 ± 0.2	9.8 ± 0.3
22:1	8.2 ± 0.7	7.6 ± 0.3
Total Monoenes	30.1	50.0
18:2	1.3 ± 0.1	2.2 ± 0.1
18:4	1.9 ± 0.2	3.4 ± 0.1
20:5	4.4 ± 0.1	3.9 ± 0.1
22:6	15.9 ± 1.9	12.2 ± 1.6
Total Polyenes	23.5	21.7
Others	1.2	2.0
Cholesterol[b,c]	60.6 ± 0.4	75.5 ± 1.2

[a] Percent distribution of fatty acids
[b] Mean and standard deviation of triplicate analyses
[c] mg Cholesterol in 100g tissue

istic feature of this species is the presence of high amounts of nonprotein nitrogen in their tissue and body fluids. Nonprotein nitrogen constitutes about 33–39% of the total nitrogen in elasmobranchs and between 9.2 and 18.3% in marine teleosts (Tarr, 1958). The NPN extractives comprise volatile bases, trimethylamine, trimethyamine oxides, urea, creatinin, and available amino acids. The NPN accounted for 1.0% in the edible portion of grayfish. A large quantity of this NPN was found to be present in the form of urea.

A systematic study of various sharks was compiled in a comprehensive report by Gordievskaya (1971) in which urea concentration for spiny dogfish was observed to be 1.6% as compared to 1.3% in our findings. However, urea levels of as high as 2.5% have been reported in certain species of sharks (Morris, 1975).

Gordievskaya (1971) also reported lower values of lipids in sharks, thus categorizing these species as lean. In this study, the lipid content of grayfish was 14.5%. Similar higher lipid values of 13.7% have been reported by Boyd et al., (1967). Protein values in sharks differ considerably, mainly due to the presence of NPN. An average protein content of 12.6% was observed for the grayfish edible portions. Low levels of protein up to 14.6% have been reported by Gordievskaya (1971) for blue and sevengill species of sharks. Hence, it is not unusual to find such low protein levels in certain sharks, such as the grayfish (Morris, 1975).

Stansby (1976) observed that the primary causes for variations in proximate composition are the extent to which energy is expended and the feed intake. This could, however, reflect upon the changes in protein and lipid values of grayfish.

Fatty acid analysis

The fatty acid profile of grayfish is shown in Table 2. The total saturated fatty acids in the male were 70% higher than in the female, with higher amounts of palmitic (16:0) and stearic (18:0) acids. Such high levels of these fatty acids have been reported by Ackman (1974) for seerfish and pomfret from the Indian Ocean. However, the levels of total monoenes in the male were 40% lower than the female. The major differences were in the 16:1, 18:1 and 20:1 fatty acids. Of these monoethylenic acids, palmitoleic (16:1) and oleic (18:1) acids are readily digested and assimilated by man, and also the dietary contribution from marine sources is not significantly different from that of most other sources such as vegetable oils. The remaining two monoenes 20:1 and 22:1, act as energy reserves in most fish species and their levels vary accordingly (Stansby, 1967). Among the polyenes present, 22:6 appeared to be the principal long-chain polyunsaturated fatty acid, followed by 20:5, in both male and female grayfish. The remaining fatty acids 22:5ω6 and 22:5ω3, 15:0, 15:1 and 16:2 were present in trace amounts.

The total amount of lipid in fish may vary with the season, sex, food intake and breeding cycle (Kinsella et al., 1975). This could reflect upon their fatty acid content and the differences among the sexes. The fatty acids of dogfish have been reported by Exler and Weihrauch (1974) in their comprehensive study of various fish species. Their profile appears to coincide with the fatty acid pattern of the female grayfish in Table 2.

Cholesterol levels

Both the male and female grayfish showed low cholesterol values (Table 2). The average cholesterol level in the male fish was 60.6 mg/100g edible portion and that in the female fish 75.5 mg/100g edible portion. Ackman (1974) reported cholesterol levels for dogfish between 28.4 and 73.0 mg/100g muscle. In comparison to other food fish, Kritchevsky et al. (1967) reported cholesterol values between 75–95 mg/100g muscle, for haddock, pollack and salmon and over 140 mg/100g muscle values for shellfish. Such high values for crustaceans have also been reported by Sidwell et al. (1974).

Mineral composition

Seven microelements (Mn, Ni, Cd, Zn, Cu, Fe and Hg) and three macroelements (Ca, Mg and Na) were determined by atomic absorption in the freeze-dried tissues of male and female grayfish. The discussion, however, will focus on the average wet weight concentrations shown in Table 3. These values are obtained by adjusting the moisture levels to 71.5% (Table 1) in the freeze-dried samples.

Manganese plays an important role in metabollic processes (O'Dell and Campbell, 1971). Manganese levels in the

Table 3—Mineral composition of male and female grayfish (Squalus acanthias) per 100g of edible portions[a]

	μg					mg				
	Mn	Ni	Cd	Cu	Hg	Ca	Mg	Na	Zn	Fe
Male	71.30	2.90	1.10	42.81	45.60	6.01	31.35	19.38	0.32	0.66
Female	102.60	2.90	1.10	71.30	31.40	3.71	15.68	13.54	0.29	0.36

[a] Average value of duplicate analyses

male and female grayfish averaged 71.3 μg/100g and 102.6 μg/100g, respectively, based on wet weight.

Nickel is considered one of the essential trace elements (Underwood, 1971) for the human body. In grayfish nickel levels averaged 2.90 μg/100g edible portion. Higher Ni levels have been reported for other finfish by Sidwell et al., (1978). Among the squalidae species, cadmium levels of 20 μg/100g have been reported (Sidwell et al., 1978). The observed Cd values were 1.1 μg/100g, which are considerably lower.

Copper levels averaged 42.81 μg/100g in the male and 71.3 μg/100g in the female fish, which appears to provide about 2% of the daily copper requirement (2.5 mg/day) for humans.

Since the Minamata Bay tragedy in Japan, the effects of chronic exposure to mercury have been well documented (Holden, 1973; Newberne, 1973; Hancock, 1976). But there is still concern regarding high mercury content for certain marine species, especially with those fish which live to a long period of years. Since grayfish is known to inhabit the oceans for as long as 25–30 yr (Jensen, 1966) they tend to accumulate heavy metals including mercury. The observed mercury levels in the male were 45.6 μg/100g and those in the female, 31.4 μg/100g edible portions. These concentrations are considerably lower than those reported by Forrester et al., (1972) where male and female grayfish contained 168 and 196 μg Hg/100g edible portion, respectively. In their comprehensive literature review, Sidwell et al. (1978) reported mercury levels of 80 μg/100g for dogfish shark. The present Food & Drug Administration limit for mercury in foods is set at 1.0 parts per million.

Generally, calcium levels are very low in finfish. The observed calcium values for male and female grayfish averaged 6.01 mg/100g and 3.71 mg/100g edible portion, respectively. Magnesium levels varied considerably in grayfish. Males and females contained 31.35 mg/100g and 15.68 mg/100g edible portion of magnesium. Very low levels of sodium were also observed, permitting its inclusion in low sodium diets.

Zinc, an important trace element which is gaining attention in human nutrition (Burch et al., 1975) was present at levels to provide 2% of the U.S. RDA (15 mg/day) per 100g edible portion. One of the most abundant elements in the body is iron (Reinhold, 1975) which was present at 0.66 mg/100g and 0.36 mg/100g edible portion in the male and female grayfish, respectively.

Keeping quality in ice

Spoilage of fish is the result of a whole series of complicated changes brought about in the tissue by its enzymes, bacteria and chemical action. Grayfish differs from other fish in that they contain large proportions of NPN mainly in the form of urea, which decomposes to ammonia, thus making the flesh inedible. This species, hence, exhibits a rapid postharvest deterioration. The chemical and microbiological changes in dressed grayfish were monitored by determining NPN, urea concentrations and bacterial populations (Fig. 2). During this storage period sensory observations were made for appearance, odor and overall condition of the fish.

The initial NPN concentration of 800 mg/100g tissue

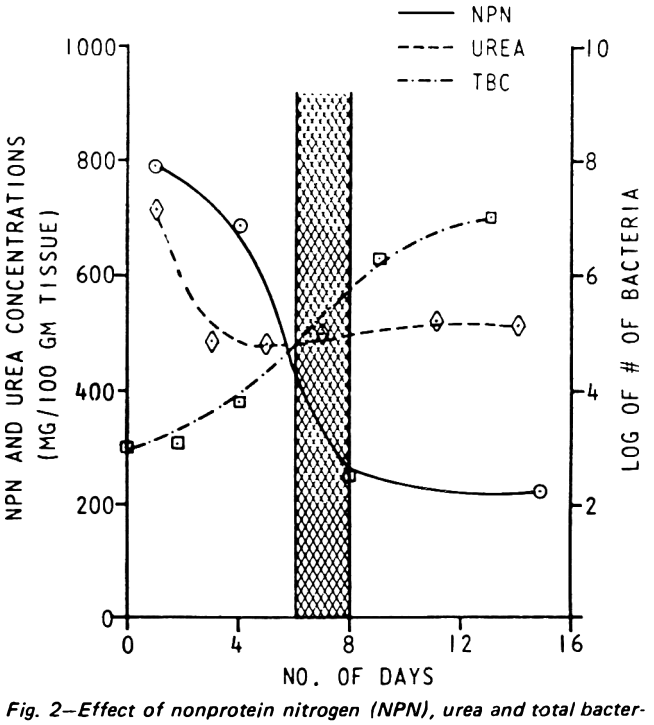


Fig. 2—Effect of nonprotein nitrogen (NPN), urea and total bacterial counts (TBC) of dressed grayfish, stored in ice. The cross-linked bar indicates the onset of spoilage, as determined by sensory observations.

was observed. These levels were depleted till the eighth day, after which there was a marginal decrease. After 4 days of ice storage urea levels decreased to 450 mg/100g tissue. following the storage up to 2 wk, no further degradation of urea was observed. Urea, a major component of nonprotein nitrogen is broken down principally by bacterial action to form ammonia (Elliot, 1952).

In the case of bacterial counts, the initial load was 1.1 x 10^3 microorganisms/g tissue (log of no. of bacteria = 3.04), which increased gradually to 1.0 x 10^7 microorganisms/g tissue (log of no. of bacteria = 7.0) by 13 days. Furthermore, the sensory observations indicated no signs of spoilage until after 7 days. During the first week, the fish appeared fresh with no signs of sliminess on the skin. At the end of 7 days, ammonia odor along with slimy skin were observed among the stored fish. Hence these observations, along with the NPN, urea and total microbial counts, was conductive to obtain the shelf life of grayfish between 6 and 8 days under these specified storage conditions.

Frozen storage stability

Among the important criteria that influence the quality of frozen fishery products are composition of fish, prefreezing treatments, methods of freezing and the environment to which the product is exposed during frozen storage and handling (Stansby, 1976). Effect of prefreezing treatment on grayfish fillets was determined by following the changes that took place during frozen storage at −15°C. Though the NaTPP and ascorbate dip was successful in

retarding the rancidity and decreasing the thaw drip (Table 4), the sensory response of the taste panel was ambiguous. After 15 wk of storage at −15°C, the panelists could detect changes in flavor, texture and odor for both the pre-treated and control samples of grayfish (Table 5). The analysis of variance showed significant differences for these characteristics at $P < 0.05$, whereas no changes were observed for appearance. Na-tripolyphosphate is known to minimize drip loss.

CONCLUSION

IN THIS RESEARCH an attempt has been made to study the chemical and technological properties of grayfish (*Squalus acanthias*). Though this fish appears low in protein because of high urea content, it could still provide a useful food resource in many parts of the world. The keeping quality in ice was determined between 6 and 8 days in ice whereas the frozen storage of fillets did not exhibit organoleptic stability. Information concerning the fatty acids, cholesterol levels and mineral distribution is also provided.

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Table 4—TBA value and percent drip of control and pretreated fillets at the end of 4 months cold storage (−15° C)

Sample	TBA[a,b]	% Drip
Control	5.3	16.8
Pretreated	3.8	10.1

[a] mg Malonaldehyde/1000g tissue of triplicate analyses
[b] Initial TBA value = 1.0 mg malonaldehyde/1000g tissue

Table 5—Sensory scores[a] of control (C) and pretreated (T) grayfish fillets during cold storage (−15° C)

	Appearance		Odor		Flavor		Texture	
Sample	0 Day	15 Wk	0 Day	15 Wk	0 Day	15 Wk	0 Day	15 Wk
C	0±0.0	−0.3±0.82[b]	−2.8±0.67	−0.4±0.84[c]	−2.9±0.33	−0.8±0.79[c]	0±0.0	−1.7±0.48[c]
T	0±0.0	−0.5±0.71[b]	−3.6±0.73	1.0±2.31[c]	−2.8±0.67	0.3±1.7[c]	0±0.0	−1.0±0.47[c]

[a] Scale rating of −4 to +4, with 0 being the optimum score
[b] No significant difference between 0 day and 15 wk storage period at $p < 0.05$
[c] Significant difference between 0 day and 15 wk at $p < 0.05$

Thermal Process Calculations for Different z and j_c Values Using a Hand-Held Calculator

J. KAO, D. NAVEH, I.J. KOPELMAN, and I.J. PFLUG

ABSTRACT

Equations facilitating the calculation of F_0 or process time used in the design and evaluation of thermal processes for low-acid foods packaged in hermetically sealed containers for j_c values of 0.4–2.3 and a z value of 10–26°F are presented. The relationships are based on the data presented by Hicks (1958) and Hayakawa (1970). The equations, although complex, can be efficiently used to calculate sterilization values and processing times using presently available programmable calculators. The equations were developed and tested so the maximum error in a calculated result will not be more than 5%.

INTRODUCTION

PROCESS CALCULATIONS and design of thermal processes for low-acid foods packaged in hermetic containers are usually performed by employing the procedures and formulas developed by Ball (1923), called the Mathematical Method, rather than by summing lethal rates by what is called the General Method of process calculations. In the mathematical method, process time and sterilization values are calculated directly using the heating medium temperature conditions, the appropriate tables that relate the ratio of the food product heating rate (f_h) to the sterilization value (U); and the difference between retort temperature and the temperature at the slowest heating zone of the container at steam-off (g). In general, two computational alternatives can be used: (1) the analysis is done manually and values of f_h/U vs g looked up in tables; or (2) a computer with a large memory core is used with appropriate programs and with the f_h/U vs g data stored in memory.

The adoption and use of analytical methods depends upon their accuracy and utility. In the process calculation area, utility is of prime importance since process calculations are carried out primarily by food technologists rather than engineers. The method of Ball [single cooling lag factor (j_c) of 1.41] is in common use today with a z value of 18°F. The utility of the method has been increased by developing simplified tables of f_h/U vs g, nomograms (Olson and Stevens, 1939), programs for computers (Sasseen, 1969), and for hand-held calculators. Vinters et al. (1975) fitted a polynomial and a hyperbolic function to these tables for the unique case where z = 18°F, applicable to a programmable calculator, thus reducing human-prone errors occurring during manual calculations. More recently, Steele et al. (1979) fitted similar functions for the same case (z = 18°F, j_c = 1.41) to a slightly modified corrected Ball table.

In the presently used calculation procedures, reasonable accuracy is attained only for values of j_c near 1.41. Using the f_h/U vs g tables of Stumbo (1973), accuracy is improved but utility is low. Consequently, there is a need for a calculation method that will have the simplicity of the presently used methods but will have the flexibility of using a range of j_c and z values.

In this paper we present functions applicable to programmable hand calculators that make possible thermal process calculation and design for a wide range of j_c and z values.

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ANALYTICAL METHODS

Calculation of the sterilization process value

The sterilization value of a thermal process is commonly denoted in terms of F_0 values, an F_0 value being the number of minutes to destroy a specified number of spores at 250°F when z = 18°F (NCA, 1968), where z is the number of °F required for the thermal death time curve to traverse one log cycle. When the z value is different than 18°F (Perkins et al., 1975; Townsend et al., 1938) and the reference temperature (T_R) is not 250°F, a nonspecific value, F (T, z), is used to express the value of the sterilization process.

The F value is obtained by integrating Eq (1) over the entire process time, t_p.

$$F(T, z) = \int_{t=0}^{t=t_p} 10^{\frac{T - T_R}{z}} dt. \quad (1)$$

Knowledge of the time-temperature history in the slowest heating zone of the container, necessary for successful evaluation of Eq. (1), is obtained using the first term approximation to the solution of the transient heat transfer equation. Two heating parameter describe this curve:

j – a dimensionless intercept value or lag factor (heating – j_h, cooling – j_c; and
f – time in minutes for the straight line of the semi logarithmic [heating (h)/cooling (c)] curve to tranverse one log cycle.

Ball (1957) evaluated this integral [Eq (1)] numerically and expressed the sterilization value obtained in terms of equivalent time at retort temperature, U. He selected a j_c value of 1.41 for positioning the straight-line cooling curve during the early curvilinear cooling period and tabulated the results for different z, g, and m + g [the difference between heating medium (T_1) and cooling medium (T_2) temperature (Ball, 1923)] values. The limitation in the use of these tables occurs: (1) when j_c is significantly less than 1.41, such as for rapid heating products (convection-heating liquids) and (2) when j_c is greater than 1.41 for slow-heating products (conduction-heating viscous or solid products). In the first case an actual j_c of less than 1.41 may result in underprocessing; in the second case a significant overdesign may occur. These deviations are more pronounced when the contribution of the lethality attained during cooling is greater, i.e., at large g values.

Hayakawa (1970) developed functions describing the initial cooling phase for j_c of 0.4–3, which can be used with the general method to calculate sterilization values when $j_c \neq 1.41$ (Holcomb and Pflug, 1976). A major limitation in the utilization of these functions is that costly numerical integration is necessary to obtain sterilization values from the time-temperature data (Table 2).

Developing the Function

The sterilization value accumulated during the linear portion of the heating curve was calculated using the first term in the Hicks (1958) integral, developed by Ball (1923).

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$$U_{hL} = f_h \frac{100 \times 10^{(-30/z)}}{2.3026} \left[-Ei\,(-2.3026g/z)\right]. \qquad (2)$$

where U_{hL} is the equivalent minutes at retort temperature accumulated during the linear portion of the heating curve and g is the difference between retort temperature and product temperature at the slowest heating zone at steam-off, and

$$-E_i\,(-x) = \int_x^{\infty} \frac{e^{-t}}{t} dt. \qquad 0 < x < \infty. \qquad (3)$$

The second term in this function, (−Ei 2.3026jI/z) (where I is the initial difference between the product and heating medium temperature), is negligible (Hicks, 1958) except when nonlinearity of the semilogarithmic heating curve occurs at temperatures above 200°F.

Contribution of the initial (curved) and linear cooling period to the sterilization value of the process was calculated by numerically integrating Eq (1) using time-temperature curves generated from Hayakawa's (1970) functions. The sterilization value obtained during heating and cooling was summed to yield an overall sterilization value (U) for a product with a given time-temperature history.

RESULTS & DISCUSSION

Function developed

Fifty-two thousand heating curves were constructed and analyzed to provide a data base matrix with values of f_h/U for j_c values of 0.4–2.3, g values ranging from 0–18°F, and z-values of 10–26°F. These were based on a single value of retort processing to cooling temperature difference of 180°F and assuming that $f_c = f_h$.

Several nonlinear functions (in the parameters) were fitted to the data using nonlinear least-square analysis; however, the stringent accuracy criterion that the function could deviate less than 5% at *all* points could not be satisfied. Thus, after partitioning the data, linear least-square data-fitting programs (CURVFIT and LSCFIT) were used in fitting a polynomial to the data for $g > 0.1$ and a hyperbolic function for $g < 0.1$.

Orthogonalization of the polynomial using Chebychev's method (Carnahan et al., 1969) could not reduce the number of terms used while maintaining the function within 5% of all data points.

The final form of the function-relating f_h/U to g and z is shown in Table 1. It should be noted that although a

Table 1—Functions relating f_h/U to g, j_c, and z

(i) $g < 0.1$: Function FOLG

$$(f_h/U) = \frac{1}{Term_1 + Term_2 - \log g}$$

where $Term_1 = \dfrac{z}{0.397408z + 23.0903}$ and $Term_2 = 2.21464E-2 \cdot j_c z^{0.480238}$.

(ii) $0.1 \leq g < 18$: Function FOHG

$$(f_h/U) = 10^Y \text{ where}$$

$x = \log g$,

$$Y = (P_6x^6 + P_5x^5 + P_4x^4 + P_3x^3 + P_2x^2 + P_1x + P_0)(1 + 0.005x),$$

$$P_6 = \frac{1}{\dfrac{z}{-1.25361E-3 \cdot z + 0.254183} + j_c\,(-2.003E-4 \cdot z^3 + 1.53064E-2 \cdot z^2 - 0.285299z + 0.514768)},$$

$$P_5 = (0.747153z^{-1.08663})_{j_c}\,(-3.69125E-3 - 3.00574E-3 \cdot z),$$

$$P_4 = \frac{1}{\dfrac{z}{5.89162E-3 \cdot z + 1.07885} + 3.03991E-2 \cdot j_c z^{1.48343}},$$

and for $0.4 \leq j_c \leq 1.0$

$$P_3 = 2.38103E-2 + \frac{1.93872}{z} + \frac{j_c}{(-311.948 + 0.912685z)},$$

$$P_2 = 9.02651E-2 + \frac{3.12655}{z} + j_c(-4.30715E-3 - 6.44606E-5 \cdot z),$$

$$P_1 = 1.92705z^{-0.433877} + j_c(-4.4481E-3 - 1.48436E-4 \cdot z),$$

$$P_0 = .767263e^{-8.90684E-2 \cdot z} + j_c(-2.5255E-3 - 263112E-4 \cdot z),$$

and for $1.0 < j_c \leq 2.3$

$$P_3 = \frac{j_c}{\dfrac{j_c z}{3.646E-3 \cdot z + 1.65847} - 1.24626E-3 \cdot z^2 - 0.128509z + 0.394849},$$

$$P_2 = \frac{j_c}{0.52037 j_c z^{0.782709} + 0.115256 - 6.41463E-2 \cdot z},$$

$$P_1 = \frac{j_c}{0.545852 j_c z^{0.484264} + \dfrac{z}{-0.848294z - 38.2992}},$$

$$P_0 = -2.92074E-5 \cdot z^3 + 2.1796E-3 \cdot z^2 - 6.37979E-2 \cdot z + 0.65144 + \frac{1}{(11.0583 - 4.37159E-2 \cdot z) j_c}.$$

relatively large number of constants are necessary to meet the 5% accuracy criterion at all points, the polynomial form minimizes calculation time.

In Fig. 1 are shown f_h/U vs log g values for three conditions and the comparative values calculated using the new function, FF (FOHG and FOLG). Solid Line I is values calculated from Ball's tables for j_c = 1.41 and a z of 18°F; the open circles are from the new function, FF. Solid Line II is values calculated using the Hicks integral and Hayakawa cooling function for a j_c of 0.5 and a z of 14°F, representing extreme parameter (j_c and z) values, where maximal deviations between fitted and calculated values are expected, especially at large g's; the open circles are values calculated using the new function. Solid Line III is values calculated using the Hicks integral and Hayakawa cooling function for a j_c of 2.04 and a z of 22°F, representing the j_c characterizing a cylindrical can filled with a conduction-heated food cooled in water, here, for a large z value; again, the open circles are values calculated using the new function. In Figure 1 we can observe the close agreement between data values calculated by the new functions. FOHG and FOLG, and by existing methods. The displacement of Curves II and III from Curve I indicates the magnitude of error that can exist if all problems are solved assuming a j_c of 1.41 and a z value of 18°F when in reality the j_c is 0.5 or 2.04 and z is 14 or 22°F. This displacement may be significant, especially when cooling contribution to process lethality is large (i.e., large j_c and g values).

Computer implementation

An algorithm for computer implementation, facilitating both process design and evaluation, is presented in Fig. 2.

Process evaluation. When the heating and cooling rate parameters (f_h, f_c, j_h, j_c) and process time, t_B, are given, the lethality of the process can be calculated, provided the z value as well as T_o, T_1, and T_2 (where these are the initial, retort, and cooling water temperatures respectively) are given, by simple substitution of the appropriate values into the function.

Process design. When a target F_o is supplied, given the heating and cooling rate parameters (f, j) and temperature (T_o, T_1, T_2) parameters, the Ball process time, t_B, can be calculated for different j values and z values using the same function. This is done by an iterative trial and error procedure in which a t_B is assumed, a resulting F_o calculated, which is compared to the target (F_o), a new t_B is assumed, and so forth until the target F_o is satisfactorily close to the calculated F_o (See Fig. 2). Convergence is usually attained after 10–15 iterations, depending on the degree of accuracy needed.

Computer programs. A program based on this algorithm was developed using a HP-41C hand-held calculator equipped with two memory modules and in Fortran using a CDC CYBER 74.

Comparison of computation times required, for process design and evaluation when using the proposed function, to those incurred in direct use of the combined Hicks-Hayakawa function using a CDC CYBER 74 and an HP-41C is presented in Table 2. The ratio of 200 in Hicks-Hayakawa/Fitted Function computation time suggests that although the large number of constants involved in the new function requires at least a programmable calculator for practical use, once the program is stored in the calculator/computer it is extremely efficient and cheap to run.

CONCLUSIONS

DESIGN AND EVALUATION of thermal processes for a wide range of values of j_c and z is facilitated by use of the two new functions, FOLG and FOHG, that have been developed. These functions are based on a combination of the Hicks (1958) heating and the Hayakawa (1970) cooling functions.

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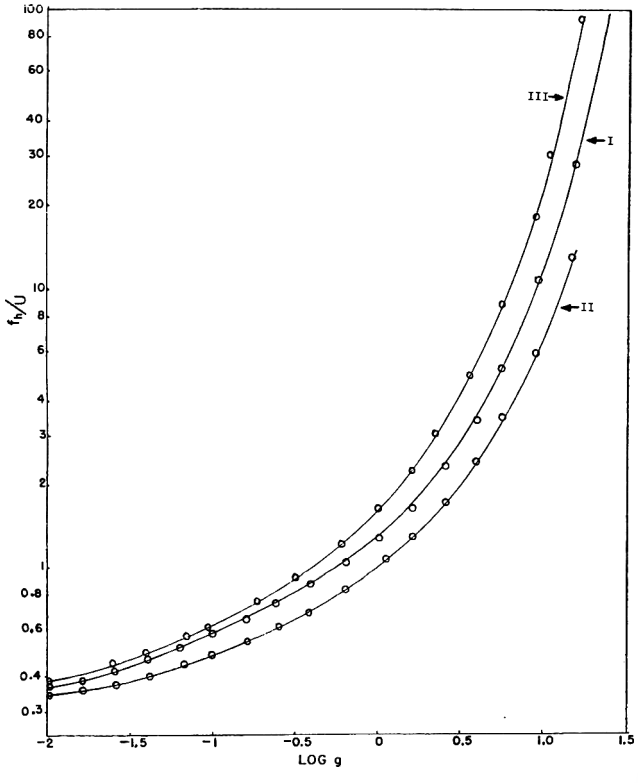


Fig. 1—f_h/U vs log g values based on: (a) Ball's tables for I (j_c = 1.41, z = 18); (b) Hicks' heating and Hayakawa's cooling for II (j_c = 2.04, z = 22) and III (j_c = 0.5, z = 14; and (c) functions FOLG and FOHG — o.

Table 2—Average computation times used in F_o (process evaluation) and t_B (process design) calculations using the functions of Hicks and Hayakawa (HH) and the Fitted Function (FF)

Method of evaluation	Computer used	Desired parameter	Avg computation[a] time x 10^3 (sec)	Comments
HH	CDC CYBER 74	F_o	72.00 cp	—
FF	CDC CYBER 74	F_o	0.36 cp	—
HH	CDC CYBER 74	t_B	1,080.00 cp	Accuracy of 1% in t_B was used as convergence criterion.
FF	CDC CYBER 74	t_B	5.40 cp	—
FF	HP—41C Hand Calculator	F_o	< 20	These calculations cannot be done on hand calculator using HH functions.
FF	HP—41C Hand Calculator	t_B	< 300	These calculations cannot be done on hand calculator using HH functions.

[a] Average of 250 runs

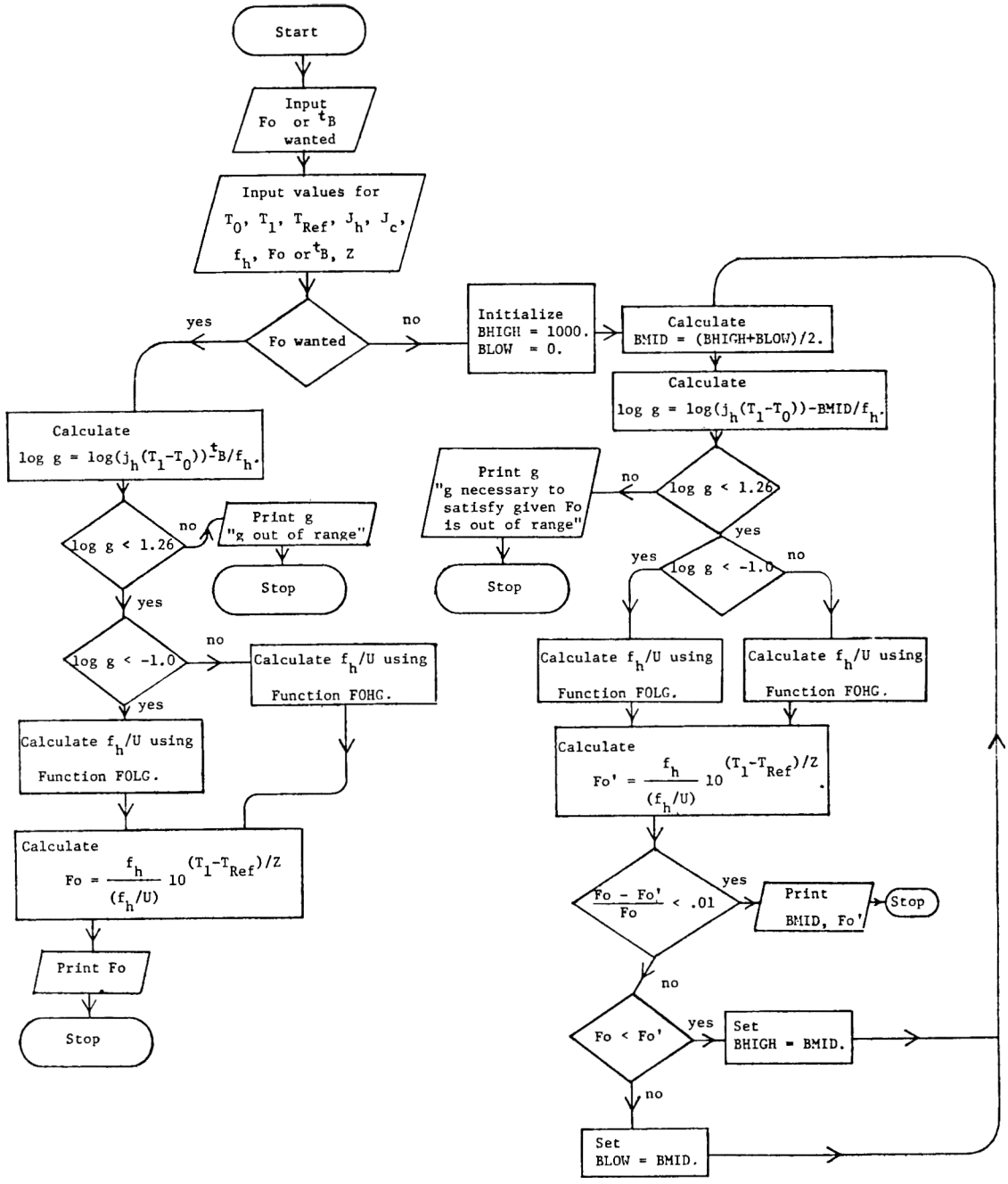


Fig. 2—Algorithm for calculation of F_O or B using hand calculator or computer.

The direct use of the Hicks-Hayakawa functions is possible only with a powerful computer. However, the fitted functions can be readily implemented using either a computer or a programmable calculator; in either mode the resulting answers have a sufficiently high degree of accuracy for practical purposes.

NOMENCLATURE

$F(T, z), F_O$

The equivalent time at temperature T delivered to a container or unit of product for the purpose of sterilization calculated using a specific value of z. F_O indicates that the temperature was 250°F and the z value was 18°F.

f_h, f_c

The temperature response parameter (f) is the time required for the straight line fitted to the log-linear portion of a heating or cooling curve to traverse one log cycle; it is the time required for the temperature difference between product and heating or cooling medium to decrease by 90%; f_h identifies the heating and f_c the cooling parameter.

g

The heating medium temperature minus product temperature at the slowest heating zone at the end of heating; $g = T_1 - T$ where T is measured at steam off.

j_c

The cooling lag factor.

m

The difference between product temperature in slowest heating zone at steam off and cooling medium temperature.

$P_0, P_1, P_2, \ldots P_6$

Coefficients in the function FOHG.

$T, T_0, T_1, T_2, T_B, T_R$

T is the variable product temperature. T_0 is the initial product temperature. T_1 is the heating medium temperature. T_2 is the cooling medium temperature. T_B is the product temperature at steam off. T_R is the reference temperature used in calculating sterilizing values.

t, t_B, t_p

Real or clock time, generally measured in minutes. t_B is Ball time. t_p is process time.

U, U_{hL}

The equivalent sterilizing value using the heating medium temperature, T_1, as the reference temperature and with a specified z value; $U = F(T_1, z)$. U_{hL} is the U value accumulated during the linear portion of the heating curve.

z

Measure of the direction of the thermal death time curve, the number of degrees of temperature change necessary to cause the F value to change by a factor of 10.

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Ms received 4/7/81; revised 6/27/81; accepted 7/2/81.

FATE OF HYPOCHLOROUS ACID IN SHRIMP PROCESSING . . . From page 187

Data indicate 2.82% was incorporated into the shrimp, 1.92% into the insoluble fraction of the immersion solution and 1.41% into the soluble organic molecules in the immersion solution. The total incorporation of chlorine was 6.15%. It follows that 74.35% of HOCl chlorine is in the form Cl^-. Of the portion of HOCl reacted, 7.4% (6.15/74.35 x 100) was incorporated and the balance (92.5%) was presumably used up by oxidation reactions.

The experiment was repeated several times. Control runs C and D were omitted in the routine experiments. The composite data are outlined in Table 3. The range for each average value is reported. The wide ranges observed are attributed to the noncontrollable variables inherent in the complex food systems.

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Agents of Proteolysis and Its Inhibition in Patis (Fish Sauce) Fermentation

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ABSTRACT

Use of specific proteinase inhibitors established a trypsin-like enzyme as the principal agent of proteolysis in Patis fermentation and this was confirmed by temperature, pH and rate studies. The trypsin-like activity developed a few days after fermentation was initiated, was maximal during the first month of fermentation and then declined rapidly and remained low throughout the remaining fermentation period. The decline is due mainly to inhibition by end product (amino acids and small peptides) accumulation. Initial inhibition of trypsin-like activity may be due to inhibitors in fish blood or substances produced by bacteria introduced with the fish. Bacteria are not responsible for proteolysis.

INTRODUCTION

FISH SAUCES of the Patis type are prepared by a natural fermentation of whole small fish in the presence of 25% or more NaCl. Proteolytic digestion due to enzyme activity is the primary process but there has been little definitive work on the nature of the enzymes involved or intrinsic factors controlling their activity. Several authors have suggested that visceral enzymes are principally responsible for the proteolysis (Alm, 1965; Voskresensky, 1965) but bacterial enzymes (Alm, 1965) and fish muscle cathepsins (Siebert, 1962; Siebert and Schmitt, 1965) have also been implicated.

Mesnard and Rose (1920) early showed that digestive enzymes extracted from fish used to make nuocnam hydrolyzed gelatin and coagulated beef albumens and peptone. Uyenco et al. (1952) observed a higher amino nitrogen content in Patis prepared from whole anchovies than from gutted fish and concluded that visceral enzymes are the main agents of digestion. They suggested that bacteria play only a minor role because of the high salt content of the Patis.

There is variability in the amount and activity ofproteolytic enzymes depending on the species of fish (Amano, 1962) and this affects the fish sauce making process. Trypsin in the pyloric caeca of fresh caught herring was a major source of endopeptidase activity and high levels of pepsin were found in herring stomach. However, pepsin is unlikely to be important in fish sauce production since the pH of the brine system is too high.

A number of authors have commented on the incomplete solubilization of proteins during enzymatic hydrolysis (Raa and Gildberg, 1975; Tarky et al., 1973). An insoluble residue remains at the end of the fish sauce fermentation which is still high in protein and may be used as a separate food item (e.g. bagoong). This suggests that some intrinsic control factors occur in the fermenting mass or develop during the process.

Finally, it should be noted that there are conflicting reports in the literature (e.g. Alm 1965, Saisithi et al. 1966) regarding the importance of bacterial enzymes in the proteolysis. Bacteria clearly do not flourish in the high salt environment but large numbers of cells are introduced with the fish and the question of residual enzyme activity has not been clearly resolved. The study described in this paper was aimed at providing a better definition of the enzymic process involved in fish sauce manufacture. Patis fermentations were conducted in the laboratory under controlled conditions and samples taken at intervals from these experimental batches and samples taken at corresponding time intervals from commercial fermentations were analyzed to determine the types of enzymes involved and their activity. In addition, the occurrence of enzyme inhibitors in raw materials and bacteria in the maturing Patis was tested. Experiments were conducted on Patis preparation using washed and untreated fish gut and inocula of bacteria to elucidate the role of bacterial proteolytic enzymes. A previous publication (Orejana and Liston, 1979) has described the principal chemical changes occurring during Patis fermentation.

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MATERIALS & METHODS

Fish

Anchovies (*Stolephorus spp.*) and round scad (*Decapterus spp.*) were obtained frozen from the Philippines. They were held at −10°C prior to use for up to 4 wk. In some experiments, locally caught herring (*Clupea pallasii*) purchased frozen were used. Commercially dried Philippine anchovies were used in one series of experiments after rehydration for 3 hr at 25°C.

Commercial fish sauce

Samples of Patis prepared commercially from round scad were obtained by sampling fermentation tanks in a Patis plant in Manila, Philippines.

Fermentation procedure

Fish were mixed with salt in a 4:1 ratio yielding final salt concentrations of 22–28% NaCl (approximately 4.8M NaCl). Fresh anchovies were used whole but scad and herring were passed through a meat grinder prior to mixing with the salt. The salt-fish mixtures were incubated at 37°C in one gallon screw-capped jars.

Samples were collected by decanting 10–20 ml of the supernatant liquid and filtering through Whatman No. 50 filter paper. This yielded a clear amber colored liquid. Samples were tested immediately or held at −5°C for later testing.

Analytical procedures

Protein. The method of Lowry et al. (1951) as modified by Chaykin (1966) was followed. Bovine serum albumin (BSA, Sigma Chemical Co.) was used to prepare standards.

Molecular size distribution. Gel filtration (gel permeation chromatography) following the methods described by Reiland (1971) and Eaker and Porath (1967) was used to analyze molecular size distribution of peptides resulting from proteolysis at various stages of fermentation. A chromatography tube, 30 x 2.0 cm (Pharmacin Fine Chemicals Co.) was packed with deaerated Sephadex gel suspension (SG-10) and equilibrated with 5% acetic acid solution at a flow rate of 0.40 ml/min for 4 hr. Samples of Patis were applied to the column and eluate fractions collected by an automatic fraction collector and transmission at 280 nm read in a self-recording UV analyzer, model UA-2 (ISCO). Standard compounds of known

molecular weight were run to obtain approximate estimates of elution volume for particular molecular weight ranges.

Trypsin-like enzyme assay. N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) was used as a specific substrate for the enzyme (Erlanger et al., 1961; Fritz et al., 1974). The compound p-nitroaniline which is quantitatively released has an extinction value at 410 nm of 8.8 cm^2/micromole and one trypsin unit corresponds to an extinction change ΔE_{410} of 2.93 cm^2/min nn 3 ml, i.e. Trypsin unit/L (TU/1) = (ΔE/min)/2.93 x dilution x 100.

Similarly, inhibitors may be assessed in terms of the inhibition unit. Where

$$\text{Inhibition Unit (IU)} = \frac{\Delta E/\text{min}_{\text{trypsin}} - \Delta E/\text{min}_{(\text{inhibitor present})}}{2.93}$$

values ΔE/min were calculated from the slope of a plot of extinction versus time. In the assay, silica cuvettes with 1 cm light path containing 3.0 ml of reaction mixture and held at 37°C were measured in a Gilford Spectrophotometer at 410 nm, using appropriate reagent blanks with readings at 1 min intervals.

Semi-purification of trypsin-like enzyme. Supernatant from a 1-month commercial Patis fermentation using round scad was treated with 2 volumes of acetone at 0°C. The precipitate was washed with Tris-HCl buffer at pH 8.2 containing 0.2M $CaCl_2$.

Enzyme inhibitors. Ethylene diamine tetraacetic acid, EDTA (Fisher Scientific Co.) and 8-hydroxyquinone-S-sulfonic acid, HQSA (Aldrich Chemicals Co., Inc.) were used to inhibit metallopeptidases. Iodoacetate, IAA (Siama Chemical Co.) was used to inhibit thiol-proteinases. Soybean trypsin inhibitor, STI (chromatographically prepared type I-S, Sigma Chemical Co.) was used to inhibit trypsin-like enzymes (serine proteinases).

Experimental procedures

Inhibitor experiments. Experiments were run using herring and round scad. In the herring experiments sufficient EDTA and IAA were added separately or together to 100 g fish:salt mixtures after 7 days incubation to yield concentrations of these agents of 10^{-3}M in duplicate sets. Samples were collected after 15, 25, and 53 days fermentation and analyzed for soluble protein, trypsin-like activity and by gel permeation chromatography.

The effect of the inhibitors IAA and STI on the semi-purified trypsin-like enzyme was tested to distinguish cathepsin-B activity.

Trypsin-like activity at different fermentation time. Commercial Patis samples from 1, 7, 18, 12, and 24 months fermentation were analyzed for trypsin activity. Laboratory samples from round scad fermentations were tested after 1, 6, 30, and 77 days. Laboratory anchovy Patis samples were tested after 15, 40, 70, and 140 days and Patis prepared from dehydrated anchovies (after rehydration) was tested after 1, 9, 15, 40, and 130 days.

Factors limiting proteinase activity in fish sauce. The development of inhibitory properties towards proteolysis in fermenting fish:salt mixtures was tested by mixing 14-month and 0 day Patis samples at the level of 10% and 20% 0-day sample. The mixtures were incubated at 37°C for 1, 9, and 30 days and analyzed by gel permeation chromatography.

Subsequently, supernatant liquid from 7, 8, 12, and 24-month commercially fermented round scad Patis was added to 1-month Patis from the same series at ratios of 1:1, 1:2, and 1:3 and trypsin-like enzyme activity assayed.

The effects of 7 and 8-month round scad Patis liquid on activity of standard bovine trypsin was similarly tested.

End product inhibition was tested for by adding an amino acid mixture at 0.2 and 0.3 μM/ml to the semi-purified trypsin-like enzume preparation and assaying trypsin activity. A similar test was run using the synthetic tripeptide glycyl-L-leucyl-L-tyrosine added at 50% and 66% level.

The presence of naturally occurring proteinase inhibitors in fish blood was tested in blood taken from round scad in the Philippines, frozen and transported in dry ice. Blood was thawed, centrifuged at 12,100 x *g* for 15 min in a Sorvall refrigerated centrifuge at 0°C. The serum was separated and an equal volume of 6% perchloric acid added. The preparation was brought to pH 4.7 by addition of 5M Na_2CO_3 and centrifuged again. The clear supernatant fluid from this treatment was tested against standard bovine trypsin.

Effect of microflora. This was tested by increasing and reducing the number of bacteria added to the original fish:salt mixture and analyzing subsequent Patis.

For the increased bacteria experiments, 50g whole herring was blended with sterile distilled water, suitable dilutions prepared and aliquots plated on Plate Count Agar and incubated at 37°C for 48 hr. Colonies randomly selected from the plates were removed using an innoculating loop and suspended in sterile water. The suspension of mixed bacteria was standardized to between 10 and 16% transmittance on a Spectronic 20 (Bausch and Lomb) at 355 nm. Seven ml of this suspension were added per 10g of a fish:salt mixture which was then allowed to ferment. A control mixture to which sterile distilled water was added was also prepared. Samples were collected after 15, 30, 49, and 70 days and analyzed for soluble protein and by gel permeation chromatography. They were also tested for inhibitory action against standard bovine trypsin.

Reduction of bacteria was achieved by dissecting out the intestines from one lot of herring and washing the carcasses thoroughly before mixing with salt and fermenting. Bacterial counts confirmed a significant reduction in bacterial numbers. A control fermentation using unwashed herring was also set up and both preparations were analyzed for soluble protein and for trypsin activity after 15, 49, and 70 days incubation.

RESULTS

Inhibitors

The effect of various inhibitors on the accumulation of soluble "protein" in round scad fermented for 30 days is shown in Table 1. Clearly, all inhibitors showed some effect but STI produced most marked results suggesting that proteolysis was due mainly to trypsin-like enzymes. However, the absorbance patterns of gel filtration fractions of the Patis do indicate a reduction in the amino acids in samples treated with HQSA and IAA suggesting that metalloendopeptidases are active in the clearing of soluble proteins (Fig. 1). This was confirmed by similar results obtained from herring Patis.

Trypsin-like activity

The results of analysis of commercial scad fermentation samples and laboratory fermentation samples for trypsin activity are shown in Table 2. The results from the commercial samples suggested a high initial trypsin-like activity followed by a decline to a somewhat variable but low level of activity after some months. Laboratory samples support this view but also indicate a low level of activity in the first few days followed by an increase with high activity through the first month which declined during the second and third months. Analysis of additional commercial samples from 5 and 7 months confirmed that the drop in activity occurred before the fifth month. Laboratory fermentations with anchovies seemed to follow a similar pattern (Table 3) though the fall-off was more rapid. These results suggest that there may be an early transient inhibition of trypsin-like activity and either a destruction of trypsin-like enzyme or a further inhibition after a few months.

—Continued on next page

Table 1—Soluble protein in 30-day old Patis from round scad treated with enzyme inhibitors[a]

Sample treatment[b]	Soluble protein mg/ml	% protein in control
Control	51.54	100.00
with HQSA (5 x 10^{-3}M)	41.59	80.69
with HQSA (7.5 x 10^{-3}M)	38.19	74.09
with IAA (5 x 10^{-3}M)	46.74	90.68
with IAA (1 x 10^{-2}M)	41.80	81.80
with STI (100 μg/ml)	32.84	63.72
with STI (200 μg/ml)	31.94	61.97
with STI (300 μg/ml)	13.61	26.41

[a] Inhibitors were added on 0 day of fermentation.
[b] HQSA: 8-hydroxyquinone-5-sulfonic acid; IAA: Iodoacetate; STI: Soybean trypsin inhibitor.

Nature of the enzyme(s)

The crude preparations showed a typical enzyme concentration aeffect when tested at various dilutions (Fig. 2). A Michaelis-Menten plot of partially purified enzyme mixture activity from 1 month commercial scad fermentation showed a typical pattern and yielded a Km of 0.42 x 10^{-3} which is similar to bovine trypsin. The temperature patterns were also similar with both enzymes showing a maximum activity about 60–65°C and rapid fall in activity above this temperature (Table 4). The pH responses were also quite close: each showed a maximum at 8.2, no activity at 1.0 and greatly reduced activity below 5.3 (Table 5).

The partially purified enzyme mixture was challenged by the inhibitors STI and IAA, each at two concentration levels; the results are shown in Table 6. It is obvious again from this that the major activity of the mixture is due to the trypsin-like enzyme though the endopeptidases are present and active also.

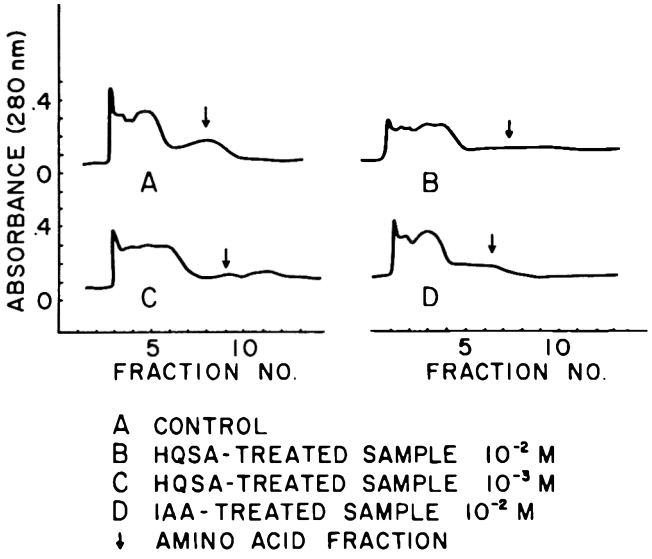


Fig. 1—The effect of inhibitors on production of amino acids in round scad Patis shown by changes in absorbance at 280 nm of fractions separated by (Sephardex G-10) gel filtration: (Arrows indicate the amino acid peaks. Inhibitors were added at 0 days and results measured after 30 days fermentation.)

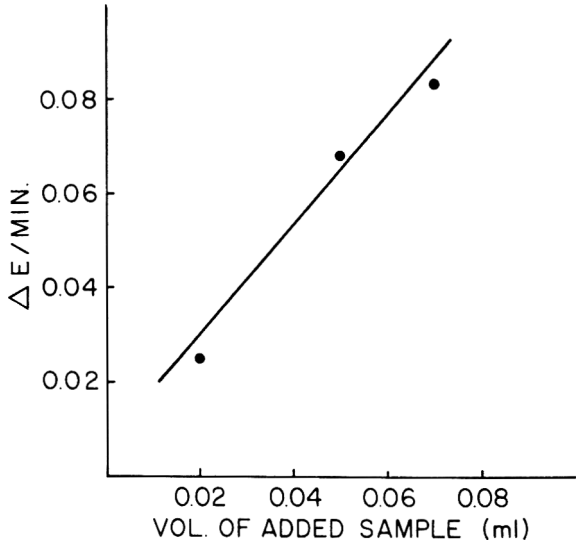


Fig. 2—Increase of trypsin-like activity from 1 month old commercial round scad Patis with increasing concentration.

Inhibition of activity in Patis during fermentation

The reduction in trypsin-like activity in the later stages of fermentation could be due to destruction of the enzyme or inhibition of its activity. Addition of Patis from later stages of fermentation to earlier stage material resulted in a marked reduction in apparent enzyme activity of the early stage sample. This is illustrated in Table 7. Moreover, the effect is also concentration related. Supernatant from 8-month Patis also inhibited the activity of pure bovine trypsin as can be seen from Table 8. Since it seemed possible that inhibition was due to the accumulation of hydrolysis end products in the Patis, the effect of a mixture of pure amino acids was tested on both fresh Patis and bovine trypsin. Results are shown in Table 9. There was considerable inhibition of the enzyme activity in the Patis but a lesser effect on the pure bovine trypsin. The effect was reversed when the synthetic tripeptide glycyl-L-leucyl-L-tyrosine was tested (Table 10) but the extent of the inhibition was somewhat limited. It seems likely from these experiments that the increase in concentration of amino acids and perhaps of small peptides which occurs during the fermentation is a major cause of the reduction in enzyme activity.

The initial low level of trypsin-like activity in fermenting fish was thought to be due to the presence of another inhibitor. Fish blood which could be expected to be present in the fresh fish/salt mixture was found to contain potent inhibitors of bovine trypsin (Table 11). However, bacteria

Table 2—Trypsin-like activity in Patis at various stages of fermentation of commercial and laboratory round scad Patis

Preparation	Time fermented	Trypsin units	Activity as % maximum
Laboratory round scad Patis	0 days	44.36	22.04
	1 "	146.75	72.88
	6 "	201.24	100.00
	30 "	109.21	52.24
	60 "	95.56	47.45
	77 "	58.02	28.81
Commercial round scad Patis	1 months	262.80	100.00
	5 "	10.20	3.9
	7 "	30.70/9.20	11.68
	8 "	6.80	2.50
	12 "	13.60	5.17
	24 "	27.30	10.39

Table 3—Trypsin-like activity from anchovy Patis[a]

Preparation	Days fermented	Trypsin units	Activity as % maximum
Rehydrated anchovy	1	71.6	67.6
	9	105.8	100.0
	15	64.8	61.2
	40	37.5	35.4
	130	23.9	22.6
Normal anchovy	15	44.36	100.0
	40	37.34	84.6
	70	6.8	15.4
	140	10.2	23.0

[a] Actual trypsin activity values for the rehydrated and normal anchovies cannot be compared directly because of differences in experimental procedures.

which are present in large numbers at the beginning of the fermentation are also known to produce trypsin inhibitors. An experiment showed that bacteria from fish would indeed significantly inhibit bovine trypsin activity in Patis within which they had been growing (Table 12). Thus it is possible that there is a variety of inhibitors of trypsin present at the very earliest stages of fermentation.

Origin of trypsin-like enzyme. Though bacteria drop quickly to very low numbers during the early phases of fish fermentation, they may be inoculated initially at a level of 10^6 or higher/gm of fish. Tests were run using herring to see whether reduction of the microflora by washing prior to preparation of Patis and enhancement by actual addition of microorganisms would affect the trypsin-like activity. As can be seen from Table 13, there was no obvious effect suggesting that the enzymes involved in the fermentation are derived from the fish.

Other factors in Patis: The concentration of metals in Patis was determined and is shown in Table 14. This was felt to be important in relation to effects on enzyme activity and stability.

The level of soluble protein produced in the Patis fermentation is related to salt concentration as can be seen from Fig. 3. This is probably due at least in part to an effect on the trypsin-like enzyme as shown by the response of bovine trypsin as shown in Table 15.

Table 4—Effect of temperature on trypsin-like activity in commercial round scad Patis (A) and on bovine trypsin activity (B)

Temp. (°C)	Trypsin Units		Activity as % Maximum	
	A	B	A	B
20	129.69	98.97	25.04	22.65
25	167.23	269.62	32.30	61.72
37	249.14	293.50	48.10	67.18
45	317.14	389.08	61.30	89.06
63	518.00	436.86	100.00	100.00
70/80	64.80	136.50	12.50	31.24

Table 5—Effect of pH on trypsin-like activity in commercial round scad Patis (A) and on bovine trypsin activity (B)

pH	Trypsin Units		Activity as % Maximum	
	A	B	A	B
1.25	–	–	–	–
5.30	88.70	98.9	29.50	36.38
6.45	136.50	116.04	45.40	43.04
6.80	163.80	129.69	54.50	48.10
7.30	184.30	218.43	61.70	81.00
7.70	221.80	228.60	73.80	84.78
8.20	300.30	269.63	100.00	100.00
10.00	279.80	228.66	93.20	84.80
11.00	136.50	139.93	45.40	51.89

DISCUSSION

THE RESULTS obtained using proteinase inhibitors specific for mechanistic sets (Walsh, 1975) clearly indicated that trypsin or trypsin-like enzyme(s) is the principal agent of proteolysis in the Patis fermentation. However, all inhibitors tested caused some reduction in soluble protein production suggesting that thiol proteinases and metalloexopeptidases are active in the system but to a much lesser degree than the serine proteases. Fish cathepsins are thiol-proteinases which have been shown to be very active in some species (Siebert and Schmitt, 1965). The pH 5–6 of fish sauce is sufficiently low to permit cathepsin activity but is too high to enable the acid-proteases such as pepsin, which is abundantly present in fish viscera (Amano, 1962), to function. The metalloexopeptidases appear to be important to hydrolysis of peptides to amino acids since the addition of metal chelating agents to the Patis interfered with amino acid accumulation. Siebert and Schmitt (1965) reported on the abundance of dipeptidases in cod muscle and Tarr (1972) has noted the isolation of decarboxylases, deaminases and transaminases from fish muscle.

Trypsin activity has been reported to be high in fish (Croston, 1965; Siebert, 1962; Cheftel, 1965). Croston (1965) reported the tryptic enzyme from the pyloric caeca of Chinook salmon was similar to mammalian trypsin and

Table 7—Inhibition of trypsin-like enzyme from commercial round scad 1-month old Patis by 7-month old Patis (CRS-3)

Sample	ΔE/min	Trypsin units (TU/1)	% Residual activity	Inhibitor[a] (Units/1)
1-mo old Patis enzyme mixture alone	0.065 ± 0.002	221.00	100.00	–
With 7-mo old Patis added at				
66%	0.050 ± 0.001	170.60	72.92	51.2
75%	0.046 ± 0.002	150.90	70.77	64.8
80%	0.033 ± 0.002	112.60	50.77	109.2

[a] $\frac{IU/1}{1000} = \frac{(\Delta E/min \text{ without I}) - (\Delta E/min \text{ with I})}{2.93} \times \text{Dilution Factor} \times$

Table 8—Effect of increasing concentration of inhibitor sample (8-month old Patis) CR-3 on activity of standard bovine trypsin

Sample	ΔE/min	Trypsin units (TU/1)	% Residual activity	Inhibitor (Units/1)
Bovine trypsin alone	0.050 ± 0.001	170.64	100.00	–
With 8-mo old Patis added at				
50%	0.044 ± 0.002	150.20	88.02	20.4
66%	0.040 ± 0.001	136.50	79.99	34.0
75%	0.036 ± 0.001	122.86	71.91	47.7

Table 6—Effect of soybean trypsin inhibitory and IAA[a] on partially purified acetone-precipitated enzyme mixture from 1-month old Patis

Sample	ΔE/min	Trypsin units (TU/1)	% Residual activity	% Inhibition
Trypsin-like enzyme mixture (TLE)	0.018 ± 0.001	61.40	100.00	–
with STI (36 μg/ml)	0.008 ± 0.001	27.30	44.40	56.00
with STI (72 μg/ml)	0.004 ± 0.001	13.60	22.20	77.80
with IAA (55.2 μg/ml)	0.014 ± 0.001	47.80	77.80	22.20
with IAA (18.4 μg/ml)	0.016 ± 0.001	54.60	88.90	11.10

[a] Inhibitors added at room temperature. Incubation time of inhibitor + enzyme is 10 minutes.

sensitive to soybean trypsin inhibitor. In general, the trypsin-like enzyme in Patis behaved very similarly to bovine trypsin even though tested only in the crude and partially purified state.

The observation that proteolysis due to trypsin-like activity takes place mostly during the first month or so of the fermentation is in accord with the reports of earlier workers (Rose, 1918; Uyenco et al., 1952; Martin and Sulit, 1955) that organic and amino N is maximal towards the end of the second month and that total dissolved combined N increases up to the fourth month. This also is in agreement with the hypothesis that the trypsin-like enzyme is responsible for the bulk of the proteolysis which is greatly reduced after two months when the enzyme is either destroyed or its activity is inhibited. Since detectable enzyme activity persists throughout the fermentation it seems likely that some inhibitory process is involved. The persistence of the enzyme suggests an unexpected degree of stability in this protease. But others have reported persistence of proteinases in food fermentations. Yong and Wood (1977) observed high protease activity still present in soy mash after 30 days fermentation at 40°C and Raa and Gildberg (1976) found protease activity in fish silage after several months at 27°C. Trypsin is known to be more stable in acid than alkaline conditions and the slightly acid pH of fish sauce may be one important factor in the persistence of the enzyme. The Ca level in Patis is quite high (Table 13) and this could help stabilize trypsin which undergoes a conformational change in the presence of this ion to yield a compact structure more resistant to autolysis (Gorini, 1951; Sipos and Merkel, 1970; Ladzunski and Delaage, 1965). However, it should be noted that trypsin may retain activity against certain substrates even after autolysis (Walsh, 1975).

Addition of old Patis with only minimal trypsin activity to Patis at an early stage of fermentation caused a marked decrease in trypsin-like activity in the latter and old Patis also caused some (but lesser) reduction of bovine trypsin activity. This clearly suggested the presence of inhibitors in the older Patis. The most likely cause of the inhibition of proteolysis is accumulation of end products. Addition of an amino acid mixture to Patis resulted in a significant inhibition of the trypsin-like activity but interestingly had little effect on bovine trypsin suggesting that a complex situation may exist in the Patis mixture. On the other hand, addition of the synthetic peptide glycyl-L-leucyl-L-tyrosine had a greater inhibitory effect on the bovine trypsin than on the Patis trypsin-like enzyme.

These observations suggest some form of end product inhibition but leave open the possibility of other causes. It is possible that the undigested protein material is naturally more resistant to enzymic digestion and it is also possible that inhibitors from blood, bacteria or other sources which may be activated in the initial phases of fermentation may be reactivated later. And again, of course, it must be accepted that some degradation of the trypsin-like enzyme occurs due to autolysis.

The brief, apparently temporary, inhibition of trypsin activity which is observed during the first few days of the Patis fermentation could well be an example of the "temporary inhibition" phenomenon described by Laskowski and Wu (1953). The reappearance of the activity would then be due to the decomposition of an enzyme-inhibitor complex which releases active trypsin and digested inhibitor. Examples of temporary inhibitors include mammalian pancreatic secretory inhibitors of the Kazel type and several pancreatic trypsin inhibitors (Laskowsky and Wu, 1953; Tsecheche et al., 1974). Blood is known to be a source of proteinase inhibitors (Heimburger, 1974) and it is not surprising therefore that trypsin inhibitors were demonstrated in serum from round scad blood. This is important because whole fish containing blood are normally used in Patis preparation.

Proteinase inhibitors have been reported from bacteria (Fossum, 1970; Wingender, 1974). Patis is frequently prepared from fish carrying large numbers of bacteria and while these organisms die off rapidly in the strong brine, their cell contents are released by autolysis into the liquid. Trypsin inhibitors were demonstrated in preparations seeded with bacteria from herring and therefore this must also be considered as a possible cause of the temporary inhibition of trypsin-like activity very early in the Patis fermentation.

The question of the involvement of bacteria in the fish sauce fermentation has long been debated. The evidence clearly indicates that living bacteria are unlikely to play more than a minor role since they rapidly die off in the high salt medium and are not replaced by large populations

Table 9—Inhibition of trypsin-like activity in Patis and bovine trypsin by amino acid mixtures

Sample	ΔE/min	Trypsin units (TU/1)	Inhibitor units (IU/1)	% Residual activity
Trypsin-like enzyme (TLE)				
Alone	0.115 ± 0.004	392.0	–	–
With AA mixture				
0.2 μM/ml	0.033 ± 0.001	95.5	296.0	24.37
0.3 μM/ml	0.028 ± 0.001	112.0	280.0	28.58
Bovine standard trypsin				
Alone	0.108 ± 0.005	368.0	–	–
With AA mixture				
(0.2 μM/ml)	0.099 ± 0.004	227.0	31.0	91.60

Table 10—Inhibition of trypsin activity by the tripeptide, glycyl-L-leucyl-tyrosine

Sample	ΔE/min[a]	Trypsin units (TU/1)	Inhibitor units (IU/1)	% Residual activity
Bovine trypsin (BT)				
Alone	0.101 ± 0.005[b]	344.0	–	–
With 50% tripeptide	0.083 ± 0.004	255.0	30.7	82.3
With 66% tripeptide	0.075 ± 0.003	283.0	44.4	74.1
Trypsin-like enzyme (TLE) (from Patis)				
Alone	0.111 ± 0.004	378.0	–	–
With 50% tripeptide	0.101 ± 0.0003	345.0	34.1	91.2
With 66% tripeptide	0.095 ± 0.004	324.0	54.60	85.7

[a] Correlation coefficient (r) = 0.986 to 0.999.
[b] Standard error

Table 11—Inhibition of bovine trypsin activity by fish blood plasma

Sample	Trypsin activity	% Inhibition
Bovine trypsin	359	–
+ Fish Plasma 0.1 ml	322	12.3%
+ Fish Plasma 0.2 ml	199	44.3%
+ Fish Plasma 0.3 ml	49	86.0%

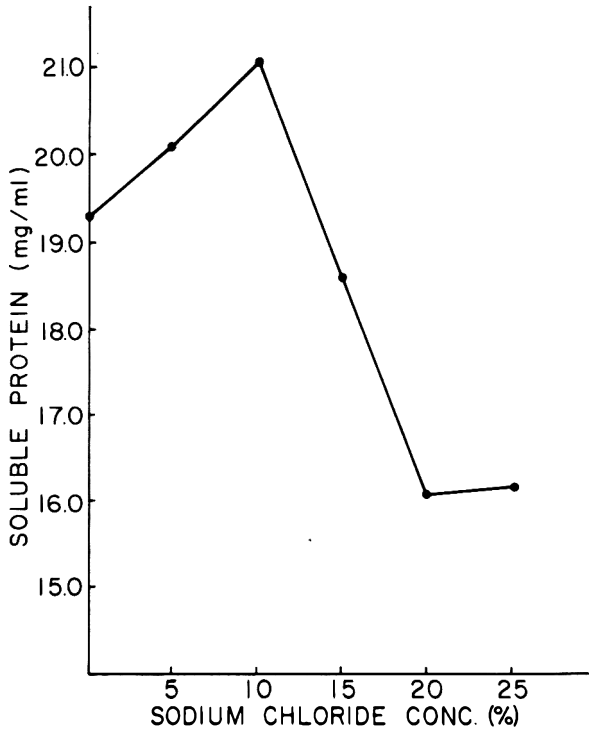


Fig. 3—Effect of increasing NaCl concentration on soluble protein produced in round scad fermentation.

of halophiles as might be expected (Orejana and Liston, 1979). The possibility that enzymes are released by autolysis from the large numbers of bacteria introduced into the fermentation mixture with the whole fish and nevertheless play a significant role in the proteolysis is largely discounted by findings of this study. Thus it appears that endogenous fish enzymes are the major and perhaps sole agents responsible for digestion in the fish sauce process.

Bovine trypsin added to Patis in which the naturally occurring trypsin-like activity had been destroyed by boiling seemed to be quite effective in restoring typical proteolysis. This raises the possibility of accelerating the fish sauce process by addition of suitable enzyme preparations. Only a little work has been done on this technique (Freeman and Hoogland, 1956; Guevarra et al., 1972; Beddows et al, 1976). While the conditions for optimum activity of the trypsin-like enzyme(s) in Patis are similar to those for bovine trypsin, as noted above, they are far removed from the normal conditions of a commercial fish sauce fermentation. The pH and the temperature are well below the optimum level and the presence of a high NaCl concentration depresses enzyme activity. While high NaCl is probably unavoidable as a protection against microbial spoilage it seems possible that pH and temperature could conceivably be adjusted nearer the optimum if a more rapid process is desired.

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Table 12—Effect of inoculating fish microflora[a] on trypsin-like activity in herring Patis and on bovine trypsin

Sample	Trypsin units	Inhibition unit	% Residual activity
Patis alone	24.00	–	
Patis + Bacteria	5.80	–	
Bovine trypsin alone	241.00	–	
Patis + Bovine trypsin	290.00	40.90	85.04
Patis + Bacteria + Bovine trypsin	197.00	133.10	57.77

[a] Fish microflora isolated from herring were inoculated in fish-salt mixture on 0 day and Patis collected after 30 days.

Table 13—Effect of fish microflora on formation of soluble protein (mg/ml) in herring Patis (LCH-3) after 49 and 70 days of fermentation

Sample	No. of days of fermentation		
	15	49	70
With added microflora	5.9	18.44	20.50
Without added microflora	4.77	17.25	19.50
Washed	19.10	24.50	26.50
Unwashed	22.69	26.50	27.00

Table 14—Concentration of metals (μg/g) in laboratory and commercial round scad Patis

	Laboratory sample LRS-1	Commercial sample CRS-3
Ca	1.5400	1.0440
Fe	0.0825	0.0574
K	9.5910	6.0870
Mg	2.8370	2.2670
Na	200.9000	146.6000
P	0.3690	0.4470
Sr	0.0010	0.0636
Zn	0.1287	0.0086
Co	–	0.0010
Mn	–	0.0033

Table 15—Effect of increasing NaCl concentration on activity of bovine trypsin standard

Time (min)	Trypsin Units (TU/1) % NaCl				
	0	10	15	20	25
10	163.80	156.99	150.17	153.58	139.93
15	111.49	106.93	100.10	100.10	86.46
25	88.73	88.73	70.98	69.62	60.07

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Heat Resistance of Proteolytic *Clostridium botulinum* Type F in Phosphate Buffer and Crabmeat

R. K. LYNT, D. A. KAUTTER, and H. M. SOLOMON

ABSTRACT

The heat resistance of proteolytic C. *botulinum* type F strains Langeland, PC and 4YRC was studied by the thermal death time tube method in phosphate buffer; 4YRC was also studied in crabmeat. Correction of exposure time for thermal lag and lethality during lag was made by the graphic method. Decimal reduction times in minutes (D) were calculated by Schmidt's probability method, using the Karber LD_{50}. For these 3 strains, D curves in phosphate buffer traversed one log cycle in 18.1–25.3°F (z); extrapolated they gave 0.14–0.23 for D_{250}. The z of the 4YRC crabmeat D curve was 23, extrapolating to 0.17–0.18 for D_{250}. Proteolytic type F, therefore, resembles other proteolytic *C. botulinum* types.

INTRODUCTION

PROTEOLYTIC *C. botulinum* type F was first isolated from a sample of home-canned liver paste that was responsible for an outbreak of botulism on the island of Langeland in Denmark (Moller and Scheibel, 1960). The Langeland strain was assumed to have a heat resistance similar to those of type A and the proteolytic strains of type B, but except for the ability of its spores to survive when heated in a boiling water bath (Dolman and Murakami, 1961), little was known about its heat resistance. Although there have been no other foodborne outbreaks caused by proteolytic type F, the organism has been isolated from raw and processed food materials (Williams-Walls, 1968; Kautter et al., 1974). Type E and nonproteolytic strains of type F were studied earlier (Lynt et al., 1977, 1979), but more information was needed about the proteolytic strains of type F. The purpose of this work, therefore, was to obtain more precise information about the heat resistance of proteolytic strains of type F.

MATERIALS & METHODS

Strains and spore stocks

The three proteolytic strains studied in these experiments were the Langeland strain, the 4 YRC strain and the PC strain. The 4 YRC strain was received from Williams-Walls in 1968, who had isolated it from a crab from the York River and designated it 4 VII (Williams-Walls, 1968). It was later renamed 4 YRC to indicate that it came from a York River crab. The PC strain was isolated from pasteurized crabmeat in the Atlanta District laboratory of the Food & Drug Administration (Kautter et al., 1974).

Spore stocks were grown at 35°C in 500-ml Wheaton bottles containing 350 ml of trypticase-glucose-yeast extract (TPGY) broth exhausted to remove oxygen just before inoculation (Lilly et al., 1971). When the cultures were fully sporulated, the spores were harvested, washed three times in distilled water and concentrated by centrifugation. The spores were finally resuspended in a small amount of distilled water for seeding 150 ml of buffer or 454g of crabmeat.

Preparation of buffer

Buffer was prepared from 0.067M solutions of Na_2HPO_4 and KH_2PO_4, which were combined with constant stirring and monitoring until a pH of 7.0 was reached. The buffer was dispensed in 150-ml quantities and sterilized by autoclaving at 121°C for 15 min. Buffer was refrigerated until used, at which time 1.0 ml of the spore stock was added to each 150 ml needed for the experiment. After being sealed and refrigerated for the duration of each experiment, at least three representative tubes of the seeded buffer were used to determine the actual spore count in that experiment. Spores were counted by the 3-tube most probable number (MPN) method, with freshly exhausted TPGY as the culture medium and one week as the incubation time. Vegetative cells were not considered to be present since the method of preparation of the spore stock makes this highly unlikely. For the majority of experiments 3 tubes were counted without heat shock and 3 after heat shock at 80°C for 10 min. Since there was no significant difference between these two sets of counts, the weighted mean of the logarithm of all counts was taken as the log of the initial spore count.

Preparation of crabmeat

Crabmeat was purchased in 1-lb (454g) cartons as needed from a local dealer as "fresh-picked crabmeat" and sterilized in 454-g portions by autoclaving 40 min at 121°C in flat, shallow pans. After being autoclaved, the crabmeat was refrigerated overnight; the following morning it was aseptically transferred to a sterile Waring Blendor and the spore stock was uniformly blended in to form a smooth paste. After being tubed and refrigerated for the duration of the experiment, at least three representative tubes, each containing 1g of crabmeat, were used to determine the spore count by the 3-tube MPN method. Counts were made without heat shock.

Experimental procedure

The thermal death time (TDT) experiments were conducted by the TDT tube technique at 210, 215, 220, 225 and 230°F. Four replicate experiments were performed for each set of conditions and the results cumulated for plotting and calculations. Each experiment was planned to cover a range of time intervals so that a gradation occurred between the last time at which surviving spores were observed in all tubes to the time beyond which no spores survived in any tube. For each experiment, 100 replicate 10 X 75 mm heat-resistant glass TDT tubes were filled with either 1.1 ml of seeded buffer from a Cornwall pipet, or 1g of the seeded crabmeat from a specially adapted grease gun (Stumbo et al., 1945). The tubes were immediately flame-sealed and refrigerated until needed. They were bundled into groups of 3 or 4, attached to a sinker to assure complete immersion in the bath, and labeled with an identifying tag. The 100 tubes were dropped, in groups of 10, into a 50-gal (378.6-L) silicone bath at the desired temperature. Precision of the bath temperature was indicated by a straight line recording produced by the monitoring copper-constantan thermocouples on a potentiometer-recorder chart. Thermocouples used for this purpose had been previously checked against a calibrated thermometer, accurate to within 0.72°F of a National Bureau of Standards thermometer, over the range of temperatures used in these experiments. Accuracy of the thermocouples was checked periodically. All tubes were removed simultaneously at the end of the experiment and immediately cooled in running tap water.

As soon after cooling as possible, but always on the same day, the TDT tubes were opened and cultured for surviving spores. Either 1.0 ml of seeded buffer or the entire contents of seeded crabmeat from each tube was cultured in a separate tube containing 15 ml of TPGY broth and a Durham tube. All cultures were incubated at room temperature; they were checked for growth every 2 or 3 days during the first 2 wk of incubation and weekly thereafter. Those which showed no evidence of growth, such as increased turbidity or gas in the Durham tube, at the end of 2 wk were sealed

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with a layer of vaspar to maintain anaerobiosis and further incubated at room temperature. If no growth occurred in 6 months, the tubes were considered to be sterile. Incubation at room temperature rather than 35°C accompanied by prolonged incubation was essential to allow enough time for delayed germination of heat-damaged spores (Dickson et al., 1925; Williams and Reed, 1942). The presence of gas in the Durham tubes was found to be a useful indicator of growth, because the medium became so turbid when crabmeat was added, that the only criterion of growth was the production of gas, which otherwise could be missed. Even when buffer was the inoculum, the period of effervescent growth might be missed because of the time between readings during prolonged incubation.

Cultures from tubes having the longest heat exposure with growth in all 10 and those with growth at longer exposure times were tested for botulinum toxin by intraperitoneal injection of 0.5 ml of undiluted culture fluid into mice. Only toxic cultures were counted. The possibility of contamination from the handling involved in filling, sealing, opening and culturing the TDT tubes required proof that the culture was from spores surviving the heat treatment. Nontoxic cultures were rarely found.

Calculations

Corrections of the time of exposure for thermal lag and lethality during lag were made by the graphic method (Anellis et al., 1954). The average of at least five heat penetration curves was plotted on inverted 3-cycle semilogarithmic paper for each temperature of the bath. Heat penetration rates were obtained by charting from a copper-constantan thermocouple located as close as possible to the geometric center of 1.1 ml of buffer or 1g of crabmeat in a TDT tube. Corresponding temperatures from these curves and the uncorrected TDT curve were the basis for the calculations. The corrected time for the last positive culture and the first time all cultures were negative at each temperature were plotted on semilogarithmic paper with time as the logarithmic (vertical) axis and temperature as the arithmetic (horizontal) axis. The TDT curve was then drawn so that all points representing the time for the last positive culture were below it and as many as possible of the points representing the first time at which all cultures were negative were above it (Townsend et al., 1938). Decimal reduction times (D) were calculated from the corrected data by Schmidt's probability method (1957). This method, based on the LD_{50} rather than the absolute end point, seemed best to describe the data because of the frequency of skips and tailing. Curves of the D values calculated from the cumulated data of replicate experiments were plotted by visual best fit on semilogarithmic paper with time and temperature along the same axes as the TDT curves. For each TDT and D curve the temperature increment in °F for one log cycle (z) was determined.

The data obtained from these experiments were subjected to statistical analysis. From the variance for each LD_{50} the upper and lower 95% confidence limits were determined for both the LD_{50} and the D values. The mean logarithm of all initial spore counts of each strain was used in calculating D values at all temperatures for that strain.

RESULTS & DISCUSSION

TABLE 1 shows the heat resistance, expressed as D values, of the PC, Langeland and 4 YRC strains of proteolytic *C. botulinum* type F. All strains were studied in phosphate buffer; the 4 YRC strain was also studied in crabmeat. Crabmeat was chosen as the food in which to study proteolytic type F because it is a possible vehicle; the 4 YRC strain was isolated from a crab and the PC strain was isolated from pasteurized carbmeat. Although the PC strain appeared to be a little more heat resistant than Langeland at the temperatures studied, the D values of the strains are nearly the same. Since the 95% confidence limits of the D values of these two strains overlap, there is probably no significant difference in their heat resistance. On the other hand, the 4 YRC strain is less heat resistant than the others at these temperatures. It is only about half as heat resistant as the PC strain at the lower temperatures, but comes closer as the temperature is raised. Compared to phosphate buffer, crabmeat has little effect on its heat resistance. The D values are very nearly the same.

Fig. 1 shows the thermal death time curves for all three strains in phosphate buffer along with the log of the initial spore count. For the PC strain it was possible to draw curves between all pairs of points representing time to the last positive culture and time to the first interval at which all cultures were negative so that the z values ranged from 20.0–21.5. The curve shown has a z of 20. However, it was impossible to draw curves for the Langeland and 4 YRC strains so that they fell between all pairs of points. They were drawn so that they were above all points representing the time to the last positive culture and below as many points representing the time to the last positive culture and below as many points representing all-negative cultures as possible (Townsend et al., 1938). Those that gave the best fit for the latter two strains have z values of 22.1 and 21.1. respectively. *–Continued on next page*

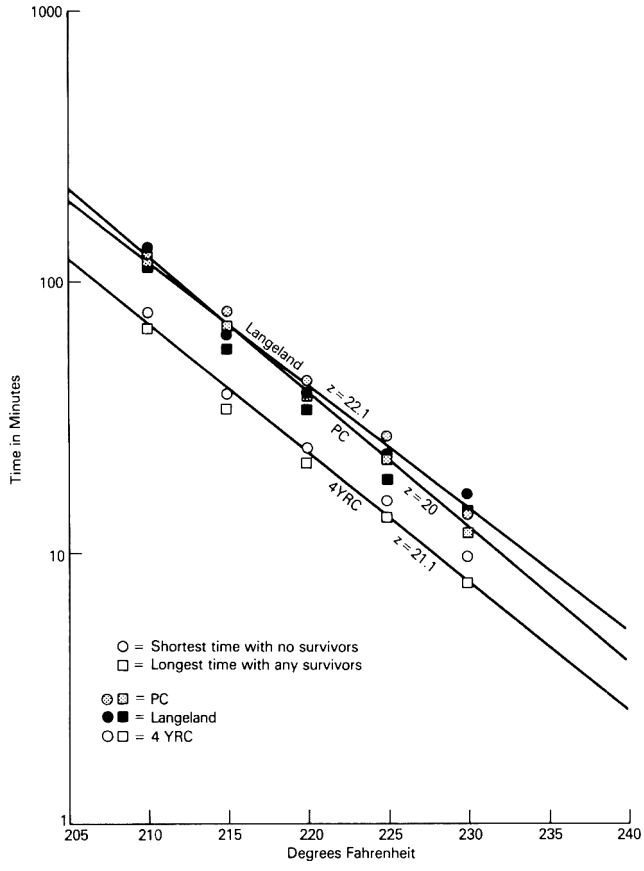


Fig. 1–TDT curves of proteolytic C. botulinum type F in 0.067M phosphate buffer, pH 7.0. Mean log of initial counts: PC, 4.036; Langeland, 4.897; 4YRC 4.159.

Table 1–D values of three proteolytic strains of C. botulinum type F in 0.067M phosphate buffer, pH 7.0

Temp (°F)	Strain: PC in 0.067M PO_4	Strain: Langeland in 0.067M PO_4	Strain: 4 YRC in 0.067M PO_4	Strain: 4 YRC in Crabmeat
210	23.22(±5.23)[a]	19.38(±5.23)	12.19(±4.16)	
215	12.02(±5.74)	8.76(±2.39)	5.35(±2.42)	5.07(±2.80)
220	6.33(±2.86)	4.74(±2.12)	3.55(±2.04)	4.02(±2.41)
225	3.33(±2.27)	2.60(±0.90)	2.09(±1.00)	2.14(±1.08)
230	1.82(±1.28)	1.79(±1.16)	1.45(±0.70)	1.35(±1.06)

[a] 95% confidence limits in parentheses.

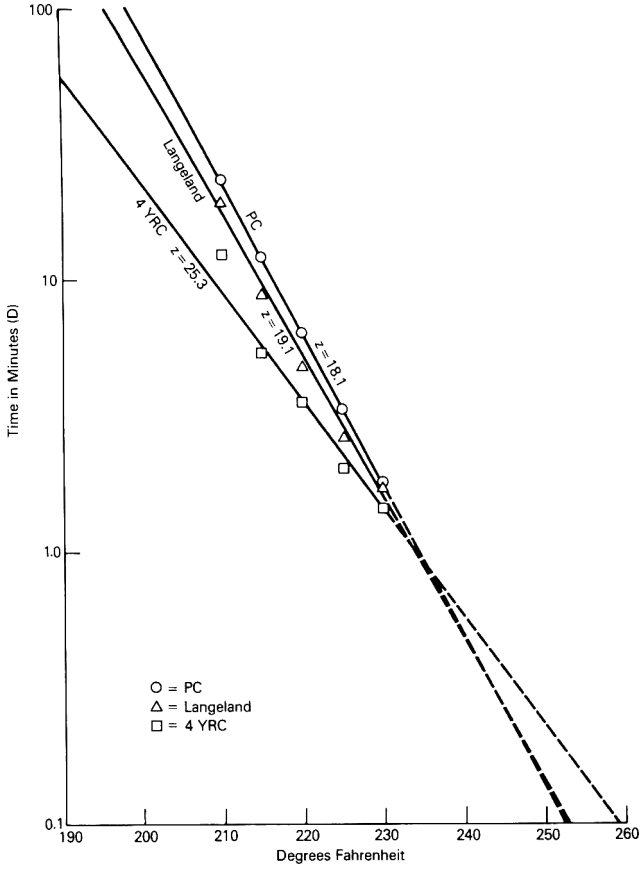


Fig. 2–D curves of proteolytic C. botulinum type F in 0.067M phosphate buffer, pH 7.0.

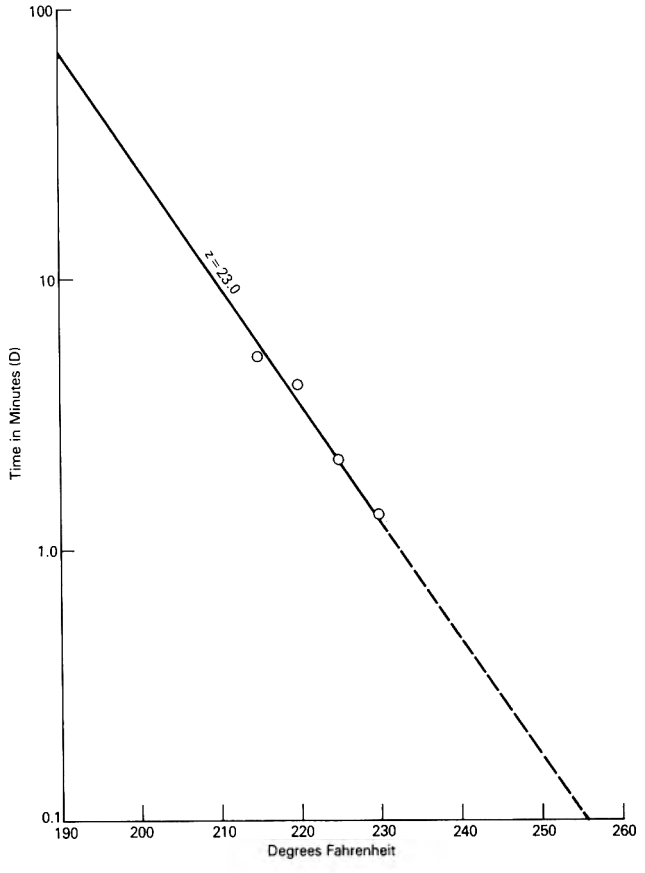


Fig. 3–D curve, proteolytic C. botulinum type F strain 4YRC in crabmeat. Mean log of initial count 4.982.

D values plotted on similar scales indicated that the thermal destruction rates for the PC and Langeland strains were nearly the same and that the 4 YRC strain was different (Fig. 2). The z values for the PC and Langeland strains are at the high end of the scale of z values reported for type A and proteolytic type B strains or those calculated from earlier published data (Esty and Meyer, 1922; Odlaug and Pflug, 1977; Odlaug et al., 1978; Perkins et al., 1975; Schmidt, 1964; Stumbo et al., 1950; Stumbo, 1973). The 4 YRC strain, although the least heat resistant at the temperature studied, has the highest z value, which is considerably above the usual range for proteolytic *C. botulinum*. However, if these curves are extrapolated to 250°F' the order of the D values is reversed and the 4 YRC strain becomes the most heat resistant with a D_{250} of approximately 0.23. The difference between the PC and Langeland strains is negligible; both have a D_{250} of about 0.14. A similar curve for the 4 YRC strain in crabmeat (Fig. 3) has a slightly lower z value of 23. This projects to a D_{250} between 0.17 and 0.18.

The results of these experiments show three proteolytic strains of type F to be less heat resistant than type A strains recently studied (Odlaug and Pflug, 1977) but considerably more heat resistant than most of the type B strains studied by the same authors (Odlaug and Pflug, 1977; Odlaug et al., 1978). Their results give a D_{230} of 2.72–2.89 for type A and 1.02–1.38 for type B, whereas our proteolytic type F strains have a D_{230} of 1.45–1.82. Earlier work (Stumbo, 1950) shows *C. botulinum*, type and strain not specified, to have a D_{220} of 10 compared to 3.55–6.33 for these type F strains and a D_{230} of 2.01 which, again, is somewhat higher than our findings for proteolytic type F.

Data from the work of Esty and Meyer, frequently used as the standard for evaluating heat resistance of *C. botulinum* spores, have been analyzed by several authors to show slightly different results for the most heat resistant strains, ranging from a D_{250} of 0.2 with a z value of 18 (Schmidt, 1964) to a D_{250} of 0.26 with a z value of 17.6 (Perkins et al., 1975). By any of these standards the heat resistance of the proteolytic strains of *C. botulinum* type F falls within the expected range for type A and the proteolytic strains of type B. Thus, thermal processes adequate to destroy other proteolytic strains of *C. botulinum* would also destroy type F. Since the z value of the D curve is relatively high, at lower temperatures type F is relatively less heat resistant than the strains of type A and B on which heat processing of canned foods is based. Consequently slight underprocessing, should it occur, would be less likely to result in a toxic product from these organisms than from type A or B. This may partly explain the rarity of botulism caused by type F.

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Sensory Attributes and Instron Measurements of Reduced-Nitrite Poultry Frankfurters With Sorbic Acid or Potassium Sorbate

EDGAR CHAMBERS IV, JANE A. BOWERS, KENNETH PRUSA, and JEAN CRAIG

ABSTRACT

Poultry frankfurters (0 and 40 ppm nitrite) with sorbic acid or potassium sorbate were studied. Flavor characteristics were influenced by nitrite but not by ascorbic acid or potassium sorbate. Frankfurters with sorbic acid were softer and those with potassium sorbate were firmer than those without. Nitrite alone increased firmness. Sorbic acid appeared to counteract that increased firmness and potassium sorbate enhanced the increase in firmness when those compounds were used in combination with nitrite in the poultry frankfurters. Simulated-teeth attachments for the Instron gave results similar to sensory firmness scores. Frankfurters with sorbic acid added had lower pH than frankfurters from other treatments.

INTRODUCTION

CONSUMERS have been concerned for 8–10 yr about the safety of nitrites in food products, with intensified concern the last 2 yr. Sodium nitrite, which delays production of *Clostridium botulinum* toxin, is responsible for developing cured-meat color and flavor. Recent investigations (Huhtanen and Feinberg, 1980; Sofos et al., 1979a, b; Sofos et al., 1980a, b) have shown that the toxin may be inhibited at lower nitrite levels (40 μg/g) when sorbic acid or potassium sorbate is present, with effectiveness partially pH-dependent (Sofos et al., 1980a; Huhtanen and Feinberg, 1980).

Adding sorbic acid or potassium sorbate to cured meat products may be a way to reduce the nitrite necessary to inhibit *Clostridium botulinum* toxin production. Adding those substances affects other quality characteristics. It is desirable to maintain inherent sensory characteristics of cured meat products while safely reducing nitrite levels.

Few studies have reported sensory characteristics of cured meat products containing sorbic acid or sorbate. Kemp et al. (1979) treated hams by dipping them into 2.5% potassium sorbate solution and then curing. Their sorbate-treated hams scored higher on a hedonic scale for flavor and overall satisfaction, and were less salty. Paquette et al. (1980) reported on consumer evaluations of bacon prepared with different amounts of sodium nitrite and potassium sorbate. Adding 0.26% potassium sorbate did not affect the "degree of liking" (hedonic scale), color, or sensory characteristics.

Chambers et al. (1981) reported that frankfurters with 40 ppm nitrite containing sorbic acid could be distinguished from those without added sorbic acid in triangle-difference testing by a trained laboratory panel. Flavor profiles showed lower flavor amplitude, more "meatiness," and lower cured character for frankfurters containing sorbic acid than for frankfurters without sorbic acid. No information on textural characteristics was found in the literature.

Sofos et al. (1980a) suggested that residual nitrite was depleted slower in chicken frankfurter-type samples with sorbic acid present than in samples with no sorbic acid, but that trend was not observed in other work (Sofos et al., 1979a).

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This study was designed to evaluate effects of sorbic acid and potassium sorbate in poultry frankfurters with 0 and 40 ppm nitrite on the intensity of sensory attributes, mechanical textural characteristics, and residual nitrite.

EXPERIMENTAL

Materials and treatments

The following was used for approximately 5–6 kg of frankfurters: mechanically deboned chicken meat (Tyson Foods, Springdale, AR), 2589g; turkey breast muscle, ground, (1/8 in. plate) 1476g; turkey leg muscle, ground, 276g; chicken livers, 92g; rendered chicken fat, 477g; dextrose, 157g; sodium chloride, 131g; ice water, 734 ml; nutmeg, 3.7g; mustard, 26.2g; white pepper, 10.0g; liquid smoke, 6.6g. Six treatment combinations in a 2x3 factorial arrangement were studied: no nitrite or 0.36g sodium nitrite (40 ppm), each containing no additional additive, 0.2% sorbic acid, or 0.26% potassium sorbate.

Frankfurter emulsions were prepared in accordance with commercial practice. Meat and salt were chopped with water (1–2°C) added during chopping; the fat and seasoning mixture was added and chopped further; the curing ingredients were dissolved in water and added last during chopping. Temperature of emulsion did not exceed 8°C. Emulsion was stuffed in casing (size 24), tied every 17.8 cm, and heated in a smokehouse with a heat cycle of 71°C for 15 min, then 82°C to an internal temperature of 68°C, chilled, peeled, vacuum packaged (six frankfurters per package), and held at −18°C until used (from 2–6 wk).

Before evaluation, frankfurters (in packages) were allowed to stand at room temperature 30 min, then were placed in a refrigerator for 1 hr to complete thawing. Thawed samples were heated in boiling water for 5 min.

Sensory analysis

A sensory panel of three trained-experienced members evaluated 1-cm radial cross-section of frankfurters according to a balanced-incomplete block design. Each panelist evaluated samples of three treatments in each presentation period (block). Ten blocks with five replications of each treatment completed the design. Color was masked using a 7 watt blue light above each panelist's booth. Flavor (cured-meat aroma and flavor, saltiness, spiciness) and textural (firmness) characteristics were scored for intensity on a 21-cm, structured linear scale, divided and labeled for intensity every 3 cm beginning 1.5 cm from the end. Panelists marked intesity characteristics on the scale and labeled their marks with the sample's code number. Scores were the distances (cm) from the beginning of the scale to the mark.

Instron measurements

To evaluate textural characteristics of the frankfurters we used a Warner-Bratzler shear attachment, a puncture-probe, a simulated incisor attachment, and a simulated molar attachment (Prusa et al., 1981) on the Instron Universal Testing Instrument. For all measurements a load cell pressure of 10 kg, a crosshead speed of 100 mm/min, and a chart speed of 200 mm/min were used. Frankfurters were placed lengthwise and the Instron attachments first penetrated skin and then interior of each frankfurter. Samples of frankfurters for sensory analysis and samples of frankfurters used for other analyses were evaluated giving 10 replications.

Residual nitrite and pH

Samples (10g) of poultry frankfurters were blended in 90 ml distilled-deionized water (5°C) for 30 sec in a Waring Blendor. The pH of the slurry was determined with a digital pH meter (Horizon

Ecology Co.). Then the slurry was filtered through Whatman 1 filter paper and 10 ml of acid buffer were added to the filtrate. An Orion nitrogen-ozide electrode (model 95–46) on a Fischer Accumet digital pH/ion meter (model 525) was used as a rapid determination of nitrite. The sample was stirred constantly for 2 min, then a reading (mv) was taken. A standard curve was run to determine residual nitrite concentration.

Statistical analysis

Analysis of variance was performed on the data according to the following statistical designs:

Sensory

Source of variation	df
Blocks (B)	9
Nitrite (N)	1
Additive (A)	2
N X A	2
B X N X A (Error A)	15
Panel Member (M)	2
B X M	18
Residual (Error B)	40

Instron

Source of variation	df
Nitrite (N)	1
Additive (A)	2
N X A	2
Period x nitrite x additive	39
Error	76

Least significant differences were calculated when significance for a treatment with more than two levels was found. Designs for sensory analysis and Instron measurements differ in that there were five replications of each treatment for sensory analysis and 10 for Instron measurements.

RESULTS & DISCUSSION

Flavor and aroma

Flavor characteristics generally were influenced by adding nitrite, but were not influenced by sorbic acid, potassium sorbate, or an interaction of those components with nitrites. Oxidation was not noted in any of the samples. Cured-meat aroma and cured-meat flavor were higher ($P < 0.001$) for frankfurters with 40 ppm nitrite than for frankfurters with no nitrite (Table 1). Although the frankfurters without nitrite had lower cured-meat aroma and flavor, the other cure-complex components (e.g. salt) that were added contributed a slightly cured character.

Price and Greene (1978) reported that the "cured" flavor could be produced in meat without nitrite but that nitrite gave a more "desirable" flavor. Using the flavor profile method, Chambers et al. (1981) found a "rounder" flavor, more amplitude, and more intense cured-flavor in poultry frankfurters with nitrite than in those containing no nitrite.

A competing cured flavor note may explain why spiciness was lower ($P < 0.001$) in frankfurters containing nitrite. Saltiness did not differ in frankfurters with or without nitrite.

Texture

Poultry frankfurters containing nitrite were firmer than those without added nitrite (Table 1). Eakes et al. (1975) observed the same trend in hams. Nitrite, a highly reactive substance, may interact with muscle proteins and affect firmness. The influence of added sorbic acid depended on whether nitrite was present. Without nitrite, frankfurters with and without sorbic acid did not differ; with nitrite, those containing sorbic acid were softer than those without added sorbic acid. Apparently, sorbic acid did not soften the frankfurters but countered nitrite's firming action. Potassium sorbate increased the firmness of the frankfurters regardless of nitrite content. In bacon, Paquette et al. (1980) reported no difference in texture "desirability."

Instron measurements

Data from the Warner-Bratzler shear and puncture-probe attachments indicated no significant differences among treatments (Table 2). But when simulated-teeth attachments (incisor and molar) were used, areas under the curves (total force) for the nitrite frankfurters exceeded that for frankfurters with no nitrite. Additionally, areas under the Instron curves with incisor and molar attachments differed when sorbic acid or potassium sorbate was added. Measured with the simulated incisor, frankfurters with potassium sorbate had larger areas under the curve than other frankfurters. Data from the molar attachment indicated that adding potassium sorbate produced firmer frankfurters, and adding sorbic acid yielded softer frankfurters than without the additives. Results from the simulated teeth attachments and from sensory analysis were similar.

Residual nitrite and pH

Residual nitrite in the 40-ppm-nitrite frankfurters was significantly ($P < 0.001$) higher than in the no-nitrite frankfurters. Frankfurters without sorbic acid or potassium sorbate but with nitrite had 11.7 ppm residual nitrite; those without nitrite, 1.2 ppm. Comparisons of residual nitrite in frankfurters with added sorbic acid or potassium sorbate are not possible because the sorbic acid and potassium sorbate apparently interfered with the electrode used. Frankfurters with no added nitrite and with no additive had 1.2 ppm residual nitrite; with added sorbic acid, 8.4 ppm; and with potassium sorbate, 12.5 ppm, although no nitrite

Table 1—Sensory characteristics of poultry frankfurters

Nitrite	Additive[a] None	Sorbic acid	Potassium sorbate	Mean within nitrite level
Cured-meat aroma				
0 ppm	6.6	6.9	7.9	7.1***
40 ppm	10.2	9.5	10.1	9.9
Mean within additive	8.4a	8.2a	8.4a	
Cured-meat flavor				
0 ppm	6.2	6.6	7.4	6.7***
40 ppm	10.8	10.0	10.8	10.5
Mean within additive	8.5a	8.3a	9.1a	
Spiciness				
0 ppm	10.0	9.3	8.4	9.2***
40 ppm	5.9	8.0	6.8	6.9
Mean within additive	8.0a	8.7a	7.6a	
Saltiness				
0 ppm	7.6	9.3	7.0	8.0
40 ppm	7.8	7.9	8.2	8.0NS
Mean within additive	7.7a	8.6a	7.6a	
Firmness[b]				
0 ppm	8.3a	9.3de	11.6c	
40 ppm	13.4b	9.8d	14.9a	

[a] Numbers within a sensory attribute followed by different letters (a—e) differ significantly ($P < 0.05$) for firmness)
[b] Main effect means are not reported because of significant interaction
***$P < 0.01$; NS—not significant

Table 2—Instron measurements (area under curve, cm^2) for poultry frankfurters

		Additive[a]			Mean within nitrite level
	Nitrite	None	Sorbic acid	Potassium sorbate	
Warner Bratzler shear	0 ppm	18.77	19.11	20.34	19.07
	40 ppm	21.90	18.13	21.90	20.64^{NS}
mean within additive		19.78a	18.67a	21.12a	
Puncture-probe	0 ppm	8.13	8.17	8.73	8.34
	40 ppm	8.76	8.14	8.93	8.61^{NS}
mean within additive		8.44a	8.16a	8.83a	
Incisor	0 ppm	7.74	8.03	9.06	8.28$_{**}$
	40 ppm	9.17	8.15	9.91	9.08
mean within additive		8.46a	8.09a	9.48b	
Molar	0 ppm	44.00	43.81	50.41	46.07$_{***}$
	40 ppm	52.07	46.10	53.91	60.59
mean within additive		48.04b	44.96a	52.16c	

[a] numbers within a measurement followed by a different letter (a—c) differ significantly ($P < 0.001$ for incisor, $P < 0.01$ for molar)
$^{**}P < 0.01$; $^{***}P < 0.001$; NS—not significant

was added. With only nitrite added, the residual nitrite was 11.7 ppm; with nitrite and sorbic acid, 17.8 ppm and with nitrite and potassium sorbate, 21.2 ppm.

Adding nitrite did not influence the pH of the frankfurters (6.5 without nitrite, 6.6 with nitrite), but the frankfurters with sorbic adic added had a lower ($P < 0.001$) pH (6.3) than those with potassium sorbate (6.7), or without sorbic acid or sorbate (6.6).

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Effects of Electrical Stimulation and Hot-Boning on Physical Changes, Cooking Time and Losses, and Tenderness of Beef Roasts

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ABSTRACT

Beef semimembranosus and semitendinosus muscles were removed prerigor (1 hr postmortem) and postrigor (7 days postmortem) after one-half of the left and right sides were electrically stimulated. The influence of electrical stimulation and hot-boning upon physical changes; cooking losses; shear force; taste panel evaluation; and time required to heat product to an internal temperature of 63°C was studied. There was no consistent influence of electrical stimulation upon physical changes of prerigor muscles or upon tenderness of pre- or postrigor roasts. Hot-boned and precooked roasts were less tender than cold-boned counterparts. Cooking yields were not altered by electrical stimulation. Prerigor roasts had 9% higher yields than postrigor roasts. Prerigor roasts from electrically stimulated sides required a longer time to cook to 63°C than roasts from the control sides. Hot-boning reduced the length of time of cooking (95 min/kg to 72 min/kg of raw weight).

INTRODUCTION

THE MEAT INDUSTRY is one of the six largest users of energy in the United States (Henrickson, 1975); consequently, processing alternatives aimed toward conserving energy are being investigated. Removal of muscle and muscle systems prior to chilling the bovine carcass has been investigated (Schmidt and Gilbert, 1970; Kastner and Russell, 1975; Kastner et al., 1976) to determine the feasibility of hot-boning as an alternative to conventional fabrication.

Hot-boning of the carcass prior to chilling saves energy by: (1) requiring chilling of only the edible portion of the carcass; (2) reducing the requirements for refrigeration facilities; (3) reducing transportation cost; (4) allowing more rapid turnover of inventory; and (5) reducing needed labor. A potential problem with hot-boning is reduced tenderness (Weiner et al., 1966; Kastner and Russell, 1975; Ray et al., 1979; Griffin et al., 1979). In addition, hot-boned muscles exhibited a distortion in shape upon cooking, but had higher cooking yields and a significant saving of energy than their control counterparts (Cia and Marsh, 1976; Marsh, 1977; Ray et al., 1979; Griffin et al., 1979) and in some instances (Cia and Marsh, 1976; Marsh, 1977), improved tenderness was noted. Interest in electrical stimulation as a means of improving tenderness has received considerable attention (Chrystall and Hagyard, 1976, Davey et al., 1976; Savell et al., 1978; Ray et al., 1978; Stiffler et al., 1978; Cross, 1979). This study was conducted to investigate the concomitant effects of electrical stimulation and hot-boning upon physical changes, length of cooking time and losses, and tenderness of roasts (semitendinosus and semimembranosus muscles) from beef carcasses.

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EXPERIMENTAL

Hot and cold-boning

Forty-four crossbred, beef cattle were used. The yearling cattle were fed a high energy ration (70–80% concentrate) for 147 days before slaughter. Within 45 min post-exsanguination, one side of each carcass was stimulated (ES). One-half of the total left and right sides were randomly selected and electrically stimulated (ES) [100 impulses (1 sec on and 1 sec off, A.C., 110 V, < 5 amps)] while the remaining paired side served as the control (NS). Semitendinosus (ST) and semimembranosus (SM) muscles were excised from the left side of all carcasses, within 1 hr postmortem (hot-boned, HB); the paired right side was processed after chilling at 2°C until 7 days postmortem (cold-boned, CB). The excised ST muscle was used intact; the SM muscle was divided longitudinally so that portions were of similar weight to that of the ST muscle. Excised muscles were weighed, temperature recorded, measured for length, width (at 25, 50, and 75% of the length) and depth at the deepest point of the muscle and then cooked immediately in a steam oven. Similar measurements were recorded after the muscles were cooked.

Cooking procedure

Preparation for cooking involved rubbing each muscle with a commercial roast beef spice preparation (B. Heller and Co.), placing muscles in L600 Cryovoc® bags, and cooking to an internal temperature of 63°C in a fabricated, low pressure steam oven. Internal roast temperature was monitored during cooking with temperature probes (Omega®) that were 0.63 cm diameter and 4.7 cm in length and a Digilloger®. The wires attached to the probe passed through the open end of the cooking bag. Subsequently, a string was utilized to close the bag around the wires to inhibit leakage during cooking. The cooking schedule was: 1st hr at 68°C; 2nd hr at 74°C; and the remaining time at 80°C. Cooking time and yield were recorded and the product was chilled to 3–4°C over a 12-hr period. Subsequently, a 3.8 cm thick sample was removed for Warner-Bratzler shear (WBS) determination. Three cores (1.27 cm in diameter) were removed from each sample parallel to the muscle fibers and all cores were sheared twice. Adjacent to the WBS samples, a 10 cm thick sample of each roast was removed from the center portion, placed in a bag with about 150 ml of juices, vacuumized and frozen (−29°C). Following 45 days of storage, the samples were thawed, reheated and evaluated for palatability traits at the USDA Meat Science Research Lab., Beltsville, MD, by an eight-member trained panel (Berry et al., 1981).

Statistical analyses

A split-split plot design with treatments arranged in a 2x2x2 factorial was assumed for statistical analyses (Fig. 1). Main plots were steers versus bulls with 48 and 40 carcasses, respectively, within main plots. The left sides (44) were assigned to HB and the right sides (44) were CB. Within method of boning, carcasses were randomly assigned to sub-sub plot treatments which included ES versus NS. Analyses of variance were computed to test for treatment effects and interactions. If interactions were significant ($P < 0.05$) simple effects were evaluated using an LSD test (Snedecor and Cockran, 1967).

RESULTS & DISCUSSION

Carcass traits

Mean carcass traits and measurements for the steer and bull carcasses are presented in Table 1. Quality grade of the carcasses from the two groups were similar, but the car-

casses from bulls exhibited a more desirable yield grade because of less fat thickness, larger rib eye areas, and less kidney, heart and pelvic fat. The mean hot carcass weights were 297 and 294 kg for steers and bulls, respectively.

Physical characteristics

Analyses of variance detected an interaction ($P < 0.05$) between sex groups and electrical stimulation for percentage change in length and depth of ST and SM roasts (fresh versus cooked). Since a stimulation by sex group interaction was noted for percentage change in length and depth, simple effect means across method of boning for stimulation within sex groups and sex groups within stimulation method were examined for the ST roast (Table 2). Within stimulation, changes in length were less ($P < 0.05$) for ST roast from steers that were ES than roasts from bulls. Within roast from steers, ES (−22.3%) roasts shorten less ($P < 0.05$) than NS (−25.3%); but within ST roasts from bull carcasses, no difference ($P < 0.05$) was observed for ES. Similar results were obtained for percentage change in depth of the ST roasts for ES versus NS within ST roasts from bull carcasses. Within stimulation, changes in depth were more pronounced ($P < 0.05$) for ES (+ 10.4%) roasts from steers than roasts from bulls (+7.3%), whereas the reverse (steers, +5.3% versus bulls, +7.9%) was observed for NS roasts.

The simple effect means for percentage change in length and depth of the SM roasts across method of boning for stimulation within sex groups and sex groups within stimulation method are presented in Table 3. Within stimulation, SM roasts from steer carcasses exhibited less ($P < 0.05$) shortening than those from bull carcasses. Within SM roasts from steers, ES (−29.9%) roasts shortened more than the NS roasts (−24.5%); however, within SM roasts from bull carcasses there was no ($P > 0.05$) difference due to ES. A greater reduction in length is noted for the SM, since it was divided longitudinally, when compared with ST (Table 2) which was utilized as an intact muscle. Simple effect means for percentage change in depth of SM roasts indicate (Table 3) that within stimulation, SM roasts from steer and bull carcasses responded differently ($P < 0.05$). Within stimulation, SM roasts from bull carcasses had a greater increase ($P < 0.05$) in depth than steers. Furthermore, within sex groups, roasts from ES steer and bull carcasses exhibited a greater increase in depth than roasts from NS carcasses. There was a trend ($P > 0.05$) for SM roast to become deeper as they shortened in length. Cia and Marsh (1976), using bovine sternomandibularis samples, reported a reduction in length (50.4%) and a concomitant increase in depth when samples were cooked prerigor. These authors, using small samples, found the prerigor cooked muscle strips, when cooked by either water or microwave, to be quite tender and suggested the improved tenderness was a

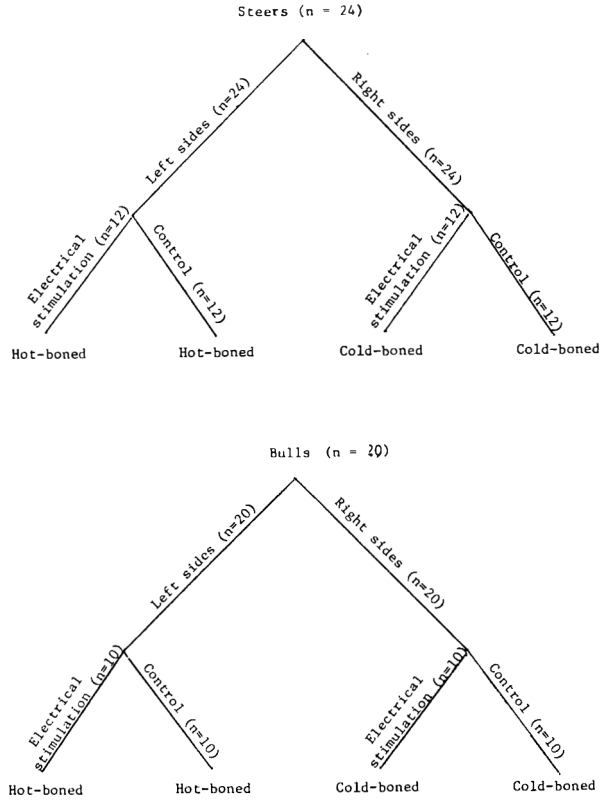


Fig. 1—Experimental design.

Table 1—Carcass traits and measurements

Trait	Steers (N=24)	Bulls (N=20)
USDA quality grade[c]	10	9
USDA yield grade	3.4 ± 0.9[a]	2.8 ± 0.7[b]
Adjusted fat thickness (cm)	1.22± 0.51[a]	.91± 0.30[b]
Rib eye area (cm^2)	66.71±10.3[a]	71.10± 9.7[b]
Kidney, heart and pelvic fat (%)	2.7 ± 0.56[a]	2.3 ± 0.42[b]
Carcass weight (kg)	297 ±23	294 ±30

[a,b]Means, with standardd deviations, having different superscripts differ significantly ($P < 0.01$).
[c] Scores based on 11 = USDA High Good; 10 = USDA Average Good; 9 = USDA Low Good.

Table 2—Physical changes of beef semitendinosus roasts as influenced by sex group and electrical stimulation[a]

Item	Sex group: Steers	Sex group: Bulls
Change in (fresh vs cooked):		
Length, %		
Electrically stimulated	−22.3 ± 0.5[b]	−24.9 ± 0.6[c]
Not stimulated	−25.3 ± 0.6[c]	−24.3 ± 0.6[c]
Depth, %		
Electrically stimulated	+10.4 ± 0.4[b]	+ 7.3 ± 0.2[c]
Not stimulated	+ 5.3 ± 0.2[d]	+ 7.9 ± 0.3[c]

[a] Means for treatments averaged across remaining treatments.
[b,c,d]Means in the same row or column, within length and depth changes, with different superscripts are different ($P < 0.05$).

Table 3—Physical changes of beef semimembranosus roasts as influenced by sex group and electrical stimulation[a]

Item	Sex group: Steers	Sex group: Bulls
Change in (fresh vs cooked):		
Length, %		
Electrically stimulated	−29.9 ± 0.5[b]	−33.6 ± 0.6[d]
Not stimulated	−24.5 ± 0.6[c]	−32.4 ± 0.6[d]
Depth, %		
Electrically stimulated	+13.9 ± 0.3[b]	+22.2 ± 0.6[d]
Not stimulated	+ 2.4 ± 0.2[c]	+14.8 ± 0.3[b]

[a] Means for treatments averaged across remaining treatments.
[b,c,d]Means in the same row or column, within length and depth changes, with different superscripts are different ($P < 0.05$).

Table 4—Mean values for physical characteristics of beef roasts (SM and ST) as influenced by method of boning and electrical stimulation

Item	Method of boning			
	Hot-boned		Cold-boned	
	Electrically stimulated	Not stimulated	Electrically stimulated	Not stimulated
Fresh muscle:				
Length, cm	34.7±0.8[a]	34.8±0.8[a]	34.4±0.5[a]	33.7±0.5[a]
Width, cm	12.5±1.2	13.0±1.2	11.3±1.1	11.2± 1.0
Depth, cm	7.5±1.1[a]	8.4±1.2[a]	7.7±1.1	8.3±1.1
Cooked product:				
Length, cm	25.8±0.54[b,c]	26.0±0.56[b,c]	28.5±0.5[b,d]	28.3±0.5[b,d]
Width, cm	13.1±1.2	13.0±1.2	11.3±1.1	11.0±1.1
Depth, cm	9.1±1.1[b,c]	10.3±1.5[b,c]	7.9±0.8[d]	7.8±0.8[d]
Change in (fresh vs cooked):				
Length, %	−34.5	−33.8	−20.7	−19.1
Width, %	+4.8	0	0	−1.8
Depth, %	+26.7	+22.6	+2.5	−6.4
No. of roasts	44	44	44	44

[a,b]Means in the same column, with different superscripts differ significantly ($P < 0.01$).
[c,d]Means in the same row with different superscripts differ significantly ($P < 0.05$).

Table 5—Influence of electrical stimulation, postmortem boning, and sex classification upon tenderness, cooking yield and cooking time (63°C, internally) for semitendinosus beef roasts

Item	Method of boning			
	Hot-boned		Cold-boned	
	Steer	Bull	Steer	Bull
Warner-Bratzler shear, kg/1.27 cm				
Electrically stimulated	4.98±0.57[a]	5.14±0.58[a]	2.90±0.23[b]	3.35±0.24[b]
Not stimulated	5.05±0.89[a]	5.42±0.94[a]	2.92±0.24[b]	3.20±0.25[b]
Cooking yield, %				
Electrically stimulated	92.4 ±0.69[a,c]	90.9 ±0.68[a,d]	83.7 ±1.65[b]	82.0 ±1.60[b]
Not stimulated	92.7 ±0.72[a,c]	90.4 ±0.77[a,d]	83.7 ±1.65[b]	84.8 ±1.75[b]
Time to cook, min/kg				
Electrically stimulated	74.9 ±5.69[a,e]	71.9 ±5.60[a,e]	88.1 ±6.04[b,e]	85.6 ±6.35[b]
Not stimulated	62.7 ±6.00[a,f]	65.8 ±6.37[a,f]	102.9 ±7.20[b,f]	85.5 ±6.40[b]

[a,b]Means with standard deviations, in the same row, within sex, with different superscripts differ ($P < 0.01$).
[c,d]Means with standard deviations, in the same row, between sex, with different superscripts differ ($P < 0.01$).
[e,f]Means with standard deviations, in the same column, within sex, with different superscripts differ ($P < 0.01$).

result of supercontraction that had resulted in fiber shattering. When increased toughness has been found in prerigor meat (Montgomery et al., 1977; Ray et al., 1980; Berry et al., 1981) these results have been attributed to heat-stimulated contraction of heat rigor without fiber shattering. Thus, the slower cooking method employed in this study could have resulted in extensive heat rigor rather than fiber shattering from supercontraction.

Table 4 presents the mean value of physical measurements, which are grouped by method of boning and electrical stimulation. Change in length of roasts (overall means) was −34.1% and −19.9% for HB and CB, respectively, which coincides with a 24.6% and −1.9% change in depth of HB and CB, respectively. Width changes were minimal.

Tenderness evaluation

The decreased tenderness ($P < 0.01$) of cooked prerigor ST (WBS) and SM (WBS and taste panel) roasts (Table 5 and 6) when compared with CB roasts, confirms the findings of Weiner et al. (1966), Montgomery et al. (1977) and Ray et al. (1979). However, Cia and Marsh (1976) found that microwave cooking improved the tenderness of prerigor muscles compared to boiling and a recent study by West et al. (1980) and Ray et al. (1981) found long-time, low-temperature cooking of prerigor meat to result in acceptable tenderness. Ray et al. (1981) suggests the slow, low-temperature (46.5°C) method of cooking may permit the muscle to proceed into and complete rigor during cooking, rather than having rigor induced by subjecting the roasts to an initial, higher cooking temperature (69°C). Neither electrical stimulation nor sex classification significantly influenced the WBS shear readings of the HB or CB roasts, but there was a tendency ($P > 0.05$) for roasts from steer carcasses to have lower WBS and slightly higher taste panel tenderness. Griffin et al. (1979) reported a difference in Instron® force for CB and HB biceps femoris muscle of ES and NS carcasses with CB roasts having lower values than HB roasts and within HB roasts the ES roasts had lower values than NS roasts. Other workers have reported ES to increase tenderness of CB product (Chrystall and Hagyard, 1976; Davey et al., 1976; Savell et al., 1978; Ray et al., 1978; Stiffler et al., 1978).

Table 6—Influence of electrical stimulation, method of postmortem boning, and sex classification upon tenderness, cooking yield and cooking time (63°C, internally) for semimembranosus beef roasts

Item	Method of boning			
	Hot-boned		Cold-boned	
	Steer	Bull	Steer	Bull
Warner-Bratzler shear, kg/1.27 cm				
Electrically stimulated	6.18±0.68[a]	6.50±0.69[a]	2.95±0.35[b]	3.92±0.34[b]
Not stimulated	5.41±0.72[a]	6.45±0.77[a]	2.96±0.35[b]	3.33±0.37[b]
Tenderness panel scores[e]				
Electrically stimulated	4.8 ±0.20[a]	4.4 ±0.41[a]	6.4 ±0.21[b]	6.4 ±0.33[b]
Not stimulated	4.9 ±0.45[a]	4.7 ±0.27[a]	6.9 ±0.16[b]	6.2 ±0.21[b]
Cooking yield, %				
Electrically stimulated	89.5 ±0.67[a]	90.0 ±0.68[a]	83.5 ±0.72[b]	80.0 ±0.69[b]
Not stimulated	90.3 ±0.71[a]	88.8 ±0.75[a]	80.2 ±0.69[b]	79.9 ±0.77[b]
Time to cook, min/kg				
Electrically stimulated	80.6 ±5.72[a,c]	77.5 ±5.71[a,c]	90.3 ±6.56[b,c]	100.0 ±6.58[b]
Not stimulated	72.5 ±6.03[a,d]	70.4 ±6.39[a,d]	114.4 ±6.79[b,d]	97.2 ±6.97[b]

[a,b]Means with standard deviations, in the same row within sex, with different superscripts, differ significantly ($P < 0.01$).
[c,d]Means with standard deviations, in the same column within sex, with different superscripts, differ significantly ($P < 0.01$).
[e] 8 = extremely tender, 1 = extremely tough.

Cooking time and losses

Cooking yield was determined as the difference in weight between the raw muscle plus seasonings and cooked weight of the roast (Tables 5 and 6). Cooking yield was influenced ($P < 0.01$) by method of boning and sex classification for the HB, ST roast; whereas electrical stimulation exerted little or no response. Roasts from HB, ST of steer carcasses had approximately 2% higher cooking yield than roasts from bull carcasses. Furthermore, HB, ST and SM roasts had 8.0% and 8.8%, respectively, higher cooking yields (overall means) than their CB counterparts.

Time required (overall mean) per unit weight (Tables 5 and 6) to cook HB roasts from the ST and SM was less ($P < 0.01$) than for CB roasts (−23.5 min/kg). When HB roasts were compared with CB roasts within ES, as to time required per unit weight to cook, the ES roasts required 18% (ST) and 20% (SM) less time per unit of weight; whereas the NS roasts that were HB required 47% (ST) and 47% (SM) less time than CB roasts cooked to the same internal temperature (63°C). Thus, HB, ES roasts required 29% and 27% ($P < 0.01$) less cooking time than CB, NS roasts of the ST and SM, respectively. Ray et al. (1979) using similar HB roasts from ES bull carcasses, which were cooked to an internal temperature of 67°C, found HB roasts required 28% and 29% less cooking time than CB, ST and SM, respectively.

CONCLUSIONS

NO CONSISTENT INFLUENCE of electrical stimulation upon physical changes of prerigor excised muscle or upon tenderness of pre- and postrigor roasts were found. Hot-boned and precooked roasts were less tender than their cold-boned counterparts. Cooking yields were not altered by electrical stimulation. Prerigor roasts had 9% higher yields than postrigor roast. Prerigor roasts from electrically stimulated sides required a longer time to cook to 63°C than roasts from the control sides. Hot-boning reduced the length of cooking time from 95 min/kg to 72 min/kg of cooking beef roasts.

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Effect of Further Processing Systems on Selected Microbiological Attributes of Turkey Meat Products

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ABSTRACT

Effects of carcass transfer and line transport, deboning, protein extraction and product wrapping, meat blending, product packaging and cooking-smoking procedures on microbiological concentrations were evaluated. Increased mesophilic bacterial numbers were associated with carcass transfer and line transport with no effects on other bacterial concentrations. Hand deboning increased bacteria on breast and thigh tissue. Mechanical deboning increased bacterial content. Protein extraction and product wrapping produced minor effects. Bacterial numbers from meat blends reflected combination of input components. Product packaging exerted minor effects on numbers of bacteria from raw product. Handling required in packaging cooked product produced increases in tissue bacterial content. Cooking and smoking procedures were equally effective in reducing bacterial contents to essentially negative concentrations.

INTRODUCTION

IN RECENT YEARS considerable attention has been focused upon the development of further processed turkey products as a means of providing alternative food sources of high nutritional quality and achieving a more efficient utilization of available food sources. Significant advances have been made by the turkey processing industry in the development of various further processed items including products such as bologna and salami, boneless turkey rolls consisting of light and/or dark muscle tissue, turkey ham, whole turkey breast products and ground turkey meat. This trend is apparent when the data for 1975 turkey meat output is compared to 1979 figures. Of the 1,716 million pounds of turkey slaughtered in federally inspected plants in 1975 (USDA, 1976), approximately 536 million pounds (31%) were marketed as cut up and further processed products compared to 1,326 million pounds (61%) of the same products from the 2,182 million pounds slaughtered in 1979 (USDA, 1980).

Several processing factors have been shown to be effective in reducing bacterial numbers on the surface of whole carcass processed turkeys (Gardner and Golan, 1974). These factors include the washing and rinsing action of the scalding, picking, washing and chilling operations. May et al. (1962) reported that the primary region of bacterial contamination of a freshly processed carcass appears to be the surface of the skin. Baran et al. (1973) indicated that the deep muscle tissue is generally considered to be essentially sterile prior to opening the body cavity by cutting.

Numerous studies have been conducted in evaluating the bacterial numbers of cooked turkey products. Froning et al. (1971) indicated increases in total bacterial numbers during refrigerated storage of turkey frankfurters. Mercuri et al. (1970) and Zottola and Busta (1971) reported significant increases in total numbers of bacteria during storage of cooked further processed turkey products. Maxcy et al. (1973) evaluated both raw hand deboned and mechanically deboned turkey meat (MDTM) and reported that both produced commercially acceptable products. However, no attempt was made to characterize the effects of processing systems on the microbiological character of the products. The objective of this study was to evaluate changes in microbiological concentrations of turkey meat products which may be associated with further processing systems.

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EXPERIMENTAL

Sampling

Samples utilized for the evaluation of further processing system effects were obtained from a turkey further processing plant. The basic procedures utilized in further processing were segregated into three major systems: (1) primary processing system consisting of (a) carcass transfer and line transport, (b) deboning operations, and (c) tissue shaping and product wrapping procedures; (2) product formulation systems consisting of (a) flaking frozen MDTM, (b) tissue trimming, (c) meat blending, (d) emulsification, and (e) stuffing procedures; (3) cooking and packaging systems consisting of individual evaluations for (a) oven roasting, (b) immersion water cooking, and (c) smokehouse procedures. Boneless breast product, comminuted white meat roll and emulsion wiener product processing procedures were utilized to evaluate the processing systems and procedures. The basic flow diagram of systems utilized in further processing with designation of sampling locations is presented in Fig. 1. Two samples were obtained from each sampling location during each of two different sampling periods in an effort to minimize error due to normal processing variation. All samples were stored in sterile containers and packed in crushed ice for transportation (2 hr) to the laboratory facility at Texas A&M Univ. Skin surface swab samples were obtained by standard swab techniques (260 cm^2 from each of four turkeys for a composite area of 1040 cm^2 per sample). Swab samples for all determinations except Shigella were placed in lactose broth for laboratory analysis. Swab samples for Shigella determinations were placed in selenite-cystine broth for laboratory analysis. Muscle tissue samples were analyzed by blending 40g of tissue in 360 ml of the appropriate media (either lactose or selenite-cystine broth). All determinations requiring plating enumeration were accomplished utilizing standard serial dilution techniques with buffered phosphate diluent. Duplicate plates were analyzed yielding N=8 for each sampling location. All MPN determinations were analyzed for each sample yielding N=4 for each sampling location.

Microbiological analyses

Mesophilic determinations were accomplished by methods outlined in the *Microbiology Laboratory Guidebook* USDA (1974) with platings on Standard Plate Count Agar (Difco) followed by incubation at 35°C for 48 hr, while psychrotrophic determinations were obtained by incubating a duplicate plating at 20°C for 4–5 days.

Coliforn numbers were obtained following the procedure outlined by Powers and Mast (1980). The method involved direct plating onto Violet Red Bile Agar with over-pour to obtain subsurface colonies followed by incubation for 18–24 hr at 35°C.

Presumptive *Clostridium perfringens* numbers were obtained by plating on Tryptose-Sulfite-Cycloserine Agar containing egg yolk emulsion as outlined in the *Bacteriological Analytical Manual for Foods* (1978). Determinations were made following incubation at 35°C for 18–24 hr in anaerobic Gas-Pak jars with hydrogen-carbon dioxide atmosphere.

Salmonella bacterial numbers were obtained by utilization of a Most Probable Numbers (MPN) technique described by Mayou

(1976), with a selective isolation technique outlined in the *Bacteriological Analytical Manual for Foods* (1978). The technique consisted of an 18-hr pre-enrichment period in Lactose Broth followed by selective enrichment in tetrathionate broth for 18–24 hr. Selective isolation on Brilliant Green Sulfa Agar plates was accomplished by incubating for 24–48 hr. Biochemical confirmation was accomplished by incubation for 18–24 hr on Triple Sugar Iron and Lysine Iron Agar slant tubes. All incubation was at 35°C temperature. Positive cultures were then tested for agglutination by use of Salmonella Poly 0 antisera by slide test.

Shigella bacterial numbers were determined by a selective isolation procedure outlined in the *Bacteriological Analytical Manual for Foods* (1978) in conjunction with a MPN technique. The procedure consisted of a selective enrichment period of 18 hr in Selenite Cystine Broth followed by an 18–24 hr selective isolation period on Xylose Lysine Desoxycholate Agar plates. Biochemical confirmation was accomplished by incubation for 18–24 hr on Triple Sugar Iron and Lysine Iron Agar tubes. All incubation was 35°C. Positive cultures were tested for agglutination using Shigella Polyvalent antisera by slide test.

Coagulase positive *Staphylococcus aureus* numbers were determined by a selective isolation procedure presented in the *Bacteriological Analytical Manual for Foods* (1978) with quantification by the MPN technique. The procedure consisted of an enrichment period of 48 hr in Tryptic Soy Broth containing 10% salt (NaCl). The procedure was modified by utilizing Vogel-Johnson Agar plates incubated 48 hr for isolation. Positive cultures were tested for coagulate production by the tube test using 18 hr Brain Heart Infusion Broth cultures. All incubation was at 35°C.

Results from plating determinations are reported as logarithm of bacterial numbers per cm^2 of surface area or gram of tissue. Counts from MPN determinations are reported as bacterial numbers per 1000 cm^2 of surface area or 100g of tissue.

RESULTS & DISCUSSION

Primary processing systems

Total mesophilic bacterial numbers from turkey breast samples obtained from primary processing systems (Table 1) increased significantly as an effect of carcass handling and line transport prior to the first cut. Handling associated with the hand deboning procedures also produced a significant increase in tissue bacterial content for both breast and thigh meat with the exception of mesophilic numbers from thigh meat. The tissue shaping and product wrapping systems exerted only minor effects on the bacterial content of breast meat with no trends which could be associated with product handling. Mechanical deboning systems produced significant increases in bacterial numbers. This is possibly an effect of the elevated product temperature and increased nutrient availability due to the increased surface area of the smaller particle sizes generated by the deboning process. These factors, when coupled with infrequent equipment sanitation procedures during periods of equipment inactivity (as long as 1–2 hr), result in increases in bacterial numbers. The intermittent use of the mechanical deboning equipment during an extended processing schedule requires more frequent cleanup efforts, especially when not in use for extensive periods of time.

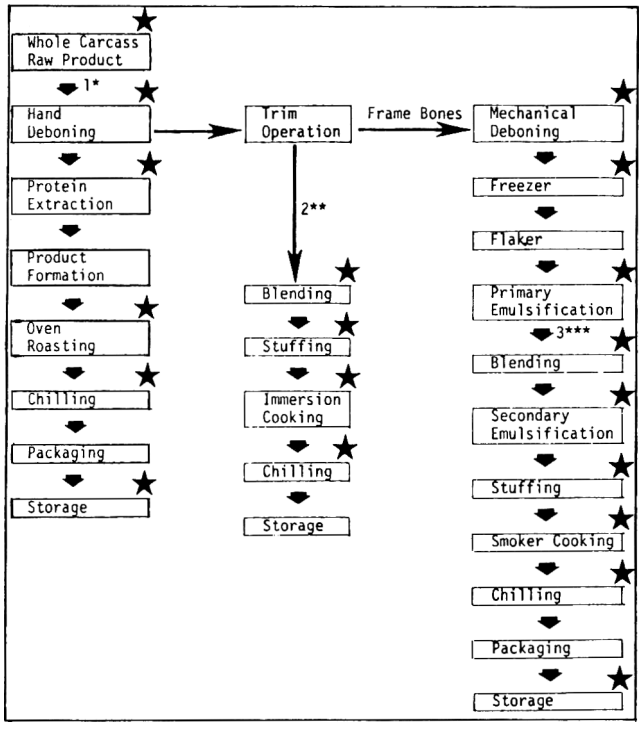


Fig. 1 — Flow Diagram of basic systems utilized in the processing of whole muscle, comminuted roll and emulsion type further processed turkey product. ★ Designates sampling location; 1—Basic procedures utilized in the production of whole muscle (breast) product; 2** —Basic procedures utilized in the production of comminuted roll product; 3*** —Basic procedures utilized in the production of emulsion (wiener) product.*

Total coliform bacterial numbers (Table 1) reflected trends similar to those noted for both mesophilic and psychrotrophic numbers. However, it should be noted that handling of the breast product during the tissue shaping and wrapping procedures resulted in a significant increase in coliform content. Only minor effects were noted for numbers obtained from thigh tissue. All coliform numbers obtained from the tissue preparation systems were relatively low. Similar trends were obtained from analysis of *Clostridium perfringens* and Shigella determinations.

Salmonella numbers for both the breast and thigh tissue increased as a result of the hand deboning procedure (Table 1). This has been associated with a deficiency in equipment and worker hand sanitation procedures which resulted in excessively high numbers obtained during one sampling period. However, it should be noted that the elevated levels associated with deboned tissue were not observed following the protein extraction and product wrapping procedures. This can most probably be explained by a redistribution of the organisms during the tissue shaping and wrapping procedure. No real increases were noted for coagulase positive *Staphylococcus aureus* numbers obtained from hand deboned breast tissue, with none detected on thigh tissue (Table 1). Mechanical deboning produced substantial increases in bacterial concentrations of both Salmonella and *Staphylococcus aureus* organisms, which is possibly due to the elevated product temperature and increased nutrient availability of the smaller particle sizes associated with the deboned meat coupled with extensive periods of equipment inactivity.

Product formulation systems

Analysis of mesophilic and psychrotrophic bacterial numbers from samples obtained from product formulation systems (Table 2) showed no trends which could be associated with primary or secondary emulsification procedures either for comminuted roll or emulsion wiener. Handling during the regular hand trim operation resulted in significantly higher bacterial numbers from these samples than in samples from trim meat obtained using electric trimming knives. Numbers from comminuted roll blended output samples reflected the blending effect of the input components, consisting of primary emulsion, regular trim and electric trim meat. Although no significant increases in bacterial numbers were noted during the product stuffing procedures for either the emulsion wiener or comminuted roll, counts from the comminuted roll indicated an increase in bacterial numbers during the stuffing procedures. This trend suggests the need for additional study with increased sample size.

Clostridium perfringens (Table 1 and Table 2) increased as a result of flaking the mechanically deboned turkey meat

(MDTM). This is probably due to the accumulation of meat product on equipment surfaces during the flaking process. Product accumulation coupled with periods of inactivity (up to 4 hr) may result in bacterial increases. This accumulation in combination with infrequent equipment sanitation may result in substantial bacterial growth. Subsequent samples from primary emulsifier output, blended output and product post-stuffing all reflected the presence of this organism to some extent. Similar trends were noted for the emulsion wiener product.

Coliform numbers (Table 1 and Table 2) also increased as a result of the flaking process for MDTM. As indicated above, this effect is associated with infrequent cleanup of equipment surfaces during processing, which suggests the need for more extensive equipment cleanup programs during the processing schedule. The additional handling required during the regular trimming procedure resulted in substantially higher numbers than were obtained from samples of electrically trimmed meat. Comminuted roll blend output samples reflected the effect of blending input components. Significant increases in coliform numbers were noted for product sampled after stuffing. This has been attributed to the extended time interval between final blending and product stuffing procedures which are inherent to the normal processing schedule. Only minor effects were noted for numbers from emulsion wiener samples with no trends which could be readily associated with product formulation systems. Similar overall effects were noted for *Staphylococcus aureus* numbers obtained from product formulation systems evaluation (Table 2). The excessively high numbers obtained from primary emulsifier output have been attributed to one sample obtained during the second sampling period, indicating that an atypical sample was obtained. This suggests the need for additional study with increased sample size. Numbers obtained were generally consistent with previous research findings for raw products (Zottola and Busta, 1971).

Salmonella numbers from comminuted roll samples

Table 1 — Effect of tissue preparation systems on bacterial concentrations of further processed turkey meat samples

Sample location	Mesophilic[a]	Psychrotrophic[a]	Coliform[b]	Salmonellae[c]	S. aureus[c]	Clostridium perfringens[b]	Shigellae[c]
			Breast				
Incoming carcass skin surface	2.04[c]*	2.26b	0.6(4/8)	3.1(3/4)	13.1(1/4)	ND(0/8)	ND/0/4)
Pre-cut surface	2.66[ab]	2.75ab	0.3(3/8)	1.1(1/4)	9.2(2/4)	ND(0/8)	ND(0/4)
Pre-cut tissue	2.08bc	1.94b	ND(0/8)	13.5(2/4)	22.3(1/4)	ND(0/8)	ND(0/4)
Hand deboned tissue	3.46a	3.68a	8.9(3/8)	383.7(3/4)	26.4(2/4)	6.2(1/8)	2.2(1/4)
Shaped tissue post-wrapping	3.00a	3.20a	132.0(4/8)	2.1(1/4)	ND(0/4)	ND(0/8)	ND(0/4)
			Thigh				
Pre-cut surface	2.84a	2.89a	1.5(5/8)	3.9(4/4)	25.0(2/4)	0.3(1/8)	ND(0/4)
Pre-cut tissue	1.63b	1.57c	ND(0/8)	17.9(2/4)	130.6(1/4)	ND(0/8)	ND(0/4)
Hand deboned tissue	1.93b	2.09b	ND(0/8)	308.6(2/4)	ND(0/4)	ND(0/8)	ND(0/4)
			MDTM				
Pre-deboner	3.47b	3.52b	53.0(8/8)	19.4(3/4)	61.3(3/4)	ND(0/8)	ND(0/4)
Post-deboner	4.34a	4.37a	276.0(8/8)	50.4(3/4)	690.3(4/4)	7.6(1/8)	4.1(2/4)

[a] Bacterial concentrations expressed as logarithm of number of bacteria per cm^2 of surface area or per g of tissue. Each figure is a geometric mean of 8 samples.
[b] Geometric mean of bacteria per cm^2 or per g of tissue; (Number of positive samples/total samples). ND=None detected.
[c] Geometric mean of MPN of bacteria per 1000 cm^2 or 100g of tissue; (Number of positive samples/total samples). ND=None detected.
* Breast, thigh or MDTM sample means in the same column not followed by the same letter differ significantly at $p \leq 0.05$.

Table 2 — Effect of product formulation systems on bacterial concentrations of further processed turkey meat samples

Sample location	Mesophilic[a]	Psychrotrophic[a]	Coliform[b]	Salmonellae[c]	S. aureus[c]	Clostridium perfringens[b]	Shigellae[c]
			Comminuted Roll				
MDTM post-flaker	4.14b*	4.28abc	2900(8/8)	10.7(2/4)	4611.8(4/4)	45.0(4/8)	2.5(2/4)
Primary emulsifier output	4.02bc	4.21abc	520(8/8)	9.7(2/4)	685.3(3/4)	40.0(5/8)	3.4(2/4)
Regular trim meat	4.98a	5.08a	7870(8/8)	2.2(1/4)	22.3(1/4)	ND(0/8)	ND(0/4)
Electric trim meat	3.26c	3.49c	50(7/8)	2.1(1/4)	80.3(3/4)	ND(0/8)	ND(0/4)
Blended output	3.74bc	4.03bc	600(8/8)	2.1(1/4)	118.5(3/4)	6.2(1/8)	3.9(1/4)
Product post stuffing	4.63ab	4.91ab	5700(8/8)	2.6(1/4)	364.4(3/4)	7.6(3/8)	ND(0/4)
			Emulsion Wiener				
MDTM post-flaker	3.93a	3.90b	44(8/8)	4.3(2/4)	782.7(4/4)	6.2(1/8)	ND(0/4)
Primary emulsifier output	4.14a	4.44a	88(8/8)	11.0(4/4)	15,675.0(4/4)	72.0(4/8)	3.4(2/4)
Secondary emulsifier output	3.88a	3.87b	36(8/8)	3.4(3/4)	255.8(4/4)	6.2(1/8)	4.3(2/4)
Product post stuffing	3.96a	3.92b	26(8/8)	7.5(3/4)	139.4(4/4)	6.2(1/8)	4.6(4/4)

[a] Bacterial concentrations expressed as logarithm of number of bacteria per g of tissue. Each figure is a geometric mean of 8 samples.
[b] Geometric mean of bacteria per g of tissue; (Number of positive samples/total samples). ND=None detected.
[c] Geometric mean of MPN of bacteria per 100g of tissue; (Number of positive samples/total samples). ND=None detected.
* Comminuted roll or emulsion weiner sample means in the same column not followed by the same letter differ significantly at $p \leq 0.05$.

Table 3 – Effect of cooking and packaging systems on bacterial concentrations of further processed turkey meat samples

Sample location	Mesophilic[a]	Psychrotrophic	Coliform[b]	Salmonellae[c]	S. aureus[c]	Clostridium perfringens[b]	Shigellae[c]
			Boneless Breast				
Pre-cook	2.40[a*]	2.64a	132.0(4/8)	2.1(1/4)	ND(0/4)	ND(0/8)	ND(0/4)
Post-cook	1.10[b]	1.41b	ND(0/8)	ND(0/4)	ND(0/4)	ND(0/8)	ND(0/4)
Post-package	2.70a	3.40a	5.8(2/4)	6.9(2/8)	ND(0/4)	ND(0/8)	ND(0/4)
			Comminuted Roll				
Pre-cook	4.84a	5.72a	5700.0(8/8)	2.6(2/4)	364.4(3/4)	7.6(3/8)	ND(0/4)
Post-cook	1.41b	1.08b	ND(0/8)	ND(0/4)	ND(0/4)	ND(0/8)	ND(0/4)
Post-package	**						
			Emulsion Wiener				
Pre-cook	3.96a	3.92a	26.1(6/8)	7.5(3/4)	139.4(4/4)	6.2(1/8)	4.6(4/4)
Post-cook	1.18b	1.04b	ND(0/8)	ND(0/4)	ND(0/4)	ND(0/8)	ND(0/4)
Post-package	2.78a	2.86a	19.9(3/8)	9.1(2/4)	ND(0/4)	ND(0/8)	ND(0/4)

[a] Bacterial concentrations expressed as logarithm of number of bacteria per gram. Lower detection limit was 1.0. Each figure is a geometric mean of 8 samples.
[b] Geometric mean of bacteria per g of tissue; (Number of positive samples/total samples). ND=None detected.
[c] Geometric mean of MPN of bacteria per 100g of tissue; (Number of positive samples/total samples). ND=None detected.
* Boneless breast, comminuted roll or emulsion weiner sample means in the same column not followed by the same letter differ significantly at $p \leq 0.05$.
** Comminuted roll was not sampled post-package since the market package is the same casing the roll is cooked in.

(Table 2) were very low in concentration as well as number of positive samples. No trends were apparent which could be associated with product formulation systems. Salmonella in emulsion weiner samples reflected a higher number of positive samples than in comminuted roll samples although the concentrations were low and no effects due to product formulation system could be identified. Similar results were obtained for Shigella numbers with a predominance of negative samples (concentrations below the lower detection limit) being obtained.

Cooking and packaging systems

Total mesophilic and psychrotrophic numbers obtained from cooking and packaging systems evaluation showed a highly significant decrease with a predominance of samples at or below the lower detection limit (10 organisms/gram) as a result of the oven roasting, immersion water and smokehouse cooking processes (Table 3). Results indicated that the three cooking procedures were equally effective in reducing bacterial numbers. However, a significant increase in tissue bacterial content of cooked product was noted for the oven roasted boneless breast and emulsion wiener products as a result of the handling associated with packaging in the final retail package. Comminuted roll product is marketed in the package utilized during the cooking phase and therefore could not be evaluated after repackaging. The increase noted for boneless breast and wiener product is probably due to contamination as a result of handling or from equipment surfaces in the cooked product packaging room. It should be noted that personnel in this department wear disposable sterile gloves in an effort to reduce contamination. In addition, a periodic cleaning with soap and hot water solutions is followed by sanitizing with iodine solution for all work surfaces which may contact cooked product.

Coliform numbers (Table 3) decreased significantly as a result of the three cooking processes with all samples after cooking being negative (below the lower detection limit). However, detection of coliform organisms following cooked product packaging was noted for boneless breast and emulsion wiener product. As previously suggested, this is probably due to a deficiency in either hand or equipment sanitation procedures in the cooked product packaging department. Sanitation in dealing with a cooked product is the most critical point in the overall control program of a turkey further processing plant. Similar trends were noted for Salmonella numbers (Table 3). Although the numbers were extremely low (<1/10g), the detection of a potential pathogen after packaging in a fully cooked product indicates the need for more extensive efforts in sanitation principles.

Clostridium perfringens numbers (Table 3) indicated that the three cooking processes were equally effective in eliminating the organisms with all counts being negative following cooking. In addition, all samples evaluated following cooked product packaging were found to be negative (below the lower detection limit). Similar results were obtained from *Staphylococcus aureus* and Shigella determinations (Table 3).

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Effect of Belly Composition on Sorbate-Cured Bacon

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ABSTRACT

A comparison was made between fat-type and lean-type bellies cured with either 40 ppm sodium nitrite/0.26% potassium sorbate or 120 ppm sodium nitrite. Residual nitrite levels were higher in fat bacon regardless of cure. Sorbate-cured bacon resulted in significantly higher TBA numbers than did regular-cure bacon. Nitrosopigment to total pigment ratio did not vary with cure, but did remain significantly higher in fat bacon and roughly paralleled residual nitrite levels. Analysis for nitrosamines indicated greater concentrations in fat bellies compared to lean. Nitrosamines were suppressed though not eliminated, by the sorbate cure in both fat and lean bellies.

INTRODUCTION

IN THE PAST DECADE since Magee and Barnes (1956) found nitrosodimethylamine to be carcinogenic in rats and Lijinsky and Epstein (1970) indicated that nitrosamines were possible human carcinogens, the use of nitrite in meat products has been closely scrutinized. With the advent of techniques able to measure nitrosamines at the low parts-per-billion levels, one cured meat product, bacon, when cooked at high temperature has consistently produced measurable amounts of nitrosamines (Gray and Randall, 1979; Bharucha et al., 1979; Gray and Collins, 1978; Hwang and Rosen, 1976). The commonly detected nitrosamines were nitrosopyrrolidine (Pensabene et al., 1979; Sen et al., 1979) and, to a smaller extent, nitrosodimethylamine (Gray et al., 1978). By decreasing ingoing nitrite levels in bacon from 156 to 120 ppm, together with the addition of 550 ppm sodium ascorbate, decreases in nitrosamine levels have occurred (CAST, 1978). Detectable nitrosamine levels, however, still remained. Alternatives to the use of nitrite have been examined (Sebranek, 1979). Unfortunately, removal of nitrite from the cured product has also resulted in an altered flavor and color (Braathon, 1980).

In 1978, an alternative cure was proposed (USDA, 1978) allowing for a 67% decrease in ingoing nitrite levels to 40 ppm, along with the incorporation of 0.26% potassium sorbate into the bacon. Several studies using sorbate have shown its effectiveness as an antibotulinal agent (Ivey and Robach, 1978; Ivey et al., 1978; Tompkin et al., 1974; Sofos et al., 1979) while some nitrite (40 ppm) was included to provide color and flavor formation.

Sorbic acid and its salt (potassium sorbate) have been used for decades as a mold deterrent in cheese, wine and bread (Melnick et al., 1954; Luck, 1976) and more recently have been successfully used as an antimicrobial dip for poultry (Robach and Ivey, 1978). A generallly recognized as safe (GRAS) food ingredient, sorbic acid and its salt have been shown repeatedly to be safe in rats, mice and dogs up to a level of 5% of the diet (Hendy et al., 1976; Gaunt et al., 1975; Food & Drug Research Labs, Inc., 1973).

Several workers have indicated that bacon can be produced using a 0.26% potassium sorbate-40 ppm sodium nitrite cure that results in sensory characteristics similar to a conventional 120 ppm sodium nitrite cure (Stevenson and Price, 1978; Ivey et al., 1978; Paquette et al., 1980). On the other hand, Berry and Blumer (1981) have reported sensory differences between bacon cured with 0.26% potassium sorbate-40 ppm sodium nitrite and that cured with 120 ppm sodium nitrite.

One area that has not been studied previously to a great extent is the effect of the proposed cure on the finished product under a variety of processing variables. Well known to cause the pink color and characteristic flavor of cured products (MacDougall et al., 1975), addition of nitrite also has been demonstrated to impart some antioxidant effects to cured meat (Watts, 1954). Removal of nitrite produces a less acceptable product as demonstrated by the results of a nitrite/nitrate ban in Norway (Braathon, 1980).

With questions concerning effects of the proposed cure and processing variables on characteristics of finished bacon, a study was initiated to determine the extent of potential product alterations. The first part of the study was designed to compare the effect of fat-type and lean-type bellies and the sorbate cure on finished bacon characteristics.

Bacon made from lean-type bellies is considered to be superior to bacon from fat-type bellies (Chant et al., 1976). This is due to a decrease in cooking losses from lean bacon (Carpenter et al., 1963) and to a recent trend toward decreasing levels of animal fats in the diet. The fat portion of the bacon also has been associated with greater residual nitrite and nitrosamine content (Sen et al., 1979) although Pensabene et al. (1979) indicated that ingoing and residual nitrite levels are much better indicators of nitrosamine content. Unfortunately, uniformity in belly composition is difficult to attain, and both industry and the consumer will have to accept a wide range of bellies resulting in a wide variation in bacon.

MATERIALS & METHODS

PAIRED BELLIES from 64 market-weight hogs were obtained from a local packing plant. Relative fatness was determined by using a 0–15 scale utilized by the packer, with 0 indicating a minimum and 15 a maximum amount of backfat. Bellies from carcasses measuring 2–4 on the 15-point scale were selected as lean representatives, and bellies from carcasses measuring 11–14 were selected as fat representatives. The bellies were skinned with a Townsend skinner and separated into two groups across pairs. One member of each pair of bellies was pumped to 110% of green weight with a brine containing 17% sodium chloride (salt), 2.6% sucrose, 2.0% sodium tripolyphosphate, 1200 ppm sodium nitrite and 5500 ppm sodium erythorbate. The second member of each pair was pumped to 110% of green weight with a brine containing identical levels of salt, sucrose, tripolyphosphate and erythorbate, but with 400 ppm nitrite and 2.6% potassium sorbate. This resulted in an ingoing nitrite level of 120 ppm in half the bellies, referred to as the regular cure, and 40 ppm nitrite and .26% sorbate in the other half, referred to as sorbate cure.

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The bellies were cooked and smoked to an internal temperature of 52°C. The bacon was then chilled at 5°C for 48 hr. After chilling, the bacon was sliced into strips approximately 3 mm thick and vacuum packaged, 14 slices per package in a shingled form by using a nylon/Saran/surlyn, oxygen impermeable pouch. Packages were subsequently boxed and held in a cooler at 5°C for 14 days. Selected packages then were removed and placed in a display cooler under 200 ft-c of cool, white fluorescent light on a 24-hr-on, 24-hr-off basis, for an additional 14 days.

For chemical analysis, package numbers 5 and 12 (slices 70–84 and 168–182 from each belly) were obtained. This was done to lessen variability between samples (Nusbaum et al., 1976). On 0, 10, 21 and 28 days after packaging, packages from each of eight bellies were opened. The eight bellies were duplicates from each of the following combinations; regular-cure, fat belly; regular-cure, lean belly, sorbate-cure, fat belly; sorbate-cure, lean belly. Each bacon strip was divided into fat and lean fractions by dissecting out the muscle tissue in the strips. Fat located within the lean portions was not removed. The fat and lean portions were frozen in liquid nitrogen and pulverized by using a Waring Blendor. One-fourth of the dissected fat and lean portions were recombined to form a fraction representing the total or average composition. Duplicate analyses on each fraction were performed to determine residual nitrite by using the 1970 AOAC method (due to the carcinogenic nature of α-naphthylamine, an alternative method is recommended). Rancidity was determined using the 2-thiobarbituric acid method of Tarladgis et al. (1960), nitrosopigment according to Hornsey (1956) and residual salt by using the Technicon (1965) autoanalyzer technique for chloride ion. The samples were also analyzed for moisture, fat and ash (AOAC, 1970). Protein was measured with a micro-Kjeldahl procedure, and pH was measured by blending 10g of frozen sample in 75 ml of distilled water. After 0, 10, and 21 days of storage the bacon samples were frozen, packed in insulated chests with dry ice and shipped to Monsanto (St. Louis, MO) for N-nitrosamine analysis. Bacon slices were fried and prepared for a gas chromatograph-thermal energy analyzer (GC-TEA) anlaysis as described by Robach et al. (1980).

Statistical analysis was performed by using the Statistical Analysis System (SAS) program. The design was a split plot over time with cure and belly type arranged in a two-by-two factorial. The standard F-ratio analysis of variance was implemented with Duncan's (1955) New Multiple Range test used between means where significance was indicated. Variation between pigs was used as the main-effect error term and was in every case larger than the corresponding residual-error term.

RESULTS & DISCUSSION

THE RESULTS of cure and belly type on proximate analysis are presented in Table 1. The lean and fat bacon differed significantly in moisture, fat and protein levels. The differences in the levels were not as great as expected, however. Chant et al. (1976) found a 15% difference in fat content between bellies from extremely lean and fat hogs. In selecting their ranges, however, several different methods of indicating fat thickness were used rather than simply a backfat measurement. Schroeder and Rust (1974) indicated that the actual fatness in bellies was highly correlated to the use of a backfat probe measurement.

The effect of cure, belly type and date on residual nitrite is shown in Table 2. Initially, the regular-cure bacon had a much higher average residual nitrite level (43.1 ppm) as compared with sorbate-cured bacon (23.6 ppm). This is as expected inasmuch as regular bacon had 3 times the ingoing nitrite content than did sorbate-cured bacon. By day 10, however, it was evident that residual nitrite levels dropped much faster in lean bacon than in fat bacon. Fat is known to have a protective effect on nitrosamines during cooking (Fiddler et al., 1974; Goutefongea et al., 1977). This presumably is due to dissolving of formed nitrosamines into the fat preventing volatilization (Fiddler et al., 1974). Our results indicate that fat may play another role by "protecting" nitrite before cooking and thus encouraging greater nitrosamine formation because of higher residual nitrite levels. In lean bacon by 21 and 28 days after processing, little difference in residual nitrite levels is seen between cures, but a sizable difference still is seen between cures in fat bacon.

Table 3 presents the result of the nitrosamine analysis. Although most of the levels are below regulatory limits and small experimental errors could significantly effect the results, two trends seem to appear. First, fat bacon regardless of cure resulted in higher nitrosamine levels. Secondly, the sorbate cure results in consistently lower nitrosamine levels. This is probably not a direct effect on nitrosamines by potassium sorbate, but more likely is a decrease in nitrosamine levels due to a decrease in ingoing nitrite.

Table 4 presents the effects of cure, belly type and date on pH. At each date tested fat bacon had a consistently higher pH value. The antibacterial effectiveness of sorbate is greatest below a pH of 6.0 and decreases in effectiveness to a pH of 6.5 (Monsanto, 1978). As a result, the high pH values found in fat bacon could be of concern with commercially processed bacon. No significant difference in pH occurred as a result of cure. This was unexpected because addition of sorbate to a meat system was reported by Pierson et al. (1979) to cause a pH drop. This lack of change may be the result of a buffer effect of the meat system and the tripolyphosphate added to the cure.

A study of the effect of a decrease in nitrite on levels of rancidity was performed. In cured meat, the modified method of Zipser and Watts (1962) is commonly substituted for the method of Tarladgis (1960) to prevent inter-

Table 1—Effect of sorbate cure and fat and lean-type bacon on proximate analysis[a]

	% Moisture	% Fat	% Ash	% Protein
Regular cure, Fat bacon	30.7 ± 1.6[a]	57.1 ± 2.3[a]	2.2 ± 0.1[a]	7.3 ± 0.6[a]
Sorbate cure, Fat bacon	30.2 ± 1.6[a]	57.0 ± 2.4[a]	2.4 ± 0.1[b]	7.3 ± 0.5[a]
Regular cure, Lean bacon	35.4 ± 1.6[b]	50.6 ± 2.4[b]	2.4 ± 0.1[b]	10.4 ± 0.6[b]
Sorbate cure, Lean bacon	35.2 ± 1.6[b]	50.4 ± 2.4[b]	2.5 ± 0.1[b]	10.4 ± 0.7[b]

[a] Averages with a common letter within columns are not different at the 5% level of significance based on Duncan's Multiple Range Test.

Table 2—Effect of sorbate cure and belly type and date on residual nitrite (ppm)[a]

Day	0	10	21	28
Regular cure, Fat bacon	40.8 ± 5.1[a]	34.2 ± 4.2[a]	22.2 ± 3.5[a]	16.5 ± 1.5[a]
Sorbate cure, Fat bacon	26.6 ± 2.6[b]	18.5 ± 1.9[b]	13.6 ± 1.7[b]	9.8 ± 0.8[b]
Regular cure, Lean bacon	45.4 ± 4.0[a]	14.3 ± 1.7[c]	7.3 ± 0.5[c]	7.4 ± 0.7[c]
Sorbate cure, Lean bacon	20.5 ± 1.7[b]	10.4 ± 0.6[d]	6.4 ± 0.6[c]	6.3 ± 0.6[c]

[a] Averages with a common letter within columns are not different at the 5% level of significance based on Duncan's Multiple Range Test.

ference by nitrite with the TBA color formation. In several comparisons done in our laboratory, it was found that the modified method had no effect, or in some instances, decreased the amount of malonaldehyde measured in the final product. Consequently, the method of Tarladgis (1960) was adopted.

The results of the TBA analysis are presented in Table 5. The type of cure had a large effect on the results of the TBA test, with sorbate-cured bacon possessing significantly greater TBA numbers than regular-cure bacon. None, however, approached levels commonly associated with detectable rancidity. This is as expected from vacuum packaged freshly processed bacon. The high TBA number appeared to be related to sorbate content in the bacon. The AOAC (1970) method for sorbate detects oxidized sorbate using TBA reagent. Luck (1976) indicated that in the presence of high salt content under acidic conditions sorbic acid is altered to form malonaldehyde which then reacts with TBA reagent.

Salt content of the bacon may play a role in combination with nitrite in the formation of nitrosamines (Hildrum et al., 1975; Theiler et al., 1981). As a result, a comparison of the two cures and belly types on residual salt concentration was performed. There were no significant differences between cures, dates or belly types with regard to salt concentrations.

MacDougall et al. (1975) reported that 25 ppm nitrite was sufficient to ensure adequate color formation. We were also interested in the duration of nitrosopigment in the product during storage. In Table 6 a difference between fat and lean bacon types is demonstrated. The nitrosopigment formation parallels the residual nitrite content of the bacon. This indicates a continuous turnover of nitrosated pigment. There were no cure differences at the 0.05 level of probability though the regular cure was usually higher.

A secondary goal of this research was to determine the effect of the curing ingredients on separated lean, fat and total fractions. The differences in composition among the three fractions is shown in Table 7. As is seen, even gross dissection resulted in formation of three different fractions, representing a reasonable estimation of the three areas. The three fractions were found significantly different in nitrite, pH, TBA number and salt levels. As seen in Table 6 the lean fraction possessed the highest values for residual nitrite, amount of rancidity and salt concentration, followed by the total fraction and then the fat fraction. All differences were significant except for rancidity for which no significant difference was realized between total and fat fractions.

Both the sodium chloride and nitrite were added as water-soluble salts and, as such, would solublize with more ease in the lean portion of the belly except where pockets of brine may form in the fat. TBA values on the other hand, agree with the data on fat and lean belly type, which showed that, even though more fat was available in the fat portion, more fat oxidation was occurring in the lean fraction. As also was seen with the lean and fat belly type, pH remained elevated in the fat portion of the belly. A significant cure by fraction interaction was seen in pH and TBA measurements. A significant difference in pH was seen between cures only in the fat fraction (6.50–6.44), with sorbate cure being lower. TBA measurements also were much higher in the sorbate-cured lean fraction when compared with the regular-cure lean fraction.

Table 3–Effect of sorbate or regular cure and fat or lean type bellies on nitrosamine content of intact bacon slices[a]

Belly type	Cure	Storage (days)	N-nitrosamines (PPb) Nitrosodimethylamine (NDMA)	Nitrosopyrrolidine (NPYR)
Lean	Regular	0	0.5	3.3
		10	0.4	1.7
		21	0.4	2.5
Fat	Regular	0	1.1	16.5
		10	0.6	6.0
		21	1.0	12.0
Lean	Sorbate	0	N.D.	0.7
		10	N.D.	N.D.
		21	0.2	1.7
Fat	Sorbate	0	0.6	5.5
		10	0.9	3.6
		21	0.5	4.5

[a] N.D. = none detected; NDMA < 0.2 ppb; NPYR < 0.5 ppb.

Table 4–Effect of sorbate cue, belly type and date on pH[a]

Days after packaging	0	10	21	28
Regular cure, fat bacon	6.51[a]	6.47[a]	6.49[a]	6.44[a]
Sorbate cure, fat bacon	6.49[a]	6.51[a]	6.44[a]	6.36[b]
Regular cure, lean bacon	6.40[b]	6.16[b]	6.26[b]	6.14[c]
Sorbate cure, lean bacon	6.26[b]	6.12[b]	6.20[b]	6.12[c]

[a] Averages with a common letter are not different at the 5% level of significance based on Duncan's Multiple Range Test.

CONCLUSIONS

BACON CURED with a brine incorporating a two-thirds reduction in nitrite, together with the addition of 0.26% potassium sorbate, compared to a regular cure, showed little effect on the chemical characteristics of cured bacon within 28 days. Residual nitrite was significantly greater in fat bacon than in lean, but was not significantly different between cures on day 28. pH was significantly higher in fat bacon than in lean bacon, but no difference was seen between cures. TBA number was significantly greater in sorbate-cured bacon, although it is doubtful this is due to a decreased level of nitrite in the product. Nitrosopigment formation was not significantly different at the 0.05 level. Nitrosamine levels were apparently decreased in both fat and lean bellies due to lower initial nitrite levels in the sorbate cure.

In the lean fraction of the bacon, increased residual levels of the soluble salts were seen, as well as a decrease in pH and an increase in TBA number as compared to the fat fraction.

Table 5–Effect of sorbate cure, belly type and date on TBA number

Days after packaging	0	10	21	28
Regular cure, Fat bacon	0.156 ± 0.016[a]	0.241 ± 0.017[a]	0.153 ± 0.019[a]	0.203 ± 0.014[a]
Sorbate cure, Fat bacon	0.530 ± 0.126[b]	0.355 ± 0.027[b]	0.237 ± 0.039[b]	0.419 ± 0.045[b]
Regular cure, Lean bacon	0.326 ± 0.019[c]	0.222 ± 0.026[a]	0.340 ± 0.024[c]	0.199 ± 0.019[a]
Sorbate cure, Lean bacon	0.490 ± 0.045[b]	0.423 ± 0.052[c]	0.406 ± 0.058[d]	0.428 ± 0.062[b]

[a] Averages with a common letter within columns are not different at the 5% level of significance based on Duncan's Multiple Range Test.

Table 6—Effect of sorbate cure, belly type and date on nitrosopigment/total pigment ratio[a]

Days after packaging	0	10	21	28
Regular cure, Fat bacon	0.813 ± 0.243[a]	0.776 ± 0.067[a]	0.670 ± 0.051[a]	0.545 ± 0.009[a]
Sorbate cure, Fat bacon	0.528 ± 0.027[b]	0.687 ± 0.098[a]	0.528 ± 0.033[b]	0.494 ± 0.027[b]
Regular cure, Lean bacon	0.441 ± 0.040[c]	0.594 ± 0.033[b]	0.424 ± 0.087[c]	0.470 ± 0.051[b]
Sorbate cure, Lean bacon	0.427 ± 0.050[c]	0.481 ± 0.051[c]	0.502 ± 0.081[bc]	0.432 ± 0.040[b]

[a] Averages with a common letter within columns are not different at the 5% level of significance based on Duncan's Multiple Range Test.

Table 7—Compositional characteristics of the lean, fat and total fractions of bacon[a]

	% Moisture	% Fat	% Ash	% Protein	Nitrite (ppm)	pH	TBA number	% Salt
Lean	52.5 ± 0.5[a]	24.8 ± 0.7[a]	3.81 ± 0.05[a]	15.3 ± 0.4[a]	28.4 ± 1.9[a]	6.21[a]	0.458 ± 0.03[a]	2.75 ± 0.04[a]
Fat	16.4 ± 0.3[b]	78.4 ± 0.4[b]	1.20 ± 0.02[b]	3.4 ± 0.2[b]	11.2 ± 0.7[b]	6.47[b]	0.227 ± 0.01[b]	0.82 ± 0.02[b]
Total	29.9 ± 0.6[c]	57.9 ± 0.9[c]	2.19 ± 0.04[c]	8.2 ± 0.3[c]	17.2 ± 1.1[c]	6.32[c]	0.279 ± 0.01[b]	1.60 ± 0.03[c]

[a] Averages with a common letter within columns are not different at the 5% level of significance based on Duncan's Multiple Range Test.

The quality of the final bacon cured with lower nitrite levels and added sorbate does not seem to be decreased significantly despite differences in belly composition. The sorbate-reduced nitrite cure does seem to decrease potential for nitrosamine formation in cooked bacon.

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Effect of Belly Handling (Chilled, Frozen, Prerigor) and Smoking Delay on Regular and Sorbate-Cured Bacon

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ABSTRACT

Bellies from three handling methods; fresh, frozen, or prerigor were cured with either 40 ppm sodium nitrite/0.26% potassium sorbate or 120 ppm sodium nitrite. After pumping, one group of bellies was processed (cooked and smoked) immediately, and the remaining group was held 72 hr before processing. Residual nitrite was significnatly higher in regular cure bacon and in bellies processed immediately. By 28 days after packaging, little difference was seen in residual nitrite as a result of any treatment. Regular bacon and immediate processing resulted in greater nitrosopigment conversion. Prerigor bacon had greater pigment conversion and greater residual NaCl concentrations while fresh and frozen bellies were similar. Nitrosamine levels were not influenced by belly handling variables.

INTRODUCTION

THE FORMATION of nitrosamines from nitrite and secondary amines in cured meats is well documented (Fazio et al., 1973; Bharucha et al., 1979; Pensabene et al., 1979). Several alternatives have been developed or suggested to decrease or remove ingoing sodium nitrite ($NaNO_2$). Alternatives include a decrease in formulated $NaNO_2$ from 156 ppm to 120 ppm, addition of sodium ascorbate in the cure, incorporation of alpha-tocopherol or lactic acid-producing bacteria into bacon (Sebranek, 1979). Removal of $NaNO_2$ increases the possibility of *Cl. botulinum* growth in abused packages, making this the primary concern in developing alternatives. In addition, products cured without nitrite have been demonstrated to be less acceptable to the consumer in flavor and color when compared with conventionally cured products (Braathon, 1980).

In 1978, an alternative cure for bacon was proposed that decreased ingoing nitrite levels from 120 ppm to 40 ppm together with addition of 0.26% potassium sorbate. In a review by Sofos et al. (1979), potassium sorbate has been shown to be an effective inhibitor of *Cl. botulinum* as well as of *Staphylococcus aureas* (Pierson et al., 1979), salmonella and *Cl. perfringens* (Tompkin et al., 1974). Commonly used as a fungistat in cheese, cottage cheese, wine and bread (Melnick et al., 1954; Monsanto, 1978), potassium sorbate is a generally recognized as a safe (GRAS) food additive. In rats, dogs and mice, up to a level of 5% in the diet (Hendy et al., 1976; Gaunt et al., 1975) is considered safe. This degree of safety has been attributed to the relative ease of sorbate digestion via α,β-fatty acid oxidation (Deuel et al., 1954).

A three-part experiment was initiated at Iowa State University to study the effects of several processing variables on bacon cured with a regular and a sorbate cure. Prior experiments have demonstrated the effect of nitrite in the formation of a "cured" color and flavor (MacDougall et al., 1975). In addition, nitrite reduces lipid oxidation in cured bacon (Watts, 1954). The purpose of the study reported here was to examine the effects of belly handling, potassium sorbate and delayed processing on chemical characteristics of sliced bacon.

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MATERIALS & METHODS

SIXTEEN PAIRED BELLIES were obtained from a local packing plant, skinned and frozen for 30 days at −30°C. After 30 days, an additional 16 paired bellies were obtained. Immediately preceding brine injection, a third group of 16 paired bellies was obtained from freshly slaughtered market weight hogs. These groups represented the three types of belly condition, frozen, fresh and prerigor.

One member of each pair of bellies was injected to a target value of 110% of green weight with a cure containing 17% sodium chloride (NaCl), 2.5% sucrose, 2.0% sodium tripolyphosphate, 5500 ppm sodium ascorbate and 1200 ppm sodium nitrite ($NaNO_2$). The other member of each pair was pumped to a target value of 110% of green weight with a brine having the same formulation, but substituting 400 ppm $NaNO_2$/2.6% potassium sorbate for the 1200 ppm $NaNO_2$. The bellies were then divided into two groups. One group was cooked and smoked immediately, and the other group was held at 5°C for 72 hr before smokehouse processing. All bellies were cooked and smoked to an internal temperature of 54°C. After being smoked, the bacon was tempered to 5°C for 24 hr before packaging.

The bacon was sliced into 3-mm slices and packaged 14 slices per package. The slices were packaged in pouches made of nylon, saran and surlyn with an oxygen permeability at 38°F, 90% RH, of 0.05 cc/100 in^2. The pouches were evacuated at 24 inches (610 mm) mercury. Evacuated packages were boxed and held in a cooler at 5°C for 14 days. Selected packages were then removed and placed in a display cooler under 200 ft-c. of cool white fluorescent light on a 24-hr-on, 24-hr-off basis for an additional 14 days.

Packages number 5, 6 and 11, 12 (slices 70–98 and 168–196) were selected for chemical analysis. This was done to lessen variation found between bellies and between different locations on the same belly (Nusbaum et al., 1976). On 0, 10, 21, and 28 days after packaging, chemical analyses were performed on the bacon. Each bacon strip from packages representing duplicates of each treatment combination was divided into fat and lean tissue by dissecting the lean and fat borders. Fat tissue located within a lean area was not separated. The fat and lean portions were frozen in liquid nitrogen and pulverized with a Waring Blendor. One-fourth of the dissected fat and lean portions were recombined to form a fraction representing average belly composition. The frozen, powdered bacon was analyzed for residual nitrite by using the AOAC (1970) method. Analysis for rancidity was performed by using the 2-thiobarbituric acid procedure of Tarladgis et al. (1960). A modified method of Hornsey (1956) was used to determine nitrosopigment conversion. Residual NaCl was measured by using the Technicon Autoanalyzer (1965) method for chloride ion concentration; pH was measured by blending 10g of frozen sample in 75 ml of distilled water and measuring with a Corning Model 125 pH meter featuring a combination electrode designed for use with slurries or emulsions. Analysis for nitrosamine content was performed by Monsanto Corporation. Slices of bacon were fried, extracted and submitted to a gas chromatograph-thermal energy analyzer as described by Robach et al. (1980).

Statistical analysis was performed by using the Statistical Analysis System for analysis of variance. Variation between pigs was used as the main effect error term and was in every case larger than the corresponding residual error. When significance was indicated, means were compared by using Duncan's New Multiple Range test of means (Duncan, 1955).

RESULTS & DISCUSSION

THE RESULT OF CURE, belly handling and processing time on moisture, fat and ash content is seen in Table 1. As is seen, moisture, fat and ash levels were significantly different with respect to belly handling variables. Regardless of cure or processing time, the prerigor bellies resulted in bacon with much greater moisture and ash levels and lower fat levels. This initially might seem to indicate that the prerigor bellies were from leaner hogs, but this was not actually the case. During belly skinning and bacon slicing, a greater fat loss was observed in the prerigor bellies than in fresh or frozen bellies.

The greater lean content in the prerigor-type bacon influences the water-soluble salt concentrations in the bacon. An earlier experiment (Amundson et al., 1981) examining the effect of lean and fat belly type on chemical characteristics indicated that greater levels of water-soluble salts, together with higher TBA numbers, were initially found in lean-type bacon. Later, the fat-type bacon exhibited a preservative effect on residual nitrite. As a result, prerigor effects may be difficult to distinguish from the effects due to increased lean content.

The effect of date, smoking delay and belly handling variables on residual nitrite level is seen in Table 2. There was no difference in residual nitrite level due to belly handling variable. This may indicate that, although an obvious difference in proximate analysis existed, lean content may not be large enough in itself to cause a difference. There was a significant difference in residual nitrite between bacon from bellies cooked immediately after pumping when compared with delay cooked bellies. Preceding 28 days after packaging, delay-processed bacon possessed significantly lower nitrite levels. By 28 days, no difference is seen. The lower nitrite content of delayed-processed bacon, however, could simply be the result of a 72-hr greater time from pumping to the measuring date. Fig. 1 shows the residual nitrite levels based on packaging date, compared with the date after pumping. As is indicated, the 72-hr delay by itself may have an effect in decreasing residual nitrite level, but a separate effect of cooking delay still exists. Further, residual nitrite was consistently higher in regular as opposed to sorbate-cured bacon as might be expected (data not shown). A significant date by cure interaction also occurred, due to the extremely large difference in residual nitrite between regular and sorbate cure bacon (39.6 ppm to 19.0 ppm) seen at 0 days after packaging and the smaller difference (10.2 ppm to 7.0 ppm) seen at 28 days after packaging.

None of the treatments had a significant effect on product pH. In an earlier experiment, it was shown that increased lean content resulted in a lower pH, presumably due to a higher metabolic capability in the lean (Amundson et al., 1982). Although the trend appears in this work (prerigor bellies pH average was 6.18 compared with 6.24 for the other belly types), a significant difference did not occur. There was no difference between cures for pH as sorbate-cured bacon had an average pH value of 6.23 compared with 6.22 for regular cure bacon. Pierson et al. (1979) indicated that a pH drop accompanied the incorporation of potassium sorbate into a meat product.

The modified TBA method of Zipser and Watts (1962) commonly has been used in measuring rancidity in cured meat because of the interference of $NaNO_2$ in TBA color development. Several comparisons in our lab indicated that

Table 1 — Effect of cure, smoking time and belly condition on moisture, fat and ash content[a]

Cure	Smoke		Moisture	Fat	Ash
Nitrite	Immediate	Fresh	29.8 ± 0.8C	59.7 ± 1.0AB	1.97 ± 0.06CD
		Frozen	26.6 ± 0.9C	63.7 ± 1.3A	1.74 ± 0.08D
		Prerigor	38.0 ± 0.7B	48.7 ± 1.0C	2.45 ± 0.08A
	Delayed	Fresh	29.5 ± 0.9C	59.4 ± 1.2AB	1.87 ± 0.06D
		Frozen	31.3 ± 1.2C	57.0 ± 1.64B	1.92 ± 0.07D
		Prerigor	40.5 ± 1.1AB	44.7 ± 1.5CD	2.29 ± 0.06B
Sorbate	Immediate	Fresh	30.9 ± 1.1C	57.6 ± 1.4AB	2.00 ± 0.11CD
		Frozen	28.7 ± 0.9C	61.0 ± 1.2AB	1.93 ± 0.10D
		Prerigor	38.9 ± 0.8B	47.1 ± 1.1C	2.87 ± 0.11A
	Delayed	Fresh	29.9 ± 0.96C	58.5 ± 1.3AB	2.22 ± 0.06BC
		Frozen	28.4 ± 1.1C	59.9 ± 1.7AB	1.84 ± 0.08D
		Prerigor	43.8 ± 1.73A	40.8 ± 2.1D	2.75 ± 0.05A

[a]Averages with a common letter within columns are not different at the 5% level of significance based on Duncan's Multiple Range Test.

Table 2 — Effect of days after packaging, smoking time and belly condition or residual nitrite[a]

Smoking Time After Pumping	Belly Condition	Days after Packaging			
		0	10	21	28
Immediate	Fresh	35.2 ± 3.8AB	15.7 ± 1.1CD	17.0 ± 1.3CD	7.5 ± 0.6D
	Frozen	31.5 ± 3.3AB	24.9 ± 2.7B	17.1 ± 1.9CD	12.7 ± 2.0D
	Prerigor	36.8 ± 2.8A	21.3 ± 1.5BC	11.2 ± 0.8D	8.8 ± 0.6D
72-Hr delay	Fresh	28.3 ± 3.4B	13.8 ± 1.4CD	8.7 ± 0.7D	10.5 ± 1.0D
	Frozen	28.8 ± 3.1AB	16.4 ± 1.9CD	10.9 ± 1.1D	7.2 ± 0.7D
	Prerigor	28.5 ± 1.7B	16.5 ± 1.0CD	10.7 ± 0.8D	8.8 ± 0.7D

[a]Averages with a common letter by column or date are not different at the 5% level of significance based on Duncan's Multiple Range Test.

the modified method was either no different or gave results slightly lower than the method of Tarladgis et al. (1960).

Sorbate-cured bacon had significantly greater TBA numbers than did regular cure bacon but at no time did the TBA number approach levels commonly associated with detectable rancidity. The use of the TBA number-malonaldehyde content has long been a favored method of determining oxidative rancidity. Luck (1976) indicated that, in an acidic environment with high NaCl content, sorbic acid was converted into malonaldehyde. In the AOAC (1970) determination of sorbic acid, the TBA method is used following oxidation of sorbic acid. It is suggested that alternative methods of measuring rancidity be used for determining the development of rancidity in cured meat that involves sorbic acid or potassium sorbate.

The effect of date, cure and processing delay on nitrosopigment conversion is seen in Table 3. As expected, the regular cure bacon had greater pigment conversion than did sorbate cure bacon at all dates. The nitrosopigment conversion also is significantly greater in bacon cooked immediately after pumping than in bacon cooked 72 hours after pumping. A significant difference in nitrosopigment conversion by date is seen, with 0 and 10 days after packaging resulting in higher conversion levels than found at 21 and 28 days. In immediately processed bacon, pigment conversion increases to 10 days after packaging. This pattern also was seen in earlier experiments. The delay-processed bacon, on the other hand, gradually decreased in conversion to 28 days after packaging. Belly handling variables had a significant effect on pigment conversion. Bacon from fresh and frozen bellies had similar nitrosopigment conversions (0.420 and 0.408, respectively) while bacon from prerigor bellies averaged a 0.502 conversion. Although other possible explanations exist, the higher lean content seen in prerigor bellies is the most likely reason for these results.

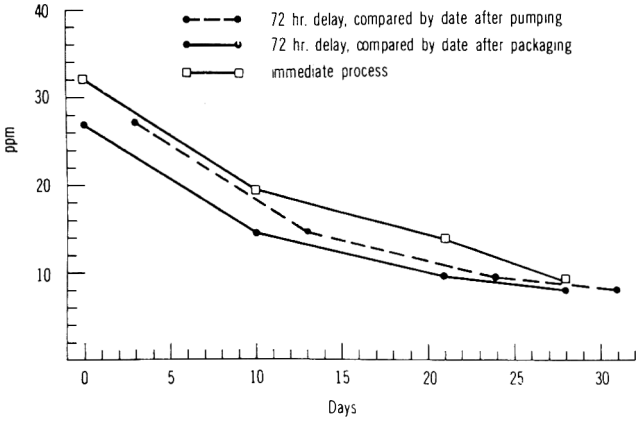


Fig. 1—Residual nitrite compared by date after packaging and by date after pumping.

The NaCl content of a product cured with $NaNO_2$ is suggested to be involved in nitrosamine formation (Hildrum et al., 1975; Theiler et al., 1981). In addition, as discussed earlier, NaCl content also is involved with the formation of malonaldehyde from sorbic acid (Luck, 1976). For these reasons, the level of residual NaCl in the bacon is of interest. No significant differences in NaCl concentration were seen because of the effects of days after packaging, processing delay or type of cure (data not shown). Belly handling did, however, result in a difference in residual NaCl concentration, with bacon from prerigor bellies having an average concentration of 2.03%, as compared with bacon from fresh and frozen bellies, 1.58% and 1.39%, respectively. This is different from the levels of the other water-soluble salt ($NaNO_2$) monitored, which suggests greater depletion of $NaNO_2$ in prerigor bacon.

Table 4 shows the nitrosamine levels in bacon from the various treatments. The reduced nitrite levels in sorbate cures resulted in suppression of nitrosamines as determined by earlier work (Robach et al., 1980). However, neither belly treatment nor processing delay produced any significant effect on the nitrosamine levels as analyzed.

CONCLUSIONS

BELLIES frozen for 30 days, refrigerated after slaughter for 48–72 hr, or obtained immediately after slaugher were injected with a brine incorporating either 40 ppm $NaNO_2$/0.26% potassium sorbate or 120 ppm $NaNO_2$. The bellies were cooked either immediately after injection or 72 hr after injection.

Prerigor bellies had a greater lean tissue content than did frozen or fresh bellies because of handling difficulties. This resulted in apparently higher nitrosopigment conversion in bacon from prerigor bellies. Bacon from prerigor bellies also had significantly higher levels of residual NaCl. Residual nitrite levels and nitrosopigment conversion were significantly greater in the regular cure bacon when compared with sorbate cure bacon, but TBA levels were higher in sorbate cure bacon. Bellies cooked and smoked immediately after pumping initially averaged higher residual nitrite levels, but by 28 days after packaging, no difference occurred as a result of delayed cooking. No difference was seen between the two in TBA number after the initial date. Bellies cooked immediately after pumping resulted in greater nitrosopigment conversion compared with delay-processed bellies.

No notable benefit in the resultant bacon was evident as a result of the handling variables examined. A processing delay of 72 hr after pumping may help to decrease residual nitrite but also may decrease color stability. The use of sorbate cure lowered residual nitrite levels, decreased nitrosopigment conversion and suppressed nitrosamine levels. Belly handling variables or delayed processing did not alter nitrosamine formation in this study.

It was found that the TBA test for rancidity was more positive for sorbate cured bacon even at a low salt content.

Table 3 – Effect of date, cure and delayed processing on nitrosopigment[a,b]

Cure		0	10	21	28
Regular	Immediate	0.507 ± 0.020A	0.558 ± 0.040A	0.454 ± 0.025A	0.466 ± 0.019A
	24-Hr Delay	0.486 ± 0.022A	0.463 ± 0.022B	0.432 ± 0.021A	0.392 ± 0.022B
Sorbate	Immediate	0.463 ± 0.016AB	0.501 ± 0.028AB	0.398 ± 0.166A	0.463 ± 0.023A
	24-Hr Delay	0.402 ± 0.021B	0.397 ± 0.027C	0.349 ± 0.013B	0.360 ± 0.028B

[a] Averages with a common letter within columns are not significantly different at the 5% level of significance based on Duncan's Multiple Range Test.
[b] Values are expressed as proportion of total pigment present in the cured form.

Table 4 — Nitrosamine levels generated as a result of experimental variables

Belly Treatment	Process	Cure	Day 0 NDMA[a]	Day 0 NPYR[b]	Day 10 NDMA	Day 10 NPYR	Day 21 NDMA	Day 21 NPYR
Fresh	Immediate	Sorbate	0.5	1.2	0.5	2.5	0.5	3.5
		Regular	1.4	4.3	1.6	5.9	1.0	7.0
	72-hr delay	Sorbate	1.1	3.3	0.6	3.2	1.0	3.5
		Regular	1.2	4.5	1.1	5.2	1.5	7.5
Frozen	Immediate	Sorbate	1.0	3.4	0.5	4.1	0.6	5.7
		Regular	1.2	6.3	1.1	5.7	0.9	7.5
	72-hr delay	Sorbate	0.4	2.5	0.4	3.8	0.7	6.0
		Regular	0.6	3.7	0.9	7.5	1.1	9.8
Prerigor	Immediate	Sorbate	0.9	1.8	0.2	1.6	1.1	2.6
		Regular	0.6	2.5	0.7	3.3	0.5	4.6
	72-hr delay	Sorbate	0.3	0.8	ND	0.7	0.7	1.2
		Regular	0.5	1.9	0.7	2.3	0.5	3.0

[a] Nitrosodimethylamine
[b] Nitrosopyrrolidine

This suggests that the use of an alternate method of determining rancidity in sorbate cured products may yield more realistic results.

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Evaluation of 54° and 60°C Holding Temperatures on Aerobic Plate Counts and Coliform Determinations of Control and Post Cooking Contaminated Beef Roast

H. W. OCKERMAN and R. DOWIERCIAL

ABSTRACT

Fabricated beef roasts were cooked to an internal temperature of 60°C and the surface inoculated with a suspension of *Escherichia coli* at a level of 10^6 cells/cm^2. Roasts were then stored in a warming oven at 54° and 60°C for 1, 2, 6, and 12 hr. Under these conditions the destruction of this strain of *E. coli* is quite rapid (less than 6 hr) at the 60°C holding temperature. The holding temperatures do allow the growth of thermoduric organisms naturally present in the product to initiate exponential growth after 6 hr at 54°C. The higher holding temperature reduced the rate of multiplication of the thermoduric organisms.

INTRODUCTION

RESTRUCTURING of meat produces a uniform, reasonably priced product that is becoming popular as a restaurant menu item (Mandigo, 1975; Cross and Stanfield, 1976) and gives almost total control of the fat content, texture, bite, shape, weight, flavor, and juiciness of the finished product (Farrington, 1975).

The muscle tissue from healthy animals contains few organisms at the time of slaughter (Ockerman et al., 1964; Romans and Ziegler, 1974; Weiser et al., 1976), but the reduction of particle size and mixing during restructuring adds and mixes microorganisms throughout the meat block (Randall and Larmond, 1977). Dennis et al. (1972), however, reported that total plate count, presumptive coliform and *Clostridium perfringens* in restructured beef roasts do not cause an increased public health hazard when compared with fresh beef cuts. A temperature above 60°C or below 6.7°C is important in controlling *C. perfringens* and most other microorganisms in the meat food products (Lechowich, 1971). Brown and Twedt (1972), however, found that a 60°C holding temperature of "Beef Served to Order" might be reduced without endangering the public health from growth of *Staphylococcus, Salmonella* or *C. perfringens.*

The coliform count is the traditional indicator of enteric pathogens in food products (Solberg et al., 1976; Thatcher and Clark, 1968). The coliform group are members of the family Enterobacteriaceae which also includes the pathogenic genera *Salmonella, Shigella* and *Yersinia* (de Figueiredo and Jay, 1976). The native habitat for *Escherichia coli* is the enteric tract of man and warm-blooded animals, and range of growth is normally considered to be between 10° and 45°C (Buchanan and Gibbons, 1974).

Maxcy (1976) held hamburgers inoculated post-cooking at 50°C and 60°C with and without covers and reported that film coverings ensured surface temperature hot enough to prevent growth. Carter (1977) held restructured beef roasts at 54° and 60°C for 12 hr and found no difference in plate count due to temperature of holding and time of holding for roasts in which most of the microorganisms were destroyed during cooking.

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The objectives of this study are the comparison of aerobic and coliform counts during 12 hr at 54° vs 60°C for control vs *E. coli* post cooking inoculated roasts.

EXPERIMENTAL

THE SAMPLES OF FROZEN, fabricated beef (3.8 ± 0.2 kg each; 23 x 17 x 9 cm each), manufactured in one production, were obtained from a commercial supplier. The roasts were defrosted for 48 hr at 3–4°C, then covered with an aluminum foil cap. Two thermopins (Pat. No. 2835480) were inserted in the bottom one-third of the roast. The roasts were cooked in a preheated (93–100°C) forced air oven to an internal temperature of 60°C. The roasts were removed from the oven and the temperature allowed to stabilize for 30 min. The thermopins were removed. Roasts were assigned randomly to contaminated and control treatments. Contaminated roasts were surface inoculated with a suspension of *E. coli* (O.S.U. strain, #455, The Ohio State Univ. Microbiology Culture Laboratory) with approximately 10^6 cells/cm^2 (1.6×10^9 cells/roast). The surface-contaminated roasts and the control roasts were placed in preheated warming ovens set at 54° and 60°C and sampled after 1, 2, 6, and 12 hr at each of the holding temperatures. Sampling was accomplished by taking a 2 mm cross-sectional entire slice (containing both internal and surface area) from the center portion of the roast with an alcohol sterilized knife. The slice (40–60g) was transferred to a sterile Stomacher bag and homogenized with 0.1% peptone diluent. Serial dilutions were made with peptone diluent and plated with Violet Red Bile Agar and Tryptone Glucose Extract Agar for coliform and aerobic plate counts, respectively. Coliform plates were incubated at 37°C for 24 hours and aerobic plates were incubated at 25°C for 4 days.

RESULTS & DISCUSSION

Effect of holding time, inoculation, and holding temperature on aerobic plate counts

Time effects were highly significant and proved to be quadratic ($P < 0.01$) in nature. However, both inoculation by time and temperature by time interactions were highly significant ($P < 0.01$). Figure 1 helps to show the interaction effects on holding time. The inoculated samples began with higher counts than the uninoculated for both holding temperatures. At the higher temperature, the final counts attained are lower than at the lower holding temperature. This is an indication of perhaps lethal effects of the higher holding temperatures on the microbial population or more favorable growth conditions at 54°C than at 60°C for the thermoduric organisms present. This may also reflect destruction of surface microorganisms accompanied by increases in the internal population.

These interactions show the presence of at least two types of microorganisms. The increase from 6 to 12 hr suggests the logarithmic growth phase of a population. This type of organism would necessarily be thermoduric, evidenced by the high growth temperature (54°C). Of course in the inoculated samples, *E. coli* is present. The inoculated samples show a decrease in counts from 1–6 hr and an increase from 6–12 hr only at 54°C. At the 60°C holding temperature, the counts remained constant after 6 hr. There is some suggestion in the data of a competitive effect of the inoculation of *E. coli* on the natural flora. For both holding temperatures, the final aerobic plate count obtained was less for the inoculated than for the uninocu-

lated samples. The differences are quite small and not significant, but may suggest a competitive effect.

Effect of inoculation on coliform counts

No coliforms were detected in the uninoculated samples at any of the holding times. As would be expected, the analysis of variance showed that inoculation had a highly significant ($P < 0.01$) effect on coliform numbers. There was also a highly significant ($P < 0.01$) interaction between inoculation and storage time, but coliforms were only detected in the inoculated samples. The coliform counts showed a steady decrease over the 12 hr holding time for the samples inoculated with *E. coli.*

Effect of holding temperature on coliform counts

The inoculated *E. coli* were destroyed at both holding temperatures. The rate of destruction appears (Fig. 2) more rapid at the higher temperature, but the difference is not significant.

Effect of holding time on coliform counts

No coliforms were detected (Fig. 2) after 6 hr holding at 60°C or after 12 hr at 54°C. The decrease appeared to be more rapid at the higher holding temperature.

Combined interpretation of aerobic plate and coliform counts

Figure 1 also shows the effect of time on the destruction of the *E. coli* inoculum. If the decrease in aerobic plate counts and the decrease in coliform counts are considered simultaneously, the evidence of the thermoduric population is clearly shown. The inoculated samples at both holding temperatures show decreases in aerobic plate counts up to 6 hr; then, at 60°C holding temperature the aerobic plate count levels off while ther e is a dramatic increase in the aerobic plate count of the 54°C samples. These decreases are mirrored in the coliform counts in Figure 2.

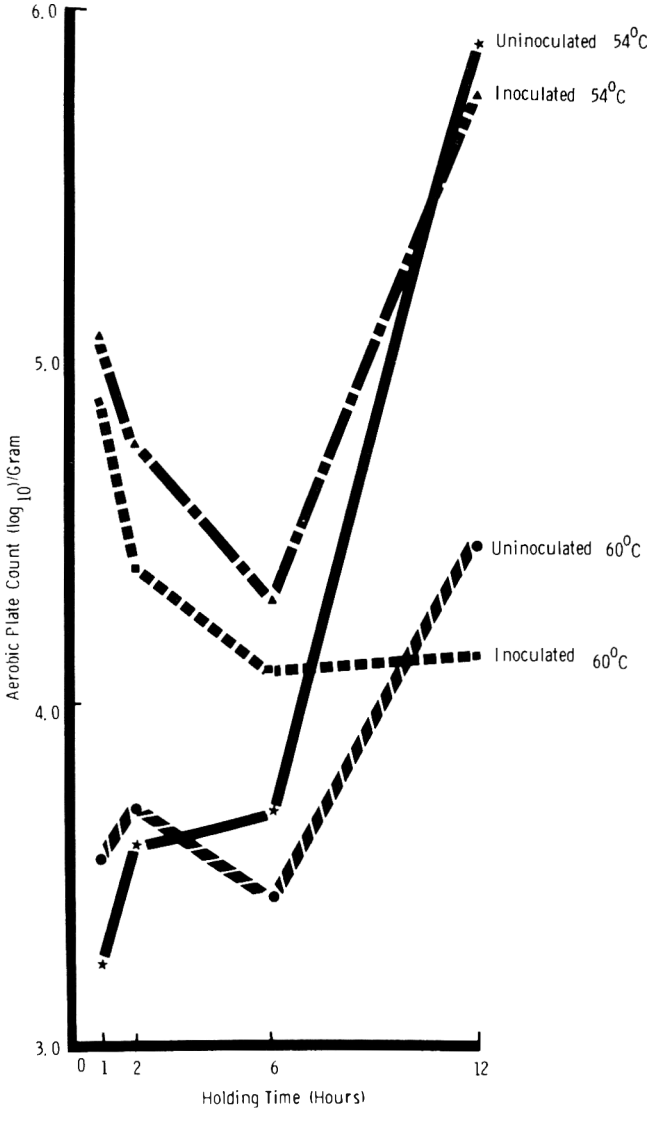


Fig. 1—Aerobic plate count for resturctured beef roasts that were uninoculated or surface inoculated (E. coli) and held at 54°C or 60°C.

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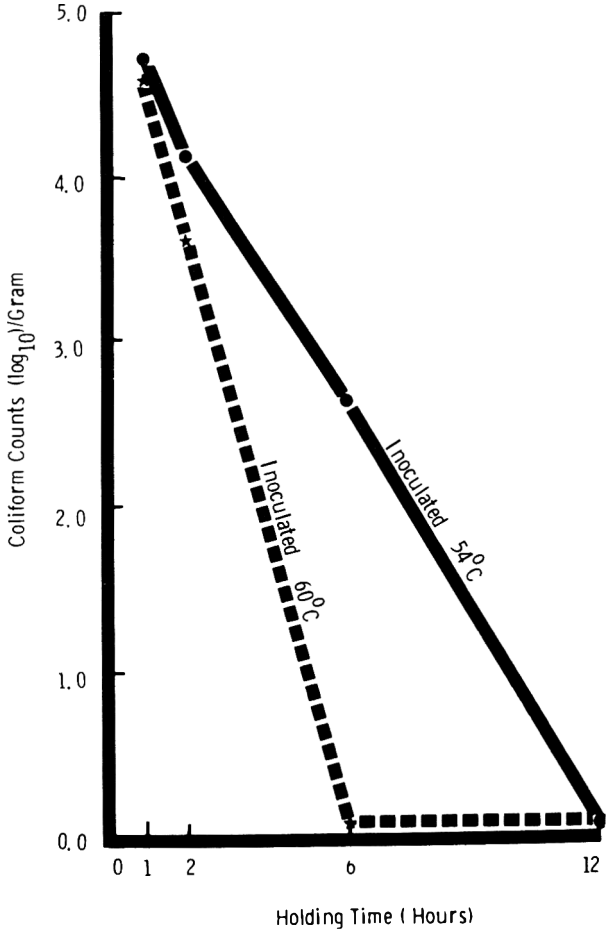


Fig. 2—Coliform counts for restructured beef roasts that were uninoculated or surface inoculated (E. coli) and held at 54°C or 60°C.

Effects of Storage on the Bioavailability and Chemistry of Iron Powders in a Heat-Processed Liquid Milk-Based Product

R. A. CLEMENS

ABSTRACT

Relative Biological Value (RBV) was determined by hemoglobin repletion after 6 and 12 mo storage of a canned milk-based product which contained 25% U.S. RDA for iron from either ferric orthophosphate (FOP), electrolytic iron (EI), or carbonyl iron (CI). RBV's for the two elemental iron sources were comparable to the RBV of ferrous sulfate and were significantly greater ($P < 0.01$) than the RBV for FOP after each storage period. Significant differences in RBV's for a given iron source were not observed between the two storage periods. A markedly greater percentage of iron from CI and EI than from FOP was solubilized and found in the ferrous form.

INTRODUCTION

RESEARCH INDICATES that the biological availability of iron can be significantly altered as a result of its interaction with food components during processing. Wood et al. (1978) found that heat and pressure processing significantly increased the relative biological value for sodium ferric pyrophosphate and ferric pyrophosphate while the RBV of ferric orthophosphate or ferrous sulfate was not altered. Clemens and Mercurio (1981) suggested that typical retort conditions improved the RBV of electrolytic iron and carbonyl iron by promoting the solubilization and oxidation of these iron sources to the bivalent form. Similar findings with iron salts were reported by Theuer et al. (1971, 1973). More recently Clydesdale (1981) suggested that reduction potential and dissolved oxygen within a product may affect ionization thereby influencing iron availability.

Changes in the physical and chemical characteristics of iron not only result from processing, but also from storage. Hodson (1970) reported that iron from ferric orthophosphate was converted to the ferrous form following six months storage in a liquid dietary. Lee and Clydesdale (1980) showed that in an ascorbic acid-fortified beverage, 17% of the iron from ferric orthophosphate and approximately 90% of the hydrogen reduced elemental iron (H_2-EI) was solubilized and converted to ferrous iron following 3 days of storage at 25°C. Nojeim and Clydesdale (1980) studying the effect of pH and ascorbic acid on iron valence reported that less than 15% of the ferric orthophosphate was ionized at pH 6.2 with or without ascorbic acid during a 4-wk ambient storage period, whereas, approximately 42% of the H_2-EI was in ionic form following the same storage period and conditions. Unpublished data of these investigators indicated less than 10% of the iron from FOP and H_2-EI was ionized during 8 wk ambient storage at pH 6.2 in the absence of ascorbic acid.

These studies suggested that alterations in the chemical and physical forms of iron in food may be explained, in part, by processing conditions, product pH, the presence of ascorbic acid, storage duration and environmental conditions. The purpose of the current investigation was to evaluate storage effects on iron bioavailability and on iron chemistry in a thermally processed liquid, milk-based product.

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MATERIALS & METHODS

Product production

A quantity of experimental liquid, milk-based product was prepared and divided into four batches. The product was fortified to contain 25% of the U.S. RDA for protein, vitamins and minerals, except iron, per 296 ml serving. To three of the batches an appropriate amount of ferric orthophosphate (Madison Industries, Inc., Old Bridge, NJ), carbonyl iron (Grade N Special, GAF Corporation, La Habra, CA) or electrolytic iron (A-131, Glidden-Durkee, Strongsville, OH) was added and mixed to provide 4.5 mg per serving. One batch was not iron fortified. Each batch was dispensed into epoxy-phenolic enamel-lined cans, retorted (6 min at 123°C and 1,055 g/cm^2), cooled to 4°C then stored either 6 or 12 mo at 22°C.

Iron analysis

After each storage period the product "Iron profile" was determined as described by Lee and Clydesdale (1980). Iron content was determined by atomic absorption spectroscopy (Heckman, 1968) using a Perkin-Elmer Model 303.

Experimental

Eighty-four weanling Wistar male rats (Hilltop Lab Animals, Inc., Chatsworth, CA) were used to assess the bioavailability of three iron sources following 6 and 12 mo of product storage at 22°C. The rats were housed individually in stainless steel cages and allowed ad libitum a purified Fe-deficient (<6 ppm Fe) basal ration and deionized water for 5 wk. The basal ration composition is shown in Table 1. Tail blood was drawn to determine hemoglobin (Hb) concentration (Sigma Tech. Bull., 1976).

The rats were then divided into 14 groups of 6 animals each based on similar hemopoietic status (Hb <6 g/dl). One group received the basal ration. Four other groups received the basal ration supplemented with powdered ferrous sulfate heptahydrate (particle size <74μ) to provide 5, 10, 15 and 20 ppm of iron. For the remaining

Table 1 – Basal ration composition (Fe content < 6.0 ppm)

Ingredients	g/kg
ANRC Casein (Sheffield)	200.0
Gelatin (U.S. Biochemical)	50.0
Corn Oil (Wesson)	50.0
Iron-Free Salt Mix[a]	27.0
Vitamin Mix[b]	10.0
dl-Methionine (U.S. Biochemical)	1.0
Choline Bitartrate (Sigma)	4.0
Cornstarch-Dextrose (1:2) (National Starch and U.S. Biochemical)	658.0

[a] Bernhart and Tomarelli (1966) prepared with AR grade chemicals without ferric citrate and modified by the addition of 0.273g chromium acetate, 0.46g sodium fluoride, and 0.045g sodium selenite to 100g trace mineral mix; cobalt II chloride (0.167g) was added per Jones and Foster (1942).

[b] Vitamin mix, units or g/kg mix: thiamin HCl, 2.2; riboflavin, 2.2; niacin, 9.9; pyridoxal HCl, 2.2; biotin, 0.176; Ca-pantothenate, 6.6; folic acid, 0.211; p-aminobenzoic acid, 15.0; inositol, 15.0; cyanocobalamin, 0.003; vitamin A, 2,100,000 IU; vitamin D_2, 220,000 IU; α-tocopherol acetate, 22,000 IU; menadione, 2.5; prepared in a hydrolyzed potato starch base.

nine test groups, freeze-dried product (Vacudyne Pilot Freeze-dryer, Model VPFD-D, Chicago, IL) was added to the basal ration to provide 5, 10 and 15 ppm of iron at the expense of the cornstarch-dextrose mixture.

After a 3-week repletion period, Hb levels were determined. Relative biological value of each iron source was determined by slope-ratio assay (Amine and Hegsted, 1974). Statistical evaluation of the Hb response data was performed by analysis of variance.

RESULTS & DISCUSSION

TABLE 2 shows the RBV's of ferric orthophosphate, carbonyl iron and electrolytic iron in an experimental thermal-precessed product stored 6 and 12 mo at 22°C. The biological value of FOP for 6 mo (RBV = 31) and 12 mo (RBV = 19) did not differ significantly ($P > 0.05$). Ferric orthophosphate showed virtually no solubilization in the pH 6.65 liquid food system (Table 3) despite an ascorbic acid concentration of 51 ppm. Hodson (1970), however, reported that in a similar type product nearly 85% of the iron from FOP was solubilized and ionized to the ferrous form after 5 mo storage. A more recent investigation by Nojeim and Clydesdale (1981) indicated that FOP in an aqueous solution at pH 6.2 was not appreciably ionized (< 1%) even in the presence of ascorbic acid. Even after 4 wk ambient storage of the FOP solution, these authors reported less than 15% of the iron was ionized. Unpublished data by the same investigators showed that in an aqueous, nonfood system less than 10% FOP was solubilized after 8 wk at pH 2.7. These results of Nojeim and Clydesdale plus physiochemical findings from this laboratory (Clemens and Mercurio, 1981), indicate that iron from FOP, when in a near neutral pH milk protein-based product, undergoes little change during thermal-processing and subsequent storage.

The RBV's of carbonyl iron and electrolytic iron did not differ from each other following each of the two storage periods and were comparable to ferrous sulfate, the reference standard (Table 2). This investigator and many others have shown that elemental iron sources generally have a good bioavailability even after processing (Clemens and Mercurio, 1981; Coccodrilli et al., 1976).

Iron profiles for the elemental iron sources are shown in Table 3. These data show that between 33% and 47% of the carbonyl iron and electrolytic iron was solubilized. Of that iron solubilized, a range of 72–100% was found in the ferrous form. Clemens and Mercurio (1981) showed that after an initial thermal processing, the same kind of product reported here had 83% ferrous iron from the solubilized elemental iron sources. Therefore, it seems that 12 mo storage at 22°C had little effect on the solubilization and ionization of elemental iron.

A comparison of the iron biological values reported in this study was made between those of elemental iron sources in the stored product and the RBV's of the same iron sources reported earlier (Clemens and Mercurio, 1981) in nonprocessed and freshly processed product (Table 4). A statistical evaluation in each study indicated the slope-ratios for controls were the same. The data indicate a significant ($P < 0.01$) difference in respective RBV's for carbonyl iron and electrolytic iron between the initial processed-6 mo stored products and the initial processed-12 mo stored product. The RBV data of 12 mo stored

Table 2 — Correlation coefficients, regression coefficients and estimation of relative biological values of three iron sources stored in a milk-based product for 6 and 12 months

	6 months storage			12 months storage		
Iron Source	Correlation coefficient	Regression coefficient	RBV	Correlation coefficient	Regression coefficient	RBV
Ferrous sulfate (reference std)	0.9133	0.3173	100	0.9460	0.4977	100
Ferric orthophosphate	0.3847	0.0983[a,b]	31	0.3444	0.0967[a,b]	19
Carbonyl iron	0.8045	0.3767[c]	119	0.7209	0.3450[c]	69
Electrolytic iron	0.7503	0.2867[d]	90	0.9212	0.5333[d]	107

[a] Linear regression is not significant at $\alpha = 0.05$.
[b,c] Within an iron source, regression coefficients do not differ at $\alpha = 0.01$.
[d] Regression coefficients differ significantly at $\alpha = 0.01$.

Table 3 — Iron profile data of ferric orthophosphate, carbonyl iron and electrolytic iron stored in a liquid, milk-based product for 6 and 12 months. (Values reported as μg Fe per gram of product)

	6 months storage			12 months storage		
	Ferric orthophosphate	Carbonyl iron	Electrolytic iron	Ferric orthophosphate	Carbonyl iron	Electrolytic iron
Total Iron	15.06	16.71	11.51	14.42	15.09	9.02
Valence Distribution[a]						
Fe^{o} (% Total)	0.16 (1.1)	0.15 (0.9)	0.22 (1.9)	0.01 (0.07)	0.01 (0.07)	0.02 (0.22)
Fe^{++} (% Total)	N.D.[d]	6.53 (39.1)	4.84 (42.0)	N.D.	3.53 (23.4)	3.59 (39.8)
Fe^{+++} (% Total)	N.D.	0.38 (2.3)	0.14 (1.2)	N.D.	0.44 (2.9)	0.22 (2.4)
Solubility Distribution[a]						
Soluble (% Total)	N.D.	6.79 (40.6)	4.79 (41.6)	N.D.	4.93 (32.7)	4.27 (47.3)
Insoluble[b] (% Total)	15.11 (100)	9.92 (59.4)	6.72 (58.4)	14.70 (100)	10.16 (67.3)	4.75 (52.7)
Complexed[c] (% Total)	N.D.	N.D.	N.D.	N.D.	0.96 (6.4)	0.24 (2.7)

[a] Valence and solubility distribution data for the three iron sources are background-corrected.
[b] Insoluble = Total – soluble.
[c] Complexed = Soluble – ionic.
[d] N.D. = not detected.

Table 4 — Estimation of relative biological value (RBV) of FOP, CI and EI in a nonprocessed and processed liquid milk-based product[a]

Product	RBV
Nonprocessed w/FOP	24
Nonprocessed w/CI	66
Nonprocessed w/EI	123
Processed w/FOP	-14
Processed w/CI	202
Processed w/EI	297

[a] Data from Clemens and Mercurio (1981)

carbonyl iron (69) and electrolytic iron (107) and the RBV for the same iron sources in nonprocessed product (66 and 123, respectively) showed no significant differences.

The biological value data indicate that the most significant changes in iron occur during thermal processing and during the subsequent 6 mo storage. The apparent RBV reversion of elemental iron to non-processed values and the seemingly small changes in iron profiles following storage suggest that factors other than solubility and ionic distribution influence iron bioavailability. It has been shown that particle size and specific surface area correlate directly with elemental iron bioavailability in a nonprocessed, nonfood system. But such may not be the case in food systems, as previously suggested (Clemens and Mercurio, 1981). Iron chemical interactions within a food matrix remain to be examined with regards to food processing and prolonged storage and how these interactions affect iron bioavailability.

This study has demonstrated that even after 12 mo of storage, perhaps an extreme time from product production to consumer consumption, carbonyl iron and electrolytic iron still exhibit excellent bioavailability while that of FOP remained low. The study has also shown that during the 12 mo storage, conversion of iron from FOP to the ferrous form is negligible and that a $Fe^{\circ} \rightarrow Fe^{++}$ conversion of EI and CI was not affected as much by storage as it was by the initial processing.

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Puffing and Textural Characteristics of Chapati in Relation to Varietal Differences in Gluten Composition

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ABSTRACT

Chapaties made from different wheat varieties differ in puffing and textural characteristics. These differences have been found to be related to the relative quantities of gliadins, glutenins and residue proteins in respective glutens. Wheat varieties having about equal quantities of gliadins, glutenins and residue proteins give fully puffed soft and pliable chapaties. Wheat varieties having more of residue protein give leathery chapaties and varieties having more of gliadin give relatively stiff chapaties. Applications of Warner-Bratzler shear press for evaluating chapati texture is discussed.

INTRODUCTION

IN INDIA, wheat flour is primarily used for making chapati. Chapati is a kind of flat bread made from unleavened dough. Typically it is rolled thin and is baked in such a way as to give a puffed ball. The puffing is due to the accumulation of steam generated during baking and consequently on cooling the ball collapses in flat chapati. In addition to full puffing, two other desirable characteristics of chapati are softness and pliability. All these three characteristics of good chapati are probably due to gluten. The assumption is based on the fact that chapaties made from other grains such as Chana (*Cicer arietinum*), bazra (*Pennisetum typhoides*) and sorghum do not puff and are brittle. A degree of softness and pliability and ability to puff can be imparted to the Chapaties of these grains by admixture with wheat.

As intervarietal differences in gluten characteristics are known to be related to gluten composition (Orth and Bushuk, 1972; Huebner and Wall, 1976; Ram and Nigam, 1979), we undertook the present study for evaluating chapati quality in relation to varietal differences in gluten composition. In addition, we also wish to report preliminary observations on the use of the Warner-Bratzler shear press for evaluating chapati texture. So far, none of the currently available instruments for texture evaluation has been used for this purpose.

MATERIALS & METHODS

THE SOURCES of wheat varieties have been reported earlier (Ram and Nigam, 1979). Wheat was milled in a laboratory Kamas Mill (Swedish Model: SLAGY 200, with 60 mesh sieve). Dough was prepared in a Farinograph (lever position 1:3) to a consistency of 600 ± 10 BU (Shurpalekar and Prabhavathi, 1976). Thirty gram pieces of dough were weighed and rolled on a wooden board having 2.5 mm high ridges on two sides. Chapaties were baked at 175°C on a griddle for 75 sec. During this time they were rotated twice. They were then turned over and baked similarly on the other side. They were turned over again and baked till puffed (1–2 min).

Subjective evaluation of texture and over-all acceptability was done by a panel of four members. These were selected from a panel of ten staff members screened for the reproducibility of score in evaluating chapaties of one variety prepared under identical conditions on different days. Quality criteria such as pliability, hand tearing, mouth feel during chewing and taste were taken into consideration. The chapaties were given marks on each quality criteria on an arbitrary eight point scale [Excellent (E), 7–8; Good (G), 5–6; Fair (F), 3–4; Poor (P), 1–2]. The marks were added and chapaties graded as E, G, F and P (Table 1). For objective evaluation the Warner-Bratzler shear press (Engineering Research Service, Canada) was used. The range selector knob was set at 20% (equivalent to 4 kg). The thickness of the blade was 1.016 mm and slot clearance was 1.143 mm. Speeds of the moving head and the chart were 7 cm/min and 76.2 cm/min respectively. Chapaties were cut into 2 cm x 3 cm pieces and six such pieces from each chapati were individually subjected to shear. The graphs were reproducible.

Whole meal (10g, 14% moisture basis) was extracted according to the procedure of Shorgen et al. (1969) except that 0.5M NaCl was used instead of water. Gluten so obtained was washed free of salt and blended in a Sorvall blendor at high speed for 1 min (blended twice for 30 sec each) with 50 ml of 0.1 M acetic acid. The suspension was centrifuged and the supernatant and the residue freeze-dried. The latter is residue protein. The freeze-dried supernatant was fractionated into gliadin and glutenin according to the procedure of Nielson et al. (1962). Approved methods of the AACC (1969) were used for determining Kjeldhal nitrogen (Method No. 46.11), microsedimentation value (Method No. 56.63), and farinograph water absorption (Method No. 54.21).

RESULTS & DISCUSSION

TABLE 1 gives the results of subjective evaluation of chapati texture, puffing character and moisture loss data. HD 4530 chapati was judged best because of its full and rapid puffing, softness and pliability, and eating quality. Chapaties made from Sonalika and HD 1949 had similar puffing characteristics (partial puffing) but different textural properties. Sonalika chapati was somewhat stiff whereas HD 1949 chapati was somewhat leathery. K 65 and K 68 chapaties were leathery like HD 1949 chapati but puffed fully. However, unlike HD 4530 chapati, puffing of K 65 and K 68 chapaties was slow.

From Table 2, it is seen that variety HD 4530 had a relatively high water absorption (70%), upper medium microsedimentation value (2.25 ml) and high protein (16.5%) content. These physicochemical characteristics of HD 4530 flour are similar to the physicochemical characteristics of good chapati making flours reported by Austin and Ram (1971).

It is further seen from Table 2 that glutens of these varieties contain roughly equal proportions of gliadins and glutenins and variable prportions of residue proteins. The latter varying from a low of 0.41% in Sonalika to a high of 2.53% in K 68. Comparing chapati characteristics (Table 1) and gluten composition (Table 2) we are led to associate low proportion of residue protein as in Sonalika with stiffness and partial puffing and high proportion of residue protein as in K 65 and K 68 with leathery texture and full but slow puffing. Thus, both low and high proportions of residue protein are detrimental to chapati quality. The vareity HD 4530 gave better chapati than the other four varieties and in this variety the residue protein occurs in about the same proportion as gliadin and glutenin. While it is not possible at present to ascribe reasons for the association of stiff and leathery characters with low and high residue protein content, a plausible explanation for the

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Table 1—Chapati Characteristics

Wheat variety	Fresh			Stored[a]		Moisture loss %	
	Puffing	Texture	Acceptability[c]	Texture[b]	Acceptability[c]	During baking	During storage
Sonalika	Rapid partial	Slightly stiff	F	Stiffer	P	9.6	13.3
HD 1949	Slow partial	Slightly tough & leathery	P	Tough leathery	P	13.3	13.7
HD 4530	Rapid full	Soft and pliable	E	Slightly tougher & leathery	F	8.3	12.6
K 65	Slow full	Slightly leathery	G	Tougher & leathery	P	7.3	10.3
K 68	Slow full	Slightly leathery	G	Tougher & leathery	P	7.9	10.1

[a] Stored for 4 hr at 30°C
[b] Compared to fresh chapati of same variety
[c] E = Excellent; G = Good; F = Fair; P = Poor.

Table 2—Physicochemical characteristics of some wheat varieties

Variety	Water absorption[a] %	Micro sedimentation value, ml	Total protein[b] %	Gliadin[c] %	Glutenin[c] %	Residue[c] protein %
Sonalika	67.8	1.25	12.5	2.92	2.70	0.41
HD 1949	70.2	1.80	14.8	3.63	3.41	1.65
HD 4530	70.0	2.25	16.5	3.69	3.36	2.90
K 65	66.0	2.34	11.3	1.57	1.37	2.06
K 68	68.0	2.40	12.3	1.62	1.42	2.53

[a] Farinograph water absorption
[b] In flour 14% moisture basis (Kjeldahl determination)
[c] Percent in flour

observed puffing characteristics of chapaties with respect to the proportions of residue proteins in various glutens is possible.

Puffing is due to the generation and accumulation of steam between the two crusts of a chapati. Separation of crusts begin at several points and as the baking proceeds, these areas enlarge and ultimately coalesce to produce one large puff. The puffing will proceed to completion only if the crusts do not develop cracks during baking. Doughs of wheat varieties low in residue protein are weak and more likely to develop cracks under increasing steam pressure. This will prevent further puffing and the chapati will be left with several small puffed areas. Sonalika gluten is poor in residue protein and it gives chapaties with several small puffs (partial puffing). In K 65 and K 68 the proportion of residue protein is high. This makes their glutens stronger and hence more resistant to breaking under steam pressure. The chapaties of these varieties puff fully, but slowly. HD 4530 chapati puffs fully and rapidly, and in this variety the quantity of residue protein is neither too high nor too low.

The case of HD 1949 is anomalous because its gluten composition is close to that of HD 4530 but in puffing it behaves like Sonalika and in texture it is like K 65 and K 68. Probably its gluten components are qualitatively very different from the gluten components of the other four varieties.

Moisture loss during baking and storage is shown in Table 1. There is a decrease in moisture loss from weak to strong varieties. The chapaties from weaker varieties will therefore be drier and this may be one of the factors contributing to the stiffness of Sonalika chapati.

Warner-Bratzler graphs of fresh chapaties (upper row) and of stored chapaties (lower row) are shown in Fig. 1. The graphs of fresh chapaties are characterized by a multiplicity of small peaks. This is indicative of minor localized breaks and can be regarded a measure of crispness (Voisey and Stanley, 1979). In all cases, the corresponding graphs of stored chapaties are smoother and in the case of varieties K 65 and K 68 also flatter. This change in the shear characteristics is indicative of the loss of crispness. This is probably due to a redistribution of moisture from the inner moist mealy portion to the drier crisp outer surfaces of the chapati. This will be so despite some loss of total moisture during storage.

Table 3—Shear force and work done as calculated from Fig 1

Variety	Fresh chapati		Stored chapati		% Increase from fresh to stored	
	Shear force (kg)	Work done (cm^2)	Shear force (kg)	Work done (cm^2)	Shear force (kg)	Work done (cm^2)
Sonalika	1.45	32	1.54	37	6.1	15.6
HD 1949	1.50	39	2.12	53	41.3	35.9
HD 4530	1.56	32	1.59	34	1.9	6.3
K 65	1.45	33	1.53	45	5.3	36.4
K 68	1.35	35	1.48	48	9.8	37.1

Table 3 presents shear force and work done calculated from the above force-deformation curves. Except for the vareity HD 1949, no significant intervarietal differences are found. However, in every variety there is an increase in shear force and work done from fresh to stored chapati. At about 2% increase in shear force and 6% increase in work done, the increase is least for the variety HD 4530. It is to be noted that in overall profile also (Fig. 1) there

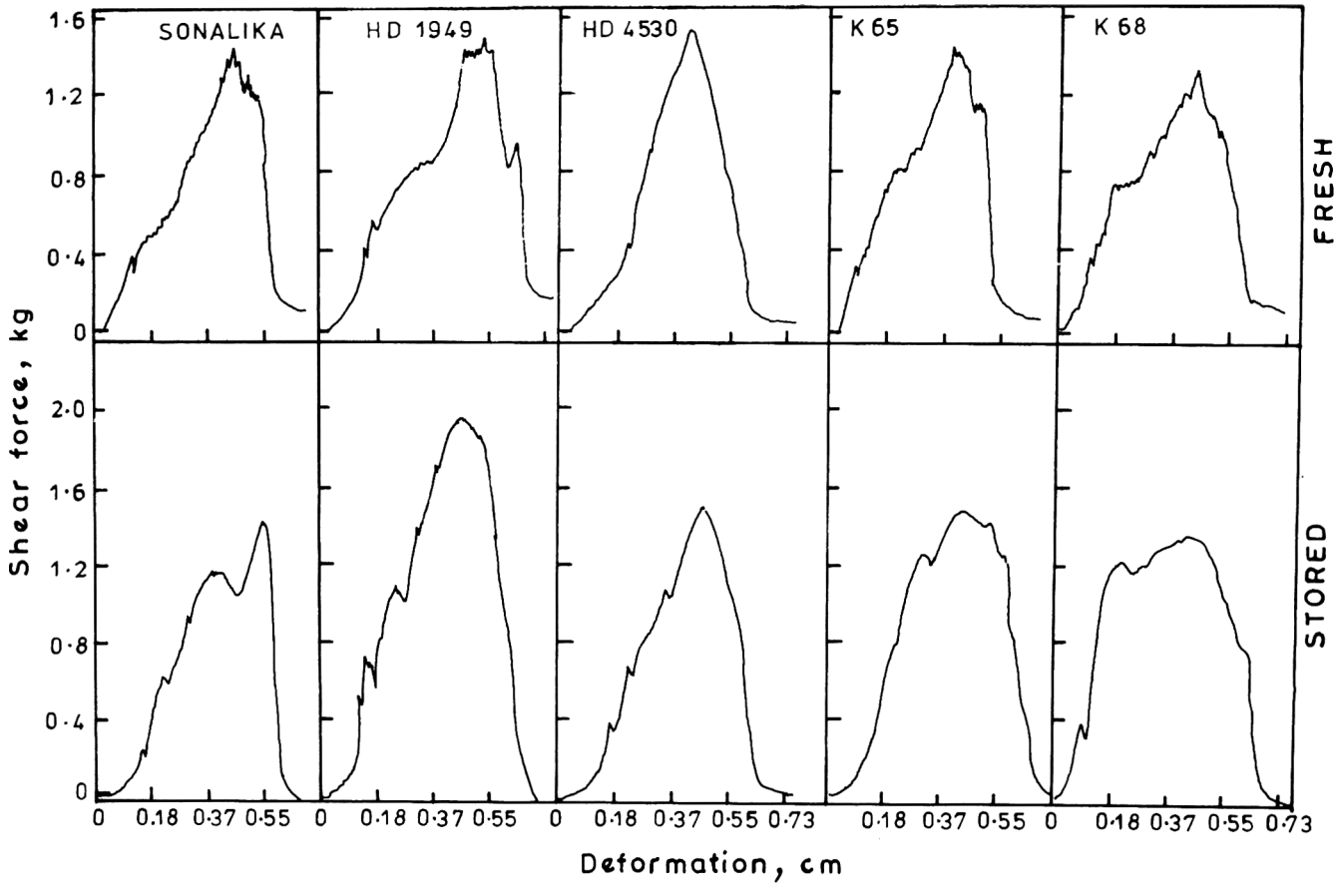


Fig. 1—Warner-Bratzler force-deformation curves of chapaties from five wheat varieties.

was least change from fresh to stored chapati in this variety. Thus, in keeping quality the chapati of the variety HD 4530 is superior to the chapaties of the other four varieties.

In conclusion, it can be said that the relative quantities of gliadins, glutenins and residue proteins play an important role in determining chapati characteristics. Wheat varieties having roughly equal quantities of these proteins will give better chapaties than varieties in which excessive amounts of one or the other gluten components are present. However, as shown by the characteristics of HD 1949 chapati, quality variation may also be present. Furthermore, Warner-Bratzler shear press has given results which though consistent with subjective evaluation show lesser variation in the texture of different chapaties. Thus, for studying varietal differences Warner-Bratzler shear press seems to be of limited use only.

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Influence of An Addition of Textured Milk Proteins Upon Physicochemical Properties of Meat Mixtures

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ABSTRACT

Meat systems consisted of beef haunch (pH 5.63) in which textured milk proteins were substituted at: 0, 10, 20, 30, 40, 50, 75, and 100%. The effects of these substitutes on the physicochemical properties of ground meats were measured for products containing no salt, sodium chloride (2%), polyphosphate mixture Hamine (0.5%) and sodium chloride and Hamine together (2% + 0.5%). Measurements included: water absorption capacity, water-holding capacity, thermal shrinkage, consistency, viscosity, fat absorption capacity, emulsifying capacity, emulsion stability, and active acidity. Addition of textured milk proteins improved the technological adaptability of ground beef, optimum substitution depending on the type of salt. In order to improve the physicochemical properties of beef, substitution of 30% meat by textured milk proteins with addition of 2% sodium chloride, is recommended.

INTRODUCTION

A DEEPENING world shortage of animal proteins in the past few years results in an increased interest in protein preparations from traditional sources as well as from unconventional ones (Horan, 1974; Towler, 1972; Worgan, 1973). New protein preparations can be used in meat and meat processing industries as meat substitutes (Bartz, 1976; USDA, 1972).

Other proteins may be substituted for meat in processed meat products if the following conditions are met (Burket, 1976):

1. The substitute is cheap.
2. It is of good hygienic quality.
3. It has high nutritional value to maintain the nutritional value of meat.
4. It has physicochemical properties that enable the theological and structural properties of meat to be retained.
5. It does not affect the sensory quality of the product.

According to Circle and Smith (1972), when substituting protein preparations for meat, it is essential both to define physicochemical properties of the preparations themselves as well as the properties of the system to which the preparation has been added. This paper assesses the effects of substitution of structurized milk proteins on the physicochemical properties of meat systems.

Textured milk protein produced as described by Poznański et al. (1980a, b) is a new protein preparation that can be used as a meat substitute in a number of meat and processed meat products or to obtain products totally imitating meat dishes with small additions of meat (Ministry of Health, 1980).

The first detailed studies of the influence of added protein preparations on the physicochemical properties of meat systems were undertaken by Hermansson (1973–1975). Further studies were carried out by Smith et al. (1973–1976), Thomas et al. (1974), Sofos et al. (1977), and others, who found that the substitutes changed the physicochemical properties of the meats. Thomas et al. (1974) also indicated that changes in functional properties of the meat product caused by substitution of other proteins depended on the pH of the meat; for example, substitution of lamb at pH 5.65 yielded a product that had more useful functional properties than when the pH of the meat was 6.15.

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MATERIALS & METHODS

Textured milk proteins

Textured milk proteins were produced by the thermoplastic pressing method (Poznański et al. 1980a, b), by which skim milk and animal fat are processed into a structurally fibrous product of a compact and elastic consistency in the form of chunks. The textured milk proteins contained 26.9% protein (N x 6.38), 51.8% water, 15.0% fat, 2.4% lactose, 2.5% ash, and had a pH of 6.48.

Meat

Beef haunch, free from tendons, 24 hr after slaughter, was used for preparing meat mixtures. The beef contained 21.7% protein (N x 6.25), 75.4% water, 2.1% fat, and 1.1% ash. To simulate practical meat processing, where meat is used at pH 5.3–5.7 (Briskey et al., 1966), meat of pH 5.63 was used in the present experiment.

In formulation, the ratio of water to protein was maintained constant at 3.47 and the concentration of substitute (textured milk proteins) was expressed as a percentage of total protein (Table 1). Since the water content of the textured proteins was less than that of the meat, water was added during cutter-grinding.

The average concentration of sodium chloride in finely ground meat products is 2% (Hamm, 1972, 1973), and the permissible maximum concentration of polyphosphates in meat products is 0.5% (Hamm, 1972). Therefore the four sets of eight experimental formulations that were studied contained (a) no added salts, (b) 2% NaCl, (c) 0.5% polyphosphate mixture (Hamine), and (d) 2% NaCl + 0.5% Hamine, making a total of 32 meat systems.

Hamine is a commercial polyphosphate mixture made in Holland. This mixture is used in the Polish meat industry for the production of ham. The chemical composition of Hamine according to Goetlich et al. (1964) is: P_2O_5, 59.3%; Na_2O, 33%; and H_2O, 0.6%. Hamine contains 10.4% orthophosphates (expressed as P_2O_5), 21.4% pyrophosphates (expressed as P_2O_5), and 32.9% triphosphates (expressed as P_2O_5).

Experimental systems

The meat was ground in a A-200 CB Seydelman grinder and then cutter-ground in a K-203 Seydelman cutter to obtain a homogenous mass. The textured milk proteins were ground twice in an EM-1 Jaga grinder with a 3-mm sieve.

The ground meat was mixed in a laboratory Spomasz cutter. After 1 min, textured milk proteins and water (ice) were added and to some of the mixtures NaCl and Hamine. All of the components were cutter-ground together for 3 more minutes. The temperature of the mixture in the final stage of cutter-grinding was below 15°C.

Samples of the ground meat mixture were put in food-grade polyethylene bags, kept in a deep freeze at −28°C until completely frozen, and stored at −18°C until analyzed. These frozen samples were kept for less than month. Before analysis at a room temperature of 20 ± 2°C, the samples were taken from the deep-freeze and kept in a refrigerator for 15 hr at 5 ± 1°C.

Chemical analysis

Water, protein, fat, and ash contents were determined by procedures as reported by AOAC (1975). Lactose was determined by the procedure of Swartling and Mattsson (1953).

Functional properties

Water absorption capacity was determined by centrifuging the homogenate of the product with water at 1800 x *g* for 9 min (Janicki and Walczak, 1954; Ozimek, 1978) (Table 2).

The water-holding capacity was measured using the Grau-Hamm pressure method modified by Pohja and Niinivaara (1957). The result (Table 2) was expressed as free water content as a per cent of the total water content in the sample.

Thermal shrinkage was determined by the method of Townsend et al. (1968) (Table 3).

Consistency was determined using an Allo-Kramer Shear Press, Model S 2HF (Table 3). Round slices of products (initial weight 100g), in which thermal shrinkages had been previously determined, were used as samples for consistency measurements. Consistency was defined as the maximum force required to shear a sample, as read off the load time curve.

Viscosity was measured using a rotation viscometer Rheotest-2, produced in GDR (Table 4). Samples were mixed with distilled water in the ratio 1:1 (by weight). Viscosity was measured 10 min after a sample had been placed in a graduated cylinder. Readings were made 15 sec after the viscometer was operated at the defined shear force. Viscosity was calculated according to the formula given in the Rheo-test service manual:

$$\eta = \frac{z \times \alpha \times 100}{D_r} \; (3 \text{ m sp}) \; z \times \alpha = \tau$$

where: η = viscosity /m·Pa·s/; z = constant value characterizing the definite graduated cylinder; α = angle coefficient, as read during the measurement; D_r = shear force; and τ = shear stress.

Fat absorption capacity was determined by homogenizing the product with oil and centrifuging the mixture at 1350 x *g* for 9 min (Ozimek, 1978) (Table 4).

Fat emulsifying capacity was measured by the method of Swift et al. (1961) modified by Webb et al. (1970) and Kwaśniewska et al. (1976) (Table 5). According to this procedure 10 ml of a dispersion prepared by dispersing 8.3g of product into 191.7 ml of 2% NaCl solution was placed in a glass beaker. This amount of dispersion contained 90 mg of protein. A set of electrodes with a resistance meter was attached. The dispersion was blended at 2000 rpm and soy a bean oil (20°C) from the graduated vessel was introduced at a constant flow-rate of 0.5 ml/sec until a substantial increase in the resistance of the system was observed. The emulsifying capacity was expressed as the amount of oil emulsified by 100 mg of protein (V_{100}).

Emulsion stability was determined using the method of Inklaar and Fortuin (1969), modified by Chojnowski et al. (1975) (Table 5).

Table 1—Composition of mixtures

			Ingredient (g)										
			Beef			Textured milk proteins							
	Substitution level[a]	Meat replacement[b]	Total	Protein	Water	Total	Protein	Water	Amount of water added	Total amount	NaCl	Hamine	NaCl and Hamine
1	0	0	100.0	21.7	75.4	–	–	–	–	100.0	2.00	0.50	2.00+0.50
2	10	11.2	90.0	19.5	67.9	8.1	2.2	4.2	3.4	101.4	2.03	0.51	2.03+0.51
3	20	22.2	80.0	17.4	60.3	16.1	4.3	8.3	6.7	102.8	2.06	0.51	2.06+0.51
4	30	32.9	70.0	15.2	52.8	24.2	6.5	12.5	10.1	104.3	2.09	0.52	2.09+0.52
5	40	43.2	60.0	13.0	45.2	32.2	8.7	16.7	13.5	105.7	2.11	0.53	2.11+0.53
6	50	53.3	50.0	10.9	37.7	40.3	10.8	20.9	16.8	107.1	2.14	0.54	2.14+0.54
7	75	78.1	24.2	5.4	18.2	60.4	16.3	31.3	25.9	110.5	2.21	0.55	2.21+0.55
8	100	100.0	–	–	–	80.6	21.7	41.8	33.7	114.3	2.28	0.57	2.28+0.57

[a] % protein exchange
[b] Amount of meat replaced by textured milk protein (%)

Table 2—Physicochemical properties of meat mixtures

Mixture	Substitution level[a]	Water-absorption capacity (g water/100g) Without salt	With NaCl	With Hamine	With NaCl and Hamine
1	0	14.5±0.41	27.3±0.47	25.2±0.85	45.3±0.94
2	10	24.3±0.47	40.7±0.94	38.8±0.85	48.7±1.25
3	20	18.7±0.94	46.0±1.63	32.0±0.00	51.2±0.62
4	30	11.0±0.82	49.7±0.47	25.8±0.85	54.3±1.25
5	40	7.5±0.41	46.0±0.82	14.0±0.82	51.7±0.47
6	50	−2.5±0.16	41.2±0.62	8.7±0.47	46.2±0.24
7	75	−9.3±0.51	26.5±0.41	3.0±0.00	30.2±1.65
8	100	−12.2±0.24	0.3±0.08	0.0±0.00	−8.3±0.48
		Water-holding capacity—content of "free water" (% total moisture)			
1	0	10.7±0.65	0.0±0.00	0.9±0.07	0.0±0.00
2	10	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
3	20	5.1±0.43	0.0±0.00	0.0±0.00	0.0±0.00
4	30	6.9±0.17	0.0±0.00	0.0±0.00	0.0±0.00
5	40	10.0±0.15	0.0±0.00	0.0±0.00	0.0±0.00
6	50	12.8±0.91	0.0±0.00	4.5±0.29	0.0±0.00
7	75	20.2±0.68	0.00±0.00	6.7±0.22	0.0±0.00
8[b]	100	–	–	–	–

[a] % protein exchange
[b] It was not possible to determine the water-holding capacity of the pure textured milk protein samples because they were not cohesive enough and spread under pressing.

Table 3—Physiochemical properties of meat mixtures

Mixture	Substitution level[a]	Thermal shrinkage (%) Without salt	With NaCl	With Hamine	With NaCl and Hamine
1	0	16.2±0.75	2.3±0.39	2.4±0.33	0.9±0.16
2	10	13.1±1.47	1.4±0.33	2.7±0.21	0.9±0.19
3	20	12.1±1.39	1.6±0.17	2.3±0.19	0.8±0.05
4	30	6.6±1.17	1.5±0.05	2.2±0.00	1.0±0.05
5	40	7.1±0.64	1.1±0.17	2.9±0.70	0.9±0.13
6	50	10.3±0.96	1.4±0.21	2.0±0.37	1.8±0.48
7	75	18.9±0.54	1.6±0.48	3.9±0.56	1.3±0.05
8	100	21.6±1.13	15.8±0.83	3.6±0.17	9.4±0.21
		Consistency (lb)			
1	0	360.0± 0.00	271.3±3.40	282.0±4.99	375.0±4.06
2	10	299.3±14.82	214.0±1.63	241.3±1.89	308.8±4.72
3	20	264.0± 8.64	239.3±9.29	199.3±5.74	259.3±7.37
4	30	236.0± 3.27	265.3±0.96	212.0±5.66	264.0±1.63
5	40	239.5±11.59	237.3±4.99	191.3±0.96	252.0±0.00
6	50	225.3± 6.22	232.0±0.00	156.8±9.57	194.0±7.12
7	75	214.8±10.50	145.3±3.78	122.6±8.22	152.0±0.00
8	100	292.0± 5.66	224.0±0.00	78.8±1.89	158.8±8.22

[a] % protein exchange

Table 4—Physicochemical properties of meat mixtures

Mixture	Substitution level[a]	Viscosity (m·Pa·s) Without salt	With NaCl	With Hamine	With NaCl and Hamine
1	0	257.7± 5.57	737.9±13.01	557.6± 5.01	1177.3± 9.75
2	10	368.7± 5.29	1517.5±17.34	586.7±13.52	1453.3±10.02
3	20	394.7±12.30	1366.6±21.81	768.5±14.44	1516.2± 7.69
4	30	195.9± 8.67	923.4±11.14	600.4± 9.55	1248.1±17.34
5	40	102.9± 2.00	677.4± 1.81	518.3± 0.00	1022.8±17.34
6	50	131.5± 8.47	524.7± 11.1	321.3± 8.70	936.1± 0.00
7	75	50.2± 1.76	138.7± 4.58	125.5± 0.58	300.5± 5.01
8	100	10.5± 0.44	30.3± 1.10	42.6± 2.50	50.1± 0.40
		Fat absorption capacity (g/100g)			
1	0	22.4± 0.90	23.1± 0.74	28.5± 1.02	24.9± 0.74
2	10	21.0± 1.56	25.0± 1.63	26.4± 1.23	25.5± 0.63
3	20	19.8± 1.56	24.2± 0.90	23.1± 0.90	25.8± 0.61
4	30	22.2± 0.70	21.1± 0.29	18.2± 1.07	18.8± 0.58
5	40	28.3± 0.24	22.2± 0.70	21.5± 1.27	20.1± 1.15
6	50	26.0± 1.43	25.0± 0.21	23.9± 0.90	28.2± 1.12
7	75	23.2± 0.80	27.0± 0.82	26.6± 0.49	24.4± 0.37
8	100	25.3± 1.31	32.3± 0.92	27.9± 0.50	30.3± 0.74

[a] % protein exchange

Table 5—Physicochemical properties of meat mixtures

Mixture	Substitution level[a]	Emulsifying capacity (V_{100}) Without salt	With NaCl	With Hamine	With NaCl and Hamine
1	0	36.9±1.84	34.9±1.53	33.0±2.07	35.1±1.02
2	10	44.0±1.50	41.4±1.50	35.0±1.17	41.0±0.74
3	20	44.5±1.04	47.9±2.12	39.3±1.66	47.8±0.79
4	30	49.6±2.14	51.9±2.50	46.0±1.27	52.6±0.86
5	40	57.6±1.16	52.8±1.83	51.7±1.82	54.6±1.64
6	50	56.4±1.73	55.4±1.64	48.9±1.40	54.0±0.70
7	75	54.8±0.84	53.2±2.77	47.8±0.92	52.0±0.93
8	100	53.1±1.00	51.5±1.20	46.5±1.21	49.6±1.46
		Emulsion stability (% emulsified oil)			
1	0	100.0±0.00	100.0±0.00	88.3±0.58	100.0±0.00
2	10	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
3	20	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
4	30	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
5	40	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
6	50	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
7	75	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
8	100	93.3±0.42	95.1±0.31	97.7±0.23	96.1±0.42

[a] % protein exchange

Table 6—Active acidity of meat mixtures

Mixture	Substitution level[a]	pH Without salt	With NaCl	With Hamine	With NaCl and Hamine
1	0	5.63±0.01	5.59±0.03	5.80±0.01	5.75±0.00
2	10	5.71±0.03	5.70±0.03	5.94±0.04	5.91±0.01
3	20	6.00±0.00	5.97±0.00	6.02±0.03	6.01±0.00
4	30	6.10±0.01	6.00±0.00	6.15±0.03	6.04±0.03
5	40	6.16±0.01	6.06±0.03	6.24±0.01	6.10±0.00
6	50	6.24±0.01	6.09±0.00	6.40±0.03	6.19±0.01
7	75	6.36±0.00	6.21±0.014	6.45±0.01	6.30±0.04
8	100	6.49±0.01	6.35±0.00	6.60±0.03	6.34±0.00

[a] % protein exchange

Active acidity of the product was determined using the universal pH-meter type N 5122 made by Mera Elmet (Table 6). Ten grams of sample were macerated with 10 cm^3 of distilled water, the pH being measured 0.5 hr after maceration.

Analysis of variance was applied to the results to determine the separate effects of salt and of concentration of textured proteins (Elandt, 1964).

RESULTS & DISCUSSION

BOTH SALTS and substituted textured proteins caused differences, significant at $p = 0.05$, in functional properties, and there were significant interactions between salt and substitute.

As indicated in Fig. 1, the highest water absorption capacity was shown by systems containing both NaCl and Hamine, decreasing progressively in systems containing NaCl, Hamine, and no salt additions.

The results indicated that addition of textured milk proteins to meat systems improved their water absorption capacity to a certain substitution level, after which, with further increase of substitution, capacity decreased. The systems without salt or polyphosphates with a substitution level of 10 and 20% showed the highest water absorption capaciteis. The water absorption capacities of systems with additions of NaCl and of NaCl and Hamine together were higher, compared to their respective controls, up to 50% protein substitution. Systems containing Hamine absorbed water better than the control, up to 30% protein substitution.

Ground meats with addition of NaCl as well as with both kinds of salts had the highest water absorption capacities, 49.7 ± 0.47 and 54.3 ± 1.25 g/100g, respectively, at 30% protein substitution. Systems without salts, as well as with addition of phosphates only, had the highest water absorption capacities, 24.3 ± 0.47 and 38.8 ± 0.85 g/100g respectively, at 10% substitution.

Thomas et al. (1974) found that addition of milk coprecipitates to lamb meat increased water-absorption capacity, the effect being pronounced at a lower pH (5.60).

From the results (Fig. 2), substitution of textured milk proteins improved the water-holding capacities of the salt-free and phosphate-containing systems. The water-holding capacities of products containing NaCl and NaCl plus Hamine, were constant, independent of the extent of pro-

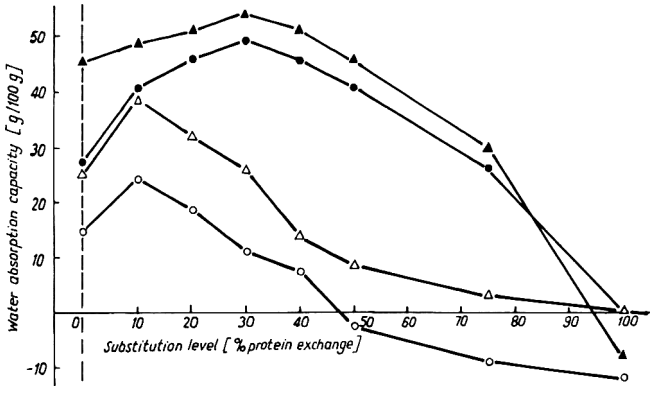


Fig. 1—Effect of protein exchange on water-absorption capacity of meat systems: ○ — ○ Without salts; ● — ● With NaCl; △ — △ With polyphosphates; ▲ — ▲ With NaCl and polyphosphates.

tein substitution. No free water was observed in these systems. Systems without salts or with the addition of Hamine alone were characterized by higher water-holding capacity than the pure-meat samples, up to 40% protein substitution. A decrease of free water content in pork systems containing 10 and 20% Promine D was observed by Hermansson and Akesson (1975a). Kozlowska et al. (1971) noticed increased water-holding capacity of beef loin systems with addition of whey protein concentrate. A similar tendency was observed by Uchman et al. (1977) in their studies of the water-holding capacity of ground meats with addition of yeast protein preparations.

Addition of textured milk proteins reduced the thermal shrinkage of systems without salt up to 50% protein substitution (Fig. 3). However, thermal shrinkage increased with increasing protein substitution to 21.6 ± 1.13% for complete substitution.

NaCl and Hamine, individually and together, reduced thermal shrinkage (Fig. 3). Shrinkage was almost independent of the degree of substitution, at between 1 and 3%. Hermansson and Akesson (1975a) observed reduced thermal shrinkage, as compared to a pure-meat sample, of ground meats with addition of Promine D and sodium caseinate at 20% substitution of protein. The decrease of thermal shrinkage caused by addition of textured soy protein to beef patties was observed by Smith et al. (1976). Hermansson and Akesson (1975b) also found that addition of NaCl reduced the thermal shrinkage of meat systems containing 30 and 50% substituted protein; this was confirmed in the present studies on textured milk proteins.

Substitution of meat by textured milk proteins reduced the consistency of the products progressively with increasing degree of substitution (Fig. 4). A similar tendency was observed by Huffman and Powell (1972) for beef patties with additions of soya meal. Drake et al. (1977) measured the texture of freeze-dried ground beef patties containing 0, 10, 20, or 30% rehydrated textured soy proteins with a Kramer Shear Press. Shear values were significantly lower at all levels of addition than for the control. The authors stated that, in general, the more tender the patty as measured instrumentally, the more acceptable the tenderness from a sensory point of view. So, it seems that the drop of shear values found in the present work for meat-textured milk protein systems is desirable, at least up to 30% protein substitution.

Hermansson (1975) observed an inverse correlation between consistency and thermal shrinkage for meat balls with addition of Promine D and whey protein concentrate. In the present work thermal shrinkage increased and consistency decreased with increasing substitution by textured

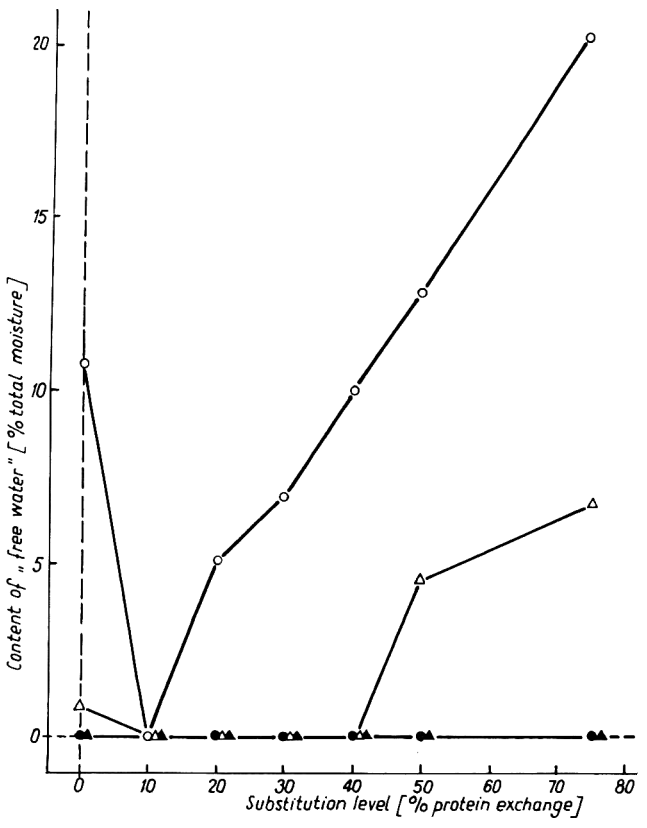


Fig. 2—Effect of protein exchange on water-holding capacity of meat systems: ○ — ○ Without salts; ● — ● With NaCl; △ — △ With polyphosphates: ▲ — ▲ With NaCl and polyphosphates.

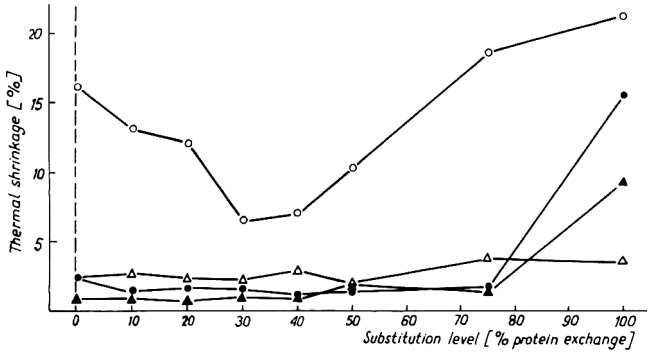


Fig. 3—Effect of protein exchange on thermal shrinkage of meat systems: ○ — ○ Without salts; ● — ● With NaCl; △ — △ With polyphosphates; ▲ — ▲ With NaCl and polyphosphates.

milk proteins in the absence of salts. Addition of salts reduced thermal shrinkage, but did nothing to maintain the consistency of the ground meat. Although these systems had low thermal shrinkage, the respective shear values were not high.

Systems containing sodium chloride and polyphosphates had the highest viscosity (Fig. 5), while those without salts were the least viscous. The highest increase in viscosity was caused by substitution of 20% of meat by milk proteins. Above the level the viscosity decreased and the lowest values were observed for the pure milk protein systems.

Uchman et al. (1975) found that the viscosity of ground meat was not changed by additions of 5% and 10% sodium caseinate and milk proteinate. Thus the effect of the tex-

tured milk protein on the viscosity of ground meats does not seem to be related only to the pH of the textured preparation, otherwise both caseinate (pH 6.6–6.8) and milk proteinate (pH 6.6) Chojnowski et al. (1975) would increase the viscosity of the product.

Fat absorption capacity also appeared to increase with increase in degree of protein substitution (Fig. 6). However, at lower degrees of substitution there was a small but statistically significant decrease in fat absorption, the minimum being at 20–30% substitution.

The emulsifying capacity of meat protein was increased by substitution of textured milk protein (Fig, 7) to a maximum at between 40 and 50% milk protein. Textured milk proteins (100% substitution) had greater emulsifying capacities than pure meat products with and without additions of salt and Hamine. This difference between the emulsifying capacities of the pure textured milk protein and the pure meat product was 44, 48, 41, and 41% of the emulsifying capacity of the pure meat product for product containing no salt additions, NaCl, polyphosphate, and NaCl + polyphosphate additions, respectively.

The substitution of textured proteins for meat proteins had little effect on the stability of the systems (Fig. 8). With the exception of the pure-meat system with Hamine, all the other systems, up to the substitution level of 75% protein, stabilized the emulsion to 100%. Improvement of emulsion stability with protein substitution agrees with the observations of Thomas et al. (1974) for meat systems containing soya and milk preparatons. Smith et al. (1973) also reported improved stability in meat products with various protein substituents.

Emulsifying capacity and pH vary similarly with the extent of substitution by milk protein (Fig. 7 and 9), suggesting that the emulsifying capacity depends to a large extent on pH changes introduced by substitution.

CONCLUSION

THESE EXPERIMENTS indicate that the concentration of salts in a manufactured meat product affects the physicochemical properties of textured milk protein substitutes and hence those of the meat product.

Substitution of textured milk proteins for meat affected the various functional properties of the product differently. Improved physicochemical properties might be caused by changes in pH of the systems, but clearly this was not the only cause of the changes in functional properties. The results suggest that interactions between meat and the milk protein during mixing engendered properties differing from those of either components.

Properties of ground beef, that affect the production of meat products, may be improved by substitution up to 20% of textured milk proteins, the limitation of further substitution being due to impaired water-absorption capacity and viscosity.

Up to 30% textured milk proteins may be added to meat containing 2% sodium chloride without being detrimental to the functional properties of the product. The upper limit of substitution is due to impaired viscosity.

Considering meat systems with addition of Hamine as well as NaCl and Hamine together, an improvement in the physicochemical properties is achieved by adding textured

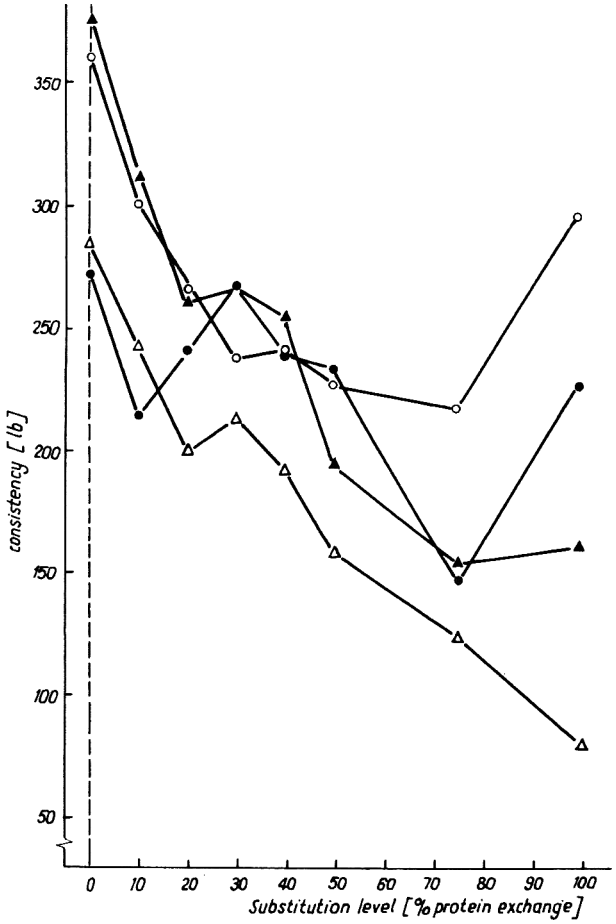


Fig. 4–Effect of protein exchange on consistency of meat systems: ○ — ○ without salts; ● — ● With NaCl; △ — △ With polyphosphates; ▲ — ▲ With NaCl and polyphosphates.

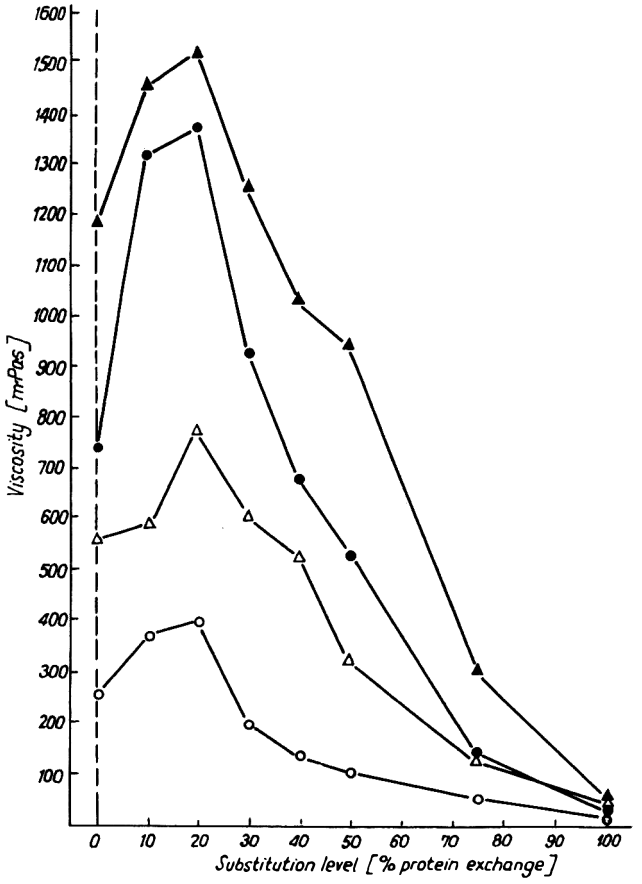


Fig. 5–Effect of protein exchange on viscosity of meat systems: ○ — ○ Without salts; ● — ● With NaCl; △ — △ With polyphosphates; ▲ — ▲ With NaCl and polyphosphates.

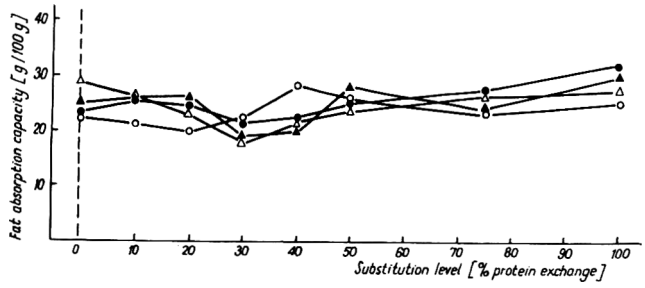


Fig. 6—Effect of protein exchange on fat absorption capacity of meat systems: ○ — ○ Without salts; ● — ● With NaCl; △ — △ With polyphosphates; ▲ — ▲ With NaCl and polyphosphates.

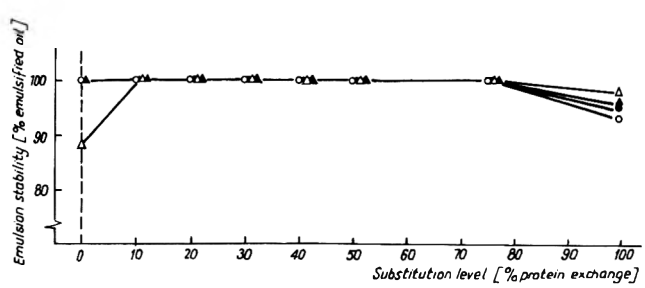


Fig. 8—Effect of protein exchange on emulsion stability of meat systems: ○ — ○ Without salts; ● — ● With NaCl. △ — △ With polyphosphates; ▲ — ▲ With NaCl and polyphosphates.

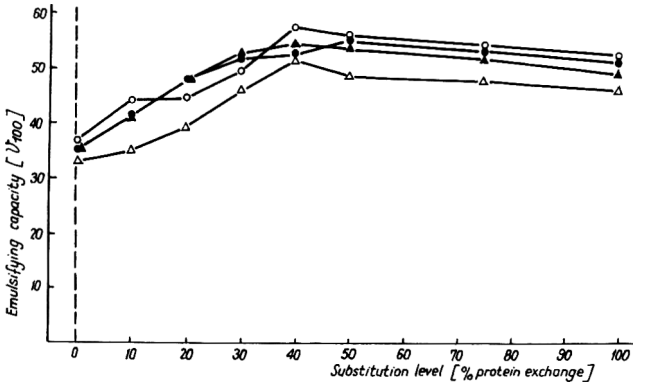


Fig. 7—Effect of protein exchange on emulsifying capacity of meat systems: ○ — ○ Without salts; ● — ● With NaCl; △ — △ With polyphosphates; ▲ — ▲ With NaCl and polyphosphates.

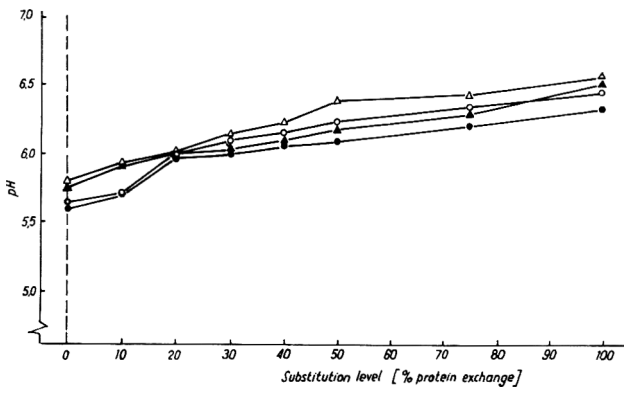


Fig. 9—Effect of protein exchange on active acidity of meat systems: ○ — ○ Without salts; ● — ● With NaCl; △ — △ With polyphosphates. ▲ — ▲ With NaCl and polyphosphates.

milk proteins up to 20%, the upper limit of substitution being due to excessive thermal shrinkage.

Summarizing the results, the greatest improvement in physicochemical properties of ground beef meat (of normal acidity pH 5.63) results from addition of 2% NaCl together with textured milk proteins up to a maximum of 30%.

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Physical and Chemical Characteristics of Extruded Rice Flour and Rice Flour fortified with Soybean Protein Isolate

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ABSTRACT

Texturized products were produced from rice flour and rice flour fortified with Soy Protein Isolate (SPI) by extrusion cooking. Water absorption capacity (WA) of the extrudates was slightly depressed in the presence of SPI. Scanning electron microscopy revealed that the fine structure of the textured products differed subtly from each other and that SPI formed fine strings in the starch matrix. Solubility tests indicated that thermoplastic extrusion processing increased noncovalent interaction and disulfide bonding resulting in decreased protein solubility. 7S subunit of the soy protein seemed more affected than the 11S component. Insolubilization was significantly less when rice flour and SPI were processed simultaneously indicating a sparing effect in their mutual presence.

INTRODUCTION

RICE (*Oryza sativa, L.*) is a principal source of calories and protein for the Asians. In Japan however, the per capita intake of rice has decreased over the years and it seems unlikely that it will increase in the near future. In addition to this, rice production has been increasing, resulting in huge surpluses. It is therefore necessary to formulate and fabricate different rice product and ingredients to encourage consumption. Dehulled rice has a protein content of 8% when measured at 12% moisture. Milled rice has about 7% protein (Juliano 1966). Since the outer layer of rice has a higher protein content than the starchy endosperm, the protein loss is higher during milling than the proportion of weight loss. Since the taste of soy bean is highly agreeable to the Japanese palate, soy protein isolate (SPI) can be incorporated with rice to improve and increase its protein content. Cereal/legume mixtures have been shown to exert a mutually supplementary effect because cereals are higher in sulfur-containing amino acids but lower in lysine than most edible varieties of legumes (FAO, 1970; Orr and Watt, 1957). Incorporation of the SPI could be accomplished with several methods. However, extrusion cooking seems to be appropriate because it produces products which can be incorporated into a wide variety of food systems. Extrusion cooking was first applied to food processing in 1935 for the continuous cooking of pasta. Since then, there has been an increased use of extrusion in the food industry for various reasons and this has stimulated numerous studies of both a technical and basic nature. To date however, the mechanism of texturization is essentially unknown and evaluation of new applications must still rely heavily on test data of previous experimentations. Numerous studies addressing this problem have been reported in the literature. Cumming et al. (1972) used transmission light and scanning electron microscopy to show the usefulness of these techniques for analyzing the structure of textured soy protein. Taranto et al. (1978) observed the structural relationship between the protein and carbohydrate fraction of the texturized products by using all the advantages of transmitted light, transmission electron and scanning electron microscopies, collectively. Unfortunately, electron microscopy did not provide data to completely elucidate the mechanism of texturization during extrusion cooking. Based on chemical analyses, Cumming et al. (1973) suggested that extrusion processing of soy flour causes dissociation and aggregation of the water-soluble proteins. Burgess and Stanley (1976) suggested that peptide crosslinks may play a role whereas Jeunink and Cheftel (1979) examined the possibility of amino acid crosslinks and found no significant formation of lysinoalanine or lanthionine after extrusion of field bean or soybean even at 200°C. The primary objectives of the present study were to prepare texturized food products from rice flour and analyze the following: (1) selected physiochemical changes in the extrudates induced by thermoplastic extrusion processing, and (2) determination of the differences in the morphology of extrudates produced from rice flour and rice flour fortified with SPI.

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MATERIALS & METHODS

Rice flour

Rice was polished to 75.5% of its initial weight to remove the husk and bran. The endosperm was then milled to a mean particle size of 100μ by brake roll milling followed by impact milling. SPI which contained 92.9% protein (dry weight basis) was obtained from Fuji Purina Co., Ltd. Both the raw materials were packed in PE and stored at 10°C and were brought to room temperature before use. Rice flour was blended with SPI using a V-mixer to increase its protein content from 6.6% to 10, 20 and 30%. The different mixtures were then premoisturized using a Hobart confectionary mixer to initial moisture levels of 20, 30 and 40%. Premoisturizing time of 30 min was found to be the most suitable. Higher premoisturizing time significantly increased stickiness (data not included) and hampered extrusion.

Extrusion processing

The extruder used in this study was a Brabender type 825601 with the following specifications: Barrel length, 320 mm; barrel diameter, 19 mm; 2-zone barrel and die assembly electric heaters; die diameter, 5 mm; and 1:1 compression ratio screw. The temperature profile was as follows: zone 1, 25°C; zone 2, 200, 220 or 240°C, and die, 200, 220 or 240°C. The screw speed was kept constant at 160 rpm as this was found to be the most suitable among all the revolutions tried (data not included). The premoisturised materials were uniformly forced to beed into the extruder using an augered feeder, the speed of which was carefully monitored to get a uniform extrusion rate. One kilogram of the material was extruded to obtain a steady-state operation before an appropriate portion was collected as a sample. All samples were vacuum dried at 25°C to around 7% moisture level. The expansion degree (ED) of the extrudates was expressed as follows:

$$ED = \frac{\text{Diameter of product}}{\text{Diameter of die (5 mm)}}$$

Bending rigidity of dry extrudated samples were examined with an Intesco-model 2005 and expressed as force per unit area (kg/cm^2). In each case, ten replications were carried out.

Determination of water absorption capacity (WA), protein solubility and gelatinization degree (DG)

For the determination of WA, the extruded material was pulverized using a compact mill and sifted through a 350μ sieve. 0.5g of the flour thus obtained was suspended in 10 ml phosphate buffer (0.1M, pH 7) for 30 min at 30°C followed by centrifugation (1600 x g, 10', 20°C). The amount of supernatant was determined and WA was calculated according to the method of the National Soybean Processors Assoc. (Anon., 1946). For the protein solubility determination, 0.5g of the finely ground samples were extracted for 2 hr at 30°C in 10 mL of phosphate buffer containing 2% sodium dodecyl sulfate (SDS) and 1% 2-mercaptoethanol (2-ME). After centrifugation (8000 x g, 10', 20°C) the nitrogen content of the paper filtered supernatant was determined by the micro-Kjeldahl method (Mitsubishi Chemical Industry, KN-01). The protein content was then calculated using a conversion factor of 6.25. To determine DG, β-amylase (Nagase Co., Ltd.) and pullulanase (Hayashibara Biochemical Laboratories Inc.) were used according to the method of Kainuma (1979). One gram of the finely ground sample was homogenized in 20 ml of acetate buffer (pH 5.2, 20 mM) and the homogenate was then diluted 25 fold in the same buffer. Enzyme solution was prepared by dissolving 5 mg of β-amylase (75 units) and 10 mg of pullulanase (20 units) in 20 mL of the same buffer as for the sample preparation and then filtered (Whatman No. 2) to remove the undissolved stabilizing materials. Two mL of the enzyme solution was then added to 1 mL of the homogenized sample and incubated for 2 hr at 40°C. Reducing sugar and total sugar content was determined by the Somogyi method (1952) and phenol-sulfuric acid method (Dubois et al., 1956), respectively. DG was calculated according to the following calculation:

$$DG\ (\%) = \frac{\dfrac{\text{Reducing sugar}}{\text{Total sugar}}\ \text{of extrudate}}{\dfrac{\text{Reducing sugar}}{\text{Total sugar}}\ \text{of initial materials after autoclaving at 120°C for 30 min}} \times 100$$

Specimen preparation for scanning electron microscopy (SEM)

Texturized samples were mounted on aluminum stubs with silver-conducting paint and coated for 5 min with platinum-palladium. Samples which were subjected to enzymatic digestion were immediately lyophilized prior to mounting. Enzymatic treatment consisted of β-amylase, pullulanase and pronase-E (1170×10^3 pu/g, Kahen Kagaku Co., Ltd.) treatment where β-amylase and pullulanase were used in the same way as for the determination of DG except that phosphate buffer (pH 7, 0.1M) was used. Pronase-E digestion was carried out as follows: 0.5g of the finely ground sample was suspended in 10 mL of phosphate buffer (pH 7, 0.1M) containing 4 mg of pronase-E and stirred gently for 4 hr at 40°C. Preliminary data showed that 4 hr was enough to get maximum supernatant absorbance at 280 nm. A Hitachi S-500A SEM was used at 15 kv for the study.

Polyacrylamide gel electrophoresis (PAGE)

SDS-PAG was prepared according to Weber and Osborn (1969). Gel and SDS concentration were 8% and 0.12%, respectively, and the gel pH was 7.0. In order to achieve denaturization of the protein, about 1g of the samples were treated overnight at 30°C with 10 mL of 2% SDS and 2% 2-ME. One mL of the sample solution was then added to an equal volume of 50% glycerol containing 0.02% malacite green and then 100 μL of the mixture was applied to the top of the gel. Electrophoresis was carried out at 7 mA per gel which were then stained for 2 hr at 60°C with 0.1% amido black 10B in 7% (v/v) acetic acid, destained by electrophoresis and stored in the same acetic acid solution. Glycoproteins in the sample were examined by the PAS staining method of Zacharius (1969). Molecular weight of the proteins was estimated from the plot of log molecular weight vs. the ratio of the distance traveled in comparison with the tracking dye. Standard proteins, RNA-polymerase, BSA and tripsin inhibitor were purchased from Berlinger Mannheim Co., Ltd. Densitometric readings of the different peaks were carried out using a Fuji Riken densitometer.

RESULTS & DISCUSSIONS

Extrusion

As can be seen in Table 1, extrusion could best be done at 200°C below which the extrudate usually did not develop the desired consistency and texture. It was observed that screw rotation below 100 rpm, was not suitable as the yield decreased resulting in occasional uncontrolled ejection. Temperature above 220°C was also unsatisfactory even when the moisture content was 30 or 40%. Excessive surface burning and irregular extrusion was sometimes observed. Product crispiness increased as temperature increased and moisture decreased. Inner structural coarseness was evident at 200°C while the surface remained smoother than it was at 220 or 240°C. Moisture content of 20% seemed to be the most appropriate while the degree of gelatinization seemed to be almost complete at temperatures above 200°C regardless of the moisture content (Table 1). As a control experiment, rice flour was autoclaved for 30 min at 120°C resulting in the hydrolysis of about 43% calculated on the basis of total sugar yielded by β-amylase and pullulanase treatment. The remaining 57% comprised of insoluble carbohydrates. Extruded rice showed the same degree of gelatinization as the autoclaved control.

Rice flour fortified with SPI

The rice flour which had an initial protein content of 6.6%, was fortified with SPI to increase the protein content to 10, 20 and 30% and extruded at 200°C and 220°C after premoisturization to 20 and 30%. The addition of SPI made the mixture stickier without significantly altering the handling quality and the product appearance changed slightly to become yellowish. The surface smoothness however, was distinctly increased. As can be seen in Table 2, SPI suppressed expansion and played a role in making the products more rigid at 20% moisture level. The tendency was opposite when the moisture level was 30% at 200°C and inconsistent at the same moisture level when the temperature was increased to 220°C. According to Aquilera and Kosikowski (1976), high moisture levels up to 35% depress the shear force of soy bean extrudate. During our

Table 1—Extrusion quality and gelatinization degree of products

	Moisture %					
	20		30		40	
Temp (°C)	Qual.[a]	DG[b]	Qual.	DG	Qual.	DG
200	A	100	A	99	C	100
220	A	100	B	100	C	100
240	X	–	C	100	C	97

[a] Quality of products was determined according to the handling for extrusion and the appearance of products: A = excellent, B = good, C = bad, X = not possible to operate.
[b] DG = degree of gelatinization (%).

Table 2—Expansion degree and shear force of products[a]

Temp (°C)	Moisture (%)		Rice	Rice + SPI		
			TP =6.6%	TP =10%	TP =20%	TP =30%
200	20	ED	2.1	2.2	2.0	1.9
		SF	2.8	3.9	4.5	5.2
	30	ED	1.0	1.1	1.2	1.2
		SF	10.0	7.8	6.0	5.6
220	20	ED	2.0	1.9	1.9	1.8
		SF	3.6	4.0	4.0	4.3
	30	ED	1.0	1.1	1.1	1.0
		SF	6.8	6.5	6.1	6.9

TP = total protein; ED = expansion degree; SF = shear force (kg/cm^2) is the mean of 10 replicates.

experimental extrusions, extrusion temperature seemed to cause this effect. It was interesting to note that ED and shear force were completely different at 200°C and 30% moisture than at other extrusion conditions. Shear force decreased abruptly to about half that of the unfortified extrudate when the protein level was 30%. As reported earlier by Cegla et al. (1978) it is not clear why the addition of rice flour to protein-rich materials before extrusion results in the improvement or depression of stress and resilience of extrudates.

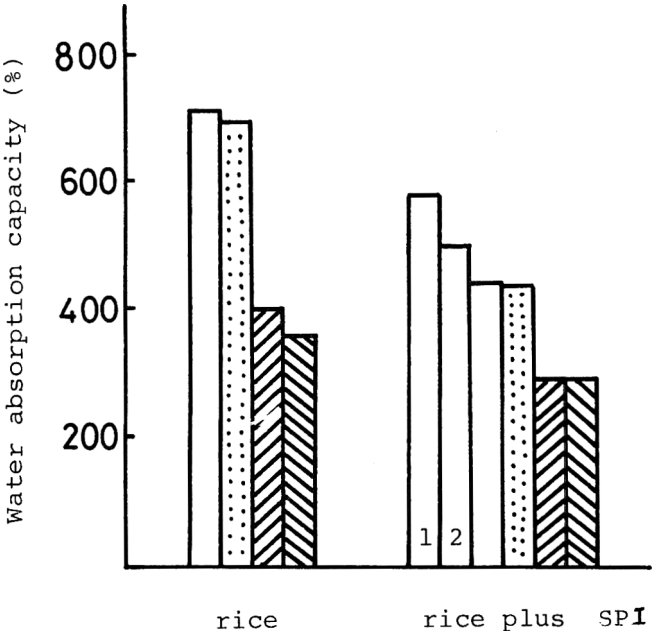


Fig. 1—Water absorption capacity of extrudates. Total protein content in rice plus SPI is 30% except 1 (10%) and 2 (20%). □ 0.1M phosphate buffer (pH 7.0); ⁘ 0.1M phosphate buffer (pH 7.0) + Pronase-E; ▨ 0.1M phosphate buffer (pH 7.0) + β-amylase + pullulanase; ▧ 0.1M phosphate buffer (pH 7.0) + β-amylase + pullulanase + Pronase -E.

Water absorption capacity

Fig. 1 shows the water absorption capacity (WA) of rice flour and fortified rice flour extruded at 200°C, 20% moisture. Since the WA decreased with the increase in SPI content, it is apparent that SPI depresses WA of rice starch. When extruded at 200°C and 20% moisture, WA of sample fortified to 30% protein level was found to be 440% in comparison with 710% for rice flour. It was further seen that β-amylase and pullulanase treatment decreased WA by 44%. As mentioned earlier, even after autoclaving, rice flour can be hydrolyzed only up to 43%. It is therefore assumed that WA is afforded not only by gelatinized starch but equally by the insoluble component. After pronase-E or β-amylase plus pullulanase followed by pronase-E treatment, neither of the extrudates showed any significant difference in WA from those extrudates not treated with pronase-E. This result suggests that protein does not contribute to WA but depresses it slightly.

Scanning electron microscopy (SEM)

The extrusion texturized products showed a sponge-like structure as shown in Fig. 2. Air cells ranged in size from 0.1–2 mm (A), 10–500 μm (B), and air cell wall thickness ranged from 10–300 μm. The inner surface of the air cells appeared very smooth. Taranto et al. (1978) reported that some particles could be found in textured soy flour and that they were identified as protein bodies because of their similarity in size and appearance to the structure described by Wolf and Baker (1975). The protein bodies of rice have been reported to be 1–4 μm in size (Mitsuda et al. 1969). We could not identify such distinct sperical bodies in our extruded samples. The protein bodies were perhaps broken, melted and fused with starch during the thermosplastic processing. Air cell localization was different for rice flour and fortified rice flour extrudates. The former had more and larger air cells even close to the outer layer in contrast to the latter, resulting in its low density. The addition of SPI made the extrudate more rigid and unexpectedly dense and thus contributed to shear force except when extruded at 200°C, 30% moisture (Table 2). After being pulverized and rehydrated in phosphate buffer, each extrudate ex-

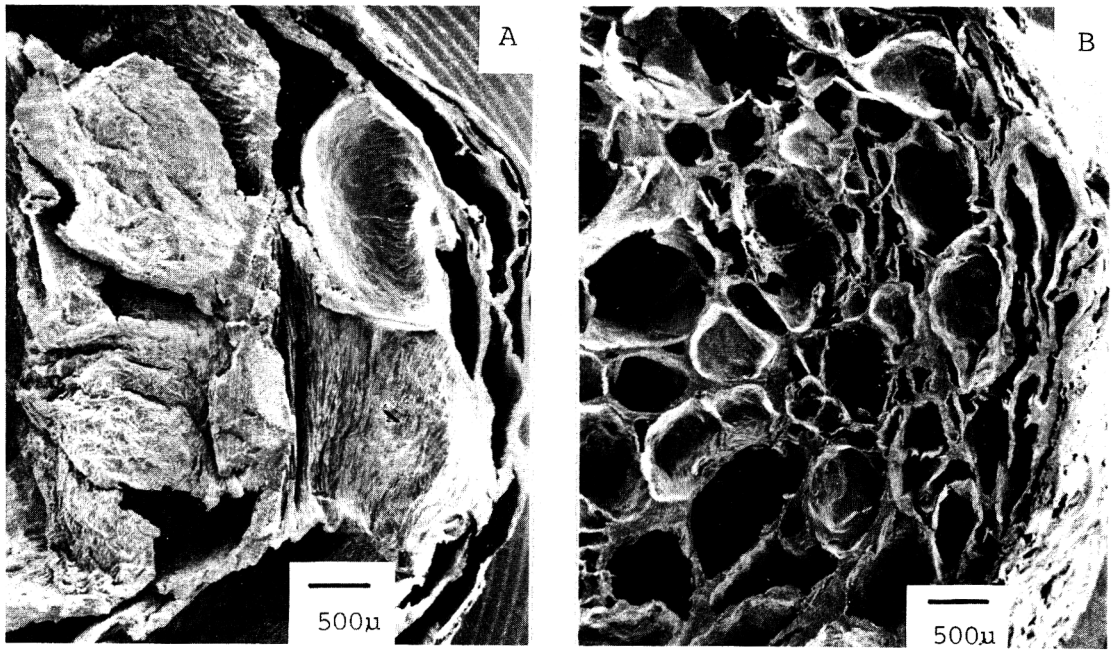


Fig. 2—Extruded sample at 200° C with 20% moisture: (A) Rice (20x); (B) Rice + SPI, total protein 30% (20x).

hibited a clearer finer structure as shown in Fig. 3. Numerous film-like structures were observed in the rice extrudate much of which could not be observed in the fortified one. It was noticeable that the fortified extrudate exhibited many fine strings throughout the rice carbohydrate matrix. On enzymatic treatment (β-amylase and pullulanase, AP; pronase-E, PR), some of these structures disappeared as can be seen in Fig. 4 and 5. The film-like structures were removed by AP treatment in the case of the rice extrudate but persisted in the case of the fortified rice extrudate. After PR, the fine strings in the fortified extrudate almost disappeared. As mentioned earlier, WA of rice and fortified extrudates was depressed to the same degree after AP treatment. Film-like structures observed in the fortified extrudate even after AP treatment, were therefore most likely to be composed of insoluble starch. The above results suggest that rice extrudate had a film-like structure composed mainly of gelatinized starch with insoluble starch skeletons and that in the fortified extrudate, fine strings were contributed by SPI.

Protein solubility

Protein solubility of the extrudates were examined by four different extraction solutions as shown in Fig. 6. Rice flour by itself exhibited a low solubility of only 4.2% in plain buffer (pH 7) and 3.2% in the buffer containing 2-ME. Significantly high solubility of 57% and 87.9% was observed when SDS or SDS plus 2-ME were added respectively to the buffer. Solubilizing capacity of the buffer containing SDS and 2-ME, always surpassed the buffer containing only SDS. It is therefore evident that rice, besides having disulfide bonds, also initially has some non-

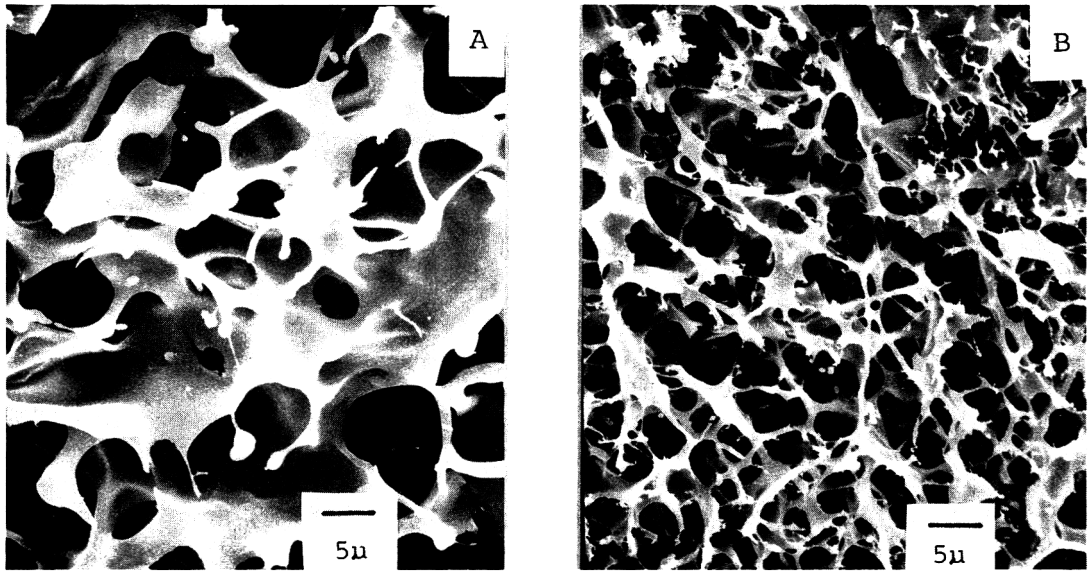


Fig. 3—Fine structure of extrudate after rehydration in 0.1M phosphate buffer (pH 7.0): (A) Rice (2000x); (B) Rice + SPI, total protein 30% (2000x).

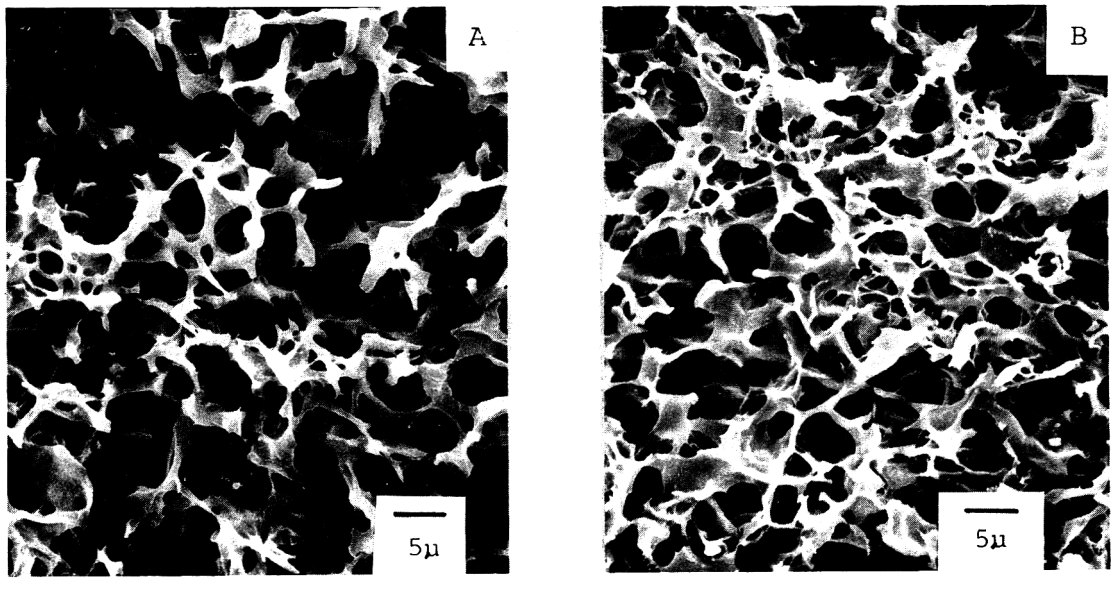


Fig. 4—Fine structure of extrudate after rehydration in 0.1M phosphate buffer (pH 7.0) followed by β-amylase and pullulanase treatment: (A) Rice (2000x); (B) Rice + SPI, total protein 30% (2000x).

covalent interaction between polypeptide chain and/or polypeptide chain and other constituents. This seems to hold true for the unextruded SPI as well. After extrusion, both rice and SPI lost their solubility considerably even in buffer containing SDS, whereas in the buffer containing SDS and 2-ME, rice still retained its high solubility and SPI exhibited it to a certain degree (39.1%). This could be because the proteins, especially rice, interacted more than before by noncovalent bonds and disulfide bonds. 2-ME could react with the disulfide bond only after SDS reduced noncovalent interaction. This agrees with the observation of Jeunink and Cheftel (1979) that low solubility is mainly due to noncovalent interaction and disulfide bonds. There was no significant difference in the solubility of extruded and unextruded rice flour in buffer containing SDS and 2-ME whereas extrusion reduced the solubility of SPI in the same buffer to almost half the initial value suggesting the increased formation of covalent bonds. There was little improvement of protein solubility in the plain buffer of unfortified and fortified rice flour after β-amylase and pullulanase treatment, indicating that rice and SPI proteins were not incorporated with the soluble starch thus protecting itself from solubility. Note that fortified rice extrudate still showed a high protein solubility of 43.1% in the buffer containing only SDS and the solubility increased to 88.5% when 2-ME was added in comparison to 39.1% and 91.5% for SPI and rice flour respectively. This indicates that rice starch or other nonprotein constituent protect SPI from noncovalent interaction, disulfide bonds, and other covalent bond formation.

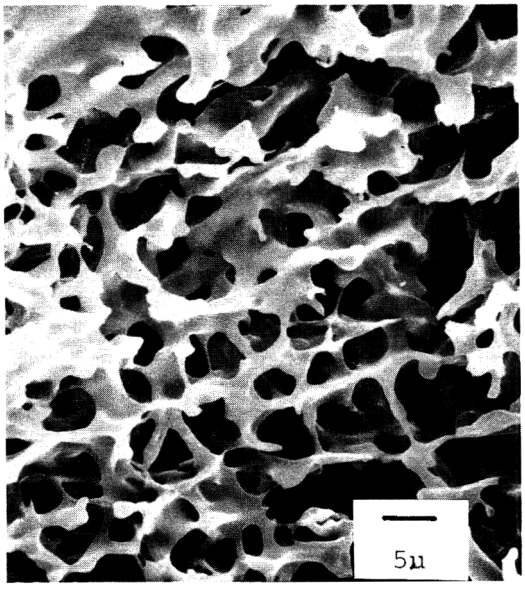


Fig. 5—Fine structure of extrudate of fortified rice with SPI after Pronase-E treatment (2000x); total protein content 30%.

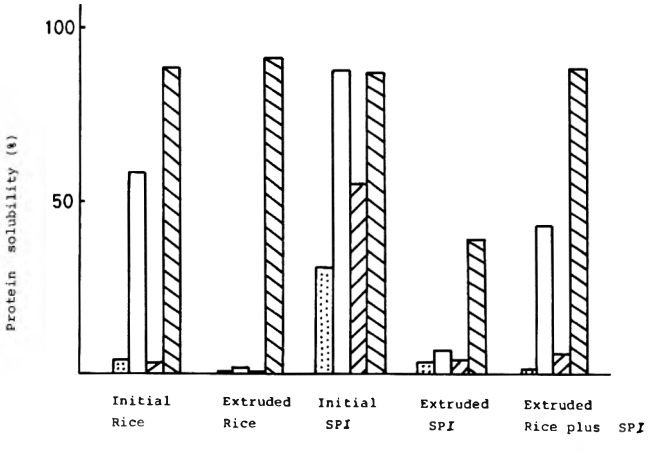


Fig. 6—Protein solubility of initial and extruded materials in different extraction solutions: □ 0.1M phosphate buffer (pH 7.0); ⊡ 0.1M phosphate buffer (pH 7.0) + SDS (2%); ▨ 0.1M phosphate buffer (pH 7.0) + 2-ME (1%); ▧ 0.1M phosphate buffer (pH 7.0) + SDS (2%) + 2-ME (1%).

Electrophoresis

The soluble extracts from five samples were submitted to electrophoresis in the presence of SDS and 2-ME. The samples were: rice flour, SPI, extruded rice flour, extruded SPI, and extruded fortified rice. All extrusions were carried out at 200°C and 20% following which the protein contents were adjusted to an equal level to determine solubility and fate of each fraction in the electrophoretic pattern. Fig. 7 shows that some protein fractions of SPI and rice had similar migration resulting in overlapped peaks, the origins of which had to be determined by PAS staining method.

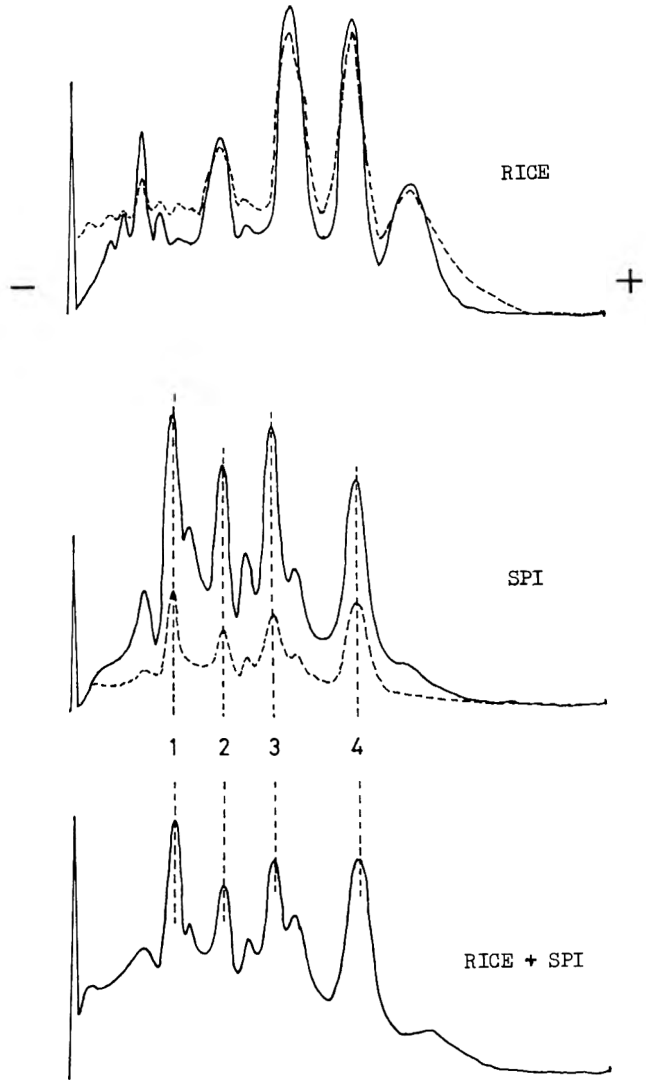


Fig. 7—Densitogram of PAGE of soluble extracts of initial and extruded samples [extraction with 2% (w/v) SDS + 2% 2-ME]. Total protein content of rice + SPI, 30%. Peak 1 and 2, 7S; peak 3 and 4, 11S of soy protein. —— initial sample; - - - - - extruded sample for rice and SPI; —— of rice + SPI is extruded one.

Rice flour extrudate showed no additional peaks and showed almost the same solubility behavior as seen during the solubility tests (Fig. 6). Each peak showed the same height and integrated value. SPI, however, showed a significant decrease of each peak height and its integrated value even though no new peaks were seen. This is consistent with SPI extrudate solubility (Fig. 6). When compared with corresponding peaks of unextruded SPI, it was noted that all the peaks did not decrease equally. Peak 1 (7S) showed a decrease of 77%; peak 2 (7S), 75%; peak 3 (11S), 70%, and peak 4 (11S), 57%. While there was little or no change at the subunit level of rice protein, soy protein underwent changes at the subunit level through the formation of new covalent bonds, besides disulfide bonds, and the 7S fraction seems to be affected more than the 11S fraction. The fortified rice extrudate did not show any new peak on electrophoresis and there was only a slight decrease in the protein solubility, indicating lack of extrusion-induced interaction between rice protein and SPI. The soy protein, especially the 7S fraction, was probably protected during the thermoplastic extrusion process by some constituent of rice. This phenomenon is now under study.

CONCLUSION

WATER-ABSORPTION CAPACITY of rice extrudate was found to be equally contributed to by the soluble and insoluble carbohydrate components. Addition of SPI to the rice flour increased its ultrastructural clarity but did not contribute to the water-absorption capacity. Protein solubility in buffer decreased significantly when SPI or rice flour were extruded separately but remained high when exturded simultaneously indicating a sparing effect in their mutual presence. The low solubility of rice flour appeared to be due to stronger noncovalent interaction and disulfide bonding whereas that of SPI appeared to be due also to the formation of isopeptide bonds affecting the 7S subunit more than the 11S. Rice protein and SPI interaction was not detected.

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Influence of Tertiary Butylhydroquinone and Certain Other Surface Coatings on the Formation of Carbonyl Compounds in Granulated Roasted Peanuts

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ABSTRACT

A 15-month shelf life study was conducted with granulated roasted peanuts (*Arachis hypogaea* L.). The nuts were coated with combinations of 0.01, 0.02, and 0.03% tertiary butylhydroquinone (TBHQ) antioxidant, two fats and an acetylated monoglyceride. Total carbonyls were measured monthly and served as an index of oxidative rancidity. TBHQ combined with citric acid was an effective anitoxidant in inhibiting carbonyl production, while the fats and monoglyceride coatings alone had no significant effect on carbonyl production. Samples without TBHQ exhibited a fivefold increase in total carbonyl content compared with samples treated with TBHQ. Total carbonyl levels of granulated peanuts treated with 0.02% TBHQ, the level presently allowed by the FDA, increased 20% during the 15-month storage period compared with an increase of 110% for those without TBHQ.

INTRODUCTION

RANCIDITY IN NUT MEATS is a familiar problem that may be attributed to many different factors. Agronomic, storage, processing, and packaging, conditions are all known to be determinants in the quality of the final product. Chemical analyses of raw and finished peanut products have indicated, that as in many other high fat foods, carbonyl compounds play an important role in the flavor chemistry of peanuts. Carbonyls contribute to the "good" aromas and flavors as well as the "off" ones detected in poor quality nuts. The beany or green aroma of raw peanuts has been ascribed to hexanal (Pattee et al., 1969), the deep fat fried flavor to 2,4-decadeinal a product of linoleic acid oxidation (Patton, 1965), and the roasted flavor to high concentrations of 2-methylbutanal and 3-methylbutanal (Brown et al., 1972). Since additive, synergistic, antagonistic and masking effects are quite common among the various carbonyl compounds (Kinsella, 1969), it is important to know the amount of total carbonyls in the peanuts and peanut products. Young and Holley (1965) found a significant increase of total carbonyls in inferior, low quality peanuts. A similar increase in total carbonyls was detected by Pattee et al. (1965) in high-temperature-cured off-flavor peanuts. Brown et al. (1972) reported a fivefold increase in total carbonyls of peanuts after roasting.

In an earlier study of salted-in-the-shell peanuts (Hoover and Nathan, 1980), flavor panels and total carbonyl determinations were conducted simultaneously. A correlation coefficient of 0.96 ($p = 0.05$) was found between the product's total carbonyl content and flavor scores, with flavor scores decreasing as total carbonyl content increased. Based on these results it was concluded that total carbonyl content is an excellent index of product flavor.

Tertiary butylhydroquinone (TBHQ) in combination with citric acid was selected as an antioxidant for the roasted peanuts. Sherwin and Thompson (1967) compared the antioxidative properties of TBHQ with butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propylgallate (PG). TBHQ was judged to be a more effective antioxidant, have better solubility characteristics and be especially effective in highly unsaturated fats and oils, in which the other three have shown inadequate potency. In another study, Sherwin and Luckadoo (1970) found TBHQ the most effective antioxidant in soybean, safflower, sunflower and cotton and seed crude oils. The oxidation products of TBHQ are also reversible, acting as antioxidants themselves (Chahine and mcNeill, 1974).

The practice of coating nuts with fat and antioxidant combinations has been reported to be an effective method of extending the shelf life of nuts. One manufacturer found that by coating granulated nuts for cake mixes with a 3–5% distilled acetylated monglyceride and antioxidant combination, the keeping qualities were improved (Anon., 1965). Peanuts were found to be stable up to 6 months by Shea (1965) when the nuts were coated with 1–5% solutions of distilled acetylated monoglyceride, BHA, BHT and citric acid.

The object of the study presented herein was to determine the changes in total carbonyl content occurring in roasted granulated peanuts during storage. Surface coatings of fats and an acetylated monglyceride, with and without an antioxidant, were used as treatments. Total carbonyl content was measured monthly to determine the effects of these treatments on shelf life and served as an index of rancidity.

MATERIALS & METHODS

Sample preparation

The peanuts used in this investigation were good quality spin blanched Florunners. In spin blanched peanuts the skins are removed by dry abrasion with a roughed surface. The peanuts were roasted in a high stability vegetable oil (Durkex 500) for 4 min at 171°C and then cooled in a forced air cooler. The roasted peanuts were granulated in a mechanical rotary granulator after which they were shaken through a 0.4 cm perforated screen onto a 0.25 cm screen. The granulated nuts trapped on the 0.25 cm screen were used in this study.

Two types of fats and one monoglyceride were used for coating the peanuts. These included a hydrogenated fat, KLX by Durkee Foods, with a melting point of 51–54°C, and a hydrogenated vegetable oil, Durkex 500, by Durkee Foods, with a melting point of 23°C maximum. Both of these fats are considered to be highly stable to oxidation. The acetylated monoglyceride was Myvacet 7-00, by Eastman Chemical, which has a melting point of 37–40°C.

Eastman's Tenox 20A, which contains 20% active TBHQ and 3% citric acid, was used as the antioxidant. Carter and Pike (1975) found the combination of TBHQ and citric acid to be more effective than TBHQ alone as an antioxidant system in unsaturated oils. In this investigation three levels of active TBHQ were used, 0.01, 0.02 and 0.03% based on the total fat content of the coated product. The amount of citric acid present at these levels is 0.0015, 0.0030 and 0.0045%, respectively. The FDA presently allows the application of 0.02% TBHQ based on the product's fat weight (Code of Food Regulations, 1977).

The TBHQ was applied in an oil medium (Durkex 500) to insure a thorough coating. A stock solution of 5% Tenox 20A, containing the TBHQ, and 95% hydrogenated vegetable oil was prepared. Two thousand gram samples of the roasted granulated peanuts were placed in a revolving coating pan. The antioxidant was applied by

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pouring the appropriate amount of the stock solution in the revolving nuts. They were then tumbled for 1 min to obtain an even coating. The hydrogenated fats and monoglycerides were heated to approximately 60°C for liquification after which 4% of each one, based on the weight of the nuts, was applied. After the hot fat or monoglyceride coating was applied, the nuts were allowed to tumble for 1 min to give a smooth coating then cooled. The coated nuts were filled into metal cans and sealed without the use of vacuum or inert gas. The samples were stored at room temperature (20–25°C) in a cabinet at atmospheric pressure until evaluations were made.

Total carbonyl determination

Total carbonyls were determined monthly using the procedure suggested by Henick et al., (1954). Certain modifications were made in the method to facilitate its usage with peanuts. Samples were replicated and two aliquots of both replicates were analyzed giving four values per sample. On the days that the total carbonyl analyses were to be made, a 100-g sample of each treatment was removed from storage and ground into peanut butter in a laboratory-size peanut mill for total carbonyl analysis. Carbonyl-free benzene was used to extract the carbonyl compounds from the peanut butter. A 2,4-dinitrophenyl hydrazine solution and trichloracetic acid were used to convert the compounds to 2,4-dinitrophenyl hydrazone derivatives, which were used in the colorimetric determination of saturated and unsaturated carbonyls. This total carbonyl analysis procedure has been described in detail previously by Hoover and Nathan (1980).

Statistical analysis

The experiment was conducted under a factorial design. Data obtained were subjected to an analysis of variance, Waller-Duncan K-ratio t-test and regression analysis, where applicable (Barr et al., 1976).

RESULTS & DISCUSSION

THE ANTIOXIDANT TBHQ was highly effective in retarding the formation of carbonyl compounds (Fig. 1). The total carbonyl content of samples without TBHQ increased approximately 110%, whereas, those containing TBHQ had an average increase of only 20% during the same period. This represented more than a fivefold increase in carbonyls for samples without the protection of TBHQ over those with it. The carbonyl build-up was less as the TBHQ concentration increased from 0.01% to 0.03%; however, this difference was not significant ($p = 0.05$).

Coating the peanuts with Durkex 500, KLX and Myvacet 7-00 alone failed to reduce the formation of carbonyl compounds, (Fig. 1 and 2). A Waller-Duncan k-ratio t-test (Barr et al., 1976) showed there were no significant differences between total carbonyl means of the coated samples and the control, over the entire 15 month storage period. However, application of the same t-test at each time interval showed that all three coatings had significantly higher ($p = 0.05$) total carbonyl contents than the control at the end of 35 wk storage. This was contrary to what one might expect to find, particularly, in view of the normally high stability of the fats and monoglyceride used.

There are two factors which may explain these results. In this study, while a 4% coating was applied on a weight basis, the surface area of the peanuts had been greatly increased by the granulating procedure, thus exposing a greater surface area for oxidation. It could be speculated that if the percent coating applied had been increased proportionately with the increase in surface area of the peanuts, the intercellular spaces would have filled and been sealed off, thus preventing the interchange of oxygen. Hence, the carbonyl formation during storage might have been contained to a much greater degree. This failure to fully impregnate the peanut might explain why the fats and monoglycerides offered no protection against oxidation.

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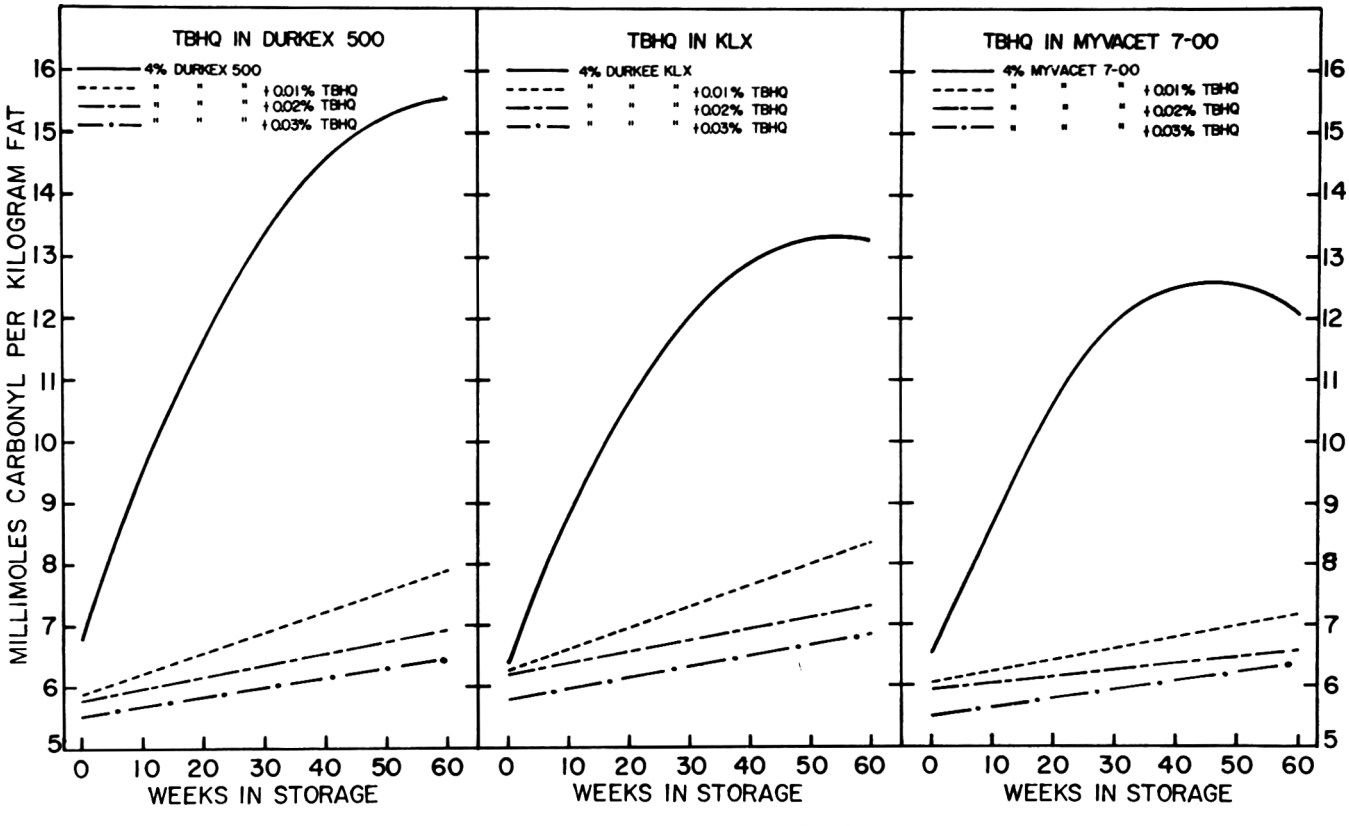


Fig. 1–Effects of combinations of TBHQ in Durkex 500, Durkee KLX, and Myvacet 7-00, on the total carbonyl content of oil-roasted Florunner peanuts.

The length of the storage period may also offer an explanation to the significant increase in the carbonyl content of the fat and monoglyceride-coated samples versus the control. Although all three coatings are highly stable, they too undergo oxidation after extended storage (Fig. 2). The increase in carbonyl compounds after 35 wk storage may be attributed to the oxidation of the coatings themselves, in addition to oxidation occurring in the peanuts, as observed in the control.

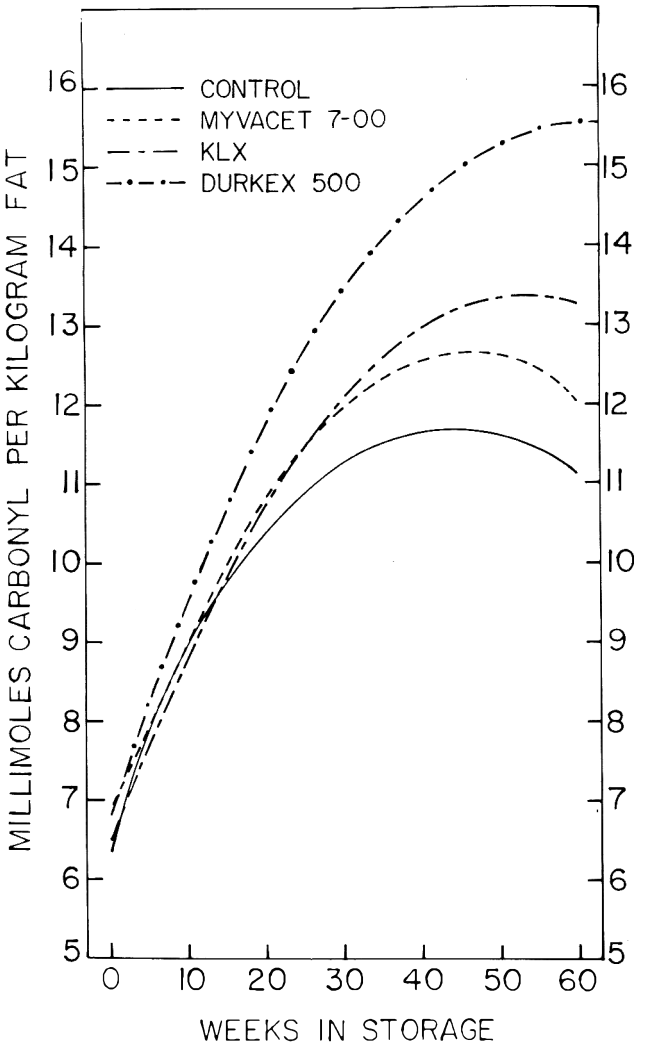


Fig. 2—Changes in the total carbonyl content of oil-roasted peanuts coated with 4% Myvacet 7-00, 4% Durkee KLX and 4% Durkex 500.

Senter and Forbus (1979) found that a 1.8% Myvacet 7-00 coating did influence the quality of pecan kernels over extended storage. However, the monoglyceride coating was only effective in seedlings. The mature Shley and Halbert varieties showed results similar to the granulated Florunner peanuts studied herein. The peroxide values for Shley and Halbert varieties coated with the monoglyceride were higher than those of their controls. Kernel deterioration during storage appeared to have a greater influence on stability than did the addition of protective coatings.

In this study TBHQ antioxidant (Tenox 20A) was found to be highly effective in prohibiting carbonyl formation in granulated peanuts during the 15 month storage period. Applications of 4% KLX, Durkex 500 and Myvacet 7-00, however, were inadequate and appeared to offer no protection against oxidation at the level applied.

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Thermal Conductivity of White Bread During Convective Heat Processing

N. UNKLESBAY, K. UNKLESBAY, M. NAHAISI, and G. KRAUSE

ABSTRACT

Thermal conductivity values of white bread were determined during heat processing to provide information for analytical modeling required during convective heat and mass transfer of the baking process. Selected physical properties of bread were determined: moisture, volume, bulk density and porosity. White bread was baked in a forced-air convection oven for 8, 16, 24 and 32 min. Thermal conductivity values ranged from 0.00072–0.00064 watts/cm-°C. Although a downward trend was observed and explained, no significant differences were revealed among thermal conductivity values. Thermal conductivity values were indirectly linearly dependent ($p<0.05$) on volume and porosity; directly, to bulk density and moisture loss.

INTRODUCTION

THERMAL CONDUCTIVITY values of foods are used for a variety of purposes, including predicting the temperature distribution within foods of various compositions and goemetric shapes when subjected to different heating conditions. This information is necessary for the optimum design of heat transfer equipment (Reidy, 1968).

Although some thermal conductivity data for raw food products are available, very little data for processed foods or mixtures are available (Sweat, 1974; Polley et al., 1980). Relatively little information is known about the thermal conductivity of baked products (Pomeranz, 1979). The relationships among the physical properties of bread, such as pore size or porosity, with thermal conductivity have not been identified. Because models of heat transfer processes cannot be developed without these data, the development of energy-efficient ovens for baked products has been hindered.

Thermal conductivity of food mixtures depends upon the thermal conductivity properties of the components; the value for water as a major food component may be used as a rough approximation of thermal conductivity of some food materials. However, when air is incorporated, a rough approximation is not feasible. The thermal conductivity of air is less by one order of magnitude of that of liquid water, and that of liquid water, is greater than that of water vapor. Furthermore, the thermal conductivity of ice is greater than water because molecules in ice are in closest proximity, but in the vapor state the least association of molecules exists (Rha, 1975). Heldman and Gorby (1974) associated low thermal conductivity values of ice cream with high overrun, thermal conductivity being lowered by the incorporation of air. Thus, consideration should be given to air content or void spaces in food materials.

This research was designed to: (1) determine the thermal conductivity of white bread during heat processing in a forced-air convection oven; and (2) analyze the relationships among thermal conductivity and selected physical properties. Any data revealed about linearly dependent relationships should help to facilitate analytical modeling of convective heat and mass transfer processes during baking of bread products.

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MATERIALS & METHODS

Development of formula

The bread formula (Table 1) was recommemded by a local baker, The Bakery, Columbia, MO. The ingredients were weighed on an electronic, digital balance, Sartorius Model 1204 Mp. The dry ingredients and water were added to a 4.5L mixing bowl and the melted shortening was added. The ingredients were combined in a mixer, Hobart Model C-11, at speed one for 12 min until the dough was elastic and did not stretch out excessively. The dough was shaped into a ball and placed into a 7.2L stainless steel bowl. The dough was proofed in an incubator, Cenco Model No. 95086-16, for 60 min at 36–38°C and 80–95% RH. The relative humidity was achieved by injecting a fine mist into the oven chamber.

After the first proofing period, the dough was punched down and worked lightly to enable the gas to escape and to redistribute the gas cells as recommended by Hamad and Fields (1979). The dough was divided into four balls, weighing 680.4g ± 1.0 and shaped to fit lightly greased baking pans (25.5 cm x 12.9 cm x 7.5 cm).

A Type K thermocouple, 32 gauge chromel-alumel wire, attached to a digital thermometer, Omega Model No. 2168, was placed into the approximate geometric center of each loaf as shown in Fig. 1. The loaf was proofed for 75 min, weighed and baked in a preheated, forced-air convection oven, Lang Model No. C-20, at 163°C.

Four pans were placed 27.9 cm from the oven top, and positioned according to Fig. 2. Pans to be heated for 8, 16, 24, and 32 min were 8.91 cm, 24.1 cm, 30.4 cm, and 54.3 cm from the oven right side wall, respectively. The temperature of the loaves was recorded at 2-min intervals. After 8 min, one loaf was removed from the oven and the oven door was immediately closed. The loaf was covered with polyethylene wrap to avoid moisture loss. This procedure was repeated for the remaining three loaves, heated for 16, 24, and 32 min, respectively. The loaves, were cooled at 20°C for 12 hr.

Moisture loss

The final weight (g) of each loaf was subtracted from the initial weight of each loaf after 8, 16, 24, and 32 min of heat processing, to calculate the moisture content (g) and loss (%).

Volume

A loaf volumeter, National Loaf Volumeter, 2475 cc loaf size, was used to determine the volume (cc) of the loaves of bread after heat processing, using the rapeseed replacement method.

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Table 1–Ingredients used in bread formula

Ingredient	Weight (g)	Percent of wt of flour (%)
Salt	35	1.84
Sugar	57	3.00
Nonfat dry milk	100	5.30
Vegetable shortening	57	3.00
Yeast, compressed	56	2.95
Water, distilled	969	51.00
Flour, bread	1900	

Bulk density

Bulk density is the density measured to include the volume of the solid and liquid materials and all the pores (Hwang and Hayakawa, 1980). The mass (g) of each loaf after heat processing was divided by its volume (cc) and the relative or bulk density was expressed as g/cc.

Thermal conductivity

A small thermal conductivity probe developed by Sweat and Haugh (1974) was used to measure the thermal conductivity of all loaves of bread. The probe was connected to a heater circuit schematically diagramed in Fig. 3. The circuit was powered by a D.C. power supply, Darcy/Behlman Model No. QS20, set at 4.0 volts. A digital multimeter, Fluke Model No. 8010A, was used as an ammeter. The thermal conductivity probe functioned as the resistance for the heater circuit. A 896-ml Dewar thermos was filled with ice and water and functioned as the reference junction for the Type T copper-constantan thermocouple which extended inside the probe. A DC amplifier, Omega Model IMNI-AMP II A, with a gain of 100, was used to amplify the thermocouple voltage.

The loaves were held at 20°C until a uniform distribution of temperature was achieved. While the loaves were covered with film, thermal conductivity measurements were taken at three positions in order to calculate a mean value for each loaf.

The top surface position of each probe in the loaves with different heating times varied slightly because the length of each loaf varied after heat processing. For example, the length after 8 min was shorter than that after 32 min. Therefore, the three probe positions were: position 1, approximately halfway between the center and the narrow (12.9 cm) end of the loaf; position 2, approximately at the center of the loaf; and position 3, approximately halfway between the center and the opposite narrow (12.9 cm) end of the loaf.



Fig. 1—Location of thermocouple into bread dough.

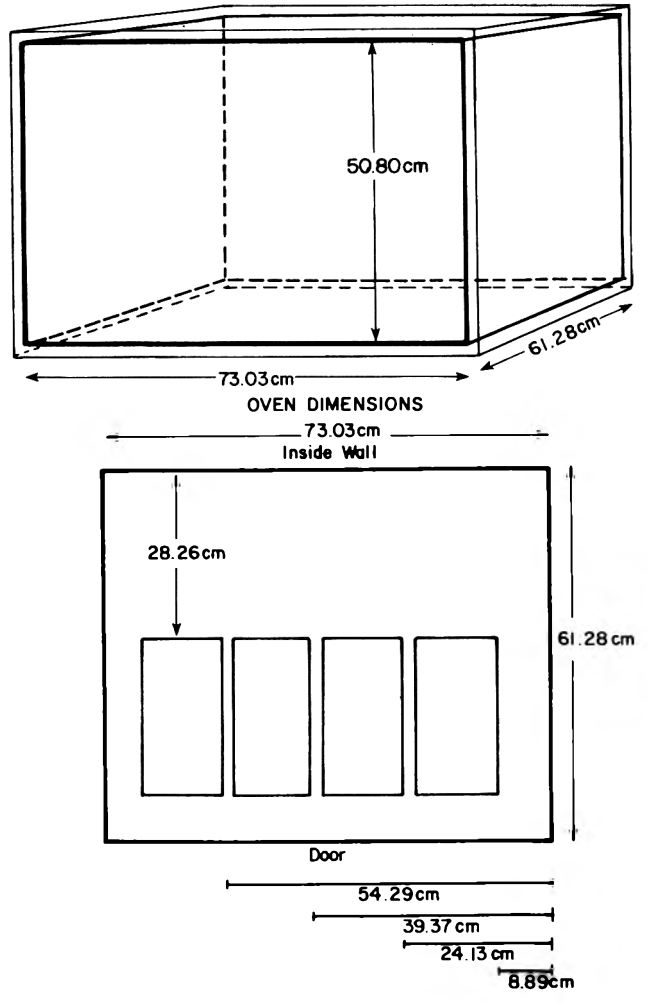


Fig. 2—Location of loaf pans in oven.

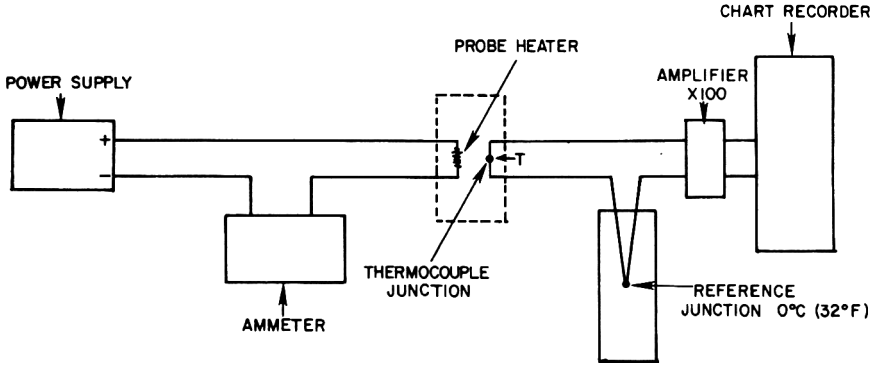


Fig. 3—Electrical schematic of thermal conductivity probe circuit.

After the probe was inserted into the bread as shown in Fig. 4, the power supply was turned on. The current ranged from 225–243 milliamperes and values for the temperature rise were recorded on a DC amplifier recorder, Sanborn Model 322, for 1 min. Because electromagnetic disturbances were amplified with the thermocouple signal, care was taken in the laboratory to eliminate sources of electronic noise during the operation of the probe.

Elapsed time and temperature values were sampled from the charts at 5-sec intervals and entered into a computer program which computed the slope between each pair of two points. From these values of slope, the clearly nonlinear segments of the curve were rejected. Linear regression was performed on the remaining values to determine the best linear fit (Snedecor and Cochran, 1967). The slope of this linear fit was used to compute thermal conductivity as recommended by Sweat and Haugh (1974).

Porosity

The porosity ratio of bread after 16, 24, and 32 min of heat processing was determined by one method. Because of the different physical properties of the dough after 8 min of heat processing, a second method for determining porosity for this product was deveoped.

In one method, the volume of crustless bread after 16, 24, and 32 min of heating was determined using the formula:

$$V = WLT$$

where: W = slice width (cm); L = slice length (cm); T = slice thickness (cm).

The bread was loaded into the cylinder of a Carver press (Fig. 5). A pressure of 267 kg/cm^2 was exerted until air was no longer escaping from the bread. When the needle of the pressure gauge was stable, the distance, L_4, was measured. The length, H, of the compressed bread was calculated by solving the equation:

$$H = L_4 + L_2 - L_1 - L_3$$

The volume of the compressed bread was calculated by the equation:

$$V = \pi R^2 H$$

where: R = radius (cm); H = length of compressed bread (cm). The porosity ratio was calculated by dividing the original volume of the bread by the volume of the compressed bread solids. Ratios were calculated at three positions in every loaf, corresponding with those for thermal conductivity measurements.

Because the dough was too soft and elastic after 8 min of heat processing to accurately measure the dimensions for volume determinations, the porosity ratio could not be determined by this method. Therefore, an alternate method was developed wherein dough was prepared to simulate dough after 8 min of heat processing by considering the moisture losses after 8 min of heating. Five grams of dough, prepared with 1.25% less water, were weighed and placed into a 60-ml volumetric flask. The flask containing the sample was filled with distilled water from a 100-ml buret and the volume of water added was recorded. The volume of the sample was calculated using the equation:

$$\text{volume (ml)} = \text{vol of flask (ml)} - \text{vol of water (ml)}$$

The density was calculated using the equation:

$$\text{density (g/ml)} = \frac{\text{mass of sample (g)}}{\text{vol of sample (ml)}}$$

The porosity ratio was calculated by the equation:

$$\text{porosity ratio} = \frac{\text{density (g/cc)}}{\text{bulk density (g/cc)}}$$

where: density = g/cc (compressed dough); bulk density = g/cc (expanded dough after 8 min of heat processing).

Statistical design

Eight replications were performed for heat processing bread to each of the four stages of doneness and for each of the selected physical properties. All thermal conductivity values and porosity ratios for 16, 24, and 32 min of heat processing were analyzed according to position of measurement and composite values were calculated. Data were analyzed using analysis of variance procedures and correlation procedures described by Snedecor and Cochran (1967) and least square means were separated using their protected Lease Significant Difference Rule. Barr et al. (1979) was used for the computerized computations.

RESULTS & DISCUSSION

THE MEAN INTERNAL PRODUCT temperatures after 8, 16, 24, and 32 min of heat processing were 33, 60, 90, and 98°C, respectively. The mean volume of bread after heat processing ranged from only 2153–2395 cc (Table 2). Several significant differences ($p<0.05$) were found in loaf volume after four different times of heat processing. The greatest increase in volume occurred between 8 and 16 min of heating.

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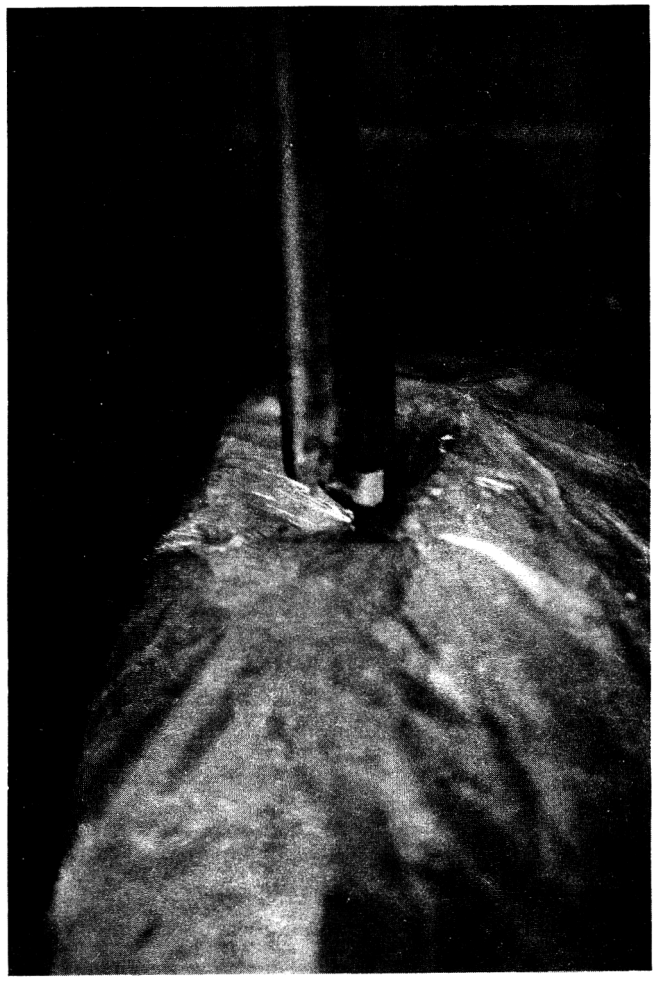


Fig. 4–Position of thermal conductivity probe in bread load.

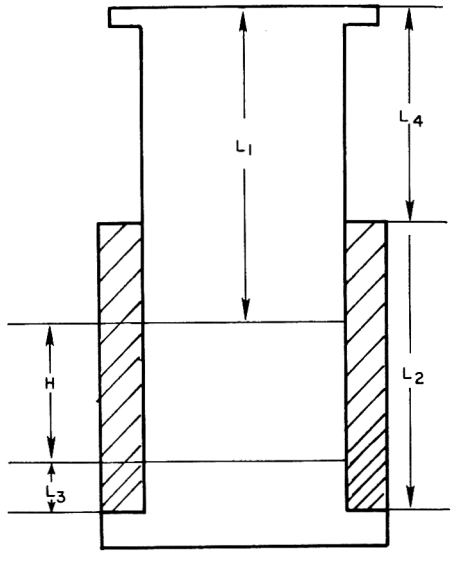


Fig. 5–Diagram of cylinder used to compress bread.

The mean bulk density ranged from 0.3073 g/cc to 0.2636 g/cc (Table 2). Significant decreases ($p<0.05$) were found after each heating period.

The decrease in the loaf bulk density can be attributed to the increase in loaf volume and to the decrease in loaf mass (Table 2). The mean loaf moisture content ranged from 665.51–635.08g, representing mean percent moisture losses of 1.25 and 3.78%, respectively. Significant increases ($p<0.05$) were revealed in moisture loss after four different times of heat processing.

The composite mean porosity ratios ranged from 3.6883–6.8000 (Table 3). Significant differences ($p<0.05$) were found in the loaf porosity ratio after eight and each of the other three heat processing periods.

No significant differences ($p<0.10$) in thermal conductivity after four different times of heat processing were revealed (Table 4). The slight decrease in thermal conductivity values could be associated with the increase in the amount of air in the bread (Tables 2 and 3). This finding tended to agree with those of Heldman and Gorby (1974) who related the incorporation of air in ice cream with thermal conductivity.

The decrease in the moisture content of the bread (Table 2) coincided with the slight decrease in the thermal conductivity values. This would tend to agree with Sweat et al. (1973) who found the water content of meat affected thermal conductivity because it was the predominent constituent, with higher values than the solid components. Furthermore, Qashou et al. (1970) found thermal conductivity values for ground chuck were higher than values for ground beef because ground chuck had a slightly higher moisture content. The decrease in thermal conductivity (Table 4) could also be associated with the decrease in the bulk density of the bread (Table 2).

Although significant differences among the thermal conductivity values were not revealed, data in Table 4 indicated a downward trend in thermal conductivity throughout heating. Significant differences were not revealed because the mean values had relatively large standard deviations, likely caused by several factors. Firstly, the bread was a nonuniform food, comprised of a system of randomly-sized air cells, unevenly dispersed throughout the dough. These air voids were surrounded by cell walls of dough which also varied in thickness. When the bread dough was moist, the thermal conductivity of the cell walls could be assumed to be greater than the thermal conductivity of the air cells themselves (Granet, 1980). As the cell walls lost moisture during heat processing, the thermal conductivity values decreased.

Secondly, the small thermal conductivity probe was located 3.8 cm into the loaf. Because of the nonuniform dough or bread conditions which surrounded the probe, it can be assumed that the probe was inserted into areas of the dough with varying heat conducting properties. Heat has different rates of conductive heat transfer through air than it does through solid cell walls of bread (Granet, 1980). Although the experimental procedure was developed to help reduce the variation in the thermal conductivity data, large standard deviations were still obtained.

Thirdly, the design of the small thermal conductivity probe itself can be used to account for the varying data points. A relatively low level of power (0.94 watts) was

Table 2–Volume, density and moisture of bread after heat processing (N=8)[a]

Heat processing time (min)	Volume (cc)		Bulk density (g/cc)		Moisture content (g)		Moisture loss (%)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
8	2153a	35.81	0.3073a	0.005	665.51	11.39	1.25a	0.03
16	2303b	52.49	0.2846b	0.007	658.14	11.50	1.87b	0.04
24	2341bc	44.05	0.2751c	0.005	647.64	11.48	2.73c	0.04
32	2395c	43.58	0.2636d	0.005	635.08	22.43	3.79d	0.12

[a] Where letters differ within a column, means differ significantly ($p<0.05$) from each other.

Table 3–Porosity ratio of bread after heat processing[a]

Time (min)	Position of sample tested 1		2		3		Composite mean	S.E.
	Mean	S.E.	Mean	S.E.	Mean	S.E.		
8	–	–	–	–	–	–	3.6883a	0.01
16	6.9525	0.17	6.0725a	0.13	6.6350	0.09	6.5533b	0.07
24	6.7225	0.10	6.1938a	0.10	6.8988	0.20	6.6050b	0.09
32	6.8214	0.22	6.5620b	0.23	6.8471	0.24	6.8000b	0.17

[a] N=8 except for the composite mean for 8 min where N=6 and positions 1 and 3 for 32 min where N=7 and position 2 for 32 min where N=5. Where letters differ within a column, means differ significantly ($p<0.05$) from each other.

Table 4–Thermal Conductivity (watts/cm-°C) of Bread After Heat Processing[a]

Time (min)	Position of probe 1		2		3		Composite mean	S.E.
	Mean	S.E.	Mean	S.E.	Mean	S.E.		
8	0.65	0.04	0.74	0.05	0.74	0.04	0.72	0.04
16	0.63	0.03	0.73	0.04	0.65	0.02	0.67	0.02
24	0.64	0.04	0.69	0.04	0.64	0.05	0.66	0.03
32	0.63	0.05	0.70	0.04	0.60	0.02	0.64	0.02

[a] N=8 for means except for position 3 at 8 min where N=6, mean scale X 1000.

used during the thermal conductivity measurement so that the probe would not burn out. Because relatively low amounts of power were used, small temperature variations were produced. Small variations are somewhat susceptible to noise, directly influencing the output data. While the regression analysis was performed on the data to help reduce this noise, it could not be completely eliminated.

Correlation coefficients revealed all of the physical properties measured were significantly related, either directly or indirectly (Table 5). Although statistically significant differences among the values for thermal conductivity were not revealed (Table 4), these correlation coefficients help substantiate the downward drift in terms of the linear relationship among thermal conductivity and other physical properties. Fig. 6 shows these relationships during heat processing.

The thermal conductivity values of bread were linearly dependent ($p<0.05$) upon the moisture content of bread. Although little moisture loss occurred before 8 min of heating (Table 2), moisture evaporated during heat processing, causing a decrease in bread moisture content. The total or greatest decrease (3.79%) was accompanied with the lowest thermal conductivity value.

The slight decrease in thermal conductivity was associated with the gradual decrease in loaf bulk density, presumably because bulk density is a function of both moisture content and volume (Fig. 6) which were correlated with thermal conductivity (Table 5).

The nonsignificant decrease in thermal conductivity was associated with an increase in loaf volume (Fig. 6). This finding indicated that increases in the amount of air incorporated into the bread were related to the decreases in the decreases in thermal conductivity values. Thus, thermal

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Table 5—Correlation coefficients among thermal conductivity, volume, bulk density, moisture loss, and porosity ratio of bread

	Thermal Conductivity[a]	Volume	Bulk density	Moisture loss
Volume	-0.487			
	30[c]			
Bulk density	0.499	-0.979		
	30	32[d]		
Mositure loss	0.356	-0.606	0.744	
	30	32	32	
Porosity ratio[b]	-0.487	0.658	-0.727	-0.705
	27[e]	29[f]	29	29

[a] Composite mean of thermal conductivity.
[b] Composite mean of porosity ratio.
[c] Represents number of observations; the absolute value of the coefficient must exceed 0.361 to be significant at the 5% level.
[d] The absolute value of the coefficient must exceed 0.349 to be significant at the 5% level.
[e] The absolute value of the coefficient must exceed 0.381 to be significant at the 5% level.
[f] The absolute value of the coefficient must exceed 0.367 to be significant at the 5% level.

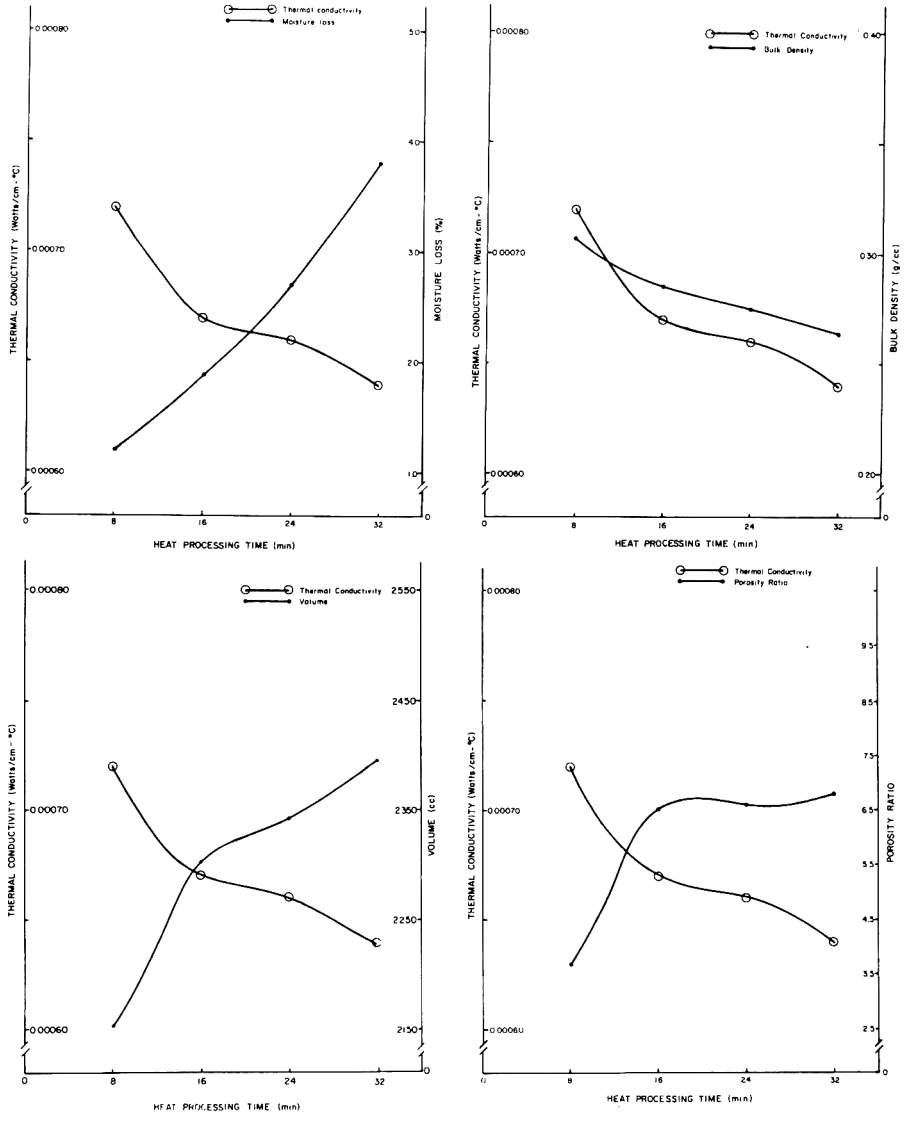


Fig. 6—Changes in thermal conductivity and other physical properties of bread during heat processing.

Influence of Dryer Infeed Matrices on the Retention of Volatile Flavor Compounds During Spray Drying

WILLIAM E. BANGS and G. A. REINECCIUS

ABSTRACT

The retention of volatile flavor compounds (octane, 2-octanone, octanal, methyl-2-octenoate, oct-1-en-3-ol, octanol, thiophene, 2-ethylthiophene, thiazole, 2,5-dimethylpyrazine, benzaldehyde, 5-methylfurfural) in model food systems during spray drying was studied. The flavor compounds were added to different model food systems (including malto dextrins DE 10 to 36.5, soy, casein and whey protein based systems and protein/fat systems) at 1 ppm and then spray dried using a Niro Utility Dryer employing centrifugal atomization. Flavor retention was found to be inversely related to DE of the maltrin carrier. Inclusion of protein in the infeed material resulted in better flavor retention than pure maltrin infeeds. Soy protein based model systems were most effective in retaining flavors followed by casein and whey protein based systems. Relative volatility of flavor compounds in different model systems was found to be an important factor pertaining to flavor retention during drying in different infeed materials.

INTRODUCTION

THE LOSS OF FLAVOR and aroma during drying of food products is detrimental to consumer acceptance and may be considered a crucial aspect in the overall technology of some spray-dried foods. Spray drying, being a simultaneous heat and mass transfer process, is quite complicated and difficult to model mathematically in terms of flavor losses. Numerous investigators have attempted to predict flavor losses with varying degrees of success by determining diffusion coefficients and relating the subsequent effect of this variable to flavor retention (Rulkens and Thijssen, 1969; Thijssen, 1969; Thijssen and Rulkens,1968; Thijssen, 1973; Menting et al., 1970a).

Several theories have been presented to explain the retention of flavors during drying. The selective diffusion theory of Thijssen (1965) is probably the most widely accepted theory. A number of factors must be considered when one attempts to air dry a food system of delicate bouquet. Such factors include the processing parameters of dryer feed temperature (Sivetz and Foote, 1963; Rulkens and Thijssen, 1972; Thijssen, 1972); powder particle size (Brooks, 1965; Boudreau et al., 1966; Sivetz and Foote, 1963; Rulkens and Thijssen, 1972; Blakebrough and Morgan, 1973); solids concentration of the dryer feed (Sivetz and Foote, 1963; Reineccius and Coulter, 1969; Menting et al., 1970b; Rulkens and Thijssen, 1972; Voilley and Loncin, 1978; Kerkhof and Thijssen, 1974); inlet/outlet air temperatures (Brooks, 1965; Reineccius and Coulter, 1969; Rulkens and Thijssen, 1972; Bomben et al., 1973; Voilley and Loncin, 1978; Blakebrough and Morgan, 1973); mass transfer coefficients controlled by gas (air) velocities and relative humidities, (Thijssen, 1972; Kerkhof and Thijssen, 1975) the nature and concentration of the flavor to be retained (Brooks, 1965; Reineccius and Coulter, 1969; Rulkens and Thijssen, 1972; Blakebrough and Morgan, 1973); and the solids type/dryer feed matrix to be used (Reineccius and Coulter, 1969; Kerkhof and Thijssen, 1977; Reineccius et al., 1978; Voilley and Simatos, 1980). Work to date has generally involved rather simple model food systems as drying matrices and a few volatiles at concentrations well above those normally found in real foods.

This work focused on the influence of infeed matrix composition on flavor retention. The drying of flavor compounds at low concentratins (1 ppm) which have a wide variety of functional groupings, molecular weights, water solubilities and volatilities in different infeed matrices was done in order to gather information as to what may be occurring in an actual food system with complex composition and structure.

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MATERIALS & METHODS

Compounds selected

Twelve organic compounds representing various classes of flavor compounds were selected including: 2-octanone (175°C bpt), octanal (171°C bpt), oct-1-en-3-ol (175°C bpt), 1-octanol (194°C bpt), methyl-2-octenoate (156°C bpt), octane (126°C bpt), thiophene (84°C bpt), 2-ethylthiophene (133°C bpt), thiazole (117°C bpt), 2,5-dimethylpyrazine (155°C bpt), benzaldehyde (179°C bpt), and 5-methylfurfural (187°C bpt). All were purchased from Aldrich Chem. Co., Inc. (Milwaukee, WI) except for 1-octanol (Sigma Chem. Co., St. Louis, MO), n-octane (Eastman Kodak Co., Rochester, NY), and methyl-2-octenoate (ROC/RIC Corp., Sun Valley, CA). Gas chromatographic (GC) analysis was used to verify the purity of each compound.

Solids selected

Model systems consisting of mixtures of carbohydrate, protein, and lipid, along with whole and skim milk were chosen for study. The primary carbohydrates selected were from a series of different DE malto-dextrins (DEs 10, 15, 20, 25, 36.5) derived from corn (Grain Processing Corp., Muscatine, IA). The proteins chosen were sodium caseinate, anhydrous sweet dried whey (Dairyland Products Inc., Savage, MN) and defatted soyflour (Cargill Inc., Minneapolis, MN). Lactose and anhydrous butteroil were obtained from Land-O-Lakes Inc. (Minneapolis, MN).

Preparations of dryer infeed materials

A standard mixture of all twelve selected compounds was prepared by adding 30 mg of each compound to about 2000 mg of 95% ethanol (to ensure their solubility) and bringing to volume with distilled water in a 50 ml Erlenmeyer flask. This mixture was then quantitatively transferred to a 1L volumetric flask and brought to volume with distilled water. Following vigorous mixing, 20 ml of the prepared standard mixture (i.e., 0.6 mg of each compound) was added to each 1,200g (50% $solids_{wb}$) of spray-drying matrix in a 1L Erlenmeyer flask (to minimize headspace). This mixture, consisting of distilled water, solids, and flavor compounds, was again thoroughly mixed and a representative 60 ml sample was removed for analysis of the amount (A_o) of flavor compounds present in the sample before drying and determination of the feed viscosity using a model RV II Haake Rotoriksso, when desired. The remaining portion was dried within one hour.

The solids/distilled water solution was always prepared approximately 12–15 hr prior to spray drying to ensure complete dissolution of the solids, with heating on a steam bath and/or blending as required. The condensed whole and skim milk samples were prepared using a four liter glass condensing unit (Precision Scientific

Co., Chicago, IL) operating under a vacuum of about 26 in. Hg. Solids content of the milk samples was determined using a Fugi hand-held refractometer (Extech, Boston, MA).

Spray dryer

The solids/water/flavor compounds matrix was dried in a Niro Utility Model (Niro Atomizers Ltd., Copenhagen, Denmark) spray dryer (inside chamber dimensions 150 cm high and 120 cm diam), equipped with 12 cm diam radial vane centrifugal atomizer operating at 24,000 rpm. Drying conditions remained constant throughout the study with the inlet temperature maintained at 200 ± 5°C and the outlet temperature at 100 ± 5°C using cocurrent air flow. Under these conditions the dryer was evaporating about 12 kg of water/hr. A positive displacement gear pump was used to feed the dryer.

Sample preparation for GC

Sample preparation involved adding 2.5g dried sample to 7.5g distilled water in a 50 ml screw-cap culture tube. Initial samples (A_0) containing dextrins were treated with 20 μl of α-amylase (Novo BAN 120, Novo Laboratories Inc., Wilton, CN) while 10 mg protease (Type V, Sigma Chemical Co., St. Louis, MO) or about 4 drops of concentrated sulfiric acid (to pH 4.5) was added to protein-containing samples. The enzyme treatments were necessary to recover compounds which were complexed with the carbohydrate or protein. The adjustment of pH was necessary to recover chemically bound flavor compounds, i.e., aldehydes.

The culture tubes, containing 2.5g solids and 7.5g water, were covered with aluminum foil to prevent leakage and capped. The samples were then placed on a gyrotory shaker (Model G10, New Brunswick Scientific Co., New Brunswick, NJ) and agitated at 150 rpm. Initial samples were agitated for 4 hr (minimal time to complete the enzyme hydrolysis) and the rehydrated dried samples were agitated for 12 hr (ensure dissolution of the solids). No loss of volatiles from the capped tubes was observed over 24 hr so losses were assumed to be due to the drying process.

GC conditions

Samples were analyzed using a Hewlett-Packard 7675A Purge and Trap Sampler coupled to a Hewlett-Packard 5840 Gas Chromatograph equipped with a flame ionization detector (FID). A 30-min purge of the liquid sample (2.5g solids in 7.5g distilled water) at a helium purge rate of 250 ml/min was selected. The purged organic volatiles were absorbed on a 10 cm by 0.32 cm o.d. SS precolumn packed with Tenax (Hewlett-Packard Co., Avondale, PA). Elution of the concentrated organic volatiles from the Tenax precolumn onto the GC column was accomplished by heating the precolumn to 200°C while flushing with helium (75 ml/min). The combined flow of helium and volatiles was split 30:1 and passed onto the GC column, a 75m by 0.25 mm Carbowax 20M glass capillary (J&W Scientific Inc., Rancho Cardova, CA). Column head pressure was maintained at 1.5 kg/cm^2 to obtain a carrier gas flow rate of 1 ml/min. The oven temperature was programmed from 50 to 170°C at 4°C/min with a 1 min hold at 170°C. The injection port and FID temperatures were 220°C and 290°C, respectively. Fig. 1 shows a typical chromatogram obtained by this procedure.

Quantification of the volatiles present in the matrix before and after the drying process was aided by incorporating an internal standard, hexaldehyde, in the sample prior to analysis. Hexaldehyde was chosen for its physical characteristics (e.g., solublity, MW, etc.) which approximate those of the compounds chosen for the study and its chromatographic separation. In order to normalize the data for variations in the amounts of the compounds added to the matrix to be dried and variations in the response factors of the compounds due to solid-type used, the % retained (A/A_0) of each compound was reported. All samples were run in triplicate.

Relative volatility

Relative volatility of 1-octanol, 2-octanone, octanal, and oct-1-en-3-ol, all similar in MW and molecular packing size but different in solubility and vapor pressure, was determined in 50%$_{wb}$ solids DE 10 maltrin and DE 36.5 maltrin, and a 46.2% solids soyflour system. The soyflour system was 7% soy protein, 18.5% lactose and 21.8% DE 10 maltrin/native soyflour carbohydrate. Relative volativity was determined by adding a known weight (about 10.0 mg) of each compound, dissolved in 0.1 g 95% ethanol, to 50 ml distilled water in a 50 ml Erlenmeyer flask. Following vigorous mixing, 0.13 ml of this mixture was added to a 50 ml culture tube containing 10g of the selected mixture of solids and water (same system used for the spray drying work) and the tube was sealed with teflon tape and capped. Following a 12-hr equilibration period at room temperature, the headspace was flushed with helium for 5 min at a helium flow of 8 ml/min. A 12-hr equilibration was found to be adequate for equilibrium to occur. The 40 ml headspace volume in the purge tube was sampled under those conditions. Because there was no mixing of the sample during the purge, an empirical estimate of the ability of the compound to escape into the headspace was quantified. Samples were prepared and analyzed in duplicate.

Free fat content

Free fat was determined for the dried whole milk samples by the method of Buma (1971).

RESULTS & DISCUSSION

Effect of carbohydrate on flavor retention

As seen in Fig. 2 the average retention of added volatile components was found to decrease with an increase in mal-



Fig. 1—Typical GC chromatogram of flavor compounds (1 ppm in 10% solids maltrin 100) purge and trap system: (1) octane; (2) thiophene; (3) hexaldehyde; (4) 2-ethylthiophene; (5) thiazole; (6) 2-octanone; (7) octanal; (8) 2,5 dimethyl pyrazine; (9) oct-1-en-ol; (10) methyl-2-octenoate; (11) benzaldehyde; (12) octanol; (13) 5-methylfurfural).

trin DE, i.e., lower average molecular weight. This observation supports the work of Voilley and Simatos (1980) who have shown increased volatile retention with increased molecular weights of propylene glycol and malto-dextrin as flavor carriers. This relationship has also been shown for other compounds and flavor carriers by Reineccius and Coulter (1969) and Kerkhof and Thijssen (1977). For certain flavor compounds this loss with an increase in maltrin DE held true over the entire spectrum of maltrins used (i.e., thiopene, 2,5-dimethylpyrazine) but, in general, DE 15 maltrin was found to exhibit the highest retentions. Statistically, using honest significant differences (HSDs; Snedecor and Cochran, 1967), it was determined that although DE 15 maltrin gave slightly better overall mean retentions than DE 10 maltrin (55.6% vs 53.5%, respectively) the retention difference was not significant at the 5% level.

Since DE 5 was not soluble at the feed solids concentration selected for study, one can only speculate whether DE 15 maltrin is the apex of the maltrin DE versus retention curve. It may be reasonable to expect that limited incorporation of small MW saccharides (e.g., $\leqslant$ trisaccharides) would be beneficial to the retention of volatile flavor components. Kerkhof and Thijssen (1974) found improved retentions of C1, C3 and C5 alcohols when dextrin was partially substituted ($25\%_{db}$) for malto-dextrin solids during extractive drying. A second consideration is that the solubility of the DE 10 carrier may have been exceeded at the 50% total solids infeed concentration. Therefore, there would have actually been less solids in solution and the lower solids level could have reduced retention during drying.

It was of interest to determine whether the better film forming properties of DE 10 maltrin could be off-set by using higher infeed solids contents of the higher DE maltrins. Solubility and viscosity are limiting factors determining infeed solids content. Therefore, two additional maltrin solutions (55% DE 10 and 72% DE 36.5) were dried. Fifty-five percent DE 10 maltrin and 72% DE 36.5) maltrin solutions were selected since they were the extremes of the DEs selected and at these solids contents they were equal in viscosity (2.96 Poise) and are near their respective solubility limits. The 55% solids DE 10 maltrin was found to give significantly greater overall mean retentions (at $\alpha = 0.01$) than the 72% DE 36.5 maltrin (79.1% vs 71.1%, respectively). This would indicate that the solids type was more important than solids content for volatile retentions in the systems studied. As seen in Fig. 3, a regression of amount of polymer added (e.g., a 50% solids DE 10 maltrin solution may be assumed to be 45% "polymer") versus overall mean retentions gave a high correlation ($r^2 = 0.87$).

A significant (at $\alpha = 0.01$) interaction between solids type and volatile compounds was also found (55% DE 10 and 72% DE 36.5 maltrins), indicating that the solids effect depended on the volatiles. By plotting (Fig. 4) the flavor compound boiling point (b.p.) vs the mean retention of each selected compound, it was noted that above a b.p. of 160°C, the 55% DE 10 maltrin yielded greater retentions in all cases. If the b.p. of the volatile components was below 160°C, the 72% DE 36.5 maltrin yielded greater retentions. It is obvious from these data that different flavor compounds interact to differing degrees with each type of flavor carrier. At this time we cannot explain the apparent reversal in retention data for compounds on either side of a 160°C boiling point. It should be noted that this experiment did not consider volatility in aqueous solution. Volatility in aqueous solutions containing different flavor carriers could present quite a different picture than is presented in the plot of retention vs. pure component boiling point.

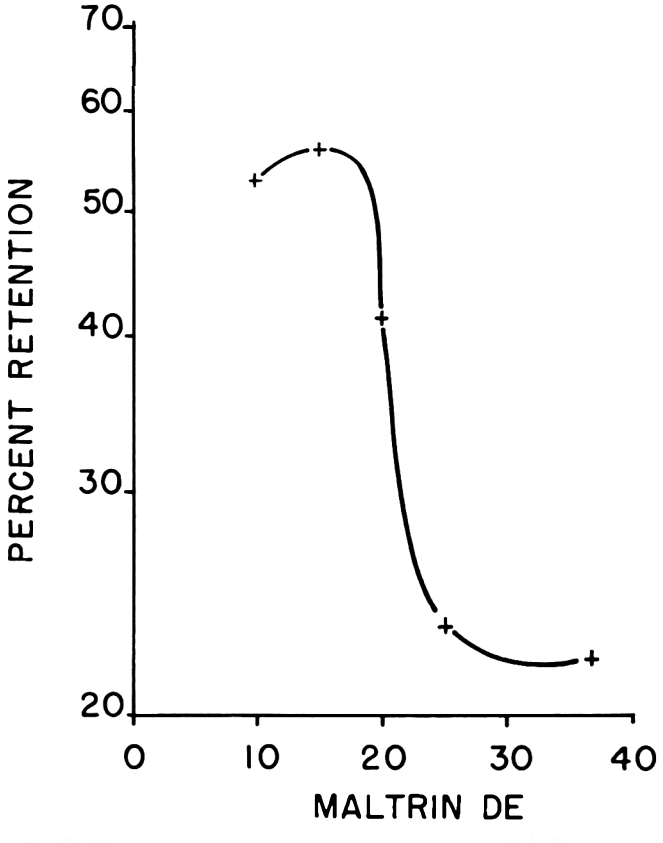


Fig. 2—Influence of maltrin De on the overall retention of flavor compounds during drying.

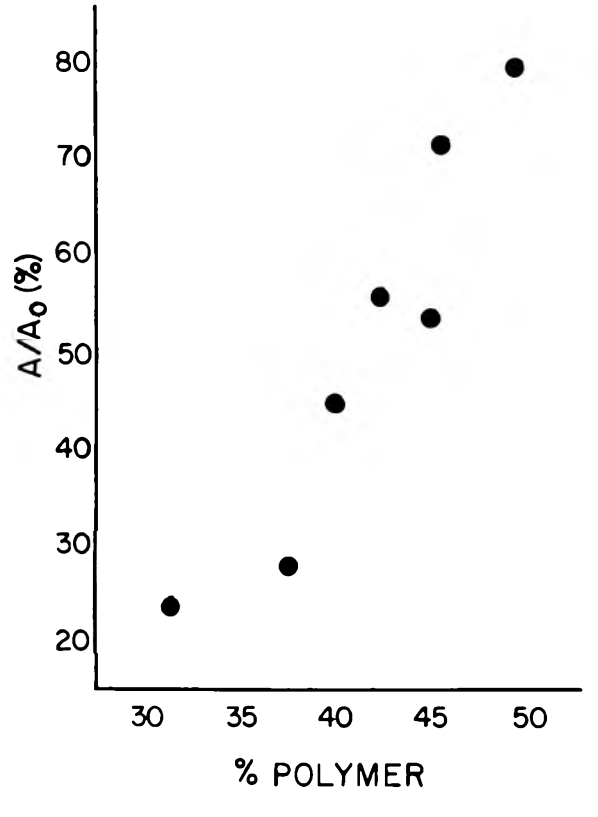


Fig. 3—Relationship of % maltrin polymer in the drying matrix solution to the overall retention of flavor compounds.

The importance of volatility in determining flavor retention was also investigated in a second manner. The relationship of "volatility" to retention was studied for octanal, octanol, 2-octanone, and oct-1-en-3-ol in 50% solids matrices of DE 10 maltrin, DE 36.5 maltrin, and a 46.2% solids soyflour system. There is the expected trend of poorer retentions for compounds which have higher headspace concentrations (Fig. 5). However, it is of greater interest that the retention of each flavor compound fell on a straight line when retention was plotted on semilog paper against headspace/total volatile ratio. These data suggest that the retention of agiven volatile is determined by one factor (probably molecular size) which is a constant irrespective of flavor carrier and that the influence of flavor carrier on retention can be explained nearly entirely by its effect on compound volatility. This hypothesis must be examined in greater detail before it can be accepted but our data suggest such a relationship.

The observation that volatility does influence retention as seen in Fig. 5 is somewhat contradictory to the previous work (Thijssen, 1971, 1972; Menting et al., 1970b; Rulkens and Thijssen, 1972; Thijssen, 1973) in that although molecular size of a compound does influence retention by influencing diffusivity, the volativity of the compound also seems to be quite important in determining retention during spray drying. Saravacos and Moyer (1968a, b) concluded that volatile retentions were dependent on the relative volatility of selected compounds during freeze drying and vacuum drying. Kieckbusch and King (1977) found that most of the volatiles lost during nozzle spray drying of water/sucrose solutions occurs within 10 cm of the pressure nozzle. This is the constant rate drying period for a drying droplet. Since there is no semipermeable membrane formed during the constant rate period, it would follow that solids systems which greatly lower volatility of flavor compounds should result in greater retentions.

The work of Voilley and Simatos (1980) is interesting in that it shows an increase in the retention of volatiles in the following order: dryer infeed matrix—polyethylene glycol, mono and disaccharides and high molecular weight malto-dextrins. They found diffusivity of their volatile compounds through each matrix to increase in the same order. If diffusivity were the only factor determining volatile retention during drying there should be an inverse relationship between diffusivity and retention. Relative volatility of the flavor compounds must be considered as an important parameter when one attempts to predict retention during drying.

It was of interest to determine whether sorbitol addition to the DE 10 maltrin infeed would improve retention of out test volatiles. U.S. Patent #1,464,616 (Polak's Fruital Works, Middletown, NY) suggests that if at least 20% of the polysaccharide solids matrix were a polyhydric alcohol, such as sorbitol, volatile retentions would be substantially improved. Therefore, an experiment was conducted where 0%, 25%, and 50% of the DE 10 maltrin solids were replaced with sorbitol, the volatiles added, and the matrix dried. Increasing levels of sorbitol addition to the drying matrix was found to significantly reduce (at $\alpha = 0.05$, again using HSDs) overall mean retentions relative to the DE 10 maltrin control (54.6% versus 40.3% and 24.8% for 25% and 50% sorbitol addition respectively). The retentions were, in fact, found to follow a similar relationship as was found for maltrin DE vs retention.

The final parameter studied for carbohydrate systems was viscosity. Since viscosities of the maltrin solutions increased with decreasing DEs and retentions also fol-

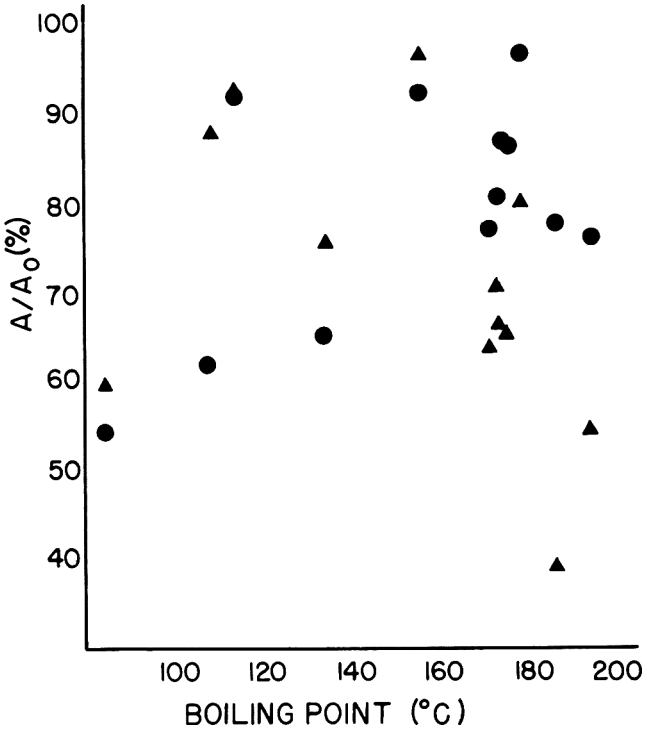


Fig. 4—Relationship between flavor compound boiling point and retention of each flavor compound (▲ 72% solids DE 36.5 maltrin; • 55% solids DE 10 maltrin).

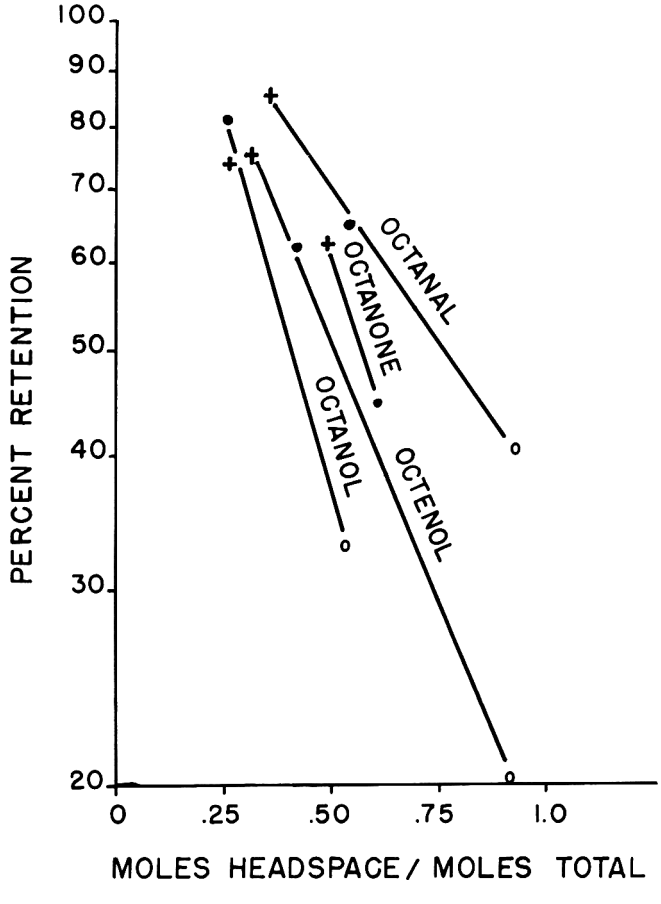


Fig. 5—Retention of octanal, 2-octanone, oct-1-en-3-ol and octanol as a function of volatile partitioning (ratio of headspace concentration to total amount added) in 50% solids DE 10 maltrin (•); 50% solids DE 36.5 maltrin (○); and a 46.2% solids soyflour system (+).

lowed this relationship, a question arose as to whether viscosity was influencing volatile retention. It is known that increased viscosity produces larger particles (Marshall, 1954). Several workers have reported that a larger particle size may improve flavor retention during drying (Boudreau et al., 1966; Brooks, 1965; Rulkens and Thijssen, 1972; Blakebrough and Morgan, 1973; Bradley and Stine, 1963, 1964; Sivetz and Foote, 1963; Tracy et al., 1951). Therefore, it is not unreasonable that the improved retention with low DE maltrins could have been partially due to the larger particle size caused by the increased viscosity. This hypothesis was tested by comparing volatile retention in a 50% solids DE 10 maltrin (1.96 Poise) to a 50% solids DE 10 maltrin which included 0.5%$_{db}$ xanthan gum (2.71 Poise). The DE 10 maltrin plus xanthan gum sample was found to give about a 10% poorer overall mean retention of the volatiles (40.1% versus 50.5% for the DE 10 maltrin). The inclusion of xanthan gum produced an opposite effect of what was expected. Apparently, the xanthan gum altered the surface properties of the dried droplet (e.g., film forming, pore size and number atomization characteristics) such that flavor retention actually decreased.

Effects of Proteins; Proteins Plus Lipid on Flavor Retention

Infeed matrices (46.2% solids) comprised of protein as whey, sodium caseinate or soy (DE 10 maltrin as control), lactose, and DE 10 maltrin or native carbohydrate present in the defatted soyflour (44% carbohydrate) were prepared and dried (Table 1). All samples were found to be significantly different in their overall mean retentions of the added volatiles (HSDs, $\alpha = 0.05$) except for the control (DE 10 maltrin) and sodium caseinate samples. The order of the overall mean retentions was soyflour, whey, sodium caseinate and DE 10 maltrin. The different proteins may exhibit different film forming abilities or varying degrees of hydrophobicity. Improved film forming or increased areas of hydrophobicity in the drying matrix would be expected to improve volatile retentions.

Milk systems (50% solids condensed whole and skim milk, and 30% solids condensed and formulated whole and skim milks) were selected for the study of the presence of a second liquid phase (butteroil) in the drying matrix (Table 1). At the 50% solids level, skim milk was found to give significantly greater overall mean retentions (treatment effect p-value < 0.001) of the added volatiles than whole milk. If the three compounds which exhibit greater than 3% solublity in water (2,5 dimethylpyrazine, thiazole, and 5-methyl-furfural) was excluded, this difference in overall mean volatile retentions between skim milk and whole milk became insignificant (88.9% for skim milk; 86.0% for whole milk). The literature is somewhat inconclusive on this subject. Blakebrough and Morgan (1973), found that skim milk solids (300 g/L maximum concentration) exhibited consistently greater retentions of selected esters than whole milk solids. Reineccius et al. (1978) however, found that the retentions of selected methyl ketones added to 30% solids condensed skim milk increased with the replacement of portions of the skim milk solids with butteroil up to a 25%$_{db}$ butteroil level. This would indicate that, for methyl ketones at least, whole milk should give better retentions than skim milk. The fat content of the whole milk used in this study was approximately 31%$_{db}$, somewhat above Reineccius et al.'s (1978) optimal lipid level of 25%$_{db}$.

A further look at the inclusion of a lipid phase was taken by drying 30% solids condensed whole and skim milk and "formulated" whole and skim milk (using dried whey, sodium caseinate, lactose, and butteroil). The volatile compounds were added to the solids/water matrix 12 hr before drying in order to allow partitioning equilibrium of the lipophylic volatiles to occur. Retention decreased in the following order: formulated skim milk, whole milk, formulated whole milk, and skim milk (Table 1). The only significant different solids type (at the 5% level using HSDs) was the formulated skim milk. The condensed skim milk gave poorer volatile retentions than the formulated skim milk. One would expect the sodium caseinate molecules to exhibit improved film forming abilities over the undenatured micellar structure. The formulated skim milk would be expected to give retentions greater than the formulated whole milk since the formulated whole milk was over the 25%$_{db}$ lipid optimum and 53.3% of the lipid was free fat. High free fat levels may allow large volatile losses via steam distillation. The lack of any significant difference between the 30% solids condensed whole (9% free fat) and skim milk samples apparently reflects the better retention of water soluble compounds by the skim milk system. If the thiazole, pyrazine and furfural were not considered, the whole milk system retained a significantly greater quantity of flavor compounds than the skim milk system. This would then be consistent with the earlier observatons of Reineccius et al., (1978) for methyl ketones. This means that some foods would benefit from the presence of a lipid phase during drying while others may not, depending upon what types of flavor compounds are carrying the flavor of the product.

Table 1—Influence of different infeed matrices on the mean retention of all volatiles during spray drying

Matrix	Infeed solids	Mean retention
Protein containing[a]	46%	
Soy flour		65.4
Na caseinate		42.6
Whey protein		51.2
Maltrin (Control)		38.0
Condensed milks	50%	
Whole		71.3
Skim milk		85.9
Condensed & Formulated milks[b]	30%	
Whole		42.0
Skim milk		36.9
Formulated whole		39.4
Formulated skim milk		58.7

[a] Composition—7% protein as whey, sodium caseinate or soy flour protein, 40% lactose, and 53% DE 10 maltrin (or native carbohydrate in case of soy flour sample).
[b] Formulated assuming a milk composition or 3.63% fat, 2.44% casein, 0.77% whey protein, 4.9% lactose and 0.9% ash (all on a wet basis).

CONCLUSIONS

THE RESULTS of this study indicate that:

(1) increasing maltrin DEs gave decreased volatile retentions, although the difference between DE 10 and 15 maltrins and between 25 and 36.5 maltrins was not found to be significantly different at the 5% level. It would appear that the addition of some small MW saccharides to the dryer feed aided retention;

(2) the addition of proteins (especially whey and soy) to the drying matrix improved volatile retention over DE 10 maltrin alone;

(3) the advantage of higher solids infeeds (made possible by high DE dextrins) did not offset the improved film forming ability of high molecular weight dextrins;

(4) the addition of gum to increase infeed viscosity (and therefore increase particle size) did not improve flavor retention;

(5) Sorbitol addition to DE 10 maltrin gave significantly poorer (at 5% level) retentions when incorporated at $25\%_{db}$ and $50\%_{db}$ solids levels. Sorbitol addition gave retentions similar to that if glucose or maltose were added;

(6) the inclusion of soyflour in the dryer infeed improved retention over milk serum proteins and sodium caseinate (soy > whey > caseinate);

(7) the effect of lipid addition to the dryer infeed was questionable, as it reduced retentions in condensed milk at a 50% solids level and a formulated milk system at a 30% solids level but slightly improved retention in 30% solids condensed milk;

(8) sodium caseinate gave improved volatile retention over undenatured casein micelles; and

(9) solids which lowered the volatilities of flavor compounds gave improved retentions.

It is evident that any model developed to explain volatile retentions in a spray-dried system must include the factors of substrate-volatile component interactions, type of solids used, flavor component volatility and diffusivity through the substrate.

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THERMAL CONDUCTIVITY OF WHITE BREAD . . . From page 253

conductivity values were linearly dependent ($p<0.05$) upon the loaf volume.

The slight decrease in thermal conductivity was also associated with an increase in the porosity ratio (Fig. 6). The porosity ratio increased during heating (Table 3) because the amount of air increased. Porosity is a function of volume and moisture loss (Table 5). As the volume increased, it can be assumed that the cell walls became thinner, also affecting the rate of heat conduction. Thus, the thermal conductivity values were linearly dependent ($p<0.05$) upon the porostiy ratios.

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Retention of Micronutrients by Polymer Coatings Used to Fortify Rice

ANITA PEIL, FRED BARRETT, CHOKYUN RHA, and ROBERT LANGER

ABSTRACT

Nutrient deficiency diseases are public health problems in developing nations. Our objective was to design a rice-fortification method using materials approved for use in foods to retain significant quantities of added nutrients during the common practice of boiling rice in excess water and draining it. We accomplished this by applying nutrients to rice in edible polymer coatings. Various starches and cellulosic polymers were evaluated with respect to flexibility, strength, stickiness and nutrient retention. Combining hydroxypropyl methylcellulose and methylcellulose (3:1) gave the best results. Nutrient retentions for 1g of coated rice cooked in 100 ml water and drained were: Vitamin A, 70%; iron, 100%; niacin, 18%; thiamin, 18%, riboflavin, 21%.

INTRODUCTION

VITAMIN AND MINERAL deficiency diseases are significant public health problems in many nations. For example, vitamin A deficiency causes keratomalacia, nightblindness, and xerophthalmia, the latter of which leads to blindness in approximately 100,000 persons, mostly children, each year (Underwood, 1978). One approach to reduce the magnitude of these problems may be through appropriate food fortification programs. Our objective was to develop a method to fortify rice, a staple food in many countries where these diseases are common.

Although rice fortification methods exist (Rubin et al., 1977; Cort et al., 1976; LaPierre, 1955; Mickus, 1955; Furter et al., 1946), they are not suitable for many countries where it is a common custom to cook rice in excess water and then drain it (Brooke, 1970). These methods usually employ various polymer coatings to apply nutrients to rice. The coatings may prevent loss of added nutrients during rinsing but they are not similarly effective when the rice is cooked (CFR, 1976). In some countries, including the U.S., rice is often cooked in water which is completely absorbed. Thus, release from coatings and subsequent absorption by cooked rice have been stressed as an assurance that added nutrients will be available when consumed (Rubin et al., 1977). However, if rice fortified in this manner is cooked in excess water and that water is drained, the released nutrients will be lost and not reach the consumer.

We sought a fortification process which would retain significant quantities of added nutrients during cooking in excess water. We felt that this could be accomplished by using edible polymer coatings which would retain nutrients during the cooking process. The polymers selected for our initial studies were approved by the U.S. Food and Drug Administration for use in foods (CFR, 1976), had good coating properties (Dow, 1974), were white in appearance, were organoleptically bland and inert, and were reported to have reduced solubility in boiling water (Fiero, 1976).

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MATERIALS & METHODS

Materials

Two starches and four celluloses were evaluated. Starches were acetate-stabilized high amylose, and propylene oxide-stabilized high amylose (National Starch and Chemical Corp.). Celluloses were methylcellulose A15, methoxyl D.S. (degree of substitution) = 1.6–1.9, M.W. = 15,000; hydroxypropyl methylcellulose E15, methoxyl D.S. = 1.8–2.0, hydroxypropyl M.S. (molar substitution) = 0.20–0.31, M.W. = 15,000; hydroxypropyl methylcellulose E50, methoxyl D.S. = 1.8–2.0, hydroxypropyl M.S. = 2.0–0.31, M.W. = 23,000; and hyroxypropyl methylcellulose F50, methoxyl D.S. = 1.7–1.9, hydroxypropyl M.S. = 0.1–0.2, M.W. = 23,000 (Dow Chemical Co.).

Solvents for the coating system were selected from those approved for use in food (NAS, 1972) and were compatible with the chosen polymers. These were distilled water, 95% ethanol (U.S. Industrial Chemical Co.) and acetone (Mallinckrodt).

Vitamin forms were selected for their stability to moisture, heat, and light. These were thiamin mononitrate, nicotinic acid, riboflavin, and gelatin encapsulated vitamin A palmitate (Hoffman-LaRoche). Electrolytically reduced iron (Baker) was used rather than the commonly used ferric orthophosphate because of its greater bioavailability (Rees and Monsen, 1973).

Apparatus

A stainless steel, spherical, 16-inch tablet coating pan (Strokes) and variable speed motor set at 60 rpm (Reliance Duty Master AC Motor; Dodge DM speed reducer) were used in the first coating process. Succeeding procedures were performed with similar but smaller equipment: a stainless steel, spherical, 8-inch tablet coating pan, 43 rpm speed reducer motor (Bodine Electric Co.), portable hairdrier (Schick), and compressed air paint sprayer (Sears).

Procedures

Preparation of films. Films of starches and celluloses were cast from 5% (wt/wt) aqueous solutions onto glass or plastic plates and dried over calcium sulfate for 24–48 hr. Films were left on supports when evaluating stickiness. Films were peeled from the supports before comparing flexibility, strength, solubility and textures.

Comparisons of starch and cellulose films. The flexibility and strength of starch and cellulose films were determined by the procedure of Morse and Hammes (1969), and stickiness by the procedure of Rutenberg et al. (1962). Solubility was determined by gravimetric analysis of weight loss after pieces of film were submerged in boiling water for 30 min.

Texture comparisons were made with films of each type of cellulose (A15, F50, E15, and E50), and two combinations of F50 and A15 (1:1 and 3:1). Pieces of each film a few cm^2 in area were submerged in boiling water for several minutes, removed, held until just cool enough to be chewed, and rated for relative firmness.

Initial rice coating comparisons. A total of fourteen polymer-solvent coating systems were prepared and used to fortify rice with thiamin. These systems were compared with respect to percent of added thiamin retained when 1g samples of fortified rice were cooked in 100 ml water for 25 min and drained.

Thiamin was used in these trials since it is highly water soluble and would therefore be a conservative indication of nutrient retentions which could be expected by the system. In fact, in earlier studies (Rubin et al., 1977) thiamin was completely released during

cooking. The assay for thiamin is also convenient (AACC, 1969).

All coating systems were composed of 5g polymer, 250 mg thiamin mononitrate (Hoffmann-LaRoche), and 100g solvent. The polymer component consisted of either pure hydroxypropyl methylcellulose E50, or mixtures of hydroxypropyl methylcellulose F50 and methylcellulose A15 (3:1 and 1:1). Choices of combinations of celluloses used were based on previous texture comparisons. The solvent component was either aqueous ethanol or aqueous acetone. The organic phase was introduced for convenience in preparation.

Solutions were prepared at 0°C by suspending cellulose powder in the organic solvent, adding water, and stirring continuously for 30 min. Thiamin powder was then added to this solution and stirring was continued for 2 min.

Samples of Texas patna long-grain rice (25g) were fortified by pouring a total of 40 ml of polymer-thiamin solution, in 10-ml increments, over the rice spread in a petri dish. Between applications, the coated rice was stirred with a glass rod to evenly distribute the solution and break up clumps, and then dried in an oven at 55°C for 10–15 min.

Retention of added thiamin was determined by cooking 1 g coated rice in 100 ml boiling water for 25 min. The rice was drained immediately and both rice and cooking water were analyzed for thiamin (AACC, 1969).

Rice fortification. The system that exhibited the highest retention in the initial coating comparisons (See Results) was then used to fortify rice with five different nutrients: vitamin A, thiamin, niacin, riboflavin, and iron. This coating system contained, by weight, 1.2% methylcellulose A15, 3.6% hydroxypropyl methylcellulose F-50, 28.5% ethanol (95%), and 66.7% water. (This coating system is designated subsequently as polymer solution.)

The procedure was designed to produce a highly fortified premix which could be blended with unfortified rice in a ratio of 1:99 to obtain nutrient levels recommended for fortified rice (NAS, 1974). In this trial, vitamin A was added at a higher concentration to compensate for anticipated processing losses (Macek, 1960).

In the first trial, 1 kg unfortified rice was coated by pouring polymer-nutrient solutions over it. Approximately 40-ml of solution were added in each increment. After each addition, warm air (40–50°C) was blown over the rice for 5–10 min to remove solvent, and the process was repeated until additions were complete. Nutrients which might react during the process (Macek, 1960) were added separately, in different layers. The coatings were as follows:

1. Thiamin (0.64g) and niacin (5.33g) were applied in polymer solution (800g).
2. Riboflavin (0.4g) was applied in polymer solution (55g).
3. Iron powder (8.8g) was dusted on by adding fractions of powder alternately with additions of polymer solution (240g).
4. A layer of unfortified polymer solution (180g) was applied, to separate iron from the next layer.
5. Vitamin A 250 SD, 250,000 I.U./g (12.86g), was dusted on by adding fractions of dry granules alternately with additions of polymer solution (550g).
6. Unfortified polymer solution (105g) was applied to seal the coating.

In three succeeding fortification procedures, polymer solutions were sprayed on continuously at a rate of 2.5 g/min, rather than being poured incrementally. Coatings were also dried continuously by operating the drier simultaneously with the sprayer. Both spraying and drying were interrupted when adding the dry nutrients (vitamin A and iron). All other conditions (drying temperature, nutrient concentrations, and layer compositions) replicated the first trial in which solutions were poured.

We tested the coated rice for retention by cooking 1g in 100 ml water for 30 min and analyzing both raw and cooked rice for nutrient content. Standard analytical procedures (AACC, 1969; AVC, 1966, AOAC, 1965) were used to determine nutrient content in samples of raw rice, raw coated rice, and coated rice cooked in excess water and drained. Retention was calculated from nutrient contents of coated rice before and after it was cooked and drained.

RESULTS

COMPARISONS of starches and celluloses on the basis of flexibility, strength, stickiness, and solubility (Table 1) indicated that, overall, the celluloses were superior to the starches. Because hydroxypropyl methylcellulose E15 was significantly more soluble than the other celluloses (23% compared to 14% for the next highest value), it was eliminated from further study.

Texture comparisons gave the following ratings for relative firmness: A15 (pure) ⩾ E50 (pure) ≃ F50 + A15 (3:1) ≃ F50 + A 15 (1:1) ⩾ F50 (pure). The pure methylcellulose A15 film was too hard to be masked in a food as soft as cooked rice. Conversely, hydroxypropyl methylcellulose F50 was very soft, which would make it more susceptible to mechanical damage during cooking. Combinations of the two had superior intermediate properties. Both of these pure hydroxypropyl methylcellulose E50 were soft enough to be undetectable in cooked rice.

Comparisons of fourteen cellulosic polymer systems (Table 2) showed that all formed adherent coatings on rice and did not alter its appearance (Fig. 1). (Coatings containing thiamin, niacin, and vitamin A also did not alter the appearance of the rice; coatings containing riboflavin appeared yellow.) Retentions of thiamin ranged between 13–38% in 40 separate tests, depending on the polymer and solvent used (Table 2). The highest retention was exhibited by the system comprised of (by weight) 1.2% methylcellulose A15, 3.6% hydroxypropyl methylcellulose F50, 28.5% ethanol (95%) and 66.7% water.

This polymer system was then used to fortify rice with thiamin, niacin, riboflavin, vitamin A, and iron (Table 3). When samples of this rice were cooked in excess water, retentions were: thiamin, 18%; niacin, 18%; riboflavin,

Table 1—Comparison of starch and cellulose films

Sample	Film density (g/cm^2)	Flexible and strong	Sticky	Solubility (%)
Starch[c]				
A	0.003	+	+	100
B	0.005	+	+	100
Cellulose[d]				
C	0.003	+	-	7
D	0.001	+	-	23
E	0.001	+	-	14
F	0.001	+	-	12

[a] Gravimetric analyses were performed in triplicate with a standard error of 2%.
[b] Solubility is expressed in percent film weight dissolved in 30 min.
[c] A = acetate stabilized high amylose; B = propylene oxide stabilized high amylose.
[d] C = methylcellulose A15; D = hydroxypropyl methylcellulose E15; E = hydroxypropyl methylcellulose E50; F = hydroxypropyl methylcellulose F50

Table 2—Retention of thiamin by fortified rice cooked in excess water[a]

Coating						
Polymer (g)			Solvent (g)			Thiamin Retention (%)[2]
E50	F50	A15	Water	Ethanol	Acetone	
5.00			30.0	70.0		16.5±4.5
5.00			40.0	60.0		19.0±1.0
5.00			50.0	50.0		19.5±7.5
5.00			70.0	30.0		18.5±1.5
5.00			30.0		70.0	24.5±1.5
5.00			70.0		30.0	20.0±6.0
	2.50	2.50	40.0	60.0		13.0±2.0
	2.50	2.50	50.0	50.0		25.0±1.0
	2.50	2.50	70.0	30.0		27.0±1.0
	3.75	1.25	40.0	60.0		20.0±1.0
	3.75	1.25	50.0	50.0		20.5±1.5
	3.75	1.25	60.0	40.0		23.5±0.5
	3.75	1.25	70.0	30.0		38.0±3.0
	3.75	1.25	80.0		20.0	25.0±0.0

[a] Thiamin content of unfortified rice = 0.62 mg/lb, dry weight basis; cooking was done by boiling 1g rice in 100 ml water for 25 min.
[b] Mean of 2 values ± 1 standard deviation.



Fig. 1—A sample of rice fortified with thiamin in hydroxypropyl methylcellulose and methylcellulose (left) and a sample of normal unfortified rice (right). Note that the two samples do not differ significantly in appearance.

Table 3—Fortified premix nutrient content and retention

Nutrient[a]	Unfortified Amount added to rice (mg/lb)	Unfortified Amount added to coating system (mg/lb)	Amount in coated rice (mg/lb)	Retention in cooked rice (%)
Thiamin	0.32	290	200	18
Niacin	0.12	2,420	1,340	18
Riboflavin	7.2	180	100	21
Iron	3.6	4,000	2.590	100
Vitamin A	0	5,840	4.360	70

[a] Nutrient forms used were thiamin mononitrate, nicotinic acid, riboflavin, and vitamin A 250 SD (all from Hoffmann-LaRoche), and reduced iron (Baker).

21%; vitamin A, 70%; iron, 100%. This procedure was repeated in the other three trials with good reproducibility, e.g., thiamin retention was 17–19%. Analyses of thiamin and vitamin A in fortified rice at 3, 14, and 31 wk showed no significant variation in nutrient concentration indicating that these nutrients are stable in the polymer coating.

DISCUSSION

THE FORTIFICATION PROCEDURE described here could be an additional step in conventional rice milling performed in developing countries. Fortification is an attractive means of preventing nutrient deficiencies because it is relatively inexpensive, it provides the opportunity for simultaneously adding several micronutrients, and it requires only passive consumer participation (Underwood, 1978; Arroyave et al., 1979; Solon et al., 1979; Paden et al., 1979).

Previously, rice fortification methods have not been aimed at retaining nutrients during cooking in excess amounts of water. For one recently developed method, release of added nutrients into excess water was reported as 100% for thiamin, 85% for niacin, and 81% for vitamin A (Rubin et al., 1977). Only if this water is absorbed completely will these nutrients be redistributed in the rice mass. Our studies demonstrate that micronutrients applied in a coating of combined methylcellulose and hydroxypropyl methylcellulose can be substantially retained, even when rice is cooked in a very large excess of water that is subsequently drained.

The bioavailability of nutrients in the fortified rice has not yet been tested. However, both hydroxypropyl methylcellulose and methylcellulose dissolve at body temperature, and tablets prepared from these polymers have been shown to disintegrate and release entrapped ingredients during the digestive process (Christenson and Dale, 1962). Thus, we expect these coatings to disintegrate and release nutrients after they are ingested.

While the present study demonstrates the feasibility of fortifying rice with a polymer coating which retards leaching of added nutrients during cooking, a clearer understanding of the rate-controlling mechanisms is required to permit modification and regulation of nutrient release from the system. The coating method would then be a practical technique for fortifying rice and other whole-grain cereals with specific micronutrients. We are currently studying the mechanism by which nutrients are released from the polymer coating. Preliminary data indicate that release is primarily via diffusion through the polymers. The high retentions of vitamin A and reduced iron, both of which are not significantly water-soluble, are consistent with such a mechanism. Several mathematical models have been developed to describe release of chemicals from various polymer systems (Rhine et al., 1980; Langer, 1980; Higuchi, 1961). Similar models may be applicable to this fortification system.

Elucidation of the mechanisms controlling release and retention in this system may also broaden its applications. There has been continuing interest in the use of polymers for sustained release in medical and agricultural applications (Langer and Folkman, 1976; Cardarelli, 1976), and for improved carriers of additives in foods (Leonard, 1978). Because of the edibility, nonreactivity, and unusual solubility characteristics of the polymer system described in this paper, it may be useful for controlled release in other food applications.

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Variations in Nutrient Content of Commercially Canned Legumes

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ABSTRACT

Nutrition information for label declaration on eight canned bean products was generated using methods of analyses recommended by the Association of Official Analytical Chemists. Variability between products and variability due to year of pack, production plant and can size was evaluated. Variability in caloric content, carbohydrates and fats per serving, was attributed to formulation and processing variations. U.S. Recommended Daily Allowances (U.S. RDA) per serving for thiamin, riboflavin, niacin and calcium fell within a range of 2–15%. All the bean products tested supplied protein and iron in amounts ≥ 20% U.S. RDA per serving. Seasonal variations in raw commodities, changes in blanching and/or processing methods, fluctuations in bean-to-sauce ratio, as well as analytical variability are probable causes of variations detected in the nutrient content of the canned products.

INTRODUCTION

FOOD PRESERVATION plays a major role in providing a year round supply of food commercially stable until it reaches the consumer. Thermal processing in hermetically sealed containers, namely, canning, also renders the food more edible by improving the quality attributes of the raw commodities such as flavor, color (Frazier and Westhoff, 1978; Lopez, 1975) and texture. Such attributes are perceptible by the consumer and are used by the consumer to make purchase decisions. Nutrient quality attributes, however, are not subjectively measured by the consumer, but may be evaluated based on quantitative measurements provided in the literature or on the food label. Although food preservation in general assures man his nutritional needs in constant supply, there is inevitable loss in certain labile nutrients which will also take place equally or more drastically if the food is not processed at all or if processed at home.

Food legumes are an important and economical source of protein, calories, vitamins and minerals in the diet of many developed and developing countries. Unfortunately, the great strides which culminated in the green revolution in the cereal crop area have not been paralleled as yet in the area of food legumes. Many efforts are still underway in the areas of genetic breeding to develop higher yields and higher protein seeds.

Many of the toxic constituents found in raw legumes are destroyed by adequate heat treatment during commercial processing. Trypsin inhibitors, hemagglutinins, antivitamins and goitrogens are heat labile and are inactivated during canning, which significantly improves the nutritional qualities of legumes. Although food legumes constitute a large number of species, the dry beans, *Phaseolus vulgaris*, have attained the greatest popularity in the United States and Europe. Such varieties as navy pea beans, pinto beans, northern beans, red beans, small red beans, and small whites, are popular in the trade. Black eyes (*Vigna sinensis*) and large lima beans (*Phaseolus lunatus*) are also produced but in lesser quantities than the varieties of *Phaseolus vulgaris*. The U.S. dry bean production by variety is shown in Table 1, which indicates the highest figure for navy beans, approximately one-half billion pounds, followed by approximately one-half billion pounds of pinto beans (USDA, 1979). Navy pea beans are used commercially in preparing canned pork and beans, baked beans and beans in tomato sauce-type products, while pinto beans are popular in products such as chili with beans and similar products. As Mexico takes its place as a consuming nation, the U.S. started exporting significant amounts of pinto beans south of the border. The U.S. rarely imports beans except when crop availability is insufficient to fill market needs. Such imports are usually made from Canada and Chile. The growing locations of beans in the U.S. are shown in Table 2, with Michigan, California, Idaho, Nebraska, Colorado and North Dakota, being the largest producers (USDA, 1979).

Environmental and horticultural practices may affect the physical, chemical, microbiological and nutritional qualities of raw beans. The nutrient content of the raw legumes will affect in turn the nutritional qualities of the finished products, but many practices in commercial processing will also determine the finished product nutrient content. Formulations such as bean-sauce ratios, soaking methods, blanching methods, type of process and length of process, are important variables which may affect the final outcome. With the advent of nutritional labeling regulations (FDA, 1973), many bean processors have accumulated nutrition information on their products for label declaration. Stokely-Van Camp, a leading processor in the U.S', has been involved in accumulating nutrition information on a variety of beanproducts. Such nutrition information is currently on the product labels. In generating such nutrition information, emphasis has been placed on the mandatory nutrients (Table 3) as spelled out in the nutrition labeling regulations, namely, calories, protein, carbohydrate, fat, vitamin A, vitamin C, thiamin, riboflavin, niacin, calcium and iron. The U.S. Recommended Daily Allowance (U.S. RDA) values listed for protein are 45g, to be used when the protein efficiency ratio (PER) of the total protein in the food product is equal to or greater than that of casein, and 65g, to be used when the PER of the total protein in the product is less than that of casein (FDA, 1973). The U.S. RDA value that applies to canned legumes is 65. Current literature data on nutrient content of commercially processed canned legumes has been limited (Watts and Merrill, 1963). Information in USDA Handbook No. 8 on nutrient content of canned legumes (Table 4) needs to be updated.

MATERIALS & METHODS

Sampling procedure

Samples of canned bean products were selected in accordance with the compliance procedure epelled out in the nutrition labeling guidelines (FDA, 1973). Samples were taken from each production location to include variability in production and regional variation in raw commodities. Although raw beans were purchased on the open market for the season through a central purchasing group, no attempt was made to identify the source of the raw beans shipped

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to production locations. Twenty-four samples of each product, representing consumer size packages, were randomly chosen from production lines to represent a lot or production code. Can codes were logged in a computer for future reference. Prior to analysis, the samples were stored under normal shelf conditions for canned foods for 6–12 months to duplicate average age in normal distribution prior to consumption.

Sample preparation

After storage for a specified time, a composite sample was prepared by homogenizing the product from 12 cans randomly selected from the lot. Homogenization was done using a Waring Blendor under nitrogen flush atmosphere for 30 sec or until a homogeneous sample was obtained. A sample was then drawn immediately and placed in metaphosphoric acid for vitamin C determination when it was indicated. Samples were drawn from the homogenate and stored in amber glass bottles for the determination of other nutrients.

Analytical procedures

The methods used for nutrient analyses were essentially those of the AOAC (1975), or with slight modification. Moisture, protein, fat, ash, and fiber were determined by proximate analysis using vacuum oven for moisture, Kjeldahl nitrogen with a factor of 6.25 for protein, Soxhlet extraction with petroleum ether for fat, digestion method in the fat extracted residue for crude fiber and muffle furnace method for ash. Carbohydrates were calculated by difference while calories were calculated using factors of 4, 4, and 9 calories per gram of carbohydrate, protein, and fat, respectively. Vitamin C and vitamin A values (essentially β-carotene) were determined by spectrophotometry, thiamin and riboflavin by fluorometric methods, and niacin by microbiological method using pH instead of titration as an index of niacin concentration. Calcium and iron were determined by atomic absorption spectrophotometry, and sodium and potassium by flame photometry. The resulting data were stored in a computer. Each analysis was performed in duplicate and the average value was used for calculations.

The resulting data, expressed on the basis of 100 grams of product, were stored in a Wang computer. Calculation of mean values and percent of U.S. RDA for appropriate nutrients per cup serving was automatically done by the computer based on nutritional labeling guidelines. Coefficients of variation and Student's t-tests were used to evaluate the data. For purposes of label declarations,

Table 1—U.S. bean production by variety in hundred pounds

Variety	Year 1978	1979
Navy Pea Bean	5795	6418
Pinto	5528	5940
Northern	1863	1998
Red Kidney	1843	1637
Blackeye	778	850
Pink	685	801
Baby Lima	512	650
Large Lima	458	520
Cranberry	375	305
Small Red	359	468
Small White	190	195
Black Turtle	171	263
Garbanzo	101	170
Flat Small White	10	4
Yelloweyes	0	0
Others	372	446
TOTALS	19040	20665

Table 2—U.S. bean production by state in hundred pounds

Variety	Year 1978	1979
Michigan	6210	6860
California	3323	3520
Idaho	2494	2460
Nebraska	1947	2160
Colorado	1530	1593
North Dakota	1243	1418
Minnesota	592	562
Washington	504	703
Wyoming	427	532
New York	428	480
Kansas	192	170
Montana	126	175
Utah	24	32
Others	0	0
TOTALS	19040	20665

Table 3—Mandatory nutrient labeling

Nutrient	U.S. RDA
Calories	
Protein	65 (45) g
Carbohydrate	
Fat	
Vitamin A	5000 I.U.
Vitamin C	60 mg
Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20 mg
Calcium	1000 mg
Iron	18 mg

Table 4—Nutrient content of dry and canned legumes per 100g and per cup[a]

BEANS	Water (g)	Food Energy (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Sodium (mg)	Potassium (mg)	Vitamin A (IU)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin C (mg)
White Beans																
Raw/100g	10.9	340	22.3	1.6	61.3	4.3	144	425	7.8	19	1196	0	0.65	0.22	2.4	–
Canned[b]/100g	70.7	122	6.1	2.6	19.0	1.4	54	92	1.8	463	210	130	0.08	0.03	0.6	2
Canned/Cup	180.3	311	15.5	6.6	48.5	3.6	138	234	4.6	1181	536	332	0.20	0.08	1.5	5
Red Beans																
Raw/100g	10.4	343	22.5	1.5	61.9	4.2	110	406	6.9	10	984	20	0.51	0.20	2.3	–
Canned/100g	76.9	90	5.7	0.4	16.4	0.9	29	109	1.8	3	264	Tr.	0.05	0.04	0.6	–
Canned/Cup	193.8	230	14.5	1.0	41.8	2.3	74	278	4.6	8	673	Tr.	0.13	0.10	1.5	–
Pinto Beans																
Raw	8.3	349	22.9	1.2	63.7	4.3	135	437	6.4	10	984	–	0.84	0.21	2.2	–
Canned	NA															
Lima Beans																
Raw	10.3	345	20.4	1.6	64.0	4.3	72	385	7.8	4	1529	Tr.	0.48	0.17	1.9	–
Canned	NA															

[a] Data from Watt and Merrill (1963)
[b] With pork and tomato sauce

the percent U.S. RDA per serving is usually incremented in accordance with the nutrition labeling guidelines. Several collaborative studies were conducted with other laboratories and the National Food Processors Association Committee of Canning Industry Analytical Chemists to test analytical reliability.

RESULTS & DISCUSSION

THE RESULTS of nutrient content for eight canned bean products are presented in Tables 5 and 6, based on one-cup servings. The caloric content of the eight bean items ranged from 170–330 calories per cup of product. The big variation in caloric content between products is a function of product formulation and consumer preference to such formulations. The coefficients of variation (C.V.) for caloric content for various products were based on data generated from 1972–1979, and represent the standard deviation as a percentage of the mean. The C.V. for calories ranged from 4.1–8.1. Calories are derived in all bean products mainly from complex carbohydrates, protein, and a small amount of fat which is either added, such as in Pork & Beans, or indigenous to the raw beans. The carbohydrate content for all the canned bean products discussed was 33–58 g/cup with Butter Beans being the lowest and Brown Sugar Beans the highest. The C.V. for carbohydrate were in the range of those of calories and the variations can be attributed to bean sauce ratio, soaking, blanching, and processing methods. Brown Sugar Beans and Mexican Style Chili Beans contain 6 and 3g of fat per cup, respectively, while the other bean products contain less than 2g of fat per cup.

All the beans outlined in the table belong to *Phaseolus vulgaris* with the exception of Butter Beans which are large, mature lima beans. The percent U.S. RDA for protein for all products was 20, which is considered a significant source of protein for all these canned bean products. The C.V. for protein among products ranged from 6.2–9.3 with overall mean C.V. of 7.4. This is considered low variability and would reflect a reasonable compliance with label declarations with least expected variability. Since protein and calories are important nutrients in canned legumes, and since both nutrients are viewed by the consumer with interest, the low variability in the results for label declaration is important. Thiamin content of various canned bean products ranged from 6–10% U.S. RDA per cup serving with C.V. from 20–35. The percent U.S. RDA for riboflavin ranged from 4–8 with C.V. from 21.2–38. The percent U.S. RDA for niacin ranged from 2–6 with C.V. from 12.4–33.8.

Table 6 shows that the calcium and iron content for the various products in terms of percent U.S. RDA per cup serving ranged from 4–15% for calcium and 20–25% for iron with C.V. of 9.1–32.1 and 16.4–29.5, respectively. All the bean products analyzed were found to be significant sources of iron.

Although sodium and potassium are not considered in nutrient labeling declaration, Table 6 also shows the sodium and potassium content in mg/cup serving for various products. Most of the sodium in these products is derived from salt added in formulation for consumer acceptance, while most of the potassium is indigenous to the raw commodities.

Vitamin A and vitamin C were present in very low amounts in all these bean products, except in the case of Mexican Style Chili Beans where vitamin A (as β-carotene) is present at 35% U.S. RDA. The source in this case is the chili pepper which is added and is rich in β-carotene. Both vitamin A and vitamin C may also be derived from tomato sauce if it is present in the formulation such as in Pork & Beans. Still, one serving of Pork & Beans provides only about 3.25% U.S. RDA for vitamin A and about 4.69% U.S. RDA for vitamin C.

One product was chosen and one nutrient was selected to investigate variability in nutrient content from year to year, production plant to production plant, and can size to

Table 5—Number of calories, grams of carbohydrate and percentage of U.S. RDA for protein, thiamin, riboflavin and niacin per cup of various products

	Calories		Carbohydrate		Protein		Thiamin		Riboflavin		Niacin	
	Per cup	C.V.	g/cup	C.V.	% RDA	C.V.	% RDA	C.V.	% RDA	C.V.	% RDA	C.V.
Pork & Beans	250	4.1	47.6	4.1	20	8.4	10	20.9	8	31.0	6	33.8
Brown Sugar Beans	330	8.0	58.1	10.5	20	6.0	6	18.1	8	24.4	4	20.3
Dark Red Kidney Beans	200	7.0	39.1	8.7	20	9.1	10	29.7	6	25.7	6	28.5
Light Red Kidney Beans	210	4.1	40.6	5.0	20	7.1	10	22.9	6	22.8	6	26.2
New Orleans Style Kidney Beans	200	8.1	39.0	10.5	20	6.2	10	25.3	6	21.2	6	26.3
Mexican Style Chili Beans	240	6.5	44.5	7.5	20	7.2	8	24.9	8	22.2	4	32.5
Red Beans	220	4.5	42.9	4.2	20	6.4	8	32.0	6	38.0	4	14.5
Butter Beans	170	6.4	33.0	7.3	20	9.3	6	35.1	4	25.3	2	12.5

Table 6—Percent RDA for calcium and iron and milligrams of sodium and potassium per cup of various products

	Calcium		Iron		Sodium		Potassium	
Product	% RDA	C.V.	% RDA	C.V.	mg/CUP	C.V.	mg/CUP	C.V.
Pork & Beans	10	14.0	25	29.5	1180	11.1	690	16.6
Brown Sugar Beans	15	15.1	25	15.3	800	7.0	640	16.4
Dark Red Kidney Beans	6	32.1	20	20.7	790	17.9	600	20.0
Light Red Kidney Beans	6	20.4	20	21.7	750	9.7	550	23.8
New Orleans Style Kidney Beans	6	20.7	20	25.2	900	25.2	690	13.2
Mexican Style Chili Beans	8	11.0	25	19.4	820	13.4	730	17.3
Red Beans	10	18.4	20	11.9	1060	12.0	620	13.6
Butter Beans	4	9.1	25	16.4	840	7.0	550	17.4

can size. Table 7 shows a summary of the results of statistical analysis of protein in Prok & Beans based on percent U.S. RDA per cup from year to year, for data collected from 1972 to 1979. The overall mean for all years was 20.05 percent U.S. RDA per cup with a total number of samples of 61, each representing a 12 can composite.

Table 7—Protein RDA per cup of Pork & Beans per year of pack[a]

Year of pack	Protein % U.S. RDA's/cup	Variability from the mean "Students" t test
1972	18.99	n.s.
1973	21.83	**
1974	20.12	n.s.
1975	20.44	n.s.
1976	20.40	n.s.
1977	19.23	n.s.
1978	18.62	**
1979	19.06	*

[a] Overall Mean = 20.05; n = 61; n.s. not significant
*Significant at 5% probability level
**Significant at 1% probability level

Table 8—Protein RDA per cup of Pork & Beans per can size[a]

Can size	% of U.S. RDA for protein/cup	Variability from the mean "Students" t test
211x300	18.76	**
300x407	20.65	**
307x409	19.56	n.s.
401x411	19.97	n.s.
603x700	21.60	**

[a] Overall Mean = 20.25; n = 61; n.s. not significant
*Significant at 5% probability level
**Significant at 1% probability level

Table 9—Protein RDA per cup of Pork & Beans per packing plant[a]

Packing plant	% of U.S. RDA for protein/cup	Variability from the mean "Students" t test
No. 1	19.85	*
No. 2	20.25	n.s.
No. 3	20.46	**
No. 4	20.01	n.s.
No. 5	19.47	**

[a] Overall Mean = 20.5; n = 61; n.s. not significant
*Significant at 5% probability level
**Significant at 1% probability level

Results of the t-test indicate no significant difference from the overall mean in the percent U.S. RDA of protein per cup for Pork & Beans for some years or differences significant at 1 or 5% level of probability. Table 8 shows a summary of the results of analysis of variance for protein in Pork & Beans based on percent U.S. RDA per cup from can size to can size ranging from 211x300 to 603x700. The t-tests indicated no significant difference from the overall mean in protein content in some can sizes or differences significant at 1 percent level of probability. Table 9 shows a summary of the results of analysis of variance for protein in Pork & Beans based on percent U.S. RDA per cup from plant to plant. The results indicate no significant difference from the overall mean in protein of Pork & Beans for some plants or differences significant at 1 and 5% levels of probability for other plants. The source of variability between years, can sizes, and plants may be attributed to seasonal variation in raw commodities, blanching methods, processing methods, and beans-to-sauce ratio, as well as analytical variability.

CONCLUSION

BASED on the data generated, it should be emphasized that canned legumes are important and economical sources of protein, complex carbohydrate, vitamins and minerals, and should play a major role in promoting a well balanced diet. It should be recognized that current available data on nutrient content of canned legume products is limited; therefore, the need exists to upgrade the USDA Nutrient Data Bank in this area. Some of the data accumulated have been submitted to USDA for incorporation in their Nutrient Data Bank.

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Effect of Steam Temperature, Speed of Cooling and Cutin Disruption in Steam and Lye Peeling of Apples

D. A. SMITH, W. A. DOZIER, W. A. GRIFFEY, and K. S. RYMAL

ABSTRACT

A laboratory model steam peeler was adapted to accept superheated steam and direct injection of cold water in order to study the effect of rapid heating with superheated steam and rapid cooling under partial vacuum on the peeling efficiency of 'Red Delicious' apples. A test was also designed to determine the effect of physical disruption of the cutin layer on the efficiency of caustic peeling. Steam peeling resulted in higher peeled yields and brighter product color than caustic peeling. The peeled yields were 97.5% for superheated steam-peeled apples with flash cooling, 91.7% for caustic peeled fruit which had a prepeel treatment to physically disrupt the cutin layer, 85.8% for conventionally caustic-peeled fruit, and 82.7% for the mechanically peeled control.

INTRODUCTION

MANY FRUITS AND VEGETABLES are not well adapted to mechanical peeling because they have irregular shapes and cavities or are sensitive to the heat treatment involved in conventional caustic peeling. Successful peeling of such produce depends upon the rapid transfer of heat to the tissues to be peeled for a minimum duration followed by cessation of heat and rapid cooling. 'Red Delicious' apples are especially troublesome to peel because of their rather truncated shape, thick skin, and waxy coating, which render them difficult to peel by mechanical or chemical means.

Steam peeling processes have been described in the literature for peeling produce by Eidt and McArthur (1944) and by Huxsol and Smith (1975). Harris and Barber (1957) showed that the depth and uniformity of the peel depends upon the steam pressure used and the rapidity of diffusion of steam among the produce being peeled. Smith et al. (1980) reported that rapid heating to peeling temperature and flash cooling by injection of cold water into the peeling chamber resulted in improved yields and quality of peeled sweet potatoes.

The common commercial method of peeling apples is mechanical peeling which results in relatively high peeling losses (Lopez et al. 1958, 1959). Several workers have been able to reduce these losses through caustic peeling of apples. Powers et al. (1977) reported that hand labor and peeling losses could be reduced by the use of warm caustics followed by steaming and washing. Powers (1975) reported KOH to be superior to NaOH for peeling apples.

A major problem in the peeling of apples by chemical methods is the penetration of the cutins (natural waxes) and the waxes which are commonly added post-harvest to increase in gloss and improve appearance. Kolattukudy and Walton (1973) reported that cutins are composed primarily of cross-esterified fatty acids with occasional low solubility triterpenoids. The commercial wax-like additives are spray emulsions of carnauba wax or shellac. Although the use of surfactants in peeling was suggested by Olson (1941), more recent workers recommend specific wax penetrants such as ethyl and isopropyl alcohols (Harrington and Hill, 1964), and sodium alkyl sulfonate (Harrington and Hill, 1968). Good results have been achieved by the incorporation of certain specific wax penetrants in warm caustic solutions (Shultz and Smith, 1968).

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MATERIALS & METHODS

Apples

All tests were conducted using unwaxed 'Red Delicious' apples grown on the Alabama Agricultural Experiment Station's Piedmont Substation. These apples were held at 0°C and processed within 2 wk of harvest. The fruit size was graded to ensure a mean diameter of 6.4 cm and a mean weight of 133g.

Steam peeling

A tumbling batch-type laboratory pilot model steam peeler of 1/4 bu (8.8L) capacity (Fig. 1) was designed to accept either saturated steam at 100 psig (7 kg/cm^2) or superheated steam at 100 psig (7 kg/cm^2) and a mean inlet temperature of 371°C. Steam feeds into the peeler through a 1/2-inch manifold at the swivel joint and enters two 1/4-inch steam diffusers which distribute the steam along the length and on opposite sides of the rotating peeler. The diffusers along with the rotation of the peeler ensure rapid and uniform distribution of steam and heating of the produce to be peeled. Another design feature provides for direct injection of cold water at 20°C into the peeling chamber through the steam diffuser system. This distributes water among the produce accelerating the reduction of pressure within the chamber and rapidly cooling the product.

During operation the peeler was preheated and then charged, in an atmosphere of steam, with 20 apples. The peeling chamber was sealed and steam was introduced into the transversely rotating peeler for 25 sec when saturated steam was used and for 20 sec when superheated steam was used. At the end of this heating period the steam inlet was closed and the exhaust was opened until the chamber pressure fell to 25 psig (1.76 kg/cm^2). The exhaust was

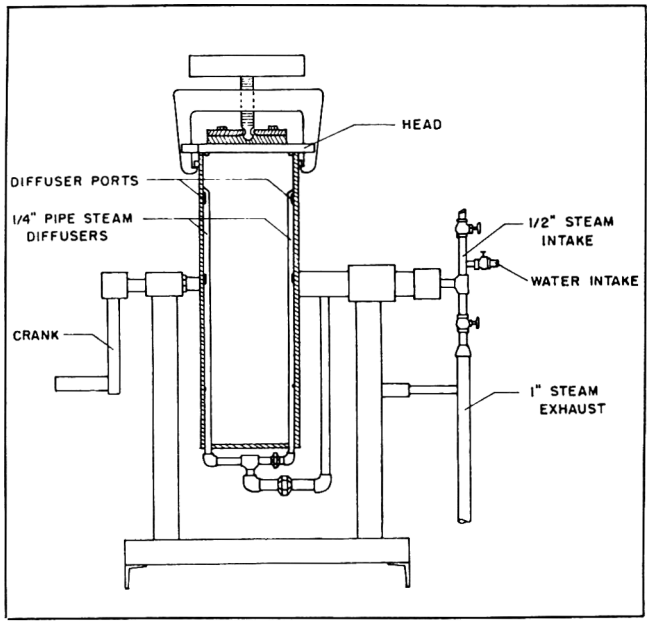


Fig. 1—Steam peeling apparatus.

then closed and cold water at a line pressure of 65 psig (4.57 kg/cm^2) was injected into the peeling chamber for 5 sec. The fruit was then discharged and washed in a rod washer. Data were taken from three replicates in each peeling test.

A further test was designed to determine the effect of a caustic pretreatment prior to peeling with superheated steam. The apples were dipped in a 40% (w/w) lye bath at 27°C and held at 27°C for 2 min prior to a 10 sec exposure to superheated steam at 371°C followed by cold water injection.

Chemical peeling

Because preliminary tests indicated that optimal peeling of apples without a dewaxing treatment could be attained at 106°C, in a 15% (w/w) NaOH solution for 4 min, all caustic treatments were run under similar conditions in a laboratory size, ferris wheel peeler varying only the time required to attain an optimal peel for the variable being tested.

A simple inexpensive method which could be used in a commercial setting was designed to vigorously prewash the apples and disrupt the waxy coating. This apparatus was designed to feed individual fruits between rotating corn silking brushes in a tank charged with highly agitated warm water and detergent. Similar tests were run using this apparatus charged with warm detergent water and size 80 grinding silica. The purpose of the silica was to disrupt the cutin layer through minute scratches which would allow the caustic solution to come into contact with the skin of the fruit. Optimal peeling periods in the lye bath were 3 min, 40 sec with a detergent prewash, and 3 min with an abrasive prewash.

Control

A mechanically peeled control was run with the peeling knives set to a peel thickness of 1.9 mm.

Evaluation

The total peeled yield was determined by weighing the apples before and after peeling without coring. Surface color of the peeled apples was determined using a Hunter Color Difference Meter standardized to a white plaque where $L = 93.8$, $a = -1.0$ and $b = 1.2$. All color readings were taken approximately 10 min after peeling. Total solids were determined by drying 10-g samples to a constant weight in a vacuum oven at 65°C. The depth of heat penetration was measured by direct measurement of the translucent zone in the peeled fruit. The heat ring was measured as the width of the dark ring that formed within 30 min at the inner boundary of heat penetration.

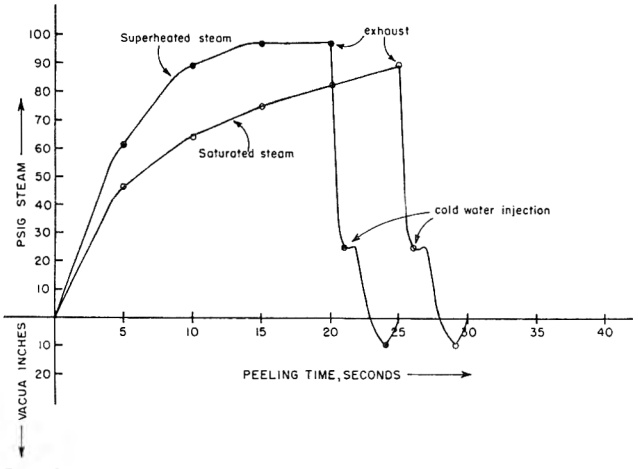


Fig. 2—Peeling chamber pressure during peeling by superheated steam with cold water injection and saturated steam with cold water injection methods. The line with open circles indicates peeling with saturated steam.

Statistical methods

Significant differences between means was determined by performing an analysis of variance and using Duncan's Multiple Range Test.

RESULTS & DISCUSSION

RAPID APPLICATION of heat to the tissues being peeled followed by rapid cooling are critical factors in achieving minimum peeling losses and tissue damage during the peeling of fruites and vegetables. Superheated steam used in conjunction with direct injection of cold water into the steam atmosphere of a steam peeling chamber resulted in a higher recovery of peeled apples than any of the other peeling treatments (Table 1). This peeling method is characterized by rapid transfer of heat to the fruit surface followed by a very rapid release of pressure and cessation of heat treatment (Fig. 2). When peeling with saturated steam the apples are exposed to lower chamber pressures and lower temperatures requiring a longer exposure time to accomplish an optimal peeling operation. Without cold water injection the steam pressure is reduced more slowly and there is no direct cooling action by cold water. These fruits leave the peeler hot and are not fully cooled until they pass through the washer. Steam peeling was accomplished in all trials without disruption of the integrity of the apples.

Peeled yields in excess of 95% were attained in peeling treatments using superheated and saturated steam with cold water injection, superheated steam without water injection and saturated steam without water injection. All of these peeling treatments yielded a smooth well blanched surface.

A dip in 40% (w/w) lye at 27°C followed by a 2-min holding period at room temperature prior to a 10-sec treatment with superheated steam removes the exocarp, a single layer of cells. The underlying parenchyma cells of the mesocarp were undisturbed and smooth. This process, however was difficult to control and areas of softer tissue on the apple were eroded away during washing resulting in a significant reduction of peeled yield.

The use of an agitated prewash with brushing resulted in significant improvements of the yield of lye-peeled fruit (Table 1). Yields were 85.8% for lye peeling, 87.4% for lye peeling following detergent prewash and 91.7%

Table 1—Effect of peeling treatment on peeled yield, heat penetration, heat ring width and total solids of 'Red Delicious' apples[a]

Peeling treatment	Mean peeled yield (%)	Mean heat penetration (mm)	Mean heat ring width (mm)	Total solids (%)
Superheated steam + H_2O	97.5a	2.5e	<0.1d	18.1b
Detergent, abrasive, superheat + H_2O	97.2a,b	3.0d	<0.1d	18.0b
Saturated steam + H_2O	96.5b	3.1d	0.1d	18.0b
Superheated steam	95.9b,c	3.4c	<0.1d	18.2b
Saturated steam	95.3c	3.4c	0.1d	18.2b
Lye + superheated steam	91.8d	0.9f	<0.1d	18.1b
Detergent abrasive lye	91.7d	5.7b	1.4c	17.8c
Detergent lye	87.4e	5.9b	1.7b	17.7c
Lye	85.8f	6.4a	2.3a	17.7c
Mechanical peeling	82.7g	–	–	18.9a

[a] Values with the same letter(s) do not differ significantly at $p < 0.05$.

Table 2—Effect of peeling treatment on the color of 'Red Delicious' apples[a]

Peeling treatment	L[b]	a[c]	b[d]
Superheated steam + H_2O	54.5a,b	−3.0d	24.8a
Detergent, abrasive, superheat + H_2O	54.6a,b	−3.5d	24.0a,b
Saturated steam + H_2O	55.8a,b	−3.4d	24.9a
Superheated steam	54.6a,b	−2.9d	24.9a
Saturated steam	56.6a	−3.5d	22.7b
Lye + superheated steam	53.8b,c	−5.4e	22.8b
Detergent abrasive lye	49.5d	−2.0c	20.2c
Detergent lye	49.0d	−2.1c	19.3c,d
Lye	49.5d	−1.6b	18.1d
Mechanical peeling	52.2c	1.7a	24.4a

[a] Values with the same letter(s) do not differ significantly at $p < 0.05$.
[b] L — total light reflectance.
[c] a — Negative values indicate greeness; positive values indicate redness.
[d] b — Indicates yellowness.

for fruit treated with a detergent and abrasive prewash before lye peeling. The use of these prewashes before steam peeling had no effect on peeling efficiency.

Mean heat penetration (Table 1) into the flesh of the apple appears to be directly related to the duration of the heating portion of the peeling process, with greater penetration occuring in lye peeling than in steam peeling processes. Superheated steam peeling with cold water injection into the peeling chamber resulted in significantly lower heat penetration than any treatment other than superheated steam following a lye bath dip.

The mean width of the heat ring was 0.1 mm or less in all of the steam-peeled apples. In lye-peeled apples the heat ring was pronounced (2.3 mm wide) but significantly narrower in those treatments receiving an agitated prewash (1.7 mm in detergent and 1.4 mm with an abrasive prewash), which permitted a shorter dwell time in the chemical peeler. The very narrow heat ring apparent in steam-peeled apples indicates that temperatures were sufficiently high to blanch tissues then dropped abruptly resulting in minimal damage to the tissues near those where temperature was sufficient for enzyme inactivation.

Color and appearance of the apples peeled by all steam peeling methods were rated excellent. The color of the lye-peeled fruit was visibly less brilliant but was still highly acceptable. The Hunter L values confirmed that total light reflectance was lower for fruit peeled by immersion in hot lye (Table 2). Hunter a values indicated that the steam-peeled apples had a slightly greener color than lye-peeled fruit. Apples peeled by superheated steam following a lye dip had the most intense green color resulting from the inner layer of skin which was left in place after removal of a single outer layer of skin. Hunter b values indicated that the lye-peeled apples were less yellow than those peeled by steam. Mechanically peeled apples darkened more rapidly than either the lye-peeled or steam-peeled fruits and lacked the smooth surface attained by lye peeling and steam peeling.

Water uptake during the peeling and washing operations as measured by total solids (Table 1) is slightly higher in lye-peeled apples than in those peeled by steam.

CONCLUSIONS

WHILE CAUSTIC PEELING of apples is a major improvement over mechanical peeling, it requires the additional step of penetration of the cutin layer by mechanical or chemical means to achieve satisfactory yields. Steam peeling appears to be a more efficient peeling method for firm ripe apples. Steam peeling with superheated steam and flash cooling by cold water increases yields, saves labor, eliminates the need for expensive caustic solutions, caustic solution disposal, and results in higher quality apples for further processing.

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Enhancement of Fruit Sensory Quality by Post-Harvest Applications of Acetaldehyde and Ethanol

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ABSTRACT

Post-harvest application of acetaldehyde vapors to blueberries, tomatoes, and pears led to the enhancement of the fruit sensory quality including an increase in the sugar content, sugar-acid ratio, and flavor changes judged to be acceptable by test panels. Applied ethanol vapors led to similar but limited enhancement of fruit sensory quality. Comparative applications of ethylene were more effective in stimulating changes in the background color, including an increase in the total carotenoid content in tomato and anthocyanines in blueberry. Ethylene, however, had little or no effect, and occasionally led to deleterious changes in the fruit sensory quality. Acetaldehyde and related volatiles may be important in the development of fruit sensory quality, as occurring normally during ripening, or as a post-harvest application for the improvement of fruit sensory quality.

INTRODUCTION

ACETALDEHYDE AND ETHANOL are usually found in fruit in trace amounts but begin to accumulate as fruits begin to ripen (Fidler 1933; Gerhart and Ezell, 1939; Romani and Ker, 1966; Janes and Frenkel, 1978). These volatiles were studied extensively in fruit with regard to their possible role in physiological disorders and although their action in metabolic abnormalities is discounted (Smagula and Bramlage, 1977) there is an interest in the effect of the compounds on fruit metabolism. Fidler (1968) observed that applied acetaldehyde led to temporary stimulation of respiratory activity in apples and oranges and our recent studies have shown that acetaldehyde vapors can stimulate respiratory gas exchange in blueberries and strawberries (Janes et al., 1978) and that acetaldehyde, ethanol, or acetic acid can trigger a respiratory upsurge in potato tubers (Rychter et al., 1979; Janes et al., 1981). In the latter the stimulation of respiration was accompanied by the turnover of respiratory intermediates (Rychter et al., 1979) and development of cyanide-resistant respiration (Janes et al., 1979; Janes et al., 1981). Acetaldehyde appears also to stimulate ripening processes in fruit even under conditions when ethylene is removed or its action antagonized (Janes and Frenkel, 1978) suggesting that the action of acetaldehyde is independent from ethylene.

In the present work we further examine the concept that acetaldehyde, and related volatiles (e.g., ethanol) act independently and differentially in the stimulation of fruit ripening by testing the effect of the compounds on the development of fruit sensory quality.

MATERIALS & METHODS

Plant material

The fruit used in this work were pears (*Pyrus communis* cv. Bartlett), tomatoes (*Licopersicon esculentum* Mill cv. Vendor, or Florida Dade 908) and blueberries (*Vaccinum corymbosum* L. cv. Jersey). Pears were grown in Amherst, MA, and harvested at the mature-green stage on Sept. 7, 1979. Some of the fruit was used immediately after harvest or kept for 10 wk at 0°C, in hypobaric storage at 1/10 of an atmospheric pressure, using air as the ventilating gas as previously described (Janes and Frenkel, 1978). Vendor tomatoes were greenhouse grown locally, and Florida Dade 908 tomatoes were obtained from a local supermarket. The Florida tomatoes were harvested at the mature green stage and kept 2 wk in storage at 10–13°C. The tomatoes used for the experiments outlined below were at the breaking stage. The blueberries were grown locally, harvested and used at the fully ripe stage.

Application of gas mixtures to fruit

Volatiles, including acetaldehyde and ethanol, and ethylene were applied to the fruit after harvest or after stoage. For treatment with the volatiles, fruits were kept in 2 liter glass jars and ventilated continuously with air at a rate of 400 ml/min. When necessary the air was supplemented with 1,000–5,000 μL acetaldehyde or ethanol/L or 10 μL ethylene/L. Ethylene was obtained from Matheson Scientific and pure acetaldehyde or ethanol from Fisher Scientific Company. The final gas compositions, indicated in the Results & Discussion section, were obtained by mixing a known concentration of a source mixture of the gases with the ventilating air. Gas cylinders containing approximately 1,000 ppm ethylene served as the source mixture for ethylene. Source of mixtures of acetaldehyde or ethanol were obtained as described previously (Rychter et al., 1979) and mixed at the appropriate proportions with the ventilating air. The concentrations of the various mixtures were estimated by gas chromatography and verified against known standards. The various volatiles were applied to the fruit for 24 hr followed by ventilation with air alone for the remainder of the experiments as outlined in the Results and Discussion section. Control sample designated as "Air" consisted of fruit ventilated with atmospheric air previously scrubbed free of ethylene or other contaminating gases.

The effect of the applied gas mixtures on fruit quality was measured by chemical and sensory methods.

Measurement of quality by chemical methods

Previously outlined methods were used to estimate the content of anthocyanine (Fuleki and Francis, 1968) in blueberries, and the total carotenoid content (McCollum, 1953) in tomato pericarp tissue. Reducing sugars and the total sugar content (Somogyi, 1952), and the titratable acidity were determined in all fruit. The sugar-acid ratio was used as an index for comparing the total sugars content (reducing sugars plus sucrose) vs total acidity expressed as citric acid equivalents of the measured titratable acidity (W/W) in 100g fresh weight of fruit tissue.

Measurement of quality by sensory methods

Flavor was used as an additional index of sensory quality. Flavor, the sensation produced by material taken in the mouth, as defined by Hall (1968), was determined by sensory evaluation using a multiple comparison procedure (Amerine et al., 1956). This method couples difference in testing with an acceptability rating. The latter designed to indicate the direction of any detected difference. In this test samples representing fruit held in air (control), in air plus ethylene, air plus acetaldehyde, or air plus ethanol, were presented to each panelist simultaneously, and in random arrangement. A labeled control was used in all tests. Judges were asked to assess differences in flavor between the labeled control and the test samples by rating each sample on a scale from none to extreme. Judges were asked also to indicate the acceptability of each sample in relation to the control by indicating whether it was better than, comparable to, or poorer than the control. A coded control was

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included where it served a dual purpose, namely, to provide a base score for the sample series and to determine the acuity of the judges. Results were statistically analyzed using analysis of variance, and the least significant difference (LSD) between panel scores was calculated. Hedonic response was measured to show differences between treatments and when LSD was exceeded, samples were designated as significantly different from each other. Coupled with the acceptability rating the analysis indicated the direction of detected differences, namely, better than or poorer than the control.

Sensory evaluations were carried out in the Sensory Evaluation Laboratory, Dept. of Food Science, Rutgers Univ. Judges were seated and performed in isolated booths. Judges were selected at random from the faculty and students of the Food Science or Horticulture and Forestry Departments. The use of untrained panels was in keeping with the objective of measuring a response to differences in fruit sensory quality resulting from the treatments (Hovenden et al., 1979). On the average, 10 to 12 judges were used for each of the different tests. The extact number of judges is indicated in each of the tests outlined in the tables.

RESULTS & DISCUSSION

FIG. 1 SHOWS the effect of ethylene and acetaldehyde on the changes in the color pigment content in ripening blueberries and tomato fruit. The application of ehtylene led to a pronounced stimulation in the anthocyanine content in blueberries and the total carotenoid content in tomato. These results are consistent with previous and widely documented data indicating that ethylene can effectively stimulate changes in the background color of ripening fruit (Burg and Burg, 1965; Sacher, 1973). Acetaldehyde, by comparison, had only a limited effect on the color changes in blueberries. In tomatoes the total carotenoid content in the acetaldehyde treated fruit was only slightly higher than in fruit held in air (control). The present and other results (data not shown) indicate that compared to ethylene the effectiveness of applied acetaldehyde in stimulating background color changes in fruit is limited.

Acetaldehyde and ethylene appear also to influence differently changes in fruit sensory quality. Fig. 2 shows that in blueberry and pear, ethylene stimulated some increase in the reducing sugar content but none in treated tomatoes. By comparison applied acetaldehyde initiated a substantial increase in the reducing sugar content in all the treated fruit. This effect was particularly noticeable in tomatoes in which ethylene had little or no effect. In a further extension of this test the effect of the applied compounds was examined as it may also influence the sugar-acid ratio. Table 1 shows that in harvested blueberries ethylene increased the sugar-acid ratio somewhat, but that acetaldehyde more than doubled this value by comparison to control fruit. In tomatoes ethylene had little or no effect, and by comparison acetaldehyde led to some increase (approximately 40%) in the sugar-acid ratio in freshly harvested fruit and to a doubling in this value in tomatoes treated after storage. A similar response was obtained in pears; more than doubling the sugar-acid ratio in acetaldehyde treated fruit, either freshly harvested or after storage, but only small changes were discernible between the control and the ethylene treated pears.

The effect of the test compounds on fruit sensory quality was examined also by subjective method using taste panels for flavor evaluation. The results (Table 2 and 3) show that in blueberries acetaldehyde stimulated significantly different flavor changes by comparison to the nontreated fruit (Table 2). This trend became even more pronounced in tomatoes and pears. In either fruit applied acetaldehyde led to a significant change in flavor, and the developed

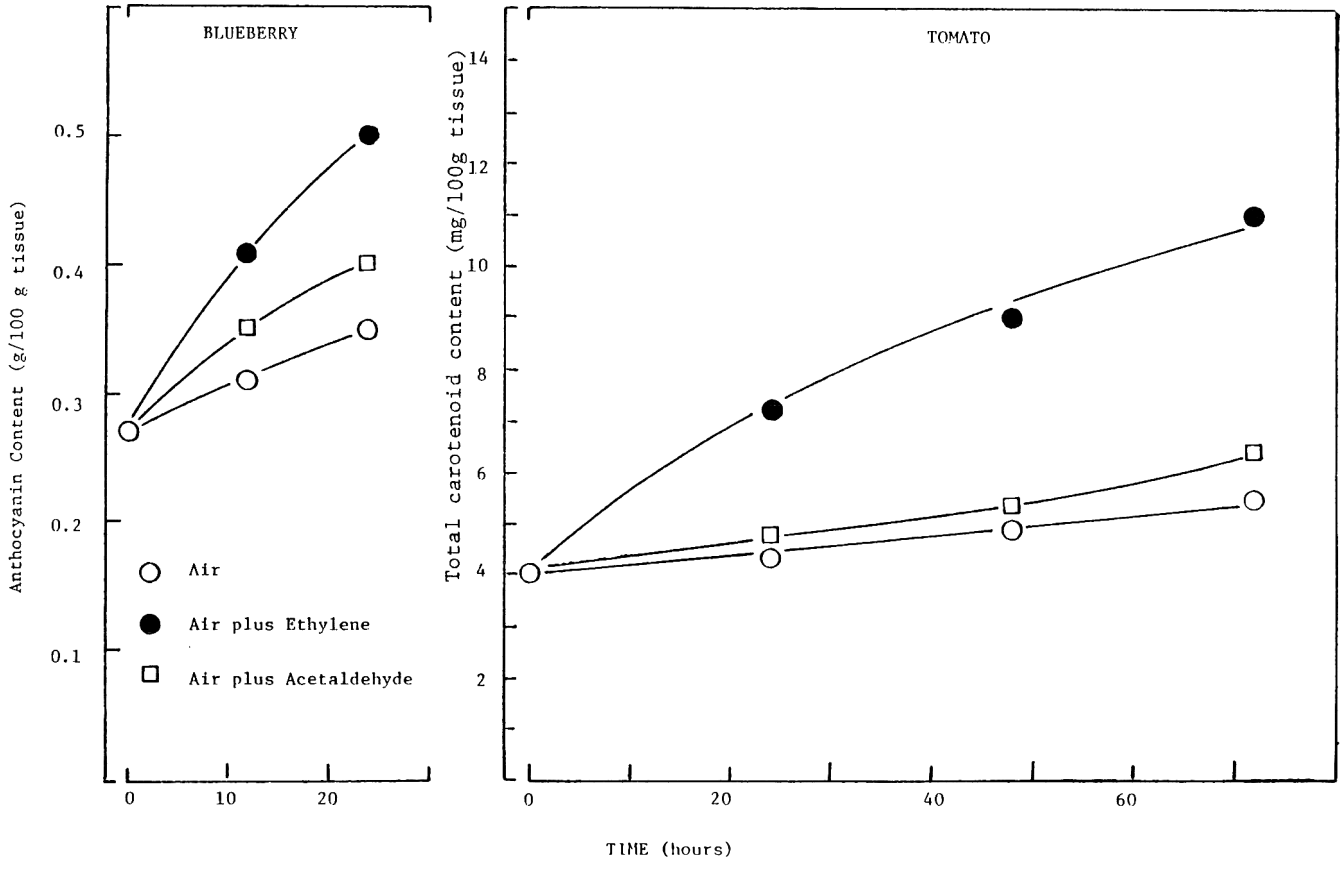


Fig. 1—Changes in the content of authocyanin in Jersey blueberries and total carotenoids in Vendor tomatoes held in air, air plus 10 ppm ethylene, and air plus acetaldehyde (5,000 and 1,000 ppm, respectively).

flavor, as judged by acceptability rating, was favored by the test panels (Table 3). Similar results were obtained by the application of ethanol although the obtained flavor differences and the acceptability rating were less pronounced by comparison with fruit treated with acetaldehyde. The application of ethylene appeared also to induce a significant change in flavor (Table 2). The trend in flavor acceptability showed, however, that the ethylene treatment resulted in poorer flavor in tomato and pear and a similar trend is suggested also in blueberries (Table 3).

The present results indicate that ethylene and by comparison acetaldehyde, or other volatiles, can influence different aspects of fruit quality. Ethylene appears to be effective primarily in upgrading the appearance of fruit by stimulating color changes associated with ripening (Fig. 1). The changes in the sugar content, the sugar-acid ratio, and flavor development in some fruit appear to be influenced only to a limited extent by ethylene as was observed also by others (Kader et al., 1978). Occasionally ethylene may have adverse effects on flavor acceptability. By comparison, the applied volatiles enhanced in fruit the development of desirable quality attributes including an increase in the sugar content, the sugar-acid ratios, and development of desirable flavor. These results further support the concept (Janes and Frenkel, 1978; Rychter et al., 1979) that acetaldehyde and related volatiles act independently and differ-

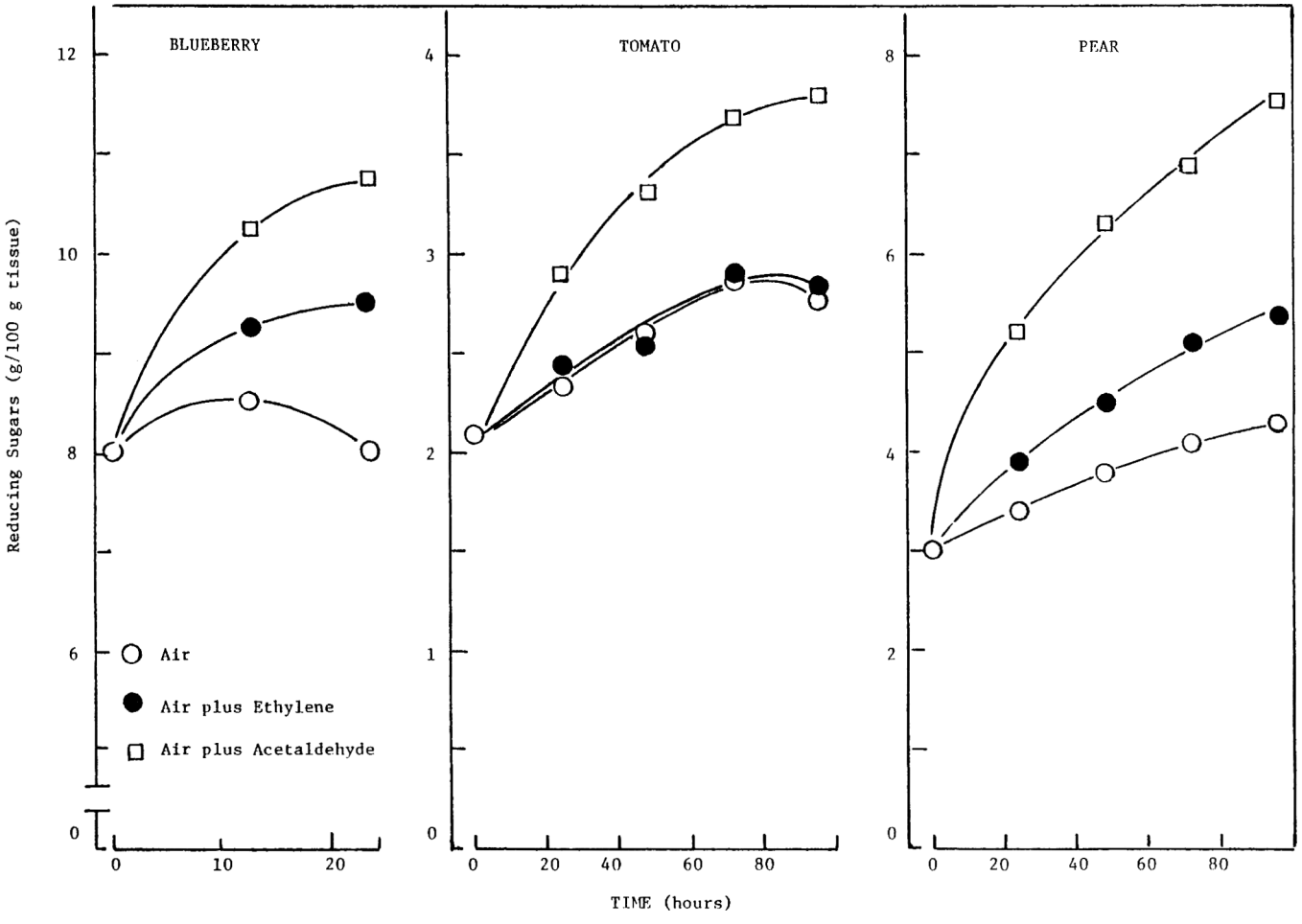


Fig. 2—Changes in the content of reducing sugars in Jersey blueberries, Vendor tomatoes, and Bartlett pears, held in air, air plus 10 ppm ethylene, and air plus acetaldehyde (5,000, 1,000, and 4,000 ppm, respectively.

Table 1—Changes in the sugar-acid ratios in fruit held in air, air plus ethylene, or air plus acetaldehyde[a]

	Sugar-acid ratio				
	Blueberry	Tomato[b]		Pear[c]	
Gas treatment	After harvest	After harvest	After storage	After harvest	After storage
Air	4.04	0.96	1.2	4.00	3.60
Air plus ethylene[d]	7.40	1.06	1.0	4.10	4.80
Air plus acetaldehyde[d]	9.76	1.37	1.9	8.90	8.20

[a] The experimental conditions were those described in Fig. 1 and 2.
[b] Vendor tomatoes were used for the "after harvest" and Florida Dade 908 tomatoes for the "after storage" experiment.
[c] Bartlett pears were used at the mature green stage for the "after harvest" and those kept in hypobaric storage were used for the "after storage" experiment.
[d] The fruit were held in the gas mixtures for 24 hr followed by ventilation in air only for the remainder of the experiment. Blueberries were assayed after 48 hr, tomatoes after 4 days, and pears after 6 days after gas application began.

entially in metabolic regulation including ripening processes in fruit.

Applications of acetaldehyde in a dip or vapor form to strawberries were used to slow down ripening process and deterioration (Prasad and Stadelbacher, 1974) and to control pests or pathogenic microorganisms (Norris et al., 1979; Aharoni et al., 1980) but the treatment is occasionally accompanied by off-flavor (Aharoni et al., 1980). In these experiments the volatile concentrations were roughly one order of magnitude higher than those employed in the present work. As the volatile concentration is increased a dose-response effect is exhibited and metabolic processes which otherwise are stimulated by acetaldehyde, ethanol or acetic acid at 1,000–5,000 ppm are pregressively inhibited (Janes et al., 1981). The use of the volatiles at supra-optimal concentrations may, therefore, be counterproductive in stimulating ripening processes, including flavor development. In addition, high volatile concentrations are apparently accompanied by metabolic disorders and consequently off-flavors in the treated fruit.

The nature of the metabolic processed elicited by acetaldehyde and leading to the development of fruit sensory quality is a complex problem (Hornstein and Teranishi, 1967). The changes in the sugar content and the sugar-acid ratio may explain, in part, the enhancement in flavor of the volatiles treated fruit. By the same token these changes may be used to illustrate the difficulties in elucidating the metabolic action of the compounds. The increase in the content of sugars may reflect their release from parent compounds, e.g., starch, cell wall constituents, or other polysaccharides. Glucoeogenesis from storage organic acid, glucogenic amino acids, or other compounds, may be an additional mechanism for the mobilization of carbohydrate metabolism. The nature of these, or other metabolic processes leading to sugar accumulation, or development of other flavor attributes are important but as yet unresolved questions.

Table 2—Flavor differences in fruit[a] held in air, air plus ethylene, and air plus ethanol or acetaldehyde

Gas Treatment	Average hedonic response[b]		
	Blueberry	Tomato	Pear
Air	1.83	1.7	1.7
Air plus ethylene	2.50	4.3	4.3
Air plus ethanol	–	3.9	3.9
Air plus acetaldehyde	3.83	4.4	4.4
LSD	1.42	1.42	1.42

[a] Freshly harvested Jersey blueberries, Vendor tomatoes, and mature-green Bartlett pears were treated as outlined in Fig. 1 and 2.
[b] Twelve judges were used for the testing flavor differences in the blueberries and ten judges for the tomatoes and the pears.

The volatiles may modulate the activity of enzymes in biosynthetic pathways of flavor constituents. The volatile mode of action may be even at a more fundamental level, since ripening processes were shown to depend on the transcriptional and the translational process (Frenkel et al., 1968). Acetaldehyde was shown to be adsorbed by apple and citrus fruit tissue (Fidler, 1968), and by potato tubers (Rychter, et al., 1979). The possibility that the applied volatiles undergo exchange reactions in tissues, and serve as precursor compounds for flavor derivatives cannot be ruled out at this time.

Acetaldehyde and ethanol were shown to accumulate during fruit ripening (Fidler, 1933; Gerhart and Ezell, 1939; Romani and Ker, 1966; Janes and Frenkel, 1978) and in turn may stimulate ripening processes (Janes and Frenkel, 1978) including fruit sensory quality in normally ripening fruit. The volatiles may thus constitute both products of and ripening stimulating compounds, and this phenomenon is analogous to ethylene metabolism and action in fruit (Burg and Burg, 1965; Sacher, 1973). As with ethylene it is difficult to discern the cause and effect relationship of volatiles and fruit ripening. In fruit which accumulate substantial levels of acetaldehyde and ethanol as part of the aroma complex (Nursten, 1970) the volatiles may lead to the stimulation of selected ripening processes, i.e., development of flavor or other attributes of fruit sensory quality. In these fruit an initial application of the volatiles may stimulate the accumulation of acetaldehyde and ethanol, and thereby enhance development of sensory quality. In other fruit not showing propensity for the accumulation of acetaldehyde or ethanol the stimulation of desirable sensory quality may depend on the continuous application of the volatiles.

The regulation of fruit sensory quality may be important particularly under the present handling practices. To confer on fruit post-harvest longevity, required in the distribution channels, fruit are harvested at early ripening stages and are often kept in refrigerated and modified atmosphere storage. In some fruit, e.g., grapes, ripening processes, including development of sensory quality, come to an abrupt halt at harvest (Winkel et al., 1974). In others, including tomatoes, some ripening process may proceed, but stage of ripeness was shown to be decisive in the development of sensory quality, since harvest at stages earlier than table-ripe restrain the ability of fruit to develop desirable sensory quality (Kader et al., 1978). Refrigerated and modified atmosphere storage also appear to be associated with losses in fruit sensory quality (Hatfield and Patterson, 1974). Clearly, the objectives of extending fruit post-harvest longevity and the attainment of desirable sensory quality by the present procedures are in conflict. The presented information indicates that applied volatiles can compensate for practices associated with losses in fruit sensory quality. Prematurely harvested tomatoes responded favorably to applied acetaldehyde and ethanol (Fig. 1, Tables 1 to 3), and simi-

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Table 3—Flavor acceptability of fruit[a], held in air, air plus ethylene, and air plus ethanol or acetaldehyde

Gas treatment	Flavor acceptability[b]								
	Blueberry			Tomato			Pear		
	Better	Comparable to	Poorer	Better	Comparable to	Poorer	Better	Comparable to	Poorer
Air	1	9	2	0	9	1	1	7	2
Air plus ethylene	0	9	3	0	2	8	1	4	5
Air plus ethanol				5	3	2	4	5	1
Air plus acetaldehyde	5	4	3	7	3	0	9	0	1

[a] Freshly harvested Jersey blueberries, Vendor tomato, and mature-green Bartlett pears were treated as outlined in Fig. 1 and 2.
[b] The number of judges were identical with those used for the tests in Table 2. The values represent the number of judges evaluating the fruit flavor as better than, comparable to, or poorer than the rest of the tested samples.

Vitamin Retention During Preparation and Holding of Mashed Potatoes Made from Commercially Dehydrated Flakes and Granules

JORG AUGUSTIN, G. A. MAROUSEK, W. E. ARTZ, and B. G. SWANSON

ABSTRACT

The retention of ascorbic acid, riboflavin, niacin, Vitamin B_6 and folic acid in dehydrated potato granules and flakes during home preparation and subsequent extended periods of holding at elevated temperatures on a steam table was investigated. Losses were insignificant with all vitamins during the preparation of the mashed potatoes except for riboflavin in the case of granules and folic acid in the case of flakes. Holding the mashed products on a steam table for up to 1 hr reduced the retentions of ascorbic acid and folic acid to 63% and 56% respectively in mashed potatoes made from flakes. Retention values for ascorbic acid in granules amounted to 66–67% and for folic acid they ranged from 78–86%. The holding period exerted little or no effect on retention of riboflavin, niacin, and vitamin B_6.

INTRODUCTION

INFORMATION on the effects of home and institutional preparation of mashed potatoes from dehydrated flakes and granules is limited. Ang et al. (1975) reported on the effects of heating and holding methods on the vitamin composition of mashed potatoes from flakes only. The study was limited to ascorbic acid, thiamin and riboflavin and did not cover such vitamins as folic acid. A recent report by Augustin et al. (1980) included home preparation of mashed potatoes made from dehydrated potato granules but not from potato flakes. Furthermore, it failed to investigate the effects of prolonged product holding at elevated temperatures on a steam table, which is a common practice with institutional feeding systems. The purpose of this investigation was to study vitamin retention of mashed potatoes made from dehydrated potato granules and flakes during home preparation as well as during subsequent holding of the product at elevated temperatures.

MATERIALS & METHODS

DEHYDRATED GRANULES fortified with ascorbic acid and packaged under nitrogen in No. 10 cans, as well as nonfortified flakes packaged in polyethylene bags under atmospheric conditions were procured from commercial processing operations in the Pacific Northwest. Within 2 wk after receiving the samples three replicate samples of each product were thoroughly and separately mixed before sampling. Mashed potatoes were then prepared from each of these samples according to label instructions as follows.

Potato granules containing whey and caseinate solids

470g of granules, 2130 ml of water and 10g of table salt. The water was first brought to a boil, then transferred to a mixing bowl; granules were then added slowly while mixing at low speed using a Kitchen Aid mixer equipped with a wire whip. After the liquid was absorbed the product was blended for an additional 3 min at high speed.

Regular potato granules

425g of granules, 266g of milk, 1775 ml of water, and 10g of salt. The water and milk were first heated to a boil, then transferred to a mixing bowl. The granules were then added, and the product was handled as outlined above.

Potato flakes

540g of flakes, 732g of milk, 1538 ml of water, 30g of salt, and 128g of butter. The water was brought to a boil. While boiling, butter and salt were added, the pan and its contents were removed from the heat, cold milk was added, flakes were added, and the mixture was stirred first gently with a fork and then fluffed.

Following preparation, all samples were held on a steam table at approximately 200°F for 1 hr. Each product was prepared in triplicate. Sampling was done on the mashed material in triplicate immediately after preparation of the mashed potatoes as well as after 15, 30, 60 min of holding on the steam table. Product temperatures increased from 150–155°F at the time of mashing to 170–180°F following holding on the steam table.

The dry mixes were sampled and analyzed without further preparation. The moist rehydrated samples were treated as follows: a roughly 30g sample was used for moisture determination, a 100g subsample was used for ascorbic acid analysis. This sample was homogenized in 200 ml of a 1:1 mixture of 5% metaphosporic acid and 5% acetic acid using a Polytron (Brinkmann Instrument) homogenizer. The third subsample was frozen by immersion in liquid nitrogen, freeze-dried, ground through a Wiley mill using a 40 mesh screen and stored under refrigeration until further analysis. Analysis of the vitamins was done as outlined by Augustin et al. (1980).

RESULTS & DISCUSSION

TABLE 1 summarizes he composition of the dehydrated potatoes, milk and butter material used in this study. The difference in the riboflavin content between the two types of potato granules is due to the presence of whey solids in the granules which were prepared without the addition of milk. Whey solids have been reported to contain significant amounts of this vitamin (Posati and Orr, 1976). Initial analysis of the dehydrated products as well as the rehydrated samples to which milk was added revealed only traces of thiamin. Because of the presence of sulfites this vitamin had obviously been destroyed during processing. The thiamin present in the milk added to the products apparently was also destroyed because of the presence of sulfites in the potato products. It is for these reasons that it was decided to exclude any further thiamin analysis from this study. Table 2 shows the changes in vitamin composition on a wet basis during preparation and extended holding of mashed potatoes from granules containing whey and caseinate solids. Although moisture values of the product decreased

Table 1—Vitamin composition of commercially dehydrated mashed potatoes, milk and butter (100g DWB)

	Granules contg. whey & caseinate solids	Granules, no added milk solids	Flakes	Milk	Butter
Ascorbic acid, mg	181.6	185.2	12.8	7.8	0
Riboflavin, mg	0.137	0.102	0.096	2.30	0.04
Niacin, mg	6.22	6.87	6.37	0.70	0.05
Vitamin B_6, mg	0.85	1.16	0.85	0.35	Trace
Folic acid, μg	24.6	32.0	24.7	41.6	3.6

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Table 2—Vitamin content of potato granules containing whey and caseinate solids during preparation and extended storage (100g WB = approx 1/2 cup or 1 serving)

	Granules water and salt	Freshly prepared	Held on steam table for		
			15 min	30 min	60 min
Ascorbic acid, mg	30.3[a] (1.8)[b]	25.3 (1.0)	23.5 (0.5)	20.3 (4.2)	17.6 (8.3)
Riboflavin, mg	0.025 (4.0)	0.022 (5.2)	0.022 (7.1)	0.023 (2.6)	0.024 (2.4)
Niacin, mg	1.12 (1.05)	1.09 (12.7)	1.05 (9.7)	1.11 (5.5)	1.10 (2.9)
Vitamin B_6, mg	0.16 (3.7)	0.17 (5.9)	0.17 (6.9)	0.17 (5.9)	0.18 (3.3)
Folic acid, μg	4.53 (2.8)	4.46 (1.5)	4.00 (5.4)	3.89 (6.0)	3.48 (4.9)

[a] Mean
[b] Relative standard deviation, %

slightly but significantly during the mashing operation and the subsequent holding periods, the data provided only indications on the vitamin retention. It is for this reason that the retention data were computed on a dry weight basis.

The resulting retention data on a dry weight basis which are compiled in Table 3 show lowest retention values with ascorbic acid and folic acid. However, in the course of the actual preparation of the mashed potatoes, retention of these two vitamins is nearly complete. The bulk of the retention loss occurred during the holding periods of the mashed product. In the case of riboflavin, retention was reduced only during the preparation step but not during the subsequent holding period of the mashed product. Since this vitamin is highly light sensitive this may have been the result of product exposure to light during the blending process.

Vitamin data on mashed potatoes prepared from granules, fresh milk, water and salt are shown in Tables 4 and 5. Essentially the same trends were obtained with regard to retention values as with the granules containing whey and caseinate solids except that in this case there was an unexplainable significant, although small, retention loss with niacin.

Composition data and retention values pertaining to rehydrated potato flakes are compiled in Table 6 and 7. Retention values in this case follow similar trends as those encountered with granules. However, in this case retention of riboflavin was not affected during the initial mashing process. This could be due to the fact that, with hand-whipping, product exposure to light may have been less extensive as with machine whipping associated with the rehydration of granules. The authors are at a loss to explain the rather large loss of folic acid which occurred during the mashing process. As for ascorbic acid and riboflavin retentions, the data are in good agreement with those reported by Ang et al. (1975). As far as thiamin values are concerned, Ang's group reported the presence of this vitamin, while in the present investigation only traces of this vitamin were detected. In an earlier study, Augustin et al. (1979) reported the presence of thiamin in flakes, but not in granules. Since sulfites are added to both products during processing, and since they are known to destroy this vitamin, the presence of this vitamin in flakes was explained by the short dwell time of the wet product in the presence of sulfites prior to dehydration in the case of flakes. It is conceivable that in some instances, occasional or regular, depending on the plant or process involved, the time lapse between the addition of sulfite to the mashed product and its dehydration could have been lengthened. This in turn could have resulted in the complete inactivation of thiamin in this study.

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Table 3—Percent total vitamin retention of potato granules containing whey and caseinates solids (DWB)

	Freshly prepared	Held on steam table for		
		15 min	30 min	60 min
Ascorbic acid	95a[a,b]	89b	75[c]	66d
Riboflavin	91a	89a	91[a]	95a
Niacin	98a	94a	97a	98a
Vitamin B_6	112a	108a	107a	113a
Folic acid	102a	91b	87c	78d

[a] Retention values are realtive to the uncooked mixture.
[b] For each vitamin retention, values followed by the same letters are not significantly different (>0.05) from each other.

Table 5—Percent total vitamin retention in potato granules with the fresh milk added (DWB)

	Freshly prepared	Held on steam table for		
		15 min	30 min	60 min
Ascorbic acid	93a[a,b]	81b	72c	67d
Riboflavin	85a	90ab	94b	89a
Niacin	91a	97a	91a	92a
Vitamin B_6	100a	98a	92b	99a
Folic acid	98a	90b	89b	86b

[a] Retention values are relative to the uncooked mixture.
[b] For each vitamin retention, values followed by the same letters are not significantly different (P > 0.05) from each other.

Table 4—Vitamin content of potato granules with fresh milk added during preparation and extended holding on steam table (100g WB = approx 1/2 cup or 1 serving)

	Granules and milk	Freshly prepared	Held on steam table for		
			15 min	30 min	60 min
Ascorbic acid, mg	28.1[a](2.1)[b]	28.8 (4.0)	25.6 (4.1)	23.0 (3.1)	21.4 (2.41)
Riboflavin, mg	0.039 (1.5)	0.036 (5.7)	0.039 (5.3)	0.041 (1.4)	0.039 (2.6)
Niacin, mg	1.05 (5.7)	1.06 (3.6)	1.15 (3.5)	1.08 (11.7)	1.10 (4.2)
Vitamin B_6, mg	0.18 (6.3)	0.22 (9.6)	0.22 (4.6)	0.21 (2.8)	0.22 (2.6)
Folic acid, μg	5.56 (6.1)	5.99 (2.5)	5.61 (3.01)	5.56 (5.1)	5.34 (3.3)

[a] Mean
[b] Relative standard deviation, %

Table 6—Vitamin content of potato flakes during preparation and extended holding on steam table (100g WB = approx 1/2 cup or 1 serving)

	Flakes and milk	Freshly prepared	Held on steam table for 15 min	30 min	60 min
Ascorbic acid, mg	2.17[a] (6.5)[a]	21.9 (22.7)	1.51 (3.1)	1.36 (1.9)	1.32 (13.9)
Riboflavin, mg	0.052 (6.7)	0.055 (17.3)	0.053 (12.6)	0.052 (7.2)	0.052 (10.5)
Niacin, mg	1.01 (10.1)	1.03 (11.5)	0.91 (2.8)	0.97 (3.7)	0.96 (4.9)
Vitamin B_6, mg	0.14 (12.4)	0.14 (0)	0.14 (7.1)	0.14 (12.4)	0.14 (4.2)
Folic acid, μg	5.50 (9.8)	4.47 (8.5)	3.86 (6.6)	3.48 (6.3)	3.11 (7.1)

[a] Mean
[b] Relative standard deviation, %

Table 7—Percent vitamin retention in potato flakes during preparation and holding on steam table (DWB)

	Freshly prepared	Held on steam table for 15 min	30 min	60 min
Ascorbic acid	98a[a,b]	71b	64c	63c
Riboflavin	103a	101a	102a	103a
Niacin	103a	93b	98ab	99a
Vitamin B_6	102a	102a	98a	98a
Folic acid	81a	71b	64c	56d

[a] Retention values are relative to the uncooked mixture.
[b] For each vitamin retention, values followed by the same letters are not significantly different ($P > 0.05$) from each other.

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lar results were obtained in grapes (results not shown). Quality attributes were enhnaced also in fruit after refrigerated or hypobaric storage (Table 1). Thus, the application of acetaldehyde, and related volatiles, may offer an opportunity to resolve, at least in part, the dilemma of a trade-off between fruit post-harvest longevity and sensory quality, and, moreover, integrate the development of sensory quality into the overall concept of fruit quality.

Objective and subjective tests showed an improvement in the sensory quality of juice extracted from acetaldehyde treated citrus, and grape fruit (data not shown). It is tempting, therefore, to suggest that the outlined concept can be extended also to the upgrading of the sensory quality of processed fruit products.

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Equilibrium pH of Home Canned Foods Comprising Combinations of Low Acid and High Acid Ingredients

G. M. SAPERS, J. G. PHILLIPS, and A. M. DiVITO

ABSTRACT

A recipe data bank and method of data processing were developed to classify tomato-based combinations of low and high acid ingredients according to product pH. Information describing the composition and preparation of more than 400 products were entered into the data bank. The extent of variation in ingredient proportions for different product categories was estimated. The ratio of low acid to high acid ingredients specified by the recipe was used as a criterion for selecting representative recipes for preparation and pH determination. High correlations were obtained between this index and product pH. The acidity of 24 common categories of combination products was characterized by the testing of representative recipes.

INTRODUCTION

A RECENT SURVEY conducted by the U.S. Department of Agriculture (Davis and Page, 1979) indicated that about 20% of home canners in the United States prepare "combination" products such as tomato sauce and vegetable mixtures which may be either low or high in acidity, depending on the proportions of low and high acid ingredients used. This distinction is critical to safe canning since low acid foods (pH $>$ 4.6) must be pressure canned while high acid products (pH $\leqslant$ 4.6) may be processed safely in a boiling water bath (IFT, 1977). Published canning instructions for combinations are frequently inconsistent and in some cases may be unsafe (Dragonwagon, 1973; Gewanter and Parker, 1976). Some canning authorities have recommended that combinations be canned by a process appropriate to the highest pH ingredient in the product (IFT, 1977; Anon., 1977). The USDA survey has shown that many home canners are using inappropriate processes for such products. It is possible that some outbreaks of botulism from such combinations as spaghetti sauce and chili (Center for Disease Control, 1979) are a consequence of underprocessing.

Ideally, the choice of canning method for mixtures of low acid and high acid ingredients should be based on the equilibrium pH of the product. However, the number of possible variations in products of this type is very large, and reliable pH data are not generally available. Our objective in this research was to determine whether the equilibrium pH of combination products could be located within a meaningful range from information on product composition compiled in a "recipe data bank" and from representative pH and acidity data so that guidelines for process selection could be developed.

EXPERIMENTAL

Recipe data bank

To compile information on products containing mixtures of low acid and high acid ingredients which might be home canned, we screened recipes found in more than 80 home canning publications and cookbooks. Product categories searched included juices, soups, mixed vegetable dishes, meatless tomato sauces, barbecue sauces, tomato-meat sauces, chilies, and condiments. Recipes from a number of ethnic and regional cuisines were represented. Insofar as possible, we selected recipes on the expectation that some products in each category would fall in the low acid pH range; consequently, we gave certain highly acidic product categories such as relishes and pickled vegetables only a superficial examination.

Information contained in each recipe was coded and entered into a computer for further processing. We converted all ingredient quantities to a weight basis by reference to standard tables (Adams, 1975; Watt and Merrill, 1975; Posati and Orr, 1976; Marsh et al., 1977) or to label declarations for commercial products. When such data were not available, we determined conversion factors experimentally (Sapers, 1981).

Recipe evaluation and comparison

We compared recipes in the data bank on the basis of their specified proportions of low and high acid ingredients. As a criterion for selecting representative recipes for testing, we calculated the ratio of low acid to high acid ingredients on a weight basis without attempting to correct for quantitative differences in acidity between ingredients in the same group. Subsequently, we calculated more exact values of this ratio, based on measurements of ingredient acidity. For this puspose, we titrated duplicate 10g samples of the major ingredients, dispersed in 40 ml distilled H_2O, either with distilled white vinegar (5% acidity) or with 0.1N NaOH, depending on the ingredient pH, to a pH 4.6 endpoint. All raw vegetables were boiled until fully cooked (London, 1972), drained, cooled, and homogenized prior to sampling for this titration. Ground dehydrated ingredients were reconstituted with boiling H_2O and cooled prior to titration. An estimate of the acidity of high acid ingredients in a recipe was obtained by computing the product of the ingredient weight in the recipe (100g basis) and the acidity of the ingredient, expressed as meq NaOH (to a pH 4.6 endpoint) per 100g ingredient, and then summing this product for all high acid ingredients. This process was repeated with low acid ingredients, computing the total quantity of vinegar (meq/100g) required to lower their pH to 4.6. We then calculated the ratio of the total meq vinegar for low acid ingredients to the total meq NaOH for high acid ingredients. We also computed a third "low acid index" from these data: the difference between the total meq of vinegar for low acid ingredients and the total meq of NaOH for high acid ingredients, per 100g of product described by the recipe.

Acidity of representative products

To obtain data on the acidity of representative products, we identified over 100 "benchmark" recipes from each product category for preparation, canning, and analysis. We used extreme values of the ratio of low acid to high acid ingredients (weight basis), unique ingredient combinations, and questionable specifications for acidulation or thermal processing as criteria for recipe selection. Each product was prepared according to recipe instructions and filled hot into pint canning jars, leaving about 3/4-inch headspace. Jars were sealed and processed for 30 min in a boiling water bath or for 60 min at 10 psi in a pressure canner, following procedures described in the USDA's Home and Garden Bulletin No. 8 (Anon., 1975). We processed each product by both canning methods since the pH was not known at the time of canning, and we expected some of the recipes to yield borderline high or low acid products.

All processed samples were equilibrated for 1–3 days before being analyzed; products that had been processed by the water bath method were refrigerated to prevent the spoilage of low acid samples. We analyzed unprocessed samples as soon as they had cooled

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to room temperature. After bringing the refrigerated jars to room temperature, we homogenized samples for 30 sec at high speed in a Waring Blendor and then determined product pH and titratable acidity, following procedures described previously (Sapers et al., 1980). Based on the measured acidity of the first set of "benchmark" products, we selected additional recipes for preparation, canning, and analysis so that each product category could be adequately characterized.

Statistical methods

The pH and titratable acidity data for unprocessed and corresponding processed products were compared by analysis of variance (ANOVA) to determine whether either method of canning altered product acidity.

The pH and titratable acidity values were correlated with the ratios of high acid to low acid ingredients (on both a weight basis and milliequivalent basis) and with the difference between milliequivalents of high acid ingredients and milliequivalents of low acid ingredients. This was done for each product category.

All calculations, file maintenance, and statistical analyses were performed with the Statistical Analysis System (SAS) at the Washington Computer Center of USDA.

RESULTS & DISCUSSION

Distribution of recipes in data bank

From an original compilation of more than 500 recipes for products containing mixtures of low acid and high acid ingredients, we selected 415 tomato-based recipes for inclusion in the data bank. Their distribution according to product category is given in Table 1. Italian, Spanish, Mexican, Creole, Southern and Western United States specialties were included in the compilation, in addition to many North American recipes not associated with a particular region or ethnic group. Approximately 25% of the recipes contained home canning information.

Table 1 — Distribution of recipes for tomato-based combination products in data bank

Product category	Number of recipes			
		Home canning		
	Total	Pressure	Water bath	Open kettle
Juice blends	26	7	9	1
Soups	48	6	1	0
Mixed vegetable dishes	54	24	4	0
Tomato sauces, meatless	165	3	27	2
Barbecue sauces	22	1	3	0
Tomato sauces with meat	54	10	0	1
Chilies	46	6	0	0

Variability within product categories

Individual recipes within each product category varied widely in the specified ingredient proportions (Table 2). The proportion of low acid ingredients varied over a 20-fold range in recipes for such product categories as tomato-vegetable soup and tomato-mushroom sauce. Less variability was seen with the high acid ingredients. However, the ratio of low acid to high acid ingredients (weight basis) varied over a 100-fold range within some product categories. Values of this ratio expressed on an acidity basis, using titration data illustrated by Table 3, were no more

Table 3 — Acidity and buffering properties of some common low acid ingredients of home canned mixtures

Ingredient	Ingredient pH	Titration to pH 4.6[a]
Low acid		With vinegar[c]
Beef (ground)	6.3	6.0
Broth (chicken)	6.3	3.7
Beans (red kidney)	5.8	10.4
Carrots	5.0	1.2
Chili powder[b]	4.6	0
Mushrooms	6.0	3.0
Okra	6.0	6.5
Onions	5.0	1.4
Peppers (green bell)	5.0	1.3
High acid		With 0.1N NaOH
Chili sauce	4.0	5.4
Lemon juice (bottled)	2.9	41.1
Tomatillos (canned)	3.8	6.2
Tomato paste	4.3	4.7
Tomato puree	4.3	2.2
Tomatoes, paste-type	4.4	1.1
Vinegar	2.7	45.0
Wine (red)	3.2	3.0
Worcestershire sauce	3.6	21.2

[a] Milliequivalents of NaOH or acetic acid per 100g ingredient.
[b] Reconstituted with boiling dist. H_2O before analysis
[c] 5% acidity

Table 2 — Variation in the proportions of low and high acid ingredients in recipes for some common combination products

Product category	Number of recipes	Range			Coefficient of variation (%)	
		Low acid ingred.[a]	High acid ingred.[a]	Low acid index[b]	Low acid index Wt basis	Low acid index Acidity basis
Tomato-vegetable juice	23	3.7–66.7	33.3–95.0	0.04–2.00	136	157
Tomato-vegetable soup	18	3.5–80.0	16.6–93.7	0.04–5.00	110	121
Stewed tomatoes	16	1.3–23.7	66.6–97.6	0.01–0.37	73	62
Tomato-bean combinations	12	18.0–78.4	15.4–69.4	0.42–3.66	60	94
Tomato-okra combinations	12	11.7–77.1	22.6–88.3	0.13–3.40	79	76
Tomato puree	6	16.0–29.4	70.6–83.7	0.19–0.42	31	35
Tomato paste	9	2.5–15.8	84.1–97.1	0.03–0.19	100	98
Tomato sauce, U.S.	32	4.8–47.0	29.7–95.1	0.05–0.91	71	79
Tomato-mushroom sauce	16	3.7–79.5	20.3–95.4	0.04–3.91	130	164
Creole sauce	20	13.6–88.4	10.8–84.7	0.16–8.20	148	123
Mexican tomato-pepper sauce	40	6.0–71.3	6.8–93.9	0.06–2.54	146	134
Barbecue sauce	22	7.8–62.9	35.2–87.5	0.08–1.79	87	137
Spaghetti sauce, meatless	16	5.7–57.3	15.4–88.5	0.10–1.69	128	166
Spaghetti sauce with meat	43	13.9–69.8	15.4–85.2	0.16–2.31	55	110
Chili con carne	34	42.6–91.0	8.7–47.6	1.09–10.45	62	61

[a] Weight percent, fat-free
[b] Low acid ingredients/high acid ingredients (weight basis)

variable than those calculated on a weight basis; coefficients of variation for these indices are included in Table 2.

Any attempt to develop guidelines for processing combinations must take such variability into consideration so that the indicated processing method (pressure or water bath canning) is appropriate for the highest pH product found within any category. Even this constraint may not be sufficient with highly variable product categories because of the probable existence of a recipe exceeding the limits of the data bank. Consequently, blanket recommendations should not be made for such highly variable categories even if all known examples fall in the high acid category.

Acidity of representative products

pH and titratable acidity data were obtained from rep-

Table 4 — Correlation between product pH and indices of recipe acidity

Product category	No. recipes Total	No. recipes Tested	Product pH range[a]	Correlation coefficient Low acid index[b] Wt basis	Acidity basis	Acidity difference
Tomato-vegetable juice	23	4	4.4–5.1	0.95[c]	0.98[c]	0.67
Tomato-vegetable soup	18	4	4.1–4.6	0.85	0.91[d]	0.95[c]
Tomato-mushroom sauce	16	7	4.3–5.0	0.79[d]	0.86[c]	0.84[c]
Spaghetti sauce with meat	43	4	4.6–5.0	0.96[c]	0.98[c]	0.95[d]
Creole sauce	20	8	4.2–5.2	0.97[c]	0.96[c]	0.84[c]
Mexican tomato-pepper sauce	40	10	3.9–4.6	0.15	0.65[c]	0.42
Barbecue sauce	22	4	4.0–4.4	0.86	0.46	0.74

[a] Determined experimentally
[b] As defined in the Experimental section
[c] Significant at 0.05
[d] Significant at 0.10

Table 5 — pH and low acid index of representative combination products

Product category	No. recipes Total	No. recipes Tested	Low acid index range[a] Weight basis All recipes	Weight basis Tested recipes	Acidity basis All recipes	Acidity basis Tested recipes	pH range	Acidity of category[b]
Juice blends								
Tomato-vegetable	23	4	0.04- 2.00	0.47-2.00	0.06- 4.50	0.33- 4.50	4.4-5.1	Low-high
Soups								
Tomato-broth	13	4	0.42- 3.67	0.99-1.66	0.58- 7.29	2.66- 3.05	4.5-4.8	Low-high
Tomato-vegetable	18	4	0.04- 5.00	0.33-2.30	0.10-15.00	0.51- 6.13	4.1-4.6	Low-high
Minestrone	9	1	2.28-14.60	2.28	16.84-41.33	16.84	5.2	Low
Manhattan clam chowder	8	2	1.26- 8.61	1.26-2.15	5.64-78.19	5.64-10.20	5.0-5.3	Low
Mixed vegetable dishes								
Stewed tomatoes	16	3	0.01- 0.39	0.11-0.24	0.02- 0.58	0.20- 0.58	4.4-4.5	High
Tomato-celery	6	2	0.30- 1.00	0.59-1.00	0.84- 2.80	1.52- 2.80	4.5-4.6	High (?)
Tomato-okra	12	4	0.13- 3.42	0.53-3.42	0.86-19.33	2.92-19.33	4.4-4.9	Low-high
Tomato-bean	12	3	0.49- 3.67	0.50-0.96	0.19-27.14	0.19- 6.04	4.9-5.3	Low
Tomato-zucchini	3	3	0.76- 1.35	0.76-1.35	2.41- 4.38	2.41- 4.38	4.7-4.8	Low
Tomato sauce, meatless								
Tomato puree	6	3	0.19- 0.42	0.33-0.42	0.26- 0.65	0.53- 0.65	4.4-4.5	High
Tomato paste	9	1	0.03- 0.19	0.19	0.03- 0.24	0.24	4.4	High
U.S. style	32	6	0.05- 0.91	0.25-0.91	0.08- 1.52	0.44- 1.52	4.4-4.6	High
Spaghetti	16	4	0.10- 1.69	0.16-1.69	0.07- 3.47	0.34- 3.47	4.3-4.5	High
Marinara	7	3	0.05- 0.38	0.17-0.38	0.09- 1.06	0.40- 0.66	4.4-4.5	High
Mexican	40	10	0.06- 2.54	0.17-2.54	0.03- 3.55	0.16- 3.55	3.9-4.6	High (?)
Barbecue	22	4	0.08- 1.79	0.48-1.79	0.02- 1.34	0.13- 1.04	4.0-4.4	High
Tomato-mushroom	16	7	0.04- 3.91	0.29-1.18	0.08-14.72	0.55- 3.14	4.3-5.0	Low-high
Creole	20	8	0.16- 8.20	0.18-8.20	0.19- 9.89	0.23- 9.89	4.2-5.2	Low-high
Spanish	5	2	0.34- 1.44	0.48-1.44	0.42- 3.90	0.82- 3.90	4.3-4.9	Low-high
Tomato-meat sauce								
Spaghetti	43	4	0.16- 2.31	0.16-0.67	0.45-22.27	0.59- 2.90	4.6-5.0	Low
Red clam	5	2	0.29- 0.89	0.29-0.70	3.18- 7.21	3.18- 6.11	4.8-5.1	Low
Chilies								
Chili con carne	34	2	0.88-10.45	1.09-1.24	4.19-44.01	5.71- 8.15	4.9-5.1	Low
Chili without beans	6	1	0.77- 9.21	0.77	3.37-42.04	3.37	5.2	Low

[a] Low acid ingredients/high acid ingredients
[b] Low acid, pH > 4.6; high acid, pH ≤ 4.6

resentative products, selected to characterize the acidity of each product category. Treatment of these data by ANOVA indicated that canning had no significant effect on product pH or titratable acidity (F values of 1.06 and 0.49, respectively).

The validity of using values of the low acid indices as criteria for recipe selection and classification was tested by correlating these parameters against product pH data. Significant correlations were obtained between product pH values and the low acid ingredient/high acid ingredient ratios, calculated on both weight and acidity bases, for a number of important product categories (Table 4). However, correlations were poor for Mexican tomato-pepper sauces and for barbecue sauces, probably because of the diversity and high acidulant content of these product categories. The acidity difference was less satisfactory as an index of low acidity than were the ratios.

pH and acidity of product categories

We have used experimentally determined pH values for products representing extreme and intermediate values of the low acid indices to classify the acidity of each product category (Table 5). With some product categories, the least acidic (highest ratio) recipes within the category yielded products with pH values in the high acid range (pH $\leqslant$ 4.6). Stewed tomatoes, tomato-celery combinations, tomato paste and puree, and several meatless tomato sauces (U.S. style, spaghetti, marinara, Mexican, and barbeque) appear to be high acid product categories. However, because correlations between the low acid indices and product pH values for Mexican sauces were poor, and also because of this category's excessive variability, we do not recommend processing the Mexican-style sauces as a high acid food. The classification of tomato-celery combinations also must be viewed with caution since this borderline high acid product category could easily be confused with other mixed vegetable dishes that are generally low acid products.

With some product categories, the most acidic (lowest ratio) recipes yielded products exceeding pH 4.6. One would infer that all recipes in such categories would yield low acid products. Recipes for tomato-bean combinations, tomato-zucchini combinations, spaghetti sauce with meat, and chili con carne yielded low acid products.

The remaining product categories (juice blends, soups, tomato-okra combinations, tomato-mushroom sauces, creole sauces, and Spanish-style sauces included recipes yielding products with pH values above and below 4.6. In the absence of a foolproof means of predicting the pH of individual products within these categories, and in the interest of consistency, one must assume that all such products are low acid foods.

These results may be used as a guide in screening published home canning recipes (or recipes submitted for publication) to assure that appropriate processing methods have been specified. Of course the adequacy of specific processing recommendations (pressures, times) would have to be verified experimentally. The approach one could take in recipe assessment would be first to locate the recipe type within our classification scheme of product categories. If the recipe falls within the scope of our data bank, one would then calculate a value of the low acid index, probably the ratio of low acid ingredients to high acid ingredients, expressed on a weight basis. By comparison of this value with the range of ratios reported in our study, one could confirm that the recipe could be represented by our pH and acidity data. If this is the case, the recipe could then be classified according to whether it would yield a high acid product or a low acid product (including borderline or ambiguous cases). Water bath or steam canning methods would be appropriate for the former situation, while pressure canning methods would be required for the latter situation.

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Effect of Sucrose Addition on the Sugar and Sorbitol Composition of Frozen Sweet Cherries and Their Derived Concentrates

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ABSTRACT

Glucose, fructose, sucrose, and sorbitol contents of Van and Black Republican varieties of sweet cherries were determined by high performance liquid chromatography (HPLC) and enzymic analytical procedures. Samples analyzed included frozen fruit, fruit packed as 3.6 parts fruit plus 1 part added sucrose, and cherry juice concentrates derived from the sugar-packed fruit. Cherry fruit showed invert patterns of glucose and fructose and contained trace amounts of sucrose, and 2.6–3.9g sorbitol/100g. Sucrose was either not detected or found in trace amounts in samples to which sucrose had been added. There is evidence that sucrose hydrolysis was caused by presence of invertase. The percent sorbitol content could be used to detect addition of sucrose. Results for sugar and sorbitol content as determined by HPLC or enzymic methods were very similar.

INTRODUCTION

A FREQUENT COMMERCIAL practice is to pack sweet cherry fruit in 55 gallon drums with added sucrose and hold in frozen storage for subsequent use in yogurts, ice creams, sauces, bakery items, etc. The free-run juice produced when the barrels are thawed is rich in color and flavor and yields a high-quality syrup when concentrated. The major objective of this study was to determine whether addition of sucrose to cherry fruit could be detected in the frozen samples and derived juice concentrates through analyses of their fructose, glucose, sucrose, and sorbitol contents. Wrolstad and Shallenberger (1981) recently published a table listing the free sugar and sorbitol content of sweet cherry fruit as reported in the literature by various workers. Sweet cherries have essentially an invert pattern, containing nearly equal quantities of glucose and fructose and either no or trace levels of sucrose. Their sorbitol content is among the highest of the various commercial fruits. Richmond et al. (1981) analyzed the sugar and sorbitol content of several fruits by high-performance liquid chromatography (HPLC) and reported a similar pattern for sweet cherry fruit. Another objective of this study was to compare the effectiveness of enzymic analytical procedures with HPLC for glucose, fructose, sucrose, and sorbitol measurements. HPLC instrumentation requires considerable investment while enzymic analyses utilize equipment which is more commonly available in industrial laboratories.

EXPERIMENTAL

Sample

Frozen samples were obtained from The Dalles Cherry Growers, Inc., The Dalles, OR 97058, and stored at −15°C until use. Samples included individually quick frozen unpitted sweet cherry fruit (Van and Black Republican varieties, 1978 season) and two lots each of the same fruit packed as 3.6 parts fruit and one part added sucrose.

Also provided were samples of cherry juice concentrate which had been manufactured from frozen Black Republican fruit packed with added sucrose. The fruit which had been frozen in 55 gal drums was thawed at ambient temperature and then heated to 60°C. The concentrate was made from the free-run juice. Three lots of concentrate were from the 1978 season, one lot was from 1977 and an additional lot was from the 1976 season. All concentrates were 51° Brix.

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Extraction and isolation of sugars and sorbitol for HPLC analysis

Fruit samples were homogenized in a Waring Blendor. Forty ml 95% ethanol were added to 10.0g of homogenate and the mixture was sonicated for 2 min through direct immersion of an ultrasonic probe (Bronwill Biosonik III Ultrasonicator; dial setting of 70). Juice concentrate samples were diluted (25.0g + 75.0 ml distilled water), and a 10.0-g aliquot combined with 40 ml ethanol. Samples were held at 2°C for 1 hr to facilitate precipitation of high molecular weight components, and then filtered by suction through a pad of 10g of hydrated polyvinylpyrrolidone (PVPP) on Whatman No. 1 filter paper; the PVPP had been washed by the procedure of Loomis, 1974. The PVPP pad and sample residue were rinsed with 25 ml 80% ethanol three times; the filtrate and washings were combined and concentrated to about 5 ml on a rotary evaporator (water bath = 37°C, pressure = 23 torr) to ensure ethanol removal from samples.

The samples were passed through two 2 x 7 cm columns in series, the first column containing 6 ml Biorad AG 50 W-X4 cation exchange resin, (200-400 mesh, Na+ form) and the second containing 6 ml Biorad AG 1 X-8 anion exchange resin (200-400 mesh, F- form). The columns were washed with deionized distilled water and the eluants collected in 50 ml volumetric flasks containing 5 ml of 10% mannitol as an internal standard and 1.0 ml 0.5% $CaCl_2 \cdot 2H_2O$. Samples were filtered through 0.45 μm Millipore filters prior to injection.

HPLC analysis of extracts

The HPLC system was composed of a Varian 5000 liquid chromatograph equipped with a column heater and refractive index detector, a 7.8 X 300 mm Aminex HPX-87 column (Biorad Laboratories), and a Hewlett Packard 3380 A integrator. Column temperature was maintained at 85°C. The mobile phase was deionized, 0.45 μm Millipore filtered water containing 0.1% $CaCl_2 \cdot 2H_2O$; the flow rate was 1 ml/min. Injection volume was 20 μl. Refractive index range was 0.5×10^{-5} RI units.

Relative detector response (RDR) factors for sucrose, glucose, fructose, and sorbitol were determined to be 1.01, 1.03, 1.04, and 1.07, respectively, using the method described by Akhavan et al. (1980). Percent recoveries (R) for standards were determined by submitting reagent sugar solutions in 80% ethanol through the isolation and separation procedures; percent recoveries for sucrose, glucose, fructose, and sorbitol were 98.7, 98.3, 95.5, and 98.3, respectively. Content of individual sugars was calculated by the following formula:

$$\text{g sugar/100g sample} = (A_s)(Wt_{is})(DF)/(A_{is})(R)(RDR)$$

where A_s = peak area of sugar; A_{is} = peak area of internal standard; Wt_{is} = weight of internal standard; DF = dilution factor; R = percent recovery; and RDR = relative detector response.

Enzymic analysis of sugars and sorbitol

Sugar and sorbitol contents were determined enzymically with Glucose/Fructose, D-Sorbitol, and Sucrose/Glucose kits from Boehringer Mannheim Biochemicals, Indianapolis, IN, according to the manufacturer's instructions. Samples (2–5g) were homogenized in a Waring Blendor and diluted with 2L distilled water; a 50-ml aliquot was centrifuged in a Sorvall RC2-B centrifuge at 12,061 x g for 10 min and a 10-ml aliquot of the supernatant diluted 25-fold. After combining 2-ml aliquots with reagents absorbance readings at 340 nm were determined on a Perkin Elmer model 550 spectrophotometer and the concentration calculated.

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Determination of invertase activity in Black Republican fruit

Presence of invertase activity in the Black Republican frozen fruit sample was determined from glucose analysis of the following samples: (1) 10.0g cherry fruit; (2) 10.0g cherry fruit plus 5.0g sucrose; (3) 5.0g sucrose. All samples were made up to 100 ml with pH 4.5 acetate buffer and incubated at 50°C for 25 min. Glucose determinations were made using the enzymic procedure.

RESULTS & DISCUSSION

FIG. 1 SHOWS typical HPLC chromatograms of cherry extract samples and Table 1 lists the free sugar and sorbitol content of all samples as determined by HPLC. The fruit samples had invert sugar patterns, containing nearly equal quantities of glucose and fructose. Sorbitol was found in

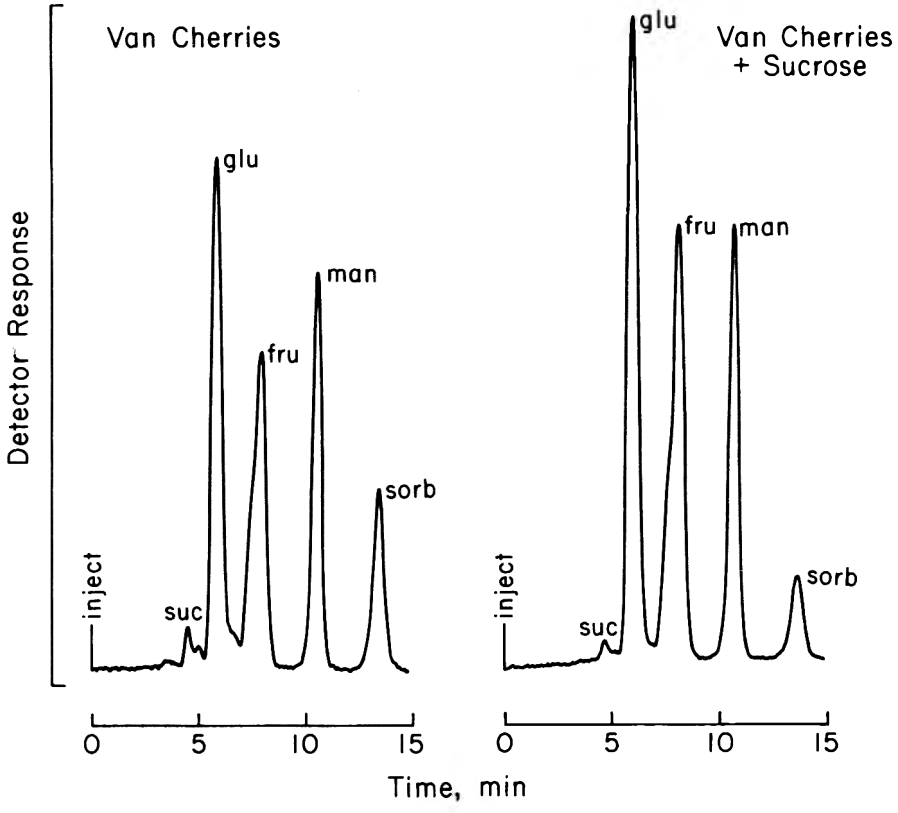


Fig. 1—HPLC chromatogram of extracts of Van cherries and Van cherries plus added sucrose. Column = Aminex HPX-87; mobile phase = water containing 0.01% $CaCl_2$; flow rate = 1 ml/min; injection volume = 20 µl. Typical retention times: Sucrose (suc), 4.70 min; glucose (glu), 5.98 min; fructose (fru), 8.08 min; mannitol-internal standard (man), 10.65 min; sorbitol (sorb), 13.53 min.

Table 1—Free sugars and sorbitol of cherry samples determined by HPLC[a]

Sample	Fructose g/100g	Fructose % C.V.	Fructose % T.S.	Glucose g/100g	Glucose % C.V.	Glucose % T.S.	Glu/Fru	Sucrose g/100g	Sucrose % C.V.	Sucrose % T.S.	Total Sugars g/100g	Sorbitol g/100g	Sorbitol % C.V.	Sorbitol % T.S.+S
Van fruit	6.38	5.01		7.32	1.78			0.27	3.70			2.61	3.45	
	6.80	1.32		7.55	1.19			0.26	3.84			2.57	3.89	
Mean	6.59	4.50	46.1	7.44	2.15	52.1	1.13	0.26	3.84	1.8	14.29	2.59	1.16	15.3
Black Republican fruit	6.66	3.15		8.04	2.11			0.16	18.7			3.94	1.01	
	6.93	1.44		8.31	0.48			0.15	13.3			3.88	5.41	
Mean	6.79	2.79	44.9	8.18	2.32	54.1	1.20	0.16	6.25	1.1	15.13	3.91	1.02	20.5
Van Fruit + Sucrose	14.99	0.93		15.55	1.03			0.04	b			1.92	7.81	
(Lot 1)	14.87	0.27		15.26	0.46			0.08	b			1.99	9.04	
Mean	14.93	0.50	49.6	15.40	1.29	51.2	1.03	0.06	50	0.2	30.09	1.96	0.05	6.5
Van Fruit + Sucrose	14.64	0.27		15.04	0.66			0.16	b			2.26	6.64	
(Lot 2)	14.55	2.09		15.34	2.48			0				2.10	8.57	
Mean	14.75	1.01	49.1	15.19	1.38	50.6	1.03	0.08	b	0.3	30.02	2.18	5.04	6.8
Black Republican fruit	14.03	2.85		14.73	2.38							2.85	9.82	
+ Sucrose (Lot 1)	14.55	4.54		14.99	2.80							3.02	6.29	
Mean	14.05	7.11	48.6	14.86	1.21	51.4	1.05	0		0	28.91	2.93	4.09	9.2
Black Republican fruit	12.95	1.16		13.99	0.79							3.08	2.59	
+ Sucrose (Lot 2)	12.97	1.54		13.74	2.69							2.76	10.14	
Mean	12.96	0.10	48.3	13.86	1.30	51.7	1.07	0		0	26.82	2.92	7.88	9.8
Syrup from Black	21.02	3.28		21.22	2.59							3.23	10.52	
Republican (Lot 1) (1978)	21.02	1.09		21.41	1.86							2.91	10.31	
Mean	21.02	0	49.6	21.31	0.61	50.3	1.01	0		0	42.33	3.07	7.49	6.8
Syrup from Black	22.26	2.92		23.22	2.24							3.18	10.69	
Republican (Lot 2) (1978)	22.13	0.95		23.16	0.99							3.30	4.84	
Mean	22.19	0.49	48.9	23.19	0.17	51.1	1.04	0		0	45.38	3.24	2.47	6.7
Syrup from Black	21.89	3.65		22.80	1.80							3.22	7.45	
Republican (Lot 3) (1978)	20.98	2.95		22.12	2.03							3.28	6.40	
Mean	21.44	2.98	48.8	22.46	2.14	51.2	1.05	0		0	43.90	3.25	1.23	6.9
Syrup from Black	19.38	2.89		19.64	3.56			0.15	b			3.52	9.37	
Republican (Lot 4) (1976)	18.68	0.58		19.03	0.68			0.11	b			3.38	1.77	
Mean	19.03	2.57	49.4	19.34	2.22	50.2	1.02	0.13	23.1	0.3	38.50	3.45	2.89	8.2
Syrup from Black	20.57	1.31		21.61	0.46			0.18	b			3.68	5.16	
Republican (Lot 5) (1977)	20.25	1.63		21.14	1.47			0				3.54	12.99	
Mean	20.41	1.12	48.7	21.37	1.54	51.0	1.05	0.09	b	0.2	41.87	3.61	2.77	7.9

[a] % C.V. = percent coefficient of variance; %T.S. = percent total sugars; total sugars = glucose + fructose + sucrose; %T.S. + S = percent total sugars + sorbitol; glu/fru = glucose: fructose ratio.
[b] Sucrose was detected in some analyses and not in others.

large amounts while only small quantities of sucrose were found. This compositional pattern is consistent with the sugar and sorbitol content for sweet cherries as recently compiled from the literature by Wrolstad and Shallenberger (1981). The fructose, glucose, sucrose and sorbitol content, the glucose:fructose ratio, and the total sugar content of the Van and Black Republican samples are within one standard deviation of the mean values reported in the literature compilation. There is a greater difference between the sorbitol contents of Van and Black Republican varieties (15.3 and 20.5% of total sugars plus sorbitol, respectively) than there is between fructose, glucose, and sucrose contents.

The most striking information in Table 1 is that either no or a very low level of sucrose was detected in the samples to which sucrose had been added. Clearly, sucrose hydrolysis is occurring during processing and/or storage. Glinskaya and Shkurina (1969) have previously reported invertase activity in cherry fruit. The results presented in Table 2 demonstrate the presence of invertase activity in the Black Republican fruit sample where 58% inversion of sucrose occurred after 25 min incubation. It is highly likely that invertase activity prior to freezing, during storage, and/or during thawing accounts for there being little or no sucrose in the various samples.

The glucose:fructose ratio in Van and Black Republican fruit, 1.13 and 1.20, respectively, is very close to the mean value for sweet cherries, 1.17 (standard deviation = 0.357), compiled from the literature (Wrolstad and Shallenberger, 1981). Sucrose hydrolysis in the samples containing added sucrose lowered the glucose:fructose ratio to values ranging from 1.01–1.07, which is still within the range reported in the literature. The glucose:fructose ratio has very limited value in detecting added sucrose in these samples as the fruit itself has close to an invert pattern.

Cherry fruit is very high in sorbitol, but there can be considerable quantitative variation with variety and season. The sorbitol content of Van and Black Republican cherries in this study was 15.3 and 20.5% of total sugars plus sorbitol, respectively. Neubeller and Stosser (1977) studied the sugar and variation of 20 varieties of sweet cherries over 3 yr and reported the sorbitol content varied from 15.5 – 24.0% of total sugars plus sorbitol. Sucrose addition has a dilution effect on sorbitol content; the percent sorbitol content in all samples with added sucrose is reduced from the percent sorbitol of the fruit samples by more than one standard deviation of sorbitol variation as compiled from the literature (Wrolstad and Shallenberger, 1981). The low percent sorbitol content of the samples with added sucrose (6.5–9.8%) is the best indication in this study that those samples are not derived 100% from fruit. Since the fruit samples with added sucrose were packed as 3.6 parts fruit and one part sucrose, the percent sorbitol would be estimated to be 5.8 and 8.4% for the Van and Black Republican samples, respectively. This is somewhat lower than was actually measured in all four lots of fruit packed with sucrose. Nonuniform distribution of sucrose in the drums and nonrepresentative sampling might account for this discrepancy. The syrup concentrates were produced from the free-run juice of the sugar packed fruit. The percent sorbitol levels of the three lots produced from 1978 season Black Republican fruit is lower than the 8.4% level of sorbitol estimated to be the percent sorbitol content of the sugar packed fruit. These results suggest that there is not complete diffusion of the sorbitol from the fruit to the juice and the free-run juice from which the concentrate is made contains a somewhat higher proportion of sucrose than the sample as a whole.

Table 2–Sucrose Inversion by Black Republication Fruit

Sample	Fruit (g)	Sucrose addition (g)	Measure glucose (g)
1	10	0	0.76
2	10	5	2.44
3	0	5	0.16

$$\% \text{ inversion} = \frac{\text{sample 2 glucose} - (\text{sample 1} + \text{sample 3 glucose})}{\text{glucose yield from 100\% hydrolysis of added sucrose}}$$

$$\times 100 = \frac{1.52\ \text{g}}{2.63\ \text{g}} = 58\%$$

Duplicate runs were carried out for all samples analyzed by HPLC and the value for each duplicate is shown in Table 1. The percent coefficient of variance (%C.V.) listed for each replicate represents the variation among the three HPLC injections of the sample extract. The %C.V. for individual sample extracts was less than five percent, except for some samples of low sucrose or sorbitol content. The %C.V. listed for the mean value of each sample is the variation between the two duplicates and would represent variation due to sampling, extraction, and isolation. Variation between duplicates is low, being less than five percent for most samples.

Table 3 lists the free sugar and sorbitol content for five of the samples as determined by enzymic methods. Duplicate runs were not made and the %C.V. represents the variation between analytical determinations for the same extract. This variation is low. Sucrose was not detected in any of the five samples by the enzymic procedure. As the enzymic assay for sucrose measures the increase in glucose concentration after sucrose hydrolysis, it will not be a sensitive procedure for determining trace amounts of sucrose in presence of large quantities of glucose. Results for fructose, glucose, and sorbitol content as determined by the enzymic (Table 3) and HPLC methods (Table 1) are very similar. The correlation coefficient for fructose content as determined by the two methods for the same five samples is 0.994; for glucose the correlation coefficient is 0.997 and for sorbitol it is 0.847. *–Continued on page 290*

Table 3–Free sugars and sorbitol of cherry samples determined by enzymic analyses[a]

Sample	Fructose			Glucose				Sucrose			Total	Sorbitol		
	g/100g	% C.V.	% T.S.	g/100g	% C.V.	% T.S.	Glu/Fru	g/100g	%C.V.	%T.S.	Sugars	g/100g	%C.V.	%TS+S
Van fruit	6.13	1.00	48.3	6.62	0.46	51.9	1.08	n.d.		0	12.7	2.65	2.64	17.2
Black Republican fruit	6.61	0.78	45.9	7.82	0.78	54.2	1.18	n.d.		0	14.4	3.61	6.46	20.0
Van fruit + Sucrose (Lot 1)	13.48	0.46	48.3	14.42	0.85	51.7	1.07	n.d.		0	27.9	2.52		8.3
Black Republican fruit + Sucrose (Lot 1)	13.00	1.89	48.3	13.92	1.97	51.7	1.07	n.d.		0	26.9	3.05	0.76	10.2
Syrup from Black Republican + Sucrose (Lot 1)	21.22	0.58	49.7	21.5	0.28	50.4	1.01	n.d.		0	42.7	3.70	1.57	8.0

[a] %C.V. = percent coefficient of variance; % T.S. = percent total sugars (glucose + fructose + sucrose); % T.S.+S = percent total sugars + sorbitol (glucose + fructose + sucrose + sorbitol); glu/fru = glucose:fructose ratio; n.d. = not detected.

Economic Comparison of Canning and Retort Pouch Systems

J. R. WILLIAMS, J. F. STEFFE, and J. R. BLACK

ABSTRACT

The economic feasibility of using the retort pouch for processing, packaging and distributing processed fruit and vegetable products during a period of rising energy prices was examined. A retort pouch packaging system was found to be the overall minimum cost packaging system among three packaging systems considered—an existing canning line, a new canning line, and a retort pouch line. Although the costs associated with acquiring and maintaining the durable machinery for retort pouch processing are significantly higher than for a new canning system or an existing canning system, other operating expenditures considered in the packaging systems are considerably lower. Lower freight costs, attributed to the lighter weight and smaller volume of pouches and the comparatively lower purchase price of retort pouches than cans, are the major contributors to the cost effectiveness of the retort pouch packaging system. Energy savings in processing the pouch versus the can are of little significance, but the comparatively lower amount of energy used in transportation and container manufacture has an important role in the cost effectiveness of the retort pouch packaging system.

INTRODUCTION

FOOD PROCESSING INDUSTRIES are relatively energy intensive in their operations and presently use a greater amount of energy per dollar of value added to finished product in their production processes than any other sector of the food system. Development of economical technologies that directly and indirectly reduce consumption of energy are important to food processing and other food system sectors. The retort pouch which is a multilayer flexible package consisting mainly of polypropylene, aluminum foil and polyester has been a prominent candidate in considering energy saving technology for use in food processing and distribution. Exploratory research on thermal processing of food in retort pouches was first conducted in the mid to late 1950's (Lampi, 1977). Pouch processing technology moved ahead rapidly and commercial retort pouch processing systems are currently available from a number of equipment manufacturers. Retortable pouches are used extensively in foreign markets and have been adopted by the United States Department of Defense as an alternative to the traditional C-ratio (Bannar, 1979). Studies by Hoddinott (1975), Anon. (1978), and Steffe et al. (1980) indicate retort pouch packaging systems offer savings of energy in production, food processing, transportation, and home preparation of food products. Additionally the retort pouch is currently cheaper to purchase and transport than its comparable size counterpart, the metal food can.

Although the retort pouch has unique advantages as a substitute package, the question of wheter it can be economically competitive with the can remains as the key to its widespread adoption in the processed fruit and vegetable industry. This study addresses the economic feasibility of adoption of the retort pouch as a processed fruit packaging system. A previous study (Steffe et al., 1980) focused only on the difference in energy utilization and container and energy costs for the can and retort pouch packaging systems. Direct energy savings do occur with the use of retort pouches as substitutes for cans; however, investment in retort pouch technology cannot be justified strictly on reduced energy costs and flows alone. This study also includes such key nonenergy costs as container prices, transportation charges, labor rates and costs associated with purchasing and maintaining the operation of processing and packaging machinery for can and retort pouch packaging systems. These costs were used to determine the minimum cost packaging system for processed peaches. Three basic processing systems were considered: an original canning line in an operating plant, a new or replacement canning line, and a retort pouch line. The design of the systems for evaluation centered around a case study (Carroad et al., 1980) of energy utilization in a peach processing plant. That study documented the energy consumption related to the unit operations involved in canning clingstone peaches. Peaches are quite commonly canned in the U.S. for retail sale and the processing and packaging operations involved are representative and common to other fruit and vegetable processing systems. Therefore, the results of this study should be general enough to make some basic conclusions concerning the economic feasibility of retort pouches for processing and packaging a wide variety of fruits and vegetables.

The major objective of this study is to evaluate the economic feasibility of retort pouches for processing, packaging and distribution of processed fruit and vegetable products. More specific objectives of the study are to compare the costs associated with:

(1) Purchasing processed food packaging containers, specifically cans, flexible retort pouches and cartons of retail size for packaging fruit and vegetable commodities.

(2) Transportation of these containers from the package producer to the food processor.

(3) Processing and packaging fruits and vegetable products in the alternative packages.

(4) Transportation of product to wholesale distribution centers from the processing location.

DESCRIPTION OF EXISTING AND ALTERNATIVE PACKAGING SYSTEMS

IN THIS STUDY operating costs are of primary concern. Three packaging system models are used to estimate the costs associated with each packaging system. These models were developed specifically for estimating the costs of processing, packaging, and transporting fruits and vegetables in accordance with the existing canning system, a new canning system and a retort pouch system.

Processing

The processing component of the model used in the economic evaluation is based on a typical fruit processing plant. A peach processing plant was selected because its many individual processing operations are typical of other

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fruit and vegetable processing and packaging operations. More importantly energy consumption and flow data to individual processing operations were available. Such data were necessary to determine if retort pouch adoption are particularly sensitive and comparably advantageous to cans under conditions of rising energy prices.

The costs of three basic processing systems were considered. Cost information including energy costs for operations common and equivalent to each system were ignored: raw product receiving, washing, pitting, peeling, grading, and waste removal. These operations provided no cost or energy saving incentive for adopting one of the alternate systems under consideration. The operation costs and efficiency of the steam delivery system (boiler efficiency, etc.) were also ignored.

The basis for comparing the costs of the three systems was a constant mass flow rate of processed peaches in 303 x 406 cans (8.1 cm diameter, 11.1 cm length) with a line speed of approximately 360 cans/min. Assuming a drained weight of 292g (10.3 oz) per can and full capacity output, the existing canning line would produce 50.45 metric tons of processed peaches in an 8-hr shift. Cost estimates for the various packaging alternatives are based on this level of output. The equipment complements for the new canning and retort pouch lines were selected so that peaches could be processed at the same rate achieved in the original canning line, i.e. 360 cans/min and 360 pouches/min. A summary of the equipment required, capital expenditure, labor and energy requirements for the processing alternative is illustrated in Table 1.

The equipment specified in the new canning and pouch lines is typical of the commercial equipment currently available on the U.S. market. The replacement canning line required one filler, two syrupers, one can closer, and two continuous retorts to achieve a processing rate of 360 cans/min. This new equipment required a total of three units of direct operator labor per 8 hr–shift and required a capital expenditure of approximately $690,000 in 1980. Retort pouch equipment is considerably more expensive and labor intensive than canning equipment. Retort pouch packaging equipment specified included eight form/fill/seal machines, four batch retorts, six dryers, and two cartoners at a cost of approximately $3,396,000. Total labor use was 20 times as great: 62 persons per 8 hr–shift.

Electrical energy use in the original canning line was calculated directly from the data reported by Carroad et al. (1980); data manipulation only required an adjustment for product flow rate. The electrical energy use of the filling, syruping and can closing machines required in the replacement canning line was calculated from the electrical ratings specified by the manufactures. This procedure was also followed for the form/fill/seal machines and the cartoners suggested for the new pouch line.

Thermal energy requirements for the original canning line were calculated from the Carroad et al. (1980) data by the method described above for the electrical energy use. Steam requirements for "steam closure" in the new canning line and in the form/fill/seal machine used in the retort pouch line were calculated from manufactures information. Retorting energy requirements in the original canning line and the new canning line were the same because the methods of operation and food volume processed were not altered.

It is generally presumed that the thermal energy requirements for retorting pouches is significantly less than that required for comparable cans. This is due to the fact that the pouch has a geometry which is more favorable to heat transfer than the can. Manufactures vary greatly in their estimates regarding process time reductions for food contained in pouches as opposed to cans. These values may range from 30–70% when an equal mass of food is being cooked. Because there is a great deal of uncertainty surrounding the use of these estimates a more conservative energy consumption estimate for pouch retoring was used for the economic analysis reported in this study. The thermal energy use for pouch retorting is initially assumed to be equipvalent to the amount used in processing cans. In addition, it was assumed that there would be no significant energy saving advantage in the pouch retorting process for peaches because the replacement of cans for pouches would involve switching from two continuous rotary retorts to four batch-type retorts for processing the same number of containers per minute. Therefore, there appears to be little if any, comparative energy saving advantage when switching from an agitating can retort to a batch retort for processing pouches. More research is needed before an accurate evaluation of retorting energy requirements can be made.

In order to illustrate the sensitivity of the results to the amount of energy used in pouch retorting the costs were also estimated under the conditions that pouch retorting used 75% less energy than cans. As will be pointed out in more detail later, the above assumption did not significantly affect the decision to adopt retort pouch or can processing technology.

Containers

Cost estimates (1980$) were obtained for 303 cans (three-piece steel with tinplate), pouches and pouch cartons. An advantage of pouch processing of products which require liquid fill is that the amount of syrup or brine may be reduced because of the ability of the flexible package to conform to the geometry of the material it contains. The idea of liquid fill reduction was incorporated into the pouch processing system by assuming a syrup weight reduction of approximately 30% in each pouch. This resulted in the estimated net weight of the pouch contents to be 341g (12 oz). Based on the net contents a pouch size of 15.3 cm x 20.3 cm (6 inch x 8 inch) would be required. The pouch cartons are made of fiberboard and designed to contain a single pouch. Nominal carton dimensions required for a filled 15.3 cm x 20.3 cm pouch are 1.9 cm x 14.6 cm (0.75 inch x 5.75 inch x 8.00 inch). The comparative weight for the contents of the 303 x 406 cans was estimated to be 431g (15.2 oz).

Transportation of empty and full containers

Transportation costs were estimated for moving empty cartons, pouches, and cans from package manufacturers to processor. Transport costs were also estimated for moving the finished processed product from processor to wholesale distribution centers. A distance of 765 miles was used as the transportation mileage. This value was reported by Barton, (1980) as the average mileage for moving manufactured food products from processing plants to the wholesale warehouses. The 765 mile figure was also used as the transportation mileage of empty containers. These costs are estimated for moving materials in trucks having a gross vehicle weight of 31,752 kg (70,000 lb) with a potential 20,049 kg capacity (44,200 lb) using diesel fuel.

THE ECONOMIC MODEL

AN ECONOMIC MODEL which consists of a capital budgeting simulator is used to estimate the costs associated with the alternative packaging systems. The total amortized value of the costs associated with the respective packging systems was estimated with the variables presented in Eq (1) and (2). All costs, which are explicitly a function of the age of the packaging equipment, were estimated with Eq (1). The other operating cost components of the

respective systems were estimated by Eq (2). Eq (3) is simply the aggregate value of the two preceding equations. The aggregate cost estimate for each new alternative packaging system, i.e. retort pouches and cans, can be compared with each other and with the amortized present value of costs for operating the existing canning system for any selected number of production periods.

$$APVFE_N = \frac{r}{1-(1+r)^{-N}}\left(C_o - C_N(1+r)^{-N} + (1-T)\left[\sum_{k=1}^{N} R_k(1+r)^{-k}\right] - T\left[\sum_{k=1}^{N} D_k(1+r)^{-k}\right] - I_N(1+r)^{-1} + T[BC_k(1+r)^{-k}]\right) \quad (1)$$

$$APVNE_N = \frac{r}{1-(1+r)^{-N}}\left((1-T)\left[\sum_{k=1}^{N} E_k(1+r)^{-k} + \sum_{k=1}^{N} L_k(1+r)^{-k} + \sum_{k=1}^{N} IN_k(1+r)^{-k} + \sum_{k=1}^{N} P_k(1+r)^{-k} + \sum_{k=1}^{N} FBP_k(1+r)^{-k} + \sum_{k=1}^{N} FAP_k(1+r)^{-k}\right]\right) \quad (2)$$

$$TAPV_N = APVFE_N + APVNE_N \quad (3)$$

where:
- $APVFE_N$ = the amortized present value of costs which are a function of the age of the packaging equipment for N years.
- $APVNE_N$ = the amortized value of costs associated with the packaging system which are not a function of the age of the packaging equipment for N years.
- $TAPV_N$ = the total amortized value of all costs associated with the packaging system for N years.
- r = after tax discount rate.
- C_o = acquisition cost of the packaging equipment.
- C_N = value of packaging equipment at the end of the Nth year in constant dollars.
- T = the marginal income tax rate.
- R_k = maintenance cost in the kth year in constant dollars.
- D_k = depreciation in k^{th} year.
- I_N = investment credit which can be taken in the first year of ownership.
- BC_k = balancing charge which adjusts for the possible difference between resale value and depreciated book value.
- E_k = costs associated with energy use in k^{th} year in constant dollars.
- L_k = costs associated with labor use in k^{th} year in constant dollars.
- IN_k = insurance cost in k^{th} year in constant dollars.
- P_k = package cost in k^{th} year in constant dollars.
- FBP_k = freight cost for transporting packages to processor in k^{th} year in constant dollars.
- FAP_k = freight cost for transporting processed product after processing in k^{th} year in constant dollars.

Although all variables in Eq (1) are applicable to the new replacement packaging systems, only maintenance costs and the discount rate appear in Eq (1) as nonzero values for the existing canning system. All variables in Eq (2) apply to the existing canning system. The cost variables in Eq (1) and (2) are a function of time; many of them represent actual cash expenditures. However, some of these, such as depreciation, investment credits, and balancing charges are noncash expenditures. These items must be considered in the analysis for adjusting actual cash expenditures for tax purposes. Cost estimates are adjusted to an after tax basis to select the packaging system with the lowest after tax costs.

Reasonable forecasts of the values of the variable that account for the cash and noncash flows are crucial but difficult to determine. The techniques for forecasting future values are based on historical trends and assumptions about the future. Although future estimates are uncertain the ones described here have been selected as appropriate and reasonable for the analysis. Any reference to a real percentage increase in costs or prices indicates the percentage increase in addition to any general inflation trends. For example an annual real increase of 1% in wages could indicate that wages increased by 12% for the year when the annual inflation rate was 11%.

Capital expenditure

The costs of purchasing new equipment in 1980 (Table 1) were based on primary data collected from a variety of equipment manufactures.

Salvage value

Salvage value of the equipment is assumed to decline with each production period. Coen (1975) suggested that the pattern of economic depreciation of equipment used in the Food and Kindred Products Industry most closely follows a sum-of-year's digits pattern. The average annual salvage value declines by an amount equal to the acquisition cost multiplied by the ratio of the remaining years of the equipment's useful life over the sum of all numbers from one through the estimated useful life. This method is used to determine the salvage value of the new canning and retort pouch processing equipment until the equipment is 15 years old. A salvage value of zero is assumed when the equipment is 15 years old or older. The salvage value of the existing equipment in the old canning lines is assumed to be zero because most of it is substantially older than fourteen years.

Maintenance

Maintenance costs are estimated for the 30-day operating period used in this study. Maintenance costs for the existing canning equipment for 1980 are based on actual operating expenditures. New canning equipment maintenance costs for 1980 are estimated at a rate which is equivalent to 2% of the original purchase cost on an annual basis. Retort pouch machinery maintenance costs for 1980 are estimated at a rate which is equivalent to 7% of the original purchase cost on an annual basis.

Depreciation and balancing charge

Straight-line depreciation was used to calculate noncash depreciation expense over a 10-yr period, the shortest depreciation year period allowed by tax regulations for industries involved in manufacturing food products. The balancing charge allows for the tax implication of depreciation recapture if, upon resale of the equipment it received a value greater than its depreciated book value.

Table 1—Equipment, capital expenditures, labor and energy requirements for processing alternatives

Operation	Number of units	Capital expenditure 1980 $	Labor required per unit	Total labor per 8-hr shift	Electrical energy use KWH's per 8-hr shift	Thermal energy use BTU's per 8-hr shift
Existing equipment						
Fillers	2	N.A.	1	2		
Seamer-Syruper	2	N.A.	1	2	16.17	
Continuous retorts	2	N.A.		2	26.95	81286450
Total				6	43.12	81286450
New canning equipment						
Filler	1	$ 40,000		0	6.0	
Syruper	2	$ 130,000		0		
Can closer (Steam Closure)	1	$ 60,000	1	1	60.0	4457950
Continuous retorts	2	$ 460,000	1	2	26.95	81286450
Total		$ 690,000		3	92.95	85744400
New retort pouch equipment						
Form/Fill/Seal	8	$2,400,000	1	8	1280.0	7132720
Retorts	4	$ 660,000		1		81286450
Loading/Unloading			4	16		
Dryer	6	$ 36,000		0	268.2	
Cartoner	2	$ 300,000		1	53.7	
Additional inspection				36		
Total		$3,396,000		62	1601.9	88419170

Investment credit

A credit for investment in depreciable property against federal income tax is allowed. This study assumes that an investment tax credit of 10% would be taken the year the equipment was acquired, if it is to be kept in production seven years or longer.

Energy expenditures

Energy consumption (KWH's per 8-hr shift) for the processing alternatives is described in Table 1. The thermal energy consumption measured in BTU's is priced as a BTU of natural gas. The prices of energy used for 1980 are national averages for industrial users. For cost projections, a 5% annual real rate (inflation adjusted), of increase in energy prices (1980$) was used (Table 2).

Labor expenditures

Labor costs were calculated by multiplying the labor charge per hour by the hours of employed labor on the food processing line. The 1980 annual average labor charge for the food and kindred products industry is estimated to be $7.02/hr. Costs associated with labor use in years beyond 1980 are projected to remain at the 1980 level in real dollars.

Insurance costs

Cash outlays for casualty insurance based on replacement value for the processing equipment are included in the analysis and calculated as 1% of the acquisition cost of the processing equipment in each subsequent year. Acquisition costs of new processing equipment are increased at a real rate of 1% per year, and is based on the historical trend (1975–1979) for costs of food product machinery.

Expenditure for containers

Prices for the containers being evaluated were obtained from package manufactures for 1980. In addition it is necessary to project package costs for several years into the future because they are an important component of the cash costs in the packaging system under evaluation. Historical trend data collected since 1973 were used to project the annual real rate of increase in the cost of the respective containers (Table 2). Metal can prices have been increasing at an average annual real rate of 3.28%. Retort pouch material is estimated to increase at an average annual real rate of 0.94%. Carton costs were assumed to remain constant in real dollars, although the historical trend indicated that they have been declining in real terms in recent years. This presents a restrictive case for comparison.

Table 2—Prices used for estimating costs

	Price (in 1980 dollars)	
	1980	1985
Cans[a]	$119/1000 Cans	$140/1000 Cans
Pouches[b]	$80/1000 Pouches	$84/1000 Pouches
Cartons	$25/1000 Cartons	$25/1000 Cartons
Electrical[c]	3.87 ¢/kWh	4.93 ¢/kWh
Natural gas[d]	$2.60/1000 ft^3	$3.32/1000 ft^3
Diesel truck fuel[e]	$1.20/gal.	$1.53/gal.

[a] Based on trend of 303 x 406 cans index in Producer Price Index Code 1031.0101, 1973–79. 1980 price information from National Can Company, Chicago.
[b] Based on trend data supplied by FMC 1973–78. 1980 price information estimated from data received from Reynolds Metals & American Can Co.
[c] Estimated from trend in prices from 1973–79. 1980 price assumes a 15% rate of inflation in CPI for 1980 over 1979. Price information from Monthly Energy Review U.S.D.O.E. 1985 price estimated using a 5% annual increase in constant dollars.
[d] Estimated from trend in prices from 1974–79. 1980 price assumes a 15% rate of inflation in CPI for 1980 over 1979. Price information from Monthly Energy Review U.S.D.O.E. 1985 price estimated using a 5% annual increase in constant dollars.
[e] 1980 price of diesel fuel estimated from 1978–79 trend assuming a 15% rate of inflation in CPI for 1980 over 1979. 1985 price estimated using a 5% annual increase in constant dollars.

Transportation costs

Freight rates were obtained from transport companies for shipping both packaging materials and processed commodities for 1980. Future years transportation rates are estimated by increasing the 1980 dollar value of transportation costs associated with energy consumption by the

forecasted annual real increase in diesel fuel prices. It is assumed that all other costs except for the energy cost component of freight charge will remain constant in real 1980 dollars although they will increase in nominal dollar terms. Therefore, the only factor which causes a real cost increase in transportation rates is the price of diesel fuel.

Discount rate

The real after tax discount rate used in the analysis is based on the interest rate on long term commercial and industrial loans, and is deflated by the gross national product deflator. The after tax discount rate is 1.07%.

Tax rate

The marginal income tax rate considered appropriate for this analysis was 46%. The tax rate is based on corporations that have a taxable income which exceeds $100,000 per year.

RESULTS OF ANALYSIS

EVALUATION of the alternative processing and packaging systems using an annual real rate of energy price increase at 5% indicated that the retort pouch processing system was the minimum cost system under the condition that it was operated for 13 or more years. Fig. 1 illustrates the present value (1980$) of the average annual cost (amortized costs) of operating the alternative packaging system from 1 through 40 yr. The total average annual costs estimated from Eq (3) for the retort pouch packaging system for a period of 20 yr are $1,048,450, which compares with $1,184,865 and $1,200,202, respectively, for the existing canning system and the new can packaging system. The estimated cost advantage of the retort pouch system using the above estimate is $13.15/1000 units when compared with the existing canning system and $14.63/1000 units when compared with the new can packaging system.

Although the costs associated with obtaining and maintaining the equipment complement for retort pouch processing are higher than that of the other systems, the other variable costs associated with the retort pouch system are lower than the other alternative packaging systems considered. The average annual costs for obtaining the maintaining the equipment complement in the retort pouch system for a period of 20 yr is $11.24/1000 units packaged, more costly than the existing canning system and $9.30/1000 units more expensive than the new canning system alternative. However, average annual costs over a 20-yr period for all other operating expenses considered indicated that the retort pouch system is $23.93/1000 and $24.39/1000 units of packaged product less expensive than the new canning system and existing canning system respectively.

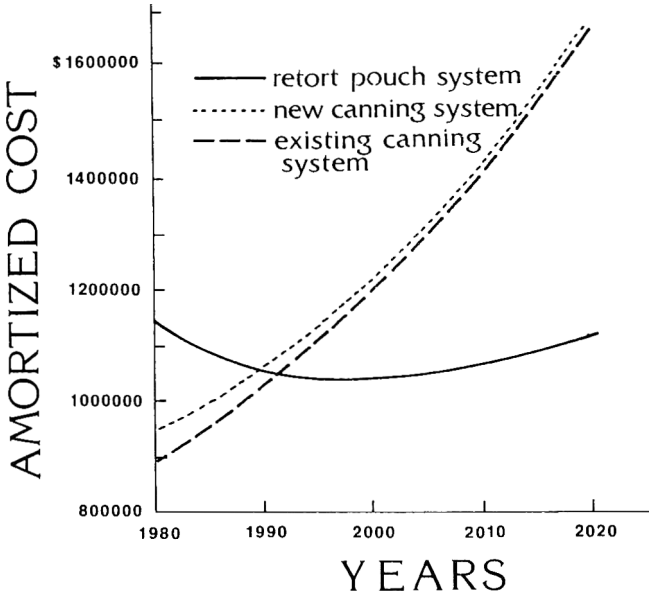


Fig. 1—Average annual cost (1980$) of all costs as a function of the number of years the system is operated.

Two major factors contribute to the increasing advantage of retort pouch packaging with time. Package costs increase less rapidly than the rate at which can costs increase for the canning system alternatives. Secondly, retort pouch processing and packaging systems are less energy intensive than either a new can packaging or an existing can packaging system (Steffe et al., 1980). Therefore, as energy prices increase, the difference between the total costs associated with the can processing and packaging systems and the retort pouch packaging systems gets larger. Further, the advantage of retort pouch processing becomes greater the faster energy prices increase. Table 2 illustrates the actual prices used for estimating the costs in 1980 and 1985. Table 3 illustrates the actual variable production costs, not associated with the age of equipment, for the three processing systems. Variable operating costs and the percentages individual cost categories account for in each alternative are illustrated for 1980 and 1985. The results indicate that package costs are the largest component of the variable costs for operating the packaging systems (Table 3). Although pouches and cartons account for a larger percentage of the total variable costs of the retort pouch packaging system than cans account for in the canning system alternative, the actual expense for the container is significantly less. Freight costs associated with transportation of the processed commodities accounted for the second largest amount in all systems. Again, freight costs of the retort pouch system were less. Energy used in processing accounted for a small amount of the total variable costs, therefore, the results of the comparative analysis are quite insensitive to the level of energy used in the processing of the retortable pouches. Further, the thermal energy used for pouch retorting was assumed to be equivalent to that amount used in processing cans even though manufacturers of processing equipment estimate that process time for an equal mass of food contained in pouches may range from 30–70% of the time required for processing of cans.

In order to test for the sensitivity of the results as a function of the amount of energy used in pouch retorting the costs were re-estimated under the condition that pouch retorting would only require 25% of the thermal energy used in retorting cans in the existing system. The results of the sensitivity analysis revealed that the cost was reduced by $0.81/1000 units processed. Any interest in retort pouch packaging as an alternative to can packaging because of its potential energy saving advantage in the retorting process appears to be economically unwarranted when compared with how other items effect the cost of retort pouch packaging systems.

Labor costs were higher and contributed to a larger percentage of variable costs in the retort pouch system than in the canning systems. If the real wage rate were to increase over time the cost advantage of the retort pouch system would be reduced.

The retort pouch packaging system is the overall minimum cost system among the alternatives considered given the acquisition and operating requirements described. Although costs associated with acquiring and maintaining the durable machinery complement for retort pouches is significantly greater than that of either a new canning equipment

complement or the existing canning system alternative, the other operating expenditures considered in the packaging system alternatives are less for the retort pouch system.

The cost of empty containers is the expenditure category which influences the analysis to the largest extent, and will be the major factor in explaining the difference in costs between retort pouch packaging systems and canning systems. If carton manufacturers and film and foil convertors are continually able to hold price increases of their products to a relatively lower rate than those of can manufacturers, the retort pouch system will have substantial cost advantages in this segment of the packaging system. The total cost of retort pouches and cartons used in this analyiss is $14 less per 1000 units than the cost of 1000 retail size cans. This is a significant factor in the base year (1980) and should prove to be even more significant over a period of years as real energy prices increase. Because the construction of the can is more energy intensive than that of the retort pouch and carton, rising energy prices will have a greater effect on the cost of production of cans than of retort pouches and cartons.

Retort pouches also have a particular advantage in the transportation sectors of the packaging system. In 1980, freight costs attributed to transporting empty retort pouches and cartons of retail size are 27% of the freight costs associated with transportation of empty 303 cans for an equivalent distance. Freight costs associated with shipment of the processed product in the retort pouch system are approximately 72% of those costs attributed to transporting the processed product in cans. This freight savings is attributed to the lighter weight of processed pouch products which is a result of the reduced brine requirements considered in this study. As freight costs and energy prices increase, the cost advantage which retort pouches hold in this area should increase because a smaller number of shipments and less energy are required for shipping the equivalent amount of product.

The results of the study are only valid for fruit and vegetable products which are currently packaged with brine or syrup. A reduction in the amount of brine needed in the package was used in estimating the size of the pouch for the retort pouch packaging systems. Less brine reduces the weight of the processed product for consideration in the calculation of freight costs. If the syrup weight for retort pouches is assumed to be equivalent to that of cans the cost advantage retort pouch products have in shipping is substantially reduced.

Although the amount of energy used in transportation and container manufacture may play an important role in the cost effectiveness of the retort pouch, the amount of energy used directly in food processing for products held in pouches versus cans appears to be of much less significance and does not significantly influence the results of the comparative cost analysis. Further details concerning sensitivity analysis related to other factors are being studied and will be reported in a forthcoming paper (Williams et al., 1981).

The preceding results illustrate the need for a broad systems evaluation of the adoption of retort pouches as a new packaging technology. Although the in plant costs may not be lower there are reduced costs to be derived from other sectors of the packaging system. Any analysis should consider the implication of retort pouch adoption from a food systems perspective.

The estimated investment and operating costs are not total system costs, because only those components of the packaging system not considered common and equivalent in terms of costs were considered. The results, should not be interpreted as actual total operating costs, but only a comparison of those costs associated with those parts of the packaging systems which are considered to be different.

OTHER ECONOMIC ISSUES

ALTHOUGH THE RESULTS reveal that the retort pouch has particular cost advantages in the subsectors of the food system considered in this study they must be interpreted carefully. A manager should consider these issues and the implications they may have on an evaluation that is conducted which concerns alternative packaging systems.

The results presented do not include comparative costs associated with cans and pouches for other subsectors of the food system such as retail marketing, home storage and

Table 3—Variable operating costs accounted for by cost category (1980$)

Cost category	Year	Existing canning system Actual	Existing canning system Percent	New canning system Actual	New canning system Percent	Retort pouch system Actual	Retort pouch system Percent
Labor	(1980)	$ 20,217	1.2	$ 10,108	0.6	$208,915	13.5
	(1985)	20,217	1.1	10,108	0.5	208,915	12.9
Packages							
Cans	(1980)	1,237,524	75.0	1,237,524	75.5	N.A.	
	(1985)	1,454,236	76.5	1,454,236	76.8		
Pouches	(1980)	N.A.[a]		N.A.		829,440	53.5
	(1985)					869,163	53.7
Cartons	(1980)	N.A.		N.A.		259,200	16.7
	(1985)					259,200	16.0
Freight							
Before processing	(1980)	84,395	5.1	84,395	5.1	23,224	1.5
	(1985)	87,861	4.6	87,861	4.6	24,742	1.5
After processing	(1980)	294,036	17.8	294,036	18.0	212,647	14.0
	(1985)	323,512	17.0	323,512	17.0	233,959	14.0
Processing energy							
Natural gas	(1980)	12,680	0.8	13,376	0.8	13,793	1.0
	(1985)	16,184	0.9	17,071	0.9	17,604	1.1
Electricity	(1980)	100	<.1	215	<.1	3,719	.2
	(1985)	127	<.1	275	<.1	4,747	.3

[a] N.A.—Not applicable

preparation, and disposal. Pouches would appear to have particular advantages in storage because of their lighter weight and cubic design. There may, however, be unforseen handling problems and additional marketing costs attributed to product promotion and consumer education which may need to be considered. Consumer reactions to products packaged with less brine or syrup need to be evaluated. Costs associated with development of the food product and technical processing characteristics may reduce the comparative costs advantage of the pouch. Initial research and development for pouch use and production start up costs could increase costs associated with pouch processing particularly when a pouch processing system was replacing an existing canning system. However, this difference in costs may be insignificant if the decision has already been made to replace an old canning line with a new processing and packaging system.

The price of the product is another important consideration. If retort pouch packaged fruit and vegetable products are of considerably higher quality than their canned counterparts and approach the quality of frozen fruits and vegetables, they may draw a relatively higher price in the market. A higher market price for pouches than cans would increase the attractiveness of the retort pouch packaging system for the processor, wholesaler, and retailer.

An additional issue that affects the results of the study is related to the replacement equipment complements that were considered. It was assumed that if retort pouch processing equipment replaced existing canning equipment, the machinery which moved the canned product from operation to operation in the processing line could also move retort pouches with a minimum of modification. The possibility of this assumption being correct would vary with the processing plants considered. The assumption was used because of the uncertainty surrounding the particular design and types of equipment available for use in these operations in any particular processing line. If the assumption is not valid the cost advantage attributed to the retort pouch system would be less. A somewhat more valid assumption is that the cost of acquiring and operating equipment which moves pouches or cans from one processing stage to another in a new processing line would be essentially equivalent although the actual equipment design may be quite different. If this is true, there is a greater level of confidence that the retort pouch processing system holds a cost advantage over a new canning system. The difference in costs estimated indicate the difference any additional costs would have to amount to for the retort pouch system to be a higher cost system than a canning system.

SUGGESTIONS FOR FUTURE RESEARCH

FURTHER QUESTIONS surrounding the use of retort pouches for packaging fruits and vegetables need to be investigated. Other types of research which are required as an input into further analysis of these questions is also needed.

1. Although the retort pouch appears to have a great deal of potential for institutional markets, further research is needed on the economic issues and problems of processing and handling large pouches in the distribution stages.
2. A better understanding of the relationship of total production costs to retort pouch product package size and length of production season would be valuable.
3. Both short and long-term evaluation of marketing costs and products desirability by retail marketers and consumers should precede investments in retort pouch processing systems.

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Oxygen Scavenging from Air in Package Headspaces by Singlet Oxygen Reactions in Polymer Media

MICHAEL L. ROONEY

ABSTRACT

Oxygen-sensitive foods are frequently packaged under vacuum or with inert-gas flushing. This paper describes a method for oxygen removal from air headspaces in transparent packs by a singlet oxygen reaction in a polymer film. Illumination of a package enclosing a coiled film containing a photosensitising dye and a singlet oxygen acceptor resulted in rapid scavenging of oxygen from the headspace. The results show that the oxygen content of plastic pouches containing air (30 ml) was reduced from 21% to 1% by 6 min illumination. The scavenging rate depended on the light intensity, and the rate of oxygen diffusion into the polymer.

INTRODUCTION

OXYGEN-SENSITIVE FOODS should be stored in packages with initial headspace oxygen contents reduced to 2% or less. Deoxygenated atmospheres are also important in areas outside food packaging such as anaerobic microbiology and free radical chemistry.

Inert atmospheres in package headspaces are usually generated by removal of air using evacuation and/or gas flushing before sealing the package. There have been very few attempts to replace the vacuum/gas-flush procedure with an in-pack deoxygenation system. Kamijo and Rikimaru (1975) used sachets containing hyposulfite, calcium hydroxide, and carbon to remove oxygen from pouches with water-saturated atmospheres. Yoshikawa et al. (1977) used iron, carbon and water as a scavenger system, which removed 13 ml of oxygen in 10 hours.

The existing oxygen scavenging techniques present problems due to the nature of the medium in which the reaction with oxygen occurs. Oxygen is removed by reaction either on particulate solids or in aqueous solutions, both of which present containment problems in food packages. Another problem is related to the reactivity of the oxygen molecule in its ground electronic state. Ground state oxygen has triplet multiplicity whereas most other molecules are in the singlet ground state. Thus ground state oxygen is relatively unreactive at room temperature so oxygen removal by conventional chemical means is slow, although ground state oxygen is reactive enough to cause off-flavor development in some foods over a period of weeks or days. The singlet excited state of oxygen which is obtained by dye sensitization of ground state oxygen using near infrared, visible or ultraviolet radiation, is highly reactive and so its chemical reaction with scavengers is rapid.

The aim of the present study was to investigate factors influencing the efficiency of oxygen scavenging from air headspaces by the singlet oxygen process. The scavenging system utilizes the high reactivity of singlet oxygen and uses a polymer film as the reaction medium. This is believed to be the first example of oxygen scavenging from air headspaces in sealed packages using organic compounds immobilized in polymer media.

The new technique involves sealing a small coil of ethyl cellulose film containing a dissolved photosensitising dye and a singlet oxygen acceptor in the headspace of a transparent package. On illumination of the film with light of the appropriate wavelength, excited dye molecules sensitize oxygen molecules, which have diffused into the polymer, to the singlet state. These singlet oxygen molecules in turn diffuse to react with acceptor molecules, which are essentially immobile in the matrix, and are thereby consumed. The scavenging process continues as long as the polymer is illuminated, and until all the headspace oxygen or acceptor has reacted.

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Photochemical scheme

The photochemical processes involved may be set out as follows:

$$\text{PHOTON} + \text{DYE} \rightarrow \text{DYE}^* \quad (1)$$

$$\text{DYE}^* + \text{OXYGEN} \rightarrow \text{DYE} + \text{OXYGEN}^* \quad (2)$$

$$\text{OXYGEN}^* + \text{ACCEPTOR} \rightarrow \text{ACCEPTOR OXIDE} \quad (3)$$

$$\text{OXYGEN}^* \rightarrow \text{OXYGEN} \quad (4)$$

where * indicates the dye or oxygen in an excited state. While light excitation of the dye initially produces the excited singlet state (reaction 1), conversion to the triplet excited state is very fast and it is principally this state of the dye which excites the ground state oxygen to the singlet state (reaction 2). Any reaction with an acceptor (reaction 3) always occurs in competition with the physical quenching of singlet oxygen to the triplet ground state (reaction 4).

Decker (1979) has used these reactions to scavenge oxygen from photopolymerisable liquids immediately before UV-initiated polymerization.

When applied in polymer films, this process may be used to prevent oxygen from permeating through polymer films (Rooney and Holland, 1979) and for the slow removal of traces of oxygen from enclosed nitrogen-flushed spaces (Rooney et al., 1981).

EXPERIMENTAL

Scavenger film preparation

Ethyl cellulose films (BDH, degree of substitution 2.5) containing dioctyl phthalate plasticiser (5% w/w) and dissolved dye and acceptor were cast as described previously (Rooney et al., 1981). Silica particles (0.5 mm diameter) were sprinkled onto the liquid film (0.1 gm^{-2}) immediately after casting to separate the surfaces of the coiled film.

Materials

Erythrosine (ICI Edicol) was recrystallized from ethanol, and difurylidenepentaerythritol (PEF) was prepared by the method of Backer and Schurink (1931). Tetraphenylporphine (TPP) was prepared by the method of Adler et al. (1967). Dioctyl phthalate (Albright and Wilson, food grade) and dimethylanthracene (DMA, Aldrich, AR grade) were used as received. L-ascorbic acid (Unilab, reagent grade) was recrystallized from 95% ethanol before use.

Cashew nuts and potato crisps were purchased in retail packs from a supermarket.

—Continued on next page

Test procedures

Air-filled pouches. A strip of scavenger film 4 cm wide and of chosen length and thickness was rolled loosely into a coil having a diameter of approximately 4 cm. It was placed in a pouch, 11 cm x 6 cm, of oriented polypropylene coated with polyvinylidine chloride described previously (Rooney et al., 1981). After the pouch was heat sealed, the air was withdrawn by means of a two-way syringe (Shorter, 1981) through a silicone rubber septum (Silastic, Dow Corning) extruded onto the pouch's outer surface. The appropriate volume of air was then injected into the pouch without the need for a second puncture.

The pouch was then hung vertically between two 500W photographic slide projectors at a distance of 35 cm from each lamp. The average illumination at each surface of the pouch was 2×10^5 lux. When the effect of light intensity was studied an increase in average illumination to 7×10^5 lux was obtained by reducing the distance between the projector lamps and the pouch to 25 cm. Samples (0.2 ml) of headspace gas were withdrawn using a gastight syringe (Precision Sampling Corp.) for analysis by gas chromatography (Rooney et al., 1981). Triplicate pouches were used for each test.

Pouches containing food. Cashew nuts (22.5g) or potato crisps (4g) were placed in pouches and a roll of scavenger film was inserted between the food and the top of each pouch. The scavenger film, 105 cm long and 12 microns thick, contained PEF and TPP at concentrations of 0.6M and 10^{-3}M respectively. The pouches were sealed and the headspace volumes were determined from the decrease in total pouch volumes after scavenging of the oxygen. Duplicate pouches were then prepared and the air volumes were adjusted to the above levels by means of a gastight syringe.

The scavenger film was illuminated by aiming the light at the top area of the pouch to avoid any unnecessary exposure of the food to strong light. The food may also be shielded from light by use of a screen, adjusting the height of the fill, or by filtering out any wavelengths which might be deleterious.

Photosensitization efficiency of the dye

Samples of the scavenger film, 3 cm x 1 cm x 12 microns, containing DMA acceptor (10^{-2}M) and TPP sensitiser (10^{-3}M) were mounted on stainless steel frames with apertures identical to that of a spectrophotometer cell holder. The samples were illuminated in air with light from a super pressure mercury source (Bausch and Lomb, Model 33863601) which had passed through a high intensity monochromator (Bausch and Lomb, Model 338676) with a 4.8 nm exit spectral slit. The samples were illuminated at each wavelength of interest and the loss of DMA was determined spectrophotometrically at 380 nm. The illumination time was varied depending on the wavelength used so that the loss of DMA was less than 10%. The emission spectrum of the mercury source was used to correct subsequent results for variation in intensity with wavelength.

RESULTS & DISCUSSION

REMOVAL of oxygen from air headspaces requires at least ten times more singlet oxygen acceptor than trace oxygen scavenging. Since the scavenging rate at low oxygen concentrations is limited by the flux of oxygen into the polymer, long strips of thin film in the form of rolls rather than short strips of thick film were chosen.

The concentration of singlet oxygen acceptor, PEF, was kept near the practical solubility limit of around 0.6 M. The PEF molecule contains two furan rings and is capable of scavenging two molecules of oxygen. The results of preliminary experiments indicated that the use of erythrosine as the photosensitiser would not be satisfactory since its high molar absorptivity at 543 nm of 72,000 would prevent light from reaching the inner layers of the film roll (Fig. 1). TPP, a commonly used photosensitiser (Matsuura et al., 1980), has four low absorbance bands and a large one at 418 nm. With TPP, therefore, there should be light available to the dye throughout the film roll provided light scattering is not excessive.

Scavenging rate

The rate of a photosensitised singlet oxygen reaction in a concentrated, air-saturated liquid solution of an acceptor is described by:

$$-\frac{dA}{dt} = I_a \cdot \phi_T$$

where A is the concentration of acceptor, ϕ_T is the quantum yield of formation of the triplet state of the dye and I_a is the intensity of light absorbed (Davidson and Trethewey, 1977). When carried out in a polymer matrix, the rate of acceptor oxidation (and therefore oxygen scavenging) will be further limited by the rate of diffusion of (a) ground state oxygen through the matrix to the triplet dye molecules or (b) the singlet oxygen molecules thus produced to the acceptor molecules.

The triplet quantum yield of the sensitizer can be measured and the intensity of light absorbed can be varied by changing the dye concentration and the incident light intensity. The access of oxygen to the triplet dye can be varied by changing the film surface area/thickness, and the diffusion distance of singlet oxygen can be changed by changing the dye concentration.

Accordingly, dye concentration was chosen as the first rate-determining variable for study.

Dye concentration

The optimum erythrosine concentration for scavenging traces of oxygen by a single layer of film containing PEF is around 5×10^{-3}M (Rooney et al., 1981). In the present context, however, there are two additional features to be considered: (a) the use of multiple film thicknesses which reduces the maximum dye concentration that can be used, and (b) the radically different absorption spectrum of TPP compared with that of erythrosine, resulting in absorption of all the light at 418 nm by the first two or three layers of film.

The rate at which oxygen was scavenged from 30 ml of air by a roll of film, 85 cm long and 13 microns thick, containing PEF (0.4M) was studied as a function of TPP concentration up to the solubility limit of 2×10^{-3}M. The results (Fig. 2) show that at 10^{-4}M TPP the scavenging rate was independent of oxygen concentration down to around 2%, after which the rate decreased with decreasing oxygen concentration. At 10^{-3}M TPP the scavenging rate was constant down to around 5%, but the initial scavenging rate was only three times greater than that at 10^{-4}M when the dye concentration had been increased tenfold. There are two variables which most commonly limit photochemical reactions, diffusion rates and light intensity, and two approaches towards explaining the results are taken in terms of these variables.

Firstly oxygen is the only mobile species and its diffusion into the film occurs from both sides so the process of oxygen removal occurs between each surface and the middle of the film strip. The greatest distance the oxygen could diffuse before scavenging is L/2 where L is the film thickness. The calculated minimum flux of oxygen entering the two film surfaces is 3.6×10^{-5} ml $cm^{-2}s^{-1}$, using the formula for flux described previously (Rooney et al., 1981). Since flux is inversely proportional to the oxygen diffusion distance, it will be greater if oxygen is scavenged closer to the film surfaces. The initial scavenging rate at 10^{-3} dye was 1.7×10^{-5} ml $cm^{-2}s^{-1}$, which is less than the minimum calculated flux value. It seems therefore that oxygen diffusion could not be the variable which limits the initial rate.

Secondly light reaching the inner turns of the film roll may be so severely limited by light scattering and extinction that the effective film area is greatly reduced. The roll used in this experiment consisted of 25 layers and, as observed visually, there was a considerable loss of light due to

scattering. Extinction is the absorption of light of specific wavelengths by dye in the outer layers so that a reduced light intensity is available at the inner layers. The outer layer absorbed 84% of the incident light at 418 nm with 10^{-3}M dye, and 17% with 10^{-4}M dye. TPP has four additional low absorbance bands in the visible (Fig. 1) and photosensitisation must be due to light at these wavelengths which penetrate and are absorbed by film layers close to the centre of the roll. The effect of wavelength of illumination on photosensitization efficiency is examined later in this paper.

The behavior of the film with 2 x 10^{-3}M dye is also consistent with the second explanation since, not only does the initial scavenging rate appear to be similar to that at 10^{-3}M dye, but the reaction rate appears to tail off slightly earlier as expected if the rate in the inner layers is light limited by increased extinction. A similar effect has been observed previously with optically dense films (Rooney et al., 1981).

The effect of increased film surface area on scavenging rate was examined in order to test the appropriateness of the light intensity argument. Increases in surface area should reduce the distance that ground state oxygen has to diffuse before reaching excited dye molecules, and faster scavenging should occur if the reaction rate is not already light limited.

Film area

Rolls of scavenger film, containing PEF (0.4M) and TPP (10^{-3}M) were formed from strips of different length and thickness so that the quantity of both compounds in each was constant. Thus the same amount of light should be absorbed by each treatment provided light scattering is not excessive.

The results (Fig. 3) show that the initial scavenging rates were similar but at low film areas (curves C-E) it decreased as the reaction proceeded, indicating that the rate became dependent on oxygen diffusion since the distance from the film surface to dye molecules surrounded by unreacted PEF molecules increases with decreasing film area. Thus with decreasing film area the reaction is not diffusion limited initially but becomes so as the PEF nearer the film surfaces is consumed. Replacement of ethyl cellulose with polymers such as silicone rubbers which are much more permeable to oxygen should eliminate the tailing-off in the scavenging rate.

Intensity of illumination

Rolls of scavenger film, 140 cm long, 7.5 microns thick, and containing PEF (0.4M) and TPP (10^{-3}M), were sealed in pouches containing air (30 ml) and illuminated at two light intensities. The results (Fig. 4) show that the larger initial scavenging rate occurred at the higher light intensity, hence the reaction is not diffusion limited but rather limited by the availability of excited dye molecules to excite the oxygen.

The superposition of the curves A and B (Fig. 3) is also consistent with a light limited reaction rate, since the increase in light scattering caused by the 34% increase in the number of film surfaces on going from curve B to A possibly negates the 34% additional film area.

The effect of higher light intensities will be studied and submitted for publication later. Use of an anti-blocking agent with a refractive index matched to that of the polymer should further reduce light scattering. It is evident that if light scattering can be reduced, a reduction in oxygen content to 2% or less in a minute or so should be feasible.

Wavelength of illumination

Since the efficient scavenging observed at 10^{-3}M dye cannot be due to absorption of light only in the 418 nm band of the TPP spectrum (see above), the photosensitization efficiency of the low absorbance bands (Fig. 1) was studied using DMA loss as a measure of singlet oxygen formation. DMA is an efficient singlet oxygen acceptor absorbing only in the UV.

The DMA losses on illumination in all five bands are shown in Table 1. The second column shows the percent rate of loss of DMA when the values obtained on illumination with the mercury source were corrected for the non-uniform light intensity distribution of the projector lamps used in the scaventing studies. The light distribution of these lamps was measured using chemical actinometry (Wegner and Adamson, 1966). Under projector lamp condi-

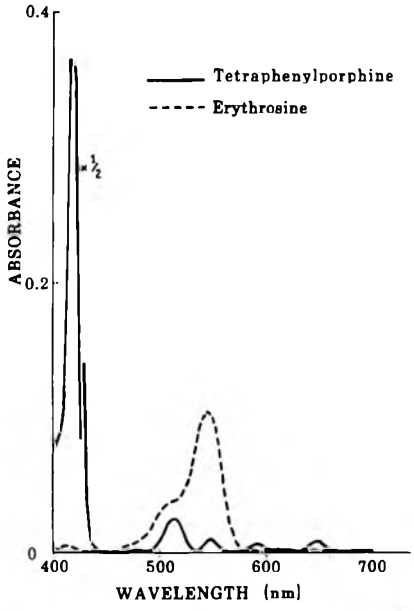


Fig. 1—Absorption spectra of dyes 10^{-3}M in ethyl cellulose 13 microns thick.

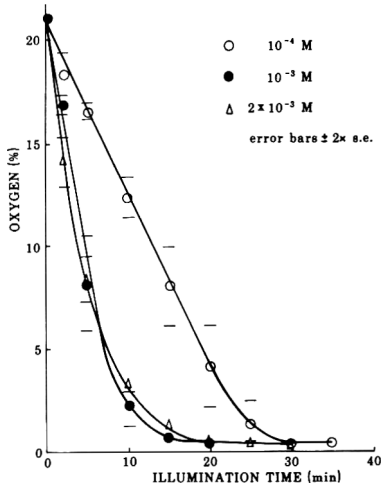


Fig. 2—Oxygen scavenging at various TPP concentrations.

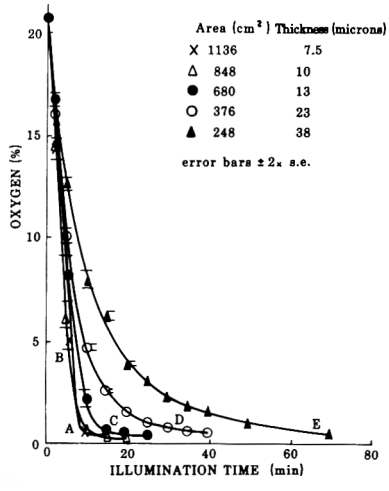


Fig. 3—Effect of film area on scavenging rate.

tions, only 25% of the total DMA loss at all wavelengths resulted from illumination in the 418 nm band. The remaining 75% DMA loss was caused by light of the other wavelengths which is not absorbed significantly due to the low absorbance values of the TPP at these wavelengths. Thus it is not surprising that the scavenging reaction occurs efficiently in the inner layers of film rolls when virtually all light at 418 nm is absorbed in the first layer or two.

The DMA loss using uniform light intensity at each wavelength (Table 1, column 3), obtained by correction of the values found using the mercury source, was used to determine the efficiency of the dye at each wavelength relative to that at 418 nm, as shown in the final column. This parameter was calculated by dividing the light-intensity-corrected reaction rate by the absorbance of the dye at the particular wavelength, and normalizing these values to that found at 418 nm. The illumination in the low absorbance bands (513 to 650 nm) provides more efficient sensitisation of singlet oxygen per quantum of light absorbed than in the most intense band at 418 nm. A detailed quantum yield and band assignment study would be needed to explain this finding.

Table 1—Efficiency of DMA photooxidation at various wavelengths

Wavelength (nm)	DMA Loss Illumination: Projector % min^{-1}	DMA Loss Illumination: Uniform % min^{-1}	Relative efficiency
418	34.2	34.2	1
513	37.0	7.4	4
551	36.0	6.0	7
592	10.8	1.8	3
650	20.0	3.3	6

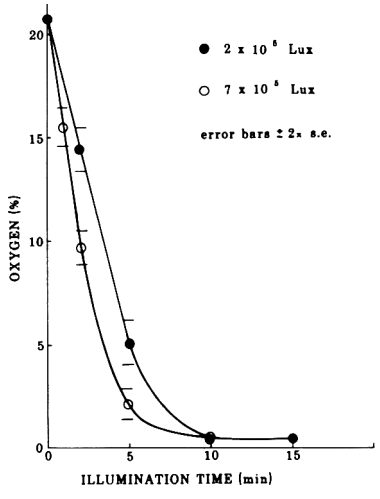


Fig. 4—Oxygen scavenging at two light intensities.

Headspace volume

Since the package headspace volume depends on the bulk density of the food and on the extent of the fill, the oxygen removal capacity of the scavenger film was examined. The air volumes were varied between 10 and 90 ml and the length of scavenger film, 14 microns thick, was varied so that the PEF and oxygen were equimolar. Since PEF is bifunctional the molar ratio of furan rings to oxygen was 2:1.

The curves in Fig. 5 show that headspace volumes up to 70 ml had little effect on the scavenging rate, and 10–15 min illumination reduced the oxygen content to 1%. With 90 ml of air the 1% level was reached after 19 min which is significantly longer (P = 0.05) than required for smaller volumes. An increase in scavenging time might be expected as the volume is increased since the number of turns in the film roll must also be increased to provide enough PEF. The results indicate that the scavenging system can handle large variations in headspace volume by use of changes in th length of scavenger film.

Pouches containing foods

Cashew nuts and potato crisps were chosen as examples of oxygen-sensitive foods with small (26 ml) and large (55 ml) headspaces, respectively. The scavenger film contained PEF equivalent to 70 ml of air in each case. There was very little difference in scavenging rate (Fig. 6) between

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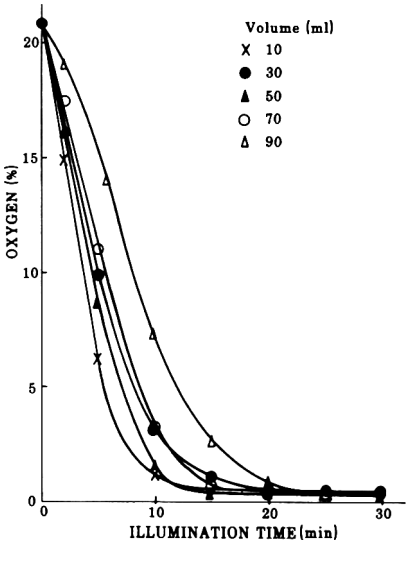


Fig. 5—Oxygen scavenging from various air volumes.

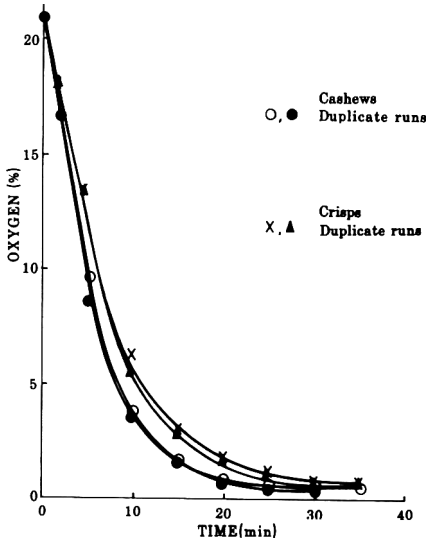


Fig. 6—Headspace oxygen content of pouches of food as a function of illumination time.

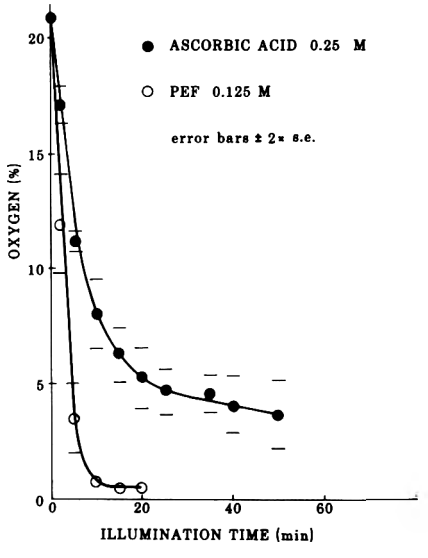


Fig. 7—Comparison of PEF with L-ascorbic acid as singlet oxygen acceptors in ethyl cellulose.

Energy Consumption in Water Blanching

THOMAS R. RUMSEY, ELAINE P. SCOTT, and PAUL A. CARROAD

ABSTRACT

The energy consumption of a tubular water blancher and a water blancher with screw conveyor were measured as a basis for suggesting energy conserving modifications. The former required 0.54 MJ/kg and the latter 0.91 MJ/kg, indicating the importance of complete steam condensation. A pilot scale experiment with a water blancher heated with heat exchange and by steam injection confirmed the energy savings potential of heating with an exchanger to minimize escape of steam rather than by steam injection.

INTRODUCTION

ENERGY ANALYSIS of spinach processing showed blanching to account for 34% of the total energy input (Chhinnan et al., 1980). The spinach processing line employed an open tank blancher, filled with water and heated by steam injection, through which spinach was transported on a mesh belt conveyor.

Energy consumption in blanching has been reviewed by Bomben (1977, 1979) with most attention having been given to conventional steam blanchers and modifications of such units. Bomben estimated blancher energy efficiencies using a theoretical requirement of 134 kg steam per 10^3 kg of raw vegetables and an arbitrary product temperature rise of 72°C. This estimate equals 302 kJ/kg of product (130 Btu/lb). On this basis a conventional steam blancher was judged to have an energy efficiency (i.e., theoretical requirement divided by actual usage) of 5%, a hydrostatic steam blancher 27%, an individual quick blanch unit 85%, and a water blancher 60%. The water blancher value represented only an estimate since no data were available and its operation was presumed to condense an appreciable amount of the steam injected into it. Applying the theoretical estimate to the spinach blancher, its energy efficiency was only 26% due to the considerable escape of uncondensed steam.

The prevalence of water blanchers in industry justified further study of energy consumption in water blanching, the results of which are presented in this paper. A tubular blancher and conventional screw conveyor unit were studied. The escape of uncondensed steam used for injection heating proved to be a significant loss, and a pilot study in which heat exchange was substituted for steam injection was also undertaken.

MEASUREMENT PROCEDURES AND EQUIPMENT DESCRIPTIONS

Thermal energy

Accutubes (Meriam Instrument Co., Cleveland, OH) inserted in steam pipes through welded couplings were used to quantify steam flow. The mass flow of steam was determined by measuring the pressure difference across the tube. The pressure difference was monitored by connecting the high and low pressure sides of the accutube to an electronic differential pressure cell (Westinghouse) by copper tubing filled with water. The cell emitted a 4–20 milliamp signal proportional to the steam flow rate. The milliamp signal was wired across a known resistance to produce a voltage signal which was recorded by a battery-operated data acquisition system (Campbell Scientific Co., Model CR5). The data-logger had capability for recording data on both paper tape and magnetic tape for subsequent analyses, and was equipped with variable scan speed and capability to integrate data over time between print-outs.

Steam characteristics of temperature and pressure were measured with thermocouples which fit into the accutubes in the steam line and with pressure gauges on the steam lines and on the differential pressure cell. Steam density was determined with such temperature and pressure data from saturated steam tables. Steam quality was determined by a calorimetric method (Chhinnan et al., 1980).

In a pilot plant experiment an external heat exchanger was used to heat water in a blancher, rather than employing steam injection. With the heat exchanger, the amount of steam used was measured by the mass of condensate exiting a steam trap. Both methods, using accutubes and the trap, correlated well.

Temperature

Temperatures of blancher surfaces and water lines were monitored during processing with copper-constantan thermocouples attached to the data acquisition system. An internal reference channel on the data logger allowed the thermocouples to be attached directly to the unit without a separate cold junction. Mass average product temperatures were determined with a metal thermometer (Bomben et al., 1975).

Electrical energy

Large motors were monitored for electrical energy use by wiring transducers (Ohio Semitronics) into the electrical circuits. The transducers emitted a millivolt signal proportional to power demanded by the motor. The signal was monitored continuously by the data acquisition system and the power demand was integrated over time to yield energy consumption.

Mass flow

Samples were taken from product lines and weighed to determine product flow rate. Product flow manually determined intermittently agreed within 3% with the processor's record of packed product. Close agreement was expected since in all cases studied, there was no sorting or waste removal between the blancher, the freezer, and the packing machine. All trimming and waste removal occurred before blanching. To systematize results, mass flow is based on final packed weight of product.

Water flow rates were determined either with accutubes as previously described or with a portable ultrasonic flow meter (Polysonics Inc.).

Analysis methodology

Energy inputs were quantified for the blanchers studied. Theoretical energy required for blanching was calculated based on product mass flow, reported heat capacity of the vegetable (ASHRAE, 1977), and the actual rise in temperature from inlet to blanching temperature. Heat transfer losses by convection and radiation were estimated by using conventional engineering correlations (Henderson and Perry, 1976).

Description of water blanchers

Physical description of tubular water blancher. The tubular blancher consisted of a convoluted stainless steel pipe, 488m (1600

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ft) in overall length and 0.10m (4 inches) in diameter, through which lima beans and heated water were pumped by a 7.5 kW (10 HP) pump. Water was heated by one steam injection port in a 3.05m (10 ft) long section of pipe 19.5m (64 ft) downstream from the pump. The hot water was separated from product in a sieve and was returned to a supply tank via a return line 52.7m (173 ft) long and 0.15m (0.5 ft) in diameter. The supply tank received make-up water as required. Product was added to the system in the supply tank, and then pumped through the tubular blancher. The product was pre-heated by the hot water recirculated to the supply tank. Blanch time was controlled by flow rate of product in the tube.

Physical description of screw conveyor water blancher. The screw conveyor water blancher was a 0.1140 m^3 (300 gallon) vessel through which product was conveyed by a turning screw. The vessel was rectangular, with a rounded bottom: length, 5.1m (16.83 ft); width, 1.0m (3.4 ft); and height, 1.25m (4.1 ft).

The screw was turned by a single motor 0.56 kW (0.75 HP) at the discharge end. The turning speed controlled the blanch time. The blancher was fed by pumping product with water over a hydrosieve. Water passed through the sieve and product passed from the sieve into the blancher. At the outlet, product was discharged from the blancher onto a screen by a paddle which turned on the screw conveyor shaft. A vibratory shaker, not attached to the blancher, conveyed the product to a moving belt for further processing (i.e., freezing). The discharging water passed through a screen and was recycled by a 3.7 kW (5 HP) pump through a 5.1 cm (2 inch) line to the blancher inlet. Steam entered the blancher through a 5.1 cm (2 inch) line and manifold system which included two independent temperature controller/recorders. The upstream controller was set at 97.8°C (208°F) and the downstream recorder at 96.1°C (205°F). Steam was sparged into the water from below. Fans above the unit withdrew uncondensed steam which escaped from the feed and discharge openings and from around the lid.

Physical description of pilot blancher with screw conveyor. The pilot scale screw blancher was equipped with a manifold system for steam injection and a water recycle line suitable for piping through a scraped surface heat exchanger. The pilot blancher resembled in all important design aspects an industrial scale unit (Swartz and Carroad, 1979).

The product feed rate of the pilot blanching operation was based on the ratio of water volume used in the pilot unit to that in the commercial screw conveyor blancher. With a water volume ratio of 24.5 liters to 1,140 liters, the throughput of cauliflower cuts was determined to be 1.32 kg/mm (2.9 lb/min). Cauliflower was chosen because it was used in the commercial blancher during the energy monitoring studies. The plant records showed the seasonal average throughput of cauliflower in the commercial blancher to be 2,720 kg/hr (6,000 lb/hr). Cauliflower cuts for the pilot study were obtained from the industrial freezer company which operated the commercial screw blancher. The cuts were taken directly from the processing line just prior to blanching and were transported under ice to Davis.

RESULTS

Results for tubular blancher

The average mass flow of lima beans was 4,980 kg/hr ± 860 kg/hr (10,970 lb/hr ± 1,890 lb/hr) over the five shifts monitored. Using the doppler flow meter the mass flow rate of water in the tube was determined to be 51,700 kg/hr (114,000 lb/hr). This latter value was confirmed within 2% by independent measurement of flow with an accutube placed in the water line filling the blancher (50,700 kg/hr; 111,700 lb/hr).

The average residence time of beans in the blancher system was 6.8 min, of which approximately 1.5 min were spent in the holding tank and 1.0–1.5 min in the cooling trough at the blancher exit. Actual blanch time was 3.8–4.3 min. Using the mean flow rate, the blanch time is calculated to be 4.2 min. Beans entered the blancher at 18.3°C (65°F), and exited to the cooling section at 40.2°C (104°F). In the immediate vicinity of the steam inlet the water temperature rose to 96°C (205°F), but water temperature dropped quickly to 79°C (175°F) as heat was transferred to the beans.

The mass flow of steam averaged 1,094 kg/hr (2,411 lb/hr), at a pressure of 5.98 kg/cm^2 (85 psia) and a temperature of 158°C (316°F), with a quality of 86%. The thermal energy input averaged 2.707 GJ/hr (2,565,600 Btu/hr). The electrical power input to the pump was measured as 2.66 kW, which on a fossil fuel basis is equivalent to only 1.1% of the total energy input (Chhinnan et al., 1980).

The temperature profile over the length of the tube blancher was measured. Based on standard correlations for convective and radiative heat transfer (Henderson and Perry, 1976), the heat losses were integrated over the length of the blancher for the measured temperature distribution. Results of the measurements are listed in Table 1.

Results for screw conveyor blancher

The average mass flow of cauliflower cuts over the seven complete shifts monitored was 2,845 kg/hr ± 690 kg/hr (6,270 lb/hr ± 1,520 lb/hr). Product entered the blancher from the hydrosieve at 20°C (68°F). The blanch water varied in temperature through the length of the unit, dropping from an upstream value of 96.1°C (205°F) to a discharge temperature of 92°C (199°F). Plant records listed the average blanch temperature as 93°C (200°F) under these conditions. The blanch time was 2.25 min. Average blancher surface temperature was 93°C (200°F).

The total mass flow to the blancher of saturated steam at conditions 7.7 kg/cm^2 (109.7 psia) pressure, 168°C (334°F), and quality 82% was 1,075 kg/hr ± 225 kg/hr (2,365 lb/hr ± 500 lb/hr). Of this total flow, the upstream manifold system averaged 68% and the manifold system at the midpoint of the blancher averaged 32%. Thus, most of the steam was injected at the point where product entered, and a smaller amount was required downstream for maintaining the desired blanching temperature. The total mass flow of steam corresponds to a total thermal energy input of 2.59 GJ/hr ± 0.54 GJ/hr (2,453,000 Btu/hr ± 514,000 Btu/hr).

Fig. 1 illustrates the thermal energy input through the feed end and downstream injection points. The steam injection inlet at the feed end exhibited greater variation in flow because the feed end controller was required to respond rapidly to changes in product feed rate in order to establish proper blanching temperature. The downstream

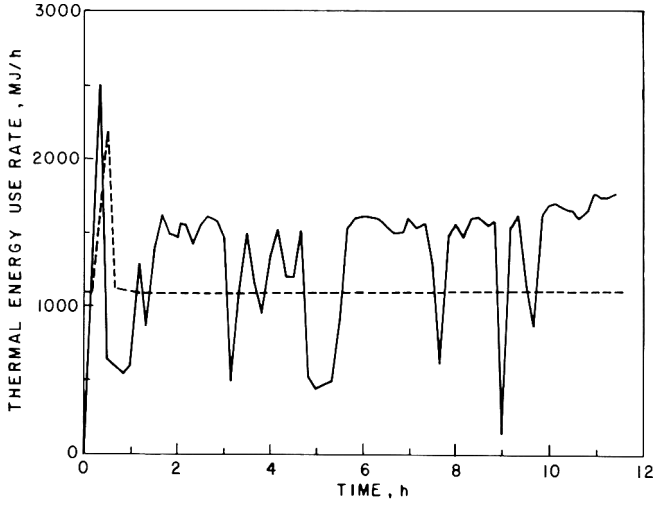


Fig. 1—Thermal energy usage rate for steam inlet (—) at feed end and steam inlet at downstream position (- - -) of water blancher with screw conveyor.

controller only maintained blanching temperature and the steam flow through it was quite uniform.

The blancher water which exited with blanched product was recycled at an average flow rate of 12,500 kg/hr ± 1,600 kg/hr (27,550 lb/hr ± 3,500 lb/hr). Water discharging from the blancher cooled to 78°C (172°F) in flowing past the discharge screen and water collector before being pumped in the return line to the blancher feed end.

Electrical energy was quantified only for the 3.7 kW (5 HP) pump used to recycle blancher water. The motor which turned the screw conveyor was judged too small to contribute significantly to overall energy use. The pump motor required 3.4 kW, which on a fossil fuel basis is equivalent to only 1.4% of the total energy input measured (Chhinnan et al., 1980).

The thermal energy results for the water blancher with screw conveyor are listed in Table 1. The energy required to heat the cauliflower cuts to blanching temperature required only 31.3% of the total thermal energy supplied. A primary injection point was close to the blancher feed opening and much steam was seen escaping. A secondary source of energy loss was the hot water which should have been recycled but which occasionally spilled from the recycle pan when excess water surged from the blancher in response to uneven product loading. This intermittent flow was not quantified.

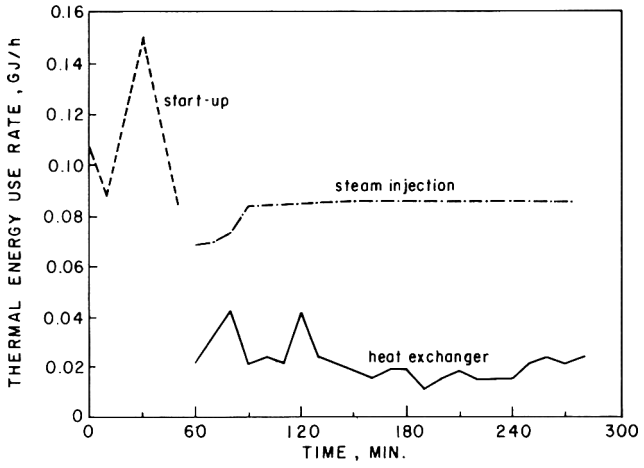


Fig. 2—Thermal energy usage rates for pilot water blancher with screw conveyor.

Table 1—Results of energy measurements for tubular and screw conveyor blanchers

	Tubular blancher	Screw conveyor blancher
Energy consumed by product for blanching (theoretical)	0.94 GJ/hr	0.81 GJ/hr
Heat transfer energy losses	0.24 GJ/hr	0.04 GJ/hr
Total thermal energy input	2.71 GJ/hr	2.59 GJ/hr
Energy required per mass	0.54 MJ/hr	0.91 MJ/hr

Results of pilot plant blancher study

Observation of the conventional water blancher with screw conveyor indicated that the practice of heating blanching water by steam injection is inefficient. The temperature gradient between the injected steam and the near-boiling water is less than required to promote complete steam condensation, given the short residence time of steam bubbles in the system. Considerable steam escaped from the blanchers. The use of a heat exchanger to maintain blanching water temperature suggested itself as an energy conserving modification.

The water temperature in the pilot blancher was maintained at 96°C (205°F). Product entered at a mass average temperature of 6°C (43°F) and exited at 75°C (167°F). Blanch time was set at 8 min to achieve a negative peroxidase test (USDA, 1975). The saturated steam used for both injection and heat exchange was 183°C (355°F) and 10.12 kg/cm^2 (144 psia). Quality was 64.2%.

The thermal energy use rates for this pilot plant study for both the steam injection and the heat exchanger cases are shown in Fig. 2. The initial peak during the first 60 min represents the heat required to bring the blanching water up to temperature. It is clearly evident that more energy is used with steam injection, by a factor of approximately four.

The amount of energy theoretically required to blanch the cauliflower was 0.0277 GJ/hr (26,350 BTU/hr) based on measured temperatures and product properties(ASHRAE, 1977). The amount of energy actually supplied to maintain blanching water temperature at 96°C (205°F) by steam injection was 0.165 GJ/hr (156,500 BTU/hr) and with a heat exchanger was 0.041 GJ/hr (38,700 BTU/hr). The amount of thermal energy used per mass of product was equivalent to 2.09 MJ/kg (900 BTU/lb) in the case of steam injection and 0.51 MJ/kg (220 BTU/lb) with the heat exchanger.

DISCUSSION

THE THREE commercial water blanchers are compared in Table 2. The table lists the measured thermal energy efficiency (i.e., theoretical requirement divided by actual input), the efficiency calculated according to the definition of Bomben (1977), and the efficiency predicted by Bomben. The tubular blancher is more efficient than the screw

Table 2—Comparison of energy efficiencies for water blanchers

Water blancher	ΔTemp °C	C_p[a] kcal/kg-°C	Thermal energy efficiency, %		
			Measured[b]	Calculated[c]	Estimated[d]
Tubular (lima beans)	61	0.74	34.7	55.8	60
Screw conveyor (cauliflower cuts)	73	0.93	31.3	33.3	60
Tank[e] (spinach)	81	0.95	30.7	26.0	60

[a] Heat capacity (ASHRAE, 1977)
[b] Efficiency equals theoretical requirement based on operating conditions divided by total measured energy input.
[c] Efficiency calculated as defined by Bomben (1977); based on theoretical requirement of 302 kJ/kg (130 BTU/lb), C_p = 4.19 kJ/[kg · °C] (1.0 BTU/lb · °F), ΔH_{steam} = 2.256 kJ/kg (970 BTU/lb), ΔT = 72°C.
[d] Efficiency estimated by Bomben (1977)
[e] Ref: Chhinnan et al. (1980)

conveyor or tank blancher. Despite greater heat transfer losses, the tubular blancher effectively condenses the steam which is injected into it, whereas the screw conveyor and tank blanchers permit a large escape of uncondensed steam. Assuming condensation of steam within a water blancher, Bomben's estimate of 60% efficiency is close to the efficiency calculated according to his definition for the tubular blancher, 55.8%. In terms of energy used per mass of product, the tubular blancher required 0.54 MJ/kg (230 BTU/lb), the screw conveyor 0.91 MJ/kg (390 BTU/lb), and the tank blancher 1.05 MJ/kg (450 BTU/lb).

The pilot plant experiment illustrates the advantage of utilizing steam to achieve complete condensation. Use of a heat exchanger rather than use of steam injection decreased energy use per mass from 2.09 MJ/kg (900 BTU/lb) to 0.51 MJ/kg (220 BTU/lb). The measured energy efficiency with steam injection in the pilot unit was only 17%, compared to 68% with the exchanger. The 17% value is smaller than that of the commercial unit (31%) and results from the shallowness of the pilot scale unit contributing to a very short residence time of steam bubbles in the blancher following injection, as well as a high surface area to volume ratio leading to more significant heat transfer losses. Nevertheless, the fourfold decrease in energy use achieved with a heat exchanger indicates that a significant conservation potential exists through heating water in an exchanger to achieve complete condensation.

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Data presented in this paper were collected to identify measures which could be taken to effect energy conservation in such processes. Because of the relatively small data base and with variation in product and operator practices in different plants, it is not possible to generalize from these results in evaluating all water blanchers of similar design or to compare directly one manufacturer's product with another.

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Reference to a company or product name does not imply approval or recommendation of the product to the exclusion of others that may be suitable.

OXYGEN SCAVENGING FROM AIR HEADSPACES . . . From page 294

the two systems, with the crisp packs requiring 4 min more illumination to reach the 2% oxygen level. This difference was probably due to the effects of differing bulk densities of the foods on the rate of diffusion of oxygen to the scavenger film.

Comparison of the results for cashew nuts with the curve (Fig. 5) for pouches filled with air, 30 ml, reveals very little effect, if any, of the nuts on the scavenging time. The results for pouches containing crisps cannot be compared directly with those obtained on the empty pouches since different lengths of scavenger film were used.

CONCLUSIONS

THE OXYGEN SCAVENGING RATE depends initially on the light intensity and subsequently on the rate of diffusion of oxygen into the film. Both of these rate limiting variables can be influenced so as to maximise the scavenging rate.

While PEF does not have FDA approval there is a wide variety of food constituents which react with singlet oxygen (Wasserman and Murray, 1979). Some suitable compounds are presently being investigated. For instance L-ascorbic acid is reported to be photooxidized by both singlet oxygen and free radical mechanisms (Davies et al., 1972; Boddanes and Chan, 1979). Preliminary results of a more detailed study of this subject are shown in Fig. 7. Examination of this figure suggests that ascorbic acid may not be a very efficient singlet oxygen acceptor.

The system as described could be subject to leaching of the photosensitizing dye, the acceptor or the reaction product into the food so polymers with dyes and acceptors as part of the polymer chain are being evaluated as a means of preventing any such extraction.

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Freeze-Thaw Stability of Illinois Soybean Beverage

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ABSTRACT

The objective of this work was to study the effects of freezing conditions, processing and formulation variables on freeze-thaw (F-T) stability of Illinois soybean beverage. Objective and subjective evaluations were made on a sample before and after freezing. Total solids content of the supernatant from a centrifuged sample was used to calculate a F-T stability index. This index was below 35% for both plain base and plain beverage. The freeze-thaw stability was affected by freezing temperature, holding time before freezing, changing of temperature during frozen storage and desludging. However, length of frozen storage and homogenization pressure had no apparent effect. Addition of sugars and salt resulted in a beneficial effect on the stability. In order to prevent visible freeze damage, approximately 7% sucrose or 5% dextrose hydrate were required in a beverage containing 0.2% salt.

INTRODUCTION

RAPID GROWTH of world population without a similar growth in animal protein supply has increased the interest in developing low-cost, high quality protein foods of vegetable origin. Soybeans are most suitable to meet this requirement. Beverages made from whole soybeans or dehulled cotyledons have been developed at the University of Illinois (Nelson et al., 1975; 1976; 1977). Illinois soybean beverages show good palatability (Kuntz et al., 1978) and excellent suspension stability (Priepke et al., 1980). A method for preparing a commercially sterile form of these beverages has been developed (Luttrell et al., 1981). However, the freezing preservation of Illinois soybean beverage has not been studied.

Although freezing is recognized as an excellent method of preserving food, many undesirable changes in properties may take place during freezing, storage, and thawing. This is especially true of liquids. For example, cow's milk shows a disruption of the fat emulsion and a destabilization of protein when the product is subjected to freezing-storage-thawing. The mechanism of such damages is complicated and not well understood. According to Nemethy and Scheraga (1962) and Scheraga (1961), the characteristic configuration and stability of proteins is governed by covalent disulfide bonds and by noncovalent interactions between side chains. The latter category includes hydrogen bonds, hydrophobic bonds, and ionic interactions. During freezing, some of the water is converted into ice, thus increasing the concentration of protein in solution. Consequently, the various types of protein bonding are altered (Fennema and Powrie, 1964). Denaturation of soybean protein by freezing and frozen storage has been studied by Hashizume et al. (1971; 1974a, b) and Ehninger and Pratt (1974). Hashizume (1971) suggested that denaturation of soybean protein during freezing and frozen storage may be caused by intermolecular reactions through disulfide bonds.

The overall objective of this work was to study the freeze-thaw (F-T) stability of Illinois soybean beverage. Initially, the effects of freezing conditions were studied. This was followed by investigation of the processing and formulation variables necessary to produce a freeze-thaw stable whole soybean beverage.

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MATERIALS & METHODS

Soybean cotyledons

Bonus variety certified soybean seed grown in Champaign County, IL, was used in this study. To prepare cotyledons, the whole soybeans were first heated for 15 min in a Proctor standard ten-tray, variable circulation dryer (Proctor-Schwartz, Inc., Philadelphia, PA) with a downward flow of air at 210°F (98.9°C). Immediately after heating, the warm beans were cracked by passing through a drum dehuller (Nelson et al., 1976). The hulls loosened by the cracking process together with the fines were removed by aspiration with a tubular separator constructed in this laboratory (Forster, 1977). After cooling to room temperature, the cotyledons were stored at 34°F in sealed 30-lb containers.

Beverage preparation

The Illinois process for soybean beverage (Nelson et al., 1977) was used for this study. The standard process is outlined in Fig. 1. Simultaneous blanching and tenderization of cotyledons was accomplished by cooking in a steam-jacketed kettle for 30 min in boiling tap water containing 0.25% sodium bicarbonate. After blanching, the solution was discarded and the cotyledons were rinsed with tap water. Then they were ground with sufficient tap water to obtain a slurry containing 10% total soybean solids. Grinding was accomplished with a Model 1 RP-6-K115 Disintegrator (Rietz Manufacturing Co., West Chester, PA) equipped with a 0.023 inch screen. Slurry pH with bicarbonate blanch was 7.2 as compared to 6.8 without. After grinding, the slurry was heated to 190°F (87.8°C) and homogenized immediately in a Model 15M-8TA single piston, two stage homogenizer (Gaulin Corp., Everett, MA). Homogenization pressure was set at 3,500 psi on the first stage and 500 psi on the second stage. The slurry, after this first homogenization, is referred to as the beverage base.

The 10% soy solids beverage base was diluted to 6% soy solids with tap water. Formulation with sugar, salt and other flavorings was usually done at this step. Also, if desired, pH adjustment could be made by adding 6N HCl or 20% NaOH with constant agitation. The diluted and formulated base was heated to 190°F (87.8°C) and homogenized as before. The resulting product was the Illinois soybean beverage. In this study, all the beverages were produced according to the standard method without salt and sugar unless otherwise specified.

Freezing of soybean beverages

After final preparation and cooling, the beverages were filled into 6 oz prescription bottles for visual evaluations and 8-oz polyethylene bottles for objective analysis. Control samples were subjected to analysis without freezing while the others were frozen in still air at −40°F (−40°C). After 1 day of storage, frozen beverages were removed and thawed at room temperature for objective analysis. All the beverages were frozen and thawed in duplicate according to the method described above unless otherwise specified.

Centrifugation

Centrifugation was accomplished with a Model V size 2 International Centrifuge (International Equipment Co., Boston, MA) equipped with a four-place No. 277 head and No. 372 carriers. Samples for centrifugation were filled into 8-oz polyethylene bottles

and warmed to 25°C prior to centrifugation. Centrifugation time was 10 min and the force was 3,000 x *g* (1,750 rpm).

Total solids determination

Total solids were determined by drying a tared sample in an air oven at 70°C for 8 hr and then in a vacuum oven at 60°C and 30 mm Hg pressure for 24 hr. Weight difference was used to calculate percent total solids.

Protein determination

Nitrogen content of samples was determined by the Kjeldahl method (AOAC, 1970). Protein content was calculated by applying a factor of 6.25 to the nitrogen content.

Freeze-thaw stability

Freeze-thaw stability of soybean beverage is defined as the tendency for suspended solid material in the beverage to remain homogeneous and smooth without any curdling or settling when subjected to freezing, low temperature storage and subsequent thawing. This tendency was determined by centrifugation of a sample before and after freezing and thawing. After centrifugation, the supernatant was carefully withdrawn and a 30 ml sample was pipetted into a beaker. Each sample of supernatant was then thoroughly mixed and subdivided into four portions. Two portions were analyzed for total solids content and the other two were analyzed for protein content. Mean values of total solids and protein content of the supernatant were used to calculate an index defined as follows:

$$\text{Freeze-thaw stability index} = \frac{\text{Total solids (or protein) content in centrifuged supernatant of frozen and thawed sample}}{\text{Total solids (or protein) content in centrifuged supernatant of the same freshly made sample}} \times 100\%$$

Sensory evaluation

Visual evaluation was carried out by an experienced panel of 8–10 members. The 6-oz glass prescription bottles of frozen beverages were removed from frozen storage, placed on a flat side, and thawed at room temperature, without disturbance. The defect to be judged was described as a gel or coagulation phenomenon. A 7-point hedonic scale was used; 7 indicated an excellent product with imperceptible coagulation and 1 as a pronounced curd.

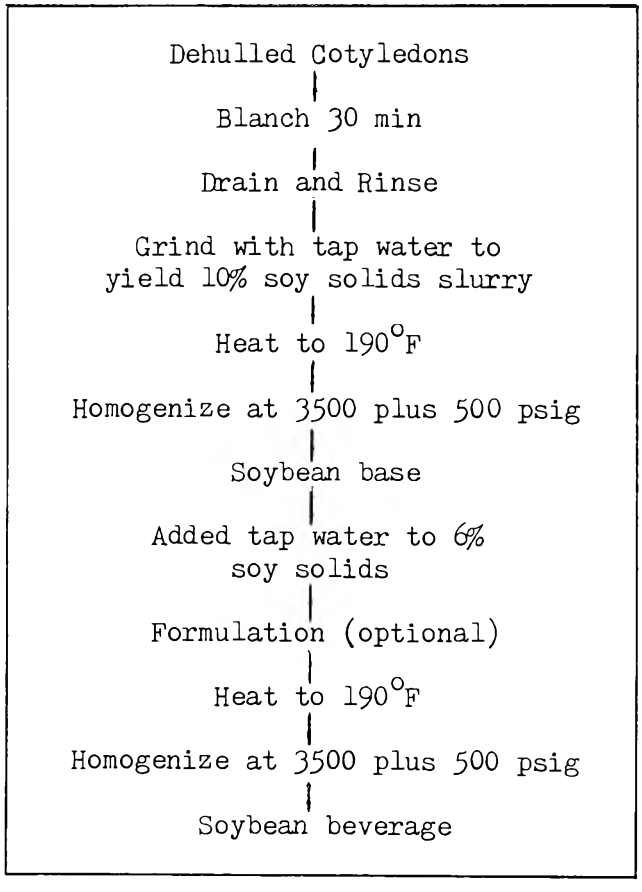


Fig. 1—Preparation of Illinois soybean beverage

Statistical analysis

The data were statistically analyzed according to Steel and Torrie (1960) for analysis of variance with F tests; a completely randomized design was used for objective measurement, and a randomized complete block design for sensory evaluation. Linear correlation equations with the coefficient of correlation (r) were calculated.

RESULTS & DISCUSSION

DATA from the initial study showed that freeze-thaw (F-T) stability indices calculated from either total solids or protein showed the same trends, i.e., they either increased or decreased together depending on freezing conditions. Therefore, a linear correlation between these two indices was calculated. Using the 54 pairs of stability indices calculated from total solids and protein, a linear correlation equation was calculated as follows:

$$\text{Protein index} = -4.8544 + 1.4826\ (\text{Solids index})$$

with r = 0.9873. For further work, only the total solids stability index was calculated.

Freezing temperature

F-T stability indices of base and beverage after 1 day freezing at −40°F (−40°C), −20°F (−28.9°C) and 0°F (−17.8°C) are shown in Table 1. In case of both base and beverage, the −40°F (−40°C) samples showed slightly better stability than the −20°F (−28.9°C) samples which were slightly better than the 0°F (−17.8°C) samples. Thus, the freezing temperature had some effect on the stability. This may be explained by the concentration effect (Fennema, 1966); in freezing a liquid, some pure water is crystallized first and the nonaqueous constituents are left behind in the unfrozen water. At higher freezing temperature, rate of freezing was slower so more unfrozen water was available to allow protein-protein interaction. However, at the very low temperature water was almost completely frozen and protein was no longer in solution. Thus, the intermolecular crosslinkage of protein was reduced. This phenomenon might also be explained by the fact that heat is more rapidly removed from the system at −40°F (−40°C) so that many ice nuclei will form and each will grow to only a limited extent (Fennema and Powrie, 1964). Consequently, the discontinuous ice phase containing many tiny crystals tended to interfere with the formation of intermolecular protein crosslinkage.

For each freezing temperature, the beverage stability index was higher than that of base. This indicated that a higher total soybean solids content decreased F-T stability. It appears that an increase in soybean solids makes it easier for protein crosslinkage to occur and curd to develop.

Frozen storage time

F-T stability index of base and beverage frozen at −40°F (−40°C) or at 0°F (−17.8°C) for 1–10 days are shown in Table 2. Increasing the time of frozen storage to 10 days at either −40°F (−40°C) or 0°F (−17.8°C) did not affect the stability. Evidently, the damage is done during freezing and/or thawing.

Upon holding the beverage at 34°F (1.1°C) for 10 days before freezing at −40°F (−40°C), the F-T stability index increased from 35.4 to 68.4% (P = 0.01). This might be explained as follows: The gel formed during freezing is caused by the formation of intermolecular S-S protein crosslinkages which requires the presence of free-SH groups

on the protein (Hashizume et al., 1971). During holding at refrigeration temperature, the free –SH groups formed *intra*molecular protein binding (Hashizume et al., 1974a). This reduced the chances of *inter*molecular S-S crosslinkages and curd formation during subsequent freezing.

Temperature change during frozen storage

Table 3 shows the F-T stability index for base and beverage frozen at −40°F (−40°C) for 1 day and then stored at 23°F (−5°C) up to 10 days. The stability index showed a large decrease after 1 day storage and continued to decrease throughout the 10 days. Thus, holding at high temperature essentially negated the beneficial effect of the low freezing temperature.

Desludging

This was accomplished by centrifuging the soybean beverage at 3000 × *g* for 10 min and discarding the sediment, which is high in fiber. The F-T stability index of desludged beverage, 39.3, was slightly higher than of the control sample, 37.0 ($P = 0.05$). Evidently the stability problem is caused by protein and not fiber since the desludged product still showed a low index. However, the sample containing fiber was even lower in stability, indicating the fiber became entrapped in the protein gel formed by freezing.

Homogenization pressure

Some effects of temperature and pressure of homogenization on suspension stability and organoleptic quality of Illinois soybean beverage were investigated by Priepke et al. (1980) and Kuntz et al. (1978). Two passes through the homogenizer are required for the processing of soybean beverages. In the present investigation, the beverages were prepared according to the standard method except that the pressures used during the second homogenization were set at 2,000, 3,500, and 5,000 psi, respectively. The F-T stability index of these beverages was 41.1, 42.0, and 42.4%. This shows homogenization pressure had no effect on stability.

Beverage pH

The formation and stability of protein gel structure may be related to electrostatic bonds (Catsimpoolas and Meyer, 1970). Thus, protein gel formation during freezing might be affected by beverage pH. F-T stability index of beverages at four pH levels are shown in Table 4. The index remained constant between pH 6.8 and 7.7. However, when the beverage pH was increased to 8.5, the stability index increased from 30% to 34%. Thus, pH must be increased above 8.0 to improve stability. At this pH the beverage would probably be unacceptable.

Sodium chloride

Since ionic bonds are involved in protein gel formation, addition of sodium chloride may affect the freeze-thaw stability (Fennema, 1966). The beverages for this experiment were prepared according to the standard method (Fig. 1) with addition of various levels of sodium chloride. F-T stability of these beverages is shown in Table 5. The stability index was increased from 36% to 42% when the level of sodium chloride was raised from 0.5% to 1.0%. However, the stability index of the beverage with only 0.2% of sodium chloride was lower than that of the beverage without sodium chloride. This was in agreement with Ehninger and Pratt (1974) who reported that adding 0.2M sodium chloride (1.16%) to soybean protein dispersion interferred with gel formation upon freezing. However, 0.1M sodium chloride (0.58%) showed little effect on this interference. According to Table 5, 1.0% sodium chloride was more effective than 0.5% in promoting stability. The mechanism of this effect is not known. Ehninger and Pratt (1974) suggested that sodium chloride neutralized the ionic charge on the protein and interfered with the gel formation during freezing when the concentration of sodium chloride was high. However, Tumerman and Guth (1965) pointed out that salts are freezing point depressants.

Sugars

The Illinois soybean beverage is essentially an aqueous dispersion of whole or dehulled soybean that is bland and

Table 1 — Freeze-thaw stability index of base and beverage after 1 day freezing at different temperatures

Freezing temp	Stability index, %	
	Base[a]	Beverage[a]
-40°F (-40°C)	29.3	35.4
-20°F (-28.9°C)	28.0	33.8
0°F (-17.8°C)	26.3	32.2

[a] Treatment effect significant at 5% level.

Table 3 — Freeze-thaw stability index for base and beverage frozen at -40°F (-28.9°C) for 1 day and stored at 23°F (-5°C) for 0, 1, 3, 5 and 10 days

Storage time (days)	Stability index, %	
	Base[a]	Beverage[b]
0	29.3	35.4
1	20.4	29.2
3	18.5	25.0
5	17.0	24.6
10	15.4	23.4

[a] Treatment effect significant at 1% level.
[b] Treatment effect significant at 5% level.

Table 2 — Freeze-thaw stability index of base and beverage frozen at -40°F (-28.9°C) or 0°F (-17.8°C) for 1, 3, 5 and 10 days[a]

Freezing storage (days)	Stability index, %			
	-40°F (-28.9°C)		0°F (-17.8°C)	
	Base	Beverage	Base	Beverage
1	29.3	35.4	26.3	32.2
3	28.3	34.0	25.8	32.0
5	30.3	36.6	26.9	32.8
10	31.1	36.6	36.6	30.6

[a] No significant differences found with time.

Table 4 — Freeze-thaw stability index of beverages at four pH levels

Beverage pH	Stability index[a] (%)
6.8	31.4
7.2	30.1
7.7	30.5
8.5	34.1

[a] Treatment effect significant at 5% level

free of bean-like flavor. This statement is correct even though flavorings such as sugars and salt have not been added. However, added sugar functions as a sweetener and flavor enhancer and also improves tactual qualities (Kuntz et al., 1978) and suspension stability (Priepke et al., 1980). When salt is added to the beverage, it tends to eliminate cereal flavor (Kuntz et al., 1978). Therefore, the effect of sugar addition was investigated.

When sugars were added, the total solids data used to calculate the index had to be corrected by compensating for the sugar in both top and bottom samples. Therefore, the F-T index reflects only soybean solids.

The corrected freeze-thaw stability index and mean visual scores of soybean beverages containing 0.2% sodium chloride and 2, 6, and 8% sucrose are shown in Table 6. Both were greatly increased by increasing the level of sucrose from 2% to 8%. The beverage containing 0.2% sodium chloride and 8% sucrose was given a score of 6.9, which represented imperceptible curdling. There appeared to be a good relationship between the objective freeze-thaw stability index and the subjective visual score, thus a linear correlation equation was calculated as follows: mean of visual score = −3.8537 + 0.1203 (stability index) with r = 0.9791. According to this equation, a beverage with a stability index of 82% should have a corresponding visual score of 6.0, which indicates a "nearly perfect" product from the stand-point of F-T damage. From the data in Table 6 it was calculated that approximately 7% sucrose is needed to obtain a stability index of 82%. Thus, in order to obtain non-visible curdling damage due to freezing, 7% sucrose and 0.2% sodium chloride is required.

Using sucrose as the protective mechanism for freeze damage is still not well understood. Fennema and Powrie (1964) stated that the adsorption of sugars on the surface of particles in a system was responsible for the inhibition of particle aggregation during freezing. However, Rose (1956) postulated that the protective effect of sucrose is due to a freezing point depression. It has been clearly demonstrated here that the severity of physical change due to freezing can be moderated by adding this freezing point depressant.

Since the beverage containing 8% sucrose was excessively sweet, dextrose which is less sweet than sucrose was investigated for its effect on freeze-thaw stability.

The corrected freeze-thaw stability index and mean visual scores of soybean beverages containing 0.2% sodium chloride and 3, 5, 7, and 10% dextrose hydrate were shown in Table 7. Both the F-T stability index and visual score were greatly increased by increasing the level of dextrose; a near perfect score was obtained at 7% dextrose. A linear correlation equation was: mean of visual score = −2.1408 + 0.1044 (stability index) where, r = 0.9938. Based on this equation and the data in Table 7, only 5% dextrose hydrate was required to prevent visible freeze damage. Thus, this study indicated that dextrose hydrate was superior to sucrose for maintaining F-T stability.

Table 5 — Freeze-thaw stability index of beverages containing various levels of sodium chloride

NaC1 conc %	Stability index[a] (%)
0.0	32.0
0.2	29.3
0.5	35.7
1.0	42.3

[a] Treatment effect signifIcant at 1% level.

Table 6 — Freeze-thaw stability index and mean visual score of soybean beverages with added 0.2% sodium chloride and various levels of sucrose

Sucrose conc %	Stability index[a] (%)	Mean visual score[a]
2	44.1	1.6
6	79.4	5.1
8	84.9	6.9

[a] Treatment effect significant at 0.5% level

Table 7 — Freeze-thaw stability index and mean visual score of soybean beverages with added 0.2% sodium chloride and various levels of dextrose hydrate

Dextrose hydrate conc %	Stability index[a] %	Mean visual score[a]
3	57.1	3.8
5	77.4	6.0
7	84.7	6.9
10	88.4	6.9

[a] Treatment effect significant at 0.5% level.

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Processing Retort Pouches in Conventional Sterilizers

R. A. ROOP and P. E. NELSON

ABSTRACT

A cylindrical rack was designed to hold four 130 mm x 178 mm retortable pouches. The outer dimensions of the cylinder were 157 mm x 178 mm, identical to a No. 10 can. These dimensions permitted pouch processing in traditional sterilizers that accept cans of this size. Dimensional considerations, pouch orientation, and heat distribution were tested in 100% steam. This racking method was found to provide equal heat treatments to all pouches regardless of horizontal, vertical or rolling orientations. These results suggest the use of various conventional sterilizers for pouch processing with little or no modification.

INTRODUCTION

LARGE CAPITAL INVESTMENTS are necessary to begin the commercial production of retortable pouches. Equipment to form, fill, and seal pouches or equipment to fill and seal pre-made pouches are absolute requirements for any production line. Because the pouch must be restrained during processing, special consideration must be given to sterilization equipment. Existing retorts must be modified or new ones must be purchased. Many sterilizers, especially continuous systems, are very expensive to modify. Therefore, a difficult decision arises: should companies spend large amounts of capital converting old systems to handle pouches or buy new equipment. If they convert old equipment, it would no longer be usable for cans. If they buy new retorts, they too would only have a single application. Davis (1981) indicated that many companies likely to enter the retort pouch field have made large investments in products and production facilities geared toward more traditional packaging. These investments would deteriorate if the retort pouch is as successful as predicted. If equipment were more versatile, companies would have less risk introducing retort pouches into the marketplace.

Though most pouches today are processed in water, processing in steam would facilitate the use of existing equipment. Many companies have sterilization equipment to process No. 10 cans (603 x 700) in 100% steam. These large cans have sufficient volume to hold several consumer-size (130 mm x 178 mm) pouches with a maximum thickness of 20 mm. The purpose of this project was to construct cylindrical racks with the same dimensions of a No. 10 can, therefore, utilizing traditional sterilization equipment for both pouches and cans without retort modification.

EXPERIMENTAL

Construction of rack

A stainless steel rack (Fig. 1 and 2) was built consisting of 4 slots, each measuring 130 mm wide, 178 mm tall, and 20 mm across. A 3 mm space separated each slot. Stainless steel rings having

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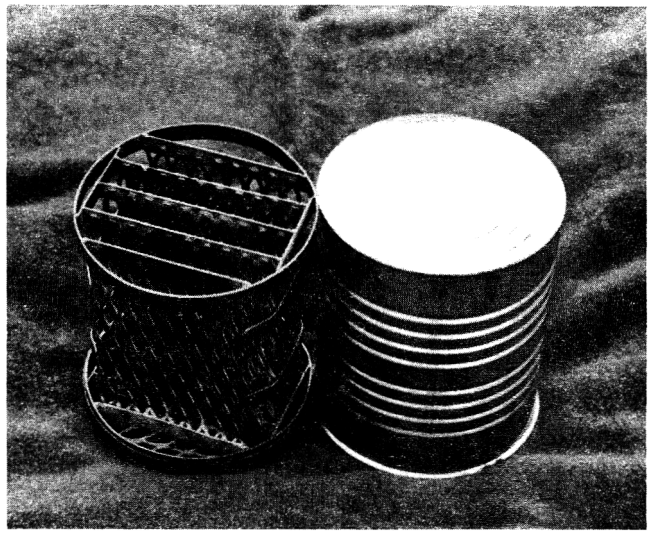


Fig. 1—Cylindrical rack for confining retort pouches in comparison to a Number 10 can.

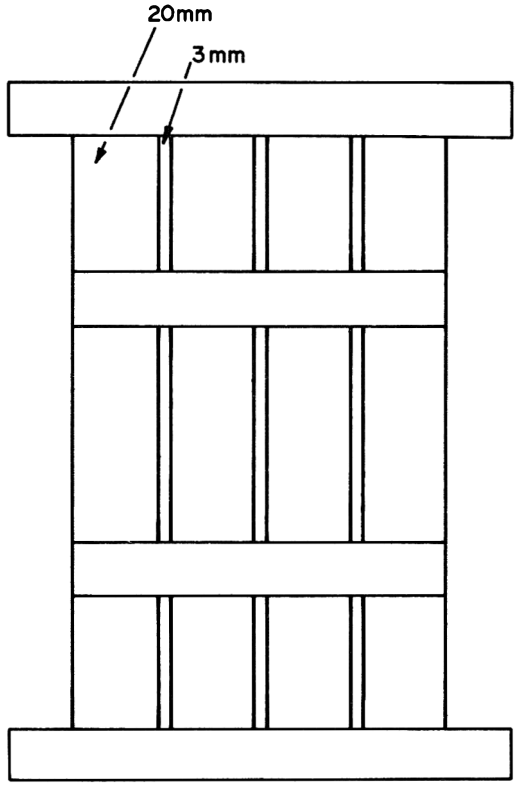


Fig. 2—Schematic of cylindrical rack for confining retort pouches during thermal processing in conventional strilizers.

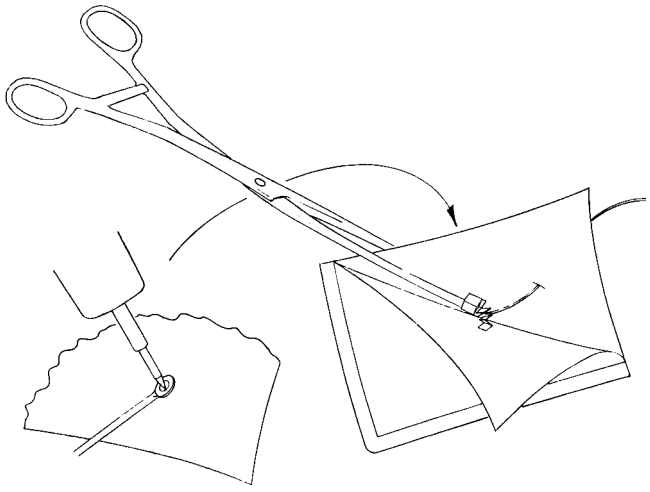


Fig. 3—Special forceps used for holding laminated web to pouch wall before heat fusion with a soldering iron.

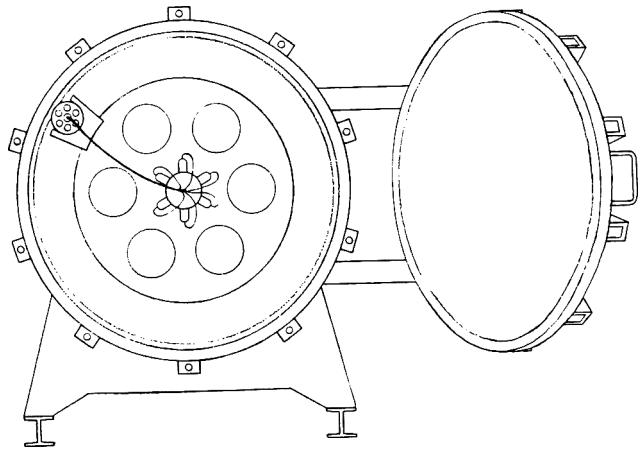


Fig. 4—Thermocouple wires attached to hub of reel in an FMC Steritort and to a rotary contacter on a cylindrical rack.

Table 1—Analysis of variance data for split-plot equipment

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-Statistic
Main Effects	985.8	8	123.2	–
Replications	37.4	3	12.5	–
Orientations	941.6	2	470.8	470.8/9.7=48.5
Slots	6.7	3	2.2	2.2/1.4= 1.6
Two-way Interactions	81.6	21	3.9	–
Replications x Orientations	57.9	6	9.7	–
Replications x Slots	12.9	9	1.4	–
Orientations x Slots	10.8	6	1.8	1.8/2.4= .75
Explained	1067.4	29	36.8	–
Residual	43.9	18	2.4	–
Total	1111.3	47	23.6	–

Table 2—Average of four heating times in minutes for 5% bentonite in retort pouches located in respective slots to heat from 82.2°C to 115°C

	Slot number			
	1	2	3	4
Vertical	16.1	16.8	17.8	15.8
Horizontal	13.3	12.8	13.2	11.5
Rolling	5.6	5.9	5.6	6.1

a diameter of 157 mm were welded to each end of the rack. These rings gave the rack identical outer dimensions of a No. 10 can. The sides of each slot were constructed of expanded stainless steel to allow efficient heat transfer.

Heat distribution tests

Experiments were performed to determine if each pouch in the rack received equivalent heat during processing. Copper-constantan thermocouples constructed from 30 gauge teflon-teflon coated wire (Omega Corp.) were positioned at the geometric center of each pouch. The lead wire entered the pouch through a small hole in the side and was sealed with silicone rubber. The thermocouple junction was held in place by a strip of pouch material folded several times to form an "accordian" web (Berry, 1979). Each end of the web was fused to the inner walls of the pouch. Newly designed forceps (Fig. 3) held the web in place while the hot tip of a soldering iron fused the web to the pouch wall.

Pouches were filled with 170 ml of 5% bentonite suspension as a model system. The bentonite was prepared and pretreated as described by Jackson and Olson (1940). The pouches, with product at room temperature, were sealed under 740 mm Hg vacuum, using a Swiss Vac sealing machine (Model 500, Transvac Maschinen Ag, 6010 Kriens/Lupen, Switzerland). The thermocouple lead wires were connected to a 5-circuit rotary contacter (Michigan Scientific Corp., Milford, MI). The lead wires from the rotary contacter were connected to the hub of an FMC Steritort. Though gold-copper, gold-constant junctions existed at the contacter, they were at the same temperature so no e.m.f. change was realized. This configuration permitted rolling of the rack while each pouch was independently monitored (Fig. 4). The thermocouples (e.m.f.) were monitored and signals were converted to temperature data by a computer-data collector (Roop and Badenhop, 1978).

A four replication, split plot, statistical design was used to test the null hypothesis that all pouches within the rack received equal heat exposure. The confined pouches were processed at 115.6°C. Timing did not begin until each had independently reached 82.2°C. At the end of each run the retort was cooled under 15 psi air pressure and water to prevent pouch bursting. The orientations and pouch positions were randomly selected using a random number table. The orientations were either vertical, horizontal, or rolling. To avoid variation, each orientation within a replication was performed using the same bentonite filled pouches. The time required for each pouch to be heated from 82.2°C to 115°C was recorded. Table 1 contains the analysis of variance (ANOVA) data for the split plot experiment.

RESULTS & DISCUSSION

THE AVERAGE TIMES required to heat from 82.2°C to 115°C within respective slots are listed in Table 2. Heating times were statistically different when comparing orientations ($F_{2,6}$ = 48.5 based on the critical value of 3.46 at α = 0.1. Student-Newman-Keuls' (SNK) range test at α = 0.05 suggested that there was no difference between horizontal and vertical orientations but a significant difference was detected between rolling and the other two orientations. Vertical pouches slump before pressurization, whereas horizontal pouches maintain a flat geometry. This could account for the apparent difference in heating rates detected in Table 2. The rapidity of heating during agitation resulted from the induced convection of the bentonite suspension throughout the process.

Table 3—Analysis of variance data for comparison of pouch heating times within vertical (V), horizontal (H), and rolling (R) orientations.

Source of variation	Sum of squares (V)	(H)	(R)	Degrees of freedom (VHR)	Mean square (V)	(H)	(R)	F-Statistic (V)	(H)	(R)
Main Effects	69.5	34.3	9.0	6	11.6	5.7	1.5	4.3	1.8	3.3
Replications	60.8	26.1	8.5	3	20.3	8.7	2.8	7.5	2.8	6.2
Slots	8.8	8.3	.50	3	2.9	2.8	.17	1.1	.88	.37
Explained	69.5	34.3	9.0	6	11.6	5.7	1.5	4.3	1.8	3.3
Residual	24.3	28.3	4.1	9	2.7	3.1	.46	–	–	–
Total	93.9	67.7	13.1	15	6.3	4.2	.88	–	–	–

Heating times for pouches in each slot were compared within each orientation. ANOVA revealed no significant difference between heating times of pouches within a given orientation, when tested against the critical value of $F_{3,9}$ = 2.81, α = 0.1. P-values are listed to demonstrate uniformity within orientations. However, the rolling orientation appeared to heat most uniformly ($F_{3,9}$ = 0.37, p-value = 0.775). The pouches in the horizontal treatment appeared to heat somewhat less uniformly among each other as indicated in Table 2 but were found not to be statistically different ($F_{3,9}$ = 0.88, p-value = 0.489). The same is true for the vertical orientation, which had $F_{3,9}$ = 1.1, at p-value = 0.406.

Using data in Tables 2 and 3, confidence intervals (C.I.) were calculated to give an indication of possible heating differences during a process. The intervals are the maximum differences expected, with 95% confidence, for the average of the outside pouches minus the average of the inside. Averages were used because the two outside slots were symmetrically equivalent to each other. The same is true for the two inside slots. For example in Fig. 5, the 95% C.I. for horizontal orientations was −3.33 and 2.14. This means that 95% of the time differences in heating times between outside and inside pouches will fall within this interval. In other words, the outside pouches could heat anywhere from 3.3 minutes faster to 2.14 minutes slower than the inside pouches. Each interval contained zero which supports the null hypothesis that there was no difference in heating times. Confidence intervals, such as these, could be used for the calculation of safety factors for process times.

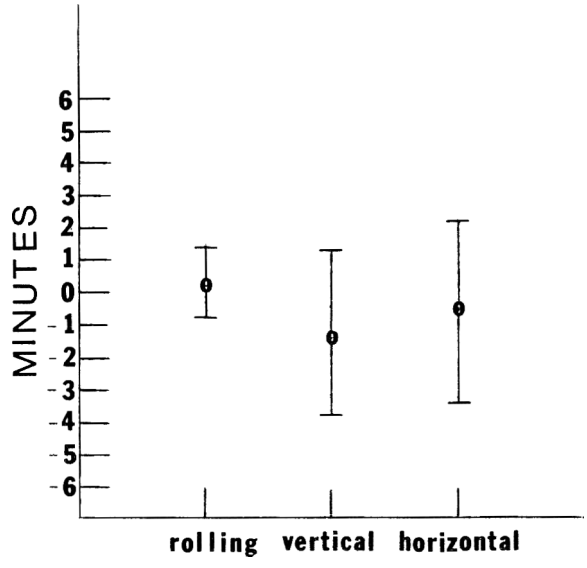


Fig. 5—Ninety-five percent confidence intervals for differences in average heating times of outside minus inside pouches with respect to orientation.

CONCLUSIONS

THE CYLINDRICAL RACK allowed equal heat treatment to all pouches within an orientation. The rolling orientation resulted in the most uniform heating. Though the heating times for pouches within vertical or horizontal orientations were not statistically different, they were not as uniform as rolling. This suggests that more care must be exercised when considering safety factors for non-rolling process times.

The use of 100% steam was satisfactory for the model systems used in this study. No pouches bursted even though they were reprocessed several times. Sealing the pouches under vacuum and cooling under pressure were found necessary to prevent pouch failure. Rolling (agitation) of pouches provided much more rapid heat transfer than the other two orientations. Agitation of products with heating characteristics similar to 5% bentonite would result in a more efficient process than non-agitating. However, heat pentration tests must be performed on specific products before any further conclusions can be made.

The design of this rack permitted pouches to be processed uniformly and warrants serious consideration for continued study by food processors as a solution to the economic problems involved in establishing a retort pouch line.

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Effect of Harvest Date, Irrigation, Maturity and Calcium Addition During Processing on Quality of Canned Summer Squash

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ABSTRACT

Soaking summer squash in calcium prior to commercial blanching at 99°C decreased sloughing, increased sensory firmness and had no effect on lightness or pH of the canned product. Although blanching squash in calcium decreased sloughing, improved lightness, decreased pH and produced off-flavors, the effect of hand blanching at 93°C with no calcium present increased sloughing, lightness, pH, and sensory firmness of the processed product. Adding calcium at the point of squash filling into cans after a commercial blanch produced no change in sloughing, decreased pH, increased sensory firmness and had no effect on lightness. Grade 1 squash exhibited the highest drained weight and lowest sloughing values. Drained weight increased at the end of the harvest season. Method of cultivation had no effect on the quality of canned squash.

INTRODUCTION

TEXTURAL BREAKDOWN causing tissue sloughing, or the loss of insoluble solids to the can liquor, occurs during the heat processing of summer squash. The economic and legal significance of this problem to the processor is (1) a loss of solid canned product and (2) the inability of the processor to control drained weight. Sistrunk et al. (1960) reported that date of harvest and irrigation affected the degree of sloughing in canned green beans. Higher percentages of sloughed skins were observed in beans from the last harvest while beans grown under irrigation exhibited less sloughing during processing. It has been shown that length of blanch, size of beans and bean variety influence the amount of sloughing in canned green beans (Sistrunk and Cain, 1960). Low temprature blanching (70–80°C) results in a lesser degree of sloughing in canned green beans (Van Buren et al., 1960) while blanching in calcium salts produces increased firmness in canned potatoes, sweet potatoes and cauliflower (Whittenberger and Nutting, 1950; Scott and Twigg, 1969; Hoogzand and Doesburg, 1961). The effect of soaking temperatures on calcium uptake and firmness values of some processed vegetables has been examined. Van Buren (1968) found little effect on firming when green beans were soaked 20 min at temperatures (2–45°C) in 4 x 10^3 ppm Ca prior to blanching, while Scott and Twigg (1969) reported that the effect of calcium on friming sweet potatoes could be improved by increasing the soaking temperature to 60°C. Calcium absorption in bean tissue was dependent upon the concentration of calcium used and the stage of processing when it was added (Van Buren, 1968). It has been reported that when calcium was used to firm tomato tissue, the drained weight of the canned tomatoes increased (Stephens et al., 1967: Saldana et al., 1971). Wilson et al. (1973) have found that green beans processed in a calcium brine yield a dull green color while Luh et al. (1975) noted an improvement in the color of lima beans during processing by adding calcium to the brine. Sistrunk and Cash (1970) reported that the presence of calcium in the blanch increased the lightness and yellow color of frozen summer squash.

According to FDA Standards of Identity (Almanac, 1978) calcium salt can be added to certain vegetables during processing to prevent sloughing and to increase the drained wwight of the canned product. Therefore, a study was initiated to determine the effect of adding calcium at different levels and at different stages of processing on the quality of canned squash. To implement an effective method of calcium addition which would not delay product flow would be beneficial to the industry. Other objectives within the study were to investigate the influence of irrigation, maturity and harvest date on the quality characteristics of squash.

EXPERIMENTAL

HYRIFIC (straightneck) variety squash, cultivated under a similar fertilization plan and insect and disease spray control program, were selected for use in this study. During the 1979 growing season (May, June, July) three batches (440 kg, each) of irrigated and non-irrigated squash were hand harvested at monthly intervals and transported by truck (31 km) to a commercial processing plant where they were segregated into U.S. Grades No. 1 and 2 (USDA, 1959). Twelve hours after harvesting crates of squash were dumped, according to grade and irrigation, into a commercial wash tank for cleaning. The squash were moved on a conveying belt past inspectors who removed any defects and prepared the squash for slicing. Squash were cut with a commercial slicer into 1.5 cm cross-sectional slices and treated by one of the following methods.

Method 1. Portions were soaked 1 min at 30°C in aqueous solutions containing 0 = no calcium, A = 8 x 10^3 ppm, B = 20 x 10^3 ppm, C = 40 x 10^3 ppm and D = 80 x 10^3 ppm calcium (in the form of $CaCl_2$) prior to blanching 3 min at 99°C in a commercial water blancher. Soaking was done by hand agitation to simulate fluming action.

Method 2. Portions were water blanched by hand 2 min at 93°C in aqueous solutions of 0 = no calcium, A = 4 x 10^3 ppm, B = 8 x 10^3 ppm, C = 20 x 10^3 ppm and D = 40 x 10^3 ppm calcium.

Method 3. Aliquots of 0 = no calcium, A = 0.8 x 10^2 ppm, B = 2 x 10^2 ppm, C = 4 x 10^2 ppm and D = 8 x 10^2 ppm calcium were pipetted into cans of squash which had been previously blanched 3 min at 99°C in a commercial water blancher.

Treated squash were filled by hand into 303 x 406 plain enameled cans, fill-in-weights recorded, a 60 grain salt tablet was added to each can and the fill completed with boiling water. Cans were sealed with an automatic closer, processed 26 min at 121°C, cooled to 38°C in a cooling tank and then stored at 25°C until analyses were made. A total of 360 cans were processed representing 120 cans per harvest date. This experiment was conducted during normal operations at a commercial canning plant using an approved scheduled process for the product.

Analytical procedures

Following a 30-day equilibration period, duplicate cans of each treatment were analyzed for percentage drained weight, sloughing, dry matter, pH, shear values, Hunter color and calcium content. Percentage drained weight was determined according to standard methods (USDA, 1959). Brine including suspended solids were collected in a shallow pan beneath the sieve used for drained weight and then poured into 100-ml graduated cylinders. Ten ml distilled water was used to make a quantitative transfer from the pan to the cylinders. The cylinders were stored overnight in a cooler at 4°C. After settling, the level of sediment in the cylinders was measured.

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The amount of sediment recorded was as an estimate of the degree of sloughing. Dry matter was determined by weighing 20g drained canned squash into tared aluminum dishes and drying to a constant weight in a vacuum oven at 60°C. The pH values were obtained from a 20g blend of drained canned squash using a Corning Model 610A pH meter, and were determined only for tissue since there was no difference in pH of tissue and brine samples. Shear force using a compression shear press was determined on an Instron Universal Testing Machine, Model 1130; a load cell calibrated to a full scale of 50 lb was used to shear 50 g drained canned squash. A crosshead speed of 5 in./min and a chart speed of 10 in./min were used. Objective color measurements were made on a Hunter Color Difference Meter, Model 0-25-A, standardized against a white plaque having an 'L' = 95.5; 'a' = −0.5 and 'b' = 1.0. Color values were determined on the rind portion selected at random and removed from 20g drained canned squash. Calcium content was determined by Emission Plasma ICP. Preparation consisted of weighing 20g of drained canned squash, drying at 100°C for 24 hr, ashing overnight at 500°C, then digesting the ash in 5% HCl. Data were reported as μg/g wet squash weight. Ten judges (6 female and 4 male) ranging in age from 21–40 years were selected for a sensory panel based on their availability and interest in the product. Since some panelists were not familiar with the canned squash, four practice sessions were held to acquaint them with its quality characteristics. Cross-sectionally cut and processed squash samples (4.1 cm) were selected at random from cans, quartered into equal sections with a knife, and served at room temperature on randomly coded paper plates. The serving order for all 18 testing sessions was randomized and tap water (room temprature) was used for cleansing the palate between samples. A descriptive 5-point scale was used for rating color, texture, taste and overall appeal. Extremely, moderately and neutral were terms used to characterize each attribute. A score of 1 indicated the sample was extremely bright in color, extremely firm in texture, extremely salty in taste and excellent in appeal. A score of 5 indicated the lowest quality or extremely dull in color, extremely soft in texture, extremely bland in taste and poor in appeal. A supplementary experiment was conducted to show changes in the structural carbohydrates due to calcium treatment by soaking and processing of fresh squash. From 20g squash blend approximately 0.25g squash (dry weight) was analyzed for pectic substances, hemicellulose and cellulose content according to the procedures described by Smittle et al. (1980). All data were submitted to statistical analysis of variance. Duncan's multiple range test was applied to the means of main effects when F values were significant. Data were graphed when significant interactions between main effects occurred.

Table 1—Effects of harvest date, cultivation, maturity and firming method on the texture and pH of canned summer squash[a]

Main Effects	% Drained weight[b]	% Dry matter[c]	Sloughing (ml)[d]	Shear press (1b/50g)[d]	Squash pH (tissue)[d]
Harvest date					
May	102b	5.4b	6.3b	17.1b	5.40b
June	101b	5.5a	5.9b	16.4b	5.42a
July	104a	5.6a	11.1a	23.1a	5.38b
Cultivation					
Irrigated	102a	5.5a	7.8a	18.8a	5.38b
Nonirrigated	102a	5.5a	7.1a	18.9a	5.42a
Maturity					
Grade 1	104a	6.8a	5.4a	16.3a	5.44a
Grade 2	100b	5.3b	10.0a	21.4a	5.35b
Firming Method					
Soak	104a	5.8a	6.5a	18.0a	5.43a
Blanch	98b	5.2c	10.1a	23.5a	5.45a
Fill	105a	5.5b	6.5a	15.2a	5.31b

[a] Mean separation within columns and main effects by Duncan's multiple range test, 5% level
[b] % Drained weight = drained weight/fill-in-weight x 100% (wet basis)
[c] Calculated as % dry weight
[d] Wet weight measurement

RESULTS & DISCUSSION

ALTHOUGH THE STAGE in processing when calcium was added had an apparent effect on the percentage drained weight of canned squash (Table 1), the higher drained weight found in the soak and fill methods and lower drained weight found in the blanch method was due to the type of blanch rather than the presence of calcium. Excessive handling during hand blanching and the fact that the squash were completely submerged under boiling water caused increased leaching of solids which accounted for a lower drained weight in the blanch method. Squash maturity influenced drained weight as exhibited by an increase in the drained weight of Grade 1 squash over Grade 2 squash. The drought conditions existing late in the harvest season may have influenced moisture absorption during blanching and processing causing an increase in squash drained weight in July.

There was an increase in dry matter as the harvest season progressed, with the lower percent in May probably being due to squash immaturity (Table 1). Dry matter was higher in Grade 1 squash than Grade 2. The fiming method used also affected dry weight. Soaking in calcium prior to blanching 3 min at 99°C in a commercial blancher resulted in the highest retention of total solids after processing, while hand blanching 2 min at 93°C in calcium resulted in the lowest retention of total solids. Method of cultivation did not have any effect on the drained weight or dry matter content.

There was no statistical difference in the sloughing of canned squash due to the firming method used (Table 1). Variation in sloughing, however, may be attributed to the significant interaction of concentration x method (Fig. 1). These data showed a decrease in sloughing (ml sediment) in canned squash when the raw product was soaked in calcium before blanching. This supports the observations made by Van Buren et al. (1968) who found that a calcium soak decreased sloughing in canned green beans. Adding

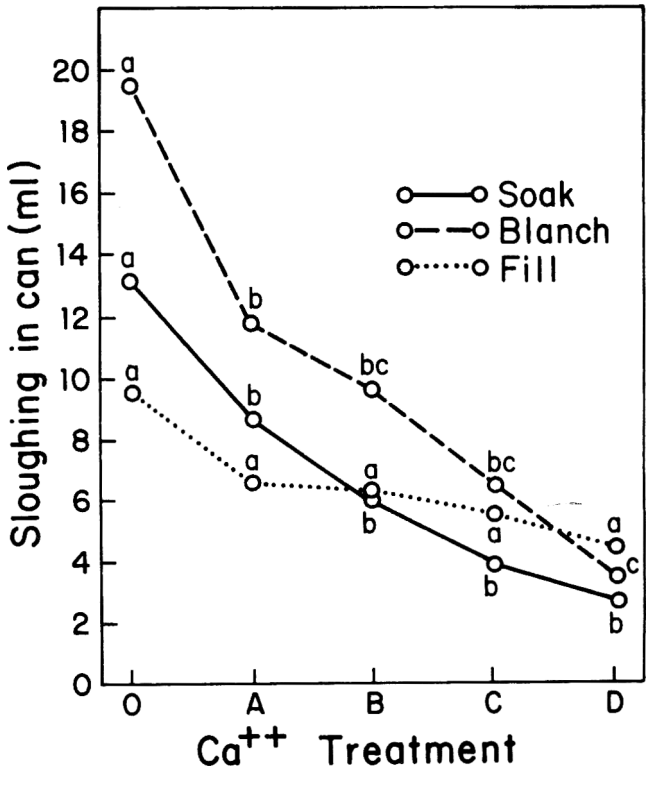


Fig. 1—Interaction of Ca^{++} treatment x firming method on sloughing of canned squash. (See Experimental for treatment concentrations.)

calcium to cans of squash prior to sealing had no effect on sloughing. Van Buren (1980) pointed out that sodium and calcium ions are freely exchanged between cells and that sodium does interfere with calcium interaction at pectic binding sites. Although blanching raw squash in calcium reduced sloughing, sediment was greater in the canned product than for squash which had been soaked in calcium prior to canning. This difference is attributed to a greater loss of tissue solids because of physical handling during hand blanching. Van Buren et al. (1960) reported sediment values higher resulting from a 93°C blanch in calcium solution than those found with beans blanched at 77°C in either water or an acidified calcium solution. Lower blanch temperatures (70–80°C) were initially used in this experiment to improve firmness, but it was discovered that 93°C was the minimum temperature needed to adequately blanch squash. Squash blanched at 80°C for 8 min did not result in an adequate blanch. An adequate blanch is important because the minimum drained weight for this product depends on properly shrinking squash for packing into cans before processing, and the achievement of a high initial temperature is an integral part of the scheduled process used for this product. Maturity had a significant influence on the degree of sloughing in canned squash, sediment values being higher for Grade 2 than Grade 1 squash. This would be expected since Grade 1 squash exhibited a higher drained weight. Sloughing of squash was highest during the month of July, which may be due to rapid growth during this month giving rise to larger more loosely bound cells. Irrigation of squash did not reduce sloughing of the canned product. Sistrunk et al. (1960) found that green beans from irrigated plots showed much less sloughing than beans from nonirrigated plots but the differences were not significant.

The results of resistance to shear appear contradictory since a higher shear value would indicate a firmer product (Table 1). This may be explained by the fact that the area sheared consisted mostly of squash rind (skin). Processing and sample handling caused much of the cavity tissue to be lost as sediment and this loss was greatest in Grade 2 squash. The higher shear for July was probably due to tougher skins late in the harvest season.

Although statistically different, the change in pH for the main effects-harvest date, cultivation and maturity was not thought to be of practical importance (Table 1). Changes in pH of squash tissue during firming (Table 1) were found for the soak and fill methods, probably due to the influence of calcium absorbed by the tissue (Fig. 2). A higher initial pH for squash blanched by hand with no calcium present in the water (Fig. 2) accounted for the higher value in Table 1. There is no ready explanation why the pH of this squash should be higher since the water used for hand blanching was obtained from the same source as that used in commercial blanching. The interaction of concentration x method for pH (Fig. 2) was significant because the calcium absorbed by soaking prior to blanching was not sufficient to change tissue pH yet did result in a decrease in sloughing, while the calcium absorbed during hand blanching and upon addition to cans was sufficient to cause a decrease in tissue pH.

The interaction of concentration x method for calcium in tissue (Fig. 3) shows that less absorption occurred by soaking squash in calcium before commercial blanching than during hand blanching or after commercial blanching. This is of practical importance because high calcium concentrations caused bitterness in the canned product.

Color differences in squash rind were found among harvest dates. Squash harvested in May exhibited higher 'L', 'b' and saturation color measurement values, indicating greater lightness and more saturated yellow color, while squash harvested in July had higher 'a' and a/b ratio values indicating greater redness in the skins (Table 2). Although color measurements for 'b' and saturation were higher for Grade 2 squash than for Grade 1, this difference was not detected by the sensory panel. Squash which were hand blanched in calcium exhibited higher 'L', 'a', 'b' and saturation color measurements indicating greater pigment color. The significant interaction of concentration x method for color measurement 'L' (Fig. 4) showed that lighness increased when squash were hand blanched in calcium. The presence of calcium and its stabilizing effect on reflectance has been demonstrated by Sistrunk and Cash (1970).

A sensory panel found that saltiness and firmness of

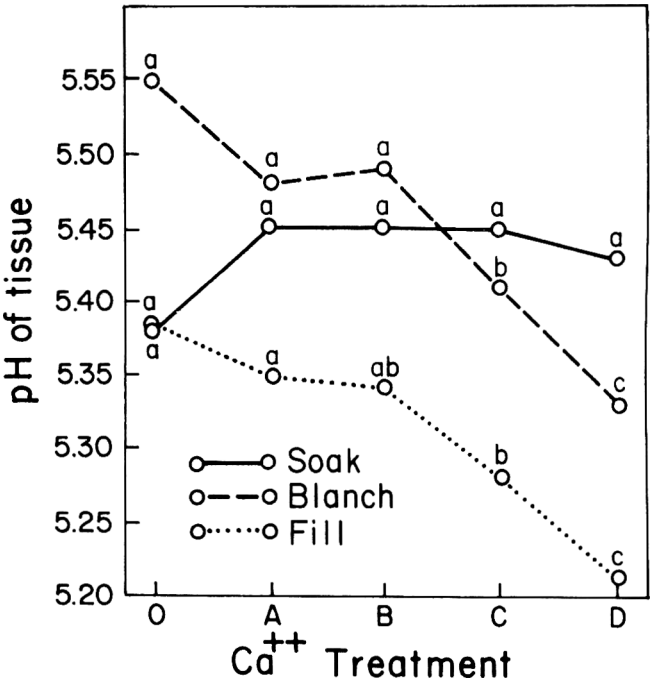


Fig. 2—Interaction of Ca^{++} treatment x firming method on pH of canned squash.

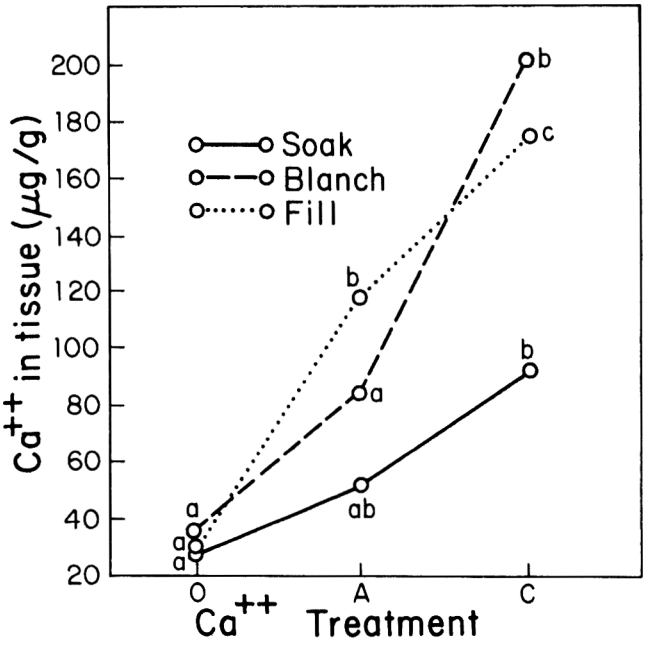


Fig. 3—Interaction of Ca^{++} treatment x firming method on calcium content of canned squash.

squash tissue increased as the growing season progressed from May to July (Table 3). Panelists rated Grade 2 squash firmer than Grade 1 squash and squash hand blanched in calcium firmer than squash treated by the other firming methods. These judgements were probably based on their observation of the surface-toughening effect on the rind of Grade 2 squash. Wittenberger and Nutting (1950) reported a similar case-hardening phenomenon in calcium treated potatoes. They found that it was more pronounced in tissues of low density rather than in those of high density. The significant interaction of concentration x method for sensory texture (Fig. 5) shows an increase in squash firmness for all the firming methods used, but hand blanching in no calcium and in the presence of calcium appeared to give the greatest improvement in texture. Such a discrepancy in firmness among the methods may be due to the temperature used to blanch squash. Hand blanching was achieved at a lower temperature (93°C) than is normally used in commercial blanching. Although this lower temperature may be the upper range of activity for the tissue enzyme, pectin methyl esterase, an increasing number of potential calcium binding sites could have been liberated due to its deesterification activities. Any free calcium in the tissue itself or calcium in the water used for blanching would be available to bind at these sites resulting in the tissue firming (calcium pectinate) phenomenon. In contrast, commercial blanching of squash probably severely limited or inactivated the enzyme and reduced the number of potentially available calcium binding sites. Although more research is needed in this area for squash, Scott and Twigg (1969) have demonstrated that calcium firming of sweet potato tissue occurs over a broad temperature range (26–100°C). There was no difference in sensory rating for appeal (Table 3), however, the interaction of concentration x method (Fig. 6) indicates that calcium soak firming was consistent in appeal over all concentrations, while the calcium blanch and calcium addition to can methods caused a decrease in appeal. This was due to the bitter taste and off-flavors reported in squash which had been treated with higher calcium concentrations of the latter two firming methods.

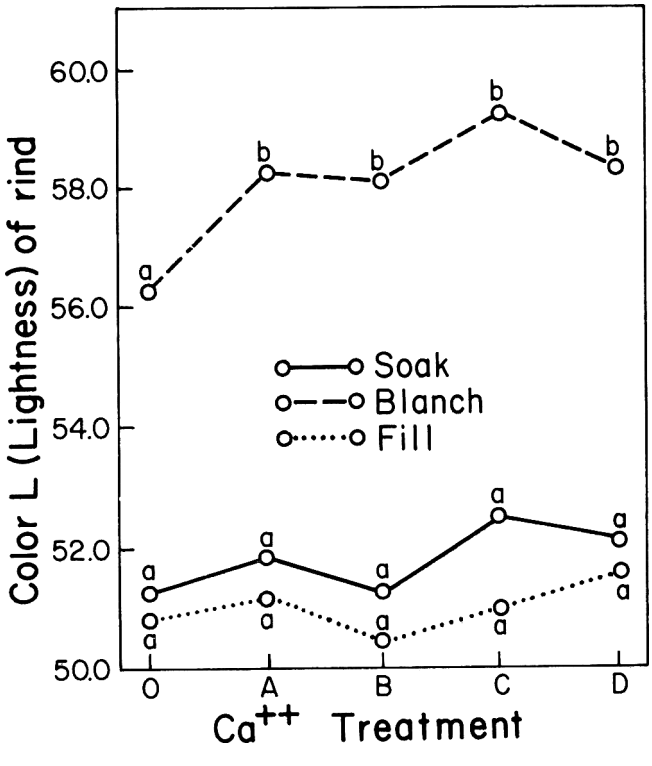


Fig. 4—Interaction of Ca^{++} concentration x firming method on color of canned squash.

Changes in structural carbohydrate fractions of fresh squash due to calcium treatment and processing are presented in Table 4. Processing caused an increase in the hemicellulose and cellulose content. Processing alone, however, may not have been totally responsible for this significant increase. The fact that immature and mature (higher cellulose content) squash were held over 12 hr at 30°C prior to processing might account for higher cellulose and hemicellulose values in the processed product. These results are similar to Smittle et al. (1980) who found increases in cellulose and hemicellulose fractions in immature squash on storage at elevated temperatures. During processing protopectin was hydrolyzed to soluble pectin, increasing the water-soluble fraction but registering no change in the calgon-soluble fraction. A significant decrease in protopectin when squash was calcium treated by soaking prior to processing led to the highest concentration of water soluble pectin. This increase in water soluble pectin, which

Table 2—Effects of harvest date, cultivation, maturity and firming method on the color of canned summer squash[a]

Main effects	Hunter color measurements[b] 'L'	'a'	'b'	'a'/'b' (ratio)	$\sqrt{a^2 + b^2}$ (saturation)
Harvest date					
May	54.7a	1.6a	27.2a	0.06b	27.3a
June	52.9b	0.7c	25.6c	0.02c	25.7b
July	53.5b	3.0a	26.5b	0.11a	27.7a
Cultivation					
Irrigated	53.7a	1.5a	26.4a	0.05a	26.5a
Nonirrigated	53.6a	2.0a	26.4a	0.07a	26.6a
Maturity					
Grade 1	53.7a	1.9a	26.1b	0.07a	26.2b
Grade 2	53.7a	1.6a	26.8a	0.06a	26.9a
Firming method					
Soak	51.8b	1.4b	25.2b	0.05a	25.3b
Blanch	58.0a	2.5a	29.0a	0.09a	29.2a
Fill	51.0b	1.4b	24.9b	0.05a	25.0b

[a] Mean separation within columns and main effects by Duncan's multiple range test, 5% level
[b] Color measured on squash rind only (wet basis)

Table 3—Effects of harvest date, cultivation and firming method on sensory attributes of canned summer squash[a]

Main effects[b]	Color[c]	Taste[d]	Texture[e]	Appeal[f]
Harvest date				
May	2.8a	3.2a	4.1a	4.0a
June	2.9a	2.8b	3.8b	3.8a
July	3.1a	2.8b	3.7b	3.9a
Maturity				
Grade 1	3.0a	2.9a	4.0a	4.0a
Grade 2	2.8a	3.0a	3.7b	3.8a
Firming method				
Soak	2.9a	3.1a	4.0a	3.8a
Blanch	3.0a	2.8a	3.6b	3.9a
Fill	2.9a	2.9a	4.1a	3.9a

[a] Mean separation within columns and main effects by Duncan's multiple range test, 5% level
[b] Sensory rating for non-irrigated squash only (wet basis)
[c] Scale = 1 (extremely bright) - 5 (extremely dull)
[d] Scale = 1 (extremely salty) - 5 (extremely bland)
[e] Scale = 1 (extremely firm) - 5 (extremely bland)
[f] Scale = 1 (excellent) - 5 (poor)

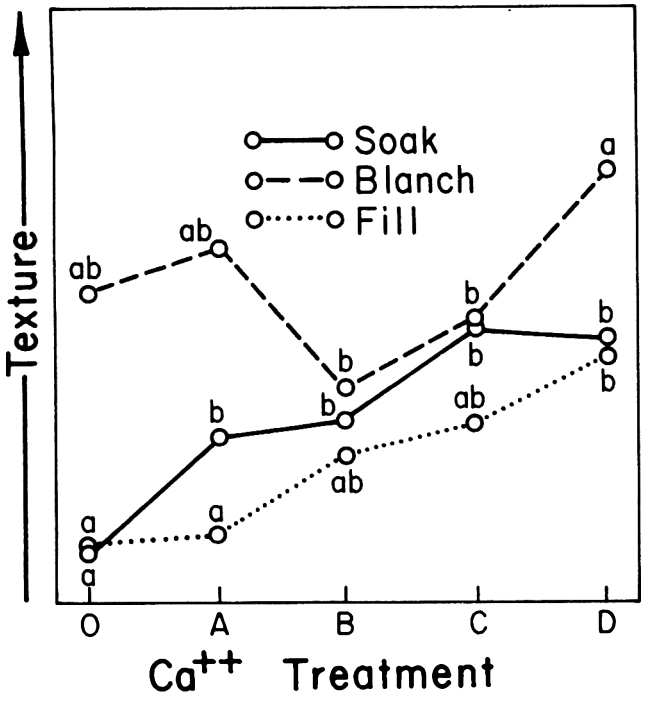


Figure 5—Interaction of Ca^{++} treatment x firming method on texture of canned squash.

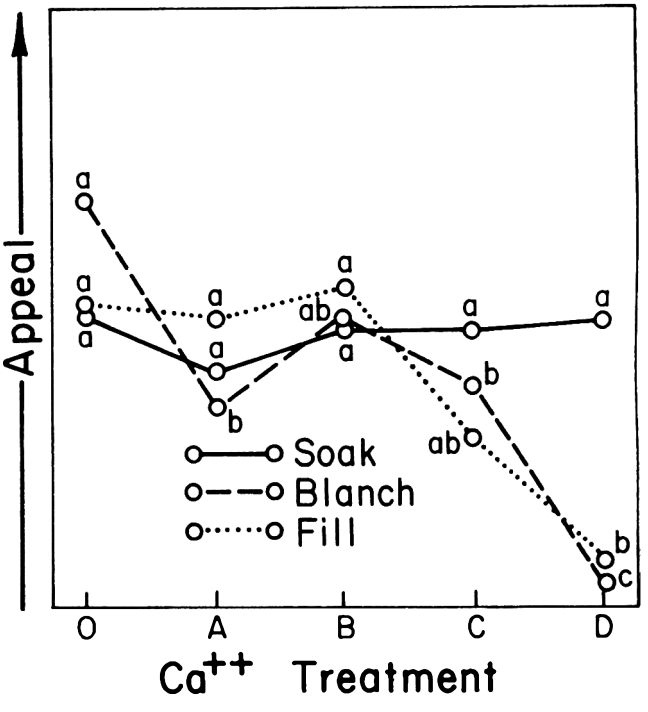


Figure 6—Interaction of Ca^{++} treatment x firming method on appeal of canned squash.

Table 4—Effect of calcium treatment and processing on structural carbohydrates, sloughing and drained weight of canned summer squash[a]

Sample[b]	% Water soluble pectin[d]	% Calgon soluble pectin[d]	% Proto-pectin[d]	% Hemi-cellulose[d]	% Cellulose[d]	Sloughing[d] (ml)	% Drained weight[d]
Fresh	1.57c	1.46a	1.29a	0.61b	4.60b	–	–
Processed	2.45b	1.54a	0.93a	1.14a	8.53a	12.3a	79.8a
Ca soaked/ processed[c]	3.10a	1.36a	0.78b	1.02a	9.07a	8.2a	81.6a

[a] Mean separation within columns and main effects by Duncan's multiple range test, 5% level
[b] Replicated five times for two grades
[c] CA content 20 x 10^{-3} ppm
[d] All carbohydrates are percent of dry weight.

provided additional binding sites for calcium firming (calcium pectate) to occur, was apparently responsible for some decrease in sloughing of the processed product.

In summary, an increase in calcium levels increased the binding of insoluble solids in squash leading to a decrease in sloughing of the canned product. A comparison of firming methods revealed that the type of blanch influenced the color, texture and pH of the processed product. Although none of the methods were effective in increasing drained weight, soaking squash in calcium prior to blanching appears to give the best firming response and results in the most acceptable canned product.

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Effect of K Fertilization and Addition of Salts on the Texture of Canned Snap Bean Pods

J. P. VAN BUREN and N. H. PECK

ABSTRACT

Increases in pod K, obtained either by fertilization or direct addition to the can, resulted in less firm canned pods. Removal of soluble solids from pods prior to canning resulted in firmer pods. Pod K and texture were highly correlated, with the greatest changes in texture with changing K occurring below 2%K in pods on a dry weight basis. The effects of K and Na appeared due to a combination of Ca displacement and an enhancement of pectin degradation.

INTRODUCTION

THE TEXTURE of canned snap beans is one of this commodity's more important quality characteristics. A variety of methods have been developed for its control. Endogenous pectin methyl esterase has been extensively used to achieve desired product texture through adjustments of the blanching temperature and time (Van Buren et al., 1960; Sistrunk and Cain, 1960). Calcium salts increase the firmness of the cooked pods and sodium salts result in a softening (Van Buren, 1980).

These results with salts indicate that the salt or cation content of pods should influence texture. Fertilization can influence mineral content, and it has been shown earlier (Van Buren and Peck, 1963) that under sand culture conditions Ca levels in the pods could be regulated by the nutrient supply, and that increased pod Ca promoted firmness.

The principal monovalent cation in bean pods is K (Peck and Van Buren, 1975) and its concentration is sensitive to the K supplied to the plant. It could be expected that augmentation of the pod K concentration would affect texture because monovalent cations are associated with softening resulting from both their replacement of Ca (Dainty et al., 1960) and their accelerating effect on β-eliminative degradation of pectins (Keijbets and Pilnik, 1974).

In this study the result of K fertilization on the texture of canned pods was determined utilizing both sand-culture and field grown plants. Further, the effects of additions of salts and removal of soluble pod components were compared with the effects of the fertilization.

MATERIALS & METHODS

SNAP BEANS were grown outdoors in either sand culture or in soil. Details of culture are given elsewhere (Peck and Van Buren, 1975). For the sand culture the K concentrations in the nutrient solutions were 0.3 and 6.0 meq K/1.

Beans grown in the soil, a Honeoye loam, were supplied with fertilizer K from K_2SO_4 as follows:

K Treatment	K Supplied, kg/hectare
Low	0
Moderate	100
High	500
Very high	1700

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Pods were harvested when 90% of the pods were sieve size 4 and under.

Snap bean pods of combined sieve sizes 3 and 4, cut to 3.81 cm lengths, were used for canning. Blanching was carried out at 82°C for 2 minutes. Pods to be leached received a second blanch at 90°C for 2 min that took place 10 min after the first 82°C blanch. For overnight leachings at 2°C blanched beans were placed in 10 times their weight of cold distilled water and stirred occasionally while being held in the cold room.

For the canning process 250g portions of pods were packed into No. 303 cans, any indicated solutions were added, and the filling completed with room temperature distilled water. The filled cans were passed through a steam tunnel raising the can contents temperature to 92°C in 3 min. The cans were immediately closed, retorted at 115°C for 20 minutes, then cooled and stored at 18°C until they were tested.

Firmness was measured on an Instron Universal Testing Machine (Instron Engineering Corporation, Canton, MA) as the maximum force reading, in kg, when a 6.50 cm diameter piston was moved at 20 cm per minute to 0.6 cm from the bottom of a 7.35 cm inside diameter heavy walled aluminum cylinder containing 100 g of randomly oriented snap beans. The procedure was a modification of that described by Van Buren (1968).

Cation concentrations were measured by atomic absorption spectrometry. (Slavin, 1968).

RESULTS

THE EFFECT of K availability in sand-culture on the firmness of canned pods was studied with two bean varieties, Wax Bonanza and Early Gallatin. Table 1 shows the firmness of the canned pods and the K concentration in the can liquid for two levels of K nutrition and for pods with and without blanching before retorting. The high K pods were 30% less firm for the blanched pods and 20% less firm for the unblanched pods as compared to the low K pods. The corresponding K concentrations in the pods and in the can liquor reflect the mineral nutrition of the plants, indicating a marked effect of nutrient K on pod K. Comparison of pods with and without blanching before retorting showed that blanching at the 82°C temperature promotes firmness in the canned pods, similar to what had been found in earlier studies (Van Buren et al., 1960).

Pods from beans grown outdoors in the soil showed the same general response to K fertilization as was seen with the sand culture beans. Although the firmness differences were smaller the effects of the K fertilization were sta-

Table 1—Sand culture K fertilization effects on firmness of canned snap beans[a]

Fertilization with K meq/L	Blanch treatment	Bean firmness Instron values[b]	K content[b], mg/g Fresh pods	K content[b], mg/g Can liquor
0.3	82°C/2 min.	59.2a	0.976a	0.445a
	None	40.5b		0.557a
6.0	82°C/2 min.	41.4b	2.60b	1.212b
	None	32.9c		1.170b

[a] Average of two varieties: Early Gallatin and Wax Bonanza.
[b] Means in columns not followed by the same letters are significantly different at the 95% level.
% dry wt = 8.0.

Table 2—Field K fertilization effects on firmness of canned snap beans[a]

Fertilization with K	Bean firmness Instron values kg	K content, mg/g		Ca content, mg/g		Bean pod yield kg/ha
		Fresh pods	Can liquor	Fresh pods	Can liquor	
Low	44.6	1.56	0.749	0.48	0.084	7130
Moderate	44.4	1.68	0.835	0.46	0.099	8050
High	41.3	1.82	0.896	0.47	0.088	7630
Very High	41.6	1.81	0.856	0.47	0.085	7900
Statistical significance	0.01	0.003	0.05	0.29	0.41	0.01
Standard error of difference	0.58	0.04	0.041	0.01	0.009	180

[a] Average values for three varieties: Early Gallatin, Wax Bonanza and Bush Blue Lake. Pods blanched at 82°C for 2 min and canned in distilled water. % dry wt = 7.1.

Table 3—Effects of salt changes during processing on the firmness of canned snap beans

Fertilization with K	Process Treatment	Bean firmness Instron values kg	Cation content of liquor, mg/g	
			Ca	K
Low	regular	44.6	0.076	0.755
Low	add KCl[a]	40.4	0.077	1.720
Low	add NaCl[c]	35.2	0.092	0.742
Low	leached[b]	53.7	0.047	0.170
Low	leached[b] then add NaCl[c]	38.5	0.059	0.163
Very high	regular	41.6	0.082	0.863
Very high	add NaCl[c]	34.0	0.089	0.850
Very high	leached[b]	55.6	0.038	0.147
Very high	leached[b] then add NaCl[c]	38.1	0.067	0.201

[a] 782 mg of K as KCl = 20 meq per can
[b] Leached in distilled water overnight between blanching and retorting.
[c] 1518 mg of Na as NaCl = 66 meq per can

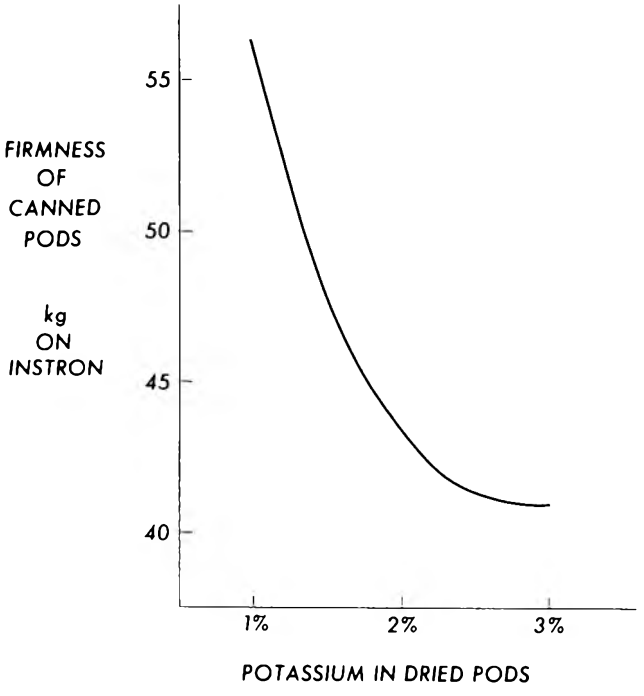


Fig. 1—Relation between pod K, as influenced by fertilization, and firmness of canned pods. Sieve size 3 and 4 pods canned at 115°C for 20 min. No salt in canning water. The equation for the curve shown is:
Firmness (kg) = 132 + 32(%K) - 108(%K$^{1/2}$),
and it fits the experimental data with an R^2 = 59.

tistically significant at the 1% level as calculated by the analysis of variance. Table 2 contains data concerning the firmness values, the K and Ca contents of the pods and liquor, as well as the yield of pods. Three varieties were used for these studies, and the values for the three varieties were combined to give the averages presented.

The field used for growing these beans had a moderate amount of K available even before fertilization, in contrast to a well depleted soil used in earlier fertilization trial (Peck and Van Buren, 1975). This may account for the small K differences obtained in the present study.

Liquor Ca concentrations were somewhat variable and the statistical analysis indicates that the differences between the treatments have 41% odds of being due to chance alone.

Analysis of data obtained over several years for %K in pods and Instron firmness values showed a good correlation through the equation:

$$F = 132 + 32(\%K)\ 108(\%K^{1/2})$$

for which R^2 = 59 and F = firmness in kg. %K = K in pods as % of dry weight. This curve is shown in Fig. 1. It indicates that firmness changes very little in the %K range 2.5–3.0. This %K range can normally be achieved only at very high, and probably uneconomical, rates of K fertilization. Levels of 1% or less K would likely require omission of K fertilization on K depleted sandy soils, but when this level of K is secured rather firm beans would be expected. The usual range of K in pods, on a dry weight basis, is 1.2–2.4%. This curve is presented as a good fit to the data obtained. Extrapolation is not warranted and, in fact, is likely to lead to error. For instance if the %K were reduced to 0 the firmness predicted by the equation would be 132. Such a high value is not to be expected in practice, as is shown by the results with leached pods (Table 3).

The relationship seen between pod firmness and %K does not describe the mechanism of interaction. Pod maturity and structure were possibilities since an earlier study (Peck and Van Buren, 1975) showed, at a given sieve size, lower % seed and fiber with increasing pod %K and higher rates of K fertilization. Chemical effects of K as a promoter of pectin breakdown (Keijbets and Pilnik, 1974) and displacement of Ca (Dainty et al., 1960) also had to be considered.

Evidence for one or more of these mechanisms was sought by processing high and low %K pods in a number of ways increasing and decreasing the K and salt content. Data are presented in Table 3 concerning the firmness and liquor K after canning the various treatments.

Removal of much of the normal pod salts by leaching resulted in firmer canned pods. Furthermore, with the leached treatments, the pods grown with low K were not firmer than the pods grown with high K. This indicated that possible pod structure or maturity differences were not affecting firmness. This conclusion was supported by the effect of K addition at the time of canning to low K pods

in reducing firmness to a level slightly below that of the regular process very high K pods. These same comparisons strongly support a chemical role for the effect of K on texture.

The similar firmness of the low K fertilization pods plus KCl and the very high K regular process beans, despite a twofold higher concentration of K in the low K fertilization cans suggests that added K was not as effective as endogenous K in causing loss of firmness.

The very soft and similar textures of the leached pods canned with NaCl are further evidence for a chemical role for the monovalent cations.

DISCUSSION

THERE WAS A highly significant effect of K nutrient supply under both sand culture and field conditions on the firmness of canned beans. Since the same sieve size of beans was used in all the canning operations the differences in texture can be attributed to the influence of potassium supply on the composition or structure of the pods.

The level of K fertilization had a direct effect on the K level found in the pods, as shown here and in earlier work (Peck and Van Buren, 1975), and it was probable that pod potassium was responsible for the texture differences seen. This was tested in experiments where K was added at the canning stage or removed prior to canning. These showed that low or lowered K concentrations gave firmer pods while high or raised K concentrations gave softer pods. This is similar to results on potato texture described by Davis and LeTourneau, 1973. The levels of monovalent cations were primary causes of variations in firmness.

There are two major mechanisms by which monovalent cations such as K^+ and Na^+ influence the texture of canned vegetables. One is through interference with the firming effect of Ca (Dainty et al., 1960; Kohn and Furda, 1967) by replacing the Ca on the pectin molecules. The other is by enhancing the β-elimination reaction whereby pectic substances are degraded (Keijbets and Pilnik, 1974).

It would appear that both mechanisms contributed to the results obtained, Table 3. More tissue Ca was released into solution when NaCl was added. This indicates some softening was due to the displacement of Ca. But the differences in firmness were much greater than one would expect from the relatively small change in liquor Ca (Van Buren, 1980). Some β-elimination pectin degradation was indicated. The lack of significance for the relationship between liquor Ca and firmness shown in Table 2 also points to an important role for β-elimination.

These studies show that it was possible and practical to affect canned pod texture by controlling the nutrient K available to the bean plant. Lower nutrient K was related to lower pod K and firmer canned product. Knowledge of soil types and fertilization practices would aid a processor in predicting the probable texture of the canned product, and adjusting processing techniques accordingly. This sort of control would be most useful for unsalted or low salt packs since the fertilization effects were masked when NaCl was added in normal process amounts (3.82g or 60 grains per can).

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A Direct Hexane Extraction Process for Glanded Cottonseed

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ABSTRACT

A modified filter funnel apparatus was used to determine mass flow velocities for extraction of uncooked glanded cottonseed flakes, and the results were compared with a continuous pilot-plant-scale extractor. A mass velocity of 2,000 lb/hr/ft^2 or higher (considered adequate for oilseed extraction) was obtained in all cases. Both the initial meat moisture before flaking and the flake moisture during extraction were found to affect the mass velocity. A meat moisture before flaking of less than 9% decreased the mass velocity, probably as a result of increased fines and thus smaller flake size. A high flake moisture (about 9%) during heated-hexane extraction caused a decrease in mass velocity compared to that of lower-moisture flakes. The correlation of filter-funnel mass velocity data to a continuous pilot-plant extractor confirmed that uncooked flakes can be satisfactorily extracted to yield low residual lipids by using a low solvent-to-flake ratio and ambient-temperature hexane solvent.

INTRODUCTION

CONVENTIONAL METHODS of defatting glanded cottonseed such as screw-pressing, direct extraction and pre-press solvent extraction requires the application of moist heat conditioning to prepare material for oil removal by solvent extraction (Harrison, 1977). But the use of moist heat causes excessive pigment gland breakage which (because of the binding of gossypol with protein) renders the defatted material objectionably dark in color and unsuitable for pigment gland removal by air classification. Residual gossypol in cottonseed protein products has also been associated with objectionable color in these products (Kadan et al., 1978).

Recent work at our Center has shown that a light-colored, edible flour product (one with less than 0.045% free gossypol) (FDA 1974) can be prepared from properly processed, defatted glanded cottonseed flour by an air classification process (Freeman et al. 1979, Kadan et al., 1979). This process requires extraction of uncooked flakes with ambient-temperature hexane. (Applying heated hexane imparts yellow color to the extracted flakes indicating pigment gland damage).

This study was conducted to determine whether sufficient mass velocity (percolation rate) could be achieved using uncooked flakes and ambient-termperature hexane to give a practical, continuous, commercially applicable extraction. The effects of (1) flake moisture during extraction; (2) initial kernel moisture before flaking, drying, and extraction; and (3) hexane temperature during extraction on the residual lipids of the extracted flakes and on the gossypol contents of the extracted flakes and extracted lipids are also reported.

MATERIAL & METHODS

SEVERAL SERIES OF EXPERIMENTS were conducted to evaluate the effects of certain processing conditions on mass velocity, which for the purpose of this study, is defined as the weight (in lb) of miscella (solvent plus oil) that flows through one square foot of filter surface in 1 hr.

Delintered, dehulled, prime quality, Texas high plains glanded cottonseed was used for this study. Seeds were dehulled to produce kernels having about 1% hulls. To prepare the meats and flakes at the various moisture levels that were required for the three series of experiments, the delintered seeds were moistened to 11.2%, equilibrated for 96 hr, and dehulled to produce kernels with about 1% hulls. The kernels were flaked to a thickness of 0.015 in. on smooth Ross rolls. One portion of the flakes was used "as is" at 11.2% moisture, and three other portions were dried to moisture levels of 8.9, 5.0, and 2.0%. Another sample of flakes was prepared from seeds that were not moistened. These flakes contained 8.1% moisture. To compare the effect of the original moisture content of the meats, the 11.2 and 8.1% moisture flakes were dried to 2% prior to extraction at 22°C and 63°C. To compare the effect of flake moisture content, the 8.9, 5.0 and 2.0% moisture, flakes were extracted at their respective moisture contents. A major portion of the 8.1% moisture flakes dried to 2% were used for the continuous pilot plant scale test runs. Drying of flakes for the laboratory scale experiments was conducted at 82°C in a high-velocity, through-flow Blue M oven, whereas, for the pilot plant scale tests, a continuous stainless-steel, mesh-type belt dryer was used.

A previously described (Graci et al., 1955), modified filter-funnel apparatus (Fig. 1) was used to determine mass velocities for the laboratory extractions. Laboratory-scale extractions were carried out as follows: a uniform sample of 600g of flakes was placed in the funnel to a bed depth of about 4 in; a 50-mesh circular

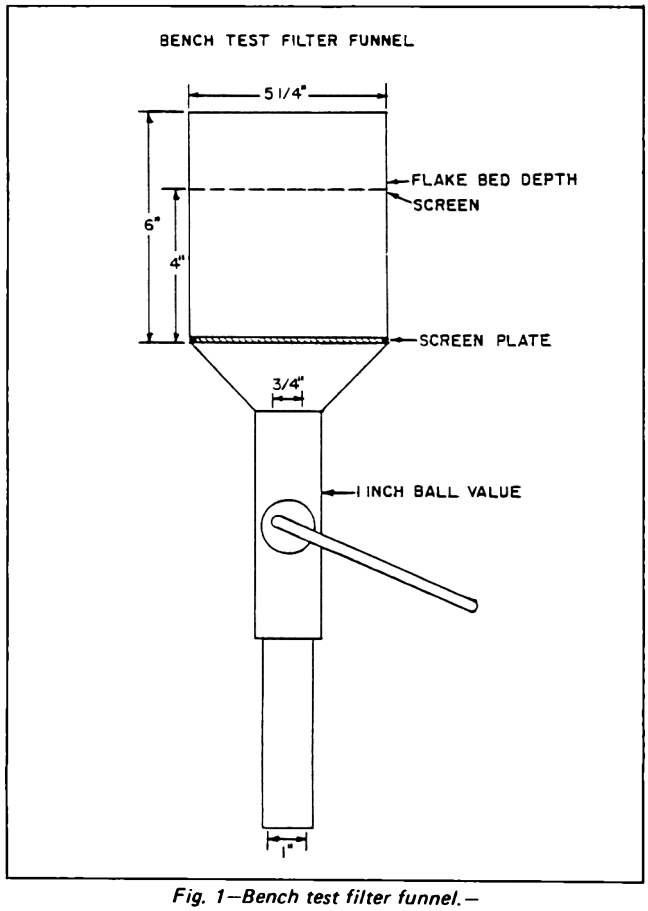


Fig. 1—Bench test filter funnel.—

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screen was placed at the top of flakes to keep the material undisturbed during extraction, and 1,000g of hexane was transferred to the funnel containing the flakes and held for 2.5 min before the drain valve was opened. The flow time was measured in seconds from the time the drain valve was opened to the break in full flow at the valve discharge with an additional 10-sec drain time allowed before closing the valve. The 10 sec drain times were not used in total flow time calculations. The miscella weight was determined. The extraction was repeated three more times using 1000 g of fresh hexane each time. The miscella weight did not include the miscella between the screenplate and the valve (261g) which was subtracted from each extraction since it did not flow through the flakes during the timed flow measurement. Thereby only the miscella that passed through the bed of flakes during the timing cycle was considered in the calculations. The 261g value was determined from the average of several physical measurements. The following formula was used to calculate the mass velocity:

$$\frac{\text{Total wt in g of miscella} - (261 \times 4)}{\text{Total filtration time in sec}} \times 71.86 = \text{lb/hr/ft}^2$$

The factor 71.86 was derived for a 114.25-mm diameter filter to convert experimental data to lb/hr/ft^2.

The mass velocities obtained with the filter funnel apparatus were compared with the calculated mass velocities for the pilot-plant-scale experiments using the continuous Crown extractor. Calculations were based on miscella density, flow rate, area of filter bed and percolation time. The amount of fines in the miscella was obtained by filtering a weighed portion and drying the recovered portion. The defatted material was desolventized at 83°C and a reduced pressure of 508-mm Hg vacuum. Miscella from several extractions were vacuum distilled to recover samples of crude cottonseed oil. The crude oil was refined by a commercial laboratory. Official AOCS methods (1976) were used for all tests, proximate analyses and gossypol assays.

RESULTS & DISCUSSIONS

THE RESULTS of multiple extraction of dried flakes with ambient-temperature hexane are shown in Table 1. About 83% of the lipid material is removed within 5 min contact time, another 7.5 min contact time removed only about 9% more lipids, confirming earlier conclusions (Othmer and Agarwal, 1955; Othmer and Jaatiner, 1959) that the contact of lipids in the material with solvent is essential, as the diffusion of lipid into the solvent does not play an important role in good lipid extraction. The flakes also showed good percolation rates; mass velocities were above 2,000 lb/hr/ft^2 throughout the extraction. A mass velocity of 2,000 lb/hr/ft^2 is considered adequate for oilseed extraction with laboratory-scale equipment, a rate that precludes filtration problems associated with pilot plant and/or commercial operations (Graci et al., 1955). The mass velocities tended to vary with the number of extractions but remained above the critical minimum mass velocity required for economical operation of oilseed solvent extraction.

The effect of initial meat moisture (before flaking) is shown in Table 2. Mass velocities were higher at both initial-meat-moisture levels with heated hexane than with ambient-temperature hexane. Flakes made from 11.2%-moisture meats had higher mass velocities than from flakes made from 8.1%-moisture meats. Physical examination of the dried flakes indicated that the average flake size from 8.1%-moisture meats was smaller than flakes from 11.2% meats, indicating that some fines were generated during the flaking and drying of 8.1%-moisture meats. Sieve analysis confirmed this observation; 71% of the dried flakes from 8.1%-moisture meats passed through the No. 12 ASTM screen, whereas only 15% of the 11.2%-moisture meast passed through this screen. The presence of fines apparently causes a reduction in mass velocity.

Test runs that had higher mass velocities extracted lipids better (as shown by lower residual lipids). Heated hexane extracted more lipids than unheated hexane (as indicated by residual lipids of defatted material). The total gossypol content of defatted flakes extracted with heated hexane are lower than that of flakes extracted with ambient hexane (at both moisture levels) indicating that heated hexane damages the integrity of pigment glands during solvent extraction and causes some pigment gland contents to be extracted in the miscella.

The effect of flake moisture prior to extraction and hexane temperature is presented in Table 3. At both temperatures, there was little difference between the 2% and 5%-moisture flakes in mass velocity, residual lipids, and the free and total gossypol content of extracted flakes. There appears to be a slight increase in gossypol content of oil extracted from 5%-moisture flakes over that from 2%-moisture flakes, but this increase did not affect the quality of the refined oils. These oils were rated prime grade and flavor based on refining, bleaching and taste tests.

The mass velocity of 8.9%-moisture flakes was appreciably different than that of lower-moisture flakes. At ambient temperature, the mass velocity increased slightly with the increase in flake moisture content, accompanied by increased residual lipids of the extracted flakes. In the case of heated hexane, the mass velocity of 8.9%-moisture flakes dropped markedly from that of lower-moisture flakes. The decrease

Table 1—Effect of multiple extractions of cottonseed flakes on mass velocity and residual lipids[a]

Number of extractions	Contact time (min)	Mass velocity lb/hr/ft^2	Residual lipids (%)	Total lipids removed (%)
1	2.5	3465	13.4	64.8
2	5.0	2249	6.5	82.8
3	7.5	2942	4.4	88.4
4	10.0	2578	3.4	91.0
5	12.5	2671	3.0	92.0

[a] Initial lipids, 38.0% on a moisture-free basis

Table 2—Effects of extraction temperature and initial meat moisture on mass velocity, residual lipids, and gossypol

Conditions			Extracted Flakes[a]			
Hexane temp (°C)	Meat moisture[b] (%)	Mass velocity (lb/hr/ft^2)	Residual lipids (%)	Free gossypol (%)	Total gossypol (%)	Total gossypol in oil (%)
22	8.1	4,900	4.42	1.49	1.82	0.016
22	11.2	5,892	2.94	1.27	1.69	0.017
63	8.1	5,408	2.87	1.48	1.82	0.065
63	11.2	7,515	1.51	1.27	1.45	0.073

[a] Moisture-free basis
[b] Prepared flakes dried to 2% moisture prior to extraction

Table 3—Effects of extraction temperature and flake moisture on mass velocity, residual lipids, and gossypol

Conditions			Extracted Flakes[a]			
Hexane temp (°C)	Flake moisture (%)	Mass velocity (lb/hr/ft^2)	Residual lipids (%)	Free gossypol (%)	Total gossypol (%)	Total gossypol in oil (%)
22	2.0	6,837	4.81	1.44	1.62	0.013
22	5.0	6,716	4.82	1.51	1.59	0.041
22	8.9	7,359	5.32	1.26	1.37	0.028
63	2.0	6,032	2.99	1.37	1.63	0.082
63	5.0	6,054	2.43	1.35	1.48	0.129
63	8.9	3,316	3.21	1.34	1.53	0.100

[a] Moisture-free basis

Table 4—Effect of cottonseed-processing parameters on mass velocity, fines, and residual lipids using continuous pilot plant extractor with ambient temperature hexane

Parameter	Pilot plant test number						
	1	2	3	4	5	6	7
Feed rate, lb/hr	90	90	140	140	209	202	197
Extraction time, min	90	90	70	70	52	52	52
Solvent-to-flake ratio	3:1	2:1	2:1	1.2:1	.97:1	.72:1	.62:1
Mass velocity, lb/hr/ft^2	9,157	5,412	6,920	5,378	4,782	5,491	3,702
Fines in miscella, % of feed	0.25	0.47	0.38	0.18	0.38	0.50	0.75
Fines in miscella, %	0.11	0.19	0.19	0.16	0.73	0.91	1.33
Residual flake lipids, %[a]	0.90	1.14	1.66	1.61	2.22	3.66	5.61

[a] Moisture-free basis

in mass velocity affected the residual lipids but not the gossypol content of the extracted flakes. We have no suitable explanation of the interaction between cottonseed components and heated hexane in the presence of high moisture; more research is needed in this area.

The effects of cottonseed-processing parameters on mass velocity, fines, and residual lipids using a continuous Crown pilot-plant extractor are given in Table 4. As can be seen, uncooked, dried cottonseed flakes can be extracted with ambient-temperature hexane with satisfactory mass velocities. Mass velocity tended to decrease with decreases in solvent-to-meat ratio, but it stayed well above the minimum needed for economical operation in commercial solvent extraction.

Some fines are generated by flakes broken during continuous extraction. Miscella fines calculated either as a percentage of feed or miscella show that fines tended to increase with decreases in the solvent-to-flakes ratios. A solvent-to-flakes ratio of about 1.2:1 appears to be critical. Below this ratio the amount of fines generated started to increase. Various techniques and devices can be used to remove the fines in the miscella, and to polish it before solvent and oil recovery operations (Graci et al., 1953).

Suitably dried cottonseed flakes prepared from uncooked glanded seed can be direct-hexane extracted to various residual lipids levels in a continuous extractor by varying the processing parameters. The mass-velocity and other data suggest that extraction cost would be reasonable for development of a commercial extraction process to produce a light colored metal for further processing to high quality, low free gossypol flours.

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A Research Note

Sensory, Physical and Chemical Properties of Canned Peaches

R. R. LIN and V. N. M. RAO

ABSTRACT

The interrelationships among organoleptic, rheological, and chemical properties of canned peaches were studied. The rheological properties were determined by relaxation and Boussinesq die loading methods with the assumption that the Hertz theory was valid under experimental conditions. The results indicated that these methods could be used to monitor the textural properties of canned peaches. Chemical analysis showed that the total titratable acidity was significantly correlated to the hardness and graininess, while alcohol insoluble solids had a significant correlation with graininess. Acid detergent fibers showed a negative correlation with the mouthfeel.

INTRODUCTION

TEXTURE of fresh peaches has been considered to be one of the main quality attributes for consumer preference. Boggess et al. (1970, 1973) reported on the suitability of several nonmelting clingstone and freestone cultivars for processing. They also found that the most preferred canned peaches were obtained from ripe peaches (Baby Gold #6) with 90–100% ground color developed (Boggess et al., 1974). Rice et al. (1977) studied the changes in composition and texture of canned peach halves inoculated with *Byssoclamys fulva*.

A well-defined measurement of the mechanical properties, texture attributes of panelists, and chemical analysis of fundamental primary entities, along with the definition of their correlation functions, may provide the basis for quality control and optimization of processed fruit products (Kapsalis and Moskowitz, 1978). Although rheology may not provide all the answers to food texture characterization (Bourne, 1975), it does offer a fundamentally sound approach to the characterization of basic properties which give rise to mechanical texture parameters, and permits expression of physical nature of the materials in terms of rheological models. The objectives of this research were to study the interrelationships of rheological, chemical, and organoleptic properties. The feasibility of using Hertz solution, in relaxation, and Boussinesq die loading to determine the rheological properties of canned peaches were also evaluated.

MATERIALS & METHODS

Samples and sample preparation

Three cultivars of peaches, Arkansas 16, Redglobe, and Elberta, were harvested from the ARS, USDA Southeastern Fruit and Tree Nut Research Station at Byron, GA. Healthy fruits of each cultivar were classified into four ripeness groups visually according to the proportion of ground color and canned separately. Canning procedures used in this study followed those reported by Boggess et al. (1973). The canned peaches were stored at room temperature and studied 2 months later.

Sensory evaluation

The sensory evaluation of canned peaches were performed by ten trained members selected from the Food Science Department, Univ. of Georgia, with previous experience on panel rating of fresh peaches. The panelists were requested to rate the hardness (measurement of force required to compress the sample between the molars), springiness (elasticity, the extent a sample regained its original shape after being compressed by molars), graininess (the number and size of fibrous particles present in the mouth after the sample had been chewed), mouthfeel (the extent of moistness or dryness of the sample), and overall texture based on a hedonic (1 to 9) scale.

An imitation peach used as reference standard was made as follows: aliquots of 200 ml of puree obtained from canned Arkansas 16 medium ripe peaches were mixed with 30 ml of the canning syrup, 50 ml of distilled water and 4g of agar (Difco) and boiled for 5 min. The product was cooled and solidified at room temperature in a sealed container for 8 hr and served in the panel session as a reference standard.

Rheological tests

An Instron (Model 1130 Universal Testing Machine) was used to perform the relaxation and Boussinesq die loading tests. All the samples were replicated five times. The Hertz theory was assumed to be valid under experimental conditions. Poisson's ratio (u) was also assumed to be 0.3.

The relaxation tests were done at a cross head speed of 25.4 mm per min and chart speed of 5.08 cm per min. The samples were compressed to a force of 3.11N to 3.56N and then allowed to relax for 4 min. The relaxation time (4 min) chosen was due to the phenomenon that some of the samples dissipated the force completely after 4 min of relaxation. The modulus of elasticity (E) was calculated from the force F, deformation D and ratio of curvature R and R^1 as:

$$E = \frac{0.531\, F(1-u^2)}{D^{3/2}} \left(\frac{1}{R} + \frac{1}{R^1}\right)^{0.5}$$

The 21 modulus of elasticity values obtained at different times were further fitted to a three element Maxwell model. The modulus of elasticity E(t) for this model is given by:

$$E(t) = E_e + E_d\, e^{-t/\tau}$$

where E(t) = modulus of elasticity at time t in N/m^2; E_e = equilibrium modulus in N/m^2; E_d = decay modulus in N/m^2; t = time in sec; τ = time of relaxation which is the time required for the stress to decay to 1/e times its original value.

A die with 0.8 cm in diameter was used for Boussinesq die loading. The load cell was set at 8.9N range. The crosshead speed and chart speed were 25.4 cm per min and 12.7 cm per min, respectively. The modulus of elasticity at failure (E_t) and work required for failure W_f were calculated.

Chemical analysis

After the rheological tests, the peach halves were collected and drained on a piece of cheesecloth. The drained halves were blended at high speed for 1 min. All the analyses were replicated six times. For total solids, 10g of puree were put into a weighed aluminum pan and dried in an oven at 70°C under 508 mm vacuum for 8 hr. Total solids were calcualted as percent wet basis. The procedure for total titratable acidity was from Rice et al. (1977). The acidity of the samples was expressed as percent citric acid.

Alcohol insoluble solids were determined by adding 50g of puree to 150g of 95% ethyl alchol and mixed thoroughly with a glass bar in a 500 ml Erlenmeyer flask. The flask and contents were heated to boiling in a water bath and allowed to simmer for 30 min before cooling. The cooled mixture was filtered through a weighed sintered glass funnel with vacuum. The residue was washed with 300 ml of 95% ethyl alcohol and then with 300 ml acetone. The washed residue was dried in a vacuum oven overnight at 50°C. The procedure used to determine acid detergent fiber (ADF) was from Hallab and Epps'

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method (1963). Both total and soluble pectin of AIS were determined by the procedure of McCready and McComb (1952, 1954).

RESULTS & DISCUSSION

THE RESULTS of all data analyses and the correlation between rheological, chemical, and taste panel parameters are shown in Tables 1 and 2, respectively. Total titratable acidity of canned peaches had a positive correlation with the hardness and graininess ratings. This is possibly due to the fact that total titratable acidity decreases as ripening progresses in fresh peaches (David et al., 1956). An increase in alcohol insoluble solids (AIS) should result in an increase of the graininess since most of these insoluble solids are polymers with high molecular weight and retain their structure generally in heat processing. A significantly negative correlation between acid detergent fiber and mouthfeel was found. Fibers, cellulose and lignin constitute the acid detergent fiber which makes up the structural network of fruits and is not broken down or hydrolyzed by heat process and hence the mouthfeel should decrease with increasing acid detergent fiber as found in this study.

Correlations between rheological and chemical properties show that all of the chemical analyses, except total solids, have some relation to some of the rheological properties. Modulus of elasticity at failure, E_f, has a significant correlation with AIS and acid detergent fibers. The work required for failure, W_f, was significantly correlated to total titratable acidity, AIS and acid detergent fiber. High correlations between E_e and acid detergent fiber, soluble pectin and total pectin were also found. Modulus of elasticity and work required for failure were highly dependent ($R = 0.72$, $p < 0.01$). Modulus of elasticity and work required for failure also correlated with the relaxation time, τ, of relaxation test ($R = 0.57$ and 0.65, respectively, $p < 0.05$). It was interesting to note that there is a relationship between the rheological properties studied by the small strain method and the destructive method. Both small strain measurement (by relaxation test) and compression-penetration measurement (by Boussinesq die loading test) provide good correlations to the hardness, springiness, and mouthfeel which indicated that sensory properties of canned peaches can be monitored by instrumental measurements either by small strain or destructive methods. The results of this experiment support the discussion of Bourne (1979) about using small strain and rupture tests to study the correlation between physical properties and panel notes.

Fridley and Adrian (1966) studied the rheological properties of peaches, pears, apricots, and apples by applying impact and compression tests on convex-shaped fruit segments. Fridley et al. (1968) also reported that the theory of elasticity gave reasonable predictions of force deformation relationships, stress distribution and bruising for plate test on whole peaches and pears. The results of this research indicate that Hertz assumptions can be approximated by careful design of experimental condition even though most fruits are not linearly elastic.

In this experiment, a scatter diagram was graphed first before studying the correlation between rheological properties and sensory ratings of the sample. Power functions

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Table 1—Chemical, rheological, and sensory properties of canned peaches

	Arkansas 16		Redglobe		Elberta	
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
Titratable acidity (%)	0.2765	0.0517	0.3548	0.0139	0.3230	0.0241
Total solids (%)	24.38	0.36	23.93	0.68	24.00	0.70
Acid detergent fiber (%)	0.41	0.04	0.0066	0.0001	0.0060	0.0002
Alcohol insoluble solids (%)	1.9725	0.1900	2.4925	0.2699	3.1000	0.6428
A_S[a] 10^{-2}	1.9095	0.2748	1.2283	0.1788	0.7559	0.1545
A_T[b] 10^{-2}	3.1600	0.3111	4.0731	0.1391	4.0723	0.8981
Hardness	4.6585	0.8724	4.9415	1.0394	6.7250	1.0352
Elasticity	4.2555	0.8731	4.7278	0.8746	5.7168	1.2235
Graininess	3.6915	0.2561	4.4473	0.6706	4.1958	0.3760
Mouthfeel	6.4498	0.3188	5.8945	0.4146	5.3753	0.5335
Overall Texture	6.3835	0.6989	5.1168	0.5085	5.4253	0.2985
Equilibrium Modulus, E_e (Pa)	2527.265	594.8043	4415.91	1712.3309	4843.8	1004.5642
Instantaneous Modulus, E_o (Pa)	12463.0	2069.1544	11621.0	1307.0312	9423.6667	107.2862
Relaxation time τ (s)	10.3125	1.6624	13.0575	2.1520	17.8633	5.2450
Failure Modulus, E_f (Pa)	22593.0	5725.9626	40610.5	9343.626	75718.0	3436.6425
Work Done, W_f (mJ)	10.8875	6.0467	19.34	3.3099	28.8475	6.5924

[a] A_S = Absorbance of soluble pectin.
[b] A_T = Absorbance of total pectin.

Table 2—Correlations between chemical, sensory, and rheological[a] parameters of canned peaches

	E_f	W_f	E_e	τ	Hardness	Elasticity	Graininess	Mouthfeel
Titratable acidity		0.742**	−0.602*		0.904**	0.743**	0.847**	−0.655**
Total solids				0.586*	−0.627*	−0.620*	−0.650**	
Alcohol insoluble solids	0.675**	0.709**	−0.578*	0.560*	0.694**	0.789**	−0.583*	
Acid detergent fiber	0.738**	0.741**	−0.720**	0.501*			0.706**	−0.807**
Soluble pectin		−0.626*	0.712**		0.692**		−0.623*	0.764**
Total pectin	0.628*		0.694**				0.759**	−0.628*
E_f								−0.841**
W_f					0.755**	0.757**		0.882**
E_e					0.888**			
τ					0.875**			−0.889**

[a] E_f and E_e are moduli of elasticity at failure and equilibrium. W_f is the work done, and τ is the relaxation time.
* Denotes significance at 5% level; ** Indicates significance at 1% level.

A Research Note

Caffeine Inhibition of Ochratoxin A Production

R. L. BUCHANAN, G. TICE, and D. MARINO

ABSTRACT

The effect of caffeine and theobromine on growth and ochratoxin A production by *Aspergillus ochraceus* was determined using microbiological medium. Caffeine produced a small decrease in growth, while reducing ochratoxin production as much as 98%. Theobromine had relatively little effect on growth or ochratoxin production. Screening of caffeine for its effect on the growth of a number of *Aspergillus* and *Penicillium* species indicated that caffeine may have biological activity against a variety of mycotoxigenic molds.

INTRODUCTION

PREVIOUS WORK in our laboratory has demonstrated that caffeine (1,3,7-trimethylxanthine) inhibits aflatoxin production in microbiological media, while theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine) had little anti-aflatoxigenic activity (Buchanan and Fletcher, 1978; Buchanan et al., 1978). Other investigations have indicated that caffeine plays a role in inhibiting aflatoxin production in coffee (Nartowicz et al., 1979) and cocoa beans (Lenovich and Hurst, 1978; Lenovich, 1981). However, it has not been determined if caffeine can influence the production of other polyketide mycotoxins. The objective of the present study was to determine the effects of caffeine and theobromine on growth and ochratoxin A production by *Aspergillus ochraceus*. Caffeine was also screened for its ability to inhibit the growth of a number of mycotoxigenic *Aspergillus* and *Penicillium* species.

EXPERIMENTAL

Aspergillus ochraceus NRRL 3174 was used to study the effect of caffeine and theobromine on growth and ochratoxin A production. *Penicillium expansum* NRRL 973, *Penicillium urticae* NRRL 989, *Aspergillus flavus* PC65, *Aspergillus versicolor* XVII/19, and *Penicillium roqueforti* M251 were also used to study the effect of caffeine on growth. The latter three species were obtained from the culture collection of the Dept. Food Science, Univ. of Georgia. Stock cultures were maintained on potato dextrose agar (Difco) slants stored at 4°C. Spore suspensions were prepared as previously described (Buchanan and Ayres, 1976), and diluted to 10^6 conidia/ml.

Growth and ochratoxin A production by *A. ochraceus* was studied using 250-ml Erlenmeyer flasks containing 50 ml of YES medium (6% sucrose + 2% yeast extract) (Davis et al., 1966) supplemented with caffeine or theobromine at concentrations of 0, 0.5, 1, 2, and 3 mg/ml. Each flask was inoculated with 10^6 conidia and incubated without agitation at 28°C. After 3, 7, 10, and 14 days, triplicate cultures of each test concentration were removed and extracted three times with 50-ml portions of chloroform. The extracts of each culture were pooled, concentrated to 10 ml, and assayed for ochratoxin A using the AOAC thin-layer chromatographic assay (Horowitz, 1975), using a fluorodensitometer for quantitation. After extraction, mycelia were collected, and mycelial mass determined gravimetrically by drying in an 85°C oven for 18 hr.

The effect of caffeine on the growth of the *Aspergillus* and *Penicillium* species was determined in a smilar manner using AMY medium (Buchanan and Ayres, 1976) supplemented with 0, 1, 2, and 4 mg caffeine/ml. Growth was measured gravimetrically using a 105°C oven without prior chloroform extraction of the cultures.

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RESULTS & DISCUSSION

THE EFFECTS of caffeine and theobromine on growth and ochratoxin A production are summarized in Table 1. Caffeine had little effect on the initial growth of *A. ochraceus*, but as the mold entered stationary growth, there was a dose-related trend toward decreased mycelial mass. Caffeine had a greater effect on ochratoxin A accumulation. A dose-related decrease in ochratoxin accumulation was evident, with the 3 mg/ml level displaying an approximate 98% inhibition. Theobromine did not appear to affect mold growth. The higher concentrations of theobromine appeared to produce a trend toward decreased ochratoxin A accumulation; however, the effect was not clearly dose-related, and small in comparison to caffeine's anti-mycotoxigenic activity. The general pattern of inhibition observed with *A. ochraceus* was similar to that noted for

Table 1—Effects of caffeine and theobromine on growth and ochratoxin A production by A. ochraceus in YES medium

Compound	Conc (mg/ml)	Day	pH[b]	Mycelium (mg)[a,d]	Mycotoxin/ mycelium (μg/g)[a]
Caffeine	0.0	3	5.8	439 ± 46	ND[c]
		7	5.9	1429 ± 44	1248 ± 274
		10	6.7	1343 ± 48	710 ± 97
		14	7.4	1230 ± 40	520 ± 220
	0.5	3	5.8	479 ± 117 (0)	ND
		7	6.0	1322 ± 41 (7)	773 ± 150 (38)
		10	7.3	1099 ± 48 (18)	913 ± 382 (0)
		14	7.5	1208 ± 36 (2)	160 ± 80 (69)
	1.0	3	5.8	487 ± 42 (0)	ND
		7	6.6	1279 ± 31 (10)	65 ± 32 (95)
		10	7.6	1124 ± 10 (16)	770 ± 452 (0)
		14	7.7	1040 ± 144 (15)	56 ± 34 (89)

continued

Table 1 — continued

Compound	Conc (mg/ml)	Day	pH[b]	Mycelium (mg)[a,d]	Mycotoxin/ mycelium (μg/g)[a]
	2.0	3	6.0	360 ± 79 (18)	ND
		7	6.5	1226 ± 130 (14)	67 ± 47 (95)
		10	7.6	1008 ± 11 (25)	149 ± 175 (79)
		14	7.7	1011 ± 173 (18)	32 ± 23 (94)
	3.0	3	6.2	321 ± 11 (27)	ND
		7	6.3	1154 ± 69 (19)	19 ± 7 (98)
		10	7.6	931 ± 95 (30)	27 ± 20 (96)
		14	7.8	774 ± 184 (37)	31 ± 24 (94)
Theobromine	0.0	3	6.1	367 ± 60	ND[c]
		7	6.1	1230 ± 8	3635 ± 611
		10	6.5	1150 ± 18	1876 ± 354
		14	7.7	1080 ± 37	466 ± 243
	0.5	3	6.0	410 ± 51 (0)	ND
		7	6.1	1243 ± 36 (0)	3693 ± 1151 (0)
		10	6.7	1129 ± 16 (2)	2035 ± 1184 (0)
		14	7.7	1105 ± 4 (0)	730 ± 388 (0)
	1.0	3	5.9	409 ± 52 (0)	ND
		7	6.0	1208 ± 27 (2)	2686 ± 1054 (26)
		10	7.4	1049 ± 57 (9)	663 ± 142 (65)
		14	7.8	1029 ± 54 (5)	534 ± 170 (0)
	2.0	3	5.9	464 ± 21 (0)	ND
		7	6.2	1244 ± 14 (0)	2310 ± 768 (36)
		10	7.5	1115 ± 11 (3)	779 ± 174 (58)
		14	7.8	1097 ± 14 (0)	405 ± 47 (13)
	3.0	3	5.9	460 ± 2 (0)	ND
		7	6.1	1249 ± 23 (0)	2959 ± 444 (19)
		10	7.4	1088 ± 64 (5)	564 ± 48 (70)
		14	7.8	1056 ± 7 (2)	346 ± 82 (26)

[a] Mean ± standard deviation; value in parentheses represents % inhibition calculated by (1-test/control) x 100%.
[b] Initial pH = 5.8
[c] No detectable toxin
[d] Dry weights determined after chloroform extraction

Table 2—Effect of caffeine on the growth of various mycotoxigenic Aspergillus and Penicillium species in AMY medium[a]

Microorganism	Caffeine (mg/ml)	Incubation time (day) 3	7	10	14
A. ochraceus	0	405 ± 74	1759 ± 55	1467 ± 22	1088 ± 95
	1	349 ± 22 (14)	797 ± 22 (55)	1410 ± 132 (4)	1395 ± 88 (0)
	2	275 ± 74 (32)	501 ± 87 (72)	1084 ± 488 (26)	1154 ± 22 (0)
	4	368 ± 13 (9)	434 ± 71 (75)	724 ± 220 (51)	611 ± 55 (39)
A. flavus	0	711 ± 233	1765 ± 141	1605 ± 180	1529 ± 32
	1	530 ± 77 (25)	1104 ± 161 (37)	1698 ± 287 (0)	1238 ± 39 (19)
	2	358 ± 105 (50)	353 ± 45 (80)	352 ± 63 (78)	720 ± 195 (53)
	4	377 ± 22 (47)	590 ± 388 (67)	410 ± 195 (74)	564 ± 120 (63)
A. versicolor	0	356 ± 55	1309 ± 7	1592 ± 45	1444 ± 39
	1	344 ± 12 (3)	520 ± 48 (60)	941 ± 135 (41)	1454 ± 45 (0)
	2	401 ± 22 (0)	369 ± 22 (72)	422 ± 84 (73)	517 ± 50 (64)
	4	409 ± 59 (0)	535 ± 81 (59)	341 ± 68 (79)	382 ± 122 (74)
P. expansum	0	402 ± 67	1413 ± 111	1530 ± 34	1485 ± 212
	1	455 ± 22 (0)	690 ± 175 (51)	1136 ± 217 (26)	996 ± 177 (33)
	2	367 ± 22 (9)	556 ± 140 (61)	581 ± 86 (62)	697 ± 67 (53)
	4	321 ± 180 (20)	475 ± 63 (66)	514 ± 22 (66)	769 ± 132 (48)
P. urticase	0	309 ± 99	1221 ± 99	1054 ± 128	952 ± 50
	1	295 ± 65 (5)	1092 ± 149 (11)	1275 ± 140 (0)	1072 ± 80 (0)
	2	411 ± 75 (0)	473 ± 139 (61)	934 ± 293 (11)	1200 ± 127 (0)
	4	413 ± 164 (0)	507 ± 154 (58)	397 ± 99 (62)	617 ± 94 (35)
P. roqueforti	0	351 ± 61	546 ± 166	1544 ± 136	1329 ± 266
	1	386 ± 81 (0)	477 ± 150 (12)	553 ± 128 (64)	1166 ± 332 (12)
	2	384 ± 32 (0)	342 ± 17 (37)	505 ± 22 (67)	572 ± 294 (57)
	4	241 ± 45 (31)	424 ± 167 (22)	599 ± 179 (61)	771 ± 395 (42)

[a] Mycelial dry weights (mg; mean ± standard deviation) determined without prior extraction with chloroform. Values in parentheses represent % inhibition = (1-test/control) x 100%.

aflatoxin production by *Aspergillus parasiticus* (Buchanan and Fletcher, 1978; Buchanan et al., 1978).

The effects of caffeine on the growth of six mycotoxigenic species of *Aspergillus* and *Penicillium* were also studied to further assess the compound's inhibitory activity. These results are summarized in Table 2. Caffeine again had little effect on initial mold growth, but did inhibit and/or delay growth in the older cultures. Of particular interest is the growth data for *A. ochraceus* depicted in Tables 1 and 2, which represent mycelial dry weights with and without extraction of lipid material by chloroform. While the values are not directly comparable since two different media were employed, the general pattern of inhibition suggests that caffeine interferred with lipid accumulation. Shih and Marth (1974) and Gupta et al. (1970) have demonstrated that *A. parasiticus* and *A. flavus* accumulate lipids as a result of secondary metabolism. Assuming the fungal species tested share a similar metabolic orientation, inhibition of lipid accumulation would account for the growth inhibition patterns observed in the present study. Furthermore, if caffeine is more inhibitory to mycotoxin synthesis than growth (as noted with aflatoxin and ochratoxin A), the growth data suggest that caffeine may be active against a wide range of polyketide mycotoxins.

Past investigations (Buchanan and Fletcher, 1978; Buchanan et al., 1978; Nartowicz et al., 1979. Lenovich and Hurst, 1979; Lenovich, 1981) characterizing aflatoxin production in caffeine-supplemented microbiological media and caffeine-containing commodities have indicated that caffeine-supplemented medium is a valid model system. Assuming the model system is also valid for ochratoxin A, the present results suggest that coffee and cocoa beans (particularly those varieties having $\geqslant$2 mg caffeine/g) should be relatively resistant to ochratoxin A contamination. Decaffeination of these commodites would be expected to increase their potential to support ochratoxin production. In the case of cocoa beans, caffeine content may prove to be a useful index of the mycotoxigenic potential of various cocoa varieties. Verification of these suppositions awaits future research employing the actual commodities.

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Reference to a brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

CANNED PEACH QUALITY . . . From page 318

seem appropriate to fit the functional relation between sensory ratings and rheological properties. Using the power function to study the functional relation between physical properties and organoleptic properties have been discussed by Moskowitz (1976), Kapsalis and Moskowitz (1977) and Szczesniak (1977). The results of this experiment support the appropriateness of using such power functions.

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A Research Note

Lead Content in Canned Foods Containing Vinegar

J. J. MORAN

ABSTRACT

The lead contents of various foodstuffs pickled and canned in vinegar were measured using ion selective analysis and microprocessor unit on pre-nitric acid digested samples. These included cucumber, sauerkraut, mixed pickles, leeks, and mustard greens. Where possible these values were compared with those of the fresh produce or the product pickled and stored in jars. In all cases studied, the fresh or bottled products showed levels well below the legal limit of 2 ppm (Food & Drug Act, Ottawa, 1975). In the case of the canned products all the lead levels were grossly in excess of the legal limit, varying from 5–10 ppm.

INTRODUCTION

LEAD is recognized as a toxic substance which accumulates in the body due to its low rate of elimination. Its potential carcinogenic nature has also been shown by Zawurska and Medras (1968) and its possible effects on neurological and psychomotor functions by Silbergeld et al. (1975) and Pueschel (1974). Unfortunately, the ubiquitous presence of lead in the environment predicates that small amounts are inevitably to be found in all raw foods. Surveys have shown, however, that these levels are well below the legal limits imposed as shown, for example by Kirkpatrick and Coffin (1974). Nonetheless, some anxiety has been felt as a result of studies carried out on canned produce. Here the levels are frequently much higher than those in the raw product and tend, furthermore, to increase with storage time. This has been attributed to leaching of lead from the solder used in can manufacture (the solder is 98% lead, 2% tin) and has been related to such factors as state of the can, duration and conditions of storage etc. A wide variety of products have been examined ranging from fruit juices to baby foods as reported by Rouseff and Ting (1980), Thomas et al. (1975), and FDA (1975). No absolute hazard has been reported.

Despite the above, no work has as yet been reported on lead levels in products canned in vinegar. It is true that such products are not as widely consumed as (say) canned fruits or juices. However, if it is borne in mind that lead acetate is only exceeded in solubility by lead nitrate, then lead leaching from cans with pickled products should be facilitated and a potential hazard could exist. It was for this reason that the present study was instituted. The main purpose of the initial study, was simply to measure lead levels of various canned pickled products as compared with the raw product (where available) or the similar product stored in jars. If necessary, it was intended to follow up with work on the effects of duration and conditions of storage on fresh produce which would be pickled and canned.

EXPERIMENTAL

Instrumentation

Beckman Selection 5000 Ion analyser with Selection 39648 Lead and 39069 Reference lectrodes.

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Yellow Springs Instruments Telethermometer model #42 SC.
Yellow Springs Instruments Teleprobe model #421.
Digestion rack.
Fisher Thermix stirring hot plate coupled with Superior Electric Powerstat model 116.

Sample selection, preparation and testing

Samples were obtained from supermarkets and delicatessens in fresh, bottled and canned forms. The cans were of the standard 28 oz size and contained approximately 75% by weight of solids. Jarred products consisted of pickled cabbage, sauerkraut, cucumber, leeks, and mixed vegetables. Fresh produce tested were cucumbers, leeks, cabbage and coleslaw. These were chosen to approximate as closely as possible the canned (mainly imported) products. Prior to opening, samples were stored at ambient temperatures. After opening, the liquor and solids were separated by vacuum filtration and stored until use in sealed plastic containers under refrigeration. Before storage, the filtered-off solids were finely ground and dispersed using a Waring Blendor (model #5011). For purposes of testing, approximately 10g of liquor or pulp were weighed accurately into 100 ml digestion flasks (containing glass beads to prevent bumping during boiling). The volume was reduced under gentle boiling almost to dryness. Eighteen ml of conc (re-distilled) nitric acid were added and the samples were gently digested for approximately 3 hr. In all cases the reflux action effectively rinsed down material adhering to the walls or neck of the flasks. At this point all solutions were clear and colorless; the protein and carbohydrate oxidize readily; the fat moeity proved to be the most intractable. Blanks using deionized water were treated similarly and all samples and blanks were prepared in triplicate. For recovery tests the same procedure was used except that 0.5 ml of a standard lead nitrate solution (200 ppm) were added to each sample and blank (this is equivalent to testing a sample weighing 10g and containing 10 ppm lead). After digestion was complete, the samples were neutralised in the flask using solid sodium hydroxide and bromophenol blue as indicator to approximately pH 7 (the median pH for electrode sensitivity). The test solutions (after cooling) were then transferred to 50 ml volumetric flasks and made up to volume using de-ionized water. The total sample volume was used for each determination.

The assays were carried out using ion selective analysis. The instrument was used with a lead ion indicating electrode and a calomel reference electrode. It incorporates a microprocessor to receive information, store it and perform the necessary calculations to provide the final result on a digital display and/or print-out. No ancillary calibration is needed. The instrument, in the standard addition mode, uses multiple additions of a standard lead nitrate solution (200 ppm) to perform a Gran's plot yielding a value of the initial sample concentration down the trace levels (limit of detection approximately 0.2 ppm in the final test solution). The method of assay, therefore, was to transfer the neutralized solution to a 100 ml beaker containing a magnetic stirring bar and place it on the table of the stirrer. Preliminary work showed that the rate of stirring had a strong effect on reproducibility of results; the production of even a slight vortex led to instability. It was found necessary, therefore, to use a Variac Powerstat in addition to the stirrer instrumental control for best results. To obviate interference from stray A.C. pick-up at the electrodes, the solution was grounded to the microprocessor unit. Temperature was monitored using the Y.S.I. telethermometer with a microprobe. The instrument was programmed in the Standard Addition mode. The slope value (millivolt electrode response per decade concentration change) used was that predicted by the Nernst equation since preliminary work showed that negligible differences between predicted and measured slopes using solutions prepared to reproduce the ionic matrix of the test solutions. Assays were then carried out using multiple additions of 0.5 ml of a standard lead nitrate solution (200 ppm). This is equivalent to

additions of 2 ppm in the final test solution volume. A stable reading indicated the original lead concentration of the test solutions. This technique could not be used to measure the lead levels in fresh and pickled-in-jar (PIJ) products as it necessitates an approximately 1:5 dilution. At this dilution the low levels of lead encountered were below the limit of sensitivity of the ion selective electrode. These products were, therefore, tested using 100g samples in 1L digestion flasks, reducing the volume as before and digesting with 50 ml of concentrated nitric acid. Once digestion was complete, neutralization and volume reduction was carried out as with the canned samples. The final test volume was 50 ml and thereafter the assay techniques were identical. In all cases tested the lead values for fresh and PIJ samples were less than 0.2 ppm.

RESULTS & DISCUSSION

TO TEST THE VALIDITY of the original premise upon which this work was based it was decided to use a digestion (and, possibly, an extraction) procedure followed by atomic absorption spectroscopy to determine lead levels. This initial work (not reported here) did show that high lead levels could indeed be found in canned, pickled products. It was suspected, however, that the extremely complex matrix of the digested samples could have caused suppression of the signal from the sensitive 283.3 nm lead line used in the measurements. This is known to occur as shown by Schrenk (1970), Roschnik (1975), and Rouseff and Ting (1980). Attempts to extract lead from the digest using diethylammonium diethyldithiocarbamate led to inconsistent (though still high) results. It was determined, therefore, to use ion selective analysis with the multiple standard addition technique. This obviates any problems due to the complex matrix since the solution itself is used in the initial calibration of the instrument during any test run. Under the conditions of test adopted, the electrode responds only to the lead ions already present or added during the multiple standard addition technique. Extensive exploratory work showed that the microprocessor technique was rapid, precise and accurate. The early (atomic absorption) work had, moreover, showed that, for these samples, simple digestion with concentrated nitric acid was the method of preference and it was adopted throughout the work reported here.

The most important precautions to be applied during the analysis were found to involve control of stirring rate, temperature (as could be predicted from the Nernst equation) and use of the optimum pH range for electrode sensitivity. Failure to observe precautions involving these parameters can lead to results so erratic as to be valueless. Fortunately, control is easily achievable and recovery result tests show that extremely reliable data can be quite simply obtained.

The values for the canned pickled products are shown in Table 1 and the recovery values of added lead are shown in Table 2. The figures represent the average of triplicate determinations on each sample. The excellent recovery results indicate that the method is a perfectly feasible one. It should be noted that the values quoted in Table 1 are of the same order though slightly higher than the values (unreported) obtained using atomic absorption spectroscopy. In studying these values, the most obvious single factor is th extremely high levels shown by all samples. Even the lowest value is more than double the legal (Canadian) limit and more than twenty times the levels shown by fresh or PIJ product; the highest value is some fifty times the fresh or PIJ samples.

Table 1—Lead levels in canned pickle products (ppm)

Product	Can #	Solids[a]	Liquor[a]
Cucumber	1	10.0 ± 0.20	8.8 ± 0.18
	2	8.2 ± 0.16	6.5 ± 0.14
	3	9.6 ± 0.18	8.2 ± 0.15
Mustard Greens	1	6.8 ± 0.14	6.5 ± 0.14
	2	6.6 ± 0.14	6.4 ± 0.14
	3	5.8 ± 0.12	5.7 ± 0.12
Sauerkraut	1	4.0 ± 0.09	4.0 ± 0.09
	2	4.4 ± 0.10	4.4 ± 0.10
	3	4.1 ± 0.10	4.0 ± 0.10
Leeks	1	6.6 ± 0.15	6.4 ± 0.12
	2	6.8 ± 0.14	6.5 ± 0.14
	3	6.4 ± 0.13	6.4 ± 0.12
Ginger and Mixed Vegetable	1	6.3 ± 0.13	6.3 ± 0.12
	2	6.5 ± 0.13	6.4 ± 0.13
	3	6.2 ± 0.13	6.1 ± 0.12

[a] Each value represents the average of triplicate determinations in the contents of separate cans.

Table 2—Percentage recovery values of added lead to samples

	Solids	Liquor
Cucumber	99.6	99.8
Mustard greens	99.7	100.1
Sauerkraut	99.6	99.6
Leeks	99.5	99.7
Ginger & mixed vegetables	99.6	99.8

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A Research Note

Coating Behavior of Suspensions

ELIZABETH ROBINSON LANG and CHO KYUN RHA

ABSTRACT

The ability of a solid suspension in liquid medium to coat a vertical surface was analyzed. Results with model suspensions, consisting of polystyrene particles (37–147 μm) in volume concentration from 15–40%, show that coating thickness increases linearly with particle concentration in suspension, and then increases sharply at a critical concentration. The coating thickness of sucrose in corn oil shows behavior similar to that of the model system.

INTRODUCTION

THE ABILITY of suspensions to coat surfaces is utilized in food processing. Coatings thicker than a monolayer result from interparticle interactions in a suspension which generate a cohesive structure. When surface adhesion is sufficient, coating thickness is determined by a balance between cohesive and gravitational forces.

Coatings are applied by spraying or pouring suspensions over the surface of the food to be coated, or by dipping food directly into the suspension (Lees and Jackson, 1975; Minifie, 1970). The suspension may flow over the food, leaving behind a surface coating, or the excess suspending medium may drain off, leaving the particles to form a coating with concentration of solids higher than in the bulk. Thus, the flow behavior of the suspension may become a factor in coating processes such as chocolate enrobing, pastry products frosting, or food battering prior to frying. Although particle size and concentration in a suspension affect coating characteristics (Pratt, 1970), the exact nature of the dependence has not been identified.

This paper is a preliminary investigation of the effects of particle size, concentration, and size distribution on the surface coating thickness obtained with simple suspensions in which liquid bridges predominate as the cohesive force.

Sulfonated polystyrene (AG MP-50, mesh size 20–50, BioRad Labs) was crushed with a mortar and pestle. The resulting powder was separated into particles of varying diameters < 37 μm, 43–67 μm, and 67–143 μm), using a sifter (Allen Bradley Sonic Sifter, Model L3P, with US standard sieves No. 100, 230, 325, 400). Particles were added to the continuous phase [50% glycerol (Fisher Scientific Co.) and 50% water] and dispersed by stirring with a glass rod for 2 min to make suspensions of 15–40% solids.

The suspensions were poured into 150-ml beakers, and ground glass plates were lowered (2 inch/min) vertically into the suspensions. The plates remained immersed for 10 min, then were raised (2 inch/sec) and allowed to stand for 3 min or until no further draining occurred. Coating thickness and area were measured with a calipers (Model 505636, Mityoyo, Tokyo, Japan) and the plates were weighed. For thickness measurement, the plate and calipers were clamped perpendicular to one another, and calipers were adjusted just until no light passed through the gap between the caliper "jaws" and the coating surface. The suspension densities were determined using 10-ml volumetric cylinders. Granulated sugar (446–463 μm diameter) and powdered sugar (22–31 μm diameter) (Domino Sugar, Amstar Corp.) in corn oil (Hunt-Wesson Foods) at 10–60% sugar were prepared. Coating thickness was determined by the vertical plate method described above.

To form surface coatings, a suspension must have sufficient surface adhesion and cohesion within the suspension. The volume concentration of particles must be high enough so the distance between particles is within the interaction ranges of interparticle forces. In food suspensions, usually several types of interparticle forces such as hydrogen bonding, ionic bonding, and liquid bridges contribute simultaneously to the cohesive network (Lang and Rha, 1979).

Coating behavior

We examined the effect of solids on coating thickness when one type of well defined attractive force, the liquid bridge (Overbeek, 1972), predominated. Surface coatings are obtained with these suspensions as the continuous phase drains from the surface allowing particles to accumulate preferentially. As the solids concentration approaches that of maximum packing, the particles, held together by liquid bridges, form a cohesive coating.

When two rigid, uniform spheres of radius r and density ρ, are held together by a liquid bridge, the minimum cohesive force necessary to overcome gravity can be estimated from the force balance ($F_{net} = F_{liquid\ bridge} - F_{gravity}$). Since the gravitational force $(4/3)(\pi r^3 \rho g)$ (g is acceleration due to gravity), is proportional to r^3 and the counteracting force of the liquid bridge, $2\pi r\gamma$, (γ = interfacial tension) is proportional to r, there is a maximum particle size that can be supported by a liquid bridge and above which F_{net} is negative.

The cohesive strength resulting from the summed liquid bridge effects can be estimated for cubic close packing. The cohesive strength is the force (F_{net}) between two particles, divided by the cross-sectional area over which it acts ($4r^2$) (Bache, 1976). The force balance analysis indicates that decreasing particle size, increasing interfacial tension, or decreasing density difference between particles and the continuous phase will increase the coating thickness by increasing cohesive strength, when surface adhesion is not limiting.

In practice, results show that suspensions with the smallest particles do not always lead to the thickest coatings (Fig. 1, Table 1). In the dilute region ($< 25\%$ solids), coating thickness increases with decreasing particle size and increases linearly with increasing concentration. The larger particles show stronger concentration dependence and, at intermediate concentrations, form thicker coatings. At higher concentrations, coating thickness increases sharply.

Table 1 shows the coating thicknesses obtained for the sugar in oil suspensions. At a sugar content of 10–30%, thickness obtained with the granulated sugar is greater than with powdered sugar. At 60% sugar, the powdered sugar eventually gives thicker coatings than granulated at equivalent solids concentrations.

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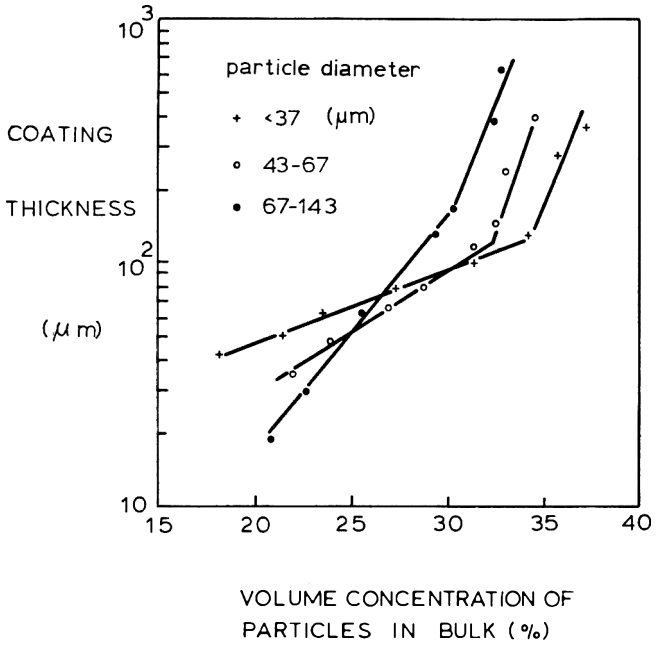


Fig. 1—Coating thickness (μm) of suspensions of polystyrene particles at concentrations of 15—40% solids, determined by vertical plate method with glass plates. Points represent the average of six determinations with error of ± 5 μm.

Table 1—Coating thickness of granulated and powdered sugar suspensions in vegetable oil

Suspension	Coating thickness (mm) (±0.02 mm)
10% granulated	0.68
10% powdered	0.52
30% granulated	0.86
30% powdered	0.74
60% granulated	2.30
60% powdered	5.40

The results show that coating thickness can be increased by increasing the volume concentration of solids in suspension. More interestingly, surface coating thickness can be maximized for a given solids content by manipulation of the particle size.

The coating thickness obtained with a suspension in which the particles are held together by liquid bridges is affected not only by surface adhesion and cohesion within the coating, but also by flow or draining off of the continuous phase during the coating process. The force balance analysis indicates that smaller particles potentially provide more cohesive coatings; however, the way in which the particles pack on a surface may determine drainage behavior, or vice versa, and thus ultimately control the coating thickness. Coating thickness can be increased, at a given solids content, by decreasing the particle concentration at which maximum packing occurs, i.e., by minimizing particle size distribution. As the maximum packing concentration of solids is approached in the bulk suspensions, surface drainage is decreased by the packing and crowding of the suspended particles, and liquid bridges form to generate a cohesive structure.

In this study, the particles of less than 37 μm in diameter have the widest size distribution, thus, the highest volume concentration at maximum packing (Lewis and Neilson, 1968). Therefore, the region where coating thickness increases significantly occurs at a volume concentration of solids higher than that for the other suspensions (Jeffreys and Acrivos, 1976). However, at very high solids content, as the concentration in bulk nears maximum packing, a minimum of drainage occurs and the coating thickness becomes limited by cohesive strength of the liquid bridges. Thus, the smallest particles eventually give the thickest coatings.

This preliminary study shows that a static force balance analysis may indicate the surface coating potential of suspensions; but in practice, drainage occurring during the coating process can also control the coating behavior. Thus, the coating thickness can be optimized in terms of volume concentration, and also in terms of particle size and size distribution at a constant solids concentration. Results with these most simple systems provide a basis for eventual optimization of surface coating thickness obtained with more complex food coatings.

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A Research Note

Partial Replacement of Wheat Flour with Yellow Field Pea Flour in White Pan Bread

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ABSTRACT

Yellow field pea flour replaced 2.5, 5 and 10% of wheat flour in white pan bread prepared by a straight dough, rapid mix home formulation. Incorporation of pea flour did not affect proofing times. No significant differences were found between Gardner L and "a" values for wheat-pea flour blends, but "b" values increased with increasing levels of pea flour; crumb color of breads did not differ significantly. Volumes of the breads were not significantly affected by pea flour addition. Pea flour breads were judged between fair and good for all characteristics by an experienced taste panel; the 10% pea flour breads were scored significantly lower for texture, color and flavor.

INTRODUCTION

RECENT STUDIES have shown that acceptable breads can be made from fababean (McConnell et al., 1974; Fleming and Sosulski, 1977a, b; Sosulski and Fleming, 1979), field pea (Fleming and Sosulski, 1977a, b; Sosulski and Fleming, 1979) and sunflower concentrates (Rooney et al., 1972; Fleming and Sosulski, 1977a, b; Sosulski and Fleming, 1979) at the 5 to 15% replacement level of wheat flour. With increasing levels of legume fortification, there was deterioration of loaf volume, crumb-grain ratings and palatability.

Partial substitution of protein fortifiers for wheat flour in breads can produce physical quality characteristics that deviate from conventional white bread. The use of pea flour in breads has the advantages of increasing protein value (Tripathi and Date, 1975; Jeffers et al., 1978), fiber content and decreasing fat content without affecting taste, baking quality or substantially increasing the cost of producing bread (Hannigan, 1979). The purpose of this study was to determine the suitability of yellow field pea flour as a partial replacement for 2.5, 5 and 10% of wheat flour in home formulation white pan bread. Loaf volume, specific volume, color differences of the flour blends and crumb were studied. Specific sensory characteristics were evaluated by an experienced taste panel.

MATERIALS & METHODS

Bread formulation and preparation

Yellow field pea flour (Dupro 300, Dumas Seed Co., Edina, MN; typical analysis: protein 24.3%; carbohydrate 61.8%; moisture 9.0%; fat 0.9%; crude fiber 4.8%) replaced wheat flour in white pan bread on a weight basis at 2.5, 5 and 10% levels with 100% wheat flour as the control. The bread dough was prepared by mixing one-third of the flour with the sugar, salt, nonfat dry milk and dry yeast in a Kitchen Aid model K-5A bowl. The water and oil were combined and heated to 52°C and added to the dry ingredients. The mixture was beaten with a wire whisk attachment for 2 min at speed 2. One-third more of the flour was added and the mixture kneaded for 2 min at speed 2 using a dough hook. The remaining flour was added and the dough kneaded with the dough hook at speed 2 for an additional 6 min.

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Five hundred grams of dough were shaped into loaves and placed into greased pans (23 x 13 x 7 cm) and proofed at 30°C (95% relative humidity) until the dough reached a height of 2.54 cm above the edge of the pan. The bread was baked in a rotary hearth oven for 55 min at 190°C and cooled for 60 min.

Volume and color measurements

Volume was measured by the rapeseed displacement method of Cathcart and Cole (1938) on one loaf of bread 1 hr following baking.

A Gardner Color Difference Meter (Model C-4) was used to measure the color of flour and crumb samples (McWatters, 1980). For color measurements, a center slice of bread (1.2 cm thick) was used. A white plate (CG-6625 with reference values L = 92.5, a = −1.0, b = +1.7 served as the standard.

Sensory evaluation

The breads were evaluated 24 hr after baking by an experienced taste panel of eight members (seven women, one man) of the Dept. of oods & Nutrition staff. The panelists were familiar with the evaluation and quality of homemade white pan bread. Shape, crust, volume, texture, grain, color, flavor and aroma were evaluated using a score card with descriptive terms for each numerical value to aid in judging. Ratings were based on a 5-point scale, ranging from 5, very good, to 1, very poor (Larmond, 1977).

Breads were tasted in a sensory evaluation laboratory with individual partitioned booths and overhead incandescent lighting. Two five-digit randomly coded samples were presented at each mid-morning session. The breads were served at room temperature and water (20°C) was provided for rinsing. Judges received half slices (1.2 cm thick) from the center third of each bread. One-half loaf of each bread was displayed for evaluation of external characteristics.

Statistical design

Two different types of bread were prepared each day according to a randomized incomplete block design. Each variation was replicated four times. Means, standard deviations of the means and analysis of variance were calculated for each parameter (Steel and Torrie, 1960). LSDs at the 5% level were computed when F values were significant.

RESULTS & DISCUSSION

Color measurements

The L, "a" and "b" values of flour mixtures used for bread preparation were not significantly different. Crumb L, "a" and "b" values were not significantly different when pea flour was used in the breads. Variance in texture and grain of the breads may have affected crumb readings.

Loaf volume and specific volume

Breads were all proofed to a given height. The pea flour dough did not require a fermentation time significantly different from the control. The volumes and the specific volumes of the breads were not significantly different. All breads had a specific volume less than six. Rooney et al. (1972) stated that 6.0 cc/g is an acceptable specific volume for yeast leavened breads.

Sensory evaluation scores

Mean sensory evaluation scores of control and pea flour breads are given in Table 1. Photographs of representative slices are shown in Fig. 1. The use of pea flour in the breads significantly affected texture, color and flavor. Shape, crust, grain and aroma were not significantly affected.

The control breads received the highest scores for volume. Sensory evaluation scores decreased slightly with increasing levels of pea flour. This is in close agreement with loaf volume and specific volume findings.

Texture scores for the 10% pea flour breads differed significantly from the control and 2.5% pea flour breads. At this level of substitution, the bread was tough and dry resulting in lower scores. Pea flour alters the water absorbing capacity of the mix. This was not accounted for in this study which was primarily concerned with suitability and acceptability of pea flour substitution in a home formulation white pan bread. This could account for differences in texture and dryness.

The crumb color of the breads became yellower with increasing levels of pea flour. At the 10% level, the breads were considered to have an unattractive, yellow off-color and significantly differed from the control and 2.5% pea flour breads.

The incorporation of pea flour in the loaves significantly affected flavor but not the aroma of the breads. Panelists detected a beany off-flavor at the 10% level and scored these breads lowest in aroma. The flavor of the 10% field pea flour bread differed significantly from all other breads.

In general, the sensory evaluation scores show all breads scored between fair and very good with 10% pea flour scoring lowest in all characteristics. The 2.5% replacement level breads were scored similarly or better than the control wheat bread. Tripathi and Date (1975) found that general

—Continued on page 329

Table 1—Sensory evaluation scores for yeast breads[a]

Type of bread	Shape	Crust	Volume	Texture	Grain	Color	Flavor	Aroma
100% Wheat	4.1 ± 0.12	4.2 ± 0.10	4.7 ± 0.06	4.6 ± 0.10*	4.1 ± 0.02	4.5 ± 0.26*	4.6 ± 0.02*	4.5 ± 0.09
2.5% Pea	4.6 ± 0.06	4.4 ± 0.03	4.6 ± 0.14	4.5 ± 0.19	4.2 ± 0.09	4.7 ± 0.13	4.6 ± 0.16	4.4 ± 0.10
5% Pea	4.1 ± 0.25	4.2 ± 0.09	4.5 ± 0.24	4.1 ± 0.11	4.0 ± 0.10	4.3 ± 0.18	4.4 ± 0.15	4.3 ± 0.09
10% Pea	3.8 ± 0.34	3.8 ± 0.24	3.9 ± 0.35	3.7 ± 0.34	3.8 ± 0.34	3.8 ± 0.15	3.8 ± 0.28	3.8 ± 0.28

[a] Means and standard deviations for four replications. Maximum possible score was 5.
*F values indicate that means in the same column differ significantly at the 5% probability level. LSD at the 5% level for texture, 0.64; color, 0.58; flavor, 0.53.

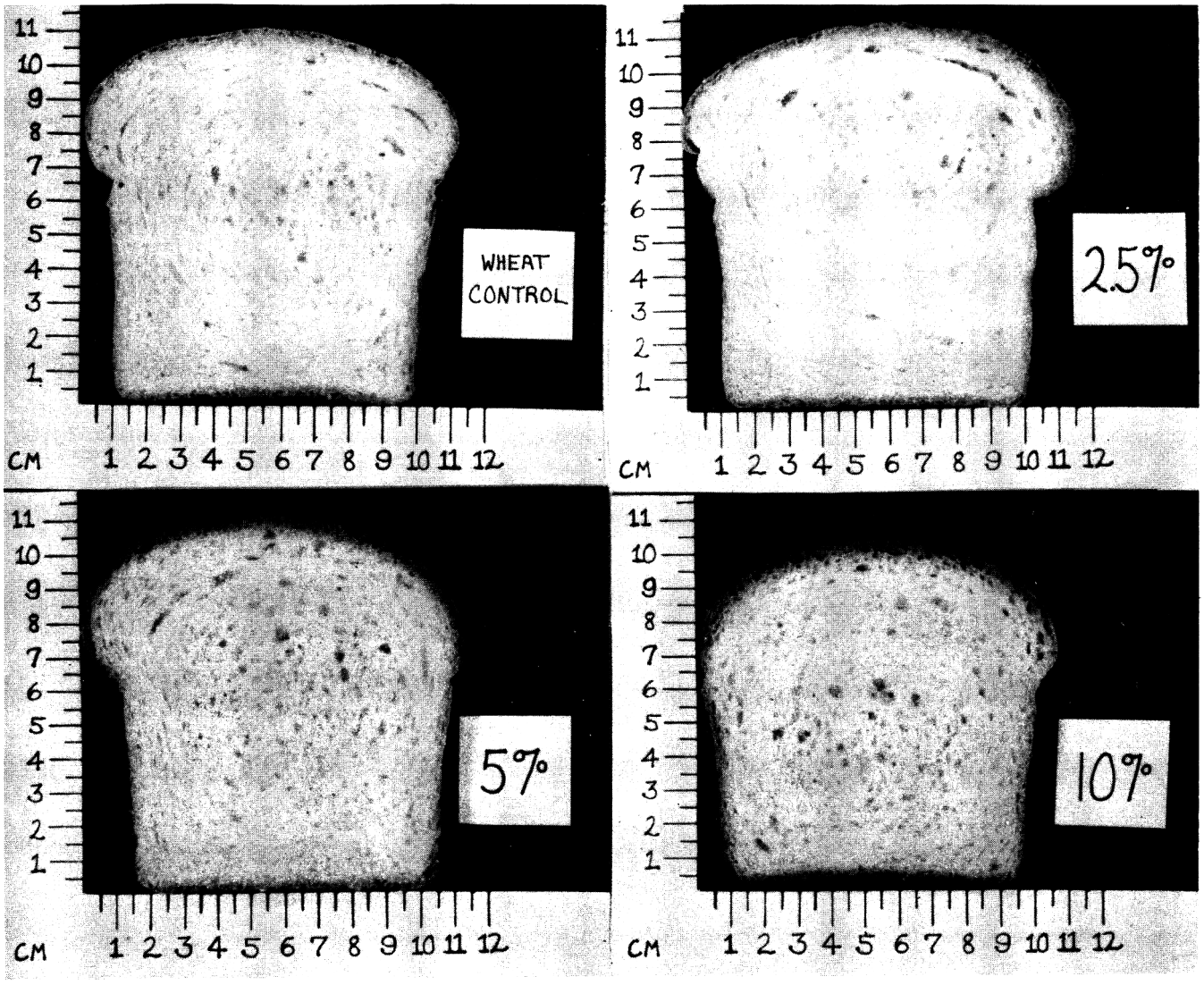


Fig. 1—Representative slices of yeast breads prepared with 100% wheat flour, 2.5% pea flour, 5% pea flour and 10% pea flour.

A Research Note

Production of Ethanol from Cellulose Using *Clostridum thermocellum*

LETICIA ZERTUCHE and ROBERT R. ZALL

ABSTRACT

Clostridium thermocellum was used to produce ethanol from cellulose in a continuous system. Batch fermentations were first performed to observe the effects of buffers and agitation on generation time and ethanol production. Continuous fermentations were carried out at 60°C and pH 7 using pure cellulose as the limiting substrate. The maximum ethanol concentrations produced with 1.5 and 3% cellulose fermenting liquid were 0.3 and 0.9% respectively. The yield of ethanol was about 0.3 grams per gram of cellulose consumed. While the continuous fermentation of cellulose with *Clostridium thermocellum* appears to be feasible, it may not be economically promising due to the slow growth of the organism.

INTRODUCTION

ETHANOL, an alcohol fuel, is among the nonpetroleum fuel options which can be produced from resources available in abundance in the U.S. and elsewhere in the world to substitute for the use of oil. The process which is being studied more intensively is the biodegradation of renewable resources into liquid fuels.

Agricultural residues such as corn stover and wheat stocks are alternative resources to produce ethanol by fermentation because they are cheap sources of polysaccharides which include cellulose, hemicelluloses and lignin. Crop residues have to be pretreated to remove lignin which is a nonfermentable material that makes cellulose unavailable for degradation. Direct fermentation of cellulose into ethanol is possible using *Clostridium thermocellum*, a microorganism which hydrolyzes cellulose and catabolizes the sugars from this fermentation to produce ethanol and organic acids. A single-step process such as this fermentation could have potential economical advantages over processes using multiple fermentation steps.

MATERIALS & METHODS

THE MUTANT S-4 of *Clostridium thermocellum* ATCC27405 (American Type Culture Collection, Rockville, MD) developed by Wang and co-workers (1978) used in this study is a strict anaerobe, gram negative, spore former, motile and thermophile with an optimum growth temperature of 60°C (Viljoen et al., 1926). This microorganism grows on cellulose and cellobiose, but, unlike the strain of *C. thermocellum* isolated by McBee (1950), it is able to grow on glucose if the amount of yeast extract in the media is tripled (1.5%). The products of cellulose fermentation include carbon dioxide, hydrogen, ethanol, formic, acetic, lactic and succinic acids, and reducing sugars.

The medium composition was that described by Wang et al. (1978, 1979), prepared under strict anaerobic conditions. The limiting substrate for microbial growth was pure cellulose supplied as Solka floc SW-100 (Brown Co. NH).

Ethanol and acetic acid produced during fermentation were determined through gas chromatography using a Varian Aerograph Series 1800 Flame Ionization Detector with a glass column, 5 ft long and ¼ inch diameter containing Porapak Q (80/100 mesh). Helium at 35 ml/min was used as carrier gas. The column was maintained at 160°C; the temperatures for the injector and detector were 190 and 200°C respectively. Reducing sugars were determined using a modification of the phenol-sulfuric acid method of Dubois et al. (1956). Cellulose was also determined using this method. To separate cellulose from the cells, a 5 ml sample of the culture was filtered through Whatman No. 1 filter paper. Cell growth was determined turbidimetrically at 490 nm using a Spectronic 20 Baush & Lomb spectrophotometer after the cellulose was separated from the suspension.

Batch fermentations were carried out under anaerobic conditions in 100 ml serum vials at 60°C. The continuous fermentations were performed in a 2L fermentor at the same temperature.

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RESULTS & DISCUSSION

BATCH FERMENTATIONS of 1% cellulose were carried out to study the effect of buffers and agitation on microbial growth and ethanol production.

Sodium bicarbonate (0.1N), sodium hydroxide (1M), and dipotassium hydrogen phosphate (1M) were used as buffers to adjust the initial pH of the media (6.7) to 7.0. Respectively, generation time, in hours, and the yield of ethanol, in g of ethanol per g of cellulose degraded, were: 7.6 and 0.22 for the bicarbonate, 10.9 and 0.19 for the hydroxide, 41.6 and 0.14 for the phosphate, and 16.3 and 0.12 when no buffer was used. The figures for the hydroxide are comparable with values reported by Wang et al. (1979) and Weimer and Zeikus (1977). The effect of the bicarbonate on the generation time may be due to the need of some microorganisms for soluble carbon dioxide for growth (Pirt, 1975).

Agitation of the cultures decreased the production of ethanol from cellulose (Fig. 1). Production of ethanol in the agitated cultures ceased earlier and at a lower concentration than in unagitated cultures. This effect may be due to the inactivation of enzymes by agitation and other mechanical forces (Bailey and Ollis, 1977). Ng et al. (1977) reported that exoglucanase of the extracellular cellulase system produced by *C. thermocellum* was inhibited by agitation of the culture. Similarly, Halliwell (1961) reported that the C_x factor of the fungal cellulase of *Myrothecium verrucaria* was inactivated by shaking.

Continuous fermentations of 1.5 and 3% cellulose were started in a batch mode to allow cell concentration to increase to avoid "washout" of the cells. On the other hand, if the dilution rate was lower than 0.01 hr^{-1}, starvation occurred and the cells began sporulation. Decrease in cell size was also observed during starvation periods. Continuous fermentations were carried out at 60°C and sodium bicarbonate was used to control pH. The fermentator was agitated using a magnetic stirrer.

Unfortunately, the system did not reach steady state although the cell and product concentrations varied within a certain range. The variation in product formation could possibly be attributed to inappropriate conditions for growth, such as the presence of oxygen. The pH changes from 6.3 to 7.2 which occurred could also contribute to the variation since pH was manually adjusted. Another factor that could contribute to the instability of the system was the formation of foam which was troublesome during the biodegradation of 3% cellulose. Table 1 summarizes the data obtained during continuous fernentation. Product

Table 1—Product concentrations during continuous fermentation of cellulose into ethanol by C. thermocellum Percentages are given by weight.

Cellulose %	Ethanol %	Acetic acid %	Reducing sugars %	Ethanol / Acetic acid	Cellulose degraded %
1.5	0.21–0.32	0.20–0.30	0.3–0.5	1.0–1.2	60–90
3.0	0.40–0.90	0.10–0.30	0.3–0.7	2.5–6.0	50–90

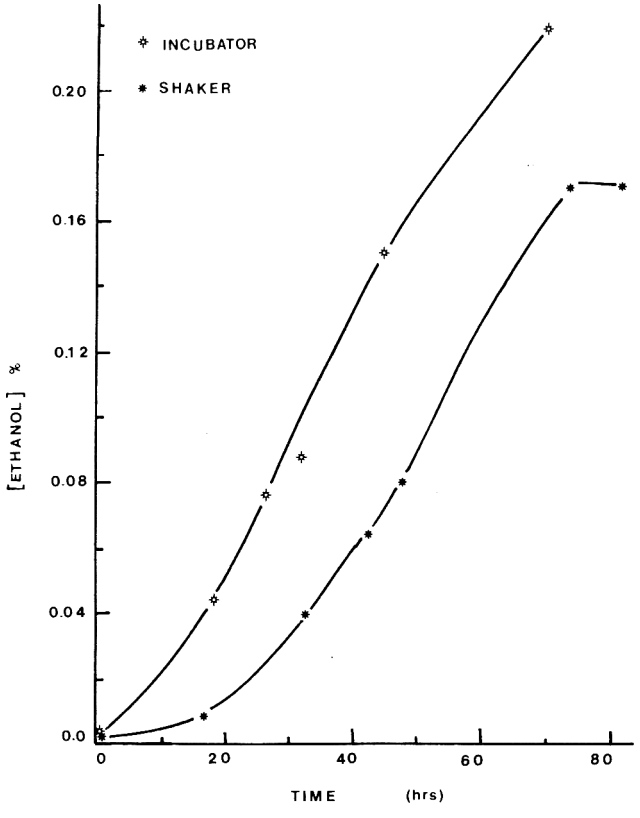


Fig. 1—Effect of agitation on ethanol concentration during batch fermentation of 1% cellulose by C. thermocellum. Temperature 60° C.

concentration as well as percentage of cellulose consumed increased with an increase in cell concentration. The ethanol to acetic acid ratio obtained during the 3% cellulose fermentation was about two to three times higher than for the 1.5% cellulose fermentation, which may be due to differences in environmental conditions such as pH. The yield of ethanol was about 0.23 and 0.3 grams of ethanol per gram of cellulose consumed for the 1.5% and 3% cellulose fermentations respectively. The yields of ethanol during these fermentations were higher than those for batch fermentations. It could also be inferred that the withdrawl of metabolites and addition of fresh media during continuous fermentations had a beneficial effect on the process.

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PEA FLOUR BREADS . . . From page 327

acceptability of the bread decreased beyond the 5% replacement level while Jeffers et al. (1978) stated that acceptable bread can be made with up to 15% yellow pea flour. The results of this study indicate that yellow field pea flour can successfully replace wheat flour in a straight dough, rapid mix home formulation of white pan bread up to the 10% level.

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A Research Note

Examination of Chili Pepper and Nutmeg Oleoresins Using the *Salmonella*/Mammalian Microsome Mutagenicity Assay

R. L. BUCHANAN, S. GOLDSTEIN, and J.D. BUDROE

ABSTRACT

Past investigations have suggested that various arylalkene spice compounds warrant further study as possible naturally occurring mutagens and/or carcinogens. The present study carried out a detailed examination of nutmeg oleoresin, myristicin, chili pepper oleoresin, capsaicin, and vanillylamine for in vitro mutagenicity using the *Salmonella*/mammalian microsome mutagenicity assay. None of the materials tested displayed significant mutagenicity over a wide range of concentrations.

INTRODUCTION

THE IDENTIFICATION of safrole (Homburger et al., 1961, 1962; Hagan et al., 1967), isosafrole (Hagan et al., 1967; Innes et al., 1969), beta-asarone (Taylor et al., 1967; Hall, 1973), and estragole (Drinkwater et al., 1976) as potential naturally occurring carcinogens has prompted investigators (Miller, 1973; Buchanan, 1979) to suggest that other methylenedioxybenzene derivatives and closely related phenolic flavor compounds warrant evaluation for possible mutagenicity and/or carcinogenicity. Two such compounds are myristicin and capsaicin.

Myristicin (1-allyl-5-methoxy-3,4-methylenedioxybenzene) is a major component of the essential oils of nutmeg and mace, being largely responsible for the characteristic aroma of these spices (Weil, 1965). The compound is also present in trace amounts in a variety of foods including carrots, black pepper, dill, parsley, parsnip, celery seed, cinnamon, and banana (Weil, 1965; Oswald et al., 1971b; Marczal et al., 1977; Fuhreman et al., 1978; Wulf et al., 1978). Myristicin has been studied extensively for its psychoactive effects (Truitt et al., 1961; Painter et al., 1971; Shafran, 1976), and is believed to be the active hallucinogenic component of nutmeg. High levels of myristicin have been reported to affect hepatic function in test animals, producing hypertrophy (Gershbein, 1977), increased microsomal enzyme activity (Fuhremann et al., 1978), and fatty degeneration (Weil, 1965). Myristicin has a molecular structure related to that of the hepatocarcinogen, safrole, and the acute and subchronic toxicities of the two compounds are qualitatively similar (Buchanan, 1979). Rockwell and Raw (1979) examined nutmeg extract for mutagenicity, but their results were inconclusive due to problems with cytotoxicity.

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the major pungent principle of chili pepper and paprika, and is noted primarily for its irritant properties (Hall, 1973; Salzer and Furia, 1977). Capsaicin produces a number of physiological effects including increased salivation and sweating, altered respiration and blood pressure, and decreased intestinal transport (Porszasz et al., 1955; Toh et al., 1955; Monsereenusorn and Glinsukon, 1979a, b). Hoch-Ligetti (1951) reported that rats fed a protein-deficient diet containing 10% chili pepper had a 54% incidence of hepatomas, and suggested that capsaicin may contribute to the etiology of liver cancer, particularly in regions where protein resources are marginal. Adamuya (1971) found that chili pepper did not produce an elevated incidence of malignant tumors in test animals, but did find that chili pepper produced an increased incidence of malignancies when fed in conjunction with a known hepatocarcinogen. Adamuyma (1971) suggested that capsaicin may act as a co-carcinogen.

In recent years, it has been demonstrated that there is often a high degree of correlation between in vitro mutagenicity and in vivo carcinogenicity. Of the various assays available for assessing mutagenicity, the *Salmonella*/mammalian microsome mutagenicity assay (Ames et al., 1975) is one of the most commonly employed, and has been reported to have an accuracy that can approach 90% (McCann et al., 1975; Purchase et al., 1978). Since the previously cited literature suggests that myristicin and capsaicin warrant investigation, the objective of the present study was to carry out a detailed examination of these compounds and the oleoresins of nutmeg and chili pepper using the *Salmonella*/mammalian microsome mutagenicity assay as a initial method of evaluating their safety.

EXPERIMENTAL

Samples

Capsaicin (Sigma Chem, Co.; Purity, 90%) and vanillylamine (Aldrich Chem. Co., purity, 98%) were obtained commercially. Myristicin (96% purity) was graciously supplied by Fritzche, Dodge, and Olcott, Inc., and subsequently repurified by thin-layer chromatography (TLC) to approximately 99% purity.

Chili oleoresin was prepared from whole chili peppers obtained at a local retail store. The chili peppers were ground, extracted according to the technique of Sass et al. (1977), and the solvent removed by flash evaporation. The capsaicin content of the oleoresin was estimated spectrophotometrically (DiCecco, 1979). The nutmeg oleoresin was prepared from whole nutmegs graciously supplied by David Michael & Co., Inc. The nutmegs were ground, extracted with 2-butanone in a Soxhlett apparatus, and concentrated with a flash evaporator. The myristicin content on the nutmeg oleoresin was estimated by separating with TLC (silica gel eluted with hexane), illuminating with short-wave UV light, and visually comparing against myristicin standards.

Mutagenicity assay

The *Salmonella*/mammalian microsome mutagenicity assay was performed according to the procedure outlined by Ames et al. (1975), employing *his*$^-$ tester strains TA98, TA100, TA1535, and TA1538. Vogel-Bonner E agar was employed as the plating medium. All samples were assayed with and without fortified microsome preparations (S-9 mix) incorporated into the top agar at a level of 0.5 ml/plate. The S-9 mix was derived from the livers of rats induced with phenobarbital. All samples except the nutmeg oleoresin were solubilized in 0.1 ml of dimethyl sulfoxide (DMSO). Nutmeg oleoresin samples were handled identically except they were solubilized in 0.1 ml of N,N-dimethylformamide (DMF). Plates receiving solvent only served as negative controls. N-methyl-N-nitro-N-nitrosoguanidine and picrolinic acid were employed as positive controls in the assays without S-9 activation, and 2-acetylaminoflourene was used in the activated assays. All assays were performed with at least four replications. All plates were incubated for 48 hr at 37°C, and then scored for revertants.

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RESULTS

THE NUTMEG yielded approximately 41% (W/W) oleoresin, and TLC analysis indicated that the myristicin content of the oleoresin was approximately 4.5% (W/W). The oleoresin was assayed for mutagenicity at concentrations of 5, 25, 50, 250, and 500 μg/plate. Higher levels were not examined since they could not be solubilized using DMF or other suitable solvent systems. Purified myristicin was assayed at concentrations of 0.5, 2.5, 5, 25, and 50 μg/plate. Higher concentrations were extremely cytotoxic. Within the concentration ranges tested, neither the nutmeg oleoresin nor the purified myristicin, with or without S-9 activation, affected the mutagenicity rate of the tester strains. No procedureal problems were evident since the positive and negative controls were within expected ranges (Ames et al., 1975; McCann et al., 1975), and microscopic examination of the background lawns indicated that they were normal.

The chili peppers yielded 12.5% (W/W) oleoresin, with the oleoresin's capsaicin content being approximately 1.7% (W/W). The concentrations of chili pepper oleoresin assayed were 2.5, 25, 250, 2500, and 25,000 μg/plate, while purified capsaicin was assayed at 0.5, 5, 50, 500, and 5000 μg/plate. Vanillylamine (α-amino-4-hydroxy-3-methoxy toluene) was also examined for mutagenicity on the basis of it being a potential metabolic or degradation product of capsaicin. The concentrations assayed were 0.5, 5, 50, 500, and 5000 μg/plate. Neither the oleoresin nor the purified capsaicin and vanillylamine produced an increased reversion rate, while negative and positive controls were within expected ranges.

DISCUSSION

WHILE NEGATIVE RESULTS in the *Salmonella*/mammalian microsome mutagenicity assay are not an absolute assurance of nonmutagenicity, the data indicates that neither chili pepper oleoresin nor purified capsaicin or vanillylamine were mutagenic. Likewise, while the lack of in vitro mutagenicity does not assure non-carcinogenicity, it does suggest that the results of Hoch-Ligetti (1951) should be re-examined. Schoental (1967) hypothesized that the chili peppers employed by Hoch-Ligetti (1951) may have been contaminated by aflatoxins, a group of highly potent hepatocarcinogens. It is also worth noting that the protein source employed by Hoch-Ligetti (1951) was derived from peanuts, a commodity often associated with aflatoxin contamination. It is possible that the test diet was contaminated with low levels of aflatoxins, and that incorporation of chili pepper promoted the activity of the hepatocarcinogen. Hendricks et al. (1980) suggested that the ingestion of aflatoxins in conjunction with a promoter, greatly decreases the amount of aflatoxin needed to induce hepatomas. This interpretation would be more consistent with the findings of Adamuya (1971) who suggested that chili pepper acted as a co-carcinogen.

The present data also suggest that myristicin and nutmeg oleoresin are not mutagenic. The cytotoxicity reported by Rockwell and Raw (1979) was not seen with the levels of oleoresin employed in the present study, but was evident with purified myristicin levels greater than 50 μg/plate. These differences probably reflect the level of nutmeg oleoresin (10,000–100,000 μg/plate) employed by Rockwell and Raw (1979). Assuming their oleoresin had approximately the same myristicin content as that of the present study, the myristicin content of their samples was greater than 400 μg/plate; a cytotoxic concentration.

Interpretation of the negative results for myristicin is difficult considering that the compound is a 5-methoxy analog of safrole. Myristicin and safrole share a number of toxicological characteristics including similar acute toxicities (Singleton and Kratzer, 1969), routes of metabolism (Oswald et al., 1971a, b), and induction of liver hypertrophy (Gershbein, 1977). Studies examining the mutagenicity of safrole using the *Salmonella*/mammalian microsome mutagenicity test have been inconclusive. McCann et al. (1975), Wislocki et al. (1977), and Swanson et al. (1979) reported that safrole was not mutagenic when assayed with and without S-9 preparations derived from Arochlor-induced rats; however, Purchase et al. (1978) found that safrole was mutagenic using liver preparations derived in a similar manner. Elcombe et al. (1975) reported that safrole was mutagenic when assayed with S-9 preparations from 3-methylcholanthrene-induced rats, while Swanson et al. (1979) reported negative results using a similar approach. Green and Savage (1978) and Dorange et al. (1977) reported safrole was mutagenic when assayed in conjunction with S-9 preparations from phenobarbital-induced mice (Green, personal communication) and safrole-induced rats, respectively. It is apparent that detecting safrole's mutagenic activity is difficult and inconsistent, possibly due to the compound's ability to inhibit cytochrome P_{450} (Netter et al., 1978). This tends to cloud interpretation of the present results for myristicin. Presumably, if myristicin could be modified to a mutagen, it would involve generation of a 2′, 3′-oxide or a 1′-hydroxy ester, in a manner similar to that hypothesized for safrole (Janiaud et al., 1976; Wislocki et al., 1977; Swanson et al., 1981). It has been suggested that the slow rates associated with the hydroxylation and epoxidation of compounds of arylalkene class (Swanson et al., 1979) coupled with high levels of epoxide hydrase activity (Swanson et al., 1981) hamper detecting mutagenicity in this class of compounds. While the present results suggest that myristicin is not mutagenic, further studies should be performed to assess its mutagenic and carcinogenic potential.

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—Continued on page 333

A Research Note

A Monolayer Equation for Use in Sorption Studies

M. CAURIE

ABSTRACT

It has been indicated that because of the assumption that $E_2 = E_L$ used in its derivation the B.E.T. infinite layer equation cannot describe adsorption beyond the first adsorbed layer. A simplification of the three constant B.E.T. equation provides a new monolayer equation which agrees more with the above assumption from which the Langmuir equation is shown to arise as a special case at low pressures. The monolayer capacity predicted by the new monolayer equation appears to maintain a more stable product than is possible with the classical B.E.T. infinite layer equation. The use of the new equation in sorption studies is recommended.

INTRODUCTION

THE BRUNAUER, Emmett and Teller (B.E.T.) multilayer adsorption equation has occupied an honored place in the family of adsorption equations for many years. It provides two useful monolayer constants: one estimates the moisture believed to be sufficient for a unimolecular layer (m_o) and another (c) from which the heat of adsorption may be calculated. Processing and stability of a food depends upon both.

The practical importance of these parameters in the food industry requires that they be accurately predicted. Results of storage studies by Martinez and Labuza, (1968) and by Strolle and Cording (1965) cast doubt on the ability of the B.E.T. infinite layer equation to fulfil this role. Results from these studies show that the B.E.T. monolayer capacity underestimates the level that gives the most stability to stored products.

However, it is pointed out (Labuza, 1975) that suboptimal predictions of m_o should probably be expected since the B.E.T. infinite layer equation was not developed for use with polar gases such as water vapor. This view overlooks the fact that limitations experienced with the use of the B.E.T. infinite layer equation are not restricted to polar gases alone but to nonpolar adsorbates as well. Thus in spite of the fact that the equation is supposed to describe infinite layered adsorption, it only applies over the monolayer range of presures for all vapors.

Many attempts (Pickett, 1945; Huttig, 1948; Smith and Pierce, 1948) have been made to modify the B.E.T. infinite layer equation to conform more to experimental data but these have met with limited success (Adamson, 1960). A major modification to the B.E.T. equation was suggested by Brunauer et al. (1938) in which they set an upper limit to the number of layers of molecules that the solid can adsorb, but the resulting three-constant B.E.T. equation was not in a form for routine use.

Recently Caurie (1981) has simplified this three-constant B.E.T. equation from which a new monolayer equation of which the Langmuir equation (1918) arises as a special case at low pressures. This note is to demonstrate the monolayer form of the classical B.E.T. equation and to show the superiority of this new monolayer equation over the classical B.E.T. equation in sorption studies.

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METHODS

CAURIE (1981) has shown that the infinite summation arrangement of the B.E.T. Eq (1) resulting from the simplifying assumption that $E_2 = E_L$

$$\frac{m}{m_o} = \frac{\frac{ca}{(1-a)^2}}{1+\frac{ca}{1-a}} \qquad (1)$$

may be stated in the more simple form

$$\frac{m}{m_o} = \frac{\frac{ca}{(1-a)^2}}{(1-a)(1+\frac{ca}{1-a})} \qquad (1a)$$

In this form the infinite summation expression in the numerator of Eq (1) reduces to the more simple form in the denominator. In Eq (1), a = water activity at moisture content m; m_o = moisture sufficient to cover one unimolecular layer of adsorbed moisture; c = constant related to heat of adsorption; E_2 = heat of adsorption in the second adsorbed layer; E_L = heat of vapourisation.

Limiting the infinite summation in Eq (1a) to only n terms now results in the more simple expression:

$$\frac{m}{m_o} = \frac{ca\frac{1-a^n}{1-a}}{(1-a)(1+ca\frac{1-a^n}{1-a})} \qquad (2)$$

It has been pointed out that the basis of Eq (1) in which $E_2 = E_L$ does not support multilayer adsorption, for when $E_2 = E_L$ there can be no adsorption beyond the first layer (Smith and Pierce, 1948; Caurie, 1981; Halsey, 1948) and therefore the only value n may assume in Eq (2) is unity and with it Eq (2) simplifies to the new monolayer Eq (3) of which the Langmuir monolayer equation arises as a special case at low pressures.

$$\frac{m}{m_o} = \frac{ca}{(1-a)(1+ca)} \qquad (3)$$

Rearranging:

$$\frac{a}{m(1-a)} = \frac{1}{cm_o} + \frac{a}{m_o} \qquad (3a)$$

The corresponding infinite layer B.E.T. form is

$$\frac{a}{m(1-a)} = \frac{1}{cm_o} + \frac{c-1}{cm_o}a \qquad (4)$$

The great similarity between Eq (3a) and (4) may be noted and the apparent success of B.E.T. Eq. (4) for estimating the constants c and m_o may be partly owed to this close similarity.

Indeed both equations give identical linear plots but with different expressions for the slope; with Eq (4) indicating the slope to be only a fraction of that of Eq (3a).

The B.E.T. infinite layer Eq (4) has previously been stated (Caurie, 1971) to underestimate the monolayer capacity on which the stability of dehydrated foods de-

pends. In this respect a comparative examination of Eq (4) and (3a) reveals that the magnitude of the monolayer capacity from the latter equation is greater than from the former equation.

We have pointed out that one of the practical applications of the B.E.T. Eq (4) in food technology is that it provides a useful constant for estimating the monolayer capacity on which the stability of the food depends. The usefulness of the new monolayer Eq (3) will therefore depend on the stability with which the larger monolayer capacity it now provides maintains the stability of the food.

To demonstrate the greater stability which the larger monolayer capacity from Eq (3) may maintain stored products, the work of Strolle and Cording (1965) and Martinez and Labuza (1968) may be used for illustration.

In their studies on mashed potato flakes, Strolle and Cording (1965) found that flakes of Idaho Russet variety of potatoes could be stored at 77°C for 1 month at a moisture level of 4%. Under identical storage conditions a moisture content of 9.6% could store for less than 1 month. On the other hand, at 6.6% moisture the mashed potato flakes stored for 6 months.

The magnitude of m_o estimated with the B.E.T. infinite layer Eq (4) for the potato flakes was 5.73% (Strolle and Cording, 1965). This level of moisture is supposed, according to Salwin (1959), to be the optimum for the storage stability of the potato flakes. The new monolayer Eq (3) on the other hand predicts an optimum moisture level of 6.51% which from the results of Strolle and Cording (1965), must provide a more stable product than the 5.73% moisture predicted with the classical B.E.T. equation. In a similar report (Martinez and Labuza, 1968) freeze-dried salmon was found to be most stable at an equilibrium relative humidity of 32% which is much in excess of 19% predicted with the classical B.E.T. equation. Using the new monolayer Eq (3) a safe moisture content of 6.84% corresponding to 31% relative humidity is predicted. Clearly the new monolayer equation aside from its logical derivation appears to be superior in its predictions of the optimum moisture capacity for the stability of dehydrated products than the classical B.E.T. equation and we suggest its use in sorption studies.

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A Research Note

Effects of Diet on Urinary 1-Ascorbic Acid in the Human

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ABSTRACT

The relationship of dietary vitamin C to that in the urine has not been established. Vitamin C intake and 24-hr urinary 1-ascorbate excretion were measured in healthy, human, Caucasian, female subjects (ages 22–26 yr). Modifications were made in the measurement of 1-ascorbic acid in the urine. The correlation of dietary vitamin C to that excreted in the urine was highly significant ($P < 0.001$; $r = 0.952$). The regression equation is: $y = -1.2970 + 0.1863x$. The average intake of vitamin C was 146.5 ± 117.6 mg (standard deviation) (range 42–424 and the average amount in a 24-hr urine sample was 26.0 ± 23.0 mg (range 5–71). The average percentage of dietary intake excreted per day was 17.04 ± 9.43 (range 6.1–29.4). Data presented here should help establish guidelines for interpreting urinary 1-ascorbic acid concentrations. If dietary data are available, it is suggested that these guidelines be expressed as a percentage of intake. Additional data are needed.

INTRODUCTION

TODAY, much interest is centered on 1-ascorbic acid (vitamin C) in both professional and lay publications. This water-soluble vitamin attracts scientific attention with unanswered questions involving health, disease, and metabolism. Laymen alike are attracted by its purported ability to cure or prevent disease when taken in larger than "normal" amounts. For this reason people may consciously alter their diets to include larger quantities of vitamin C rich foods.

Much of the dietary vitamin C is believed to be excreted in the urine as 1-ascorbic acid or as various metabolites. Twenty-four hour urinary levels of 1-ascorbic acid have been determined by several investigators (Johnson et al., 1945; Davey et al., 1952; Roderuck et al., 1958; Keltz et al., 1978). The majority of these workers included either fixed or variable amounts of an ascorbic acid supplement in the diets of their subjects. Roderuck et al. (1958) published the only paper that reported data showing how urinary 1-ascorbic acid was affected solely by natural food sources of the vitamin.

Since the determination of vitamin C in the urine has been subject to technological problems, it has been difficult to relate levels of urinary 1-ascorbic acid to that in the diet for purposes of nutritional assessment. Consequently, data in this area are limited thereby delaying the establishment of guidelines for interpreting urinary data (Sauberlich et al., 1974).

The objectives of this research were: (1) to modify current analytical techniques for determining 1-ascorbic acid in urine to give a high recovery of an internal standard; and (2) to employ this modified technique to further explore the relationship between quantities of the vitamin excreted in the urine and amounts consumed in the normal diet.

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EXPERIMENTAL

Subjects

Five healthy, Caucasian females, ranging in age from 22 to 26 years with a mean age of 24 years, volunteered for this study. All subjects were well versed in urine sample collection techniques and in procedures for collecting dietary information.

Materials and Reagents

A Hansen model 1440 dietetic scale, daily food diaries, food composition tables and diet record forms were used by each subject for tabulating food intake.

A picric acid solution (saturated: Fisher for automatic analyzers), sodium hydroxide in distilled water (10% w/w: ACS grade) and a creathinine standard solution in distilled water (1 mg/ml: Fisher Certified Grade) were used in the determination of urinary creatinine. All solutions were stored at room temperature.

The following chemicals were used in the determination of vitamin C: trichloracetic acid (TCA) in distilled water (4% w/w: Fisher ACS grade; store at room temperature), 2,6-dichloroindophenol solution in distilled water (0.2% w/v: Fisher ACS grade; filter and store for no longer than one month at 5°C), 2,4-dinitrophenylhydrazine solution (2% w/w: Matheson, in one part concentrated sulfuric acid and three parts water; store at −5°C and filter before use), thiourea solution (1% w/w: Fisher ACS grade, in 4% TCA; filter if turbid; store at 5°C), Norit carbon decolorizing agent (Eastman), stock ascorbic acid solution (0.1% w/v: Mallinckrodt USP grade, in 4% TCA; store frozen), and hydrochloric acid/85% phosphoric acid solution (3/2 v/v: Fisher ACS grades; prepare just before use). A 20 μg/ml working standard was made from the stock standard by dilution with 4% TCA.

Procedures

The six subjects weighed all food consumed each day for a total of 7 days. All food and liquid intake data were recorded. Vitamin C values of foods were tabulated from Agricultural Handbook No. 456 (Adams, 1975). In this study an attempt was made to minimize certain stress factors. Physical activity was kept light and constant, no supplements or drugs were allowed, and the subjects were nonsmokers. All subjects started and ended the study in apparent health. Three 24-hr urine samples were collected from each subject on days three, five, and seven. After total volume was measured, portions of urine were frozen at −32°C for later testing.

The preformed creathinine in the sample urine was determined by the Jaffe reaction (Albanese and Orto, 1963). Creatinine coefficients were then calculated for each subject as a check on the completeness of the urine collections (Harper, 1971).

The basic procedure used for assaying 1-ascorbic acid was that of Schwartz and Williams (1955) with technical modifications taken from Schaffert and Kingsley (1955). with modifications made by the authors. Metaphosphoric acid and acetic acid were replaced by trichloroacetic acid (TCA) in preparation of the standards and samples. Also, Norit was utilized as a decoloration and oxidizing agent for the urine samples and was beneficial in obtaining clear, colorless solutions. Standards were prepared as follows: 25 ml of working ascorbic acid standard and 25 ml of 4% TCA were each mixed with ½ teaspoon Norit for 1 min. These solutions were filtered through Wattman No. 42 filter paper. A range of concentrations were then prepared to a final volume of 2 ml. Preparation of samples was as follows: 1 ml of urine was added to 19 ml of 4% TCA. One-half teaspoon Norit was added, the solution mixed for 1 min, and then filtered through Wattman paper No. 42. Two ml of the filtrate were used for color development. A blank from only one urine sample needs to be prepared for each experimental run. For osazone formation, the 37°C incubation temperature was followed as speci-

fied in an original paper on the osazone method by Roe and Kuether (1943) and as reemphasized in 1961 by Roe, who mentioned that using a temperature of 100°C to carry out the coupling reaction as proposed by Schaffert and Kingsley, results in values that are erroneously high, probably due to the formation of ascorbate-2-sulfate. Incubate for 3 hr. After addition of the hydrochloric/phosphoric acid solution, a final incubation period at 37°C for at least 20 min was added to the procedure to enhance dissolving of the osazone crystals. It was necessary to check carefully to be sure the osazone crystals were completely dissolved and that the solution was clear. Erroneous results occurred if crystals were still present. Since undissolved osazone crystals are difficult to see without holding the tubes up to a light, frequent errors may be made in determining vitamin C in urine by this method. All tubes were read at 540 mμ on a Coleman spectrophotometer, model 295.

The data were analyzed by correlating ascorbate intake with excretion for the same day and plotting a least squares line. Any significant relationship between intake and excretion was determined by a t-test.

RESULTS & DISCUSSION

RECOVERY EXPERIMENTS verified the validity of the above procedureal modifications. The addition of 1 μg/ml and 5 μg/ml of ascorbic acid to urine samples resulted in an average recovery of 99.5%. All urine samples were complete as determined by creatinine coefficients.

The data obtained from measuring the l-ascorbic acid levels of urine exhibit a high degree of correlation between ascorbic acid intake and excretion ($r = 0.952$) (Fig. 1). The correlation is significant ($t = 10.297$, $P < 0.001$, $df = 11$). The 95% confidence bands for the regression are extremely narrow. The regression line is represented by: $y = -1.2970 + 0.1863x$. Two points were eliminated. One represented an extremely high vitamin C intake and the other fell outside the 95% confidence belt for a single observation. This relationship is theoretical with vitamin C intake estimated from food values reported in food composition tables. In the practical sense, nutrient intake will usually be assessed using tables; nevertheless, relationships, as the one reported here, need to be confirmed by direct chemical analysis of food. Data used in preparing the regression line are shown in Table 1. The correlation of dietary vitamin C to the urinary vitamin C/creatinine ratio was 0.962. Little gain was made over the correlation of dietary l-ascorbic acid with total ascorbate excretion.

The average daily intake of vitamin C was 146.5 ± 117.6 mg (range 42–424) and the average amount in a 24-hr urine sample was 26.0 ± 23.0 mg (range 5–71). The percentage of l-ascorbic acid excreted was 17.04 ± 9.43% (range 6.1–29.4).

The effect of diet on urinary excretion of ascorbic acid is clearly shown by the results. There is a direct and significant relationship between the amount of vitamin C taken in the diet and excreted in the urine over the range of intake reported in this study. The direct relationship between diet and urinary levels of vitamin C is supported by Roderuck et al. (1958).

The value of 17% for the average percentage of ascorbic acid excreted (recovered) in this study agrees well with another percent recovery guideline given as 12–14% by Kutsky (1973). The data reported here also agree well with an average percentage recovery of 26 (range 8–49) as reported by Keltz et al. (1978). Johnson et al. (1945) reported a percent recovery of 24. The data of Davey et al. (1952) (percent recovery of 46 with a range of 37–82) seem in error on the high side. Data presented here should help establish guidelines for interpreting urinary l-ascorbic acid concentrations. If dietary data are available, it is suggested that these guidelines be expressed as a percentage of intake rather than mg/24 hr. Hopefully, it may be possible to correlate vitamin C intake with excretion so that a determination of vitamin C status can be done by an analysis of

—Continued on page 337

Table 1—Dietary and urinary Vitamin C (in mg) for five human subjects. Data for days 3, 5, and 7 were used in preparing the regression line

Subject	Days 1	2	3	4	5	6	7
1	40[a]	65	69 20[b]	117	89 14	67	63 12
2	28	154	131 20	94	81 8	116	90 24
3	284	100	79 5	99	42 6	79	99 6
4	200	304	351 71	103	213 45	198	424 69
5	169	1344	—[c] —[d]	1246	167 2[e]	138	176 38

[a] Dietary vitamin C in mg
[b] Urinary vitamin C in mg
[c] Diet record in error
[d] Urine data not included since diet record was in error
[e] Urine value not included since the data point was shown to be a statistical outlier

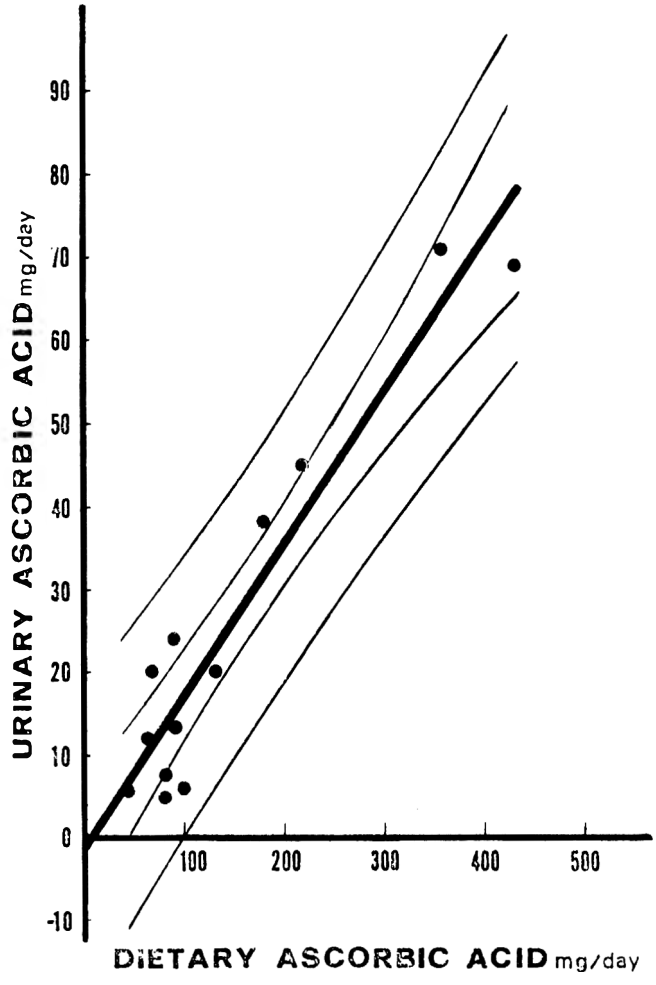


Fig. 1—Relationship of dietary to urinary ascorbic acid in a studied sample of young, female subjects. ($r = 0.952$; $t = 10.297$; $P < 0.001$; $df = 11$). The equation for the regression is: $y = -1.2970 + 0.1963x$. The lines on either side of (and closest to) the regression represent the 95% confidence belts for the regression and the lines on either side of (and further from) the regression represent the 95% confidence belts for a single observation.

A Research Note

Comparison of Sample Storage Methods for Vitamin B_6 Assay in Broiler Meats

CATHARINA Y. W. ANG

ABSTRACT

Five methods were compared for storage stability of vitamin B_6 in broiler meat samples. Maximum retention of vitamin B_6 was observed in the N_2-packed freeze-dried samples at −34°C (101–103% up to 20 mo). Good retentions occurred in the intact tissues of half birds at −34°C (90% in 16 mo) and in ground meat samples (200g portions) at −34°C (91% up to 12 mo). Significantly lower retentions were found in freeze-dried samples stored at room temperature (85–77% in 1–5 mo, respectively) and for ground meat small portion samples (5g) at −34°C (84–72% in 3–5 mo, respectively).

INTRODUCTION

THE STABILITY of some micronutrients in food products during storage depends on the type of food, packaging method and time and temperature of the storage employed. It is customary to preserve fresh food samples for later analysis for a few days to several months in a freezer, at −18° to −40°C or by freeze-drying.

For some nutrients, the changes during frozen storage may be appreciable. Fennema (1975) summarized in a review that the losses of B-vitamins in animal tissues during 6 months storage at −18°C ranged from 0–59%. Richardson et al. (1961) found that frozen raw boned chicken retained 77% of its vitamin B_6 after 15 months of storage at −20°C.

The objective of the present study was to assess the effectiveness of frozen storage of fresh and freeze-dried raw broiler meat for later vitamin assays. Vitamin B_6 was studied because it is labile to heat, oxygen, and light and because chicken meat is an important source of this vitamin.

MATERIALS & METHODS

Storage treatments

Ready-to-cook broilers from a local processing plant were collected and prepared further for five storage treatments. Various periods (2–20 months) were tested. Three to eight replicate samples were analyzed for each storage period of each treatment.

Treatment 1 involved storage of frozen intact half carcasses at −34°C for 4, 8, and 16 months. Broilers were cut into halves and the breast meat of one-half of each bird was removed, and freed of bone and skin. The lean meat was ground two times with a 4.8 mm plate meat grinder and the moisture and vitamin B_6 contents were analyzed immediately to provide initial values. The other half of each bird was placed in an moisture-tight plastic bag and stored at −34°C until used. At the end of each storage period, 3–8 frozen halves were thawed for 20–24 hr at 2°C and then prepared in the same manner as for the initial halves.

Treatment 2 consisted of storage of 200g samples of ground raw breast meat at −34°C for 2, 4, 8 and 12 months. The ground meat was packed in 8-oz (227 ml) screw-capped glass jars. At each designated period, jars were thawed in a 2°C refrigerator overnight and the contents of each jar were thoroughly mixed before aliquots were taken for analyses.

Treatment 3 duplicated Treatment 2 except that 5g samples in 2 oz (56.8 ml) tightly capped plastic vials were stored at −34°C for 3 and 5 months. At the designated periods, the entire 5g sample was used directly for vitamin B_6 assay without thawing.

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Treatment 4 consisted of storage of freeze-dried samples at −34°C under N_2. Ground meat samples were freeze-dried, finely ground in a mortar with pestle, and then stored in glass sample jars (approximately 50g) with nitrogen at −34°C for 2, 5 and 20 months.

Treatment 5 consisted of storage of freeze-dried samples at room temperature (20°C) for 1, 2 and 5 months. Dried ground samples (approximately 5g) were placed in 2 oz (56.8 ml) tightly capped plastic vials. No nitrogen was used.

Analytical methods

Moisture content was determined by the vacuum oven method at 90°C and vitamin B_6 content was determined by a microbiological method using *Saccharomyces uvarum* ATCC No. 9080 as the test organism (AOAC, 1975). Pyridoxine·HCl from U.S.P. References Standards (The United States Pharmacopeial Convention, Inc., Rockville, MD) was used as the standard. At least triplicate aliquots were taken from each sample for the vitamin B_6 determination.

For statistical calculations, a pooled error variance of each treatment was used and paired comparison between the initial and final values were made at each storage period for each treatment (methods of Steel and Torrie, 1960).

RESULTS & DISCUSSION

THE AVERAGE VITAMIN B_6 content and retention values of broiler breast meat stored under different conditions are presented in Table 1.

Since the meat samples may lose moisture (in the case of wet samples) or gain moisture (in the case of freeze-dried samples) during storage, moisture content was determined along with vitamin B_6 content before and after storage. Vitamin B_6 values thus expressed on a dry weight basis would more accurately show the actual changes of vitamin B_6 content in the samples. In the case of Treatment 3 where vitamin B_6 values were determined on a wet weight basis, the weight of each sample was determined before storage.

For intact half carcasses stored at −34°C (Treatment 1), the retention of vitamin B_6 ranged from 99–90% in 4–20 months. The observed reductions were not statistically significant.

Freeze-dried samples packed in nitrogen, and stored at −34°C showed excellent retention of vitamin B_6 activity up to 20 months. Slightly higher than 100% retentions were observed in some cases, but they were within the analytical errors.

Ground meat samples packed in glass jars and kept at −34°C lost 5–9% vitamin B_6 during 12 months storage. However, the reductions were not statistically significant.

Large portions of frozen meat either as ground or as intact half carcasses had to be thawed before samples (5g portions) could be taken for vitamin assay. To determine the effect of freezing-storage only without the subsequent thawing, 5g of ground meat samples weighed in vials were frozen and analyzed from the frozen state (Treatment 3). Surprisingly, significantly ($P<0.01$) lower vitamin B_6 activity was found in these samples (84 and 72% retentions in 2 and 5 months, respectively). One possible explanation might be that the small portion samples (5g) were exposed to more air during storage because of the increased surface area per unit of weight.

For freeze-dried ground meat samples stored at room

Table 1—Vitamin B_6 content and retention in broiler breast meat during storage

No.	Treatment	Storage period months	Vitamin B_6 Initial (mg/100 g, dry wt)	Final (mg/100 g, dry wt)	Mean retention[a] (%)	Statistical significance[d]
1	Intact half carcass, −34°C	4	2.35 ±0.09[b]	2.33 ±0.18	99	ns
		8	2.36 ±0.04	2.23 ±0.17	95	ns
		16	2.42 ±0.12	2.18 ±0.21	90	ns
2	Ground meat (200g) large samples, −34°C	2	2.36 ±0.12	2.23 ±0.16	95	ns
		4	2.37 ±0.17	2.26 ±0.22	95	ns
		8	2.41 ±0.20	2.21 ±0.21	92	ns
		12	2.22 ±0.32	2.00 ±0.09	91	ns
3	Ground meat (5g) small samples, −34°C	3	.591±0.034[c]	0.497±0.046	84	**
		5	.602±0.029	0.429±0.021	72	**
4	Freeze-dried ground meat −34°C, N_2	2	2.38 ±0.05	2.39 ±0.03	101	ns
		5	2.43 ±0.11	2.46 ±0.13	101	ns
		20	2.32 ±0.09	2.39 ±0.06	103	ns
5	Freeze-dried ground meat 20°C, air	1	2.35 ±0.08	2.00 ±0.11	85	**
		2,5	2.35 ±0.08	1.82 ±0.10	77	**

[a] Retention = (Final/Initial) x 100
[b] Average and standard deviation
[c] For this treatment only, the vitamin B_6 values are expressed on wet weight basis.
[d] ns—No significant difference from initial
**The final values are significantly lower than the initial values at 1% level.

temperature without N_2, the retention of vitamin B_6 was not satisfactory. Apparantly air or oxygen still had adverse effect on the vitamin content of dried samples.

The present study suggests that broiler meat samples can be preserved for later vitamin B_6 assays by storage at −34°C as intact tissues, as ground meat large-portions (200g) packed in jars, or as freeze-dried ground meat packed with N_2. Since vitamin B_6 is one of the more unstable B-vitamins, it seems reasonable to expect that preservation methods suitable for this vitamin might also be suitable for other B-vitamins such as, riboflavin, niacin, pantothenic acid and thiamin.

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The mention of firm names in trade products does not imply that they are endorsed or recommended by the Dept. of Agriculture over firms or similar products not mentioned that also may be suitable.

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the urine. More work needs to be done along these lines to ascertain the validity of the observed regression line using direct chemical analysis of food. Also, future work will have to look at: (1) the effects of larger dietary ascorbate intake and supersaturation; (2) the influence that other dietary variables may have on the regression line; and (3) specific stress variables that may alter vitamin C assessment.

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A Research Note

Modification and Evaluation of a Reverse Phase High Performance Liquid Chromatographic Method for Amino Acid Analysis

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ABSTRACT

A reverse phase high performance liquid chromatographic method was used to separate the o-phthaldialdehyde derivatives of of amino acids from food protein hydrolysates. Comparison of hydrolysis with hydrochloric, methanesulfonic and mercaptoethanesulfonic acids revealed that the latter could not be used because it interfered with derivatization. Methanesulfonic acid is preferable to hydrochloric acid since it protects the tryptophan in samples low in carbohydrates. Hydrolysates were prepurified with a Sep Pak C18 cartridge prior to derivatization. A linear gradient was used for combining the binary solvent system composed of acetonitrile and phosphate buffer. The derivatives were measured fluorometrically and histamine was used as an internal standard. Results obtained with this method were similar to those obtained with an ion exchange chromatography except that threonine and glycine coeluted and cysteine, cystine and proline were not detected.

INTRODUCTION

HIGH PERFORMANCE liquid chromatography (HPLC) has recently been applied to amino acid anysis since it is rapid and utilizes equipment that is more economical and versatile than a traditional ion exchange amino acid analyzer. This research was undertaken to determine the feasibility of using the reverse phase HPLC procedure of Hill et al. (1979) for the analysis of amino acids in foods. Specifically the study was designed to determine the best acid hydrolysis and solvent elution procedures and to select a suitable internal standard.

EXPERIMENTAL

THE HPLC UNIT (Waters Associate Co.) consisted of two model 6000 A pumps, a μ bondapack C18 reverse phase column (30 cm x 3.9 mm i.d.), a model 660 solvent programmer and a U6K injector. The model FS 950 fluoromat fluorometer (Schoeffel Instrument CO.) with a time constant setting of 0.6 sec., a 1.0 μA full-scale range setting and a sensitivity dial setting of 750 mV. The fluorometer output was connected to a Spectra-Physics 4100 Chromatographic Computer Integrator.

Chicken egg white lysozyme (3X crystallized from Sigma Chemical Co.) was used to study the acid hydrolysis procedure. The following samples were analysed by both conventional ion exchange chromatography and reverse phase HPLC: (a) freeze-dried egg white; (b) unenriched, unbleached, spring wheat flour; (c) pressure cooked, freeze-dried, blackbeans; (d) sesame seed α globulin; (e) Tarhana, a fermented Turkish food, prepared from yogurt, milk, tomato, wheat flour, salt, pepper, monosodium glutamate and yeast. These samples were chosen to give a range of nonproteinaceous material and varying ratios of amino acids.

The traditional 6N HCl hydrolysis was compared to two sulfonic acid hydrolysis methods (Simpson et al., 1976; Penke et al., 1974). These acids were 4N methanesulfonic acid with 0.2% 3-(2 amino ethyl) indole and 3N mercaptoethanesulfonic acid respectively. Samples containing 1–2 mg proteins were hydrolyzed by 1 ml of acid under vacuum (<50μ) at 115°C for 22 hr. Before acid hydrolysis, histamine ($\sim$1 μ mole) was added to the sample as an internal standard.

After acid hydrolysis, sample was neutralized with 6N KOH to $\sim$ pH 10 and the solution was filtered through a 0.45 μm membrane filter with a sample filtration kit (Millipore Co.). The filtrate was diluted to 50% with methanol and filtered through a small column cartridge (Sep Pak C18 Cartridge, Waters Assoc. Co.) to remove nonpolar materials.

An amino acid standard containing all the amino acids except tryptophan, glutamine and asparagine was purchased from Pierce Chemical Co. Solutions of tryptophan (2.5 mM) and histamine (7.5 mM) were prepared and 1 ml of these two solutions were added to 1 ml of 2.5 mM amino acid standard solution and the mixture was diluted to 5 mL The standard solution was stored in plastic vials in a freezer when not in use since histamine is absorbed on glass at sub-freezing temperature (Redlich and Blick, 1969).

Separate solutions containing 100 mg o-phthaldialdehyde (OPA) and 100 μl ethanethiol (97%) in 10 ml methanol were prepared daily and kept in ice water. A 0.8M potassium borate buffer (pH 10.5) containing 12% Brij 35 (Sigma Chemical Co.) was prepared from boric acid and KOH. For amino acid derivatization, 0.4 ml each of OPA and ethanethiol solution was combined with 0.1 ml borate-Brij solution. The desired amount of sample was added and the volume adjusted to 2 ml with methanol. The final concentration of the amino acids was about 4μ mole/ml. After exactly 2 min reaction time 25 μl of the derivative was put on the column.

Two solvents were used to develop the chromatograph. Solvent A was a 15 mM sodium phosphate buffer and solvent B was a 15 mM sodium phosphate buffer in 55% acetonitrile/45% aqueous solution. Both A and B solvents were prepared from a 300 mM sodium phosphate stock solution (pH 7.2) and filtered through a 0.45 μm membrane filter prior to usage (Millipore Co.). HPLC grade acetonitrile and high purity water from a Milli Q purification system (Millipore Co.) was used. Before starting the chromatograph, solvent A and B were carefully degassed under vacuum and sonification. A linear gradient of eluting solvents was used with the concentration of solvent B being increased from 15% to 75% at the rate of 1% per minute. The gradient was controlled with a M660 solvent gradient programmer. The flow rate was set at 2 ml/min and the total chromatograph time was 60 min.

Acid hydrolyzed samples were also analyzed by the Beckman Model 119 CL analyzer (6 mm bore, single column). Norleucine was used as an internal standard.

RESULTS & DISCUSSION

THE DERIVATIZATION and chromatographic procedures of Hill et al. (1979) were modified to simplify the procedure and to optimize the separation. Brij 35 was included in the derivatizing reagent to increase lysine fluorescence (Gardner and Miller III, 1980). Use of a single linear solvent gradient instead of a three-step gradient gave more reproducible separations. A representative chromatogram of the standard amino acids is shown on Fig. 1. Threonine and glycine coeluted. Cysteine gives very low fluorescence with OPA (Chen et al., 1979) and proline does not react with OPA. Asparagine and glutamine are not included in the chromatograph because they degraded to aspartic acid and glutamic acid upon acid hydrolysis. Preliminary trials showed that asparagine eluted before serine and glutamine eluted between serine and histidine if they were present in the sample. The other amino acids were fully separated. The elution order was the same as that of Hill et al. (1979) except that the order of tyrosine and arginine was reversed. Histamine, the internal standard, appeared before lysine.

Solvent composition, buffer concentration, and pH are major factors affecting reverse phase chromatography.

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Varying the concentration of sodium phosphate buffers from 6 mM to 21 mM altered the relative elution order of alanine, arginine and tyrosine. The relative retention times of the other amino acids were not affected by phosphate concentration although all retention times increased slightly with increasing buffer concentration. The same phenomena was observed with variations in pH. The parallel response of most of the amino acids upon changes in pH and buffer strength indicate that this procedure is more reproducible than the separation of phenylthiohydantoin (PTH) amino acid derivatives. The elution order of the PTH derivatives was greatly affected by the pH and buffer concentration of the eluting solvent (Somack, 1980).

Although food samples contain components which might interfere with amino acid analysis no extra peaks were observed on the chromatogram (Fig. 1). Presumably the specificity of the fluorometric procedure and selectivity of the solvent system eliminated this type of interference. However, nonpolar contaminants were observed coming off after the amino acids. These contaminants could interfere with column performance, therefore a Waters Sep Pak 18 cartridge was used to remove the nonpolar contaminants. Complete recovery of the amino acids from the Sep Pak was achieved by eluting them with 50% ethanol.

Histamine appears to be a very satisfactory internal standard since it was not lost during acid hydrolysis and it forms a stable OPA derivative (Davis et al., 1978). Norleucine, the internal standard normally used in ion exchange amino acid analyses, coelutes with Jeucine when HPLC separation is used (Gardner and Miller III, 1980).

Since Penke et al. (1974) and Simpson et al. (1976) reported that hydrolysis of proteins with methanesulfonic acid and mercaptoethanesulfonic acid gave better recoveries of tryptophan than HCl, all three acids were tested. Egg white lysozyme was used for this test since its amino acid content is known. Hydrolysis with mercaptoethane sulfonic acid gave results that agreed with an ion exchange amino acid analyzer but erratic results with the HPLC method. Presumably this acid interferes with the formation of the OPA derivatives.

The amino acid composition of several food samples determined by HPLC with methanesulfonic acid hydrolysis are compared with the data obtained with the Beckman 119 CL automatic amino acid analyzer in Table 1. The samples for the ion exchange (IE) automatic amino acid analyzer were hydrolyzed with HCl or mercaptoethanesulfonic acids. Neither of the sulfonic acids appeard to protect the tryptophan in the samples with high carbohydrate content which confirms the findings of Simpson et al. (1976)

—Continued on page 341

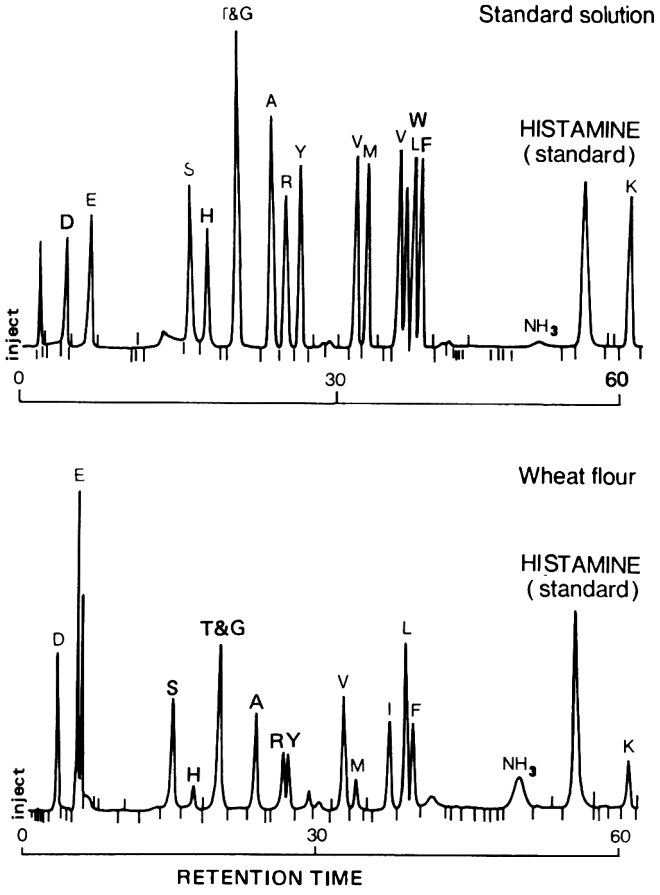


Fig. 1—Separation of 14 OPA derivatives of amino acids on a Waters μ Bondapak C18 Column. (top) A standard solution. (bottom) A wheat flour methanesulfonic acid hydrolysate. (25 μl of OPA derivatives of hydrolysate with a sample concentration equivalent to 40 ng/μl). Abbreviations; D—Aspartic acid, E—Glutamic acid, S—Serine, H—Histidine, T—Threonine, G—Glycine, A—Alanine, R—Arginine, Y—Tyrosine, V—Valine, M—Methionine, I—Isoleucine, W—Tryptophan, L—Luecine, F—Phenylalanine, K—Lysine.

Table 1—Comparison of Amino Acid Values Obtained with HPLC and an Amino Acid Analyzer[a]

Amino acids	Egg white		Sesame α globulin		Wheat flour		Blackbean		Tarhana-1		Tarhana-2		Geometric means	
	HPLC[b]	IE[c]	HPLC[b]	IE[d]	HPLC[b]	IE[c]	HPLC[b]	IE[c]	HPLC[b]	IE[c]	HPLC[b]	IE[c]	HPLC	IE
Aspartic acid	707	676	713	735	42	37	223	236	49	44	48	40	149	140
Glutamic acid	810	768	1197	1359	206	297	261	263	244	216	203	211	370	394
Serine	555	579	442	486	59	56	143	157	53	54	45	44	131	134
Histidine	112	113	160	179	17	18	43	41	17	15	14	14	39	39
Thr & Gly	610	729	858	1003	80	82	211	227	70	74	64	66	185	201
Alanine	591	580	580	564	39	41	117	119	44	45	37	38	117	118
Arginine	269	269	784	815	28	28	101	97	25	25	24	21	84	82
Tyrosine	179	188	200	218	24	25	55	53	24	24	20	19	53	54
Valine	275	477	410	388	41	42	107	98	46	45	37	37	107	104
Methionine	204	231	129	174	12	16	23	23	14	16	11	13	32	38
Isoleucine	313	320	292	273	32	32	86	78	30	30	27	26	77	74
Tryptophan	55	62	75	78[e]	0	0	13	8	0	0	0	0	—	—
Leucine	524	550	511	541	64	65	150	155	65	65	53	53	144	148
Phenylalanine	290	305	283	299	38	38	89	87	31	32	27	27	78	80
Lysine	344	354	118	138	17	19	104	110	30	30	20	21	59	63

[a] Values expressed as μmole/g.
[b] Sample was hydrolyzed by 4N methanesulfonic acid.
[c] Sample was hydrolyzed by 3N mercaptoethanesulfonic acid and determined by Beckman 119-CL automatic amino acid analyzer.
[d] Sample was hydrolyzed by 6N hydrochloric acid.
[e] Tryptophan determined by NBS method (Spande and Witkop, 1967) and Edelhoch's method (1967).

A Research Note

Sensory Evaluation of Forage- and Grain-Fed Beef

M. F. CHASTAIN, D. L. HUFFMAN, and S. BERTRAM

ABSTRACT

Rib roasts, rib steaks, and shoulder roasts from forage and grain-fed cattle were compared. By means of triangle testing, panelists were able to detect differences between beef from the two feeding regimes. However, no significant preferences were shown for flavor of lean, flavor of fat, tenderness, or juiciness nor were there significant differences in cooking losses. The differences shown did not affect acceptability of forage-fed beef.

INTRODUCTION

INCREASING COSTS of animal grain finishing, as well as concerns for utilizing more of our available grain for direct human consumption, emphasizes the importance for research in the area of forage-fed beef. Research dating as far back as Wanderstock and Miller (1948) and Meyer et al. (1960) reported that grass-fed beef was lower quality, less tender, flavorful and juicy, trimmer and yellower in fat color than grain-fed beef. More recent studies (Bowling et al., 1977; Kropf et al., 1975) have shown similar findings indicating that grain-fed beef is more tender and has a more desirable flavor and overall palatability than forage-fed beef. The objectives of the present study were to determine: (1) if differences between forage and grain-fed beef of similar weight, grade and age could be detected by sensory evaluation and (2) if lean flavor, fat flavor, tenderness, juiciness and cooking losses differed among rib roasts, shoulder roasts and rib steaks from the two feeding regimes.

EXPERIMENTAL

TEN 18-month old cross-bred steers were divided into two groups of five each. The forage group was finished to market weight on rye-ryegrass-Yuchi Arrowleaf clover pastures. The feedlot-finished animals were grown out in the same pastures and then given a 90-day period of feedlot finishing on a conventional high-energy finishing ration. All cattle were slaughtered at about 475 kg body weight. Age of cattle at slaughter was about the same for both groups, although rate of gain averaged slightly higher for those finished on grain than those slaughtered directly off pasture.

Grades for individual animals ranged from low USDA Good to low USDA Choice. The following forequarter retail cuts were used for this study: rib roasts (1–7th ribs, 1 from each side); rib steaks (2.54 cm thick from 10th, 11th, and 12th ribs, 3 from each side); and shoulder arm roasts 5 cm thick, taken from the arm side of the standard square cut chuck (2 from each side). All meat was freezer wrapped, frozen and stored at −26°C for subsequent evaluation.

Cooking

Steaks and roasts were tempered at −3°C for 24 and 48 hr, respectively, prior to cooking, then held for 1 hour at 25°C to standardize starting temperatures. Rib roasts were prepared on open racks by oven-roasting at 163°C. Steaks were broiled in electric ranges with the surfaces of the steaks approximately 13 cm from the broiler element. Both rib roasts and steaks were cooked to an internal temperature of 65°C as determined by a probe thermister thermometer. Shoulder roasts were placed on racks in roasting pans which contained 50 ml of water, covered with heavy duty aluminum foil and cooked at an oven temperature of 163°C to an internal temperature of 72°C.

Sensory panel training and evaluation

Six panelists were selected from a group of 15 staff members of the Animal & Dairy Sciences Department by the use of the triangle test and sequential testing (Amerine et al., 1959). Samples were evaluated after 2 storage time periods, 5–6 months postmortem and 7–8 months postmortem. Sensory evaluation was done in a specially constructed room having controlled atmosphere and freedom from noise and odors. Individual booths were lighted with low-intensity red light to mask possible cooked color differences. Water for rinsing was served at all evaluation sessions. Triangle tests were conducted for all comparisons to determine if differences existed between each of the treatments. At each session, three triangles were presented to each judge. Not every steak or roast prepared was evaluated by every judge; each panelist was randomly assigned samples from the various animals. Since triangle testing indicated that panelists were able to detect differences between all comparisons, a scaling technique was used to evaluate rib steaks and shoulder roasts for flavor of lean and flavor of fat (9 = extremely flavorful, 1 = extremely bland), tenderness (9 = extremely tender, 1 = extremely tough), and juiciness (9 = extremely juicy, 1 = extremely dry). The same trained 6-member sensory panel was used.

Statistical analysis

Triangle test results were analyzed for significance by use of a table given by Roessler et al. (1948). Scores from sensory ratings were tested by analysis of variance (Amerine et al., 1959). Student's t-test was used to test for possible differences between cooking losses (Steel and Torrie, 1976).

RESULTS & DISCUSSION

TABLE 1 shows the results of triangle tests done on each of the meat cuts. Significant differences were found between forage-fed and grain-fed beef rib cuts after both storage periods. The shoulder roasts showed significant differences after the shorter time (5–6 months), but not with longer storage (7–8 months).

Judges were able to distinguish between forage-fed and grain-fed rib cuts in approximately 57% of the total judging sessions (Table 1). In contrast, the differences in shoulder roasts were apparent in only 46% of the judging sessions. These findings indicate the possibility that cooking methods emphasized flavor differences. Rib cuts were cooked by dry heat to the medium done stage whereas shoulder roasts were cooked by moist heat to the well-done stage. Another explanation for the flavor difference between shoulder and rib cuts could be the fat content. Published information (Adams, 1975) indicates that cooked rib roast has a fat content of 39.4% whereas cooked shoulder roast contains 14.6–19.2% depending on grade. Since most flavor components are fat soluble (Wasserman and Talley, 1968; Shorland et al., 1970) a higher fat content could mean a greater concentration of substances contributing to flavor.

Rating tests done on rib steaks and shoulder roasts after completion of triangle testing are shown in Table 2. The steaks from grain-fed animals were not significantly different from steaks from forage-fed animals for the sensory

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Table 1—Triangle test results comparing forage and grain-fed cuts of beef

Cut	5–6 mo Storage		7–8 mo Storage	
	Responses[a]	%[b]	Responses[a]	%[b]
Rib roast	36/66 ***	54	22/36 ***	61
Rib steak	29/57 **	57	30/54 ***	55
Shoulder roast	24/51 *	47	24/54	44

[a] Number of correct responses out of total responses
[b] Percent correct responses
*Significant at $P < 0.05$
**Significant at $P < 0.01$
***Significant at $P < 0.001$

Table 2—Sensory scores comparing cuts of beef from forage-fed and grain-fed cattle[a]

Feeding treatment	Flavor[b] of lean	Flavor[b] of fat	Tenderness[c]	Juiciness[d]
		Rib steaks		
Grain	5.81±2.00	4.51±1.92	5.69±1.55	6.00±1.61
Forage	5.60±2.17	4.14±1.86	6.42±1.47	6.57±1.73
		Shoulder roasts		
Grain	5.70±1.11	3.63±1.47	5.43±1.83	3.40±1.30
Forage	5.03±1.56	3.46±1.27	5.60±1.92	3.20±1.52

[a] Figures are means ± standard deviation.
[b] Extremely flavorful = 9; extremely bland = 1.
[c] Extremely tender = 9; extremely tough = 1.
[d] Extremely juicy = 9; extremely dry = 1.

traits flavor of lean, flavor of fat, tenderness or juiciness. Studies by Williams (1976) showed a preference for steaks from grain-fed cattle, with the flavor of those steaks being preferred over the flavor of meat from forage-fed cattle. In the present study, shoulder roasts from grain-fed animals were rated slightly higher than those from forage-fed cattle for all attributes except tenderness.

Rib cuts (roasts and steaks) from forage-fed cattle showed slightly higher total cooking losses than did similar cuts from grain-fed cattle. Cooking losses were very slightly higher for shoulder roasts from grain-fed cattle as compared to shoulder roasts from forage-fed cattle; however, none of the cooking loss differences was significant.

Shoulder roasts from both feeding regimes showed higher cooking losses than rib cuts. Rib roasts lost more weight than did rib steaks. The higher losses with the shoulder roasts can be attributed to longer cooking times and the moist heat method of cookery.

CONCLUSIONS

A DETECTABLE palatability difference does exist between forage-fed and grain-fed cattle, as shown by triangle testing; however, it was not possible to determine the reason for the differences in the present study. Although it is apparent that differences do exist between forage-fed and grain-fed cuts the eating quality of beef from both treatments was in the acceptable range.

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and Penke et al. (1974). The geometric means (Table 1) of the HPLC and IE results were evaluated by a 3-way factoral analysis of variance. (Geometric means could not be computed for tryptophan because of the zero values). The HPLC method gave significantly lower results if the methionine values are included. The lower methionine values for HPLC may be due to the fact that methanesulfonic acid is a weaker antioxidant than mercaptoethanesulfonic acid. If the methionine values are excluded, the HPLC and IE results are not significantly different. In spite of the limitations of this HPLC method, it does provide a relatively rapid and inexpensive method for measuring most of the amino acids.

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A Research Note

Humectant Analysis of Intermediate Moisture Meat by High Performance Liquid Chromatography

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ABSTRACT

A quantitative extraction and high performance liquid chromatography (HPLC) procedure for analysis of four common humectants (sucrose, sorbitol, glycerol and propylene glycol) in intermediate moisture meat was developed. The procedure includes removal of ionic species from an extract with cation and anion columns prior to HPLC analysis with an Aminex HPX-87 column. The total time for HPLC analysis was 20 min. Total mean recovery of the humectants added to the meat ranged from 97% for sucrose to 110% for glycerol.

INTRODUCTION

INTERMEDIATE MOISTURE (IM) foods are characterized by water activities of 0.65–0.90 and moisture contents of 10–40% (Karel, 1976). IM foods contain enough moisture to impart a plastic mouthfeel, yet the water activity is low enough to ensure storage stability at room temperature. One of the techniques used to adjust water activity of IM foods is infusion. Food pieces are soaked in an infusion solution containing humectants, such as glycerol, sorbitol, propylene glycol, (1, 2-propanediol), sucrose, sodium chloride, and other components. Since water activity of the infusion solution is lower than that of the food, water diffuses out of the food into the solution, and solutes diffuse from solution into the food until an equilibrium is reached. This method has been used to prepare a variety of IM foods (Brockmann, 1970; Collins and Yu, 1975; Lee and Kraft, 1977; Jayaraman and Gupta, 1978; Dymsza and Silverman, 1979; Webster et al., 1979; Herrera, 1981). In most infusion studies, the quantity of humectants diffused into the foods was estimated on the basis that the humectant concentration is the same in the solution and the food (Kaplow, 1970). Little research has been done to analyze humectants quantitatively in IM foods directly.

Recently, high performance liquid chromatography (HPLC) using refractive index detection has been used to analyze sugars and other carbohydrates in foods (Macrae, 1981). Schwarzenbach (1977) separated several polyhydric alcohols using an aminoalkylsilica gel column with a mobile phase of water-acetonitrile. Scobell et al. (1977) utilized different Aminex columns to analyze the carbohydrate components of sweetners. Using a silica column and an acetonitrile-water partition HPLC-system, Aitztmuller et al. (1979) separated polyols in food emulsifiers. Recently, Brandao et al. (1980) developed a technique to separate sorbitol, glucose and other mono- and disaccharides by joining two bonded phase carbohydrate columns in tandem and by using a ternary mobile phase (acetonitrile-water-ethanol). This technique was later applied to fresh fruits (Richmond et al., 1981).

The objective of this study was to demonstrate the feasibility of HPLC in quantitative analysis of four of the common humectants used in IM meats.

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MATERIALS & METHODS

Infusion solutions

Four infusion solutions were used for this study. The solutions differed in humectant concentration, but the ratio of humectants was constant in solutions 1-3. All humectants were reagent (ACS) grade except propylene glycol ('Baker' grade, J.T. Baker Chemical Co., Phillipsburg, NJ) and sorbitol (Sigma Chemical Co., St. Louis, MO). Solutions were prepared on a weight basis:

- Soln 1: Distilled water, 50%; sucrose, 11%; sodium chloride (NaCl), 12%; sorbitol, 9%; glycerol, 9%; propylene glycol, 9%.
- Soln 2: 4 parts soln 1 + 1 part distilled water.
- Soln 3: 1 part soln 1 + 1 part distilled water.
- Soln 4: Distilled water only (control).

Preparation of infused meat

A beef top round roast (semimembranosus muscle) obtained from a local store was used as the source of meat for infusion. The meat was trimmed of excess fat and connective tissue and cut into about 8 cc cubes. A 450-g portion of the cubed meat was placed into each of four glass canning jars, an equal weight of one of the infusion solutions was added, and the jars were capped and sealed. The jars were placed in a boiling water bath and heated for 20 min,

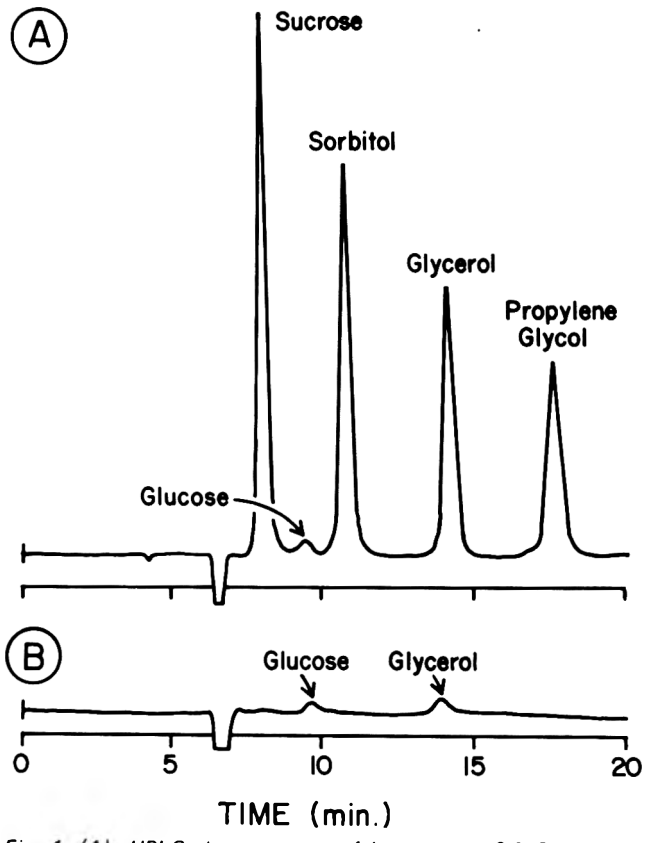


Fig. 1–(A): HPLC chromatogram of humectants; 0.013N H_2SO_4 mobile phase, RI detection at 8X, 0.6 ml/min flow rate. (B): HPLC chromatogram of meat extract from control sample. Conditions same as (A).

removed, and placed at 55°C for 24 hr to allow equilibration of the meat with the infusion solution. The infusion solution was then drained from the meat through a Rapid-Flo milk filter (Johnson and Johnson, New Brunswick, NJ) into a one liter volumetric flask, and the meat and filter were thoroughly rinsed with distilled water. After complete draining, the meat was weighed. Excess solution was squeezed from the filter, and the flask was filled to volume with distilled water.

Sample preparation

The meat was sampled by blending 100g with 300 ml distilled water in a Waring Blendor for 2 min, and diluting to 500 ml. Samples of either the diluted infusion solution or blended meat sample were centrifuged at 10,000 x *g* for 5 min to remove particulate matter. An aliquot of the centrifuged sample was used for ion exchange treatment. The sample size was selected such that it contained 10–100 mg of each humectant. In order to remove interfering ions (NaCl) and proteins, the sample was run through a Dowex 50 H^+ column (20–50 mesh, 1 cm x 8–10 cm) which was connected to an Amberlite IR 45 CP OH^- column (20–50 mesh, 1 cm x 8–10 cm). Distilled water was used to wash the sample from the columns into a 100 ml volumetric flask. After adjusting to volume, an aliquot was filtered through a 0.45 μm Millipore filter (Bedford, MA) prior to analysis by HPLC. After use, the columns were disconnected and regenerated with at least 10 column volumes of 1N HCl for the Dowex 50 and 1N NaOH for the Amberlite IR 45. The columns were washed with distilled water until the excess acid or alkali was removed and reconnected.

Analysis of samples

The samples were analyzed by HPLC using a Waters Associates Model 6000A pumping system, Differential Refractive Index Detecter (model R401) and the Wisp 710B Autosampler (Milford, MA). A Spectraphysics Minigrater (Santa Clara, CA) was used to determine peak area while a Houston Instruments Omniscribe Recorder (Austin, TX) was used to trace detecter response. The column used for separation of the humectants was a BioRad Aminex HPX-87 column (300 x 7.8 mm, Richmond, CA). The eluting solvent was 0.013N H_2SO_4 at a flow rate of 0.6 ml/min. The injection volume was 20–100 μl depending on the humectant concentration. Linear standard curves were obtained with concentrations of 15–125 μg.

RESULTS & DISCUSSION

A TYPICAL TRACING of the HPLC separation of the humectants is shown in Fig. 1A. The order of elution of the humectants is sucrose, sorbitol, glycerol and propylene glycol. The small peak following the sucrose peak is attributed to glucose. One problem encountered with this column is that fructose has a retention time similar to that of sorbitol. Therefore, if there is significant hydrolysis of the sucrose, some interference is observed. However, corrections could be made for sucrose hydrolysis by determining the amount of glucose. Since the glucose to fructose ratio should be one to one, a correction could be made for sorbitol. However, the meat itself may contain some glucose.

Table 1—Percentage recovery of humectants after infusion of meat with solutions 1, 2 and 3

Soln		Sucrose	Sorbitol	Glycerol	Propylene glycol
1	Soln	75.0	74.6	73.7	67.5
	Meat	22.2	24.5	28.1	27.2
	Total	97.2	99.1	101.8	94.7
2	Soln	74.9	78.0	81.1	74.1
	Meat	24.7	26.8	31.0	30.4
	Total	99.6	104.8	112.1	104.5
3	Soln	73.3	79.5	86.2	67.4
	Meat	23.1	24.6	28.6	27.7
	Total	96.4	104.1	114.8	95.1

The recovery of humectants from a standard solution which had been passed through the columns in duplicate runs was: sucrose, 97.3 and 97.3%; sorbitol, 104.4 and 102.2%; glycerol, 101.1 and 100.0%; and propylene glycol, 101.1 and 101.1%. Thus, the reproducibility of the technique is quite good. Again the slight loss of sucrose could be due to hydrolysis, which in turn would result in the slightly increased recovery of sorbitol, due to interference by fructose.

The recovery of the humectants from the infusion solution and infused meats for solutions 1, 2 and 3 are shown in Table 1. The mean recoveries for the three treatments are: sucrose, 97.7 ± 1.6%; sorbitol, 102.6 ± 3.1%; glycerol, 109.5 ± 6.8%; and propylene glycol, 98.1 ± 5.5%. In general the recoveries were adequate, with the exception that the glycerol content was unusually high in two samples. The control meat sample, which had not been infused with any glycerol, showed a glycerol peak (Fig. 1B). Thus, some of the fat may have been hydrolyzed during the heating of the meat, releasing glycerol. This accounted for approximately 8% of the total glycerol recovered in sample 3. Presumably, the other peak is glucose. Approximately 20–30% of the four humectants diffused into the meat, with the lower molecular weight compounds diffusing to a greater extent.

The data presented here show that extraction and HPLC can be used to estimate the content of the humectants sucrose, sorbitol, glycerol and propylene glycol in IM meat samples. Whether additional heat processing and/or smoking would result in production of artifacts that would interfere with the analysis for these compounds remains to be determined.

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A Research Note

Properties of Frankfurters Made From Electrically Stimulated Beef

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ABSTRACT

Frankfurters (3 replications of 4.54 kg meat batches) were conventionally made. Paired sides from beef were used for control (NES) and electrically stimulated (ES) meats (clods, flanks and plates). All-beef frankfurters (100% ES beef or 100% NES beef) and beef-pork frankfurters (80% ES beef-20% pork or 80% NES beef-20% pork) were made. The pork was not electrically stimulated. Proximate composition within cut of beef (plate, flank, clod) was not affected ($P > 0.05$) by ES. However, ES clods had (numerically) greater percentages of expressible juice loss, greater percentages of salt-soluble protein and lower percentages of juice loss during cooking than did NES clods (not statistically different). Use of ES beef alone or in combination with 20% pork did not affect external visual color, off-flavor, overall desirability or processing shrinkage of frankfurters. ES may affect certain properties (raw and cooked muscles) as measured in the laboratory, but when these ES muscles are used to make frankfurters, no real advantages or disadvantages were suggested by this study.

INTRODUCTION

ELECTRICAL STIMULATION (ES) of beef has recently been used to improve tenderness, color and muscle firmness of beef (Savell et al., 1978a, b; Bouton et al., 1980; Houlier et al., 1980). Quality indices of such beef to be used as fresh retail meats are known to vary according to muscle and methods of electrical stimulation (voltage, amperage and number of impulses per unit of time); however, possible effects that such meat may have on cured/cooked sausages have not been identified. Because of the increased rate of pH decline and other biochemical as well as structural changes which occur as a result of ES (Dutson et al., 1980), characteristics associated with utilization of these ES meats in sausages need investigation. The aim of this research was to determine effects of ES on processing properties of raw muscles and to determine effects that ES may have on chemical and sensory properties of frankfurters made from electrically stimulated beef alone and in combination with pork.

MATERIALS & METHODS

TEN PAIRED SIDES (right and left) from cattle varying in sex classification, age and live weight were used. Right sides were electrically stimulated (ES) within 45 min postmortem; left sides were not stimulated and served as controls. Stimulation was accomplished by use of a prototype (LeFiell & Co.) unit (17 impulses at 550v, 5 amps, 1.8 sec impulse duration with 1.8 sec intervals between impulses). Carcasses were placed in a cooler which had a temperature of 5°C at the beginning and −1°C at the end of a 24-hr chilling period. Two days postmortem, wholesale cuts (clod-shoulder arm, flanks and plates) were removed, ground (plate with 12.7 mm holes) and these meats were sub-sampled for proximate composition (AOAC, 1975) and determinations of processing properties (expressible juice loss, Grau and Hamm, 1953; juice loss during cooking, Weirbicki et al., 1957; salt soluble protein, SSP, Saffle and Galbreath, 1964). In addition to these beef cuts, pork picnic shoulders (not stimulated) were boned 24 hr postmortem, ground and subsampled in a manner similar to that used for beef.

Three days postmortem, three replications (4.54kg meat batches) of frankfurters were made (bowl chopper without vacuum) according to the following formulations: 100% ES beef; 100% NES beef; 80% ES beef-20% pork; and 80% NES beef-20% pork. Lean meats, salt, sodium nitrite (156 ppm), sodium erythorbate (550 ppm) and 1/3 of the total water was added and this mixture was chopped for 2 min. Fatter meats, sweeteners (dextrose and corn syrup solids), seasonings and the final 2/3 of the total water was then added and chopping continued for a total of 12 min (batter temperature = 18.3°C). Batters were stuffed into 25 mm clear cellulose casings, linked (14.6 cm in length) and smoked and cooked to 71.1°C (1½ hr, Alkar-DEC 2-truck electric oven). After heat processing, frankfurters were chilled (14–18 hr at 3°C), peeled, randomly sorted and vacuum packaged in moisture-impermeable pouches (6 links/pouch). These packaged products were refrigerated (3°C) for 7 days prior to sensory evaluation.

A 5-member experienced panel evaluated unheated links for external and internal visual color (7 = excellent cured-pink color, 1 = poor cured-pink color). An 8-member experienced panel evaluated reheated links (steeped in boiling water for 7 min) according to the following scales: moistness, 8 = extremely moist, 1 = extremely dry; off-flavor, 8 = extremely weak to no off-flavor, 1 = extremely strong off-flavor: texture, 8 = extremely firm exterior and interior, 1 = extremely soft exterior and interior, and; overall desirability, 8 = like extremely – would repeat purchase, 1 = dislike extremely – would not purchase.

Analysis of variance (Steel and Torrie, 1960) and mean separation (Duncan, 1955) were used to analyze these data.

RESULTS & DISCUSSION

PROXIMATE COMPOSITION and processing properties of flanks, plates and clods from ES and NES beef cuts used in formulations are shown in Table 1. Among these cuts, clods had higher ($P < 0.05$) percentages of moisture and lower ($P < 0.05$) percentages of fat than plates or flanks. However, there were no differences in proximate composition within wholesale cuts due to ES versus NES. Although ES and NES clods did not differ ($P > 0.05$) from each other in expressible juice or juice loss during cooking, ES clods had numerically higher percentages of expressible juice loss and numerically lower percentages of juice loss during cooking than NES clods. Salt-soluble protein percentages were not different ($P > 0.05$) among all wholesale cuts; however, within wholesale, ES numerically increased these percentages when compared to those that were not stimulated (NES).

Data of the present study and those of Terrell et al. (1981a, b) suggest that electrical stimulation of beef carcasses has no statistical effect on either expressible juice loss or juice loss during cooking of clod muscles. Conversely, data of the present study and those of Terrell et al. (1981a, b) have consistently shown a numerical advantage for ES vs not-ES beef, with regard to yields of salt-soluble protein; again, however, differences in SSP percentages between ES and not-ES clods were not statistically significant. ES may alter protein structures in such a manner as to make them more salt soluble but this numerical increase in soluble protein does not necessarily result in less juice loss during cooking of raw muscles or in higher processing

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Table 1—Proximate composition and processing properties of ES and not-ES beef used to make frankfurters[a]

	Boneless wholesale cut					
	Flank		Plate		Clod	
Percentage	ES	NES	ES	NES	ES	NES
Moisture	54.08	53.67	56.88	57.42	68.57[a]	67.40[a]
Fat	29.68[a]	30.27[a]	28.00[a]	25.28[a]	11.47	10.25
Protein	12.04	14.31	13.42	13.49	15.63	15.83
Expressible juice loss[b]	–	–	–	–	65.73	60.45
Juice loss during cooking[c]	–	–	–	–	38.77	39.97
Salt soluble protein[d]	36.30	34.23	35.64	35.36	33.97	32.78

[a] Means within wholesale cuts were not different ($P > 0.05$). Means within a row bearing a superscript letter are different from those without a superscript letter ($P < 0.05$).
[b] Carver Laboratory press. Based on percentages of total moisture in raw meat. Flanks and plates were not analyzed due to high fat content which distorts measurement.
[c] Meat samples placed in centrifuge tubes and heated to 70° C. Based on percentage of total moisture in raw meat.
[d] Based on percentage of total protein (N x 6.25) in raw meat.

Table 2—Mean values for chemical, sensory and processing properties of frankfurters made with ES and not-ES beef

	Frankfurters				
	100% beef		80% beef 20% pork		
Trait	ES	NES	ES	NES	Overall SD
Moisture (%)	50.46[c]	52.88[c]	51.42[c]	51.25[c]	1.89
Fat (%)	30.94[c]	28.03[d]	29.90[cd]	29.60[cd]	0.95
Protein (%)	12.25[c]	12.13[c]	11.64[c]	12.34[c]	1.55
External visual color[a]	5.1[c]	5.2[c]	4.4[c]	4.7[c]	0.78
Internal visual color[a]	5.5[c]	5.4[cd]	4.4[d]	5.0[cd]	0.82
Moistness[b]	6.6[c]	6.1[d]	6.3[cd]	6.2[cd]	0.27
Off-flavor[b]	7.1[c]	7.0[c]	7.0[c]	7.1[c]	0.23
Texture[b]	6.8[c]	6.8[c]	5.8[d]	6.5[c]	0.43
Overall desirability[b]	6.8[c]	6.9[c]	6.4[c]	6.6[c]	0.38
Processing shrinkage (%)	16.1[c]	16.0[c]	15.2[c]	16.5[c]	1.34

[a] Five-member panel: 7 = excellent cured-pink color, 1 = poor cured-pink color.
[b] Eight-member panel: moistness, 8 = extremely moist, 1 = extremely dry; off-flavor, 8 = extremely weak to no off-flavor, 1 = extremely strong off-flavor; textures, 8 = extremely firm exterior and interior, 1 = extremely soft exterior and interior; overall desirability, 8 = like extremely—would repeat purchase, 1 = dislike extremely—would not purchase.
[c,d] Means within a row bearing a common superscript letter are not different ($P > 0.05$).

yields of frankfurters made from such meats. Whiting et al (1981) also reported that ES did not affect press-juice loss, cooking shrinkage, or SSP contents of lamb muscle and that ES had an inconsistent and minimal effect on these functional properties.

Mean values for chemical, sensory and processing shrinkage of frankfurters made with ES or NES beef are shown in Table 2. Percentages of moisture and protein did not differ ($P > 0.05$) among formulations. Franks made with ES beef had higher ($P < 0.05$) fat contents and numerically lower moisture contents than those made with NES beef, when only beef (100% beef frankfurter) was used in the formulation. Sensory properties of external and internal visual color (within formulation) was not affected by use of ES beef, but those frankfurters made with 20% pork had less intense cured-pink color (numerically lower scores) than those made with 100% beef. Frankfurters made from 100% ES beef were more moist ($P < 0.05$) than those made from 100% NES beef, but this difference is probably due to differences in fat content. Although frankfurters made with 80% NES beef-20% pork were firmer in texture than those made with 80% ES beef-20% pork, this difference was not observed for 100% beef franks (ES vs NES). There were no differences within or among formulations for off-flavor, overall desirability or processing shrinkage.

These data (Table 2) suggest that electrical stimulation of beef to be used in 100% beef frankfurters does not affect ($P > 0.05$) processing shrinkage or sensory properties except for moistness (differences in moistness were more likely due to differences in fat content). When ES beef is combined with 20% pork (not stimulated) in frankfurters, texture was decreased ($P < 0.05$) but no other sensory properties or percentages of processing shrinkage were affected ($P > 0.05$). These results are in agreement with those of Whiting et al. (1981) who reported a lack of differences in sensory properties and processing shrinkage for frankfurters made from ES lamb and pork backfat. Hence, there appears to be no real advantages or disadvantages for using ES beef in the manufacture of frankfurters.

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