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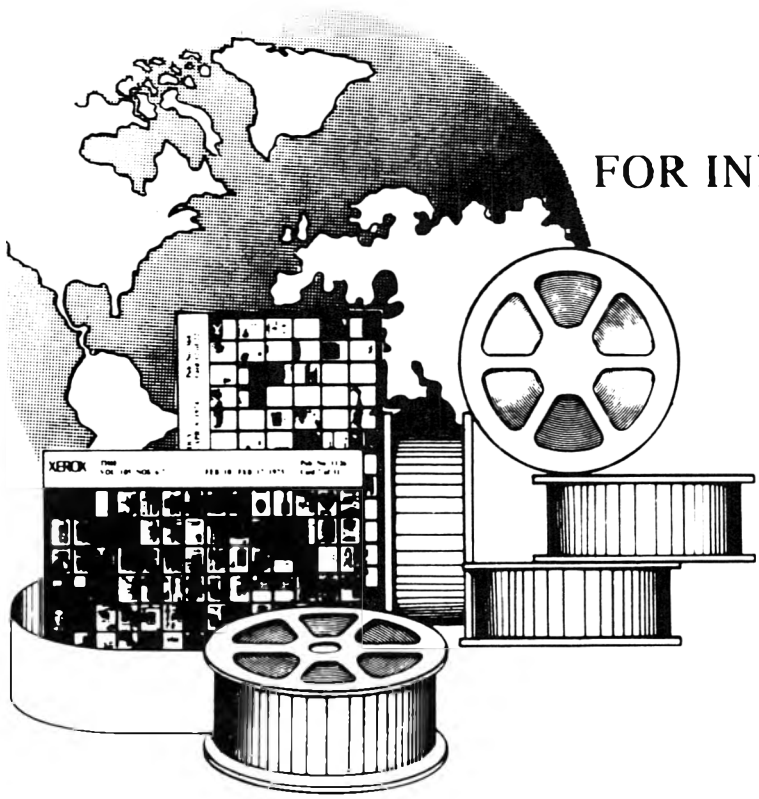

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Memo *FROM THE SCIENTIFIC EDITOR*

During 1980 the number of manuscripts submitted increased to about 750. This is a significant increase over 1979. Six issues of the Journal contained 457 manuscripts in 1848 pages. There was a reduction in the number of pages in the first two issues of the Journal, because of typesetting problems. However, by increasing the size of later issues, by year end we were only 8 pages behind the 1979 total.

Reviewers are performing their task well and in most cases with good speed. There are some members who feel review of sensory evaluation manuscripts is not adequate. This is a rapidly developing area with many views existing on how sensory research should be done and reported. I hope the Sensory Division will get its act together and help the new editor in improving this part of the Journal.

Yes, I said new editor. I have been editor since January 1971 and feel it is time for a change by January 1981. The Journal has about doubled in size over the ten years. Manuscripts submitted have increased from about 300 to 750 per year. Over the ten years this translates into about 6000 manuscripts received and processed. I have enjoyed contact with the many researchers around the world and hope my efforts have made the Journal a better source of information on our food supply.

Again we need to recognize the excellent support for the Journal from Anna May Schenck, John Klis, Barney Schukraft, and Gladys Anderson. They really are responsible for the final form and rapid publication schedule for the Journal. Any editor would be helpless without their great support.

The Editorial Board has had a busy year with the increase in the number of manuscripts. I wish to express my thanks to the retiring Board members and welcome the new Board members starting three-year terms.

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Letters

EFFECTIVE TEMPERATURE IS INDEPENDENT OF REACTION ORDER IN A THERMALLY FLUCTUATING SYSTEM

The extension by Labuza (1979) to reaction product yield of the theories of Hicks (1944) and ourselves (Schwimmer et al., 1955) for prediction of effective rates and temperatures in reaction systems undergoing temperature fluctuations, constitutes a valuable contribution to rationalization of storage practices and prediction of food quality during storage. We do wish, however, to clarify what appears to be a semantically associated misapprehension concerning the role of order of reaction in such systems. Firstly, Labuza ascribed to our treatment an implied zero order reaction. Secondly, he attributes to us an "incorrect" statement that the solution of the relevant equations is independent of the order of the reaction.

The facts are that neither Hicks nor we assumed or implied any order of reaction. We stated in our paper that the parameter α—defined by $\alpha = (T_e - T_o)/a$ where T_e is the effective temperature, T_o is the average temperature, and a is the temperature fluctuation—is independent of the order of the reaction and incidently also of the temperature scale. Therefore, the effective temperature is independent of the order of the reaction. This fact is demonstrated by the following arguments.

If we assume that the rate constant of a reaction increases by a factor Q_a for a temperature change of a degrees then we can write the rate constant, k_i at temperature, T_i, in terms of the rate constant, k_o, at temperature, T_o.

$$k_i = k_o\, Q_a^{(T_i - T_o)/a} \tag{1}$$

We can also write the average or effective rate $\bar{R}$ as a sum of n components of rates, R_i, each weighted by the factor W_i.

$$\bar{R} = \frac{\sum_{i=1}^{n} W_i R_i}{\sum_{i=1}^{n} W_i} \tag{2}$$

In general this expression will be an integral over the temperature fluctuation. The rates, $\bar{R}$ and the R_i, are functions of both the rate constant, k_i, and the concentrations of reactants, C_j. The effective rate constant is designated by $\bar{k}$.

$$\bar{R}(\bar{k}, C_j) = \frac{\sum_{i=1}^{n} W_i R_i(k_i, C_j)}{\sum_{i=1}^{n} W_i} \tag{3}$$

Eq. (3) reduces exactly to Eq (4) because each term has the same dependence on the C_j's.

$$\bar{k} = \frac{\sum_{i=1}^{n} W_i k_i}{\sum_{i=1}^{n} W_i} \tag{4}$$

This reduction to Eq (4) makes the assumption that the number of fluctuations for the time course of the reaction is large. Thus concentration changes can be neglected over one cycle of the fluctuation.

Combining Eq (1) and (4) and defining the effective temperature, T_e as the temperature that produces the rate $\bar{R}$, one obtains Eq (5),

$$Q_a^{(T_e - T_o)/a} = \frac{\sum_{i=1}^{n} W_i\, Q_a^{(T_i - T_o)/a}}{\sum_{i=1}^{n} W_i} \tag{5}$$

which reduces to:

$$Q_a^{T_e} = \frac{\sum_{i=1}^{n} W_i\, Q_a^{T_i}}{\sum_{i=1}^{n} W_i} \tag{6}$$

The solution of Eq (6) for T_e depends on the relationship between W_i and T_i and in general requires integration, but does not depend on the order of the reaction. This statement is true for any type of waveform.

Thus the effective initial rate or the rate at any one time is independent of the order of reaction as stated in our paper. However, it is clear that the decrease in rate with time will obviously depend upon the order of the reaction. It is also clear that after the effective temperature is calculated, the amount of products depend on the order of the reaction as Labuza found in his calculation.

However, Labuza's analysis led to the conclusion that for most food deteriorative situations the effective temperature is, in effect, independent of reaction order. Indeed this conclusion has been fairly well confirmed experimentally by the application of our theory to results obtained in the extensive time-temperature-tolerance study of frozen foods conducted at USDA's Western Regional Research Laboratory, the results of which were published in at least 24 papers between 1957 and 1962 (Van Arsdel, 1957; Dietrich et al., 1962). Where tested and with few exceptions, the study showed remarkable and consistent correspondence between effective temperature predicted on the basis of theory from observed changes (considered as effective initial rates) occurring at nadir and zenith of various sinusoidal fluctuations for a variety of foods and measurements, and the observed effective temperature calculated from experimental data obtained during the temperature fluctuation. This correspondence, summarized in part by Schwimmer (1980), held for foods and measurements diverse as losses of chlorophyll from green vegetables, ascorbic acid and anthocyanin-associated color from fruits, and even water from frozen chickens.

Not only could our theory be used to predict effective temperatures, but other predictions of our theory survived confrontation with experimental data, i.e., that effective temperature increases with increasing amplitude (illustrating Labuza's conclusion that temperature sensitivity is critical) but is independent of oscillation frequency. These predictions held for ascorbic acid loss in frozen strawberries

(Guadagni and Nimmo, 1958).

Labuza (1979) states that there is "no magic in fluctuating temperature systems" and that we do not wish to introduce a "new art into food science." While we can generally agree with these statements, we wish to point out again (Schwimmer et al., 1955) that if a food product is undergoing two different reactions with different temperature coefficients, the effective temperatures for these reactions will be different and product ratios will be formed under fluctuating temperature conditions that are not possible under any one constant temperature.

This latter circumstance, along with the meaningful, significant work of Powers and colleagues (Wu et al., 1974, 1975a, 1975b) on the response in model systems of enzyme catalyzed reactions undergoing temperature cycles, suggests that the latter is a source of diversity of enzyme generated food constituents in addition to those categorized previously (Schwimmer, 1978, 1980). These and further consequences of temperature fluctuations in frozen food systems, elaborated in further detail in the later publication, strongly indicate that further study of food systems stored under fluctuating temperature conditions will reveal new aspects and is thus important to our understanding of food storage problems.

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–*Lloyd L. Ingraham*, Dept. of Biochemistry & Biophysics, Univ. of California, Davis, CA 95616.

I am not sure what Dr. Schwimmer means by a misapprehension concerning the role of order of reaction in food systems undergoing sequential temperature fluctuations. I agree that using the derived equations, and as shown in my calculations, the effective temperature T_e is independent of order and dependent only on the amplitude of the fluctuation, the mean temperature, and the activation energy if one uses the proper equations. This is not inherent in reading either the original Hicks (1944), or Schwimmer et al. (1955) article. In the Hick's article the only mention of rate says that the rate is:

$$R = R_o e^{b(\theta - \theta_o)}$$

where rate is reaction at temperature θ; R_o = rate at temperature θ_o; and b = a constant.

This would be true for a zero order reaction at any concentration but would not be correct for a first order reaction unless it is stated that the concentration at θ and θ_o for R and R_o are the same. It also does not indicate that the rate changes with time. Thus, I incorrectly gave credit for stating the solution was independent of order.

In solving for what I call the Γ function, Hicks says:

$$\Gamma = \frac{\bar{R}}{R_o}$$

where $\bar{R}$ is the effective mean rate for the fluctuation. If one measured concentration with time, the value of Γ would change depending on which order was used since (by using the integrated value for the rate constant) at a given time of reaction:

Zero	First
$\Gamma_o = \frac{A_o - A_f}{A_o - A_c}$	$\Gamma_1 = \frac{\ln A_f/A_o}{\ln A_c/A_o}$

If there were 50% loss under constant conditions and 75% loss under fluctuating conditions at a given time then:

$$\Gamma_o = \frac{75}{50} = 1.5 \qquad \Gamma_1 = \frac{\ln 0.25}{\ln 0.5} = 2.0$$

Thus, the square wave would give a calculated lower effective mean temperature if it is calculated based on the amount of loss which is the way most people would do an experiment. Of course, in the Hick's paper for a sine wave, the calculation is made with the equation below which does not use the concentration terms or order so that the right hand side of the equation is independent of order.

$$\Gamma = \frac{\bar{R}}{R_o} = 1 + \frac{(ab)^2}{2^2} + \frac{(ab)^4}{2^2\,4^2} + \frac{(ab)^6}{2^2\,4^2\,6^2} + \ldots$$

The problem is that the left hand side changes in calculating the amount lost in a fluctuating situation vs a constant temperature. The order must be specified as indicated above if amount of loss is to be evaluated. The same situation exists in the Schwimmer et al., (1955) paper in which they state that the equation is independent of order, which I said was incorrect. Similar Γ functions were developed for saw tooth and square waves. If T_e is calculated, then the order does not have to be specified as noted in the letter.

One of the basic points of my paper was to point out that if one is to predict the amount lost, then the order must be specified to calculate the left hand side. This was the major error in the series of papers from the Power's group. They discussed growth rate in hours, yet cells grow by a logarithmic order (first order) and the rate is:

$$R = \frac{dN}{dt} = k_1 N$$

N = # of cells at time θ

They showed poor comparison of actual growth rates to predicted values because of using a zero order rather than a first order. The same was true for the loss of ascorbic acid, in which they discounted the difference because of experimental error as discussed in my paper.

As Dr. Schwimmer points out, they published extensive data on use of the equations for frozen foods and found good correlation. This is fortuitous since all my analyses show that frozen foods degrade by a zero order, so use of constant rates works out well. It should be noted that in a series of papers we presented this year at the 40th Annual Meeting of the Institute of Food Technologists, we have tested the equation for first order reactions in terms of prediction of times to a given amount of loss (thiamin in pasta, lysine in whey powder) and have found excellent correlation to actual times.

As to Dr. Schwimmer's last statement about systems with two different reactions, I agree with him. The equations do not hold. That is why I have always taught in my classes and short course that a single temperature or a fluctuating cycle is not the proper way to do accelerated shelf life testing.

–*T.P. Labuza*, Dept. of Food Science & Nutrition, Univ. of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108.

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CHEMICAL AND PHYSICAL CHARACTERISTICS OF FRANKFURTERS PREPARED WITH MECHANICALLY PROCESSED PORK PRODUCT

K. W. McMILLIN, J. G. SEBRANEK, R. E. RUST, and D. G. TOPEL

ABSTRACT

Frankfurters were prepared with frozen beef trim, pork fat, and 0, 10, 20, 30, 40, and 50% mechanically processed pork product (MPPP) produced by a hydraulic press deboner. Beef trim, salt, and ice were chopped to 8°C in a laboratory chopper, the appropriate amount of MPPP and pork fat added to maintain 25% fat, and the mixture was chopped to a final temperature of 13.5°C. Emulsion stability tests indicated that formulations with 30% or less MPPP were similar in smokehouse yield and percentage of water and fat lost during processing. Water-holding capacity increased with increasing levels of MPPP, but frankfurters containing greater than 30% MPPP were softer in texture as determined by Warner-Bratzler shear force values.

INTRODUCTION

MORE THAN 1.9 billion pounds of frankfurters are produced each year in the U.S. (National Hot Dog and Sausage Council, 1979). As the demand for meat trimmings increases, alternative sources of materials for processed meat become more important (Brown, 1975). The possible use of mechanically processed (red meat species) product has been reported in cooked salami (Joseph et al., 1978), dry fermented salami (Berry et al., 1979), ground beef (Cross et al., 1977, 1978; Seideman et al., 1977; Emswiler et al., 1978), and bologna (Field, 1974; Chant et al., 1977; Misock et al., 1979). These studies have indicated that low levels of usage are acceptable in comminuted products.

The USDA has published a final ruling on mechanically processed (species) product, setting production requirements, labeling rules, compositional guidelines, and permitted uses, including usage in frankfurters (Federal Register, 1978). Where mechanically processed pork product has been used in frankfurter production (Meiburg et al., 1976; Marshall et al., 1977), research has centered on storage stability, consumer acceptability, flavor, and subjective processing traits. The present study determined the processing characteristics, emulsion stability and chemical composition of frankfurters which contained progressively increasing levels of mechanically processed pork product (MPPP).

EXPERIMENTAL

FRANKFURTERS were manufactured using frozen beef trim, pork backfat, and mechanically processed pork product (MPPP). MPPP was produced by prebreaking young, commercially trimmed pork backbones in a Weiler grinder (2.54 cm plate), then passing the ground bone through a Kartridg-Pak Meat Removal System, a hydraulic press deboner. The Meat Removal System was operated at a pressure of 27600 kilopascals/cm², dwell time of 5 sec for pressure application, and 0.23 mm opening between the concentric separation rings. After the deboning process, the MPPP was quickly frozen in a CO_2 cryogenic tunnel and stored at −15°C for 2 wk. In formulating the frankfurters (Table 1), thawed (0°C) beef trim was ground through a 0.95 cm plate, weighed and then analyzed for fat with an Anyl-Ray (Kartridg-Pak, Inc.) machine. The proper amount of beef trim determined to contribute 21% fat to the final formulation, 3% salt, and 10% added ice were chopped to 8°C in a 45 cm diameter Hobart laboratory silent cutter. Thawed MPPP (1°C) was added at 0, 10, 20, 30, 40, or 50% levels and pork backfat was used to adjust the 2.5 kg batches to a constant formulation of 25% fat. Chopping was continued to a temperature of 13.5°C. After hand stuffing and linking into 26 mm cellulose casings, the raw meat emulsions were weighed and then processed in a conventional smokehouse to 70°C internal temperature according to the following schedule: 15 min at 40% RH and 54°C, 30 min at 63% RH and 70°C, 10 min at 78% RH and 80°C, and 5 min at 80°C and 100% RH followed by a 3 min cold shower. Frankfurters were chilled to 4°C, weighed, and peeled before analysis of the finished product.

Table 1—Formulations of frankfurters

Ingredient	Level of MPPP (%)					
	0	10	20	30	40	50
Beef trim (kg)	2.28	2.04	1.81	1.58	1.34	1.11
Pork backfat (kg)	0.22	0.21	0.19	0.17	0.16	0.14
MPPP[a] (kg)	0	0.25	0.50	0.75	1.00	1.25
Other ingredients[b] (kg)	0.46	0.46	0.46	0.46	0.46	0.46

[a] MPPP composit on was 56.7% moisture, 26.3% fat, 14.1% protein, 3% ash, 0.56% calcium, and 3% crude bone.

[b] Ice 375g, salt 75g, Heller Premier Weiner Seasoning 12.5g, sodium erythorbate 0.6834g, and sodium nitrite 0.1984g for each kg batch.

Smokehouse yields were calculated by weighing the stuffed casings before smokehouse processing and after chilling. Moisture, crude fat, Kjeldahl protein, and ash were determined on raw emulsions and finished frankfurters by AOAC procedures (AOAC, 1975). Crude bone content was measured by the papain digestion and acetone: carbon tetrachloride procedure of Hill and Hites (1968). Water-holding capacity determination was made by modifying the procedure of Wierbicki and Deatherage (1958) as follows: samples (0.3g) of raw emulsions and frankfurters were placed on dried, #1 Whatman filter discs and pressed for 3 min in a Carver press at 20690 kilopascals/cm². Meat and juice areas were measured using both a plastic grid calibrated for 20 dots/6.45 cm² and a compensating polar planimeter. Water-holding capacity was expressed as the ratio of juice area to meat area. Expressible juice was calculated by the formula reported by Briskey et al. (1960). Values for the mg of water/square cm of juice area, 5.72 for raw emulsions and 5.55 for frankfurters, used in the formula were determined by the method of Connell (1955). A modified Rongey (1965) stability test was employed to estimate the amounts of fat and water lost during processing. Raw emulsion samples were collected immediately after chopping and placed into weighed Wierbicki tubes. After heating for 30 min at 70°C in an open water bath, tubes were cooled 10 min and centrifuged at 250 × G for 20 min. Volumes of fat and water lost were expressed as a percentage of sample weight. Warner-Bratzler shear force values were obtained on chilled frankfurters after measuring the diameter with a vernier caliper.

Statistical analysis was performed using the SAS analysis of vari-

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ance procedure (Barr et al., 1976), Duncan's multiple range tests (Duncan, 1955), and t-tests (Steel and Torrie, 1960). Three replications of each of the six formulations (0, 10, 20, 30, 40, and 50% MPPP) were processed in a completely randomized fashion.

RESULTS & DISCUSSION

THE PROXIMATE ANALYSES for raw emulsions are listed in Table 2. Fat and protein composition was held fairly constant for all raw formulations by adjusting the pork backfat ingredient levels. Moisture level, ash content, and bone content increased as level of MPPP used in the formulations increased. The increase in ash and bone contents would be expected, as minerals and bone particles are forced through the deboner along with the muscle tissue (Field et al., 1975). The finished frankfurter composition (Table 3) showed no difference in protein content among treatments, but frankfurters with 40 and 50% levels of MPPP contained less moisture and more fat than in frankfurters with the other levels of MPPP. Ash and bone content increased as level of MPPP increased in the frankfurters since MPPP contains a greater ash and bone content than muscle tissue. As seen in Tables 2 and 3, moisture:protein ratios of 40 and 50% MPPP formulations were higher than other treatments in raw emulsions but the ratios were lower in the finished frankfurters.

Raw emulsions which contained 20% MPPP resulted in the greatest smokehouse yield of frankfurters (Fig. 1). The vertical lines at each level of MPPP in Figure 1 represent the wide ranges of yields over the three replications of each MPPP level in the formulations. The yield of frankfurters with 0 or 20% MPPP was significantly ($P < 0.05$) higher than yields of frankfurters with 10, 40, or 50% levels of MPPP (Table 4). Smokehouse yield was significantly correlated ($P < 0.05$) with the amount of fat present in finished frankfurters (Table 5). Simple correlation coefficients between smokehouse yield and water and fat loss in the emulsion stability test were −0.34 and −0.53, respectively, indicating the emulsion stability test explains some but not all of the variation in yield. No large differences in water, fat, or total fluid loss were observed among levels of MPPP to 30% (Table 4; Fig. 2), but a significantly greater ($P < 0.05$) increase in weight loss occurred for the 40 and 50% levels of MPPP. The amount of water lost was highly correlated ($r = 0.70$) with the amount of fat lost in the stability test. Morrison et al. (1971) and Brown and Toledo (1975) found that the amount of water bound to the interface of the fat

Table 2—Proximate analysis values for raw emulsions

	Level of MPPP						
Component[a] (%)	0	10	20	30	40	50	S.E.M.[b]
Moisture	60.51a	60.65a	60.91ab	61.21ab	61.71b	61.85b	0.08
Fat	21.99a	21.76a	21.88a	21.96a	21.76a	20.97b	0.17
Protein	14.13a	13.19bc	13.32bc	13.53b	13.01c	13.14c	0.12
Ash	3.08a	3.12a	3.27bc	3.21ab	3.37cd	3.42d	0.02
Bone content	0.04a	0.11ab	0.18bc	0.25d	0.22cd	0.35e	0.01
Moisture:protein ratio	4.28a	4.60bc	4.57bc	4.53b	4.77d	4.71cd	0.04

[a] Means for each component with the same lower case letter are not significantly different ($P < 0.05$).
[b] Average standard error of mean for all treatments

Table 3—Composition of finished frankfurters

	Level of MPPP						
Component[a] (%)	0	10	20	30	40	50	S.E.M.[b]
Moisture	54.58a	54.69a	54.31a	54.91a	52.98b	53.18b	0.24
Fat	24.89ab	24.91ab	24.27a	24.81ab	26.60c	25.83bc	0.40
Protein	16.44a	16.38a	16.45a	16.35a	16.38a	16.25a	0.11
Ash	3.49a	3.58a	3.74b	3.81b	3.99c	3.84b	0.05
Bone content	0.09a	0.08a	0.18ab	0.22b	0.41c	0.46c	0.03
Moisture:protein ratio	3.32ab	3.34ab	3.30abc	3.37a	3.23c	3.28bc	0.03

[a] Means for each component with the same lower case letter are not significantly different ($P < 0.05$).
[b] Average standard error of mean for all treatments

Table 4—Mean values[a] and standard errors of means[b] for physical characteristics of frankfurters

	Level of MPPP						
Characteristic	0	10	20	30	40	50	S.E.M.[b]
Smokehouse yield (%)	90.69ab	85.73c	92.45a	88.00bc	85.47c	81.73d	1.22
Emulsion stability							
Water released (%)	5.14a	5.11a	4.61a	5.47a	7.96b	9.98b	0.84
Fat released (%)	1.02ab	1.12ab	0.73a	1.45b	2.12c	3.03d	0.21
Total released (%)	6.15a	6.23a	5.34a	6.92a	10.08b	13.01c	0.84
Warner-Bratzler shear force (kg)	2.79a	2.75a	2.16b	1.44c	1.08d	0.99d	0.04
Frankfurter diameter (mm)	22.80a	22.78a	22.42a	21.10b	21.45b	20.92b	0.22
W-B shear/diameter (kg/mm)	0.122a	0.121a	0.096b	0.067c	0.050d	0.047d	0.002

[a] Means for the same characteristic with the same lower case letter are not significantly different ($P < 0.05$).
[b] Average standard error of mean for all treatments

Table 5—Simple correlation coefficients for processing characteristics

	1	2	3	4	5	6	7	8
1 Smokehouse yield	1.00	−0.34*	−0.53**	0.35*	0.28	0.36*	−0.20	−0.40*
2 Emulsion stability water lost		1.00	0.70**	−0.28	−0.50**	−0.60**	0.20	0.42*
3 Emulsion stability fat lost			1.00	−0.48**	−0.59**	−0.68**	0.28	0.42*
4 Raw emulsion water-holding capacity (grid)				1.00	0.55**	0.57**	−0.28	−0.43**
5 Frankfurter diameter					1.00	0.77**	−0.26	−0.48**
6 Frankfurter Warner-Bratzler shear force						1.00	−0.42**	−0.47**
7 Raw emulsion moisture: protein ratio							1.00	0.43**
8 Frankfurter fat composition								1.00

** $P < 0.01$
* $P < 0.05$

and protein film influenced emulsion stability. In the present study, raw emulsions with lower moisture:protein ratios produced more stable frankfurters than raw formulations (40 and 50% MPPP) with higher moisture:protein ratios, but low correlation coefficients of −0.20, 0.20, and 0.28 were obtained between raw emulsion moisture:protein ratios and yield, water lost, and fat lost, respectively (Table 5). Marshall et al. (1977) previously reported that frankfurters containing MPPP had more "fatting-out" problems than control frankfurters and use of 25 and 40% MPPP levels resulted in greater smokehouse shrinkage percentages than control or 10% level MPPP frankfurters.

Water-holding capacity (WHC) and expressible juice percentage for raw emulsions and frankfurters are shown in Figure 3. Values for WHC closer to 1 indicate a greater ability to retain or hold water. Lower values for expressible

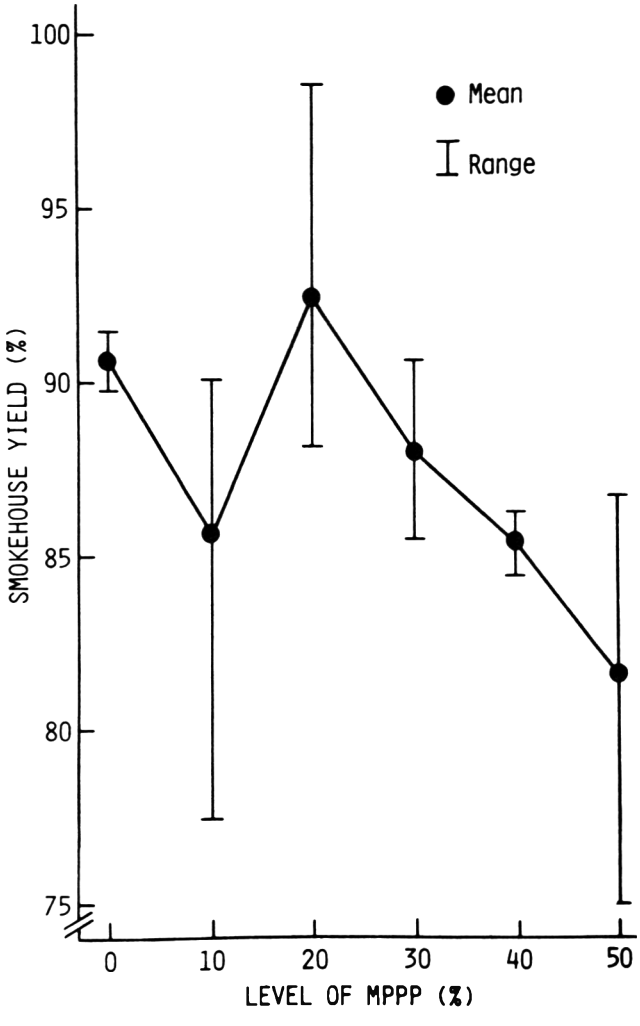

Fig. 1—Smokehouse yield means and ranges for increased levels of MPPP.

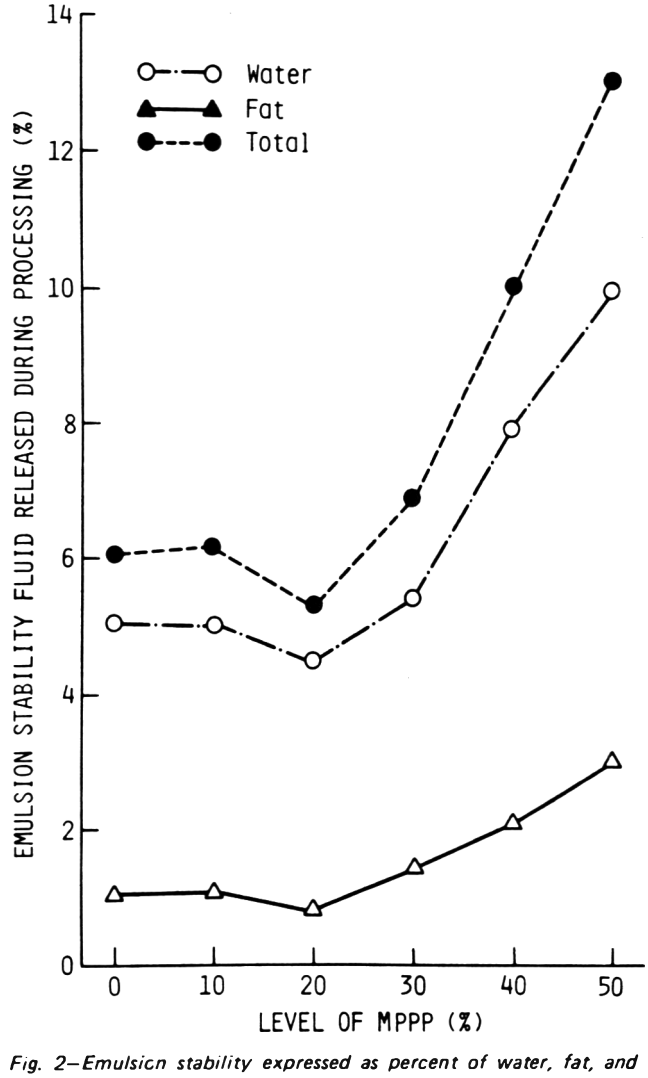

Fig. 2—Emulsion stability expressed as percent of water, fat, and total fluid released with increased levels of MPPP.

juice also indicate increased water retention by the samples. At each level of MPPP, raw emulsions held water more tightly than frankfurters of the same formulation. Hamm and Deatherage (1960) reported less water bound with meat heated to temperatures greater than 40°C than meat which remained at a lower temperature. In the present study, internal temperature of the frankfurters reached 70°C during the smokehouse heating. The WHC increased as the level of MPPP increased for both raw emulsions and frankfurters. The ability of the 0, 10, and 20% MPPP formulations to hold water during the application of pressure was significantly less ($P < 0.05$) than for the 30, 40, and 50% formulations in both raw emulsions and frankfurters (Table 6). Expressible juice measurement also showed the decreased ability of the control, 10%, and 20% MPPP formulations to hold water (Fig. 3). The great difference in WHC or juice retained may be explained by comparing the MPPP structure to meat which has been ground. Hamm (1959) stated that grinding of meat increases hydration because more polar groups of proteins become available for the binding of water dipole molecules. Most MPPP is produced in the form of a paste (Froning, 1970) since the sieving device must have an opening less than 0.5 mm (Federal Register, 1978). The increased size reduction of MPPP muscle tissue which occurs during deboning compared to grinding of the muscle tissue may cause more protein side groups to be available for water binding. Formulations with increased amounts of MPPP would then exhibit greater WHC, as was observed in this study. The raw emulsion WHC was correlated with smokehouse yield ($r = 0.35$) and the amount of fat lost in the stability test ($r = -0.48$), but WHC was not highly correlated with the amount of water lost in the stability test or the moisture: protein ratios of raw emulsions (Table 5).

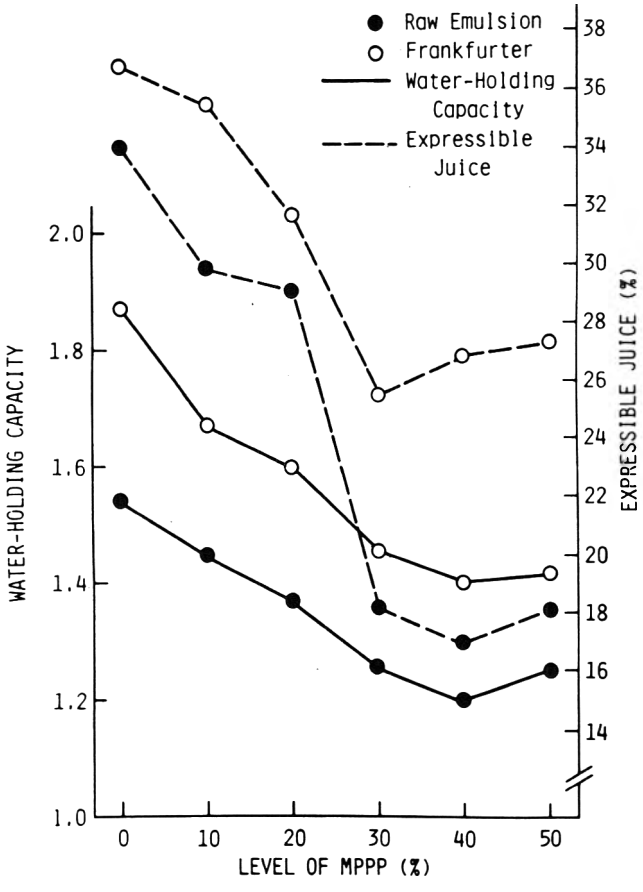

Fig. 3—Water-holding capacity and percent expressible juice by grid area measurement for raw emulsions and finished frankfurters.

The meat and water areas used for WHC and expressible juice calculations were measured with a plastic grid and compensating polar planimeter. No difference between the two methods of area measurement was observed for WHC or expressible juice determinations on raw emulsions or finished frankfurters (Table 6) when the measurements were compared at each formulation level of MPPP. Simple correlation coefficients between raw WHC and raw expressible juice measurements were equal to or greater than $r = 0.88$,

Table 6—Mean values[a] and standard errors of means[b] for planimeter and grid area measurements of water-holding capacity and expressible juice

	Level of MPPP					
	0	10	20	30	40	50
Water-holding capacity						
Raw emulsion						
Planimeter	1.57a	1.43b	1.36bc	1.22d	1.21d	1.21d
	(0.09)	(0.09)	(0.05)	(0.04)	(0.02)	(0.02)
Grid	1.54a	1.45b	1.37c	1.26d	1.20d	1.25d
	(0.08)	(0.10)	(0.04)	(0.07)	(0.02)	(0.03)
Frankfurter						
Planimeter	1.91a	1.71b	1.65bc	1.44d	1.43de	1.36e
	(0.06)	(0.06)	(0.05)	(0.07)	(0.02)	(0.04)
Grid	1.87a	1.67b	1.60c	1.46d	1.40e	1.42de
	(0.05)	(0.04)	(0.04)	(0.08)	(0.02)	(0.04)
Expressible juice						
Raw emulsion						
Planimeter	33.16a	27.16b	27.41b	15.82c	16.71c	14.78c
	(3.75)	(5.52)	(4.53)	(2.89)	(1.51)	(1.97)
Grid	34.00a	29.84b	29.23b	18.13c	16.95c	18.13c
	(3.71)	(5.74)	(3.97)	(5.16)	(1.94)	(1.97)
Frankfurter						
Planimeter	37.35a	33.21ab	32.60b	24.89c	27.00c	23.03c
	(1.99)	(2.70)	(1.34)	(2.05)	(1.38)	(1.77)
Grid	36.77a	35.43a	31.67b	25.42c	26.89c	27.34c
	(2.85)	(2.76)	(0.91)	(3.92)	(1.20)	(2.67)

[a] Means for the same measurement with the same lower case letter are not significantly different ($P < 0.05$). Levels of MPPP were compared by Duncan's multiple range tests and the two area measurements by t-tests.

[b] Standard error of each mean is in parentheses below the mean

Table 7—Simple correlation coefficients for water-holding capacity and expressible juice percentage on raw and finished frankfurters using grid and planimeter measurements of area

	1	2	3	4	5	6	7	8
1 Raw water-holding capacity grid	1.00	0.94**	0.56**	0.60**	0.95**	0.89**	0.31	0.49**
2 Raw water-holding capacity planimeter		1.00	0.63**	0.71**	0.88**	0.94**	0.42*	0.63**
3 Finished water-holding capacity grid			1.00	0.85**	0.51**	0.57**	0.87**	0.76**
4 Finished water-holding capacity planimeter				1.00	0.57**	0.66**	0.60**	0.93**
5 Raw expressible juice grid					1.00	0.93**	0.29	0.48**
6 Raw expressible juice planimeter						1.00	0.38*	0.60**
7 Finished expressible juice grid							1.00	0.61**
8 Finished expressible juice planimeter								1.00

** $P < 0.01$
* $P < 0.05$

and correlations were equal to or greater than r = 0.60 between frankfurter WHC and expressible juice determinations (Table 7). These simple correlation coefficients suggest the measurement of WHC is comparable to the expressible juice method of measuring the ability of meat to bind or hold water. WHC was shown earlier in this study to influence the yield and fat loss during processing. The simple method of measuring WHC with a plastic grid would enable meat processors to estimate the ability of raw emulsions to bind water more quickly than the measurement of expressible juice.

A significant ($P < 0.05$) decrease in frankfurter diameter was observed in frankfurters with higher levels of MPPP (Table 4). The 30, 40, and 50% levels of MPPP produced frankfurters with smaller diameters, reflecting the greater losses of fat and water during processing. Warner-Bratzler shear force values also decreased with increasing levels of MPPP in frankfurters. Marshall et al. (1977) found that increased levels of MPPP resulted in frankfurters very susceptible to deformation and less desirable in texture. In the present study, the decrease in Warner-Bratzler shear force indicates a softer frankfurter texture. Expressing shear force values as a measurement of fixed shear area did not alter the differences seen for Warner-Bratzler shear values in Table 4. The diameter and shear force were highly correlated with the raw emulsion moisture:protein ratio, amounts of fat and water lost during processing, and raw emulsion WHC (Table 5).

These results indicate that stable, satisfactory emulsions may be produced utilizing MPPP at levels of 30% or less. Increased fat and water loss during processing of 40 and 50% MPPP formulations resulted in lower yields, smaller frankfurter diameters, and less force required to shear frankfurters. Water-holding capacity may be evaluated by different methods, but formulations with increased levels of MPPP exhibited greater abilities to bind water in raw emulsions and frankfurters. The use of MPPP appears to be acceptable as an alternative source of meat protein in frankfurters, but differences in the water-binding of different formulations deserve further study.

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—Continued on page 1462

INFLUENCE OF MICROWAVE REHEATING ON SELECTED QUALITY FACTORS OF ROAST BEEF

MARY BETH JOHNSTON and RUTH E. BALDWIN

ABSTRACT

Roast beef slices (longissimus muscle), when reheated by microwaves after 2 days storage (4°C), compared favorably with conventionally reheated samples. Sensory evaluation indicated significantly less intense "warmed-over" aroma in microwave reheated than in conventionally reheated samples. No differences in "warmed-over" flavor of the slices or in lipid oxidation (TBA numbers) were evident in the meat reheated by the two methods. No significant losses of thiamin or riboflavin resulted from either microwave or conventional reheating.

INTRODUCTION

CURRENT TRENDS are for greater utilization of precooked meat products rather than traditional products prepared immediately before consumption (Sink, 1979; Herz and Chang, 1970). The use of precooked meats in both institutional and home meal preparation can be a means of reducing waste, saving time, energy, and labor as well as providing an element of convenience. It generally is conceded, however, that the reheating of precooked meats results in undesirable changes in flavor, primarily due to oxidative reactions, and in increased destruction of heat labile vitamins.

Copson (1975) indicated that the predominant quality factor at issue in precooked foods is the freshness of flavor which probably is most difficult to maintain in meats due to the unsaturated fatty acid content. Chang et al. (1961) noted that precooked slices of beef develop rancid off-odors and correspondingly high TBA numbers upon exposure to air and room or refrigerator temperatures. However, Copson (1975) proposed that the usual flavors produced in precooked meats are absent when re-thermalization is by the microwave method. Additionally, reheating by microwaves is fast and permits substantial energy savings (Copson, 1975; Smith, 1977).

The increasing popularity of microwave heating is evidenced by the appearance of directions for microwave heating on the package of a variety of items including prepared refrigerated and frozen foods. Also, food products are being developed specifically for the microwave oven market (Decareau, 1979).

There is only limited evidence supporting Copson's (1975) proposal that the flavor of microwave reheated meat is superior to that of conventionally reheated meat. Also, data on vitamin retention in meats heated by microwaves is not conclusive. Therefore, this study was conducted to compare the aroma, flavor, lipid oxidation, and retention of thiamin and riboflavin of precooked beef reheated by microwave and conventional methods.

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METHODS & MATERIALS

Procurement and preparation of meat

Trimmed beef rib eye (longissimus muscle) roasts from eight animals (eight replications) were obtained from the Univ. of Missouri meat laboratory. The roasts were equivalent to USDA Good grade and averaged 3.1 kg in weight (range 2.5–3.7 kg). All roasts were frozen (−20 ± 5°C), held for 2 days, then transferred to a freezer (−17°C) for 3–15 days. Forty-eight hours prior to cooking, the roasts were allowed to thaw at refrigerator temperature (4°C).

The roasts were cooked on a rack in an open pan (40 × 26 × 6.5 cm) in preheated (162 ± 2°C) electric ovens. The average time required for roasts to attain a final internal temperature of 70 ± 2°C was 62 min per kg. Roasts were wrapped in aluminum foil until they were cut (approx 8 min) into slices of 0.6 cm thickness. Groups of slices were randomly assigned to moisture, thiobarbituric (TBA) and vitamin determinations, to sensory analyses or to storage (2 days, 4°C) prior to conventional or microwave reheating. Eight slices were designated for each reheating treatment. The slices were placed in a doughnut arrangement around the sides of 2-L Pyrex baking dishes and covered with tight fitting lids. By placing the slices in this pattern, they were ready for reheating without further handling. The doughnut arrangement makes optimum use of the heating pattern in microwave ovens and is also suitable for conventional reheating.

Reheating procedure for meat

Following the 2-day refrigerator (4°C) storage period, the slices were reheated to 70 ± 2°C. One set of eight slices from each roast was reheated in an electric oven preheated to 162 ± 2°C. The other set of eight slices from the same roast was reheated in a domestic Litton microwave oven (Model 416, Litton Industries, Minneapolis, Minn.) with the control set for "reheat." This oven operates at 2450 MHz with a cooking power of 525W. Operation on the "reheat" setting reduced the cooking power by about one-sixth. Endpoint temperatures for the microwave reheated samples were determined with a telethermometer (Model 42SC, Yellow Spring Instrument Co., Yellow Springs, Oh.). For conventionally reheated samples, temperatures were determined with iron-constantan microthermocouples attached to a recording potentiometer. Average reheating times for conventionally and microwave reheated samples were 34 and 6.8 min, respectively.

Sensory analysis

Testing was conducted in an air-conditioned sensory testing facility equipped with individual booths, each illuminated with a red light. The panel consisted of seven judges (one male and six female) with previous experience in sensory evaluation. One week prior to the collection of data, four consecutive orientation sessions were conducted. This served to familiarize the judges with "warmed-over" aroma and flavor and the procedures for evaluation.

Slices from freshly roasted, microwave and conventionally reheated roasts were cut for serving on a Plexiglass template (1.3 cm grid) as recommended by the American Meat Science Association (1978). Two of the 1.3 cm × 1.3 cm × 0.6 cm cubes were placed into randomly coded, preheated 50-ml beakers. Pyrex covers were placed over the beakers, and they were served immediately. Samples from each of the three treatments were presented one at a time to each panelist. The order of serving for each treatment was randomized for each day of serving.

Upon receiving the sample, each judge lifted the Pyrex cover and evaluated the aroma, then proceeded to evaluate flavor. "Warmed-over" aroma and flavor were scored on a 5-point intensity scale. To prevent first-sample bias, a "warm-up" sample was always served first. This sample was scored, but these data were not included in the statistical analysis. Each panelist was supplied a glass of tap water at room temperature for palate cleansing before and between tasting samples.

TBA, vitamin, and moisture determinations

The slices of meat assigned to TBA, vitamin, and moisture analyses were twice ground (0.3-cm aperture, stainless steel plate) with chips of dry ice. Samples were prepared from raw, freshly roasted, and microwave and conventionally reheated treatments. TBA tests were performed according to the extraction method developed by Witte et al. (1970). Thiamin and riboflavin contents of the samples were measured by the thiochrome and fluorometric methods, respectively (Association of Vitaman Chemists, 1966). Moisture was determined by vacuum drying 8 hr at 95–100°C (AOAC, 1975).

Statistical analysis

For statistical analysis, the descriptive phrases for each sensory attribute were assigned a numerical value. The phrases no "warmed-over" aroma and no "warmed-over" flavor were given a value of 5, whereas the phrases intense "warmed-over" aroma and intense "warmed-over" flavor were given a value of 1, Analysis of variance was used to determine if significant differences existed among treatment means (Snedecor and Cochran, 1967). To locate differences among means, Duncan's New Multiple Range Test was applied (Duncan, 1955).

RESULTS & DISCUSSION

Sensory analysis

Mean sensory scores for aroma indicated that microwave reheated roast beef slices had significantly ($P < 0.05$) less intense "warmed-over" aroma than the conventionally reheated samples. Scores for "warmed-over" aroma of the microwave reheated samples were, however, significantly ($P < 0.05$) more intense than those of the freshly roasted samples and were scored as having a distinct "warmed-over" aroma. The same trend was apparent in the scores for "warmed-over" flavor although the difference between the samples reheated by conventional and microwave methods were not statistically significant (Table 1).

TBA values, vitamin content and retention and moisture content

The extent of lipid oxidation, as measured by the TBA test, was significantly ($P < 0.01$) greater for the reheated samples than for the raw or freshly roasted samples. Although lipid oxidation of microwave reheated samples appeared to be less than that of the conventionally reheated samples, the difference was not statistically significant (Table 1).

The thiamin and riboflavin content of the freshly roasted and reheated samples did not differ significantly, but the content of these three treatments was less ($P < 0.05$) than that of the raw meat. The percent retention of thiamin and riboflavin on a dry weight basis revealed no significant differences among the freshly roasted and reheated samples, indicating little loss of either thiamin or riboflavin upon reheating (Table 2).

Moisture content of the conventionally reheated samples and that of the microwave reheated meat did not differ significantly (Table 2). Since samples for reheating were taken from adjacent slices, the fat and protein contents would have been similar within each replication.

General discussion

Results of this study support the findings of Bowers (1972), provided that rancid aroma and "warmed-over" aroma can be interpreted as denoting the same characteristic. These notes were less intense in the products reheated by microwaves than in products reheated by the conventional method. In contrast, research conducted on conventionally and microwave reheated pork loin slices, by Penner and Bowers (1973), revealed no significant differences in either stale aroma or flavor among freshly cooked, conventionally reheated or microwave reheated samples. Panelists, however, noted sulfurous and fleeting rancid-oily flavor components in the conventionally reheated samples on several occasions.

The difference in intensity of "warmed-over" aroma of the microwave and conventionally reheated samples could be due to the difference in time required for reheating by the two methods. Since adjacent slices were used for reheating by the two methods, differences in the composition of the meat was not a contributing factor. Although MacLeod and Coppock (1976) studied primary heating, not reheating, they also attributed the differences in aroma of volatile components to the longer cooking time required by the conventional heating as compared to the microwave method. In the present study, it was apparent that neither flavor evaluation nor TBA numbers were as sensitive a measure of "warmed-over" quality in reheated beef as was aroma evaluation.

Since the time required for conventional reheating of samples was about six times that for microwave reheating, differences in retention of thiamin would be expected. Thiamin is unstable to heat, particularly in the pH range found in meat (Beadle et al., 1943; Rice and Beak, 1945). However, as in this study, other researchers (Causey and Fenton, 1951; Bowers and Fryer, 1972; Ang et al., 1975)

Table 1—Mean[a] sensory scores[b] and TBA numbers for freshly roasted and reheated roast beef

Treatments	Sensory scores		TBA numbers
	Aroma	Flavor	
Raw			0.479a
Freshly roasted	4.9a	4.9a	0.386a
Conventionally reheated	1.8b	1.7b	1.980b
Microwave reheated	2.3c	2.1b	1.947b

[a] N=8. Where letters differ within a column, means differ significantly (sensory scores $P < 0.05$, TBA numbers $P < 0.01$).
[b] Range of scores: 1, intense "warmed-over" aroma or flavor to 5, no "warmed-over" aroma or flavor.

Table 2—Mean[a] moisture content and thiamin and riboflavin content and retention[b] of raw, freshly roasted, and reheated roast beef

Treatments	Moisture content %	Content, wet weight basis mg/100g		Content, dry weight basis mg/100g		Retention %	
		Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin
Raw	70.8a	0.103a	0.156a	0.352a	0.538a	100a	100a
Freshly roasted	63.0b	0.072b	0.160a	0.190b	0.424b	54.0b	79.7b
Conventionally reheated	60.8bc	0.072b	0.156a	0.184b	0.398b	52.0b	74.8b
Microwave reheated	59.7c	0.071b	0.163a	0.177b	0.404b	50.3b	76.4b

[a] N=9. Where letters differ within a column, means differ significantly ($P < 0.01$) from each other.
[b] Retention (%) was calculated by dividing the vitamin content (dry weight basis) for each cooked sample by the vitamin content (dry weight basis) of the raw sample and multiplying by 100.

also found little or no loss of thiamin in meat products reheated by conventional or microwave methods.

Of greater importance to nutrition and helath professionals are the data illustrating thiamin and riboflavin content on a wet weight basis. As illustrated in Table 2, from a dietary standpoint no significant differences in the intake of these two vitamins would result from any of the reheating treatments.

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FACTORS AFFECTING PALATABILITY AND COOKING PROPERTIES OF GROUND BEEF PATTIES—FROZEN LEAN, PATTY SIZE, AND SURFACE TREATMENT

H. R. CROSS and B. W. BERRY

ABSTRACT

The objective of the study was to evaluate the effects of frozen lean addition, patty size, and surface perforation on the palatability and cooking properties of ground beef patties. Eighteen ground beef formulations were prepared at a commercial plant from various raw material sources. Frozen lean from domestic, Mexican, and Australian sources were added at the 40% level. Control formulations were comprised of 40% unfrozen U.S. Cutter cow lean and 60% unfrozen U.S. Choice trimmings. Immediately after the patties were formed, the surface was treated in one of three ways: (1) "knife-like" perforations; (2) "waffle-like" perforations, and (3) no perforations. Patties were evaluated for differences in sensory, cooking, and physical properties. Use of frozen lean had no important effects on ground beef palatability or cooking properties. There was no advantage to subjecting the surface of the patty to a knife or waffle treatment.

INTRODUCTION

THE CONSUMPTION of ground beef has increased steadily over the past few years. It has been estimated that, by 1980, 50% of the beef consumed in the U.S. will be consumed as ground beef. Numerous factors can affect the palatability, cooking properties, and ultimate consumer acceptance of ground beef patties. For example, Cross et al. (1976) reported that ground beef from U.S. Utility or Cutter grade carcasses was unacceptably high in connective tissue. Connective tissue appears to be one of the major problems associated with the consumer acceptance of ground beef. New technology, such as mechanical desinewing, can remove most of the objectionable connective tissue, as reported by Gillett and Tanatikarnjathep (1976) and Cross et al. (1978a). The method of grinding can also affect the quality of cooked ground beef. Cross et al. (1980) reported that the larger the initial grinding plate, the more connective tissue found in the final product.

Other factors that may affect ground beef palatability include the source of the frozen lean (domestic and imported), patty size, and surface treatment. Some purchase specifications preclude the use of frozen lean, whereas others allow up to 30 or 40% to be added. Little or no research has been reported in this area. The meat industry at times treats the fresh patty with a "knife" or "waffle" perforation or impression on each surface. Research is needed to determine if these treatments have an effect on palatability and cooking traits. Therefore, as a part of the USDA's continuing effort to develop optimal methods for the production of ground beef, the objective of this study was to evaluate the effects of frozen lean addition, patty size, and surface perforation on the palatability and cooking properties of ground beef patties.

EXPERIMENTAL

Formulation

Eighteen ground beef formulations were prepared at a commercial plant from various raw material sources as outlined in Table 1. Formulations 1–15 were prepared by adding 40% frozen lean (Mexican, Australian, or domestic) to 60% Choice lean trimmings (unfrozen). Frozen lean was equivalent in grade to U.S. Cutter. Frozen lean was derived from the major and minor cuts of the entire carcass. The control formulations (no. 16–18) were comprised of 40% unfrozen U.S. Cutter cow lean (major and minor cuts) and 60% unfrozen U.S. Choice trimmings. Each formulation contained a minimum of 1400-kg. Frozen meat blocks were passed through a Rietz grinder with a 0.95-cm plate. Unfrozen U.S. Cutter lean and Choice trimmings were passed through a Weiler grinder with a 0.95-cm plate. Samples were randomly pulled after a 3-min mix and fat contents were determined with an Anyl Ray Instrument. If necessary, the fat content was adjusted to 21 ± 2%. The average temperature of the unfrozen lean and fat after the first grind was 4°C. After the course ground trimmings were mixed for 3 min with the frozen lean and ground through the 0.32-cm plate, the average temperature of the ground mixture was −2°C.

Two 115-kg batches were randomly selected from the 1400-kg batch after the final grind. Each 115 kg batch was randomly divided into three equal parts and assigned to one of three patty sizes (227, 114, or 102-g). Ground beef was passed through a FORMAX 24

Table 1—Sample formulations

Formulation no.	Raw meat material	Patty size g(oz)	Patty surface treatment
1	Mexican frozen lean + Choice trimmings	227(8.0)	Plain
2	Mexican frozen lean + Choice trimmings	114(4.0)	Plain
3	Mexican frozen lean + Choice trimmings	102(3.6)	Plain
4	Australian frozen lean + Choice trimmings	227(8.0)	Plain
5	Australian frozen lean + Choice trimmings	114(4.0)	Plain
6	Australian frozen lean + Choice trimmings	102(3.6)	Plain
7	Domestic frozen lean + Choice trimmings	227(8.0)	Plain
8	Domestic frozen lean + Choice trimmings	114(4.0)	Plain
9	Domestic frozen lean + Choice trimmings	102(3.6)	Plain
10	Mexican frozen lean + Choice trimmings	227(8.0)	Knife
11	Mexican frozen lean + Choice trimmings	227(8.0)	Waffle
12	Mexican frozen lean + Choice trimmings	114(4.0)	Knife
13	Mexican frozen lean + Choice trimmings	114(4.0)	Waffle
14	Mexican frozen lean + Choice trimmings	102(3.6)	Knife
15	Mexican frozen lean + Choice trimmings	102(3.6)	Waffle
16[a]	U.S. Cutter lean + Choice trimmings	227(8.0)	Plain
17[a]	U.S. Cutter lean + Choice trimmings	114(4.0)	Plain
18[a]	U.S. Cutter lean + Choice trimmings	102(3.6)	Plain

[a] Unfrozen control formulations.

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Table 2—Effect of raw lean source, patty size and surface treatment on palatability traits of ground beef patties

Palatability trait	Raw lean source[a]				Patty size, g[b]			Surface treatment[c]		
	Mexican frozen	Australian frozen	Domestic frozen	Control unfrozen	227	114	102	knife	waffle	plain
Tenderness[d]	6.1h	6.0h	5.8h	6.1h	6.1h	6.0h	5.6h	5.7h	5.7h	6.0h
Juiciness[e]	5.8h	5.5hi	5.3h	5.2h	5.7h	5.5h	5.3i	5.1i	5.3i	5.8h
Connective tissue[f]	5.4h	5.6h	5.3h	5.2h	5.6h	5.4h	5.2i	5.2h	5.2h	5.4h
Flavor intensity[g]	5.5h	5.3h	5.2h	5.6h	5.6h	5.5h	5.1i	5.3h	5.3h	5.5h
Instron shear, kg	8.37h	6.97i	7.22i	7.01i	7.91h	7.33h	7.22h	8.27h	8.04h	8.37h

[a] n = 18 observations per mean. Means in the same row within the same treatment factor with different letters are different.
[b] n = 48 observations per mean.
[c] n = 18 observations per mean.
[d] Tenderness: 8 = extremely tender and 1 = extremely tough.
[e] Juiciness: 8 = extremely juicy and 1 = extremely dry.
[f] Connective tissue: 8 = none and 1 = abundant.
[g] Flavor intensity: 8 = extremely intense ground beef flavor and 1 = extremely bland flavor.

patty machine. The average pressure during patty formulation was 34–36 kg/sq cm.

Immediately after the patties were formed, the surface was treated in one of three ways (Table 1). The surface was either (1) plain (no surface treatment), (2) perforated with a "Knife-like" blade, or (3) perforated with a "waffle-like" blade. The knife cut was approximately 0.65 cm deep and 0.25 cm wide, and the waffle cut was approximately 0.25 cm deep and 0.25 cm wide.

After formation, the patties were immediately passed via conveyor belt into a Northfield Spiroblast ammonia freezer (−50°C). The conveyor belt speed varied depending on the patty size. The internal temperature of the frozen patties averaged −7°C after 12–16 min. One hundred frozen patties were randomly selected from the conveyor belt for each treatment. Patties were placed in plastic bags and stored 48 hr at −10°C. Patties were shipped 2000 km to Beltsville, Md., by truck at −10°C and stored at −20°C until evaluation for sensory, physical, and chemical properties (4 wk).

Cooking method and properties

Frozen patties were broiled on electric Farberware broilers (model 350A). The cooking time varied with the patty size. Preliminary trials indicated that, in order to achieve the same approximate degree of doneness, the 227-g (8.0-oz) patty required 8 min on each side; the 114-g (4.0-oz) patty, 7 min; and the 102-g (3.6-oz) patty, 6 min. Degree of doneness was evaluated 5 min after cutting sections, using an 8-point photographic scale with 8 = rare and 1 = well done. Total cooking loss was determined as the weight loss from the frozen to the cooked state. Measurements were obtained for raw and cooked height and diameter for evaluation of dimensional changes during cooking.

Panel selection and training

A 10-member descriptive attribute panel was selected and trained by the procedures of Cross et al. (1978b). The panel rated the following attributes on an 8-point structured scale: (a) tenderness and juiciness, with 8 = extremely tender and juicy and 1 = extremely tough and dry; and (b) connective tissue with 8 = none and 1 = abundant. Ground beef flavor intensity was rated with 8 = extremely intense and 1 = extremely bland. Panelists rated six samples at each of 18 sessions; thus, each treatment was replicated six times. Samples to be evaluated in each session were selected via a table of random numbers.

Instron shear

Ten patties from each treatment were broiled, using identical cooking procedures as described for the sensory panel patties, for determination of maximum shear force values. The cooked patties were cooled for at least 1 hr before being trimmed into 2.5-cm squares. Shear force was measured on each square with the single-blade shearing device attached to the Instron as described by Cross et al. (1978c). Data were recorded for the maximum force required to shear through the meat patty.

Fat and water

Fat and water content of raw patties were determined according to AOAC (1975) procedures. Ten patties per treatment were thawed until pliable and then passed through a 0.3 cm grinding plate, mixed and reground through a 0.2 cm plate. Water content was determined as weight loss of two 3-g samples after drying for 24 hr at 102°C. Fat content was determined as the weight loss of the dried samples after 16 hr of extraction with diethyl ether.

Statistical analysis

For each type of data, the analysis of variance was obtained using a sequential sums of squares approach. Since many of the contrasts were correlated, it was also necessary in the means analysis to use Scheffe's method to confirm, in several instances, that a difference between means was significant (Scheffe, 1959).

RESULTS & DISCUSSION

ANALYSIS OF VARIANCE revealed that few interactions were significant. Thus, in order to simplify comparisons, data were combined when considering frozen lean source, patty size, and surface treatment, with the significant interactions noted. From the practical viewpoint, frozen lean source had no effect on palatability (Table 2). There were a few significant effects, but the magnitudes of the differences were not large enough to be considered important. All palatability values were 5.0 or above, the level usually considered "acceptable" by consumer panels. Thus, addition of frozen lean from domestic or imported sources had no important effect on palatability.

Frozen lean from Mexico and Australia and the unfrozen control had less ($P < 0.05$) total cooking loss than ground beef prepared from domestic frozen lean (Table 3). Frozen lean from Australia had significantly less percent diameter change than patties prepared from domestic lean. As expected, percent fat and percent water were not significantly affected by sources of frozen lean. Thus, for unexplainable reasons, ground beef containing domestic frozen lean had greater cooking losses and percent diameter change than the remaining formulations. It is important to be able to control the diameter of the patty so that hamburgers sold by a fast food operation cover a constant area of the bun.

The 102-g patties received significantly lower ratings for tenderness, juiciness, amount of detectable connective tissue, and flavor intensity (Table 2) when compared to the 114-g and 227-g patties. Patty size may be closely related to cooking effects, i.e., the ability of the patty to withstand high cooking temperatures. Additional research is needed to determine the optimal cooking method for patties of varying sizes. Future research may indicate that it is impossible to prepare an acceptable patty below a certain size.

As patty size decreased, the percent cooking loss and percent diameter change increased (Table 3). Main effect interactions were highly significant ($P < 0.01$) for percent height change; thus further means analysis is inappropriate. Degree of doneness ratings decreased (i.e., patties became more well done) as patty size decreased though only the 227-g and 102-g patties differed significantly.

Type of patty perforation (knife or waffle) did not en-

Table 3—Effect of raw lean source, patty size, and surface treatment on cooking and cnemical properties of ground beef patties

Trait	Raw lean source[a]				Patty size, g[b]			Surface treatment[c]		
	Mexican frozen	Australian frozen	Domestic frozen	Control unfrozen	227	114	102	Knife	Waffle	Plain
Total cooking loss, %	32.94d	33.85d	36.24e	33.15d	32.58e	33.75de	34.02d	33.19d	31.59d	32.94d
Diameter change, %	14.30de	13.86e	15.53d	14.23de	12.19f	14.02e	16.02d	13.72d	13.16d	14.30d
Height change, %	18.44d	15.36e	15.89e	16.15de	18.46*	14.05*	16.78*	20.80*	24.91*	16.96*
Raw fat, wet %	21.52d	22.65d	21.76d	21.24d	20.92e	22.82d	22.37d	23.77d	21.34e	21.52e
Raw water, %	61.35d	58.42d	62.35d	61.98d	62.24e	59.33d	59.41d	58.85d	61.20e	61.35e
Degree of doneness[d]	3.0d	2.9d	3.0d	2.8d	3.0d	2.4de	2.0e	2.6d	2.3d	2.5d

[a] n = 18 observations per mean. Means in the same row within the same treatment factor with different letters are different
[b] n = 48 observations per mean.
[c] n = 18 observations per mean.
[d] Degree of doneness evaluated with a photographic scale, with 8 = rare and 1 = well done.
* Interactions were highly significant ($P < 0.01$), thus, further means analysis is inappropriate.

hance any palatability trait (Table 2). Untreated patties (plain) were juicier than patties receiving the knife or waffle treatment. The differences in tenderness, connective tissue, and flavor intensity were not significant, but trends favored untreated patties. Patty treatments had nonsignificant effects on all cooking traits (Table 3). Knife treated patties had less water and more fat ($P < 0.05$) in the cooked patty than did the untreated (plain) or the waffle treated group. The surface treatments may make the patty more suceptable to the effects of cooking with no advantage (other than appearance) to subjecting the surface of the patty to a knife or waffle treatment.

CONCLUSIONS

1. Use of frozen lean had no important effects on ground beef platability or cooking properties. Frozen lean source (Mexican, Australian, or domestic) had no practical effects on palatability or cooking properties.

2. Patty size had significant effects on all palatability traits and cooking properties. The 102-g (3.6 oz) patty appeared to be too small to produce an acceptable patty.

3. There was no advantage to subjecting the surface of the patty to a knife or waffle treatment. These data indicate that patty perforation may actually have negative effects on palatability and cooking properties, i.e., juiciness, moisture, etc..

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EFFECTS OF FROZEN STORAGE ON FUNCTIONALITY OF MEAT FOR PROCESSING

A. J. MILLER, S. A. ACKERMAN, and S. A. PALUMBO

ABSTRACT

Frankfurter quality was monitored to evaluate effects of frozen storage on meat components. After fresh meat controls were tested, beef, pork, and pork fat were frozen and stored at −17.8°C for 1–37 wk. At 6-wk intervals, functional and quality tests were performed on thawed and control meat samples and on frankfurters made from the samples. Frozen storage significantly affected beef and pork lean (drip loss, % solids and N in drip, extractable protein, water binding, emulsifying capacity) and fat (beef-thiobarbituric acid and pork-peroxide values); frankfurters produced from these ingredients were also affected (cooking tests, penetration force, sensory panel scores).

INTRODUCTION

FREEZING AND FROZEN STORAGE can produce profound effects on the structural and chemical properties of muscle foods, including changes in muscle fibers, lipids, and proteins, all of which have the potential for significantly influencing the quality attributes of meat and meat products.

Early studies by meat researchers focused on relationships between drip volume and freezing conditions of beef muscle (Cook et al., 1926; Moran, 1932; Ramsbottom and Koonz, 1939, 1940). A concurrent line of investigation that continues today is the effect of freezing on meat tenderness (Paul and Child, 1937; Hankins and Hiner, 1941; Winger and Fennema, 1976).

The structural changes that occur in muscle were reported by Luyet (1959), who, in a series of experiments, demonstrated the damaging effects of ice crystal growth on muscle fiber morphology.

Lipids have been studied with regard to oxidative and degradative changes that result from frozen storage. Awad et al. (1968) reported a slight increase in extractable lipids from beef during 8 wk of frozen storage at −4°C. Cholesterol levels were unaffected by the treatment, but free fatty acids (FFA), thiobarbituric acid (TBA), and peroxide values (PV) increased. Keller and Kinsella (1973) observed no change in lipid extractability but did observe increases in carbonyls, TBA, and PV values. Also, phospholipid extraction decreased during the −18°C storage of ground beef.

Alterations in proteins have been observed previously in frozen meat. Sarcoplasmic proteins are less soluble after freezing of muscle tissue; moreover, there is a loss of specific components separated by electrophoresis and ultracentrifugation (Kronman and Winterbottom, 1960). Awad et al. (1968) found that total extractable protein (TEP), sarcoplasmic protein, and actomyosin extractability decreased with frozen storage. These investigators observed significant changes in banding patterns on electrophoretic polyacrylamide gels for sarcoplasmic and urea-treated actomyosin proteins. These degradative transformations appear to be evidenced by changes in the hydration of meat (Deatherage and Hamm, 1960) and its water-holding capacity (Wierbicki et al., 1957a, b).

To date, investigators have studied these alterations mostly with regard to fresh meat products. Little attention has been focused on the relationship between frozen storage and the use of meat for processing. This study reports on the functional changes that occur in the storage of frozen meat block components and the relationship of these properties to changes within a comminuted meat product.

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EXPERIMENTAL

Materials

Boneless beef chuck, boneless pork shoulders, and pork back fat were randomly selected from freshly cut lots from two commercial slaughterhouses. The beef came from ungraded mature dairy cows, while the pork and pork fat were obtained from United States #1 young swine. The raw materials were transported to the U.S. Department of Agriculture's Meat processing Laboratory where they were ground through a 1.91 cm plate on a Butcher Boy B-52 grinder. Aliquots of each were ground 2× through a 0.31 cm plate and were retained for proximate analysis. Eight sets of beef (6.5 kg), pork (5 kg), or pork fat (5 kg) were packaged in polyethylene (Cry-O-Vac) bags.

All samples which were to be frozen were placed in a −31.7°C freezer for 24 hr and then transferred to a −17.8°C freezer for the allotted storage time. Thawing was accomplished by transferring frozen bags to a 4.4°C cooler for 72 hr. Unfrozen meats were stored in the 4.4°C cooler until utilized. Frozen meat thaw exudate (drip) was measured, and a 50 ml sample was retained for Kjeldahl nitrogen and moisture determinations.

Product preparation

Proximate analyses for moisture, lipid, and protein were performed according to AOAC procedures (1975). Frankfurters were formulated to meet the following constraints: lipid ≤30%, protein ≥11% (65% of the protein to come from beef), % moisture ≤4X protein + 10%, NaCl 2.5%, sugar 1.5%, commercial spice mixture 1% (Baltimore Spice Co., #FF3118), 150 ppm $NaNO_2$, and 550 ppm sodium ascorbate.

Meats were ground through a 0.48 cm plate immediately prior to the manufacture of the frankfurters. The beef and pork, spices, cure, salt, and 3/4 water (added as ice) were comminuted for 2 min at 2500 rpm in a Koch-Alpina Pb-50 chopper. The remaining ice and the pork fat were then added, and the mixture was comminuted to a temperature of 15.6°C. The total time required for comminution was recorded. Emulsion was stuffed into #29 No-Jax casings (Union Carbide Corp.), linked into 15 cm lengths, and cooked and smoked for 90 min in an air conditioned smokehouse according to the following schedule: 10 min at 54°C dry bulb (DB), 30 min at 63°C DB and 54°C wet bulb (WB), and 45 min at 74°C DB and 63°C WB. The products were cooked to an internal temperature of 71°C and then showered to 32°C. Processing losses were determined as the difference in weight of raw and cooked sausage expressed as percentages. The frankfurters were stored in plastic bags in a 0.5°C cooler until they were tested within 48 hr.

Chemical and physical tests on meats and fat

Functionality and quality tests were performed on the raw components in investigating their relationship to the characteristics of finished products as follows:

The pH values of beef, pork, and pork fat were determined in duplicate on a Radiometer model 25 pH meter, with the electrode inserted into the tissue.

Water-holding capacity (WHC) was measured in triplicate according to the method of Wierbicki and Deatherage (1958).

Emulsifying capacity of the meats was determined in triplicate by the method of Swift et al. (1961).

Total extractable protein (TEP) was measured in duplicate by the method of Awad et al. (1968).

Thiobarbituric acid (TBA) values of fresh meat samples were obtained in triplicate according to the method of Witte et al. (1970). Values are expressed as absolute absorbance units.

Peroxide values (PB) of pork adipose tissue were determined in triplicate by the AOAC procedure (1975).

Product evaluation

Frankfurters were tested for cooking losses by the consumer cooking and severe cooking tests of Tauber and Lloyd (1947). The pH was measured with a combination electrode inserted into two sections of randomly selected product. A penetrometer (Marine Colloids, Inc.) was used to determine peak force required to penetrate cross sections of 2.54 cm frankfurter sections. A 7.8 mm blunt plunger was utilized at a crosshead speed of 20 cm/min. In addition, subjective evaluation of fat capping was conducted.

Sensory evaluation

Nine days after the products were manufactured they were evaluated by a taste panel. Frankfurters were cooked in boiling water for 10 min and kept between 50–55°C in an apparatus similar to that described by Caperaso (1978). A panel of 10 experienced tasters determined differences between control (fresh raw materials) and treated (frozen raw materials) frankfurters by a triangle test. Additional comments were solicited from the panelists concerning the basis for determining differences between test samples. Results were analyzed by use of tables published by Roessler et al. (1978).

Experimental design

Experiment 1 was conducted with a batch of beef, pork, and pork fat divided into 8 lots. Seven lots of samples were stored frozen for 1, 7, 13, 19, 25, 31, and 37 wk prior to manufacture. The fresh and frozen lots were analyzed as indicated above. All tests except the sensory evaluation were performed. A second experiment was initiated in which the fresh meat and fat controls were included at each of the seven frozen storage testing increments. The controls were purchased fresh from the same sources and were subjected to the full complement of chemical and physical tests; frankfurters were also manufactured from these samples. Sensory triangle tests were performed on controls and treated products. This brought the total number of replicates to n = 9 for control samples and to n = 2 for experimental samples. Except for taste panel determinations, the results are expressed as the means of the two experiments.

Statistical analysis

Data were analyzed by analysis of variance and polynomial regression statistical treatments. Significance was determined by the F-test at the 5% level of probability (Steel and Torrie, 1960).

RESULTS

THE EFFECTS of frozen storage on pork and beef thaw exudate volume are shown in Figure 1. Both species lost increasing amounts of exudate as storage time progressed, although beef drip losses remained higher ($P < 0.05$) throughout the majority of the testing increments. Previous reports have shown that there is no simple relationship between frozen storage time and drip volume. The data presented here are in general agreement with Rahelic et al. (1974), Jakobsson and Bengtsson (1973), Awad et al. (1968), Law et al. (1967), and Moran (1932). Investigators have also attributed thaw exudate losses to other factors. These include pH of meat (Empey, 1933; Sair and Cook, 1938; Ramsbottom and Koonz, 1940), storage temperature (Moran and Hale, 1932), time that meat spends in the range of −1 to −5°C (Reay, 1934), and time of freezing postmortem (Rahelic et al., 1974).

The relationship between drip volume (Fig. 1) and meat pH (Table 1) was determined by a linear regression analysis of the data, and correlation coefficient was calculated; the correlation coefficients for beef and pork were r = 0.32 and r = −0.09, respectively. These values were not significant at the 95% level.

The percentage of solids in the thaw exudates are presented in Figure 2. The values were expressed as the difference between 100 and % moisture in the thaw exudate. Increases in % solids in thaw exudates in beef and pork were closely parallel, both being significantly correlated

Table 1—Effect of frozen storage on meat and product pH and processing parameters

Wk in frozen storage	Beef pH	Pork pH	Frankfurters pH	Comminution time (sec)	Processing loss (%)
0	5.8 (0.0)*	6.2 (0.1)	6.0 (0.0)	594 (41)	8.4 (0.6)
1	5.8 (0.0)	6.1 (0.1)	5.9 (0.2)	527 (117)	8.8 (0.4)
7	5.9 (0.1)	6.0 (0.0)	5.9 (0.1)	430 (30)	7.6 (0.1)
13	5.9 (0.0)	6.0 (0.1)	6.0 (0.1)	485 (63)	8.0 (0.5)
19	5.9 (0.0)	6.1 (0.0)	6.0 (0.1)	573 (92)	7.5 (2.3)
25	6.0 (0.1)	6.1 (0.1)	5.7 (0.1)	543 (53)	8.3 (1.7)
31	5.9 (0.1)	6.0 (0.1)	5.8 (0.1)	622 (22)	8.4 (0.4)
37	5.9 (0.0)	5.9 (0.0)	5.7 (0.0)	563 (39)	8.4 (2.1)

* Standard error (SE) in parentheses.

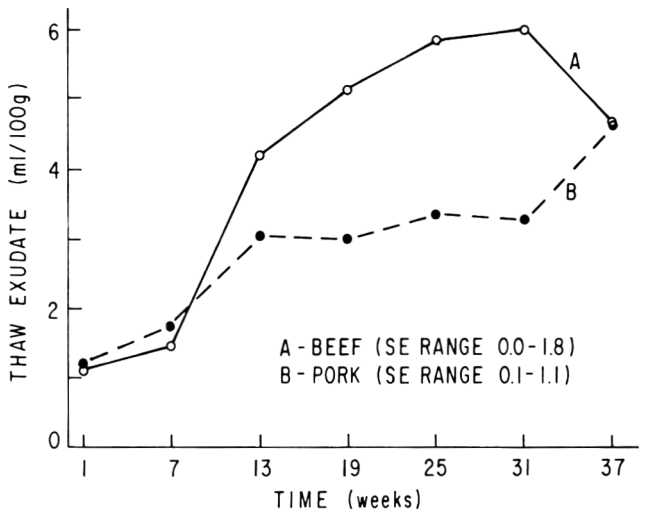

Fig. 1—Effect of frozen storage of meats on thaw exudate volume.

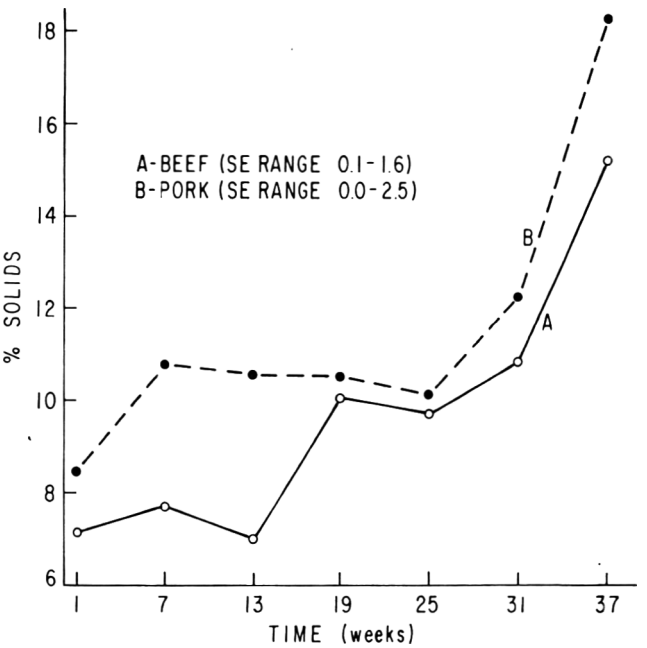

Fig. 2—Effect of frozen storage of meats on solids in thaw exudate.

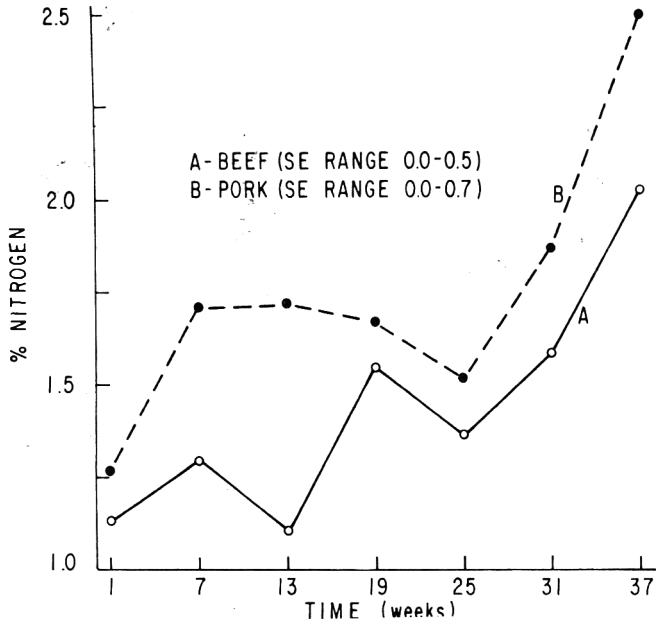

Fig. 3—Effect of frozen storage of meats on nitrogen in thaw exudate.

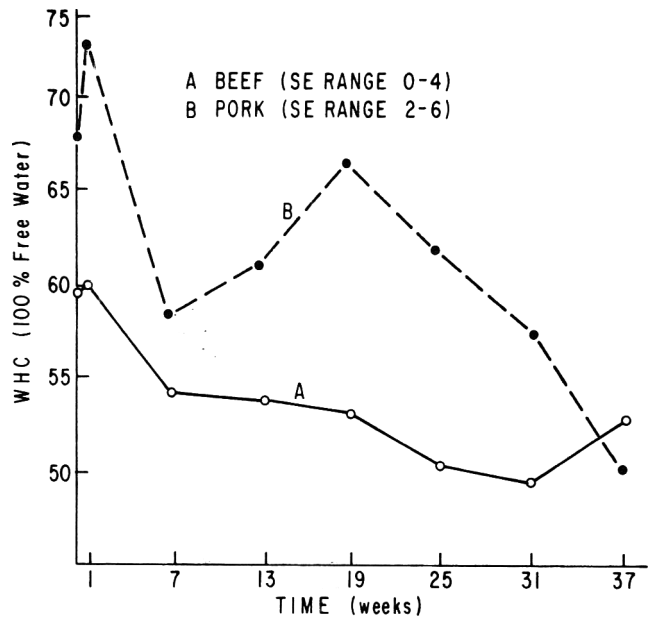

Fig. 5—Effect of frozen storage of meats on water holding capacity.

with longer freezing time ($P < 0.01$). These levels ranged from 8.5% in pork and 7.1% in beef exudates after 1 wk to 18.3% in pork and 15.2% in beef after 37 wk. Toward the latter storage times there was an accelerated increase of solids in the thaw exudate. This appeared between 25 and 31 wk of storage of sample pork, and 31–37 wk of storage of sample beef.

The changes in the concentration of dissolved nitrogenous compounds in the thaw fluid are shown in Figure 3. As with drip volume (Fig. 1) and percent solids in the exudate (Fig. 2), contents of nitrogen in the exudate increased with frozen storage time ($P < 0.05$), with the increasing nitrogen losses in the meat closely following the course of solids formation. In the pork samples there were two periods during which nitrogen losses sharply increased. This occurred between the first and seventh week and then after 25 wk of storage. Nitrogen content of beef thaw exudate gradually increased up to 19 wk, then there was a very sharp rise between the last two test periods. According to Lawrie (1979), the solubilized nitrogenous compounds in this fluid are sarcoplasmic proteins, creatine and creatinine, free amino acids, peptides, nitrogenous bases and their corresponding nucleosides and nucleotides, purine and pyrimidine degradation products, porphyrin containing compounds, and metabolic cofactors. Of these, amino acid, peptides, proteins, and vitamins represent potential nutritional losses.

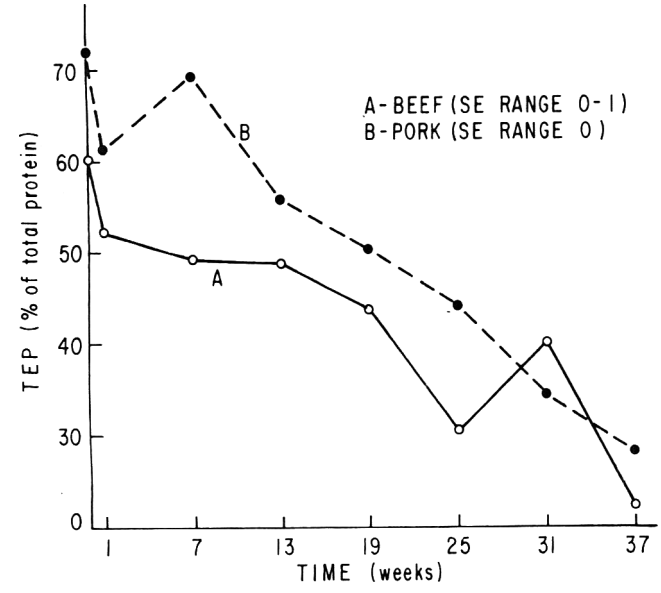

Fig. 4—Effect of frozen storage of meats on total extractable protein.

The total extractable protein (TEP) values determined from the eight sampling increments are presented in Figure 4. From initial means of 72% TEP for pork and 60% TEP for beef muscle, decreases to 28% ($P < 0.01$) and 22% ($P < 0.01$), respectively, were observed. For both species of meat a gradual, almost linear, decrease in TEP was observed. Other investigators likewise have found that the extractable protein or nitrogen decreases with increased storage time. Awad et al. (1968) observed a change from 90.99% to 50.84% TeP in beef muscle over an 8 wk storage period at $-4°C$. According to Khan et al. (1963), the% totalextractable nitrogen of chicken pectoralis muscle decreased from 88 to 56% over a 50 wk period at $-4°C$.

The water-holding capacity (WHC) of the meat of both species decreased with frozen storage time ($P < 0.01$). These data are presented in Figure 5. WHC of both beef and pork samples produced an increase after 1 wk of freezing. This was followed by a sharp decrease in samples frozen for 7 wk. Beef WHC decreased gradually throughout the remainder of the testing periods. Pork WHC increased between weeks 7 and 19. The significance of this observation is not clear. From 19–37 wk pork WHC markedly decreased. Deatherage and Hamm (1960) reported decreased WHC for beef which had been slow frozen. This phenomenon has been attributed to a mechanical loosening of the muscle tissue by the formation of ice crystals inside the cells. Extensive splitting of the sarcolemma has also been observed (Hiner et al., 1945).

Figure 6 shows a decrease in emulsification capacity in

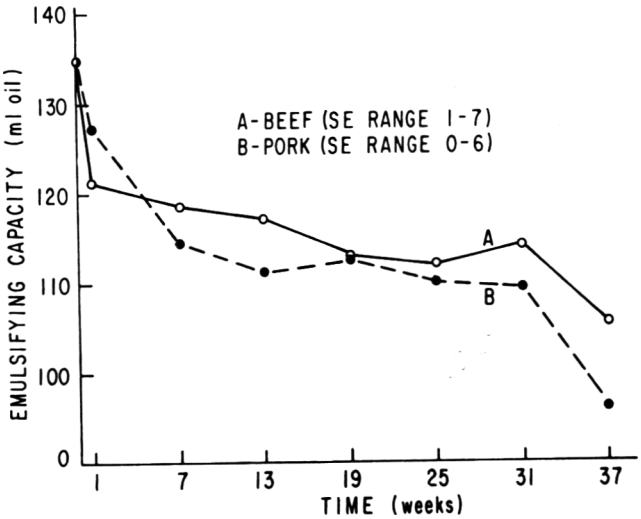

Fig. 6—Effect of frozen storage on meat emulsifying capacity.

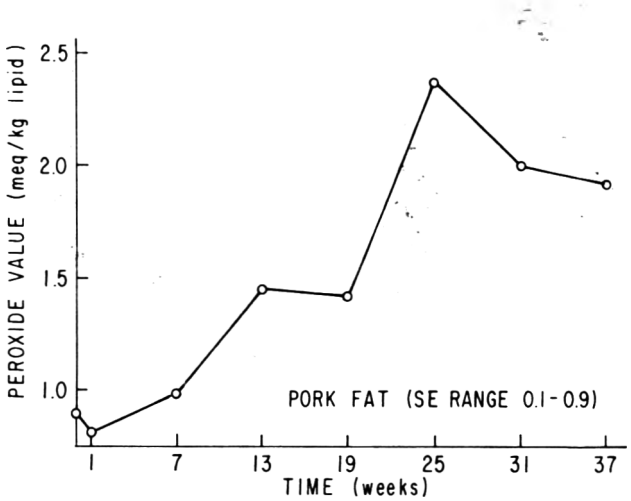

Fig. 7—Effect of frozen storage of pork fat on peroxide value.

beef ($P < 0.01$) and pork ($P < 0.01$). Dramatic diminution of emulsifying capacity occurred between 0 and 7 wk in pork and 0 and 1 wk in beef. Swift et al. (1961) showed that higher levels of salt soluble protein increased emulsifying capacity. The concurrent decrease in muscle protein solubility explains the decrease in emulsifying capacity observed.

It has been recognized for some time that lipids in frozen meat are likely to undergo oxidative deterioration (Ramsbottom, 1947). The common indicators of these changes are PV and TBA tests. In this investigation, the peroxide values (meq peroxide/kg lipid) of adipose tissue from pork rose from initial measurements of 0.9 to 2.37 ($P < 0.05$) after 25 wk of $-17.8°C$ storage (see Fig. 7). The remaining 14 wk of storage resulted in a decrease of detectable peroxides. A rise followed by a decrease in PV was noted by Awad et al. (1968, 1969) on bovine and fish muscle. Degradation of hydroperoxides to carbonyl compounds has been postulated (Awad et al., 1968. 1969). TBA values, a measure of malonaldehyde and rancidity, of stored frozen pork and beef are shown in Figure 8. Observed values from both species remained quite close up to 13 wk in storage; thereafter, beef malonaldehyde production in relation to time of freezer storage accelerated ($P < 0.01$) at a higher rate than that of pork ($P > 0.05$). This phenomenon is contrary to expected results since pork lipids contain a higher percentage of unsaturated lipids than those of beef. However, evidence has also been presented by Shamberger et al. (1977) which agrees with our results. Recently, Brown et al. (1979) found that TBA values increased in beef muscle stored 180 days at $-18°C$. This increase is perhaps explained by the greater concentration of iron-containing pigments in beef muscle, which can act as prooxidants.

Table 1 contains results of observations of meat pH and tests made during and after frankfurter manufacture. Frozen storage duration did not appear to have an effect on variations in chopping time to $15.6°C$. The variations observed in this parameter could be a function of ingredient temperature. Average comminution time was 544 sec (standard error, SE = 21.7). Processing losses averaged 8.1%, with an SE of 0.1. Mean frankfurter pH was 5.9. These parameters were not affected by ingredient freezing. The size and incidence of fat caps increased as frozen storage progressed. However, there was no evidence of severe emulsion breakdown.

Cooking tests showed that frozen storage did affect product quality. The consumer cooking test (Fig. 9) demonstrated that as storage time increased the comminuted products lost their ability to retain moisture and fat ($P < 0.05$). This trend was more evident in the severe cooking test ($P < 0.01$). The greatest decrease in product quality appeared between weeks 1 and 19.

Cross sectional peak force required to puncture cores showed a significant decrease ($P < 0.01$) with greater storage time (Fig. 10), indicating a marked textural change. From an initial value of 900g, a decrease to 555g was recorded after the 37 wk testing period. Lee and Toledo (1976) reported that factors contributing to textural characteristics of a comminuted fish muscle product included presence of NaCl and/or polyphosphates, temperature of cooking, and type of heating medium used. In addition, these investigators found a significant relationship ($P < 0.01$) between product texture and comminution time and WHC. To test the hypothesis that decreased penetration force was the result of variations in chopping time, the linear regression and correlation coefficient between these factors was determined. Results indicated that the relationship was not significant at the $P < 0.05$ level ($r = -.44$) for beef and pork frankfurter manufactured with commercial

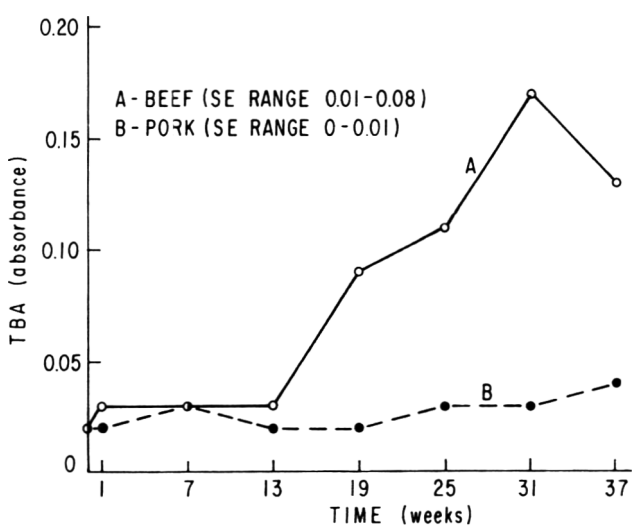

Fig. 8—Effect of frozen storage on meat thiobarbituric acid.

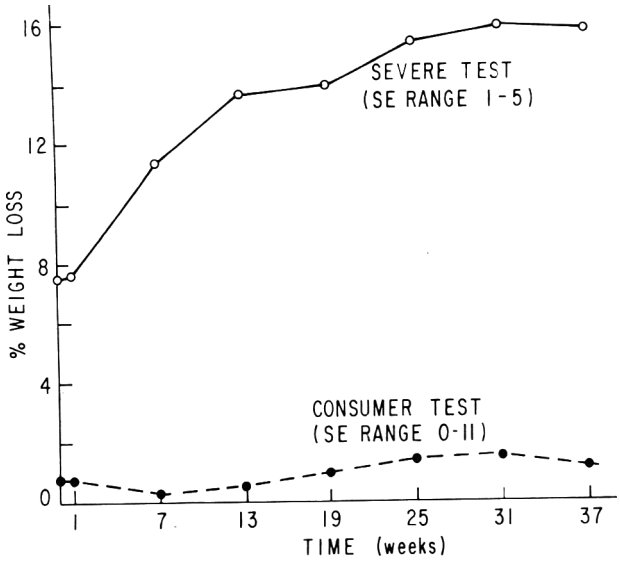

Fig. 9—Effect of frozen storage of meats and fat on frankfurter cooking tests.

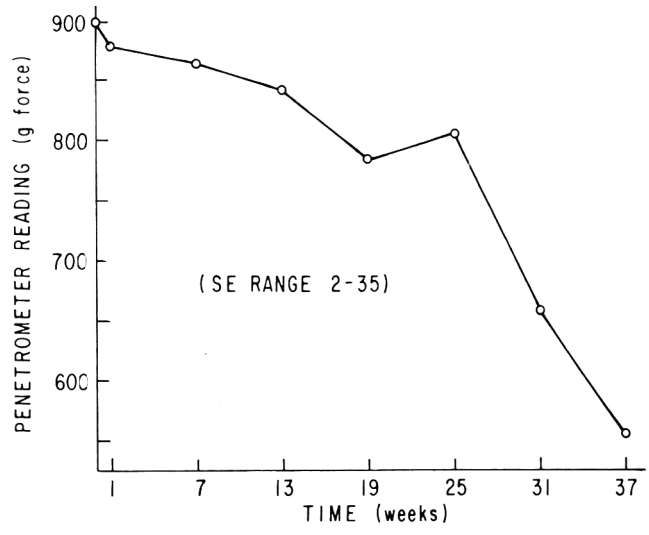

Fig. 10—Effect of frozen storage of meats and fat on frankfurter texture.

equipment. Correlation coefficients for various factors compared to penetrometer readings are presented in Table 2. The following characteristics were shown to relate significantly with these scores: beef emulsifying capacity ($r = 0.75$, $P < 0.05$), pork emulsifying capacity ($r = 0.81$, $P < 0.05$), pork WHC ($r = -0.75$, $P < 0.05$), beef TEP ($r = 0.83$, $P < 0.05$), pork TEP ($r = 0.93$, $P < 0.01$), beef TBA ($r = -0.87$, $P < 0.01$), severe cooking test ($r = -0.75$, $P < 0.05$), and triangle test ($r = -0.77$, $P < 0.05$). These comparisons suggest that the state of the myofibrillar proteins directly affect textural quality of emulsified meat products.

Triangle sensory tests, comparing fresh meat (control) frankfurters with frozen meat (treatment) frankfurters, upheld the evidence that significant changes had occurred during the 37 wk freezing period (see Table 3). After meat and fat were stored for 7 wk at $-17.8°C$, the resulting frankfurters were discernable ($P < 0.01$) from controls. This tendency continued throughout the course of storage, progressing by 31 wk to differences which all of the panelists were able to distinguish. Solicited comments added insight into the factors that panelists used to discern differences between samples. The majority of the comments were related to differences in texture between frozen and fresh product. In addition, there were comments related to rancid flavor in the treatment samples and a lack of overall flavor as time progressed. Two panelists were especially sensitive to oxidative notes, and one of these members detected rancidity as early as 1 wk of storage of meat materials. The other panelists did not, by and large, recognize an oxidative note before 31 wk of storage.

Comparing PV and sensory scores (% correct choice) by regression analysis it was determined that a highly significant ($P < 0.01$) correlation coefficient (0.89) existed between these parameters. Panelists, however, did not, on the whole, perceive rancidity until the 31 wk sample, suggesting that lipid alterations were perceived as secondary defects until the later stages of the experiment. During this time textural changes were the predominant characteristics recognized by panel members. It is quite possible that these changes were induced by the lipids.

DISCUSSION

IN THE PRESENT STUDY, we evaluated changes in beef, pork, and pork fat stored at $-17.8°C$ for 37 wk. The data

Table 2—Correlation coefficient between mean penetration scores and various parameters

Parameter	Correlation coefficient (r)
Beef emulsifying capacity (ml)	0.75*
Pork emulsifying capacity (ml)	0.81*
Beef WHC (%)	0.47
Pork WHC (%)	0.75*
Beef TEP (%)	0.83*
Pork TEP (%)	0.93**
Beef TBA (absorbance)	−0.87**
Pork TBA (absorbance)	0.47
Comminution time (sec)	−0.44
Consumer cook test (%)	−0.62
Severe cook test (%)	−0.75*
Triangle test (% correct responses)	−0.77*

* Correlation coefficient significant ($P < 0.05$).
** Correlation coefficient significant ($P < 0.01$)

Table 3—Triangle sensory evaluation of frankfurters made from frozen beef, pork, and pork fat

Wk in frozen storage	% Choosing correct pair[a]	Comments
0	—	—
1	50	Flavor—1[b], texture—5, rancidity—1
7	80**	Flavor—1, texture—6, rancidity—2
13	80**	Flavor—1, texture—3, rancidity—1
19	80**	Flavor—3, texture—5, rancidity—2
25	90***	Flavor—1, texture—8, rancidity—2
31	100***	Flavor—1, dryness—1, texture—9, rancidity—5
37	100***	Texture—6, rancidity—4

[a] n = 10.
[b] Number of responses.
** Significant at probability level $P < 0.01$.
*** Significant at probability level $P < 0.001$.

presented here indicate that alterations occurred in the chemical and physical properties of these components, especially in proteins and lipids. Theories regarding the frozen storage induced deterioration of muscle have been proposed. Callow (1955) attributed the phenomenon to the presence of high concentrations of natural salts in the juice of frozen meat. A second explanation postulates interaction between proteins and lipid degradation products. Free fatty acids, the products of hydrolytic rancidity, have been shown to bind to proteins in model systems (Bull and Breese, 1967a) and will denature proteins (Bull and Breese, 1967b). In addition, Buttkus (1969) has shown that malonaldehyde, a product of polyunsaturated fatty acid oxidation, reacts with muscle proteins to produce a denaturation. Moreover, observations we have made on the possible effects of peroxides in meat emulsion systems suggest that these compounds may exhibit deleterious effects on emulsion stability, implying a protein alteration. Sikorski et al. (1976), in a review of protein changes in frozen fish, included partial dehydration and the formation of formaldehyde as additional factors influencing the denaturation of muscle proteins. The observed changes in the present study support the theory that degradation products formation and interaction with muscle tissue result in decreased protein solubility, emulsifying capacity, and WHC. Moreover, it is plausible to suggest that the observed increases in solids and nitrogen in the thaw exudates were the result of the endogenous salt solubilization of these substances.

The factors involved in the stabilization of emulsified meat products include the solubilization of myofibrillar proteins, especially myosin, and their inclusion as an emulsifier, interfacing hydrophobic fat globules, and a hydrophilic aqueous phase. Once heat denaturation of the proteins occurs during cooking, a stable water, protein, and lipid matrix is set. Any factors upsetting this balance could result in deleterious alterations in the final product (Kramlich, 1971). Evidence obtained here indicates that the quality of proteins and fat deteriorated and that the comminuted products manufactured from the frozen meat block components gradually decreased in quality characteristics. While we found no acute emulsion instability during the 37 wk study, the trend indicated an eventual failure through further storage. The first signs of change were evident after only 1 wk of freezing, and by 25 wk all observed characteristics showed significant degradative changes. By 7 wk, panelists could readily discern differences between treatment and control products. For this reason it is recommended that short frozen storage periods be used for meat block components intended for use in comminuted meat products.

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Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TIME AND TEMPERATURE, MICROBIOLOGICAL, AND SENSORY ASSESSMENT OF ROAST BEEF IN A HOSPITAL FOODSERVICE SYSTEM

M. L. CREMER and J. R. CHIPLEY

ABSTRACT

Time and temperature, microbiological and sensory assessments were made of roast beef in a "Ready-Prepared" type foodservice system to determine critical points in operation. Time from the end of roasting to heating for service was about 45 hr. This included 3.5 hr holding at room temperature and two periods where after 15–20 hr refrigerator storage, mean temperatures were 8.2 and 7.6°C respectively. After microwave heating at service, temperatures ranged from 43–93°C. Total plate counts indicated <200 microorganisms per g before and after roasting but a 3–11 fold increase occurred from roasting to heating for service. Unusually large numbers of *Staphylococcus epidermidis* were found in cooked meat due to contamination introduced during the slicing operation and the time of storage at temperatures which would permit active growth of this organism. Also, cooked meat was found to contain *Bacillus* sp., *Clostridium sporogenes, C. perfringens, S. aureus*, as well as small numbers of *Penicillium* sp. Overall mean scores for sensory characteristics ranged from 6.6–7.5 (9-point scale). Data indicated that prevention of microbial contamination and control of internal temperatures during storage, holding, and heating for service are critical in this system of operation.

INTRODUCTION

"READY-PREPARED" foodservice systems are an established type of operation where food is cooked and then stored either chilled ("cook-chill") or frozen ("cook-freeze") for service at a future time (Unklesbay et al., 1977). Inadequate cooling and lapse of a day or more between food preparation and service have been identified (Bryan, 1978b) as primary factors contributing to outbreaks of foodborne disease. Both of these factors are inherent in the ready-prepared system, but despite potential hazard, operational data on time and temperature conditions and on sensory and microbiological quality of food are quite limited.

Nicholanco and Matthews (1978), in an actual operation, studied product flow and food quality in order to develop methods for system evaluation. Glew (1970) considered use of the cook-freeze system for hospital foodservice and found no reduction in food acceptability or increased numbers of microorganisms compared to a conventional system. Bunch et al. (1976) and Bunch and Matthews (1977) found that processes of holding and heating beef loaves, as might occur in a ready-prepared system, were not lethal to microorganisms; but product acceptability (Bunch et al., 1976) was not affected by length of storage time. Because of potential health hazard and limited information on the ready prepared system, this study was undertaken in an actual hospital operation to determine (a) time and temperature conditions during the various phases in product flow, (b) sensory and microbiological quality of food, and (c) critical points in system operation.

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MATERIALS & METHODS

THIS STUDY was conducted in a 700-bed general, metropolitan, teaching hospital in which the cook-chill system had been in operation for at least 10 yr. Selection of this particular operation was based upon relatively long use of the system and high reputation of both the professional staff and the institution. Roast beef, an entree, was chosen for study because of the frequency with which the item was served and because entrees would generally be subjected to all phases in product flow in a cook-chill system.

Through preliminary investigation, a flow chart was developed to provide a basis for time and temperature recording and to determine sampling points for microbiological analyses. Phases in product flow identified were: receiving, refrigerator storage, roasting, refrigerator storage, slicing, refrigerator storage, assembly, transportation, refrigerator storage, microwave heating, and service. The study was conducted in terms of these basic phases. For ease in identification, phases and holding periods studied were numbered as are indicated in Table 1. All data collection was replicated 3 times with conditions as occurred within the operating system and as roast beef appeared on the menu.

The sampling scheme is indicated in Table 1. Selected pieces of raw beef roasts were followed through the various phases of product flow to the point of ultimate service on 3 (10 total) selected galleys. Galleys were areas, external to the central kitchen, where food was stored, heated in microwave ovens, and then carried to patients. Galleys studied were the first, middle, and last for which food was portioned at assembly (Phase 8) and were chosen to give indication of the overall parameters of time and temperature in the total system.

Product

Meat that was roasted was U.S. Choice Grade aged bottom beef round and weighed approximately 7.2 kg per piece. In trials 1 and 3, 10 pieces of meat (72 kg total) were roasted in 5 pans with 2 pieces of meat per pan. From the first, middle, and last pans to be placed in the oven, 1 piece of meat, selected at random, was tagged for service on the first, middle, and last galleys in the assembly process. In trial 2, only 4 roasts (28.8 kg total) were used. Tagged meat for sampling was for one roast in the first pan and both roasts in the second pan as placed in the oven. In all instances, one 7.2 kg roast was essentially sufficient meat for one galley.

Meat was roasted in convection ovens with thermostats set at 135°C. Actual temperatures, read at the beginning and end of the roasting period from Taylor oven thermometers, Model 5928, ranged from 135–154°C. Ovens were preheated in all instances and some of the temperature variability indicated may be attributed to variability in the thermostatic setting during the preheating period. Roasting times and end-point internal temperatures of meat are indicated in Table 2.

During Phase 5, after slicing, hot beef broth was poured over meat. Broth was prepared from equal parts of commercially prepared canned beef consomme and kitchen-prepared beef stock. The quantity of broth used was sufficient to cover sliced meat. For two 25 × 30 × 6 cm pans, this was approximately 2.8L. During storage (Phase 6), the broth formed a gel around meat and at assembly, when meat was portioned onto plates, gelled broth adhered to meat. The adhering gel was used to provide for a moist product after heating in microwave ovens.

Meat portions were not weighed either at the time of slicing or portioning for service. Generally, two slices of meat were served per plate, and the approximate weight of the total was 113–142g. Slices were fairly uniform as based on conformation and trimming of meat and the setting of the meat slicer. Green beans and mashed potatoes

Table 1—Sampling scheme for time, internal temperature, and microbiological evaluation

Phase	Internal temperature	Time	Microbiological
1 Roasting	Center of 1 piece of meat from first, middle and last pans	First, middle and last pans	End slice (before and after roasting) from same piece of meat as used for internal temperature readings
2 Holding		Removal of first, middle and last pans from oven to placement in refrigerator	
3 Storage	Center of same 3 pieces of meat as in (1) roasting	Placement of first, middle and last pans in storage until removal for slicing	
4 Slicing	Center of 3 pans of meat (after slicing) to be used for service on 3 selected galleys	Slicing and panning each of the 3 pans of meat for service on 3 galleys	End and middle slices from each of 3 pieces of meat sliced for service on 3 selected galleys
5 Holding		Removing first, middle and last pans (all pans removed at one time) for slicing to returning pans of sliced meat to refrigerator (all returned at one time)	
6 Storage	Center of same 3 pans of meat as in (4) slicing	Placement of meat for selected galleys in refrigerator until removal for assembly	
7 Holding		Removal of 3 pans of meat, for 3 selected galleys, from refrigerator to beginning of assembly (all generally removed at one time)	
8 Assembly, overall		First tray on assembly line to last tray removed	
Selected individual galleys (3)	At beginning of assembly for 3 selected galleys in center of same 3 pans of meat as in slicing	Beginning to end of assembly for 3 selected galleys	Slice removed from center of 3 pans of meat as this area was reached during assembly for 3 selected galleys
9 Transportation, selected galleys		Removal from assembly line of last tray for each of 3 galleys to placement of carts, containing these trays, in galley refrigerators	
10 Beginning assembly to beginning of galley storage[a]			
11 Galley storage	At beginning and end of storage on each of the galleys for meat on trays in first (top) position on first cart, two middle positions[b] in middle cart, and bottom position in last full cart as both stored and heated	First cart placed in storage to removal of first tray for heating on each of 3 galleys	Taken at the end of galley storage from meat on middle cart from tray in position 9[b] on each of the 3 galleys
12 Heating for service	Recorded at the end of heating for same meat as in (11) beginning galley storage except for sample 9[b] which had already been removed for microbiological evaluation	Heating time was 60 sec in all instances as determined by push button control on equipment	Samples were taken from the same meat as used for internal temperature reading on the 3 galleys

[a] Procedure self explanatory in terms of Table 2
[b] Middle positions were trays 8 and 9 in a total of 16

were other meal components which were portioned onto plates with meat and were the same throughout study.

Time and temperature recording

Time and internal temperature readings were made as is indicated in Table 1. Environmental temperatures in rooms were read, at the beginning of food holding, from Taylor utility thermometer, Model 5135; and in refrigerators, at the beginning and end of food storage, from instruments built into the equipment. A Taylor Model 5974 refrigerator-freezer thermometer was used for reading temperature in one refrigerator. Internal temperatures of food were recorded from dial-type thermometers with 1 or 2° gradients. Thermometers used were Taylor Model 6072-1 and Weston Model 2292 for heated food; and Weston Models 2261 and 61048 for cold food. Thermometers were checked for accuracy and calibrated in either ice water or boiling water depending upon the temperature range involved. For internal temperature readings in Phases 1 through 8, thermometers were inserted into the center of pieces or pans of meat. In Phases 10 and 11, covers on plates of food were tipped slightly, and the thermometer was inserted between the two slices of meat. Readings were made 45 sec after insertion. Time recording was made relative to a single clock to which watches were syncronized.

To provide additional information on internal temperature of meat at Phase 6, a Leeds and Northrup Speed-o-max G recording potentiometer, with 4 lead wires, was used for continously monitoring temperatures over time. This was after completion of 3 trials of study with procedures and product the same as previously described for Phases 1 through 5. The total amount of product involved was 9 pans which were placed on 5 shelves in the refrigerator. Probes were placed in the center of 4 pans on 4 different shelves including the

Table 2—Internal temperatures of beef and time in phases of product flow

Phase in product flow	Internal temperature		Time			
	Mean °C	Range °C	Mean	Range	Unit	Observations[a]
1 Roasting[b]	5.7	5 – 7	4	2.9 – 4.6	hr	9,9
	75.4	66 – 88				9
2 Holding*			26.2	13 – 84	min	3
3 Storage[c]	8.2	7 – 9	15	14.2 – 15.6	hr	9,3
4 Slicing for[c]*	10.2	9 – 12	5.2	3 – 7	min	9,9
selected galleys						
5 Holding* (Includes time in Phase 4)			62.7	53 – 70	min	3
6 Storage[c]	7.2	7 – 8	24.4	24.2 – 24.6	hr	9,4
7 Holding*			47.3	26 – 66	min	4
8 Assembly, overall*			60	54 – 68	min	3
Selected individual galleys (3)	8.9	7 – 11	21	13 – 28	min	9,9
9 Transportation* selected galleys			5	1 – 12	min	9
10 Beginning assembly* to beginning galley storage (includes transportation time)						
First galley, first cart			9	7 – 11	min	2
Middle galley, middle cart			39	36 – 45	min	3
Last galley, last cart			1.1	1.0 – 1.2	hr	3
11 Galley storage[b]						
First galley	13.9	12 – 17	2.5	2.0 – 2.8	hr	12,3
Middle galley	13.3	11 – 15	2.2	2.2 – 2.2	hr	12,3
Last galley	15.1	14 – 17	1.9	1.8 – 2.1	hr	11,3
Overall	14.1	11 – 17	2.2	2.2 – 2.8	hr	25,9
First galley	5.8	4 – 7				12
Middle galley	7.8	7 – 10				12
Last galley	6.6	4 – 8				12
Overall	6.7	4 – 10				36
12 Heating for service[c]			30[d]		sec	27
First galley	68.7	60 – 78				9
Middle galley	57.1	43 – 82				9
Last galley	63.3	43 – 93				9
Overall	63.9	43 – 93	26.8[e]	16 – 36	min	27,9

* Indicates stages where meat was held at room temperature

[a] Where two numbers are given, the first refers to temperature and the second to time

[b] First set of internal temperature readings were for the beginning of phase; second set for the end

[c] Internal temperature readings were taken at the end of phase

[d] Heating time was the same for all observations of heating individual meals

[e] Refers to total time for heating all meals on galleys

top and bottom. Recording was done continously for a 13.5 hr period beginning with placement in storage. After an additional 7 hr storage, before removing meat from the refrigerator, end-point temperature determinations were made using the same instrument. At the end of Phase 5, before adding broth, dial-type thermometers were used to record internal temperature in 4 pans of meat and in the broth.

Sensory

Sensory evaluation of roast beef was made to give indication of food quality which might be expected from an operating system of this type and to determine effectiveness of preparation procedures. Evaluations for all replications of study were made by the same 10-member trained panel of female dietitians and dietetic interns. Judgements were made through use of a score sheet with a 9-point scale and corresponding descriptive terms ranging from 9 "excellent" to 1 "extremely poor" as patterned after Peryam and Shapiro (1955) and Amerine et al. (1965). Characteristics scored were: appearance, color, texture, tenderness, juiciness, flavor, and general acceptability.

Evaluations were made within the hospital with environmental conditions as might be encountered by a hospital patient. Panelists received food from each of the 3 selected galleys as food was heated for patient service. This was for the same beef and trays as were used for recording internal temperatures at Phases 11 and 12. Each panelist always received a tray with meat for evaluation from the same position in storage and in the heating process.

Microbiological

Samples of both raw and cooked roast beef (approx 100g) were aseptically removed for microbiological examination according to a predetermined sampling plan (Table 1) and were placed in presterilized plastic bags which were packed in ice in plasticfoam ice chests for transport to the laboratory. Total time of transport for any of the samples was less than 2 hr. Ten gram portions of the meat were immediately blended in a sterile laboratory blender for 30 sec with 90 ml of sterile distilled water containing 0.05% (wt/vol) Tween 80 (polyoxyethylene sorbitan monooleate) to emulsify and disperse the blended samples, and 0.1% (wt/vol) peptone (Cremer and Chipley, 1977a, b; 1979).

Ten ml from each of the blended samples were added to flasks containing 20 ml of Trypticase Soy Broth and incubated 1 hr at 25°C. This is basically the procedure reported by Warseck et al. (1973) and Ray and Speck (1973) for the repair and enumeration of injured coliforms in frozen foods. Serial dilutions were then immediately prepared in peptone-water-Tween 80 diluent and plated onto the appropriate media.

Total microbial counts were obtained using Plate Count Agar, yeast and mold counts with acidified Potato Dextrose Agar, and staphylococcal counts with Baird-Parker Medium. In addition, coliform counts were obtained using Violet Red Bile Agar with an agar overlay and clostridial counts using Sulfite-Polymyxin-Sulfadiazine Agar with an agar overlay. Isolation and quantitation of staphylococci and clostridia were performed by the methods of Baird-Parker (1962) and Angelotti et al. (1962), respectively. In addition, isolates of staphylococci were submitted to the Ohio Department of Health for confirmation; and isolates of clostridia were streaked onto plates of tryptose sulfite cycloserine agar according to the procedure of Duncan and Harmon (1976) for subsequent identification. Proce-

dures for incubation and enumeration of all plates were the same as those given in the *Bacteriological Analytical Manual* (Olson, 1976), *Recommended Methods for the Microbiological Evaluation of Foods* (APHA, 1966), and the *Compendium of Methods for the Microbiological Examination of Foods* (Speck, 1976). All subsequent identification of microorganisms was made through procedures outlined in Riemann and Bryan (1979) and *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974).

RESULTS & DISCUSSION

Time and temperature

Time and internal temperatures of roast beef in the various phases of product flow are indicated in Table 2. Between the two phases (1 and 12) of heat processing involved within the total system, time was about 45 hr. Within the 45-hr period, meat was held at room temperature for a total time which amounted to 3.5 hr in Phases 2, 5, 7, 9 and 10 (last Galley, last cart). Influence of room temperature holding is indicated in internal temperature readings between Phases 3 and 4, 6 and 8, and 8 and 11. Temperatures in Phase 11 also reflect, to an extent, the variation in time at assembly (Phase 10). In all instances, room temperatures were 26°C.

Average internal temperatures of meat were 8.2 and 7.2°C respectively, after an average of 15 or 24-hr storage (Phases 3 and 6). However, random sampling of internal temperatures indicated that a greater temperature hazard might exist than was revealed by the end-point temperatures. Thus, subsequent to completion of 3 trials of study, a continuous recording of internal temperature was made at Phase 6. Mean values (4 probes) at the beginning of storage, at 1-hr intervals, and at the end of a 13.5-hr observation period were as follows: 25.3, 23.8, 22.8, 20.4, 18.9, 17.5, 16.2, 14.4, 13.5, 12.5, 11.7, 11.2, 10.5, 10.2, and 9.4°C. After 20 hr total storage, the mean temperature was 7.6°C. The mean internal temperature of meat (4 pans), before adding broth at the end of Phase 5, was 13.3°C and temperature of broth, immediately before adding to meat, was 40°C.

For microbiological control, recommended cooling time standards are 7.2°C in 2 hr (Rowley et al., 1972) or in 4 hr (HEW, 1978; Longree, 1972). Temperatures at Phase 6 within this operation were in gross violation of those standards and would indicate conditions highly favorable for microbial growth.

Some additional data were also collected for Phase 3. After 4 hr storage, the mean temperature for 5 pieces of meat was 27.7°C. This coupled with the fact (Table 2) that the mean temperature at the end of Phase 3 was 8.2°C suggests that the same cooling rate problem existed in the 15-hr storage at Phase 3 as in Phase 6. Data collected subsequent to completion of 3 trials of study and those in Table 2 indicated that food was held within this operating system for about 45 hr and that food temperatures reached 7.2°C for only brief periods of time, if at all.

Internal temperatures of food suggest that refrigeration equipment was not adequate for effective temperature reduction in food. Environmental (air) temperatures in refrigerators (recorded at the time of placement and removal of meat) ranged from 1.7–4.4°C at Phase 3, from 1.7–5.6°C during storage at Phase 6, and from 1.7–6.7°C at Phase 11. Longree (1972) recommended air temperatures of not more than 4.4°C during refrigerator storage.

Air temperatures alone probably did not account for the slow cooling rate of meat. This is more likely related to poor air circulation and storage of fairly large masses of food. Blast chillers were not used within the operation. Meat at Phase 3 was stored in a conventional walk-in type refrigerator and, at Phases 6 and 11, in roll-in type refrigerators built to accommodate carts with pan or tray glides. Pieces of meat at Phase 3 were fairly large and, in Phases 6 and 11, space for circulation of air around pans was limited. Refrigeration equipment utilized was built by a major manufacturer and was designed for use in the ready-prepared system. However, data on internal temperatures of food point to need for writing equipment specifications in terms of time in which a specific mass of food must reach a specific temperature, since performance of refrigeration equipment would appear to be a major factor in safe operation of this system.

As indicated in Table 2, only 2 phases of heating and no hot holding were involved in the system of operation. At Phase 1, roasting, end-point temperatures were variable, but the average temperature was above 74°C as recommended by Rowley et al. (1972). From a sensory quality perspective, a mean temperature of 75°C (Table 2) indicates well-done meat and may be an important consideration in sensory quality.

End-point temperatures at the end of heating for service in microwave ovens, Phase 12, were highly variable (Table 2), and both means and ranges in temperatures were well below 74°C as recommended for reheating cooked food (Rowley et al., 1972). Relatively low temperatures and only 60 sec heating time provide little assurance that microorganisms which may have developed during 45 hr holding would be destroyed.

Factors which have been identified as influencing heating in microwave ovens are dielectric properties of food, quantity of food heated at one time, positioning of food, and power within the oven (Copson, 1975). In this operation, food composition, portion sizes, and placement on plates and in ovens were fairly uniform but not totally controlled, since food was not weighed and food composition was somewhat variable in terms of quantities of fat, lean, and broth in portions of meat. The microwave ovens used incorporated 2 magnetron tubes and were designed to operate with 2860W of power. This should and did, in some instances, provide sufficient power for effective heating. However, a major manufacturer (Anonymous, 1978) indicates that fluctuations in line voltage will affect power output within the oven and that within health care facilities this may vary as related to location near surgical suites, intensive care units, or time of day. Temperature variability points to need for actual power checks in ovens and for insuring adequate voltage availability in installations of this type.

Microbiological

Results of the microbiological evalution of roast beef are presented in Table 3, Part A. Total plate counts indicated that the microbial quality of the meat, both raw and cooked, was good but variable among replications of sampling. After roasting, 1000–1500 fold reductions in the total microbial population were observed. However, there was a 3–11 fold increase in total numbers following storage and slicing of cooked roasts, assembly, and before heating for service after transport and storage of meals in the three galleys. These increases are logically explained by the relatively long storage times at temperatures conducive to microbial growth (Table 2) and the significant amount of contamination introduced during the slicing operation (Table 3, Part B). Following heating for service, there was a 10–25 fold reduction in the microbial population. Results, similar to those described above for total plate counts, were observed for coliforms, *Clostridium*, and yeast and molds.

Unusually large numbers of staphylococci were detected in cooked roast beef slices in the present study. An attempt was made to determine at which phase or phases an increase in the numbers of these microorganisms occurred. The data indicate (Table 3, Part B) that at the time of slicing, ample

Tabie 3—Microbial evaluation of roast beef and of meat slicing equipment

	Total plate count[a]	Staphylococci	Coliform	Clostridia	Yeast and mold
(A) Phase in product flow					
1 Roasting					
Before	1,600,000	340	19,000	104,000	220
After	1,200	10	0	270	40
4 After slicing	3,900	460	20	430	240
8 Assembly	6,300	1,600	80	510	130
12 Heating for service					
Before heating					
First galley	13,000	780	180	720	110
Middle galley	11,000	580	100	890	80
Last galley	10,000	1,100	160	720	95
After heating					
First galley	1,200	520	0	380	10
Middle galley	500	240	0	280	0
Last galley	600	480	0	310	15
(B) Description of surface swabbed					
Feed trough at base	15[b]	10	0	0	0
Carriage at base	0	0	0	0	0
Top of knife (blade)	560	330	0	150	5
Bottom of knife (blade)	2,400	630	0	850	10
Receiving platform (where sliced meat would fall)	20	0	0	0	40
Cutting board (used for trimming prior to slicing)	3,000	120	0	760	240

[a] Expressed as numbers of microorganisms/10g of product; average of 3 experiments.
[b] Expressed as numbers of microorganisms/2.5 cm^2 of surface area.

opportunity existed for the contamination of cooked meat slices by staphylococci. It was also observed that during the slicing operation, employees transferred sliced meat by hand (without gloves) into containers for subsequent storage and that these containers were placed in refrigerators in positions which would not allow for adequate air circulation and cooling during the time of storage. *Staphylococcus epidermidis* was subsequently isolated and identified from swabbed surfaces of the meat slicing blade, from the cutting board adjacent to the meat slicer, from the hands of the employees operating the meat slicer, and from meat after slicing. This was confirmed when isolates from the above sources were submitted to the Ohio Department of Health. The isolation of unacceptably large numbers of this microorganism from these sources is of concern since it is well-established that *S. epidermidis* can cause infections in humans and can become resistant to multiple antibiotics.

Subsequent identification procedures revealed a wide variety of microorganisms associated with all samples of raw meat. These included *Clostridium sporogenes, C. perfringens, Bacillus* sp., micrococci including *Micrococcus luteus, Staphylococcus aureus, S. epidermidis, Escherichia coli, Enterobacter aerogenes, Salmonella typhimurium, S. enteriditis, Proteus* sp., *Pseudomonas* sp., *Alcaligenes* sp., and *Falvobacterium* sp. Yeasts and molds included species of *Cladosporium, Penicillium*, and *Alternaria*, as well as *Aspergillus niger*. Microorganisms identified in the present study have previously been described by Weiser et al. (1971) as occurring naturally in raw meats.

Samples of meat after roasting and after heating for service were found to contain *Bacillus* sp., *Clostridium sporogenes, C. perfringens, Staphylococcus epidermidis*, and *S. aureus*. Small numbers of *Aspergillus niger, Aspergillus* sp., *Cladosporium* sp., and *Penicillium* sp. were isolated from both meat after roasting and after heating for service (an average of 1/g of cooked meat) as well as from swabbed surfaces of the meat slicer and cutting board (averages of 1–24/2.5 cm^2 of surface area). Since cooked meat should have been processed at temperatures sufficiently high to kill both staphylococci and fungi, their presence would indicate post-processing contamination. Also the internal temperatures reached during the roasting phase were, in actuality, end-point temperatures (Table 1); after reaching these temperatures, products were removed and placed in a storage refrigerator for cooling. Thus, while the end-point temperatures were sufficient to kill staphylococci and fungi, the actual time of exposure of roasts to these temperatures was minimal. A similar situation existed when sliced roasts were microwave heated for subsequent service where wide ranges of end-point internal temperatures were noted (Table 2).

Bacillus sp. and *Staphylococcus* sp. have previously been isolated from several frozen cooked meat entrees that had been reheated for service (Causey and Fenton, 1951). Tuomi et al. (1974) reported that *Clostridium perfringens* could grow and multiply in ground beef gravy at 50°C. However, when 74°C was the final temperature to which the gravy was heated, no viable cells were found. Reports of the survival of low numbers of coliforms and staphylococci in spaghetti, chili, precooked hamburger patties, and meat loaf have also been published (Cremer and Chipley, 1977a, b; 1979).

Bryan (1978a, b) stated that inadequate refrigeration and preparing foods far in advance of planned service (when coupled with inadequate holding or cold storage of foods) are the two most common factors that contribute to foodborne disease outbreaks. Data from the present study clearly indicate the need for establishment of precise microbiological standards and thorough monitoring of phases within the operating system.

Sensory

Data on sensory evaluations of beef are presented in Table 4. For each characteristic evaluated, least squares analysis of variance was used to determine differences in scores according to judge and trial of study. No significant ($P < 0.05$) differences were found. Scores indicated that

Table 4—Mean scores[a] for sensory evaluations of roast beef

Characteristic	Trial 1	Trial 2	Trial 3	Overall
Appearance	7.2	6.5	7.1	6.9
Color	6.9	7.3	7.1	7.1
Texture	7.1	6.6	7.0	6.9
Tenderness	7.2	6.6	6.0	6.6
Juiciness	7.7	7.4	7.5	7.5
Flavor	7.8	7.5	7.3	7.5
General acceptability	7.2	6.9	7.1	6.8

[a] Scale of 9—1 with 9 being optimum; mean of 10 observations per trial with 30 overall

quality was generally "good, 7" as related to the scale used for evaluation (Peryam and Shapiro, 1955; Amerine et al., 1965). Relatively high scores for juiciness and flavor (Table 4) may indicate a positive effect of the broth added and allowed to gel on meat. Although differences were not significant, the range in mean scores for tenderness were relatively wide and may reflect differences in roasting time and end-point temperature. For Trials 1, 2, and 3 respectively, mean internal temperatures were 82.8, 73.9, and 69.4°C with respective roasting times of 4.6, 4.0, and 3.4 hr. Although scores are not at the top of the evaluation scale, data on sensory quality of beef were generally positive for this system of operation. Control of temperature and microbiological quality of meat may be more critical factors in the use of this system of operation.

Recommendations and critical factors for control

Data from this study indicated that for safe, effective operation of the ready-prepared type foodservice system, the following practices should be observed: 1. Food should be thoroughly cooked or heated; this involves (a) selection of microwave ovens with adequate power, (b) routine check on actual power within microwave ovens, and (c) uniformity in food portion sizes, composition, and placement. 2. Rate of cooling food after initial or subsequent heat processing, prior to heating for service, must be controlled; (a) equipment should be selected which is adequate to cool the required volume of food in an appropriate period of time. 3. Contamination of food by either food handlers or equipment must be prevented; particularly in the stages after initial cooking; (a) employees should wear gloves and (b) equipment should be sanitized. 4. Time when food is held at room temperature during processing, transportation, and holding should be quite limited or equipment should be provided to keep food cold. Careful monitoring of these factors is critical if public health hazard is to be avoided.

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MINERAL CONTENT AND PROXIMATE ANALYSIS OF BROILER MEAT FROM TWO STRAINS AND THREE REGIONS OF PRODUCTION

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ABSTRACT

Contents of moisture, fat, protein, and eight minerals in commercial broiler breast and thigh meat were defined by analyzing 15-bird lots in a factorial design of two sexes × two commercial strains × three widely separated regions of production. Small but statistically significant differences due to strain, sex, and region were found for many of the variables examined. Region significantly influenced all the element levels except phosphorus in both breast and thigh flesh. Calcium levels found were about half those previously reported. The differences are probably not nutritionally important under normal consumption patterns.

INTRODUCTION

CHICKEN MEAT constitutes an important protein source in the American diet. Approximately 23% of the meat consumed by the average American in 1978 was chicken (USDA, 1979), and of this over 90% was broilers or fryers. Consumers are currently expressing a strong interest in food compositional data, but the data on broiler meat in the literature are limited and are not adequately representative of modern broilers and production practices.

Modern processing and marketing technology are such today that broilers and/or broiler meat products consumed in any given area at any given time may have been produced at different and/or distant points and, further, the feedstuffs consumed by the birds may have come from even more diverse areas and have different compositions. The influence of certain ingested fats or fat-soluble materials on depot fats has been known for some time (Cruickshank, 1934; Fritz and Wharton, 1957). Likewise, the grow-out area or the environment may influence body composition (Kubena et al., 1974).

It therefore seemed desirable to determine what, if any, important differences exist in nutrient content between broilers of different strains produced in different geographical areas of this country that may be of significance to the consumer. This report provides proximate analyses and mineral contents for two strains of broilers grown out in three geographical areas by the same general management system, information that, to our knowledge, has not been available up to this time.

EXPERIMENTAL

BROILERS were obtained from a large integrated broiler processor with production facilities in several areas. Fifteen male and 15 female broiler carcasses of the same age from two strain crosses and from production facilities in East Texas (TX), North Carolina (NC) and Delmarva (DMV) were obtained. All birds were fed regular commercial feed that was formulated to supply exactly the same level of nutrients per pound of feed. However, ingredients were from different sources, and the feed was manufactured in three different mills. Carcasses were individually placed in polybags, packaged 15 birds of same sex and strain to the case and stored at −34.4°C until needed. Before analysis, carcasses were removed from the cold room in case lots, and placed in a 2.2°C room until they had thawed enough to be cut up. Carcasses were divided into 5-bird lots of as equal gross weight as possible. Breast meat was removed after removing the breast skin and cutting the meat loose from the bones. Thigh flesh was collected in a similar manner. Skin and bone were left attached to the remainder of the carcass. Care was exercised to leave most of the visable fat tissues with the skin portion. The meat in 5-bird lots was diced into 2-cm cubes, run through a home kitchen-type meat grinder with a coarse blade, then again with a fine blade.

Proximate analysis

For dry weight determination about 10g of raw meat was placed in a tared beaker and heated at 102°C until the residue maintained a constant weight. The remaining meat was frozen in shallow containers, then freeze-dried. The freeze-dried meat was crumbled, placed in 55-mm dried tared Soxhlet thimbles, and the thimbles with contents dried and weighed. Fat was removed by 18-hr extraction with petroleum ether. The ether-fat mixture was dried over a steam bath and the fat weighed. As a check, the thimbles and extracted contents were dried and weighed. The dried defatted meat sample was ground in a Wiley mill, placed in glass containers, flooded with anhydrous nitrogen gas, and stored at −34.4°C for further analyses. From this defatted, dried sample, aliquots were taken for determinations of Kjeldahl nitrogen, ash, and the selected elements.

Ash and elemental analyses

Two to three grams of the dried defatted samples were weighed (to 0.1 mg) into Vycor crucibles. Samples were charred at 250°C for 2 hr. then ashed at 500°C for 16 hr. After cooling, a measured amount (usually 1 ml) of 50% HNO_3 was added. The sample was again charred, then ashed at 500°C for 1 hr. If the ash was white, it was cooled in a desiccator and weighed for ash determination. If specks of grey or black remained, an additional ml of HNO_3 was used and the drying and ashing steps repeated. A blank was carried to determine residue from HNO_3 additions. The ash was solubilized in concentrated HNO_3, then diluted so that final dilution of 100 ml contained 10% HNO_3. The diluted solution was then analyzed for 8 elements (see Table 2) with a Perkin Elmer 403 atomic absorption Spectrophotometer operated according to manufacturer's recommendations, except for P. Phosphorus was determined spectrophotometrically (at 880 nm) as the blue antimony-phosphate-molybdate complex (EPA, 1974).

RESULTS & DISCUSSION

AVERAGE CARCASS WEIGHTS w/o giblets for the 56-day old birds used in this study were 1481g (± 66) for males and 1147g (± 23) for females. The means (duplicate runs on 3 groups of 5 birds each) of all groups for proximate analysis of breast and thigh meat are given in Table 1. One lot of birds (female H&H cross from Delmarva) was lost and could not be replaced. Data on elemental analyses for the same groups are given in Table 2. The high Fe value of 11.8 ppm found in the breast meat of sample NC HH F birds may have been due to contamination from the grinder, since this sample was the first to be ground.

Input data from Tables 1 and 2 were subjected to least squares analysis of variance using the system of Barr et al. (1976). No statistical comparison was made between breast and thigh. Small but significant differences were noted between the means for many of the values found. These significant differences including interactions are recorded in Table 3 for proximate analysis and in Table 4 for the elemental analysis. That so many significant differences were found may be partially explained by the large sample size

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Table 1—Proximate analysis of breast and thigh meat (on wet weight basis) of birds grown under similar conditions at different locations[a]

Region/Strain/Sex			Breast meat (%)				Thigh meat (%)			
			Water	Fat	Ash	Protein	Water	Fat	Ash	Protein
NC	HH	F	72.7	0.90	1.18	24.9	73.2	6.92	1.03	18.7
NC	HH	M	72.6	1.07	1.26	25.2	72.7	7.24	1.06	18.9
NC	RA	F	73.9	0.86	1.11	23.9	72.9	8.47	0.95	17.4
NC	RA	M	75.2	0.88	1.16	22.9	73.3	8.37	1.00	17.3
TX	HH	F	74.8	0.70	1.07	23.3	73.4	7.24	0.95	18.1
TX	HH	M	74.7	0.97	1.13	23.2	72.7	7.62	1.01	18.4
TX	RA	F	74.7	0.94	1.11	23.5	72.5	8.53	0.91	18.0
TX	RA	M	75.0	0.81	1.12	23.1	72.8	7.89	1.01	18.3
DMV	HH	M	74.2	0.95	1.11	23.8	72.5	7.04	1.04	19.3
DMV	RA	F	74.1	0.83	1.23	23.8	72.4	7.47	1.11	18.3
DMV	RA	M	73.7	0.87	1.14	24.3	72.2	7.62	1.05	18.9
	mean		74.2	0.89	1.15	23.8	72.3	7.67	1.02	18.32
coefficient of variation			0.53	12.8	2.5	1.70	0.39	7.6	2.7	2.9

[a] Each value is the mean of duplicate analyses of three 5-bird composite samples. Strains: HH = Hubbard X Hubbard, RA = Ross X Arbor Acre. Regions: NC = North Caroline, TX = Texas, DMB = Delmarva.

Table 2—Elemental analyses of breast and thigh meat (wet weight basis) of broilers grown under similar conditions at different locations[a]

Region/Strain/Sex			Breast meat (ppm)								Thigh meat (ppm)							
			Cu	Fe	Zn	Mg	Ca	Na	K	P	Cu	Fe	Zn	Mg	Ca	Na	K	P
NC	HH	F	0.45	11.8	6.5	328	31	450	3300	2370	0.49	14.0	14.9	240	32	640	2620	1790
NC	HH	M	0.39	6.7	7.4	300	32	500	2985	2260	0.72	11.4	17.1	230	39	670	2460	1800
NC	RA	F	0.72	6.7	6.8	290	40	510	2790	2210	0.76	13.4	15.7	200	45	680	2180	1660
NC	RA	M	0.38	6.2	6.6	280	35	500	2600	1990	0.53	9.5	15.7	210	39	660	2110	1500
TX	HH	F	0.40	4.5	5.8	300	38	360	2700	2180	0.80	9.7	14.8	220	41	510	2280	1720
TX	HH	M	0.58	4.6	6.0	290	41	380	2720	2220	0.72	10.0	15.9	220	43	540	2330	1810
TX	RA	F	0.55	5.6	6.1	290	49	480	2630	2390	0.83	11.5	14.7	210	49	650	2150	1810
TX	RA	M	0.67	4.8	6.0	290	51	420	2620	2110	0.84	8.9	15.3	220	54	540	2300	1630
DMV	HH	M	0.41	7.8	7.3	300	32	550	2800	2450	0.67	13.1	17.6	240	39	720	2500	1830
DMV	RA	F	0.50	8.0	6.9	300	44	470	2870	2230	0.68	12.9	16.0	220	43	580	2420	1780
DMV	RA	M	0.37	7.1	7.2	300	32	510	2940	2210	0.62	12.0	17.2	230	35	640	2470	1830
mean			0.49	6.7	6.6	297	39	465	2810	2240	0.70	11.5	15.8	220	42	620	2350	1740
coefficient of variation			23	24	3.7	2.6	8.4	4.4	2.5	5.4	12	11	3.5	3.8	7.3	5.2	5.0	6.4

[a] Each value is the mean of duplicate analyses of three 5-bird composite samples. Strains: HH = H ubbard X Hubbard, RA = Ross X Arbor Acre. Regions: NC = North Carolina, TX = Texas, DMV = Delmarva.

Table 3—Levels of significance of strain (S), sex (Sx), and production region (R) on proximate analysis of broiler breast and thigh meat

Variable	Water	Fat	Ash	Protein
Breast				
S	**			**
R	**		**	**
Sx		*		
S X Sx	**	**		*
S X R	**		**	**
R X Sx	**		**	*
S X R X Sx				
Thigh				
S		**		**
R		*	**	*
Sx				
S X Sx				
S X R			**	*
R X Sx			**	
S X R X Sx				

* $P \leq 5\%$
** $P \leq 1\%$

Table 4—Levels of significance of strain (S), sex (Sx), and production region (R) on element analysis of broiler breast and thigh meat

Variable	Cu	Fe	Zn	Mg	Ca	Na	K	P
Breast								
S	*			**	**	**	**	*
R	*	**	**	**	**	**	**	
Sx		*	**		*	*	**	
Sx X S			**			**		*
S X R		*		**	*	**	**	
R X Sx	**			*	**	**	**	
S X R X Sx		*						
Thigh								
S			*	**	**		**	
R	**	**	**	*	**	**	**	
Sx		**	**					
S X Sx	*					**		*
S X R				*	*	*	**	*
R X Sx		*			*	**		
S X L X Sx	**				**			

* $P \leq 5\%$
** $P \leq 1\%$

Table 5—Comparison of values from this report with earlier reports on proximate and select elemental analyses (wet weight basis) of broiler breast and thigh meat[a]

	Breast				Thigh			
	A	B	C	D	A	B	C	D
Moisture (%)	74.2	74.8	74.3	76.0	72.8	75.8	72.1	75.5
Fat (%)	0.89	1.24	1.82	2.4	7.67	3.91	7.77	5.6
Protein (%)	23.8	23.1	22.4	20.8	18.3	18.1	18.3	18.1
Ash (%)	1.2	1.02	–	0.8	1.02	0.96	–	0.8
Ca (mg/100g)	3.9	11.0	8.7	11	4.2	10	9.8	12
P (mg/100g)	224	196	230	214	174	168	295	186
Fe (mg/100g)	0.67	0.72	0.55	1.2	1.1	1.04	0.95	1.6

[a] A = Means for this paper; B = CFEI USDA Handbook 8-5, Aug. 1979; C = Hamm et al., (1980) weighted means from 2 samples of local broilers (Athens, GA); D = USDA Handbook 8 data (1963).

and the low coefficients of variation found for most items. Of 24 variables, only 5 had coefficients greater than 10% and of these only 2 were above 12.5%. With our pooled 5-bird samples, at a coefficient of variation of 10%, a sample size of 16 would be needed to develop a mean within 5% of the true mean at 95% probability level. The influence of the region of production was statistically apparent in levels of all elements checked except P. In both thigh and breast meat, strain influenced the levels of Mg, Ca, and K and the interactions of strain and region influenced Mg, Ca, Na, K, and P levels.

The mean values found in this study are in general agreement with those for corresponding fryer pieces reported elsewhere (Watt and Merrill, 1963; CFEI, 1979, 1979; Hamm et al., 1980) except for fat and Ca. In Table 5 we list those elements for which comparisons can be made. That the fat content is different is not unexpected considering the high variability found in this report and since to some extent the fat content reported could be influenced by method of sample preparation (Hamm et al., 1980). The reduced levels of Ca found in this study remain unexplained at this time. The significant influence of region on Ca levels points to the involvement of some regionally varying factor on Ca levels.

There is probably no effect, from a consumers standpoint, on the nutritional quality of broilers produced in these different regions. One hundred grams of raw broiler breast meat from any of the areas checked will yield from 22.6–24.7g of high quality protein which will satisfy 42–46% of the Recommended Daily Allowance (RDA) for protein for the average 19- to 22-year-old male. The thigh, with its lower protein content, would provide less than this amount. Of the minerals checked and for which RDAs have been established, P is of greatest interest. One hundred grams of broiler breast meat will supply between 24.9 and 30.6% of the RDA for the male mentioned above. For rapidly growing youth and pregnant or lactating females, the P requirement is increased by 50%, resulting in a 33% decline in the percentage of RDA met by broiler meat. Thigh meat, which is slightly lower in P than breast meat, will supply from 20.4–22.9% of RDA. Other minerals on the RDA list for which we made analyses are supplied at less than 10% adult RDA per 100g chicken meat.

Although we found that breast and thich meat from broilers of two strains raised in different areas contain statistically significant differences in amounts of various elements and proximate analysis, we do not believe that these differences are nutritionally significant for the consumer. That those differences do exist must be accepted, and further, it remains to be determined if the differences due to sex, strain, or region are magnified or reduced in cooked products.

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PRODUCT QUALITY AND ENERGY USE IN HIGH TEMPERATURE SMOKING OF FISH

P. KOSAK and R. TOLEDO

ABSTRACT

A process for heating smoked fish was developed such that the lethality of the heating process is equivalent to a 12 decimal reduction of spores of *Clostridium botulinum* Type E. This lethality is similar to the specifications in the Food & Drug Administration smoked fish Good Manufacturing Practice regulations. The requirements for a fish smoking oven that would be necessary to carry out the process were also determined. The staged temperature process, which started at 160°F (71.1°C) dry bulb and 140°F (60°C) wet bulb, with wet and dry bulb temperatures increases of 10°F (5.6°C) in 10 min increments until the internal temperature of the fish reached 194°F (90°C), minimized undesirable defects in the smoked fish product when compared with other smoking (process) schedules. The energy consumption of the recirculating air oven, for the high temperature staged process (186.482 MJ) was found to be twice that for a constant temperature 71.1°C process (92.337 MJ) with most of the energy used attributed to the generation of steam used for humidification, and heat losses through the uninsulated ductwork. A heat balance on an oven with steam humidification and a heating element capacity of 35.5 kW showed that only 45.5 kg of product can be processed effectively using the staged temperature process.

INTRODUCTION

AMONG THE FOOD poisoning organisms, the nonproteolytic strain of *Clostridium botulinum* Type E is considered to be the most hazardous in refrigerated food products because of its ability to grow and produce toxin at temperatures as low as 5°C. In smoked fish, toxins were produced by the organism at 10°C regardless of whether the fish were packaged under a vacuum in plastic film, or in open packages (Kautter, 1964). Thus, oxygen availability around the product cannot be used as a method for controlling the hazards from the organism. Botulism outbreaks from smoked fish occurred in 1960 and 1963, and *Clostridium botulinum* Type E was identified as the causative organism in these outbreaks (Pace et al., 1972).

In the years 1965 to 1972, the concept of using heat as a means of reducing the incidence of viable spores of *Clostridium botulinum* Type E in smoked fish products was explored extensively (Roberts and Ingram, 1965; Christiansen et al., 1968; Crissley et al., 1968; Pace et al., 1972; and Pace and Krumbiegel, 1973). Based on data generated by these investigators, the time and temperature relationships necessary to reduce the incidence of spores of the organism in the processed products were defined and promulgated in regulations passed by the city of Milwaukee and the state of Wisconsin (Pace and Krumbiegel, 1973). In 1972, the Food & Drug Administration issued a Good Manufacturing Practice regulation (GMP) for smoked and smoke flavored fish, that specified a heat treatment schedule (Federal Register, 1972). Ever since the adoption of the smoked fish GMP, the smoked fish industry has consistently argued that the times and temperatures specified in the regulation result in a virtually unmarketable product. In December 1977, the Court of Appeals for the Second Circuit ruled against the FDA on enforcement of the smoked fish GMP. However, the hazards from botulism in smoked fish are real, and every effort must be made to minimize this hazard. The present study was conducted to determine a process for heating smoked fish such that the lethality of the heating process would be equivalent to a 12 decimal reduction of spores of *Clostridium botulinum* Type E. The requirements for a fish smoking oven that would be necessary to carry out the process are also determined.

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MATERIALS & METHODS

Smokehouse

A recirculating (10 air changes/min) gas-fired smokehouse (Alkar, Lodi, Wis.) was used. The smokehouse had inside dimensions of 1.37 × 1.70 × 2.44 m. The surface areas available for heat transfer to the surroundings were 13.4 m^2 of 16 gage thick carbon steel wall, uninsulated ductwork for air recirculation, 2.3 m^2 of 0.635 cm thick steel plate resting directly on a concrete slab floor, and 17.3 m^2 of walls consisting of a double wall of 18 gage type 304 stainless steel with 7.62 cm fiberglass insulation between the inner and outer walls. Exhaust air velocity measured at the 20.32 cm diameter exhaust duct averaged 4.63 m/s when the smokehouse was operating with both intake and exhaust dampers fully open, and 0.8 m/s when both intake and exhaust dampers were at their minimum opening. A gas meter was installed to monitor the gas consumption. Maximum heating capacity for the gas burners was 120,000 BTU/hr (35.35 kW). Gas in the Athens, Ga. area has a reported heating value (Georgia Natural Gas Company) of 1030 BTU/ft^3 or 38.34 MJ/m^3. The steam supply for humidification delivers 0.0227 kg/s of steam at 60 psia pressure with the control valve fully open.

Preparation of fish for smoking

Mackerel, mullet, and croaker were purchased from a local fish market. All fish were purchased on the day of arrival of shipments from suppliers and were verified to be ice-packed unfrozen fish. The fish were cleaned, and were either filletted, or butterfly cut. The croaker was pan dressed. During the development of the staged temperature process it was observed that fish processed at high temperatures showed reduced incidence of shape distortion when the skins were slit longitudinally with at least 3 slits spaced uniformly at each side. The slits were shallow and had just enough depth to barely penetrate the skin. The fish were then immersed in 24% (w/w) salt solution at 20°C for 3.5 min. The brined fish were hung on hooks from the larger end of the fish, and allowed to drip overnight in a refrigerated room at 34°F (1.1°C).

Smoking

The fish were transferred to the smokehouse. Thermocouple made from standard thermocouple wires (Type T, 24 gage, Teflon insulated, Thermoelectric Inc., Saddlebrook, N.J.) by fusing the measuring junction using an oxyacetylene torch were installed in the center of the thickest portion of the largest fish in each batch. The thermocouples were connected to a multipoint recorder (Honeywell). The fish were then cold smoked, with the smokehouse heater off, for 3.5 hr. Heat from the smoke generating unit and from the recirculating blower raised the smokehouse temperature to 112°F (44.4°C) dry bulb and 102°F (38.9°C) wet bulb temperature during this cold smoking process.

Following the cold smoking, the fish were hot smoked using either of the following four schedules:

1. 160°F (71.1°C) dry bulb and 140°F (60°C) wet bulb for one hour (Deng et al., 1974).

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Table 1—Hunter color values for butterfly mullet and mackerel fillets

	Process	Butterfly mullet Hunter color values			Mackerel fillets Hunter color values		
		L	a	b	L	a	b
1.	160°F 60 min	29.5 ± 1.4	10.8 ± 1.4	10.2 ± 2.2	29.1 ± 2	12.1 ± 1.2	12.9 ± 1.2
2.	160°F 30 min 200°F 35 min	26.6 ± 2.6	10.3 ± 2.2	5.4 ± 2.2	26.0 ± 2.6	9.8 ± 1.7	6.4 ± 1.3
3.	200°F 55 min	20.3 ± 1.9	6.7 ± 1.2	2.8 ± 0.7	21.8 ± 2.9	7.2 ± 1.2	2.2 ± 0.8

2. 160°F (71.1°C) dry bulb and 140°F (60°C) wet bulb for 30 min followed by 200°F (93.3°C) dry bulb and 180°F (82.2°C) wet bulb to an internal temperature of 192°F (88.9°C, 45 min) (Chan et al., 1975).
3. 200°F (93.3°C) dry bulb and 180°F (82.2°C) wet bulb for 80 min.
4. Staged temperature process. Starting at 160°F (71.1°C) dry bulb and 140°F (60°C) wet bulb, the dry and wet bulb temperature settings were raised in 10°F (5.6°C) increments every 10 min until dry bulb temperature reached 210°F (98.9°C). Heating was continued at this temperature until the internal temperature of the fish reached 194°F (90°C). Smoke generation was discontinued when the smokehouse dry bulb temperature exceeded 180°F (82.2°C). The total process took 55–75 min depending upon thy size of the fish.

Analysis

Moisture was analyzed using a vacuum oven (80°C at 25 in. Hg vacuum for 12 hr).

Color was evaluated by excising a 2 cm × 2 cm area of the surface, placing this thin sample area over a glass plate, overlaying with transparent plastic film and determining reflectance readings on a Hunter color difference meter.

Defects were evaluated by visul comparison with the moderately heat processed (160°F, 60% RH) controls.

RESULTS & DISCUSSION

Defects in high temperature processed smoked fish

Smoking fish at high temperatures result in excessive darkening. Table 1 shows Hunter color values for fish smoked using smoking schedules 1, 2, and 3. Visual inspection of the products showed that schedule 1 gave the best color and this was in agreement with the results of Deng et al. (1974). The data for schedules 2 and 3 show progressive darkening (decrease in L value), a shift from yellow towards blue (decrease in b value) and a shift from red to green (decrease in a) with prolonged exposure to high smoking temperatures.

A major problem that results from using high heat treatments for smoking is deformation of the product. Figure 1 shows various types of defects in high heat processed smoked fish. Figure 2 shows what normal forms of the products are like and are shown for comparison. Curling is a common occurrence, but this is not a serious defect since laying the fish on a flat surface while it is warm and allowing it to cool while in this position usually allows the product to regain its normal shape upon cooling. The most serious defect is splitting. If the fish are processed with the backbone (pan dressed or butterfly cut), splitting usually occurs by separation of the flesh from the backbone. In a very severe case where splitting and curling both occur, the product takes on a grotesque appearance. With fillets, a common problem is tearing of the flesh at the point where they are hooked for hanging during smoking, and cracking at the longitudinal line where the two groups of muscles that make-up the two halves of the fillet, meet.

Table 2 shows the percentage of defective fish in batches processed using the different smoking schedules. The number of fish in each batch during the test was at least 50 fish or 100 fillets. The totals do not add to 100% because some fish exhibited both curling and splitting. The designation "slight" for the defect indicate that the product is still acceptable and the defect can be alleviated by laying the fish flat while warm and cooling them while in this position. The fish used in these tests were small (450–700g in the round), therefore there was no problem of fish falling off the hooks during smoking. Again, the severity of the heat treatment is reflected in the higher percentage of defects. The extent of defects was highest in whole fish (pan dressed) and least in fillets. Schedule 3 resulted in almost all fish showing some type of defect. Schedule 2 could be used for fillets, but the process induced a large percentage of unacceptable defects in fish that contain the backbone (pan dressed or butterfly cut).

Fig. 1—Product distortion during high temperature smoking of fish.

Fig. 2—Fish smoked at 71.1°C and 60% relative humidity for 1 hr showing normal appearance.

Table 2—Percentage of defective fish

		Abnormalities Butterfly Mullet/Mackerel Fillets/Whole Croaker				
		Hook lose	Slt. curl	Sev. cur	Slt. split	Sev. split
1.	160° F 60 min	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
2.	160° F 30 min 200° F 35 min	0/0/0	40/40/50	30/0/50	70/30/10	30/0/80
3.	200° F 55 min	0/0/0	0/50/20	100/10/80	30/60/0	70/10/100

Development of the staged heating process

Temperature of fish exposed to constant smokehouse temperatures (Fig. 3) show the most rapid rates of increase the first 10 minutes of exposure to air at a given temperature. It was hypothesized that the slow rate of heating at the later stages of a constant temperature process result in wide temperature variations between the interior and the exterior of the product resulting in uneven dehydration and shrink and could be responsible for the shape distortion. The ideal heating curve that would subject both interior and exterior of the fish to the least difference in severity of heat treatment, would be one where the process temperature is staged in such a manner that the internal temperature of the product increases at a constant rate throughout the process. Based on this premise, the process represented by the dry and wet bulb temperatures shwon in Figure 4 was developed. The temperature was staged in 10°F (5.55°C) increments every 10 min. The internal temperature of the fish showed an almost constant rate of increase following a short lag, during the time the smokehouse temperature was increased in stages. Figure 5 shows the lethality of this process towards spores of *Clostridium botulinum* Type E. The area under the curve is log (N_o/N), the number of decimal reductions of spores and is represented by Eq (1):

$$\log \frac{N_o}{N} = \int_0^{\theta} \frac{1}{D_T} d\theta \qquad (1)$$

D_T is the decimal reduction time at temperature T, and is evaluated using Eq (2) θ = heating time.

$$D_T = D_{82.2} (10)\, 82.2/z \qquad (2)$$

The values for D at 82.2 and z were taken from Crissley et al. (1974) and these were 2.5 min and 6°C, respectively. These values were the basis for the smoked fish GMP (30 min at 180°F, equivalent to a 12 decimal reduction of spores of *C. botulinum* type E) therefore, the lethality of the heat treatment for the process shown in Figure 5 (Processing schedule 4) is equivalent to that specified in the smoked fish GMP.

Table 3 shows Hunter color values and extent of defects for fish processed using processing schedule 4. The color is lighter than those obtained using the other smoking schedules, and there is not as much change in Hunter color value for a and b compared to the control process (schedule 1) as those processed using schedules 2 and 3. Table 3 also shows that the defects resulting from the heat treatment in the staged temperature process, were the least when compared to schedules 2 and 3. However, although the process shows feasibility for commercial processing, careful handling of the smoked fish must be done after smoking to correct the slight curling and prevent break-up of the smoked fish.

—Continued on next page

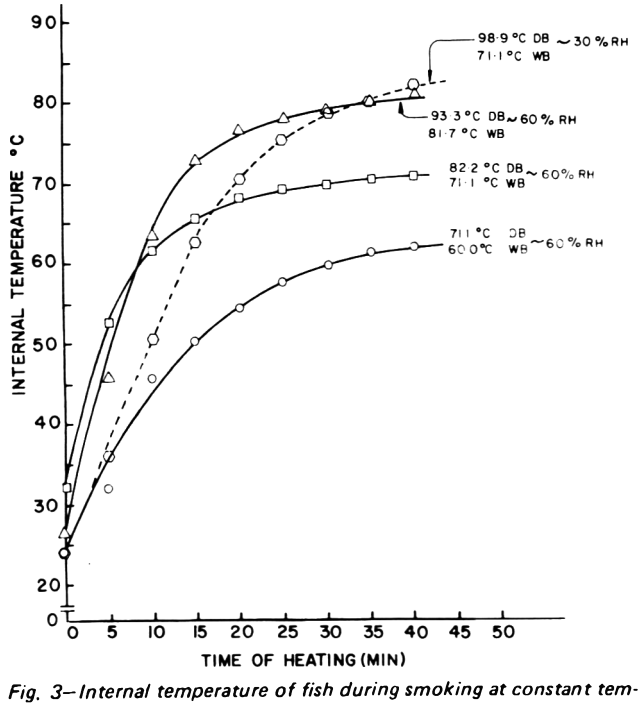

Fig. 3—Internal temperature of fish during smoking at constant temperature and humidity.

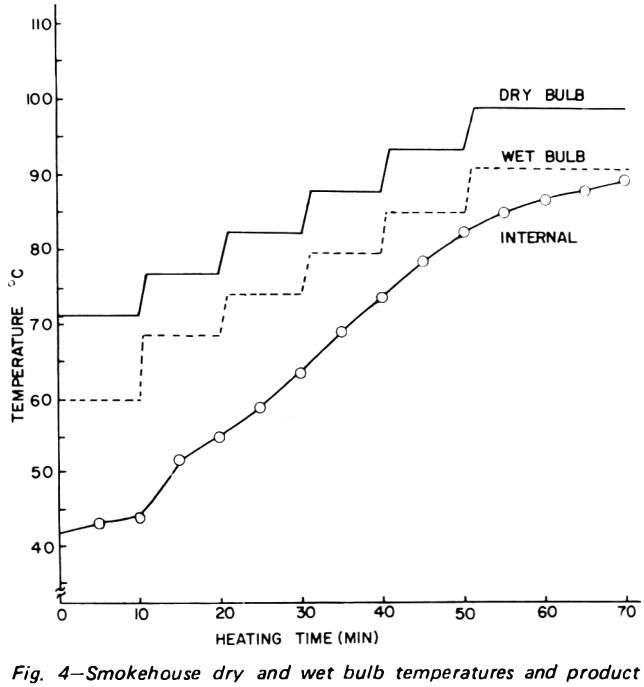

Fig. 4—Smokehouse dry and wet bulb temperatures and product internal temperatures in a staged heating schedule.

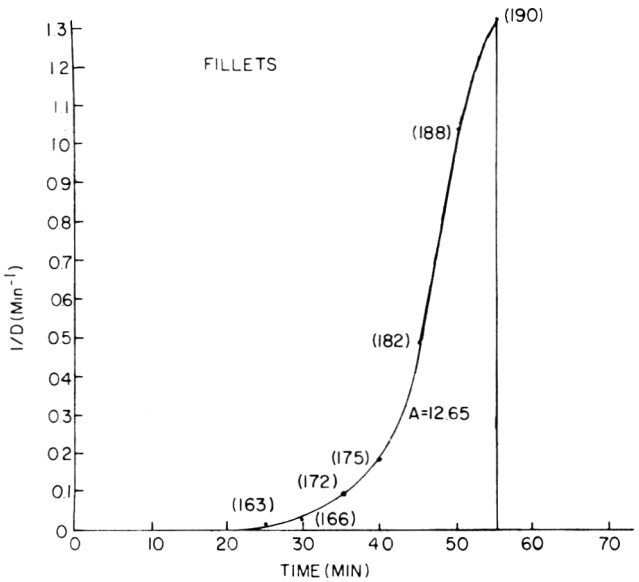

Fig. 5—Lethality of the heat treatment in the staged smoking schedule towards spores of C. botulinum type E.

Requirements of fish smoking ovens for high temperature processing

In order to reach product internal temperatures high enough for microbiological inactivation within a short period of exposure to high air temperatures in the smoking oven, the oven must have adequate heating capacity, and adequate temperature and humidity controls. Control of humidity is essential because product temperatures level-off at the wet bulb temperature of the air. Very little information is available in the literature on the heating requirements for smokehouses, particularly for processes at high temperatures and high humidities. Traditional techniques for estimating overall heat transfer coefficients are unreliable because of the uncertain airflow patterns in the oven, which makes it difficult to estimate the heat transfer coefficients. Furthermore, with direct fired gas heated smoking ovens, venting is required to discharge the products of combustion and the rate of venting cannot be readily estimated.

For an empty smoking oven equipped with a blower and ductwork for air recirculation, the data in Table 4 show energy consumption patterns, and these data can be used to calculate the heat conductance of the oven walls. The value of the heat conductance (product of the overall heat transfer coefficient, U, and the total area available for heat transfer, A) is 258 ± 14 W/K. This value is close to a calculated value of 250 W/K if an inside local heat transfer coefficient of 200 $W/m^2 \cdot K$ and an outside local heat transfer coefficient of 20 $W/m^2 \cdot K$ is used in the calculation, and the oven floor area is assumed to be transmitting heat at the same rate as the oven walls.

To estimate the heat capacity of the oven walls for estimation of the sensible heat gain during a step change in temperature, the data shown in Table 5 were collected. The whole system was found to have a heat capacity (product of the specific heat C_o, and the weight of the metal that contain the air within the oven, W_o) of 0.265 ± 0.04 MJ/K. This experimentally determined value compares with a value of 0.270 MJ/K calculated for both inside and outside oven walls steel plate floor and ductwork if specific heats of 447 and 502 J/kg·K and densities of 7860 and 8020 kg/m^3 are used for carbon steel and stainless steel, respectively.

The data on heat conductance and heat capacity of the oven walls, can be used in a heat balance to determine the maximum quantity of product that can be processed in the oven knowing the heating capacity. A similar procedure can be used on other types of smoking ovens, to determine the product loading capacity for a particular oven.

Table 3—Hunter color values of butterfly mullet and mackerel fillets

		Buterfly mullet Hunter Color Values			Mackerel fillets Hunter Color Values		
	Process	L	a	b	L	a	b
3.	Staged	33.5 ± 2.4	7.5 ± 1.2	8.3 ± 1.4	33.7 ± 1.7	8.1 ± 1.6	8.4 ± 1.9

Abnormalities
Butterfly Mullet/Mackerel Fillets/Whole Croaker

	Process	Hook lose	Slt. curl	Sev. curl	Slt. split	Sev. split
3.	Staged	0/0/0	90/10/90	0/0/0	20/0/30	0/0/0

Table 4—Energy balance for an empty oven at constant air temperature

Temperature °C			Gas consumed	Heat quantities (kW)			
Internal T	Ambient T_a	ΔT $T-T_a$	m^3/min V_g	input $(q_c)^c$ $(V_g \Delta H_g)/60$	vent $(q_v)^b$ $WC_p\Delta T$	net (f) q_c-q_v	Conductance[a] UA, W/K $q/\Delta T$
71.1	26.6	44.5	0.0256	16.358	4.115	12.243	275
76.6	29.4	47.2	0.0309	19.719	7.338	12.38	262
82.2	33.3	48.9	0.0297	18.978	7.337	11.64	238
87.8	33.3	54.5	0.0332	21.215	8.050	13.16	241
93.3	33.3	65.5	0.0380	24.282	8.730	15.55	259
98.8	33.3	65.5	0.0424	27.094	9.389	17.71	270
104.4	33.3	71.1	0.0449	28.691	10.041	18.65	262

[a] Mean US = 258 ± 14 W/K

[b] W = weight of vented air calculated from measured air velocity (u) at exhaust duct. $W = \frac{12(u)}{T + 273}$; Cp for dry air is 1005 J/kg.K

[c] ΔHg = heat of combustion of natural gas, 38.34 MJ/m^3

Table 5—Energy balance for an empty oven with a step change air temperature

Temperature °C				Time (min)	m^3 Gas	Heat quantities (MJ)				
T_1	T_2	Ambient	$\overline{\Delta T}_L$[b]	θ	consumed	Input q_c $V_g\Delta Hg$	Vent q_v $WCp\overline{\Delta T}_L$	Surface loss (q_s) $UA\overline{\Delta T}_L\theta$	Net[c] q	W_oC_o[a] (MJ/K) $q/(T_2 T_1)$
29.4	71.1	26.6	15.1	9	0.368	14.13	1.376	2.104	10.65	0.255
71.1	77.8	26.6	47.7	2.08	0.106	4.06	0.914	1.536	1.61	0.209
76.6	82.2	33.3	46.1	2.88	0.127	4.87	1.209	2.055	1.606	0.286
82.2	87.7	33.3	51.6	2.63	0.127	4.87	1.213	2.100	1.557	0.283
87.7	93.3	33.3	57.2	3.72	0.169	6.48	1.873	3.294	1.313	0.234
93.3	98.8	33.3	62.7	5.05	0.246	9.43	2.748	4.901	1.781	0.323

[a] Mean $W_oC_o = 0.265 \pm 0.04$
[b] $\overline{\Delta T}_L = (T_2-T_a) - (T_1-T_a)/\ln(T_2-T_a)/(T_1-T_a)$
[c] $q = q_c - q_v - q_s$

A heat balance on a smoking oven can be represented by Eq (3):

$$q = W_pC_p\frac{dTp}{d\theta} + W_oC_o\frac{dTo}{d\theta} + C_a\frac{dWa}{d\theta}(T-Ta) + (H_a\frac{dWa}{d\theta} - D\frac{dx}{d\theta})(h_2-h_1) + D\frac{dX}{d\theta}(L) + UA(T-T_a) \quad (3)$$

where

q = rate of energy used by the system.
W_p = weight of product processed.
C_p = specific heat of product processed, 3349 J/kg·K for fish.
$\frac{dT_p}{d\theta}$ = rate of temperature increase by the product, °K/min. From Figure 4, this is 0.926 °K/min for the majority of the smoking process.
W_oC_o = heat gain by the oven walls, 0.265 MJ/K.
$\frac{dT_o}{d\theta}$ = rate of increase in temperature of the oven walls with a step change in air temperature. Ideally, the 5.55 °K step change in temperature should be traversed by the system within 3 min, and $dT_o/d\theta$ should be 1.85 °K/min.
C_a = the specific heat of dry air, 1005 J/kg·K.
T = dry bulb temperature and T_a = ambient air temperature.
$\frac{dW_a}{d\theta}$ = rate of venting of dry air. This is calculated at the minimum vent rate, 1.557 m^3/min. Using the ideal gas equation for an atmospheric pressure of 101.3 kPa.

$$\frac{dW_a}{d\theta} = \frac{101.3(1000)(1.557)(29)}{8315(T+273)}\left(\frac{H_a}{1+H_a}\right) = \left(\frac{550}{T+273}\right)\left(\frac{H_a}{1+H_a}\right).$$

H_a = humidity of air in the smoking oven.
D = weight of dry material in the product.
$\frac{dX}{d\theta}$ = rate of moisture loss, $\frac{\text{kg water}}{\text{kg dry matter (min)}}$
= $\frac{X_1 - X_2}{\text{total process time}}$. X_1 and X_2 are initial and final moisture contents, kg H_2O/kg dry matter. For mackerel and mullet, $X_1 = 3$ and $X_2 = 1.985$, and for a 60-min process, $dX/d\theta = 0.0169$ kg H_2O/(min)(kg dry matter).
h_2 = enthalpy of water in air leaving the vent, and h_1 = enthalpy of steam used for humidification.
L = latent heat of water at the wet bulb temperature.

The first term on the right hand side of Eq (3) is the sensible heat gain by the product. The second term is the sensible heat gain by the oven walls. The third term is the heat loss with the dry air vented from the oven. The fourth term is the heat attributed to the humidifying steam. If the term yields a negative quantity, this represents a heat input. If the term yields a positive quantity, such as if atomized liquid water is used for humidification, it will be an added heating load to the system. The fifth term is the heat of vaporization of water that leaves the product. The last term is the heat loss to the surroundings. Table 6 shows the values of these various quantities on a smoking oven loaded with varying quantities of product. The analysis was done during the point in the staged smoking schedule where rate of gas consumption of the oven was maximum, and this was during the period when the oven air temperature was step changed from 93.3 to 98.9°C.

The values for the maximum rate of heat input calculated using Eq (3) agreed with the values determined from the rate of gas consumption monitored during the process, for the case when the oven was empty, and when the oven had 4.5 kg of product. The particular oven used in these experiments, had a heating capacity of 35.35 kW, and the conditions for product temperature increase shown in Figure 4 can be achieved if the product loaded into the oven does not exceed 45.5 kg. Some slowdown in heating rate can be expected when this load is exceeded.

The data in Table 6 also show that heat losses to the surroundings constitute a major heat load. For the oven used in this study, 95% of this heat loss can be attributed to the 13.4 m^2 of uninsulated ductwork for air recirculation in the oven. Insulating the ductwork with the same insulation used for the oven walls could result in a 92% reduction in the heat loss values shown in Table 6. When selecting a smoking oven to perform at a given product loading capacity, Eq (3) can be used to determine the size of heaters required.

Another important requirement for a smoking oven is the ability to maintain the humidity. The steam injection system used for humidification must be able introduce steam at the rate sufficient to replace the water present in the vented air. This can be calculated from the highest temperature expected in the process, the humidity of the air at that temperature, and the venting rate. To maintain a relative humidity of 60% at 104°C (absolute humidity = 2.351 kg H_2O/kg dry air) at a venting rate of 0.0243 kg dry air/s, the steam injection system must be capable of providing at least 0.017 kg steam/s.

Energy use in high temperature smoking processes

Energy use in high temperature smoking processes can be calculated using Eq (3), by multiplying each term on the right with the time span for which the analysis was con-

Table 6—Heat balance on smoking oven showing contribution of various heat quantities to the heating load

Product weight (kg)	Heat quantities (MJ/min)						Max heat input (kW)	
	Sensible heat gain oven walls	Sensible heat gain product	Sensible heat vented air	Latent heat water vapor	Surface heat loss	Input from humidifying steam	Calculated	Measured
0	0.490	0	0.0577	0	1.078	0.0342	26.5	26.7
4.5	0.490	0.0139	0.0577	0.0435	1.078	0.0331	27.5	27.2
22.7	0.490	0.0837	0.0577	0.218	1.078	0.029	31.6	–
45.5	0.490	0.141	0.0577	0.437	1.078	0.0228	35.4	–
90.9	0.490	0.282	0.0577	0.072	1.078	0.0113	46.1	–

ducted. In the case of a staged temperature process, the analysis will have to be carried out for each constant air temperature segment of the process and the heat quantities summed for the total process. Table 7 shows an analysis of energy consumption in smoking 45.5 kg of fish using the staged temperature process. For comparison, the energy consumption for a 60-min constant temperature process at 71.1°C and 60% RH is also shown. The high temperature process requires twice as much energy as that for the 71.1°C process to carry out. Most of the enrgy used in the high temperature process is used in the generation of steam used for humidification. Using current natural gas rates of 20¢/therm (a therm is 1000 BTU or 1.055 MJ), energy cost for the staged temperature process would increase product cost by 39¢/kg compared to the constant temperature 71.1°C and 60% RH process.

CONCLUSIONS

HIGH TEMPERATURE processing to the same degree of heat lethality to *Clostridium botulinum* Type E as specified in the Food & Drug Administrations smoked fish GMP induced undesirable color changes and deformed the product. The incidence of defects were minimized by making shallow slits on the skin and processing using a staged temperature heating schedule. Recirculating air ovens such as that used in the meat industry, have uninsulated ductwork and large quantities of energy are dissipated through the ductwork. Heat conductance through the oven walls measured from the gas consumption is equivalent to that calculated if heat transfer coefficients of 200 W/m^2K and 20 W/m^2K for the inside and outside wall surfaces respectively, are used, and the floor is assumed to transmit heat at the same rate as the walls. A heat balance on an oven with steam humidification and a heating element capacity of 35.5 kW showed that only 45.5 kg of product can be processed effectively using the staged temperature process. Heat capacity of the oven walls calculated from the gas consumption rate is equivalent to that calculated from literature values of the density and specific heat of the materials of construction, with the weight of both walls in double-wall construction, included in the calculation. Energy consumption for a high temperature staged process is twice that for a constant temperature 71.1°C process, with most of the energy used attributed to the generation of steam used for humidification, and heat losses through the uninsulated ductwork.

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Table 7—Energy use in fish smoking

Process time (min)	Energy expended (MJ)								
	Sensible heat oven	Sensible heat product	Sensible heat vented air	Surface heat loss	Latent heat water vapor	Heat input steam	Heat input heater	Enthalpy steam	Total energy use
0–10	8.825	0.339	0.713	6.90	4.528	0.050	21.255	1.124	22.469
50–20	1.484	1.608	0.787	7.74	4.496	0.130	15.987	3.417	19.404
20–30	1.458	1.355	0.863	8.58	4.463	0.213	16.506	5.646	22.152
30–40	1.484	1.608	0.935	9.48	4.441	0.321	17.627	10.335	27.962
40–50	1.472	1.264	1.004	10.32	4.415	0.465	18.010	12.330	30.340
50–70	2.944	1.183	2.144	22.38	8.769	1.373	36.047	56.09	92.137
TOTALS	17.667	7.357	6.448	65.40	31.112	2.522	125.432	89.032	186.482
0–60 process 71.1°C	8.825	3.809	4.277	41.238	27.206	0.302	85.053	7.284	92.337

STABILITY OF LEAN AND FATTY FISH FILLETS IN HYPOBARIC STORAGE

S. VARGA, R. A. KEITH, P. MICHALIK, G. G. SIMS and L. W. REGIER

ABSTRACT

Fillets of lean (cod) and fatty fish (herring and mackerel) were stored in a hypobaric chamber at −1.1, −0.55, and 0°C under a pressure of 10 mm Hg. The atmosphere was changed twice hourly, and had a relative humidity greater than 95%. The growth of bacteria in the fillets was slowed, and a qualitative shift in the microflora occurred, compared with fillets held at 0°C on ice. The rate of development of rancidity in the fatty fillets was also decreased. Because of these changes, a 10–15% extension in keeping times of hypobarically stored fillets was observed at 0°C. The storage life of fillets at low pressure was further extended by the decrease of storage temperature and by treating the fillets with Na_3H EDTA. EDTA did not increase the storage life of the fatty fillets.

INTRODUCTION

HYPOBARIC STORAGE was devised by S.P. Burg (1976) for the storage of plants, fruits, and vegetables. In this storage environment, the atmosphere is constantly vented, its pressure is reduced, the humidity is nearly saturated, and the temperature is controlled. The evolving ethylene gas, responsible for the accelerated ripening of fruits, and the carbon dioxide as well as other respiratory volatile compounds adversely affecting some vegetable materials are out-gassed and constantly removed. The high relative humidity of the atmosphere precludes desiccation of materials, and the pressure reduction minimizes the air volume intake and consequent refrigeration demand. The reduced oxygen tension slows the oxidation of lipids and hemoglobin, and the activities of some undesirable microorganisms. Even though the partial pressures of oxygen and respiratory gases are low, the respiration of vegetables and microflora remains oxidative rather than fermentative, so that the appearance of putrefactive compounds in the foods is avoided.

Chilled lean fish, such as cod, is spoiled by the action of visceral ferments, endogenous enzymes, and the microbial flora. The rate of proteolysis caused by visceral enzymes often is so high that keeping most kinds of ungutted fish in a chilled environment is not practical. The autolytic degradation of fish flesh has been shown to be quite slow (Meyer, 1965; Wojtowicz, 1972). The storage life of wet fish fillets is thus limited primarily by the activities of the bacterial flora, especially the proteolytic, psychrophilic groups (Castell et al., 1949).

Development of rancidity in chilled fatty fish such as herring and mackerel often appears earlier than the proteolytic spoilage of muscle tissue. Lipids of these fish are highly unsaturated and subject to rapid auto-oxidation. The first products of oxidation are the hydroperoxides (Ke et al., 1977). These are further decomposed into various carbonyl compounds which are responsible for the rancid flavor of the flesh (Ackman, 1967, 1974).

From the foregoing, it follows that the rates of microbial activity and lipid oxidation in the fish could be affected by storage in a hypobaric environment. A measurable change in the speed of these processes should influence the keeping qualities of chilled fish flesh. This study therefore, was undertaken to determine the stability of lean and fatty fish fillets stored in a hypobaric chamber.

MATERIALS & METHODS

Sample preparation

Atlantic cod (*Gadus morhua*), kept alive in the Laboratory aquarium, were killed, then filleted and skinned by hand. The fillets were washed in potable tap-water and packed in 10- or 20-lb polyethylene boxes. A polyethylene film was heat-fastened at random points to the rims of the boxes so that access of air to the fillets was possible. The full boxes were buried in ice in plastic containers which in turn were placed either in the hypobaric chamber or in an insulated cabinet (control samples) for the storage study. Unskinned herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) fillets, taken from freshly caught, pre-rigor fish, were handled in a similar manner.

Storage

Access of air to the control fillets was ensured by open ports and drain holes in the insulated cabinet. Temperature of the fish remained at 0.5–0°C during the storage. The hypobaric environment was provided in an experimental storage trailer leased from Grumman Allied Industries, Inc. The pressure during storage was 10 mm Hg, the humidity was adjusted to not less than 95%, and the atmosphere was changed twice each hour. Experiments were conducted at 0, −0.55, and −1.1°C. Temperatures were measured by copper-constantan thermocouples with an accuracy of ±0.1°C, and recorded automatically.

Sampling

Sampling frequency was intentionally low to minimize the possible effect of changing pressures. Samples were analyzed immediately after sampling. Each sample consisted of about 4.5 kg of fillets. Subsamples from this sample were taken for sensory, chemical, and microbiological analyses.

Objective measurements

Moisture content was determined by standard procedures (AOAC, 1975). Lipid content was measured by the method of Bligh and Dyer (1959), trimethylamine nitrogen (TMA-N) by the official AOAC (1975) procedure, and the total volatile basic nitrogen (TVB-N) by the method of Lucke and Geidel (1935). Peroxide values (POV) were obtained by the AOCS official method (1960), free fatty acid (FFA) by the method of Ke et al. (1976), and thiobarbituric acid values (TBA) by the technique of Tarladgis et al. (1960).

Total aerobic bacteria counts (TABC) were obtained from decimally diluted homogenates on Bacto (B2) tryptone glucose extract agar (TGEA) complemented with 0.5% NaCl. *P. putrefaciens* was enumerated on Bacto (89) peptone iron agar (PIA) with added 3% gelatin and 0.5% NaCl. Cell densities of fluorescent pseudomonads were estimated on Bacto Psuedomonas Agar F augmented with 3% gelatin and 0.5% NaCl. The fluorescent colonies were identified with the aid of a UV lamp radiating with peak power at 366 nm. Proteolysis was revealed by the clear zones around the colonies after the gelatin was precipitated with saturated $(NH_4)_2SO_4$. Plates were surface-inoculated and incubated at 25°C for 72 hr. Fish homogenates were prepared with a Stomacher blender using sterile saline and 30–40g of fillets.

–Continued on next page

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Table 1—Results of sensory and chemical examinations of cod fillets stored in hypobaric chamber at 0° C or on ice for periods of time

Storage time in days	Mean taste panel score & Conf. limits		mg-TMA-N 100g		mg-TVB-N 100g		% H_2O	
	Iced	Hypobaric	Iced	Hypobaric	Iced	Hypobaric	Iced	Hypobaric
0	9.1 8.2–10	9.1 8.2–10	0.4	0.4	21.11	21.11	80.7	80.7
12	5.2 4.4–6.1	6.9 6.3–7.5	26.4	11.6	60.24	48.23	80.3	80.5
15	3.0 2.3–3.7	3.9 2.8–4.9	42.6	24.3	80.80	65.64	80.5	80.6
18	–	2.3 1.5–3.1	–	19.2	–	80.55	–	80.3

Table 2—Results of microbiological analyses of cod fillets stored in hypobaric chamber at 0° C or on ice for periods of time

Storage time in days	Log means (and conf. limits) of TABC/g		Log means (and conf. l mits) of P. putrefaciens/g		Log means (and conf. limits) of fluorescent Pseudomonads/g		Log means (and conf. limits) of proteolytic bacteria/g	
	Iced	Hypobaric	Iced	Hypobaric	Iced	Hypobaric	Iced	Hypobaric
0	5.01 4.95–5.08	5.01 4.95–5.08	2.00	2.00	2.34	2.341	4.83–5.00	4.83–5.00
12	7.92 7.68–8.16	7.51 7.01–8.01	6.80 6.22–7.38	6.64 6.26–7.02	6.31 5.93–6.69	4.40 3.41–5.39	7.35 6.94–7.76	6.64 6.63–6.66
15	7.78 7.71–7.85	7.81 7.73–7.90	6.19 5.93–6.44	7.19 6.99–7.39	6.46 6.16–6.74	6.12 5.94–6.30	7.37 7.21–7.52	7.54 7.36–7.73

Subjective measurements

For sensory assessment the samples were baked at 230°C for 25 min in covered containers lined with aluminum foil. They were coded and served hot on paper plates to a 7–8 member taste panel of experienced assessors. The samples were rated on the basis of appearance, odor, flavor, texture, and overall quality, using a 10-point scale. A mean score of 7.5–10 signifies top quality; a score of 4.5–7.5, medium quality; and a score of less than 4.5 denotes inedible sample. The mean taste panel scores with confidence limits at $P < 0.05$ were calculated for each examination. The assigned mean scores and the storage times of the samples in days were correlated, and the storage life calculated.

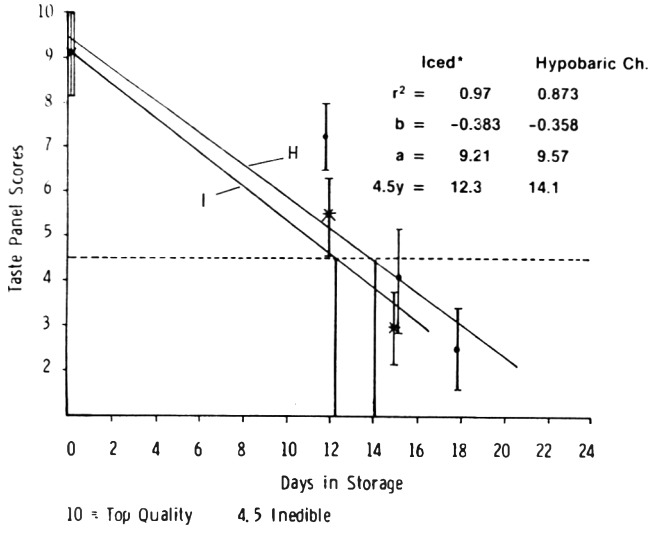

Fig. 1—Spoilage rate and storage life of cod fillets in hypobaric chamber at 0° C and on ice.

RESULTS

TABLE 1 shows that cod fillets stored on ice had lower taste panel scores, and higher TMA-N and TVB-N values than fillets stored in the hypobaric chamber. There was no difference in the moisture contents of fillets from different treatments at different sampling times. Table 2 shows the mean log values of bacteria counts and confidence limits at $P < 0.05$. The sample size of replicate TABC and proteolytic counts (PC) was 10, while the sample size of replicate *P. putrefaciens* and fluorescent pseudomonas counts was 5. The results show that at 12 days of storage the TABC and PC was significantly higher in the samples stored on ice compared with samples held at low pressure. After 15 days there was no significant differences in the counts. The mean of *P. putrefaciens* was significantly higher in hypobarically-stored fillets than in fillets stored on ice at the end of storage. The mean counts of fluorescent *Psuedomonas* were higher in the fillets stored on ice than in fillets kept in the hypobaric chamber. These results indicated that increase in the microflora in the fillets stored in the chamber was slowed and that a qualitative shift in the population had taken place. Figure 1 illustrates the difference in spoilage rate and storage life of fillets from the two treatments. The spoilage rate of the control fillets was higher and the storage life was 15% shorter.

Results for herring fillets (skin on) at 0°C are shown in Tables 3 and 4, and Figure 2. The POV and TBA values were markedly lower in fillets stored in the chamber compared with fillets stored on ice. Microbiological results in Table 4 are similar to the results obtained for cod fillets; the multiplcation of aerobic microflora was slowed in fillets stored at low pressure, and a qualitative shift took place in the population. A high percentage of the microflora on the fillets was proteolytic. Figure 2 shows that the spoilage rate of herring fillets in low pressure storage was lower and the storage life was 9% higher than for fillets stored on ice. The

Table 3—Results of sensory and chemical examinations of herring fillets stored in hypobaric chamber at 0° C or on ice for periods of time

Storage time in days	Mean Taste Panel Score & Conf. limit		mg-TMA-N 100g		mg-TVB-N 100g		% H_2O		% Lipid		Perox. value meq H_2O_2 1000g		Free fatty acid % oleic acid		TBA mg-MA 1000g	
	I[a]	H[b]	I	H	I	H	I	H	I	H	I	H	I	H	I	H
0	9.3	9.3														
	8.7–9.9	8.7–9.9	0.6	0.6	20.1	20.1	67.2	67.2	13.2	13.2	0.4	0.4	0.58	0.58	0.31	0.31
12	5.2	6.8														
	4.5–5.9	6.1–7.5	3.0	2.0	21.2	25.1	66.8	67.1	15.0	15.0	8.41	2.0	1.34	1.62	2.32	1.84
15	4.0	4.0														
	3.3–4.7	2.8–5.2	26.3	28.6	30.1	28.3	–	–	–	–	10.84	2.43	3.04	1.53	3.03	1.34
18	*	3.0														
	–		–	32.6	–	39.5	–	–	–	–	–	12.83	–	3.1	–	3.11

[a] I = Iced
[b] H = Hypobaric
* = Not tested for reasons of spoilage.

Table 4—Results of microbiological analyses of herring fillets stored in hypobaric chamber at 0° C or on ice for periods of time

Storage time in days	Log means (and conf. limits) of TABC/g		Log means (and conf. limits) of P. putrefaciens/g		Log means (and conf. limits) of fluorescent Pseudomonads/g		Log means (and conf. limits) of proteolytic bacteria/g	
	Iced	Hypobaric	Iced	Hypobaric	Iced	Hypobaric	Iced	Hypobaric
0	4.60	4.60						
	4.6–4.7	4.6–4.7	2.0	2.0	2.0	2.0	4.1–4.4	4.1–4.4
12	7.78	7.27	7.05	6.53	4.70	4.12	7.42	6.43
	7.5–8.0	7.1–7.4	6.7–7.4	6.2–6.9	3.5–5.9	3.9–4.3	7.1–7.8	6.1–6.7
15	7.86	7.76	7.21	7.41	6.65	6.25	7.61	7.70
	7.7–8.0	7.7–7.8	7.0–7.4	7.4–7.5	6.5–6.8	6.0–6.5	7.3–7.9	7.6–7.8

various bacteria counts and chemical indices are consistent with the quality assessment by the taste panel. Either the lag phase or the generation time of the microflora—most likely the former—was extended in the low pressure environment. The rate of oxidation of the herring fillets was also slowed in the chamber.

The slower growth of bacteria and the slower oxidation of the lipids resulted in a measurable increase in storage life of fillets of lean and fatty fish held in the hypobaric environment. The initial bacterial load on the fillets exceeded 10^5 cells/g, which indicates that the bacterial load on the fish should be lowered before filleting. Washing the fillets to lessen the load has not been successful. Fillets intended for chilled storage should be processed with a bacteria count of less than 10^4 cells/g. Good washing of fish prior to filleting and good processing methods designed to lessen the contamination of fillets with proteolytic psychrophiles is indicated. The TABC of fillets was not affected markedly in the hypobaric chamber. This is not surprising since fillets stored in evacuated containers have been reported to be spoiled by *Pseudomonas* (Pelroy and Eklund, 1976; Licciardello et al., 1966). The percentile increase of *P. putrefaciens* and the decrease of fluorescent *pseudomonas* in the microflora of fillets stored in an environment in which the partial pressure of oxygen is low could be attributed to the differences between the abilities of these organisms to utilize bound oxygen as hydrogen acceptor.

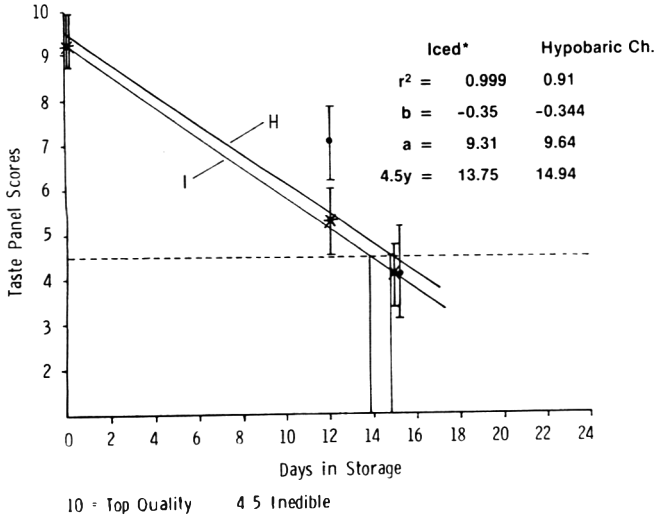

Fig. 2—Spoilage rate and storage life of herring fillets in hypobaric chamber at 0° C and on ice.

The storage life of cod and herring fillets on ice at 0°C was 13 and 13.3 days, respectively. Figures 3 and 4 show that the spoilage rate of fillets in the hypobaric chamber was further reduced, and the storage life increased, at a storage temperature of −0.55°C. At this temperature, the cod fillets kept for 17.2 and herring fillets for 18.5 days. A further lowering of the storage temperature in the chamber to −1.1°C resulted in another extension of keeping time to 18.6 days for cod and 21.5 days for mackerel fillets. Considerable quantities of ice formed in the fillets during storage at −1.1°C in the hypobaric chamber so that the samples resembled frozen fish (Fig. 5 and 6).

Since EDTA has been shown to extend the storage life of chilled fillets (Levin, 1967; Pelroy and Seman, 1969), and particularly of vacuum-packed fish (Laycock et al., 1971), cod and mackerel fillets were treated by dipping in a

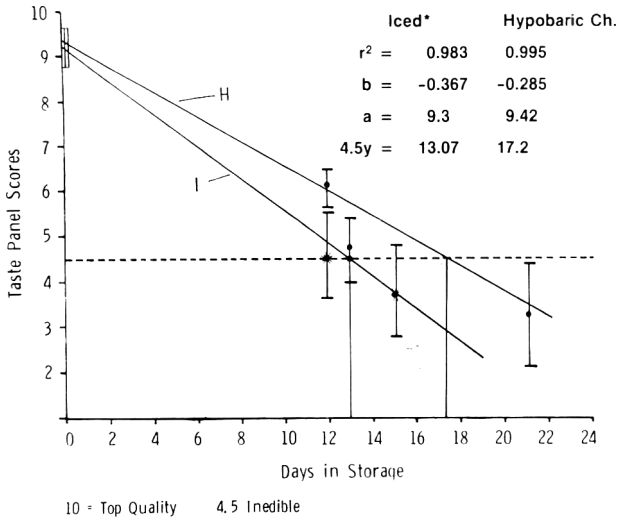

Fig. 3—Spoilage rate and storage life of cod fillets in hypobaric chamber at −0.55° C and on ice.

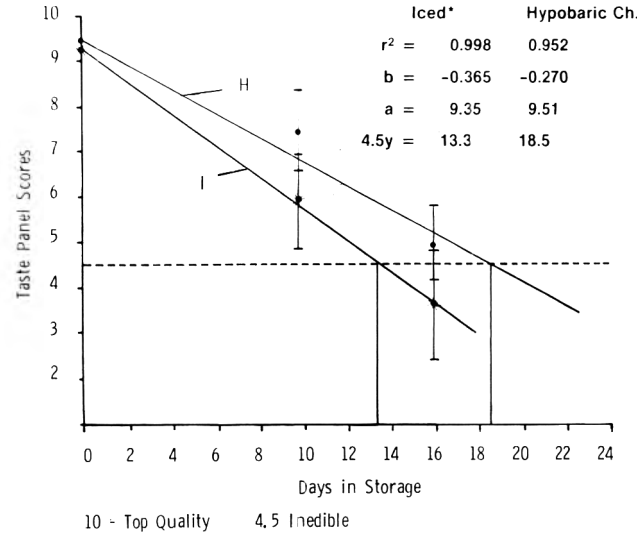

Fig. 4—Spoilage rate and storage life of herring fillets in hypobaric chamber at −0.55° C and on ice.

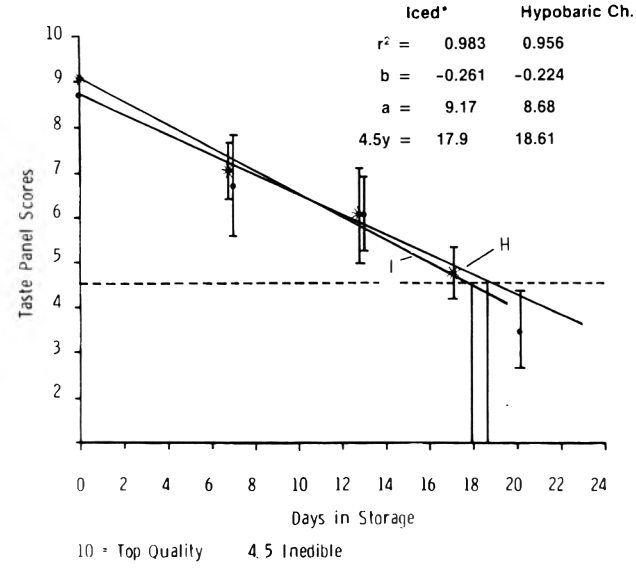

Fig. 5—Spoilage rate and storage life of cod fillets in hypobaric chamber at −1.1° C and on ice.

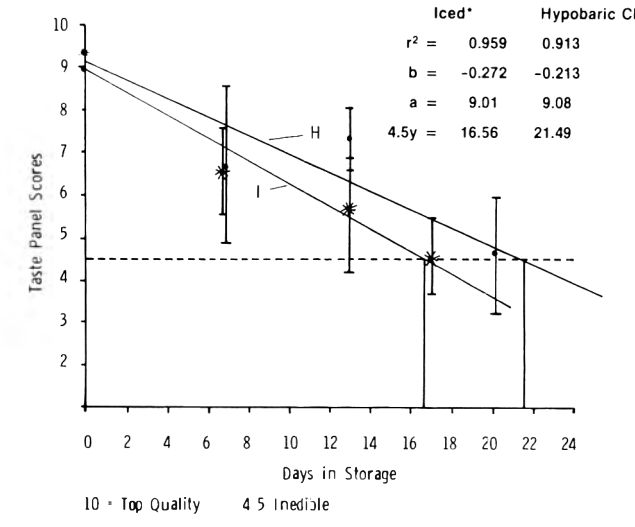

Fig. 6—Spoilage rate and storage life of mackerel fillets in hypobaric chamber at −1.1° C and on ice.

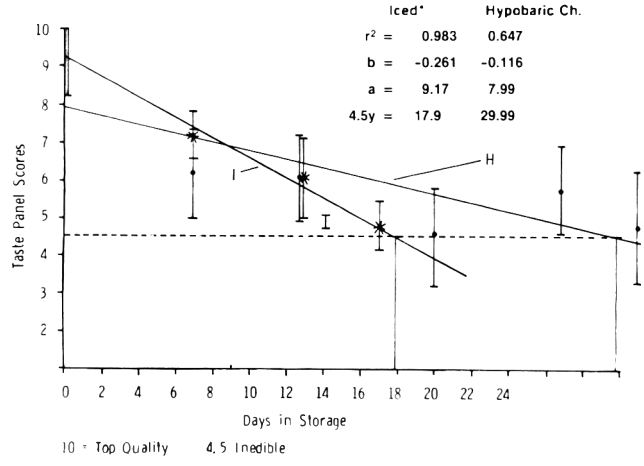

Fig. 7—Spoilage rate and storage life of Na_3H EDTA treated cod fillets in hypobaric chamber at −1.1° C and untreated fillets on ice.

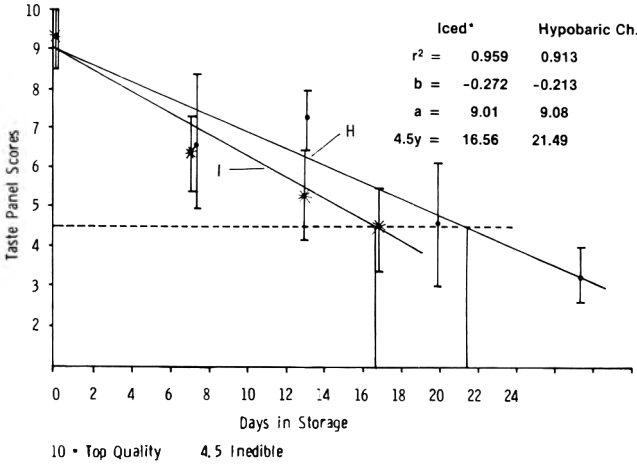

Fig. 8—Spoilage rate and storage life of Na_3H EDTA treated mackerel fillets in hypobaric chamber at −1.1° C and untreated fillets on

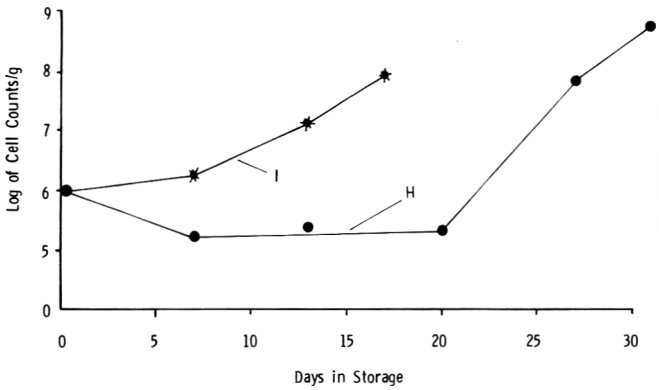

Fig. 9—Log of total aerobic plate counts from Na_3H EDTA treated cod fillets stored in hypobaric chamber at −1.1°C and untreated fillets held on ice.

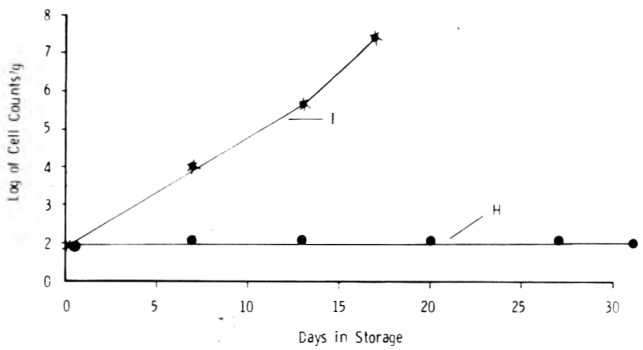

Fig. 10—Log of P. putrefaciens counts from Na_3H EDTA treated cod fillets stored in hypobaric chamber at −1.1°C and untreated fillets held on ice.

1% solution of Na_3H EDTA for 10 sec to achieve an uptake of about 300 ppm (mg/kg) (Varga et al., 1979). The treated fillets were stored at −1.1°C in the hypobaric chamber. Figures 7 and 8 show that the storage life of the cod fillets was extended to about 30 days, while that of the mackerel fillets was 21.5 days. The control samples held on ice at 0°C were the same as those shown in Figures 5 and 6. While the effect of EDTA treatment on storage life was marked with cod, it was negligible with mackerel. This might be expected since EDTA influences chiefly the activities of microflora, and not the oxidation of lipids. The influence of EDTA treatment on the TABC and cell densities of *P. putrefaciens* on cod fillets is illustrated in Figures 9 and 10. The lag phase of microflora on samples stored at low pressure was longer than that of the microflora on control fillets stored on ice at 0°C. Figure 10 shows that the growth of *P. putrefaciens* on EDTA-treated fillets was completely inhibited. This result is similar to the one observed by Laycock et al. (1971) on EDTA-treated, vacuum-packed haddock fillets. The EDTA probably affects the enzyme system that enables *P. putrefaciens* to reduce the bound oxygen in trimethylamine oxide. Since this organism is a highly proteolytic psychrophile, its selective inhibition markedly influenced the spoilage of chilled fish. Laycock and Regier (1971) speculated that the formation of TMA in marine fish is entirely due to activities of *P. putrefaciens.* In EDTA-treated cod fillets in which the *P. putrefaciens* were inhibited, the TMA-N increased during storage from 0.1 to 15 mg/100g. This evidence would tend to indicate that other members of the microflora of chilled fillets may also reduce TMAO. Even though the initial TABC of iced fillets was high, the cell counts of *P. putrefaciens* and fluorescent *pseudomonas* were low ($<10^2$ cells/g). These counts increased during storage, and appeared to have a good correlation (90%) with the storage time of the fillets. Cell counts of these organisms, therefore, may be useful for the estimation of the storage age of chilled fillets, provided there is no preservation process other than refrigeration applied.

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SEPARATION OF MONO- AND DI-SACCHARIDES AND SORBITOL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

S. C. C. BRANDAO, M. L. RICHMOND, J. I. GRAY, I. D. MORTON, and C. M. STINE

ABSTRACT

A simple yet rapid high performance liquid chromatography (HPLC) technique has been developed to separate and quantitate mono- and di-saccharides and sorbitol in foods. The technique differs from previously described HPLC methods in that (a) a pair of HPLC columns are joined in tandem for better resolution of the glucose-sorbitol peak, and (2) a ternary solvent system (acetonitrile/water/ethanol) with isocratic elution is used to increase resolution and decrease total analysis time. Elution order is fructose, glucose, sorbitol, sucrose, maltose, and lactose, all within 22 min.

INTRODUCTION

THE DETERMINATION of sugars in foods can be achieved by a number of techniques involving physical and chemical determinations (AOAC, 1975), thin-layer chromatography (Lara and Yabiku, 1974), paper chromatography (Lee et al., 1970), gas chromatography (Sweeley et al., 1963), and high performance liquid chromatography (HPLC) (Linden and Lawhead, 1975).

The rapid development of HPLC has been of great importance for the analysis of sugars, which can be isolated and estimated quantitatively with accuracy and without sample derivatization. However, the analysis of certain foods, specifically fruits and diatetic foods, where sorbitol occurs in the presence of its parent sugar glucose and other monosaccharides, has proved difficult. An HPLC method has been reported (Woidich, et al., 1978) in which sorbitol can be determined in the presence of glucose; however, the use of two different systems is required to determine fructose, glucose, sorbitol, sucrose, maltose, and lactose.

We report here a single system in which it is possible to separate and determine fructose, glucose, sorbitol, sucrose, maltose, and lactose.

EXPERIMENTAL

Apparatus

The HPLC system was assembled from a Waters model 6000 pump, a Waters model RI-401 differential refractometer detector, and a Whatman Partisil PXS 10/25 PAC column (4.6 mm × 25 cm) connected in tandem to a Waters prepacked μBondapak/Carbohydrate® column (4.2 mm i.d. × 30 cm). Samples were loaded onto the column with a Waters U6K septumless injector containing a 2 ml sample loading loop. Volumes injected ranged from 1–10 μl. Detector attenuation was held constant at 8×, and a Linear Instruments model 232 recorder was used for detector response recording.

Standards

Sugars (analytical reagent grade) were dried in vacuo at 65°C for 24 hr, and a 29% w/v standard solution (3% fructose, 3% sorbitol, 4% glucose, 6% sucrose, 8% maltose, 5% lactose) prepared. Before injection all solutions were filtered through a 0.45 μm Metricel membrane filter (Gelman Filtration Products, Ann Arbor, Mich.).

Mobile phase

Isocratic separations were achieved with the mobile phase, acetonitrile/water/ethanol [80/15/5 v/v/v; nonspectro grade acetonitrile, Burdick and Jackson (Muskegon, Mich.); reverse-osmosis, ion-exchange, deaminated water; absolute pure ethyl alcohol, reagent quality, U.S. Industrial Chemicals Co. (Tuscola, Ill.)]. The flow rate was adjusted to deliver 1.8 ml/min. The mixed ternary solvent was degassed under vacuum on a stirring plate for 5 min. Deionized water was passed through the reference valve of the pump every morning or whenever necessary to remove any gas bubbles present so that a steady recorder baseline resulted.

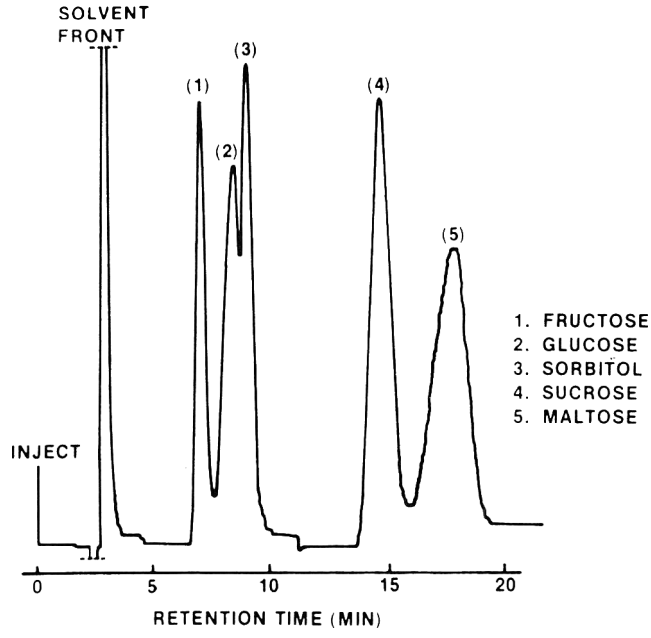

Fig. 1—HPLC chromatogram: 'Waters carbohydrate column'; Acetonitrile/H_2O (85/15); flow rate, 1.5 ml/min; Attenuation, 8X.

RESULTS & DISCUSSION

COMMONLY USED carrier solvents for carbohydrate analyses in HPLC include various ratios of acetonitrile and water (Linden and Lawhead, 1975; Woidich et al., 1978). However, if sorbitol is present in the sample as in fruits of the Rosaceae family (peaches, pears, apples, and berries), and in certain dietetic foods, the glucose-sorbitol peak is not resolved sufficiently to accurately evaluate either compound (Fig. 1). If a high ratio of acetonitrile/water (95/5) is used, resolution will be improved but the retention will be so increased that is is impossible to quantify the dissaccharides (Linden and Lawhead, 1975). When a second column was placed in tandem ahead of the Waters CHO column, glucose-sorbitol resolution was improved (Fig. 2 and 3). By using a flow rate of 1.8 ml/min (Fig. 2) with the columns joined tandemly the glucose-sorbitol peaks are adequately resolved, but the retention time for maltose is

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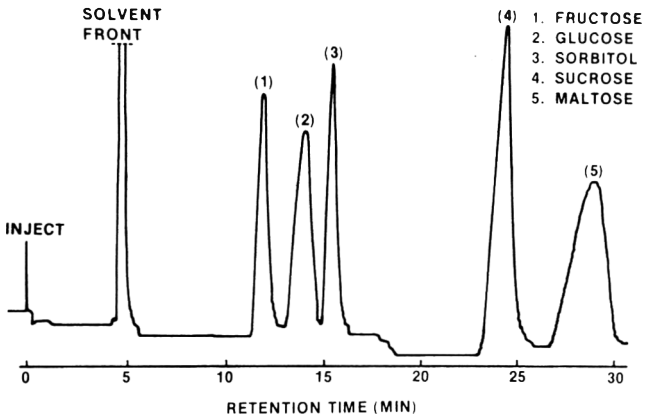

Fig. 2—HPLC chromatogram: Dual column arrangement; Acetonitrile/H_2O (85/15); flow rate, 1.8 ml/min; Attenuation, 8X.

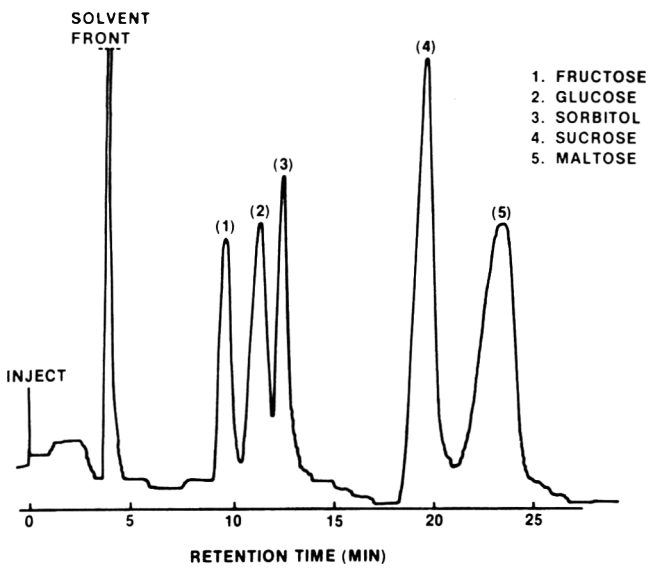

Fig. 3—HPLC chromatogram: Dual column arrangement; Acetonitrile/H_2O (85/15); Flow rate, 2.2 ml/min; Attenuation, 8X.

30 min; increasing the flow rate to 2.2 ml/min (Fig. 3) decreased the total analysis time.

The data in Figure 4 clearly demonstrate the advantages of the ternary solvent system: decreased total analysis time and increased sample resolution. Two possible explanations for this improvement are:

(1) the ethanol competes with the sugars for the active sites of the stationary phase but still permits a high degree of interaction and selectivity. This would then decrease the retention time of all sugars present in the system.

(2) the ethanol decreases the available water for solubilization of the sugars and this would in turn increase retention time.

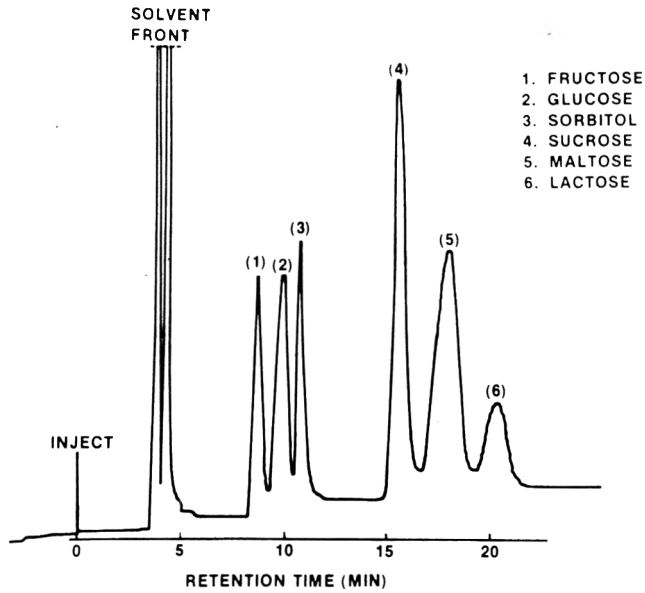

Fig. 4—HPLC chromatogram: Dual column arrangement; Acetonitrile/H_2O/ethanol (80/15/5); Flow rate, 1.8 ml/min; Attenuation, 8X.

A combination of these hypotheses may result. The best solvent mixture was determined to be 80/15/5 (acetonitrile/water/ethanol). Moreover, with this system the analysis of a mixture of simple mono- and di-saccharides and sorbitol can be achieved in 22 min.

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FOOD SOUNDS: HOW MUCH INFORMATION DO THEY CONTAIN?

ZATA M. VICKERS

ABSTRACT

Subjects listened to tape recorded sounds made by crushing each of 18 different foods. After listening to each sound, they selected from a list of the 18 foods the four foods most likely to have produced that sound. Many of the subjects were able to use the information present in the sound to identify the food; however, their performance was far from perfect. The foods differ in the recognizability of their crushing sounds. The recognizability of a food's sound does not appear to depend on the familiarity or the class (e.g., vegetable, cracker) of food.

INTRODUCTION

STUDIES by Drake (1963; 1965a, b), Drake and Hallidin (1974), Andersson et al. (1973), Vickers and Bourne (1976), Sherman and Deghaidy (1978), Vickers (1979) and Vickers and Wasserman (1980) have all provided evidence to support the idea that sounds may be important for the eating quality of foods. However, there are several unanswered questions concerning this auditory aspect of food, such as: (1) How much information can one obtain about a food from its sound? and (2) Can one obtain enough information to identify the food? The objectives of this study were to observe the ability of individuals to identify a food by the sound produced when the food is crushed, and to determine if certain foods can be differentiated by their sounds.

MATERIALS & METHODS

Subjects

Thirty judges, 10 males and 20 females, participated in Part One of this study. Nineteen of these 30, 7 males and 12 females, participated in Part Two. Subjects were students and staff (ages 20–30) from the University of Minnesota.

Preparation of tape

The tape recording consisted of sounds made by crushing the following foods: hard candy (Reeds, root beer); fresh celery (1 cm piece cut crosswise from a stalk); blanched celery (cut like the fresh celery and immersed in rapidly boiling water for 30 sec); saltines (Sunshine); unripe pear (1 cm wedge); peanut (Fisher, Virginia-style whole); gingersnap (Nabisco); fresh carrot (crosswise section approximately 1 cm in length and 1.5 cm in diameter); blanched carrot (cut like the fresh carrot and immersed for 1 min in rapidly boiling water); regular potato chips (Pringles); ruffled potato chips (Pringles); ripe golden delicious apple (1 cm wedge); unripe golden delicious apple (1 cm wedge); cold milk chocolate (Hershey's mini bar); water chestnut (Geisha, canned); shortbread cookies (Lorna Doone, Nabisco); and mini shredded wheat (Nabisco). These foods were chosen because they represent a wide range of sound qualities. The recorded sounds were produced by crushing each of the foods by hand with rubber-coated pliers. No attempts were made to control the rate of force application or the rate of deformation. (Sounds produced in this manner can be expected to differ from the sounds heard when biting or chewing food. The sounds heard during chewing are influenced by the size and shape of the oral cavity, bone conduction, damping of the sounds by the soft tissues, accompanying tactile information, absorption of saliva, rate of deformation, etc.)

During crushing, the foods were held 3 cm from a microphone (Bruel and Kjaer 4133). Sounds were recorded on Scotch brand 207 tape using a Nagra IV-SJ tape recorder (tape speed: 38.1 cm/sec). A Bruel and Kjaer 2619 pre-amp and a QSJA-BK microphone amplifier were connected between the microphone and the tape recorder. Five samples of each food were crushed and recorded. The tape segment containing the best of these five sounds ("best" defined as the segment that sounded to the author most characteristic of the food product) was removed. These 18 "best" segments were then spliced in random order into a single tape.

Two copies of this tape were spliced together. Individual sounds were separated by segments of nonmagnetic tape. The second set of 18 sounds was arranged in the same order as the first.

Experimental procedure

The subjects listened to the sounds through AKG K240 earphones. Each subject was tested separately by an experimenter present throughout the test. The experimenter operated the tape recorder, gave directions, and answered procedural questions. Each judge was told how the recorded sounds were produced. Subjects listened to the 18 sounds beginning at a randomly chosen position on the first half of the tape. Subjects were allowed to hear each sound as often as they wished. After listening to each taped sound, subjects selected (from a list of the 18 foods) the four foods most likely to have produced that sound and ranked them from 1, most likely to have produced that sound to 4, fourth most likely to have produced the sound.

About 6 months after the first test 19 of the 30 original subjects were asked to bite and chew each of the 18 foods, paying close attention to the sounds. Before sampling the foods they were told they would be repeating the first part of the experiment. Subjects were allowed to sample as much of each food as they wished. Within 1–7 days after sampling the foods the subjects repeated the first test.

Analysis

The number of times each judge correctly identified a sound (ranked it number one) and the number of times each judge correctly ranked a sound in the top four (ranked it number one, two, three, or four) were tabulated. The binomial distribution was used to calculate the number of samples a subject would need to correctly identify or correctly rank in the top four, assuming that the judges were purely guessing (i.e., had not learned) such that, in repeated random sampling, these events would occur, at most, 5% of the time (Snedecor and Cochran, 1965).

The number of times each food sound was correctly identified by the subjects and the number of times that sound was correctly ranked in the top four were also tabulated. These numbers were then compared with the critical levels calculated from the binomial distribution to determine which foods were recognized by their sounds at a "statistically significant" ($\alpha = 0.05$) rate.

An estimate of the proportion of people able to correctly identify each food from its crushing sound was calculated as follows:

$$P[\text{correctly identifying the sound}] = P[\text{correctly identifying the sound} \mid \text{random guessing}]$$

Now:

$P[\text{correctly identifying sound} \mid \text{random guess}] = 1/18$

$P[\text{random guessing}] = 1 - P_I$

$P[\text{correctly identifying the sound without random guessing}] = P_I$

Therefore:

$$P[\text{correctly identifying the sound}] = 1/18\,(1 - P_I) + P_I \quad (1)$$

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An estimate of the proportion of people able to correctly rank each food in the top four was calculated in a similar manner.

$$P\,[\text{correctly ranking the sound in the top four}] = 4/18\,(1 - P_R) + P_R \quad (2)$$

where: P_R = P [correctly ranking the sound in the top four without random guessing]; $1 - P_R$ = P [random guessing]; 4/18 = P [correctly ranking the sound in the top four|random guess].

The assumption was made that the subjects' judgements were binomially distributed and the individual judgements were independent. The probabilities of a correct judgement are estimated by Eq (1) and (2) using estimates of P_I and P_R.

To determine whether a subjects's performance improved on the second test the number of responses changing from incorrect on the first test to correct on the second test was compared to the number of responses changing from correct to incorrect. An estimate of the proportion of changers who changed from an incorrect response on the first test to a correct response on the second test, $\hat{p}$, was calculated as follows:

$$\hat{p} = \frac{C_{ic}}{C_{ic} + C_{ci}}$$

where C_{ab} = the number of subjects whose response changed from a to b, where a, b = c (correct) or i (incorrect).

The following standardized version of $\hat{p}$ was used as a test statistic for testing the hypothesis that c/(b + c) = 1/2 (Anderson and Sclove, 1978):

$$(\hat{p} - 1/2) \,/\, [(1/2)\,(1/2)/C_{ic} + C_{ci})]^{1/2} \quad (3)$$

Since $C_{ci} + C_{ic} > 75$ in both instances, it is appropriate to assume that the standardized test statistic (3) is approximately standard Normal. The significance levels for the tests were computed under this assumption.

Table 1—Number of subjects showing an ability to successfully identify a food from its sound or successfully rank a food in the top four foods most likely to produce that sound

	Number of subjects		
Test	Total	Correctly identifying 4 or more foods	Correctly ranking 8 or more foods
1	30	10	14
2	19	7	11

RESULTS & DISCUSSION

TEN of the thirty judges participating in the first test (6 females and 4 males) correctly identified four or more foods from their sounds. The significance level for a performance this good or better is p = 0.016. Fourteen of the same thirty judges (10 female and 5 male) correctly ranked eight or more of the foods in the top four. The significance level for a performance this good or better by chance was p = 0.029 (See Table 1).

Seven of the nineteen judges participating in the second test (4 females and 3 males) correctly identified four or more foods from their sounds (p = 0.016). Eleven of these nineteen subjects (7 females and 4 males) correctly ranked eight or more of the foods in the top four (p = 0.031). Thus, many of the judges appear to be able to use the information present in the sound of a food being crushed to identify the food. However, while a significant number of the subjects could identify the foods, their performance was far from perfect. At most, eight of the eighteen samples were correctly identified and the most samples correctly ranked was thirteen out of eighteen.

Most of the subjects improved their performance on the second test. Fifty-three percent of the subjects correctly identified more foods whereas sixteen percent showed no change and thirty-two percent correctly identified fewer foods on the second test. This improvement was statistically significant (p = 0.042). Seventy-four percent of the judges correctly ranked more foods in the top four whereas

Table 2—Recognizability of foods

	Test 1 (n = 30)				Test 2 (n = 19)			
Food sound	No. of judges correctly identifying the food	P_I[a]	No. of judges correctly ranking the food 1–4	P_R[b]	No. of judges correctly identifying the food	P_I	No. of judges correctly ranking the food 1–4	P_R
Hard candy	8 ***	0.22	13 ***	0.27	6 ***	0.28	11 ***	0.46
Fresh celery	12 ***	0.36	19 ***	0.53	2	0.05	8 *	0.26
Blanched celery	3	0.05	9 *	0.10	2	0.05	7	0.19
Saltine	1	0	12 ***	0.23	2	0.05	8 *	0.26
Unripe pear	1	0	6	0	0	0	6	0.12
Peanut	4 *	0.08	10 **	0.14	2	0.05	5	0.05
Gingersnap	1	0	7	0.01	1	0	4	0
Fresh carrot	5 **	0.12	13 ***	0.27	3	0.11	13 ***	0.59
Blanched carrot	1	0	4	0	1	0	7	0.19
Regular Pringles	4 *	0.08	9 *	0.10	4 *	0.16	8 *	0.26
Ruffled Pringles	5 **	0.12	13 ***	0.27	5 **	0.22	7	0.19
Ripe apple	8 ***	0.22	17 ***	0.44	5 **	0.22	11 ***	0.46
Unripe apple	5 **	0.12	15 ***	0.36	2	0.05	6	0.12
Graham cracker	0	0	14 ***	0.31	2	0.05	9 **	0.32
Milk Chocolate	8 ***	0.22	17 ***	0.44	1	0	6	0.12
Water chestnut	7 ***	0.15	19 ***	0.53	7 ***	0.33	12 ***	0.53
Lorna Doone	6 ***	0.19	13 ***	0.27	6 ***	0.28	11 ***	0.46
Shredded wheat	3	0.05	12 ***	0.23	5 **	0.22	13 ***	0.59

[a] P_I = the proportion of subjects able to correctly identify the food from its crushing sound See Eq (1). Negative estimates of P_I are forced to be zeroes.

[b] P_R = the proportion of subjects able to correctly rank 1–4 the food from its crushing sound See Eq (2). Negative estimates of P_R are forced to be zeroes.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

five percent showed no change and twenty-one percent ranked fewer foods correctly during the second test. This improvement was statistically significant ($p = 0.022$). This improvement might have been greater had the subjects crushed the foods with pliers instead of biting and chewing them.

Inspection of Table 2 reveals that the foods differ in the recognizability of their crushing sounds. Some foods, e.g., water chestnut, ripe apple, hard candy, fresh celery, and Lorna Doone cookie, appear to be more easily identified by their crushing sounds. Others, e.g., gingersnap, unripe pear, and blanched carrot, do not appear to be identifiable from their sounds. A list of the 18 foods in order of the recognizability of their sounds is shown in Table 3.

Table 3—Foods arranged in order of their recognizability

Food	Recognizability index[a]
Water chestnut	0.39
Ripe apple	0.34
Hard candy	0.31
Fresh celery	0.30
Lorna Doone	0.30
Fresh carrot	0.27
Shredded wheat	0.27
Ruffled Pringles	0.20
Milk chocolate	0.20
Unripe apple	0.17
Graham cracker	0.17
Regular Pringles	0.15
Saltine	0.18
Blanched celery	0.10
Peanut	0.08
Blanched carrot	0.05
Unripe pear	0.03
Gingersnap	0.00

[a] Recognizability index = (the sum of P_I and P_R for both Tests 1 and 2) ÷ 4.

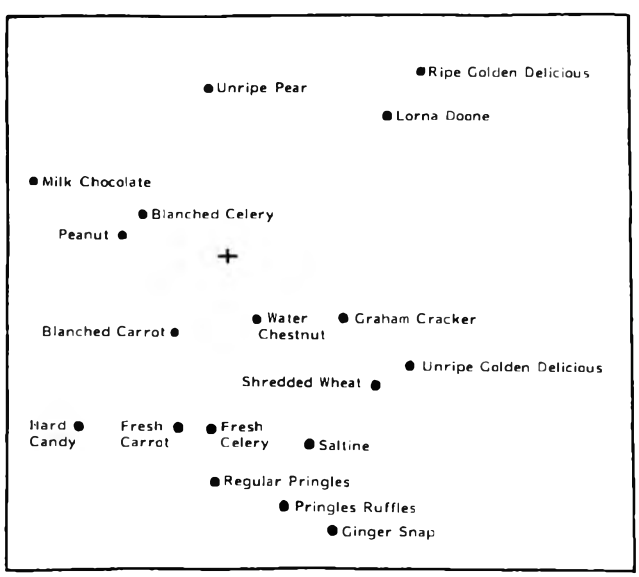

Fig. 1—Two-dimensional multidimensional scaling solution for dissimilarities of paired food sounds. Origin indicated by + (From Vickers and Wasserman, 1980). The MDS data analysis technique arranges stimuli (in this instance, the 18 food sounds) in a spatial map on the basis of a quantitative measure of dissimilarity. Stimuli judged similar in quality are arranged close to one another in the space.

As yet no explanation for why some food sounds are more recognizable than others exists. No particular class of foods such as vegetables or crackers appears to be more identifiable than any other. The blanched carrot and blanched celery may have produced more noise than most subjects expected; this might account for their being placed towards the bottom of the list. The foods towards the top of the list in Table 3 do not appear to be more or less familiar than foods at the bottom although the familiarity of the foods was not measured. Perhaps the sound of a water chestnut (top of the list) is somewhat unique or distinctive while that of a peanut or saltine (lower on the list) is similar to many other food sounds and therefore more difficult to identify.

Vickers and Wasserman (1980) used multidimensional scaling (MDS) to arrange these same 18 food sounds in a psychophysical space on the basis of their similarities. One might speculate that the more recognizable sounds would be relatively isolated in this MDS space, whereas unrecognizable sounds would be located in close proximity to other sounds. An MDS scatter plot shown in Figure 1, however, illustrates that this is not the case. The most recognizable sounds (water chestnut, ripe golden delicious apple, and hard candy) appear to be no more isolated than the least recognizable sounds (blanched carrot, unripe pear, and gingersnap). The positions of the food sounds on the MDS map appear unrelated to the recognizability of the sounds.

Although no directly comparable studies of the identification of foods by visual, olfactory, taste, or tactile sensations alone exist, one can speculate on the relative power of these senses to identify foods. In a similar study, vision or olfaction alone should be sufficient for a nearly complete identification of foods. Taste or tactile sensations alone would likely be no more useful for identifying foods than auditory sensations. Acoustical input may, therefore, contribute as much as taste or tactile sensations to the eating quality of foods.

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THIAMIN AND RIBOFLAVIN ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

J. F. KAMMAN, T. P. LABUZA, and J. J. WARTHESEN

ABSTRACT

A technique utilizing high-performance liquid chromatography (HPLC) was developed for the analysis of thiamin and riboflavin in enriched and fortified foods. The vitamins were extracted from cereals and processed cereal products and simultaneously assayed by reverse-phase HPLC. The vitamins eluted as separate peaks within 8 min and were detected by absorbance at 254 nm. The developed technique was compared to the semi-automatic modification of the AOAC method for seven cereal products. There was no statistical difference found in the values obtained from the two procedures.

INTRODUCTION

HIGH PERFORMANCE liquid chromatography (HPLC) has emerged in recent years as an effective analytical tool in determining the nutrient and compositional characteristics of food products.

Although studies utilizing HPLC in the analysis of thiamin and riboflavin have been presented (Van de Weerdhof et al., 1973; Mauro and Wetzel, 1976; Roser et al., 1978), they have relied upon fluorometric measurement of the vitamins. Due to the amount of additional manipulation required to fluorometrically measure thiamin, it was felt that these techniques offered little advantage over conventional, approved methods (AOAC, 1975; AACC, 1962; Assoc. Vit. Chemists, 1966).

The approved techniques require considerable purification or conversion, as well as separate handling and measurement equipment for each vitamin. The principle of these assays is an extraction with acid followed by separate quantitation of riboflavin by a fluorometric procedure and thiamin by oxidation to thiochrome with analysis by fluorometry.

The purpose of this study was to develop a rapid, accurate, simultaneous and comparatively simple method of thiamin and riboflavin (vitamin B_1 and B_2, respectively) extraction and analysis, utilizing HPLC. The HPLC technique was then compared to the semi-automatic method. The focus of this study centered upon the analysis of cereals and processed cereal products.

EXPERIMENTAL

Sample preparation

A variety of cereals and processed cereal products were selected for examination in this study. These foods represented both enriched and fortified levels of vitamin nutrification in commonly encountered foods. All of the products, with the exception of the noodles, were purchased at retail outlets. These products included three ready-to-eat (RTE) breakfast cereals; two wheat flake cereals at 25% and 100% USRDA; and a corn flake cereal fortified at 25% USRDA. Enriched all-purpose wheat flour and enriched precooked rice were the other foods obtained at the retail level. The fortified and enriched noodles were made on a custom built laboratory extruder with a 0.64 mm × 0.64 cm die. The noodles were cut to a 10 mm length by a rotary face knife and dried in a forced air tray dryer to 6% moisture content (wet basis). These noodles were made from durum semolina (International Multifood Corp., Mpls., Minn.) and water. Enriched and unenriched semolina were obtained from the same feed line in the mill to ensure identical raw composition. Fortified noodles were made with the addition of thiamin mononitrate and riboflavin (Hoffmann-La Roche, Inc., Nutley, N.J.) to unenriched semolina. Prior to extraction and analysis, the food products (except flour) were ground to pass a 40 mesh screen to facilitate vitamin extraction. All manipulations and analyses were carried out in subdued light and/or amber glassware.

Conventional measurement of thiamin and riboflavin

The measurement of thiamin and riboflavin was made utilizing an AutoAnalyzer II system (Technicon, Tarrytown, N.Y.). This method utilizes a fluorometric determination of thiamin and riboflavin under a continuous flow technique. This method automates the steps of the approved method (AOAC, 1975) for the analysis of the vitamins. Separate fluorometric measurement for thiamin and riboflavin were made following a single extraction step for both vitamins.

For extraction, a 5-g sample was added to a 100 ml amber volumetric flask. Fifty ml of 0.1N HCl was added and the sample was fully dispersed. The flask was covered with foil and autoclaved for 30 min at 15 psi (121°C), then cooled to room temperature. Following the addition of 6.0 ml 1.25N sodium acetate, 5 ml of Clarase solution (Fisher Scientific Co., Fair Lawn, N.J., 5g in 100 ml pH 4.3 metaphosphoric buffer) was added and the extract was incubated overnight at 37°C. The flask was cooled to room temperature and brought to volume with pH 4.3 metaphosphoric buffer. The sample was thoroughly mixed and separate aliquots removed for individual analysis of thiamin and riboflavin. The samples were then filtered through either Whatman 2 filter paper (thiamin) or Whatman GF/A glass fiber filter paper (riboflavin). The automated thiamin analysis was based on the flow scheme presented by Pelletier and Madere (1975). Separate automated analysis of riboflavin was based on the flow scheme of Egberg and Potter (1975). The semi-automatic technique has been shown to have equal or better measurement precision of the vitamins than the manual AOAC method (Dunbar and Stevenson, 1979; Egberg, 1979, 1980; Pelletier and Madere, 1975).

HPLC measurement of thiamin and riboflavin

The extraction technique developed for HPLC measurement was based on the approved method, but with some modifications. A finely ground sample of 5g was dissolved with only 30 ml 0.1N HCl (compared to the 100 ml of final dilution in the approved method). The flask was covered with foil and then autoclaved for 30 min at 15 psi. Following cooling to room temperature, the sample was centrifuged for 15 min at 1500 rpm (375 gravities relative centrifugal force). The centrifugation step was deemed necessary since normal filtration was not possible due to the high solids content of the digest. The decanted supernatant was then filtered through a 0.20 μm micropore membrane filter (Gelman Instrument Co., Ann Arbor, Mich.).

Filtered samples ranging in volume from 10–50 μl were injected into a Waters Associates, Inc. (Milford, Mass.) liquid chromatograph. The chromatograph was equipped with a Model U6K injector, a Model 6000 A solvent delivery system, and a Model 440 absorbance detector fitted with a 254 nm wavelength filter. Detector output was recorded on a Hewlett Packard 3380A recorder-integrator. Vitamin concentration was measured from peak height at 0.008 absorbance units full scale. Separation by reverse phase was accomplished with a Waters Assoc. μBondapak C_{18} column. Mobile phases of various compositions were examined for optimal separation of the vitamins in the types of cereal products tested. The mobile phase determined to be optimum for simultaneous measurement of the vitamins consisted of 12.5% nanograde acetonitrile (Mallinckrodt,

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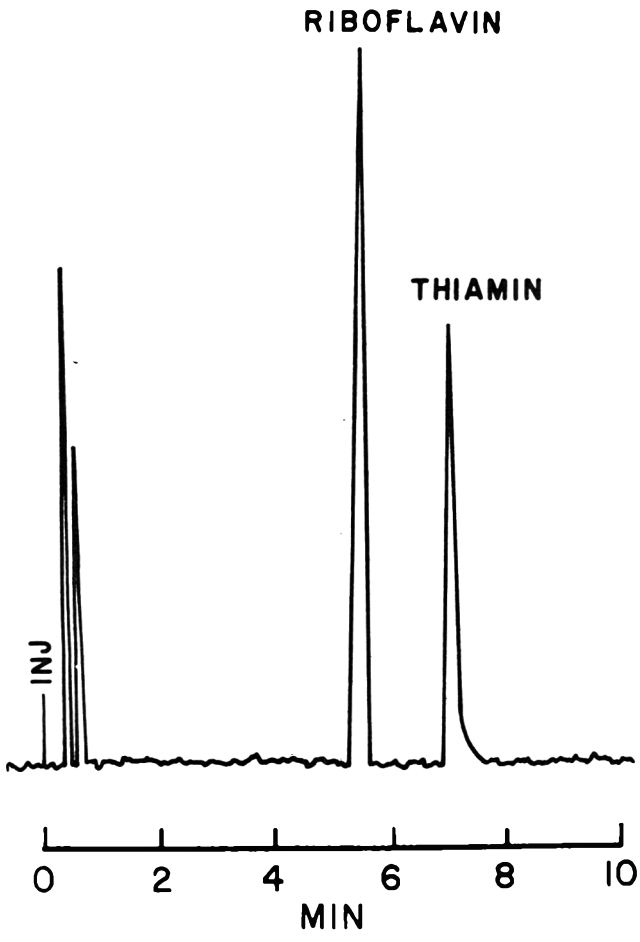

Fig. 1—Chromatogram of thiamin and riboflavin standards.

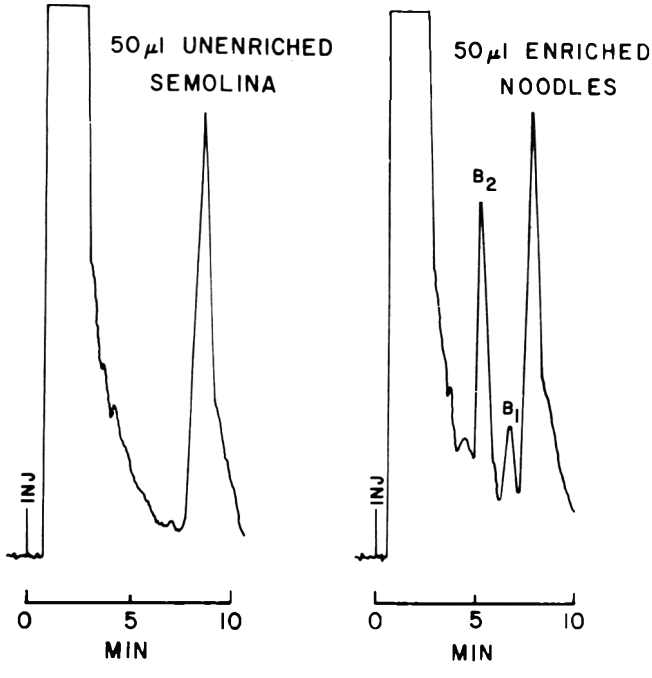

Fig. 2—Chromatograms of unenriched semolina and enriched noodles.

Paris, Ky.) and 87.5% 0.01M phosphate buffer (pH 7.0) containing 0.005M of the sodium salt of heptane sulfonic acid (Eastman Chemicals, Rochester, N.Y.). At a flow rate of 2.5 ml/min, the elution of both vitamins was completed within 8 min. A standard solution of thiamin and riboflavin containing 0.20 mg/ml of each vitamin in 0.1N HCl was used as an external standard for identification and quantitation. The solution was prepared weekly and stored at 4°C in amber bottles between use. Quantification was accomplished by comparing the peak heights of the vitamins in the samples to the peak heights of the standards. The baseline for peak height measurement was made from the point where the vitamin elution began to the end of the peak in line with the elution trail prior to the peak. Peak height was measured from the midpoint of this line. Neither the range of vitamin concentrations studied or the volume of material injected (10–50 μl) affected the linearity of the detector response.

Following completion of a day's analyses, 25 ml of 20% nanograde acetonitrile/80% glass distilled water were pumped through the column to prevent precipitation of the solvent saltsand column malfunction. Another 25 ml of 80% acetonitrile/20% glass distilled water was then used for final column clean-out and storage.

RESULTS & DISCUSSION

THE EXTRACTION TECHNIQUE developed in this study resulted in a greater vitamin concentration in the supernatant aliquot than that which results from the normal approved method. This increased concentration allowed detection of the vitamins by ultraviolet (UV) absorbance whereas vitamin concentrations resulting from approved methods of extraction for food products are below the limits of UV detection for most samples. Detection of the vitamins at 254 nm, a wavelength near the absorbance maxima of both vitamins, precluded the need to convert thiamin to thiochrome and permitted simultaneous measurement with riboflavin, thereby simplifying the entire procedure.

Figure 1 is a chromatogram of vitamin standards resulting from the conditions established by this study. The detection limits for thiamin and riboflavin were approximately 30 ng and 5 ng, respectively, per injection.

The solvent phase selected for use in this study was the result of the examination of elution profiles for the vitamins as affected by acetonitrile concentration, buffer pH, and alkyl chain length of the sulfonate. Buffer pH appeared to affect only the sharpness of the peaks, whereas acetonitrile concentration affected the retention time of riboflavin and, to a lesser degree, thiamin. Alkyl chain length of sul-

Table 1—Comparison of HPLC values to the semi-automated technique for riboflavin and thiamin[a]

	Riboflavin (mg/100g)		Thiamin (mg/100g)	
	HPLC	Semi-automated	HPLC	Semi-automated
Wheat RTE cereal (100% RDA)	6.96 ± 0.17	7.28 ± 0.28	6.94 ± 0.13	7.25 ± 0.28
Wheat RTE cereal (25% RDA)	1.65 ± 0.06	1.85 ± 0.10	1.88 ± 0.13	1.73 ± 0.04
Corn RTE cereal (25% RDA)	1.57 ± 0.08	1.57 ± 0.02	1.25 ± 0.11	1.19 ± 0.04
Fortified noodles	21.25 ± 0.95	20.34 ± 0.53	20.05 ± 0.52	20.64 ± 0.43
Enriched noodles	0.45 ± 0.02	0.51 ± 0.01	0.98 ± 0.04	1.00 ± 0.03
Enriched wheat flour	0.52 ± 0.07	0.55 ± 0.01	0.89 ± 0.11	0.92 ± 0.05
Enriched precooked rice	0.17 ± 0.07	0.05 ± 0.02	0.72 ± 0.07	0.55 ± 0.02

[a] Mean ± S.D., N=5

fonic acid, pentane and/or heptane, affected only the elution of thiamin in the system. By adjusting the acetonitrile concentration and the type or ratio of sulfonic acid used for ion pairing with thiamin, it was possible to select specific retention times for each of the vitamins.

The reason for selecting the mobile phase conditions utilized in this study is demonstrated in Figure 2. This figure compares unenriched semolina to noodles made from the same semolina after having been commercially enriched. The resultant chromatograms are nearly identical except for peaks at 5.5 min and 7 min, those being riboflavin and thiamin, respectively. The peaks were also verified as to identity by the addition of vitamin standards to enriched semolina at various concentrations (coelution). Mobile phase conditions were selected which provided separate, sharp peaks for the vitamins. These peaks were free from background interference with a total analysis time of less than 10 min. The solvent phase selected and utilized in this study facilitated the development of a rapid and simultaneous procedure. Figures 3 and 4 present typical chromatograms for other food products analyzed by this technique. For enriched samples, such as the flour and rice in Figure 3, a 50-μl injection was necessary to give measurable vitamin peaks on the chromatogram. Injection volumes of 10–20 μl were sufficient for fortified samples, such as the wheat RTE cereals in Figure 4.

The HPLC technique developed in this study was compared to the semiautomated technique. Seven different cereal products were analyzed. The data obtained for riboflavin and thiamin analysis are presented in Table 1. There appears to be close agreement in the values between methods. Mean recovery values for the vitamins with the semiautomatic technique has been shown to be 100.7% and 96.3% for riboflavin and thiamin, respectively (Egberg, 1980; Egberg and Potter, 1975). For example, for enriched noodles, the HPLC value for riboflavin was 0.45 mg/100g whereas the AutoAnalyzer value was 0.51 mg/100g. A paired t-test of sample means showed no significant difference between methods at the 5% level. The disparity for the enriched rice may reflect levels close to the detection limits for riboflavin.

There is also close agreement in values between techniques for thiamin. For example, values determined for enriched noodles were 0.98 mg/100g by HPLC and 1.00 mg/100g by the semi-automated method. A paired t-test of sample means showed no significant difference between the methods at the 5% level. The variation observed between the methods appears typical for the approved method between laboratories and may be considered normal experimental variability.

Techniques such as the one described in this study can be useful to food manufacturers and researchers as an analytical tool, for example, in the examination of vitamin shelf-life, validation of nutritional label claims, or fortification underage/overage during processing. Table 2 presents a comparison of measured vitamin values to percentage RDA declaration on the nutritional label for the three RTE breakfast cereals analyzed in this study.

This study has presented a reliable and rapid technique for measuring enriched and fortified levels of thiamin and riboflavin in cereal products. A modified extraction technique which enabled UV detection of the vitamins, in combination with a selected mobile phase, resulted in simultaneous measurement by HPLC. Although analysis time is slightly shorter with the semi-automatic technique, this HPLC method offers certain advantages including simulta-

—Continued on page 1504

Table 2—Comparison of measured thiamin and riboflavin values by HPLC against nutritional label declaration (for RTE cereals)

	USRDA (1 oz serving)		
	Declared	Measured	
		B_1	B_2
Wheat RTE Cereal	100%	130%	115%
Wheat RTE Cereal	25%	36%	27%
Corn RTE Cereal	25%	24%	26%

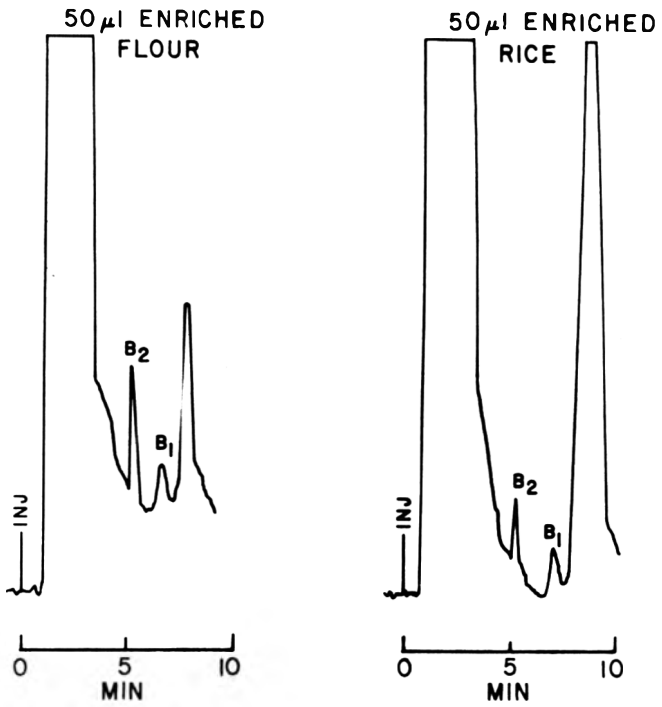

Fig. 3—Chromatograms of enriched flour and enriched precooked rice.

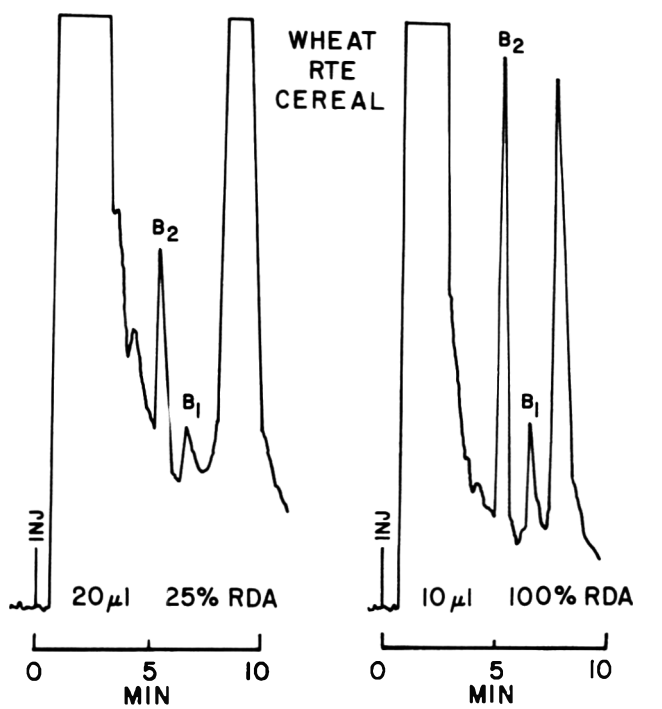

Fig. 4—Chromatograms of fortified wheat RTE breakfast cereals containing 25% and 100% of the USRDA for riboflavin and thiamin.

EFFECT OF BAKING ON THE FORMS OF IRON IN IRON-ENRICHED FLOUR

KEN LEE and F. M. CLYDESDALE

ABSTRACT

The effects of baking processes on the chemical forms of iron were investigated. Various iron enrichment sources were shown to exist initially in distinct chemical forms. These sources were then added to flour and baked as biscuits, and the resulting distribution of iron forms, or "iron profile," measured. The baking generated large amounts of insoluble iron, independent of the identity of the iron source added. Thus the large differences found between iron sources prior to baking had vanished in the final product. Similar iron profiles were shown to exist in commercial samples of flour and bread.

INTRODUCTION

IRON IN FOODS may change chemically as a result of interactions with the food or food process. This hypothesis has wide implications as the chemical form of iron is known to influence the biological availability of the nutrient iron (Lee and Clydesdale, 1979a). Thus knowledge of the forms of iron present after processing may serve as a predictive measure of the utility or futility of enrichment of a particular food with iron.

It has been postulated that chemical changes occur during baking that detract from the superior availability that ferrous sulfate has over other iron sources (Moore, 1970). Leichter and Joslyn (1967) found the iron in various breads was mostly in the ferric state, but the original form of iron was not reported. Narasinga Rao and Prabhavathi (1978), studying human iron absorption from various foods and diets, proposed that the ionizable iron at pH 7.5 is a reliable measure of bioavailability of nonheme iron from foods. However, solubility of iron under simulated gastric conditions did not correlate well with the availability of iron to rats from soda crackers (Ranhotra et al., 1973).

Numerous studies demonstrate dramatic changes in bioavailability of iron after processing. Wood et al. (1979) reported that a combined heat and pressure process increased the RBV (relative biological value, which is the bioavailability expressed as a percent of a ferrous sulfate control) of all three ferric phosphates tested. The authors speculate that the processing caused the formation of some form of chelate which enhanced bioavailability of certain of the iron compounds evaluated. Shah et al. (1979) demonstrated an increase in bioavailability from iron phosphates in cereals and infant foods, concluding that the physical-chemical characteristics of the iron phosphates which affect bioavailability require investigation. The effect of the baking of bread on iron is of interest as bread is a common vehicle of iron enrichment in several nations. Unfortunately, the sources of iron which have the best functional stability are generally the least bioavailable, while the most bioavailable iron sources have the greatest potential for decreased product quality (Ranum and Loewe, 1978). Ranhotra et al. (1979) found bioavailability of iron in 10 commercial varieties of bread to range in RBV from 32–80%. They conclude that the bread interferred with the availability of iron, but the magnitude of the interference was unrelated to the phytate or fiber content in the breads. Lee and Clydesdale (1980) have shown that iron added to a beverage product may not remain in the original form after storage or drying processes. In addition, the final form of iron found in the food was dependent upon the iron compound added prior to the process.

Thus numerous reports indicate significant changes in bioavailability due to processing, including baking. Many authors speculate that these changes are due to some alteration of the chemical form of the iron initially added to the food. However, this speculation has never been adequately investigated in baked products. It is our intent to quantify the chemical forms of iron which are present before and after baking.

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MATERIALS & METHODS

Sources

Each iron source is listed here with the specified iron content and manufacturing source. The manufacturer's specification agreed with values determined by atomic absorption analyses conducted upon receipt of shipment. All iron sources are food-grade and meet Food Chemicals Codex specifications. Ferrous sulfate monohydrate, containing 32.1% iron, was obtained courtesy of George Uhe Inc., N.Y. Ferric ortho phosphate, white, containing 28.6% iron and 100% passing through 200 mesh, was obtained courtesy of Joseph Turner & Co, N.J. Ferric sodium EDTA, trihydrate, containing 13% iron, was obtained courtesy of W.R. Grace & Co., N.H. Both hydrogen reduced elemental iron, consisting of 96.9% iron with 97% less than 44 microns, and electrolytically reduced elemental iron, consisting of 98.3% iron with 98% less than 44 microns, were obtained courtesy of Glidden-Durkee, N.Y. Carbonyl processed elemental iron (Grade N Special), consisting of 99% iron with 99% less than 10 microns in diameter was obtained courtesy of G.A.F. Corporation, N.Y. Unenriched all-purpose white wheat flour was obtained courtesy of General Mills Inc., Minn. Commercial samples of enriched white wheat flour and enriched white bread were purchased from local markets.

Baking

Biscuits, chemically leavened with baking soda, were selected over yeast-leavened breads to simplify procedures and eliminate any possible effect of fermentation on the iron. A standard procedure was employed for the baking of biscuits (Swanson, 1957), using the following composition: 230g flour, 244g whole milk, 2.2g salt, and 7.5g baking soda. Each iron source was added to the flour at a level of 40 mg iron per pound of flour, corresponding to the once proposed increased level of flour enrichment (Fed. Regist, 1977). The dry ingredients were sifted together, and then hydrated to a dough. The dough was dropped onto aluminum pans, weighed, and baked at 230°C for 15 min. The cooled biscuits were weighed, dried under vacuum at 25°C for 2 days, then ground into crumbs in a blender at lowest speed. Thirty-gram aliquots of biscuit crumbs were mixed with continuous stirring in 170 ml water for 30 min and then analyzed for the distribution of iron forms.

Design and analysis

The percent distribution of chemical forms of iron, or the "iron profile," was measured in 20 different samples as follows:

1–6 Aqueous profiles. Each of the six iron sources listed above was first analyzed in an aqueous system. The six aqueous iron pro-

files serve as controls to which the baked samples may be compared.

7–12 Laboratory enriched biscuits. Each of the six iron sources was added to unenriched flour and baked as biscuits.

13–14 Unenriched flour and biscuits. Unenriched white flour was analyzed as supplied for its endogenous iron profile prior to baking. Biscuits were then produced from this flour without addition of an iron source to ascertain the endogenous iron profile after baking.

15–16 Commercially enriched flour. Commercially enriched white flour was purchased locally and analyzed immediately. Biscuits were then baked in the lab from the commercially enriched flour. The label indicated that this product contained reduced iron as the enrichment source.

17–18 Drying. Biscuits were baked from commercially enriched flour and either freeze-dried or vacuum-dried to determine if the drying procedure had any effect on the final iron profile.

19–20 Breads. Two samples of commercially enriched and baked bread, known to contain either ferrous sulfate or reduced iron as the enrichment source, were analyzed.

Analysis for the distribution of iron forms, or "iron profile," was by the method of Lee and Clydesdale (1979b). Twenty-five gram aliquots were used for the determination of total and soluble iron forms. Calculations employed on these data were delineated previously (Lee and Clydesdale, 1980).

Statistics

Data were analyzed using the University of Mass. IDAP statistical program package (Wiedmann and Hosmer, 1979). A one-way analysis of variance was used to compare means of each form of iron in each treatment. A Duncan's multiple range test was used to examine differences between iron profiles after baking. A 0.05 level of significance was employed in all tests.

RESULTS

Controls

All of the food-grade iron sources were analyzed when received for their initial distribution of iron forms, or "iron profile." Each source was placed in solution and analyzed as supplied with results shown in the top of Figure 1. Although seven forms of iron are measured in this analysis (Lee and Clydesdale, 1979b), the five forms presented in Figures 1 and 2 sum to 100% and the remaining two forms (soluble and ionic iron) may be calculated by difference. The iron sources used in this work were chosen from an intial determination of the iron profiles of all of the current GRAS-listed iron sources. Six iron sources were selected

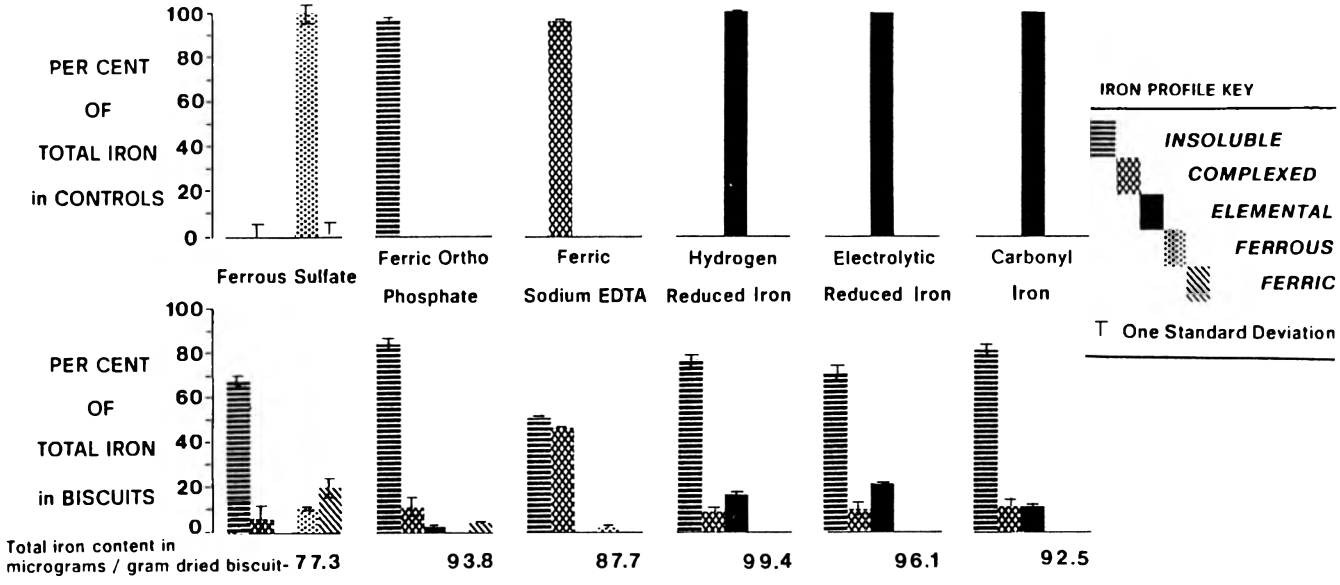

Fig. 1—Iron profiles for six major iron enrichment sources as supplied (top) and after addition to flour and subsequent baking as biscuits (bottom). T-lines indicate plus and minus one standard deviation for six replicates of each bar.

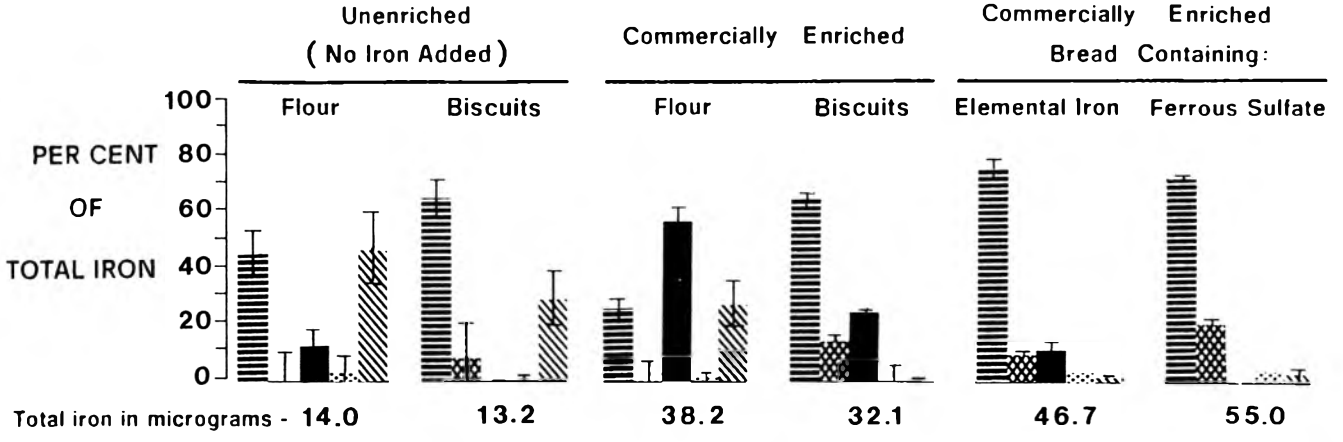

Fig. 2—Iron profiles for unenriched and enriched white flour, biscuits baked from each flour, and two samples of commercial white bread containing either elemental iron or ferrous sulfate as an enrichment source. Key is identical to Fig. 1.

Table 1—Comparison of vacuum versus freeze-drying. Data are the means in percent of each form of iron in biscuits baked from flour enriched with reduced iron

Preparation:	Total iron: µg/g	Percent of total iron						
		Elemental	Soluble	Insoluble	Ionic	Ferrous	Ferric	Complexed
Freeze-dried biscuit[a]	37.7 ± 0.96	21.2 ± 1.62	14.1 ± 1.55	64.7 ± 2.84	0.9 ± 1.35	0.2 ± 1.09	0.7 ± 1.63	13.2 ± 2.15
Vacuum-dried biscuit[a]	38.8 ± 1.41	22.1 ± 0.70	13.2 ± 2.50	64.8 ± 1.81	0.3 ± 0.50	1.0 ± 0.25	−0.7 ± 0.58	12.9 ± 2.00
f-Value, $P < 0.05$	1.243	0.660	0.323	0.005	0.447	1.636	1.829	0.055
Probability (f)	0.33 n.s.	0.46 n.s.	0.60 n.s.	0.95 n.s.	0.54 n.s.	0.27 n.s.	0.25 n.s.	0.82 n.s.

[a] Means plus or minus one standard deviation for six replicates; n.s. = no significant difference.

which best distinguish the five forms of iron shown in Figure 1. Thus ferrous sulfate is used as it is 99 ± 4.5% in the ferrous form and ferric ortho phosphate is used as it is 96 ± 0.9% insoluble. Although ferric EDTA is not approved as an iron enrichment source, it has approval for other uses and was used as it is a strongly complexed iron source. It contains 95 ± 0.7% complexed iron. Hydrogen, electrolytic, and carbonyl reduced iron consisted of 100 ± 0.5%, 99 ± 1.1%, and 100 ± 0.7% elemental iron, respectively. All the above iron sources are also frequently employed in bioavailability studies.

Laboratory enriched biscuits

Each of the iron sources was added to unenriched flour from which biscuits were baked. The resulting iron profile is shown on the bottom of Figure 1. The major effect of the baking process was the generation of insoluble forms of iron, ranging from a mean of 50 ± 1.4% for ferric EDTA to 79 ± 3.0% for carbonyl iron. 82 ± 3.8% of the ferric ortho phosphate remained in the insoluble form. In addition, there was a small but significant proportion of complexed iron produced in the baking of biscuits containing added iron, with the exception of those containing ferrous sulfate. Approximately half (46 ± 0.86) of the ferric EDTA complex remained as such after baking.

The results with ferric EDTA are noteworthy as ferric EDTA has been studied as a fortification source. Viteri et al. (1978) found labeled ferric EDTA in milk was absorbed 2.5 times better than iron in ferrous sulfate fortified milk. The ferric EDTA was also less hampered by inhibitors present in foods. Based on these findings as well as the results of this study some consideration may be given to complexed forms of iron as enrichment sources in baked products.

Unenriched flour and biscuits

The unenriched flour was analyzed as supplied to ascertain the iron profile of the endogenous iron prior to baking. As shown in Figure 2, almost all of the 14.0 ± 0.95 micrograms endogenous iron per gram of flour was either in the insoluble or in the ferric form. A small yet significant amount of the endogenous iron was in the elemental state. This is presumably from processing machinery as elemental iron does not occur naturally in biological systems. After baking, the insoluble iron increased with a corresponding decrease in ferric iron and loss of the elemental iron which was present in the flour (Fig. 2). There was a higher proportion of free ferric iron in the biscuits with no iron added, than in any of the enriched biscuits shown in Figure 1.

Commercially enriched flour

A locally purchased commercial sample of flour which was known to be enriched with elemental iron was analyzed. The results are shown in Figure 2. The total iron content was 38.2 ± 1.00 micrograms per gram of flour, corresponding to the upper limit for flour enrichment of 16.5 mg per pound of flour. The majority of the iron was still in the elemental form. The remaining iron was divided almost evenly between insoluble and ferric forms, much like the unenriched flour described above. It is apparent that nearly half of the elemental iron added to the flour by the manufacturer had changed during handling and storage. The iron had changed into the same forms which were identified in the unenriched flour.

Biscuits were then baked in the lab from the commercial flour. The resulting profile (Fig. 2) shows that 22 ± 1.2% of the iron remained in the elemental state, whereas all of the ferric iron present prior to baking was rendered insoluble. As with the laboratory enriched samples, the iron from the commercial flour was primarily insoluble after baking. Note that the iron profile of the biscuits made from the commercial sample of flour was remarkably similar to the biscuits containing electrolytic iron in Figure 1.

Drying

The commercial flour was also used to examine the possibility of changes in iron due to the drying process. Biscuits were formulated from a commercial sample of flour and either vacuum-dried (an evaporative process) or freeze-dried (a sublimation process). Table 1 is an analysis of variance of each form of iron found after either drying procedure. There was no significant difference ($p < 0.05$) between vacuum- and freeze-drying for all seven forms of iron determined in the analysis. Freeze-drying has been previously shown to have no effect on the forms of iron (Lee and Clydesdale, 1980). Thus the vacuum evaporation of water does not have any effect on the chemical forms of the iron contained in the biscuits.

Breads

Two types of commercially enriched yeast-leavened white wheat bread were analysed with results as shown in Figure 2. The first bread was enriched with elemental iron, the second with ferrous sulfate. Whereas the elemental iron enriched bread was not significantly different from elemental iron enriched biscuits, the ferrous sulfate enriched bread contained considerably more complexed iron than the ferrous sulfate enriched biscuits.

DISCUSSION

FOR EACH of the six iron enrichment sources selected for this study, a very large change in iron profile resulted from the baking process. It is apparent that the baking resulted in distributions of iron forms which were quite similar regardless of the original form of iron used for enrichment. Thus a range test was employed to examine differences between all of the biscuits and breads analyzed in this study. As shown in Table 2, every mean was tested for a significant level of iron (difference from zero) and then compared with other

Table 2—Percent distribution of seven iron forms found in biscuits or bread enriched with various iron sources. Data are the means in percent of six replicates for each product

Products:	Distribution of forms as a percent of total iron						
	Elemental	Soluble	Insoluble	Ionic	Ferrous	Ferric	Complexed
A. Unenriched control	1.9[a]	35.1	63.0	28.1	0.9[a]	27.2	7.0[a]
B. Ferrous sulfate enriched biscuits	0.3[a]	32.6	67.1	28.1	8.7	19.3	4.6[a]
C. Ferric ortho phosphate enriched biscuits	3.0[a]	15.4	81.7	4.0	1.1	2.9	11.4
D. Ferric sodium EDTA enriched biscuits	0.8[a]	49.0	50.2	2.9	3.1	− 0.2[a]	46.2
E. Hydrogen reduced iron enriched biscuits	15.4	8.1	76.5	0.3[a]	0.2[a]	0.1[a]	7.8
F. Electrolytic iron enriched biscuits	20.5	10.0	69.6	1.1[a]	0.2[a]	0.9[a]	8.9
G. Carbonyl iron enriched biscuits	10.3	10.8	78.9	0.5[a]	−0.1[a]	0.6[a]	10.3
H. Commercial flour with reduced iron	21.6	13.7	64.7	0.6[a]	0.6[a]	0.0[a]	13.0
I. Bread enriched with reduced iron	10.2	12.6	77.2	5.0	3.0	1.9	7.6
J. Bread enriched with ferrous sulfate	0.9[a]	26.9	72.2	7.3	4.3	3.0	19.7
lowest-	B	E	D	E	G	D	B
Rank order of means	D	F	A	G	F	H	A
and	J	G	H	H	E	E	I
Multiple Range Test[b]	A	I	B	F	H	G	E
	C	H	F	D	A	F	F
	I	C	J	C	C	I	G
	G	J	E	I	I	C	C
	E	B	I	J	D	J	H
	F	A	G	B	J	B	J
highest-	H	D	C	A	B	A	D

[a] Does not differ from zero percent ($p < 0.05$).
[b] Means in the same column adjacent to a common line segment are not significantly different ($p < 0.05$).

product means via a Duncan's multiple range test.

The iron profile of the ferrous sulfate enriched biscuits (B in Table 2) was quite similar to the profile of the biscuits containing only endogenous iron (A). There was no difference in soluble, insoluble, or ionic forms of iron. Also, there was no elemental or complexed iron in either the ferrous sulfate enriched or unenriched biscuits. However, the two products did differ slightly in the relative distribution of free ferrous and ferric iron. When ferrous sulfate was added to the flour, 8.7% remained in the ferrous form. In contrast, no ferrous iron remained in the unenriched biscuits. Thus the two products were not significantly different except for the amount of ferrous iron remaining in the enriched biscuits. It is apparent that when biscuits containing ferrous sulfate are baked, an iron profile results which is nearly identical to the endogenous iron profile of biscuits baked from unenriched flour.

It is interesting to note the similarity between the profiles of the biscuits containing the three elemental iron sources after baking. The hydrogen reduced, electrolytic, and carbonyl iron-enriched biscuits (products E, F, and G respectively, in Table 2) contained similar levels of soluble and complexed forms of iron. Likewise, there were insignificant levels of ionic, ferrous, and ferric iron in all three of the elemental iron enriched biscuits. The only significant difference between products E, F, and G was the amount of the elemental iron remaining after baking. Note that products E, F, and G were enriched in the laboratory with elemental iron. Product F had a significantly lower level of insoluble iron due to the larger amount of elemental iron remaining in the sample. A striking result is the nearly identical iron profiles of electrolytic iron enriched biscuits (F) and the biscuits made from a commercial flour sample containing an unspecified form of reduced iron (H). For every iron form measured, there was no difference ($p < 0.05$) between products F and H. It is therefore likely that the commercial flour contained electrolytic iron as the original enrichment source. Another close similarity was observed between the bread commercially enriched with an unknown type of reduced iron (I in Table 2) and the carbonyl iron biscuits (G). These were identical in every iron form, except for a small but significant amount (5.0%) of ionic iron in the bread, which was mostly (3.0%) in the ferrous form. Even though products I and G were so alike, no conclusions should be drawn about the original enrichment source in the bread, since the methods of production were different.

The bread enriched with ferrous sulfate (J in Table 2) was similar to the ferrous sulfate enriched biscuits (B) only in the percent insoluble iron present. Whereas there was no elemental iron in either product, there were different levels of soluble, ionic, ferrous, ferric, and complexed forms of iron. The iron in the yeast-leavened bread was distinctly different from the iron in the chemically leavened biscuit, even though the same iron source was added to each prior to baking. However, as with all other products, the majority of the iron was in an insoluble form after baking.

CONCLUSIONS

IRON SOURCES when added to flour and baked as biscuits or bread do not remain in the original chemical form. The overwhelming net effect of the baking process is the formation of insoluble forms of iron.

The large differences found between iron sources prior to baking all but disappeared in the final products, with the exception of ferric EDTA. The baking process altered the

ferric EDTA complex less than it altered other sources of iron. Thus there is a need for consideration of complexed iron as a potential iron enrichment source for baked flour products.

These data reveal that baking produces insoluble forms of iron, even when a highly soluble form of iron, such as ferrous sulfate, was used for enrichment. The generation of insoluble forms of iron from each of the iron sources tested fits the general pattern of decreased bioavailability of these same iron sources observed in bread and other baked goods (Ranhotra et al., 1979). However, more work is needed to establish the relationship between these chemical tests and bioavailability. Future work may enable the prediction of bioavailability of iron after processing on the basis of chemical tests.

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neous assay, wide equipment availability, and rapid changeover for alternative applications. This method also affords greater handling simplification as compared to approved manual techniques by eliminating both the thiochrome conversion step and the need to remeasure for background fluorescence.

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COTTONSEED AND SOY PROTEIN INGREDIENTS IN SOFT-SERVE FROZEN DESSERTS

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ABSTRACT

Six vegetable protein ingredients (glandless cottonseed flour, liquid cyclone process deglanded cottonseed flour, glandless cottonseed storage protein isolate, soy flour, soy protein concentrate, and soy protein isolate) were evaluated as partial replacements for milk solids-not-fat (MSNF) in soft-serve frozen desserts. Physical tests and sensory evaluations were conducted with the experimental desserts. All ingredients tested produced satisfactory results at low levels of substitution for MSNF. At higher levels of substitution, glandless cottonseed storage protein isolate appeared to be the best of the materials tested.

INTRODUCTION

IN 1976, 285 million gallons of ice milk were sold in the United States. Of this amount, 60% was in the form of "soft-serve" (Lindsay and Wesson, 1979). Sales of frozen desserts have continued to increase since that time, and some manufacturers predict an increase of 25% in 1979 alone (Futch, 1979).

In a survey of frozen dessert manufacturers, Futch (1979) found that 94% of the respondents considered price an important factor influencing sales of frozen desserts and that all of the respondents felt that quality influences sales. Rising costs of ingredients, however, are making high-quality, low-cost frozen desserts difficult to produce.

Milk solids-not-fat (MSNF) are an important part of many frozen dessert formulas (Arbuckle, 1972a), but they are expensive. For this reason, whey solids have been investigated extensively as a replacement for MSNF. Arbuckle (1972b), Singleton (1973), and Arnold et al. (1976) all reported that using whey to replace MSNF in dairy products contributed to loss of firmness and smoothness in the frozen dessert. "Pinking," a color change associated with the use of annatto as a colorant under certain conditions, also occurred in some cases with the use of whey products (Singleton, 1973). However, Bhusri and Jordan (1977) and Arnold et al. (1976) agreed that whey solids can be used successfully to replace part or all of the MSNF in frozen desserts.

One of the trends in the dairy industry is toward nondairy products (Grey, 1979). Since nondairy products have been gaining popularity, an alternative to the use of whey solids to avoid the high cost of MSNF might be the use of vegetable proteins in part-dairy, part-vegetable protein frozen desserts. The purpose of this study was to investigate the performance of cottonseed and soy protein ingredients in a food of this type.

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Table 1—Basic formula for soft-serve frozen desserts

Butterfat	804.42g
Milk solids-not-fat MSNF)[a]	727.71g
Sugar	814.47g
Stabilizer	28.20g
Coloring	2.55 ml
Vanilla	11.55 ml
Water	3.88 kg

[a] Or MSNF plus appropriate oilseed protein-lactose blend

MATERIALS & METHODS

Sources of ingredients

Defatted glandless cottonseed flour (GF) and glandless cottonseed storage protein isolate (GI) were prepared at the Food Protein Research & Development Center, Texas A&M University, College Station, Tex., according to the procedures described by Clark et al. (1978) and Berardi et al. (1969), respectively. Deglanded cottonseed flour (LCP), produced by the Liquid Cyclone Process, was obtained from the USDA Southern Regional Research Center, New Orleans, La. Soyafluff 200W soy flour (SF), Promosoy 100 soy protein concentrate (SC), and Promine F soy protein isolate (SI) were obtained from Central Soya Co., Chicago, Ill. Foremost Edible Lactose ±305 was obtained from Foremost Foods, San Francisco, Calif. Nonfat dry milk, Jersey cream, Go! Southern Special Stabilizer (containing mono- and diglycerides, guar gum, carrageenan, calcium sulfate, polysorbate 80, polysorbate 65 and CMC), ice cream color (an alkaline extract of annatto seeds), and vanilla extract were obtained through Lilly Ice Cream Co., Bryan, Tex. Granulated sugar was purchased from a local supermarket.

Formulation of blends and mixes

Each cottonseed and soy ingredient was blended with lactose to yield a protein-lactose blend with a protein content of 35.5%, the same as that in the nonfat dry milk used. (To eliminate confusion, the term "nonfat dry milk" will refer hereafter to the nonfat dry milk powder purchased, and the term "MSNF" will refer to the milk solids-not-fat component of the mix. These are different entities, since the nonfat dry milk consisted of MSNF, but not all MSNF in the frozen dessert mixes was obtained from nonfat dry milk—some was obtained from the cream.)

Table 1 contains the basic formula used in preparing the frozen dessert mixes. To incorporate a cottonseed or soy protein ingredient into this formula, the protein-lactose blend containing that ingredient was substituted on a weight-for-weight basis for the desired percentage of MSNF in the formula. By using the blends described above, substitution of oilseed ingredients on a protein-for-protein basis was also accomplished.

Preparation of mixes

All dry ingredients in the mix formula except the sugar were blended, then divided into three approximately equal portions. Using the highest speed of a 7-speed blender, each portion was blended with approximately 250 ml tap water (65°C) until all particles were dispersed. These dispersions were then poured into a 400 ml stainless steel beaker. To prevent loss of material, all containers used so far were rinsed with 65°C tap water, and the rinse water was added to the contents of the beaker. The remaining water, along with the cream, coloring, and vanilla, was added; then the sugar was dissolved in the mixture.

The stainless steel beaker containing the mix thus prepared was placed in a boiling water bath, and the mix was heated to 78°C. After the mix had reached this temperature, it was left in the boiling water bath for another 25 sec to effect pasteurization. The beaker containing the mix was then placed in a cold water bath, and the mix was stirred slowly by hand until it had cooled to 65°C. The cooled mix

was homogenized at 2500 psi, using a Gaulin Type 15M homogenizer, and then it was cooled to approximately 4°C, using an APV heat exchanger, type Jr. H.E. The mix was then placed in covered plastic containers and stored in a refrigerator at 4–6°C for aging.

Evaluation of formulas

After 5–7 days storage, 2.4L of mix were placed in the freezing chamber of a Swedette Model A500 soft-serve freezer and frozen until the freezer automatically stopped. At this point the temperature of the frozen dessert was −7°C. The freezer had an automatic cycle that maintained the dessert at approximately this temperature.

After the dessert had been frozen for 30 min, 79 ml was drawn and weighed. At this point 79 ml of liquid mix were also weighed. Percent overrun of the frozen dessert was calculated using the following equation (Arbuckle, 1972a):

$$\% \text{ overrun} = \frac{\text{wt of mix} - \text{wt of frozen dessert}}{\text{wt of frozen dessert}} \times 100$$

After the dessert had been frozen for 45 min, a sample was drawn into a 50 ml beaker in such a manner that the beaker was filled beyond the top. The excess dessert was then removed by scraping the flat edge of a kitchen spatula across the rim of the beaker. This provided a sample with a level top.

Penetrability of the sample was measured using a Precision Universal Penetrometer equipped with a cone 3.5 cm in diameter and weighing 10.8g. A 50-g weight was added to the loading bar, bringing the total weight of the cone, weight, and loading bar to 108.3g. In measuring penetrability, the cone was allowed to penetrate the dessert for 3 sec, and the depth of penetration was measured.

After the dessert had been frozen for 60 min, color (L, a) was measured using a Hunterlab Digital Color and Color Difference Meter, Model D25D2.

An eight-member, in-house panel of judges familiar with soft-serve ice milk evaluated the dessert for texture, color, flavor, odor, and overall acceptability, using a three-point scoring system: 3=good; 2=fair; 1=poor. Because the study was conducted over a period of 5 months, two of the panelists had to be replaced due to employee turnover. New panelists were of the same sex and approximate age as those whom they replaced.

Since only one mix could be frozen at a time, each sample was evaluated singly. In order to minimize the effect of age on the mixes, they were evaluated in the same order in which they were prepared. Samples for sensory evaluation were drawn into 1-oz white paper souffle cups, and actual sample volume was approximately 45 ml. Panelists were allowed to swallow the samples.

The formula evaluation process described thus far was repeated when the mix was 11–13 days old, and the results of the two freezings were averaged to form one replication.

When the remainder of the mix was 14 days old, it was allowed to warm quiescently to room temperature (25°C). Its color was then measured in the same way as that of the frozen dessert. Mix viscos-

Table 2—Physical characteristics of soft-serve frozen desserts containing cottonseed protein ingredients[a]

Protein type and level of replacement	Mix viscosity (cp)	Overrun, 30 min (%)	Penetrability 45 min (mm)
Glandless cottonseed flour (GF)			
Control (0%)	100.90	72.5	19.00
5%	73.00	68.5	19.75
20%	100.35	48.0*	16.00
40%	92.90	48.0*	15.20
60%	84.50	50.0*	16.65
80%	88.75	56.5*	16.40
Liquid cyclone process flour (LCP)			
Control (0%)	114.20	71.0	20.20
5%	94.90	74.0	21.90
20%	104.90	43.0*	15.20
40%	92.60	45.0*	14.80
60%	92.20	46.0*	15.60
80%	74.70	54.0*	17.15
Storage protein isolate (GI)			
Control (0%)	121.65	70.5	19.35
5%	53.40*	71.0	20.50
20%	50.75*	68.5	19.00
40%	48.75*	64.0	18.00
60%	45.65*	62.0*	16.20
80%	51.25*	61.5*	17.15

[a] Each mean followed by an asterisk is significantly different (α = 0.05) from the control mean for its series.

Table 3—Physical characteristics of soft-serve frozen desserts containing soy protein ingredients[a]

Protein type and level of substitution	Mix viscosity (cp)	Overrun, 30 min (%)	Pentrability 45 min (mm)
Soy flour (SF)			
Control (0%)	140.35	74.0	20.00
5%	95.00*	70.5	19.00
40%	131.10	65.5*	18.50
80%	187.10*	59.0*	17.20
Soy protein concentrate (SC)			
Control (0%)	131.10	73.0	19.75
5%	131.10	74.0	19.10
40%	130.05	65.5*	16.55
80%	124.45	50.0*	15.60*
Soy protein isolate (SI)			
Control (0%)	102.45	77.0	22.75
5%	116.15	74.5	23.95
40%	108.40	67.5*	20.35
80%	165.10*	65.0*	18.40*

[a] Each mean followed by an asterisk is significantly different (α = 0.05) from the control mean for its series.

Table 4—Hunter color values for soft-serve frozen desserts containing cottonseed protein ingredients[a]

Protein type and level of replacement	Mix "L"	Mix "a"	Frozen "L"	Frozen "a"
Glandless cottonseed flour (GF)				
Control (0%)	82.55	2.80	89.00	0.85
5%	80.80	2.95	88.70	0.75
20%	79.05*	2.40	86.45*	1.00
40%	76.60*	2.20	84.30*	0.95
60%	73.70*	2.55	83.00*	1.20
80%	72.65*	2.55	82.30*	1.25*
Liquid cyclone process flour (LCP)				
Control (0%)	81.10	3.00	89.20	0.90
5%	77.80*	2.60	87.40*	0.70
20%	72.70*	1.80*	83.80*	0.90
40%	68.50*	2.00*	80.60*	0.80
60%	65.50*	2.20	79.00*	0.90
80%	64.00*	2.70	78.70*	0.70
Storage protein isolate (GI)				
Control (0%)	82.00	2.55	89.65	0.80
5%	81.05	2.75	89.65	0.65
20%	79.05*	2.55	88.70	0.55
40%	77.10*	1.80*	87.15*	0.10*
60%	77.55*	1.15*	86.30	0.00*
80%	74.05*	0.75*	85.45*	−0.55*

[a] Each mean followed by an asterisk is significantly different (α = 0.05) from the control mean for its series.

ity was then measured at 25°C, using a Brookfield Model LVT-E Viscometer equipped with T-shaped spindle A at 60 rpm. The spindle was allowed to rotate in the mix for 30 sec before a reading was made.

Experimental design and analysis of data

Since too many mixes were handled to allow processing of all at one time, they were prepared in groups according to protein type (the cottonseed or soy ingredient used). In each group, or series, there was an all-dairy control mix, plus a mix incorporating each level of substitution of that ingredient for MSNF. Mixes within a series were prepared in random order. The cottonseed ingredients were substituted for 5, 20, 40, 60, and 80% of the MSNF in the formula, and the soy ingredients were substituted for 5, 40, and 80% of the MSNF. Each series except the LCP series was replicated twice. The LCP series was prepared only once.

Analysis of variance of the data was computed using the GLM procedure of the SAS-76 package (Barr et al., 1976). For each quality factor measured, the data from each series of mixes were analyzed using Dunnett's test (Steel and Torrie, 1960) to determine whether any of the levels of substitution in each series produced values significantly different from those of their control.

RESULTS & DISCUSSION

Physical characteristics

Physical characteristics of the frozen desserts are shown in Tables 2 and 3. In the GF, LCP, and SC series, none of the unfrozen mixes was significantly different in viscosity from its controls. In the SF series, two of the viscosities were significantly different from the control viscosity: at 5% substitution the mix was less viscous, and at 80% substitution it was more so. At 80% substitution, SI also significantly increased viscosity. These data suggest that for the cottonseed flours and soy ingredients, there were no discernible trends in mix viscosity. However, all of the mixes containing GI were significantly less viscous than their control.

Overrun was significantly reduced by GF and LCP at 20% and higher substitution, but the GI overrun was not affected until 60% substitution was reached. With all three soy products, the overrun was significantly reduced at 40% and higher substitution. Since 20% substitution was not tested for these products, it is not known whether the soy products would have affected overrun at that level. According to England (1968), MSNF depresses overrun, while whey solids enhance it. All of the ingredients tested here reduced overrun, though none of the products had overrun below the minimum of 40% for soft-serve products specified by Arbuckle (1972a).

Penetrability, which is inversely related to firmness, was affected only by SC and SI, each of which significantly reduced penetrability at 80% substitution. Reduced penetrability indicated that the products were more firm; therefore, none of the products tested in this study had the tendency of whey solids to reduce firmness that was reported by Arbuckle (1972b), Singleton (1973), and Arnold et al. (1976).

Color

Hunter color values for the unfrozen mixes and the frozen desserts are shown in Tables 4 and 5. GF and GI significantly reduced the "L" value of the unfrozen mix at and above 20% substitution, and LCP significantly reduced it even at 5% substitution. All soy ingredients reduced "L" values at 40 and 80% substitution. The reduced "L" values indicated darkening of the mixes.

Mixes containing SF and SC at 40 and 80% substitutions had significantly higher "a" values than did their controls. Since a higher "a" indicates a shift toward redness, this indicated that some "pinking" may have occurred similar to that reported by Singleton (1973) with regard to whey solids. None of the other products appeared to cause increases in "a", and, in fact, LCP reduced "a" at 20 and 40% substitution and GI at 40% and higher.

As would have been expected, the desserts after freezing displayed trends in color similar to the ones described above for mixes. For all series except the GI series, significant differences in "L" values appeared at the same levels of substitution as they did while in mix form. With the GI product, however, significant difference in "L" did not appear until 40% substitution, one level higher than with that series in mix form. Differences in "a" were the same as with the mixes except that with LCP, the differences did not

Table 5—Hunter color values for soft-serve frozen desserts containing soy protein ingredients[a]

Protein type and level of replacement	Mix "L"	Mix "a"	Frozen "L"	Frozen "a"
	Soy flour (SF)			
Control (0%)	81.35	2.45	89.90	0.65
5%	79.75	2.30	89.55	0.60
40%	77.25*	3.90*	87.10*	1.75*
80%	75.00*	3.80*	84.50*	2.25*
	Soy protein concentrate (SC)			
Control (0%)	83.45	1.80	89.75	0.50
5%	83.20	2.05	89.80	0.65
40%	80.95*	3.20*	88.80*	1.30*
80%	78.35*	3.15*	85.90*	1.60*
	Soy protein isolate (SI)			
Control (0%)	83.55	1.75	90.35	0.30
5%	82.75	1.55	90.30	0.20
40%	80.80*	1.90	89.35*	0.30
80%	78.60*	1.90	87.95*	0.45

[a] Each mean followed by an asterisk is significantly different (α = 0.05) from the control mean for its series.

Table 6—Mean sensory scores for soft-serve frozen desserts containing cottonseed protein ingredients[a]

Protein type and level of replacement	Texture	Color	Flavor	Odor	Overall
	Glandless cottonseed flour (GF)				
Control (0%)	2.95	3.00	2.95	3.00	2.95
5%	2.90	3.00	2.95	2.95	3.00
20%	2.95	2.80	2.55	2.85	2.70
40%	2.95	2.50*	1.95*	2.80	2.15*
60%	2.55*	2.10*	1.35*	2.60*	1.50*
80%	2.80	1.90*	1.20*	2.60*	1.20*
	L quid cyclone process flour (LCP)				
Control (0%)	2.90	3.00	3.00	3.00	3.00
5%	2.90	3.00	2.90	3.00	3.00
20%	3.00	2.60	2.70	2.90	2.80
40%	2.70	1.90*	1.90*	2.80	2.10*
60%	2.50	1.60*	1.60*	2.70	1.70*
80%	2.80	1.30*	1.30*	2.20*	1.10*
	Storage protein isolate (GI)				
Control (0%)	3.00	3.00	3.00	2.90	3.00
5%	3.00	3.00	2.95	3.00	3.00
20%	2.90	3.00	2.90	3.00	2.95
40%	2.70	2.95	2.80	2.95	2.80
60%	2.60*	2.70	2.65	3.00	2.75
80%	2.45*	2.50*	2.45*	2.90	2.45

[a] Each mean followed by an asterisk is significantly different (α = 0.05) from the control mean for its series.

Table 7—Mean sensory scores for soft-serve frozen desserts containing soy protein ingredients[a]

Protein type and level of replacement	Texture	Color	Flavor	Odor	Overall
		Soy flour			
Control (0%)	2.95	3.00	2.95	2.95	2.95
5%	2.95	2.90	2.85	3.00	2.95
40%	2.90	2.90	1.80*	2.70*	2.05*
80%	2.80	2.30*	1.50*	2.80	1.70*
		Soy protein concentrate			
Control (0%)	2.85	3.00	2.90	2.95	2.90
5%	2.95	3.00	2.85	3.00	2.95
40%	2.30*	3.00	1.80*	2.80	1.90*
80%	2.15*	2.70	1.50*	2.65*	1.55*
		Soy protein isolate			
Control (0%)	2.75	3.00	2.90	2.95	2.95
5%	2.85	3.00	2.90	3.00	2.95
40%	2.85	3.00	2.40*	2.95	2.65
80%	2.60	2.55*	1.90*	2.90	2.00*

[a] Each mean followed by an asterisk is significantly different ($\alpha = 0.05$) from the control mean for its series.

appear, and with GF, an increase in "a", possibly indicating "pinking" in the frozen product, appeared.

Sensory evaluations

Results from sensory evaluations of the frozen desserts are shown in Tables 6 and 7. LCP, SF, and SI did not appear to affect texture acceptability. However, GI significantly reduced texture acceptability at 60 and 80% substitution, and this acceptability was reduced significantly when SC was incorporated to replace 40 or 80% of MSNF. At 60% substitution with GF, texture acceptability was significantly lower than that of the control, but at 80% substitution it was not significantly different.

Flavor acceptability, one of the areas of major concern with using oilseed ingredients to replace MSNF, was significantly reduced by all of the products at one or more levels of substitution. With the cottonseed flours and soy products, the difference appeared at 40% and higher substitution. However, with GI, the difference appeared only at 80% substitution.

Neither isolate appeared to affect odor acceptability, and the other products affected it only at higher levels of substitution. Significant differences from the control appeared at 60 and 80% substitution with GF and at 80% only with LCP and SC. The only significant difference in odor in the SF series was at 60% substitution.

GF, LCP, SF, and SC all appeared to affect overall acceptability at 40% substitution and higher, and SI affected it at 80% substitution. However, GI did not significantly affect overall acceptability.

CONCLUSIONS

BASED on the results of the sensory evaluations, and supported by the data from the physical tests, it appears that all ingredients tested could be used to replace MSNF in soft-serve frozen desserts at low levels of substitution (under 40%). However, acceptability and functionality in the desserts of all ingredients tested except glandless cottonseed storage protein isolate tended to fall at 40% substitution or higher. Though glandless cottonseed storage protein isolate caused significant differences in several quality factors, it appears that this product might be used successfully to replace up to 80% of the MSNF in soft-serve frozen desserts.

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SOUR CASSAVA STARCH PRODUCTION: A PRELIMINARY STUDY

O. S. CÁRDENAS and T. S. de BUCKLE

ABSTRACT

Sour cassava starch is produced in Colombian rural areas by traditional small-scale technology, which includes a 20–30 days fermentation stage. A study was carried out with the purpose of analyzing the fermentation mechanisms, in an attempt to shorten the above mentioned step. Samples taken from five plants and produced by acid treatment in the laboratory, were evaluated. Results showed that lactic acid bacteria and yeast are involved. Sweet, sour, and acid-treated starches showed differences in molecular weights and in the surface appearance, under SEM. Although acid-treated and sour starches showed similar amylogram curves, only the latter produces good specific volume and crumb structure in a baked product called "pan de yuca."

INTRODUCTION

THE CASSAVA (*Manihot esculenta, Crantz*) starches, commercially known as sour starches, are the product of the natural cassava starch, acid fermentation. In Colombia this type of acid-modified starch is widely used in the elaboration of some baked products widely accepted in the local market. The acids and the modification produced in the starch during the fermentation process impart certain characteristics of flavor, texture, and volume to the baked product denominated "pan de yuca." ("Pan de yuca" the most common baked product prepared mainly with sour cassava starch and salty nonripened cheese, has an inflated appearance, typical alveolar crumb structure, and a crispy crust.) The above mentioned characteristics cannot be obtained when the sour starch is replaced by the natural nonfermented (sweet) cassava starch.

The sour starch process mechanisms and the nature of the acidity, have not been studied yet, although the process has been used for many years in some rural areas of the country.

This paper will describe the small scale cassava starch extraction processes in rural Colombian areas as well as the characterization of the products. These processes probably have much in common with those used in other countries.

EXPERIMENTAL

The fermentation process

The sour cassava starch process, includes starch extraction, fermentation, and sun drying (Fig. 1). After peeling, washing, and grating of roots, starch granules are separated by filtering the cassava mash with the aid of water through a sieve with a fine cheese cloth. The water starch suspension is collected in the sedimentation tanks, which are constructed only with cement or lined with ceramic tile. The supernatant water is drained periodically (at least every 2 hr) depending on the filtration and sedimentation speed.

Once the tank is full, the settled starch is allowed to ferment, at environmental conditions (15–25°C), covered with some of the supernatant liquid. No starters are added and this fermentation period takes 1 or 2 wk. During this time the dark upper layer of the starch is scraped off, until the surface looks clean. The residue produced from the scraping is denominated "Mancha" and consists of cassava starch and proteinaceous materials; it is utilized for animal feed. After 2 wk, the wet starch is transferred to another tank where fermentation continues for 1 or 2 wk more, without supernatant water. Finally, the wet starch (44–46% moisture content), is placed on wooden trays or on cement yards, provided with a moving roof (to protect starch from rain). Then the starch is sun-dried for a period of time ranging from 2–5 days, to a moisture content of 12–15%. The entire fermentation process takes from 20–30 days and in some cases longer, depending upon duration of the final sun drying period for the starch which can be greatly affected by weather conditions.

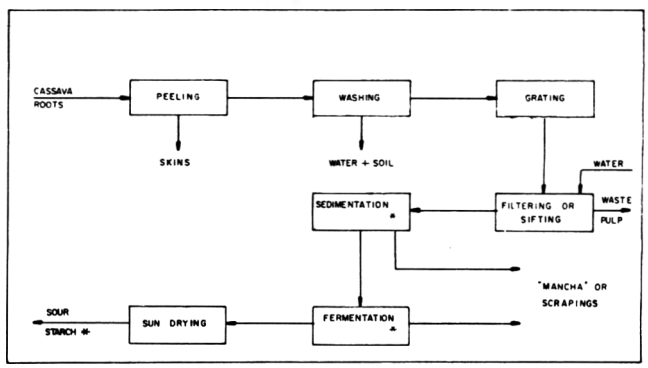

*Fig. 1—Process of extraction, fermentation, and drying of the sour cassava starch. (*Sample sites)*

Materials

During the course of this investigation five rural plants were visited, and samples of sweet and sour starches were taken during the process. Those samples were used for the starch characterization and for microbiological studies. Supernatant water samples from 20-days fermentation tanks were also taken for organic acids identification and quantification. (A 20-days fermentation periods is empirically considered the fermentation end point, since after this time the starch acidity declines.)

Once the acids produced during fermentation were identified, fresh extracted cassava starch was modified using the same type of acids (alone and in mixtures) as those detected in fermentation. The starches obtained in this manner were characterized and used to elaborate "pan de yuca," replacing the commercial sour starch, in order to evaluate the functional properties of both fermented and acid-modified cassava starches.

Fresh cassava starch was obtained in the pilot plant by means of the wet extraction method described by Shipman (1967). After drying, the isolated starch was utilized for the acid-modification experiments.

Starch acid modification

The acid treatment of starch was carried out at 37°C for periods of 10–20 days.

Acid solutions (0.5–1% v/v) were prepared with lactic, acetic, and butyric acids, alone and in mixtures of 1:1 lactic-acetic, lactic-butyric, and 2:1:1 lactic-butyric-acetic and acetic-lactic-butyric. The supernatant acid solution was renewed daily and starch samples were taken for amylographic determinations every 5 days. Treatment was stopped when starch paste characteristics were similar to those of sour starches; starch was then filtered, washed, and dried.

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Isolation and identification of microorganisms

For the sour starch microorganisms isolation and enumeration, the following methods were used: The procedure recommended by Hobbs and Greene (1976) for cereals and flours, for total bacteria, yeast, and mould counts. It includes inoculating two series of standard plate count (SPC) agar. The selective medium according to Davison and Croning (1973) was used for the isolation and enumeration of lactic acid bacteria. All the cultures were incubated at 25°C, in aerobic conditions except one of the series of SPC and the lactic acid bacteria selective medium, which were placed in a microaerophilic atmosphere enriched with CO_2.

The identification of lactic acid bacteria was performed as indicated by Sharpe and Fryer (1966).

Chemical analysis

Standard AOAC (1965) methods were used for moisture and starch. Total acidity was measured by direct titration with NaOH of the supernatant fluid samples, taken from 20-days fermentation tanks. Acetic, lactic, and butyric acids were measured according to the AACC (1969) method recommended for rye flour.

The reducing end-groups of the different starch samples were measured by the Alkali Number determination, according to Schoch (1964), and molecular weight was determined by the potentiometric method recommended by Von Ceh et al. (1976).

Physical and functional characteristics

Brabender viscosity curves were determined, using a Brabender Viscoamylograph. Starch suspension was prepared at 5.5% (dry basis) and if necessary pH was adjusted to 3.5 in order to avoid pH influence on starch pasting. The special procedure followed for the determination has been described by Smith et al. (1979).

Microscopy. A Reichert Zetopan microscope was used to study the starch appearance, to determine granule size and distribution, and to measure the gelatinization temperature, using a Kofler hot stage according to Schoch and Maywald (1956).

Scanning electron microscopy (SEM), was also used to study some of the sweet, sour, and acid-modified starch samples. The samples were coated with a 150 Å gold-palladium layer, and the specimens were viewed and photographed using a Cambridge 54-10 steroscan electron microscope.

Baking tests were performed on "pan de yuca" rolls prepared with sweet, sour, and acid-modified cassava starches, according to the ususal formulation:

Starch	125 parts
Cheese	125 part (Salty type, fat 20–24%)
egg	16 parts
milk	100 parts
sugar	2 parts

The ingredients were mixed into a dough and 30g "pan de yuca" rolls were molded and baked at 200°C for 30 min in a rotating laboratory oven with an initial wet atmosphere. The finished products were observed for appearance, flavor, and texture, but main emphasis was placed upon the loaf volume measurements.

Table 1—Wet sour starch—bacterial, mold and yeast counts. (samples taken during fermentation process)

Total count microorganisms/g	Range[a]	Isolated microorganisms
Microaerophilic bacteria CO_2 enriched atmosphere	$1.0\times10^7-3.0\times10^7$	*Lactobacillus casei, L. casei* v. *Rhamnosus L. plantarum*
Yeast	$2.0\times10^4-1.5\times10^6$	*Saccharomyces* type
Mould	$0.5\times10^3-1.1\times10^5$	*Geotricum candida*

[a] Range of 5 samples taken in different plants.
[b] Few gram-positive sporulated rods were also isolated.
NOTE: Microbial growth curves were not determined, as it was considered beyond the scope of the present study.

Table 2—Concentration of organic acids present during the fermentation process in five rural factories[a]

Plant[c]	Total acidity g/100 ml	(% of total acidity[b]) Lactic acid %	Butyric acid %	Acetic acid %
1	0.57	66.0	17.0	16.0
2	0.57	75.0	N.D.*	24.0
3	0.69	82.7	2.0	13.8
4	0.42	73.8	2.8	23.0
5	0.64	76.6	N.D.*	23.4

[a] Measured on the supernatant after 20 days
[b] g/100 ml as latic acid
[c] Plants located in Cauca, Colombia
* Not detected

Table 3—Some characterics of sweet, sour, and acid-treated cassava starches

	pH (20°C) 10% water suspension	Starch %	Alkali no.[a]	Mol. wt[b] g/mole
Sweet starch	6.0–6.5*	97	1.2	215.000
Sour starch	3.5–4.0*	96–99*	8.2	30.000
Acid treated[c] (A B L)	3.5	97	4.8	136.000
Acid treated[d] (A)	3.6	97	5.1	–

[a] Reducing end-groups determination by the alkali number
[b] Potentiometric method
[c] 20 days at 37°C with a mixture of acetic, butyric, lactic 2:1:1, pH 3.5
[d] 10 days at 37°C with acetic acid, pH 3.5
* Range determined in five samples from different plants

RESULTS & DISCUSSION

Microbiological studies

Microbial population of sour starches from different sources is represented by a high density of microaerophilic bacteria (Table 1). The predominating microorganisms found in all samples were Gram-positive rods which were later identified as Lactic acid bacteria in the bacterial isolates. *Lactobacillus plantarum* was the most common in the various fermented samples examined. This homofermenta-

Table 4—Characterization of natural and fermented cassava starch granule

Sample	No. avg diam[a]	Wt avg diam.[a]	Gelatinization temp[a]	Microscopic examination (Polarizing-Microscope)
Sweet cassava strach	11.95μ	14.9μ	61.0°C	Round granule with centric hilum 100% birefringence
Sour cassava strach	11.75μ	15.36μ	60.5°C	Nearly 10% of starch granules show changes in morphology and birefringence loss. There is a tendency to aggregation
Acid-treated starch (ABL 2:1:1)	11.20μ	15.07μ	61.0°C	100% Birefringence. The granules exhibit tendency to aggregation
Std dev.[b]	3.86μ	3.24μ	0.69	

[a] Microscopic examination according to Schoch (1956). Average calculated from granule size distribution curves of 600 measurements, taken on single samples of sweet, sour, and acid-treated starches.
[b] Between several measurements in the same sample

tive lactic acid bacterium was found alone or accompanied by *Lactobacillus casei*.

Few sporulated Gram-positive rods were also found but due to their very small number their importance was ignored. Numerous yeast colonies were also found in all sour starches. Microscopic observations of the fresh yeast isolates showed that the yeast cells morphology was similar to that described for the *Saccharomyces* group, but no further identification was carried out. The fungus *Geotrichum candida*, a common mould frequently associated with lactic acid-fermented foods (Alexopoulos, 1962), was the only type of mould found. It could be a contaminant or it could play an important role in the amylolytic action upon the starch, supplying the fermentable sugars for the lactic acid bacteria fermentation. Since no inoculum is added, microorganisms involved, such as lactic acid bacteria, must be present in the tanks, in other pieces of equipment and/or in the environment. It is important to point out that lactic acid bacteria, mainly *Lactobacillus plantarum* has also been recovered from "gari," a traditionally fermented cassava product, original to some African countries (Ngaba and Lee, 1979). The lactic acid microorganisms seem to be responsible for the characteristic flavor and aroma of both "gari" and sour cassava starch.

Nature of the acidity

An acidic environment in the sour cassava process is reached after 2 or 3 fermentation days; during this period pH values drop from 6.5 to 3.5 and then remain stable for about 20 days. After this time the pH value increases, indicating the end of the fermentation period.

The role of the lactic-acid bacteria in this process, was confirmed by analysis of the organic acids (Table 2) in which lactic acid represented from 66–82% of the total acidity produced. The remainder was acetic acid (samples 2 and 5) or an acetic and butyric acid mixture.

Chemical characteristics

Chemical composition of sweet and sour cassava starches is similar. Fiber, protein and ashes are very low (ranging from 0.1–0.4% for any of the three components). The pH values of the starch suspension (10%), the alkali number, and the molecular weight are different for sour and sweet starches (Table 3).

A remarkable increase in the alkali number was observed in the fermented samples, probably due to the starch chain cleavage produced by the amylolytic action of both, acids and enzymes, with the subsequent exposure of reducing groups. This breakdown of the molecule is also confirmed by the reduction of the average molecular weight in the sour starch. In the acid-treated starches the reducing end groups increased (Alkali number) and the molecular weight dropped, but a 20-days treatment was not sufficient to obtain similar values as those observed in sour starch.

Physical characteristics

The shape, dimension, and gelatinization temperature of sour and sweet starches did not show significant differences (Table 4). Fermented starches when viewed through polarized light indicated a partial loss of birefringence and a marked tendency to form aggregates. This last characteristic was also observed on acid-treated starches.

The sweet, sour, and acid-modified cassava starches (Fig. 2–5) reveal some differences. The surface of granules, which are round or oval in shape, some with truncated concave edges, appear smooth and homogeneous in the sweet starch, whereas the sour (fermented) cassava starch surface shows some indentations and slight evidence of pitting. The acid-treated cassava starch compared with the sweet starch, does not show much difference. It seems that in the sour starch case, there is not only an acid modification but also an enzymic attack as indicated by the pitting

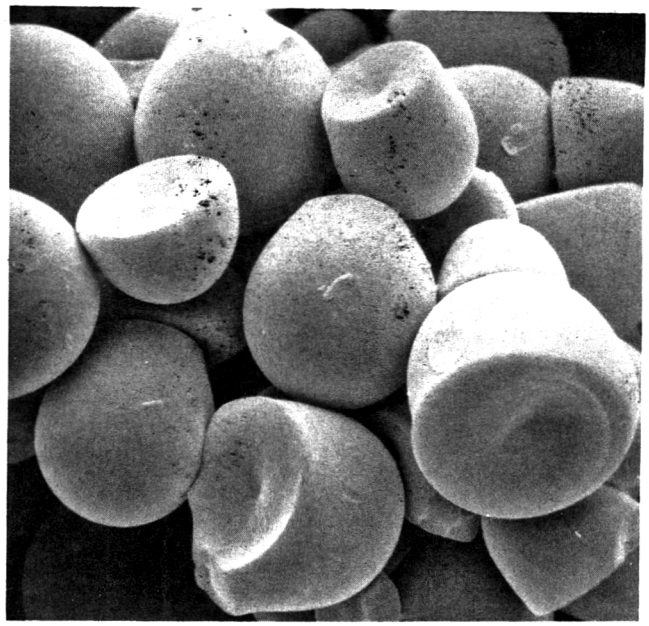

Fig. 2–SEM showing smooth surface of sweet cassava starch granules. Magnification: 1700X.

of the granules, which apparently is not as severe as that described by Evers and McDermott (1970) for alpha amylolysis on wheat and by Evers and Juliano (1976) on rice.

Functional behavior

Figure 6 shows characteristic patterns of paste behavior of sweet, sour, and acid-modified cassava starches. The Brabender viscosity curves reveal extensive thinning in all starches when cooked in the Brabender at 90°C. Significant differences are observed in the viscosity between sweet (nonfermented) and sour or acid-treated samples; this property is higher in the sweet starch. Both sour and acid-treated starch pastes behave in a similar manner during

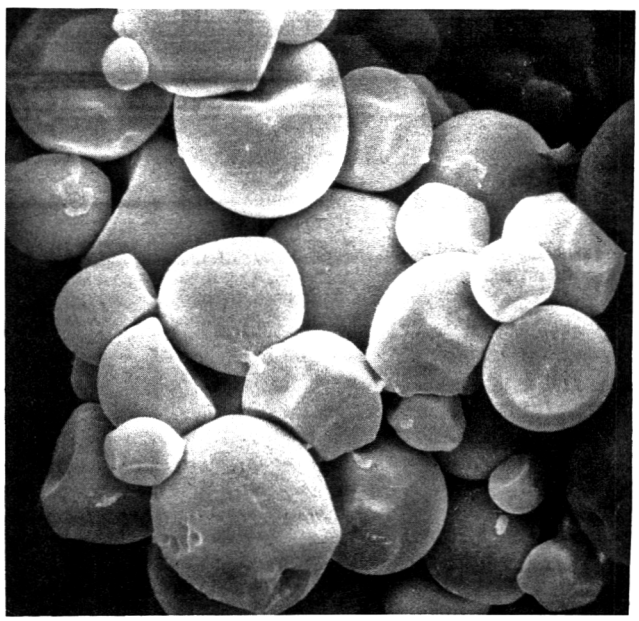

Fig. 3–SEM showing slight pitting on some granules of sour cassava starch. Magnification: 1700X.

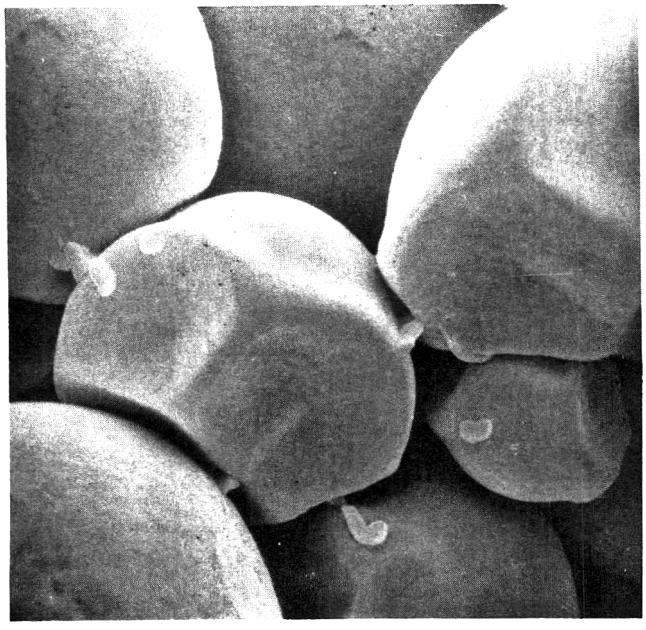

Fig. 4—Pitting on a single sour cassava starch granule as observed in SEM. Magnification: 4000X.

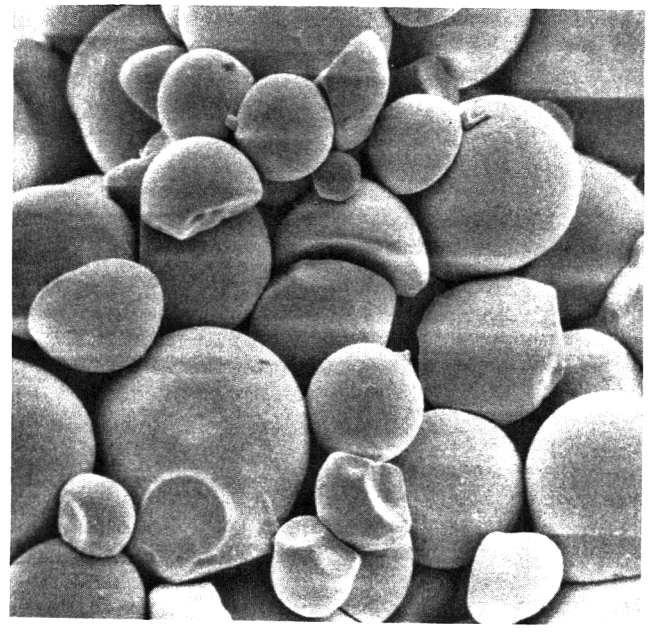

Fig. 5—Surface of some granules of acid-treated cassava starch (lactic acid, 10 days, 37°C, pH 3.6), as viewed in SEM. Magnification: 1700X.

Table 5—Functional properties of sweet and sour starch in "pan de yuca"[a]

Type of starch used	Specific volume cc/g	Crumb structure	Other Props
Sour starch	4.2	Loose, large alveola	Thin and crispy crust
Acid treated (A-B-L) (See Table 3)	2.2	Dense, small alveola	Thick crust. Characteristic flavor diminished
Acid treated (A)	2.3	Dense, small alveola	Thick crust. Characteristic Flavor diminished
Sweet starch	2.0	Very poor	Flavor and appearance not characteristic

[a] Baked product (200°C) made from sour cassava starch, hard, salted unfermented cheese (1:1) (1 egg per pound of starch) and water. No leavening agent is added. Final weight (22g)

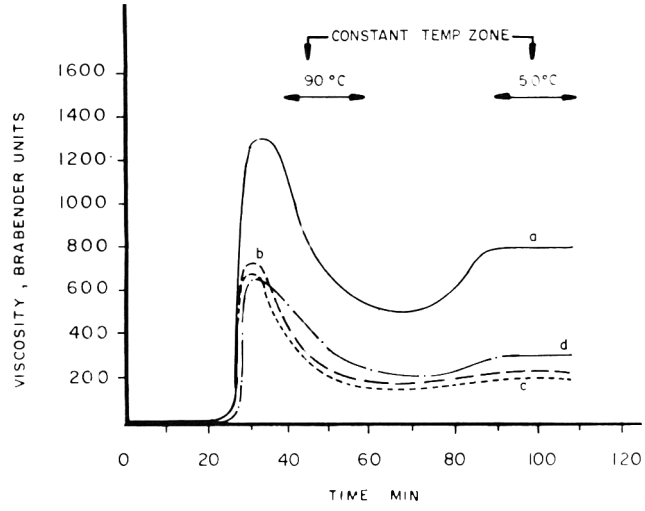

Fig. 6—Amylograms of sweet, sour, and acid-modified cassava starches: a = sweet starch; b = acid-modified starch (acetic, butyric, lactic 2:1:1, 20 days, pH 3.6); c = commercial sour starch; d = acid-modified starch (acetic 10 days, 37°C, pH 3.5).

heating and cooling, and show less tendency to retrograde than that observed in sweet starch.

Baking test. Table 5 illustrates the results of baking tests for "pan de yuca." Independently from the degree of acidity, fermented or sour starch produces a baked product with an excellent volume, alveolar loose crumb structure, and a thin and crispy crust. On the contrary, "pan de yuca" prepared with acid-treated or sweet starches, did not exhibit such good volume, the crumb was dense, and the crust thick. The possible explanation for the difference in behavior of sour and acid-treated starch during baking, could be that during the fermentation process there is not only an acid modification, but also an enzymic attack, giving to the sour starch certain properties which in turn produce a baked product with typical and acceptable characteristics. The few pits observed on some granules show some evidence of the enzymic attack, those enzymes probably have a microbial origin, produced during fermentation.

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PREDICTION OF NUTRIENT RETENTION IN THERMALLY PROCESSED HEAT CONDUCTION FOOD PACKAGED IN RETORTABLE POUCHES

P. F. CASTILLO, J. A. BARREIRO, and G. R. SALAS

ABSTRACT

A model was developed to predict the retention of nutrients with first order kinetics of thermal degradation in foods packaged in retortable pouches and sterilized by thermal conduction. The validity of the model was verified experimentally on a simulated food. The model was effective in predicting the temperature at the center of the container at the end of the heating period and, to a lesser extent, at the end of the cooling period. The prediction of the fraction of nutrient retained at the end of processing compared favorably to the fraction obtained experimentally, and they were within the 90% confidence intervals of the experimental fractions.

INTRODUCTION

THE EFFECT of the heat sterilization process for canned foods on the quality and nutrient retention of the food has been a major concern of food processors since the beginning of the canning industry. This concern has constantly prompted studies into ways to reduce nutrient and quality destruction in the sterilization process, and different means to predict these losses according to the conditions of processing.

The formulation of mathematical models for the prediction of bacterial destruction and loss of nutrients has been tried in the past, utilizing different approaches. Stumbo (1948) presented certain bacteriological considerations in which greater attention was given to the kinetics of microorganism inactivation when subjected to moist heat.

A number of graphical and mathematical procedures have been proposed (Stumbo, 1949; Gillespy, 1953; Stumbo, 1953; Stumbo, 1973) in order to estimate the effectiveness of thermal processes in terms of their capacities to reduce the number of viable microorganisms in containers for which the heating characteristics were known.

Ball and Olson (1957), Teixeira et al. (1969) and Hayakawa and Ball (1969) presented methods for estimating nutrient degradation in canned foods in cylindrical containers during thermal processing.

A computer model was proposed by Teixeira et al. (1969) who developed a numerical solution to simulate the thermal processing of canned foods, that would simultaneously predict the lethal effect of a heat process on bacteria and its destructive effect on thiamin.

The accuracy and validity of Stumbo's modified mathematical method and the computer model of Teixeira et al. (1969) were studied by Teixeira et al. (1975). The experimental evidence obtained showed agreement with the calculated values, in all cases, and confirmed the validity of both the computer model developed by Teixeira et al. (1969) and Stumbo's method as modified by Jen et al. (1971).

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Jen et al. (1971) modified Stumbo's original method to obtain one that would apply equally well to both thiamin and bacterial inactivation, or to the inactivation of any other thermally vulnerable factor exhibiting first order kinetics. In devising the method, a new equation was derived for integrating heat effects throughout the food in the container during heating and cooling.

Lenz and Lund (1977a) developed the lethality-Fourier number method for calculating the center point sterilizing value of a thermal process applied to conduction heating foods by combining first order destruction kinetics with the Arrhenius equation. The lethality-Fourier number method was extended by the same authors (Lenz and Lund, 1977b), so that mass-average retention of heat-labile quality factors (e.g., color and nutrients) could be calculated for conduction heating foods.

Another theoretical model for the prediction of the loss of nutrients during thermal processing was developed by Barreiro et al. (1977). This model was developed by solving analytically the heat conduction equation for a finite cylinder under nonstationary conditions, and its validity was experimentally verified.

Manson et al. (1970) presented a numerical solution to predict lethality and nutrient retention in packaged conduction-heating food in rectangular shaped containers utilizing an approach similar to that of Teixeira et al. (1969). However, the model was not experimentally verified with an actual nutrient. A similar procedure was followed for pear-shaped containers (Manson et al., 1974).

Herrera (1978) developed and verified experimentally an analytical solution for the prediction of nutrient retention in rectangular cans.

The use of retortable pouches is becoming very popular in the food industry due to their convenience and better preservability of the food qualities as a result of faster heat transfer due to the configuration of the package. Several experimental studies have been carried out on the heat transfer rates and sterilization of foods in retortable pouches (Yamano and Komatsu, 1969; Yamano et al., 1969; Yamaguchi et al., 1971; Yamaguchi, 1974; Terajima, 1975).

There were not found in the literature any studies in which a theoretical model is developed for the prediction of nutrient retention in foods heated by conduction in retortable pouches. Therefore, the objectives of this investigation are, mainly, to develop such a model assuming the geometry of the pouch as rectangular, and to verify its validity experimentally in a simulated food packaged in a retortable pouch.

DEVELOPMENT OF THE MODEL

THE FOLLOWING limitations and assumptions were taken into consideration prior to the development of the model:

1. Heat is transferred by conduction in the product inside the pouch, and the thermal diffusivity of the product is constant during the heating and cooling periods.
2. The product is packaged in retortable pouches whose dimensions are approximated to a rectangular parallelepiped of dimensions a, b and c.
3. Heat is transferred through all sides of the pouch.
4. The heat resistances of the three-ply components of the pouch

are negligible compared to the thermal resistance of the product.

5. The surface temperature of the product is constant and equal to the temperature of the heating or cooling medium. This implies that during heating and cooling the external heat transfer coefficient is large and the resistance to heat transfer small as compared to the internal one. For the present study, it was calculated in the order of 100 to 150 Btu/h ft² °F during cooling (Kreith, 1973), and in the order of 1000 Btu/h ft² °F during heating.

6. The initial temperature of the product, T_0, and the initial concentration of the nutrient in the food, C_0, are constant and uniform throughout the product.

7. The temperature at the beginning of the cooling period is the same as the temperature at the end of the heating period which is distributed as a function, f (x,y,z).

8. The degradation of the nutrient follows first order kinetics.

9. The nutrient degradation during the come-up occurring at the beginning of the heating and cooling periods is negligible.

Temperature prediction during the heating period

The temperature at any point of the rectangular pouch for a time, t, less than or equal to the total heating time ($t \leq fht$) is obtained from the equation presented by Carslaw and Jaeger (1959) for a rectangular parallelepiped of dimensions a, b and c.

$$T_1(x,y,z,t) = \frac{64}{nmp\pi^3} \sum_{n=0}^{\infty} \sum_{m=0}^{\infty} \sum_{p=0}^{\infty} \sin\frac{n\pi}{a}x \sin\frac{m\pi}{b}y \sin\frac{p\pi}{c}z \exp\left[-\alpha\left(\frac{n^2\pi^2}{a^2} + \frac{m^2\pi^2}{b^2} + \frac{p^2\pi^2}{c^2}\right)t\right] \quad (1)$$

where n, m and p are odd numbers for x, y and z, respectively, in the intervals $0<x<a$, $0<y<b$, $0<z<c$.

This equation is governed by the following initial and boundary conditions:

$$T_1(x, y, z, 0) = 1 \quad (2)$$

$$T_1(a, y, z, t) = T_1(0, y, z, t) = 0 \quad (3)$$

$$T_1(x, b, z, t) = T_1(x, 0, z, t) = 0 \quad (4)$$

$$T_1(x, y, c, t) = T_1(x, y, 0, t) = 0 \quad (5)$$

A change of variable of the following order is necessary in order to obtain the true temperature at any point of the container as a function of time, t:

$$T_1(x, y, z, t)_R = T_1(x, y, z, t)(T_0 - T_h) + T_h \quad (6)$$

From Eq (6) the true initial and boundary conditions are calculated:

$$T_1(x,y,z,t)_R = T_0 \quad (7)$$

when $T_1(x,y,z,0) = 1$.

$$T_1(a,y,z,t)_R = T_1(0,y,z,t)_R = T_h \quad (8)$$

when $T_1(a,y,z,t) = T_1(0,y,z,t) = 0$.

$$T_1(x,b,z,t)_R = T_1(x,0,z,t)_R = T_h \quad (9)$$

when $T_1(x,b,z,t) = T_1(x,0,z,t) = 0$.

$$T_1(x,y,c,t)_R = T_1(x,y,0,t)_R = T_h \quad (10)$$

when $T_1(x,y,c,t) = T_1(x,y,0,t) = 0$.

Temperature prediction during the cooling period

The temperature at any point in the pouch for a time, t, greater than the total heating time ($t > fht$) is obtained in a similar form (Carslaw and Jaeger, 1959):

$$T_2(x,y,z,t) = \frac{64}{nmp\pi^3} \sum_{n=0}^{\infty} \sum_{m=0}^{\infty} \sum_{p=0}^{\infty} \sin\frac{n\pi}{a}x \sin\frac{m\pi}{b}y \sin\frac{p\pi}{c}z \exp\left[-\alpha\left(\frac{n^2\pi^2}{a^2} + \frac{m^2\pi^2}{b^2} + \frac{p^2\pi^2}{c^2}\right)t\right] \quad (11)$$

where n, m, p are odd numbers for x, y and z, respectively, in the intervals $0<x<a$, $0<y<b$, $0<z<c$.

This equation is governed by the following initial and boundary conditions:

$$T_2(x, y, z, 0) = f(x, y, z) \quad (12)$$

$$T_2(a, y, z, t) = T_2(0, y, z, t) = 0 \quad (13)$$

$$T_2(x, b, z, t) = T_2(x, 0, z, t) = 0 \quad (14)$$

$$T_2(x, y, c, t) = T_2(x, y, 0, t) = 0 \quad (15)$$

Since, in general, the distribution of the temperature is not uniform at the end of the heating period, the initial distribution of the temperature during the cooling period is given by Eq (1), with t=fht, being in general, a function f (x, y, z).

A change of variable of the following order is necessary in order to obtain the true temperature at any point of the container during the cooling period:

$$T_2(x, y, z, t)_R = T_2(x, y, z, t) + TCW \quad (16)$$

Prediction of nutrient retention at any point of the container

In the case of nutrients, enzymes, colors, and microorganisms whose thermal destruction follows first order kinetics, the following reaction must hold (Daniels and Alberty, 1970; Stevens, 1971):

$$-\frac{dC(x,y,z,t)}{dt} = kC(x,y,z,t) \quad (17)$$

According to Stumbo (1973)

$$D = D_0 10^{\left[\frac{250 - T_i(x,y,z,t)}{Z_0}\right]} \quad (18)$$

Substituting for k in Eq (17),

$$-\frac{dC(x,y,z,t)}{C(x,y,z,t)} = \frac{\ln 10}{D_0 10^{\left[\frac{250 - T_i(x,y,z,t)}{Z_0}\right]}} dt \quad (19)$$

Integrating between C_0 and C (x, y, z, t), and 0 and ϕ

$$\frac{C(x,y,z,t)}{C_0} = \exp\left[-\frac{\ln 10}{D_0}\int_0^{\phi} \exp\left(\frac{T_i(x,y,z,t) - 250}{Z_0}\right) \ln 10\, dt\right] \quad (20)$$

which gives the remaining fraction of nutrient at any point of the pouch at any time.

Prediction of nutrient retention after processing

In order to calculate the quantity of nutrient at any point of the container at any time of processing, Eq (1) and (11) are plugged into Eq (19).

At the end of the heating period (t=fht), the concentration of the nutrient at each point of the container, C(x,y,z,t) will vary due to the decreasing concentration gradient from the center to the walls of the container. The nutrient concentration at each point of the container at the end of the heating period will be equal to the initial concentration of the nutrient at the beginning of the cooling period.

$$\frac{C(x,y,z,t)}{C_0} = \exp\left[\frac{-\ln 10}{D_0}\int_0^{fht} \exp\left(\frac{T_1(x,y,z,t) - 250}{Z_0}\right) \ln 10\, dt\right] \exp\left[\frac{-\ln 10}{D_0}\int_0^{\phi - fht} \exp\left(\frac{T_2(x,y,z,t) - 250}{Z_0}\right) \ln 10\, dt\right] \quad (21)$$

where (ϕ−fht) is the total cooling time.

Taking an average of the fraction of nutrient remaining in the pouch after a total processing time, ϕ:

$$\frac{\bar{C}}{C_0} = \frac{1}{abc}\int_0^a \int_0^b \int_0^c \exp\left[\frac{-\ln 10}{D_0}\int_0^{fht}\left(\frac{T_1(x,y,z,t) - 250}{Z_0}\right)\ln 10\, dt\right]$$

$$\exp\left[\frac{-\ln 10}{D_0}\int_0^{\phi-fht}\exp\left(\frac{T_2(x,y,z,t) - 250}{Z_0}\right)\ln 10\, dt\right] dx\, dy\, dz \quad (22)$$

This equation represents a model for the prediction of nutrient retention in foods packed in retortable pouches after being sterilized by thermal conduction. A computer program was developed in order to solve this equation.

EXPERIMENTAL VERIFICATION OF THE MODEL

A SET of experiments was conducted in order to verify the validity of the model. The experiments were conducted on a simulated food in which thiamin was included as one of its components with the purpose of determining its destruction after processing.

Thiamin was selected because it is thermolabile and its degradation follows first order kinetics. This degradation was studied by Feliciotti and Esselen (1957) who established that the most pronounced changes occur at neutral pH's. A pH of 6.0 was selected throughout the experiments for this particular reason. The kinetics parameters D_0 and Z_0 were calculated by Barreiro et al. (1977) from the data presented by Mulley et al. (1975). Values of 157.2 min and 45°F were obtained for these parameters.

Simulated food

The simulated food was prepared by mixing corn starch (U.S.P. grade), carboxymethylcellulose and thiamin (5 mg/100g) in a pH 6.0 buffer solution of potassium phosphate, monobasic, at 0.1N and sodium hydroxide at 0.1N. The mixture was homogenized for uniform distribution of the vitamin and immediately heated with continuous agitation on a stove until it reached the boiling point (197.6°F) with the purpose of gelatinizing the starch. Then it was packed into the retortable pouches, sealed and placed into a refrigerator until processing (about 24 hr).

The thermal diffusivity of the mixture was determined by Herrera (1975) utilizing the formula developed by Olson and Jackson (1942). The value obtained was 0.01576 in²/min.

Retortable pouches

The retortable pouches used to conduct the experiments were supplied by Continental Flexible Packaging. These pouches are produced from a lamination of 48 polyester, 70-gauge aluminum foil, and 300 polypropylene.

The size of the pouches utilized in the experiments was 5 1/2 in. × 7 in. with a capacity of 9 ounces and 4 3/4 in. × 7 1/4 in. with a capacity of 6 ounces. The thickness of the pouches was measured in the center after they were filled and sealed.

A correction was made to these dimensions in which the seals forming the edges of the pouch were substracted from the above mentioned dimensions. The new dimensions obtained were 4.791 in. × 6.252 in. × 0.819 in. for the 5 1/2 in. × 7 in. pouch and 3.976 in. × 6.437 in. × 0.709 in. for the 4 3/4 in. × 7 1/4 in. pouch. The corrected values were used for the computer solution of the model.

Thermal processing

After the simulated food was packaged into the pouches and cooled overnight, the samples were processed in a still retort ("Dixie", model RDSW-3). The procedure used is described as follows: the retort was loaded, closed and the vents opened; the steam was turned on; when the retort had reached the desired temperature the process timing started, at the end of the process time, the vents were closed, air at a pressure of 17 psig was admitted into the retort, the steam valve was closed and the cooling water admitted from the bottom. The pressure was released and the process terminated when the temperature at the center of the pouch decreased to 100°F.

The pouches were removed quickly from the retort and placed in a water and ice mixture to avoid further destruction of the vitamin, then they were placed in a refrigerator overnight prior to the vitamin analysis. One of the samples was not processed but saved as control in order to determine the initial quantity of thiamin present in the food.

Heat penetration studies

The heat penetration studies were performed following the procedure detailed by Alstrand and Ecklund (1952). The package gland unit was installed at the midpoint of one of the sides of the pouch and a copper-constantan needle thermocouple (Ellab, model TG 67) was threaded through the unit. The time-temperature relationships at the center of the pouch, as well as the temperature and time of heating, the temperature and time of cooling and the total processing time were recorded by means of a time-temperature recorder (Leeds and Northrup, Speedomax model).

Experimental design

Four experiments were conducted utilizing different combinations of time, temperature, and size of pouches. The processing time and temperatures were chosen arbitrarily according to the sterilization procedures that are ordinarily used for retortable pouches.

A total of five pouches were used for each experiment. One was saved in the refrigerator as control. Another one was used for the heat penetration study and discarded at the end of processing.

After processing, the thiamin content of the samples was determined by the method recommended by the AOAC (1975). The analysis of the vitamin in the control sample was conducted on four replicates obtained after three aliquots were taken from the sample. A similar procedure was used for the processed samples in which four replicates were analyzed for each of the three samples. This made a total of 12 determinations for the control and 12 determinations for the processed samples.

RESULTS & DISCUSSION

Temperature prediction at the center of the pouch

The experimental temperatures obtained at the end of the heating and cooling periods are compared to the model predictions in Tables 1 and 2. These tables show the percent deviation between the predicted and experimental temperatures at the end of the heating and cooling periods for each experiment.

The percent deviations of the predicted temperatures with respect to the experimental ones, at the end of the heating period, ranged from 0.0–1.3%. The percent deviations at the end of the cooling period were higher, ranging from 2.0–16.0%.

It may be observed that the predicted values at the end of the heating period are not significantly different than those obtained experimentally. The higher deviations at the end of the cooling period could be due to the assumption

Table 1—Predicted and experimental temperatures at the end of the heating period

Exp	Processing temp (°F)	Heating time (min)	Temp at center of pouch (°F) Predicted	Exp	Dev. (%)
1	246.0	16.5	241.1	238.0	1.3
2	240.0	39.0	240.0	240.0	0.0
3	248.5	28.3	248.2	247.0	0.5
4	238.5	60.4	238.5	238.5	0.0

Table 2—Predicted and experimental temperatures at the end of the cooling period

Exp	Processing temp (°F)	Cooling period (min)	Temp at center of pouch (°F) Predicted	Exp	Dev. (%)
1	246.0	11.0	103.6	100.0	3.6
2	240.0	18.0	92.8	91.0	2.0
3	248.5	18.6	87.7	95.0	7.7
4	238.5	9.0	106.7	91.0	16.0

Table 3—Predicted and experimental fractions of thiamin retained after thermal processing

Exp	Size of pouch (in.)	Processing temp (°F)	Total processing time (min)	Fraction of thiamin retained		90% Confidence intervals of avg exp values
				Predicted	Experimental	
1	5 1/2 × 7	246.0	27.5	0.878	0.929	± 0.054
2	5 1/2 × 7	240.0	57.0	0.747	0.719	± 0.039
3	5 1/2 × 7	248.5	46.9	0.737	0.767	± 0.044
4	4 3/4 × 7 1/4	238.5	69.4	0.631	0.619	± 0.018

of a very high heat transfer coefficient at the surface of the pouch during the cooling period. The heat transfer coefficient, in this case, may be affected by the admission of air into the retort to raise the degree of pressurization. If too much air is admitted into the retort, the heat transfer to the contents may decrease.

Prediction of nutrient retention

Table 3 presents a comparison between the predicted and experimental average fractions of thiamin retained after the thermal processing of the simulated food. This table also shows the intervals which contain the true mean fraction of vitamin retained at the end of processing with 90% confidence. These intervals were calculated by the statistical analysis of the results obtained from the experimental determination of the vitamin before and after thermal processing. The "t" distribution was used for the statistical evaluations.

The vitamin fractions predicted by the model were within the 90% confidence intervals obtained by the statistical analysis of the experimental fractions from each determination. These results lead to the conclusion that the model is valid for the prediction of the retention of nutrients in foods packaged in retortable pouches and sterilized by thermal conduction which degrade according to first order kinetics.

NOTATION

a : Dimension of the container on the x axis
b : Dimension of the container on the y axis
C : Nutrient concentration at a point of the container, (mg/g)
C_0 : Initial concentration of the nutrient in the container, constant (mg/g)
$\bar{C}$: Concentration of the nutrient in the container (mg/g)
c : Dimension of the container on the z axis
D : Time required at any temperature to destroy 90% of the nutrient at T_i (min)
D_0 : Time required to destroy 90% of the nutrient at 250°F (min)
fht : Final heating time (min)
k : Reaction velocity constant (=ln 10/D) (min^{-1})
m : Positive integer number
n : Positive integer number
p : Positive integer number
T_h : Temperature of the heating medium (°F)
T_i : Temperature at any point of the container (°F); i=1 for heating; i=2 for cooling
T_0 : Initial temperature of the mixture, constant (°F)
TCW : Average temperature of the cooling water (°F)
t : Time (min)
x : Rectangular cartesian coordinate
y : Rectangular cartesian coordinate
Z_0 : Number of Fahrenheit degrees required for the thermal destruction curve to traverse one log cycle (°F)
z : Rectangular cartesian coordinate

Greek symbols

α : Thermal diffusivity (in^2/min)
ϕ : Total processing time period (min)

Subscripts

R : Real values

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OPTIMAL STERILIZATION TEMPERATURES FOR SENSORY QUALITY IN CYLINDRICAL CONTAINERS

THOMAS OHLSSON

ABSTRACT

Optimization calculations are presented for sterilization of foods in cylindrical cans. The C-value is used to describe the sensory quality changes during processing. The optimal temperatures for sensory quality were the same as for maximal thiamin retention. They were 116–119°C for conduction heated foods of commonly used can sizes. The calculations showed that variations in the retort temperature program or starting temperature have minor effects on the optimal temperatures. The results are generalized using the f-slope-value of heat penetration measurements. It is proposed that optimal sterilization temperatures may be determined for any product or can size using presented diagrams. Its practical application is examplified.

INTRODUCTION

THE OPTIMIZATION of the quality retention in thermally processed foods is based on the differences in temperature dependence between the spore inactivation and the changes in the sensory and nutritional quality. The markedly higher temperature dependence of the spore inactivation suggests that processing at high temperature for a shorter time (HTST-processing) is advantageous in minimizing the heat-induced quality changes (Lund, 1977).

The advantages of HTST-processing have previously been both theoretically and experimentally shown. Also, the limited possibilities to utilize the HTST-principle, due to the slow heat conduction in solid foods packed in cans of normal sizes have been demonstrated. Teixeira et al. (1969) found that the optimal processing temperatures were close to 119°C, for maximal thiamin retention in cylindrical cans. Bauder (1974) determined optimal sterilization temperatures, using a browning reaction as evaluation parameter for a number of F_o-values, can volumes, and can surface-to-volume ratios. For commonly used can dimensions the optimal surface color was reached in the temperature range 117–120°C. Based on experimental results, Reichert (1974), has presented guidelines for choice of optimal temperatures for rotational sterilization of many ready-to-serve foods. The optimal temperatures ranged from 120°C for, e.g., meat chunks in thick sauce to 140°C for, e.g., onion soup.

To describe the sensory quality changes during heat processing Mansfield (1962) proposed a cook- (or C-) value defined similar to the well-known F_o-value (Ball and Olson, 1957):

$$C = \int_0^t 10^{\frac{T-100}{Z_c}} dt$$

Experimental determination of the temperature dependence of the C-value (Z_c) has been presented by Ohlsson (1979b). Using the C-value as an evaluation parameter, Ohlsson (1979a) calculated the optimal sterilization temperature ranges for flat containers. Studies by Teixeira et al. (1975) and Sjöström and Dagerskog (1977) have demonstrated that variations in the retort temperature program influence the results of the optimization to a small extent, only up to 10% in thiamin retention or C-values.

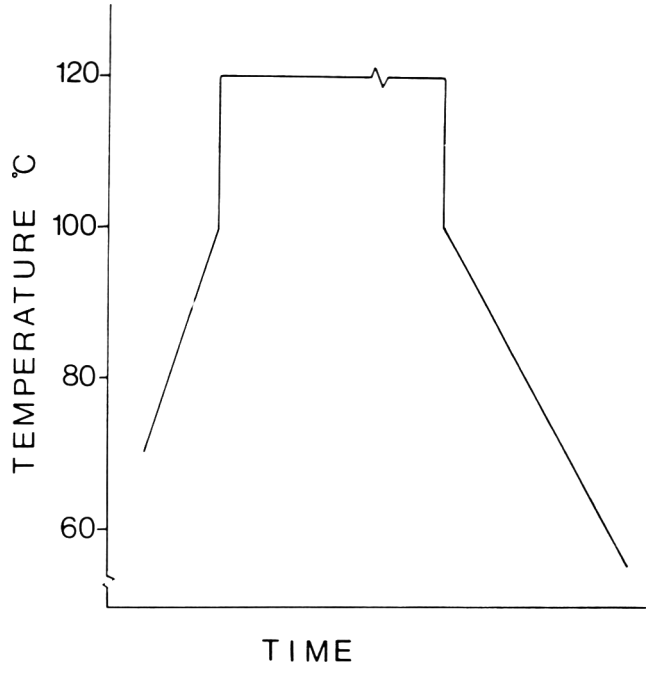

Fig. 1—Principle diagram of the temperature variation with time in the calculations of the simulated sterilization in hydrostatic sterilizers.

As pointed out by many authors, the most important parameter by far is the can dimensions. These together with the thermal diffusivity constitute the f-slope value, which can be determined experimentally by heat penetration measurements (Ball and Olson, 1957). With the aid of the f-slope value, the optimization results can then be generalized (Ohlsson, 1979a).

In this paper, optimization calculations of the sterilization of foods packed in cylindrical cans of different dimensions are presented. C-values with different temperature dependences are used as optimization parameters. The influence on the optimal temperatures of lower initial temperature, higher F_o-value, and processing according to temperature programs of hydrostatic sterilizers is also studied. The optimization results are generalized for applications involving practical can sizes and products, also those heated by combined conduction and convection. The practical use of the method is exemplified.

METHODS

THE CALCULATIONS were performed using a FORTRAN pro-

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Table 1—Heating times to a F_o-value of 6.0

Can size (mm) diam x ht	Retort temperature, °C					
	110	115	120	125	130	135
ϕ73 x 31	–	38	22	15	12	10
ϕ73 x 58	–	64	44	35	29	25
ϕ73 x 107	138	84	61	49	42	36
ϕ99 x 119	178	119	91	75	65	57

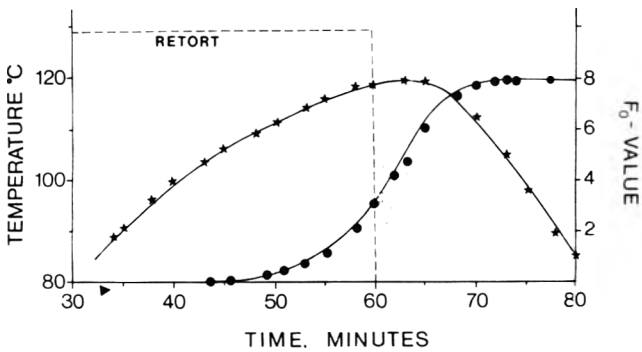

Fig. 2—Calculated (lines) and experimental can center temperature (stars) and accumulated F_o-value (dots) for rotary sterilization of a fish-stew product at 129° C.

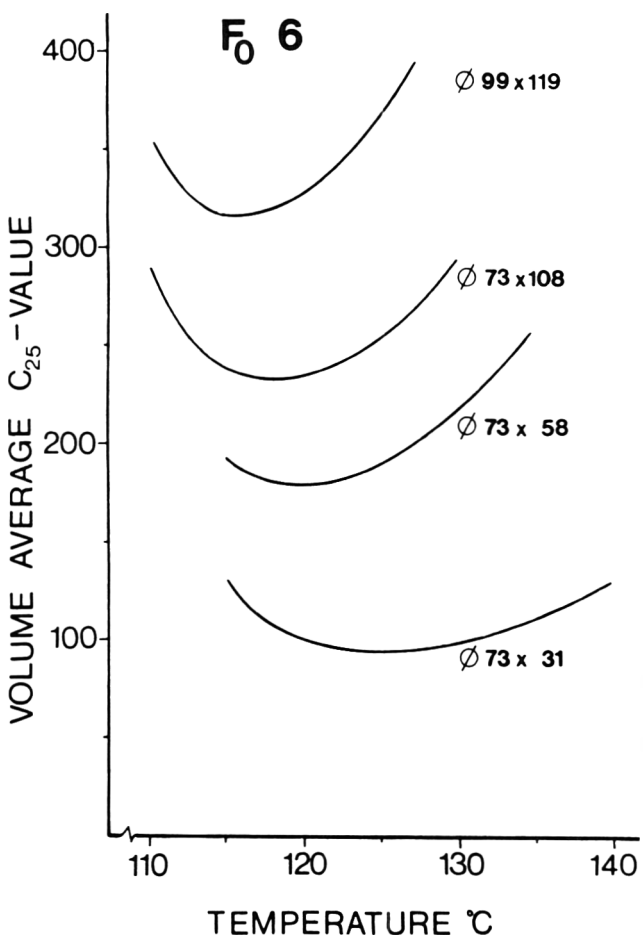

Fig. 3—Volume averaged C-values calculated with a z-value of 25° C as a function of temperature and can size, (diameter x height in mm).

gram based on the finite difference solution of the unsteady, heat conduction equation for two dimensions, with negligible surface heat transfer resistance, similar to the program presented by Teixeira et al. (1969). However, the heating time required to reach a prescribed final F_o-value in the center was determined by an iteration method. An error of ± 0.10 in F_o-units was normally considered acceptable. The final F_o-value was chosen to be 6.0 in most calculations. Examples of heating times to a F_o-value of 6.0 are given in Table 1.

The calculations for the hydrostatic sterilizer were performed using the temperature program procedure outlined in Figure 1, based on information from manufacturers of sterilizers.

Calculations were performed for can dimensions chosen from the list of European standardized sizes. They were ϕ 73 × 31, ϕ 73 × 58, ϕ 73 × 107 and ϕ 99 × 119 mm outer dimensions. The C-values were calculated with z-values of 17, 25, and 33°C (Ohlsson, 1979b). For the C-value both a volume-average and a surface-average C-value were calculated at each time interval, based on the C-values of each individual increment and its contribution to the total volume or surface.

Calculations were performed at intervals of 2.5°C, ranging from 110–140°C. A thermal diffusivity value of $0.16 \cdot 10^{-6}$ m²/s was used for the main part of the calculations. For generalization of the optimization results the f-slope value is calculated according to (Ball and Olson, 1957):

$$f = \frac{0.0995}{a\left(\frac{1}{D^2} + \frac{0.427}{L^2}\right)}$$

where a = thermal diffusivity in m²/s; D = can diameter in m; L = can height in m. The f-slope value is the time required to reduce the difference between the retort temperature and the temperature at a given point (usually the center) to one-tenth of its value at the beginning of the time interval.

Some additional calculations were also made with higher thermal diffusivity values, as a basis for extending the generalization of the results.

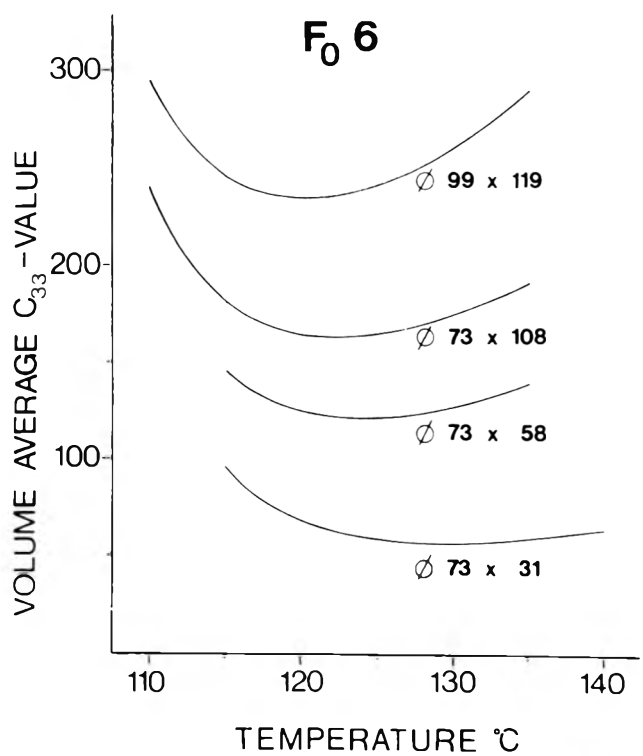

Fig. 4—Volume average C-values calculated with a z-value of 33° C as a function of temperature and can size, (diameter x height in mm).

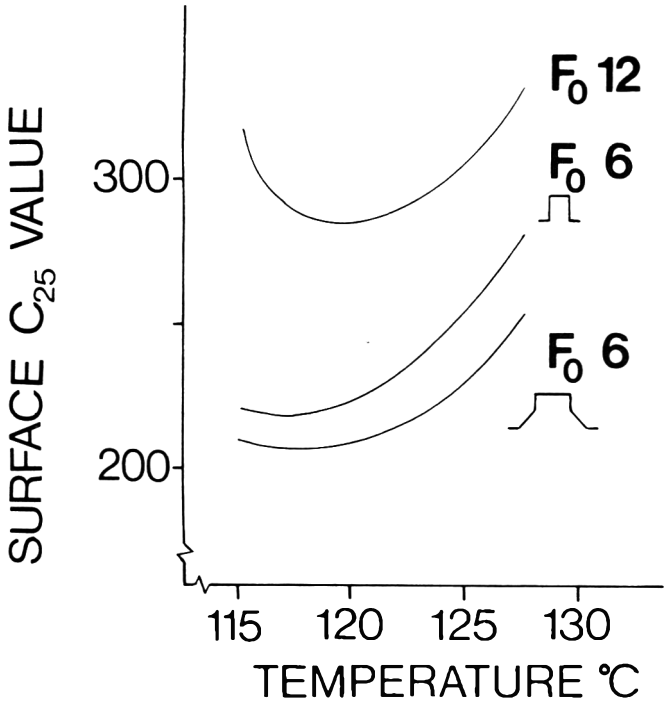

Fig. 5—Comparison between surface C-values calculated for different F_o-values and different retort temperature programs.

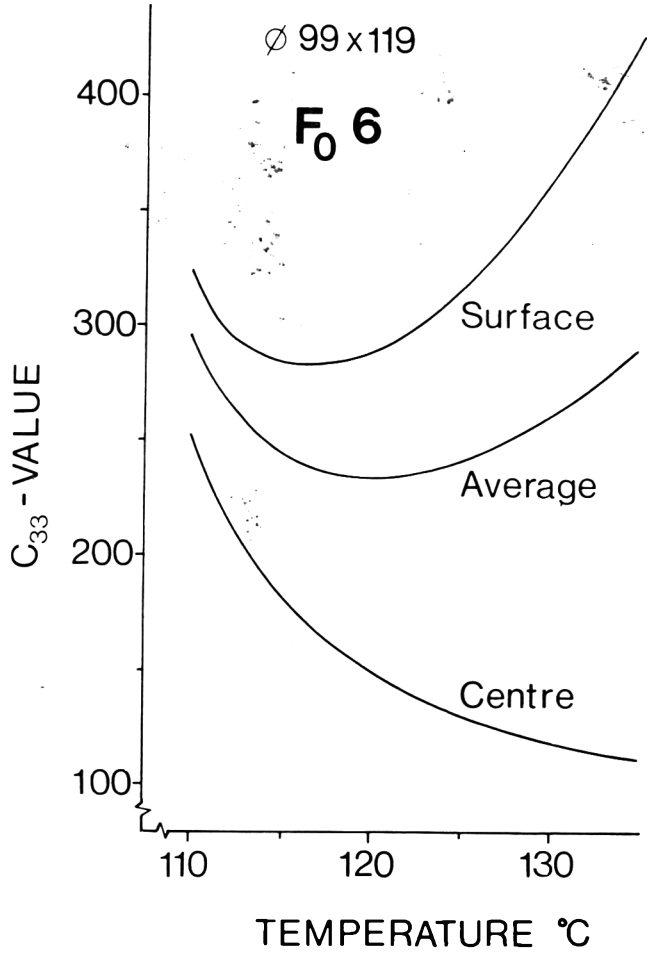

Fig. 6—The C-values of surface, volume, and center calculated for a z-value of 33°C, a F_o-value of 6 and a can size of ϕ 99 x 119 mm, (diameter x height) as a function of retort temperature.

RESULTS & DISCUSSION

THE RESULTS calculated by the computer program correlated well with results of experimental sterilizations of typical conduction-heated products. This is illustrated in Figure 2, where both the calculated and the experimental development of temperature and F_o-value with time are shown for sterilization at 129°C of a can (ϕ 73 X 103 mm) of minced meat.

The volume averaged C-values for sterilization of cans of various diameters to a F_o-value of 6 are given in Figure 3 calculated with a z-value of 25°C and in Figure 4 calculated with a z-value of 33°C of the C-value. The very large influence of the can dimension on the C-value is clearly shown. For better quality retention, that is lower C-values, small cans or cans with a length-to-diameter ratio far from unity should be chosen, as previously pointed out by Teixeira et al. (1975). It should also be noted that for many food products C-values in the range 200–250 and above means that the quality has been so severely deteriorated that the product is unacceptable (Ohlsson, 1979b).

The diagrams also show that the optima are fairly wide, with only a few per cent quality difference for a temperature change of 2.5°C. Figures 3 and 4 also show that with increasing size of the can the optimal temperatures, e.g. where the C-values are minimal, shift towards lower temperatures. For the same can size the optimal temperature is about 5°C higher for a z-value of 33°C as compared to 25°C. In the original definition of the C-value by Mansfield (1962) a z-value of 33°C was used. Later experimental investigations (Ohlsson, 1979b) have shown that the z-values often are close to 25°C. A z-value in the range 25–33°C should therefore be used as representing most quality parameters in heat-processed foods. It is important to note that the Z-value for thiamin retention is close to 27°C. This means that optimization results for maximal thiamin retention (Teixeria et al., 1969) will also be valid for optimal sensory quality and vice versa.

The optima determined for the surface-averaged C-values should be considered when quality parameters such as appearance or odor are of prime interest. For surface averaged C-values the optimal temperatures are about 2.5°C lower compared to the volume-average values.

For sterilization to higher F_o-values, the optimal temperature as based on C-values increases. Figure 5 shows that for an increase in the F_o-value from 6 to 12, the increase is about 2.5°C. With a low initial temperature of the can the heating time will of course be longer. The C-values will be some 10–15% higher as compared to using high initial temperature, but the position of the optimal temperature is not affected. The calculations involving processing in hydrostatic sterilizers with temperature program according to Figure 1 show that some 5–10% lower C-values are obtained as compared to sterilization with rapid changes of the retort temperature, e.g. in a spiral-type continuous pressure cooker. In the "hydrostatic" calculation more uniform temperature profiles with lower maximum temperatures were obtained. The advantage of using lower temperature during the initial part of the sterilization has also been shown by Teixeira et al. (1975) and Sjöström and Dagerskog (1977). Of course, slowly increasing retort tempratures may also be used in other types of sterilizers e.g. a batch retort, but at the expense of longer heating times and lower production.

The calculations have shown that the slow heat conduction in the food will lead to excessive temperatures and overprocessing of the surface parts, especially for large cans at higher temperatures. This is illustrated in Figure 6. The severe overprocessing of the surface is also evident from the differences between the retort heating temperature and the

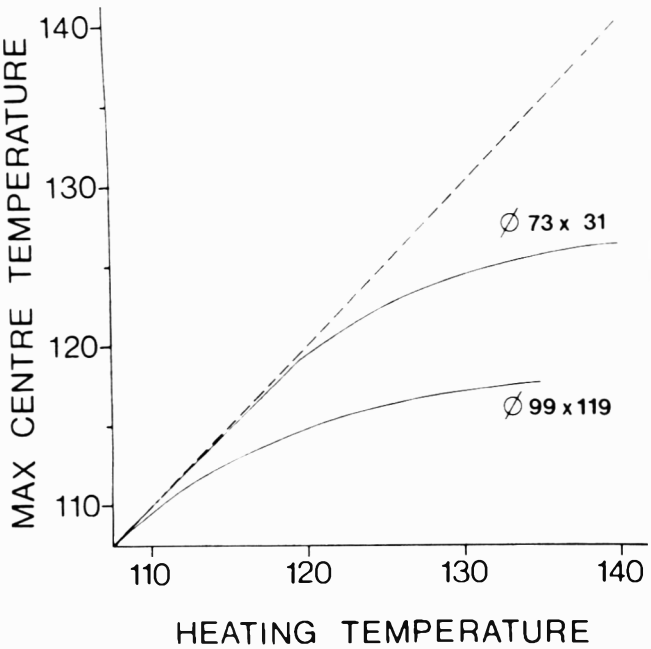

Fig. 7—Maximal center temperatures as functions of heating temperatures for sterilization to a F_o-value of 6 for two can sizes, (diameter x height in mm).

maximal center temperature, shown in Figure 7. Increasing the heating temperature from 120 to 135°C will increase the maximum center temperature by only 2.5°C but the surface temperature will increase substantially.

The results obtained from the optimization calculation agree with the results reported by Bauder (1974). Both these results show that the optimum temperature for sterilization of a ϕ 73 X 55 mm can is close to 117°C, if the optimization parameter is the surface browning of the product with the browning reaction having a z-value of around 25°C (Ohlsson, 1979b). Comparison with the results of Teixeira et al. (1969) confirm these findings. Based on optimization of thiamin retention they found 117–119°C to be the optimal range for a can of U.S. size 307 X 409 which is in agreement with our results for a ϕ 73 X 99 mm can, where the optimal temperature for the volume-averaged C-value with z = 25°C is 117.5°C.

To make the optimization results applicable also to other can dimensions and other thermal diffusivity values, the f-slope values as defined by Ball and Olson (1957) were computed. The optimal temperatures for the calculated examples are plotted against the f-slope values in Figure 8 for volume-averaged C-values with z-values of 25 and 33°C, for F_o-values of 6 and 12. With the aid of this diagram the use of the results may be extended to other f-slope values, e.g., other can dimensions. For foods with unknown thermal diffusivity values and with a combined conduction and convection heat transfer, the f-slope value can be determined by conventional heat penetration measurements (Ball and Olson, 1957), well-known to canned food manufacturers. This f-slope value method may make it possible for the producer of canned foods to find the sterilization temperature where the optimal quality retention can be expected just by using diagrams such as Figure 8 and the f-slope value determined from the heat penetration measurement from the actual product and can size. Previously presented optimization calculations of this kind have been restricted to only conduction heated foods and the can sizes given in the examples.

Application of this method will be illustrated by sterilization of a junior food fish-stew product. The fish-stew cans were sterilized at different temperatures using end-over-end rotation at 45 rpm. From the heat penetration plot in Figure 9, the f-slope value was determined to be 5.0 min. According to Figure 8 for a F_o-value of 6, the optimal temperature should be 127–128°C based on a volume averaged C-value with a z-value of 25, chosen according to the results of Ohlsson (1979b). The panel scores from the sensory evaluations of the sterilized fish-stew samples are

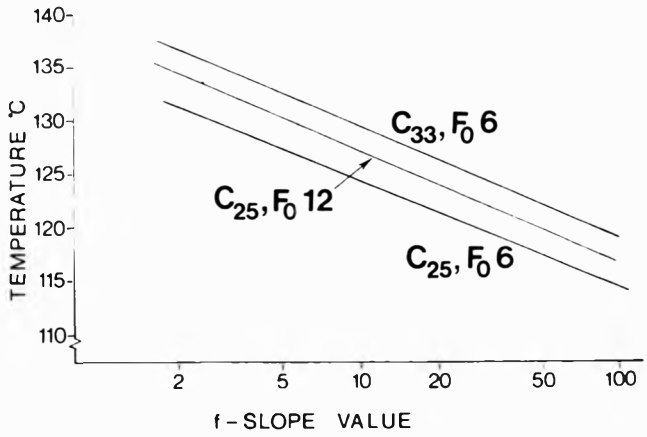

Fig. 8—Optimal sterilization temperatures as a function of the f-slope value, determined from calculations of volume average C-values using z-values of 25 and 33°C and to F_o-values of 6 and 12 (only z = 25°C).

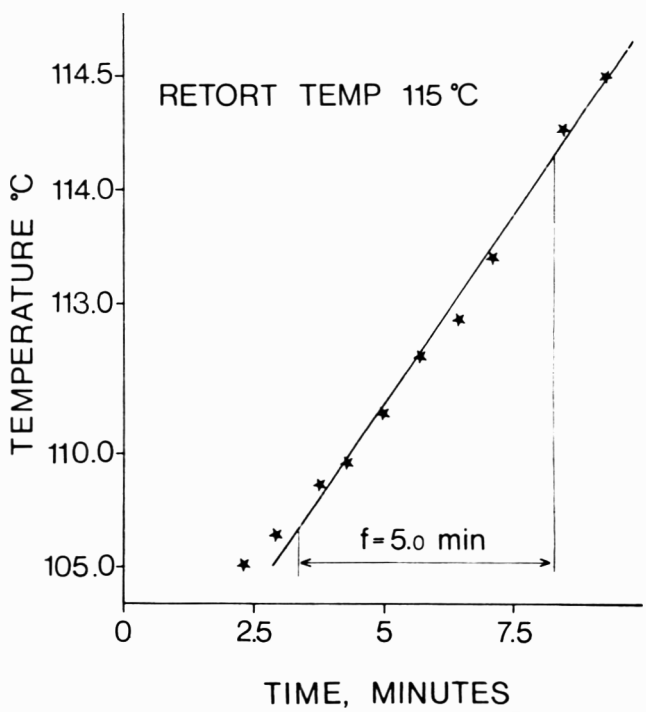

Fig. 9—Time-temperature plot of the f-slope-value determination based on heat penetration experiments with a fish-stew product. The temperatures on the ordinate axis are labeled on an inverted logarithmic scale, where the 1.0 label of each log cycle corresponds to a temperature determined from the retort temperature substructed by 0.01, 0.1, 1, 10, 100, e.g. 114.99, 114.9, 114.0, 105, 15°C.

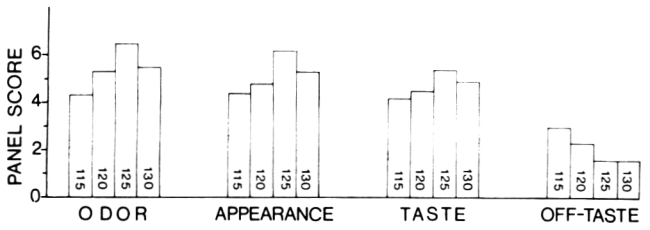

Fig. 10—Panel score values from sensory evaluation of a fish stew product sterilized to a F_o-value of 6 at four different temperatures. For odor, appearance and taste the scores go from 1 (extremely bad) to 9 (extremely good), whereas the scores for off-taste increase from 0 to 8 with increasing off-taste.

given in Figure 10, from which it may be concluded that the optimal temperature—where the highest panel scores were obtained—is slightly higher than 125°C. This is in good agreement with the optimal temperatures found in Figure 8. It should be pointed out that, in addition to this single experiment, the validity of this optimization method must be proven by more investigations.

CONCLUSIONS

THE C-VALUE, which is easily computed in the evaluation of sterilization experiments, was found to be a useful parameter in optimization of the sensory quality in heat sterilization of cylindrical cans. The optimal sterilization temperatures for reaching the minimal C-value decreased when the can size increased, so that for normal can sizes optimal temperatures were in the 117–119°C range for solid, conduction-heated foods. Only for substantially smaller can diameters or heights is sterilization at higher temperatures an advantage.

The optimal temperature range does not change for sterilization from lower initial can temperature or processing with progressively increasing and decreasing temperatures (e.g., in hydrostatic sterilizers), according to our calculations. For higher F_o-values the optimal temperature range increases. In order to improve the quality retention the sterilization should be done using lower f-slope values. This can be achieved either by choosing other can dimensions or by improving the heat transfer within the food e.g. by agitation. Only by changing the parameters determining the f-slope-value (can height and diameter, thermal diffusivity), may the C-value be substantially reduced, and thus the food quality substantially increased. The sensory quality changes and the thiamin degradation during heat sterilization has essentially the same z-values (23–27°C). Thus, it is important to note that the determined optimal temperatures will be valid both for maximal thiamin retention and optimal sensory quality.

The results in terms of optimal sterilization temperatures could be generalized using the f-slope. This f-slope value can be determined by heat penetration measurements with the actual product and can size of the canned food. Combined with the diagrams for optimal sterilization temperatures presented in this paper it may be possible to optimize the quality retention of both conduction and conduction-convection heated canned foods. However, further investigations are needed to prove the validity of the method.

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FLUIDIZED BEDS AS TURBULENCE PROMOTERS IN THE CONCENTRATION OF FOOD LIQUIDS BY REVERSE OSMOSIS

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ABSTRACT

Fluidized beds offer a potential improvement of reverse osmosis processes for food liquids, less fouling of the membrane, and reduced energy consumption. Our experiments were concerned with tubular systems in which fluidized beds of glass, steel, and lead beads were used. Glass beads appeared to be preferable, since they caused little damage to the membrane. Only with the larger glass beads (3 mm) did the membrane skin become corrugated, so that the rejection decreased. The permeate flux for Gouda cheese whey was almost equal to that of an empty tube for which the velocity was about thirty times higher. The erosive action of the glass beads on the fouling layer was partially responsible for this effect. For reverse osmosis of skim milk and potato fruit water the bed did not reduce the fouling layer to a sufficient extent and, therefore, had a lower permeate flux than an empty tube.

INTRODUCTION

AFTER the energy crisis in 1973, reverse osmosis (RO) of cheese whey became a point of interest in the Netherlands. One of the main reasons for this interest was the reduction in energy costs that can be reached by RO, in comparison with the costs of evaporation (De Boer et al., 1977). At the moment a number of industrial plants have been installed which concentrate the Gouda cheese whey two to three times. Concentration is done at the temperature of cheese making (about 30°C) or after cooling to 10 or 18°C. After this treatment the whey is transported directly to centralized plants, or, occasionally, additional evaporation is carried out. In addition to the dairy industry, the potato starch industry shows growing interest in RO, in order to solve the big effluent problems that are encountered in the northern part of this country (Meindertsma, 1980). In this process the potato fruit water is concentrated to half its volume in a tubular system at ambient temperature, before being processed into denatured potato protein.

Our investigation concerning food liquids was a continuation of the work conducted by Van der Waal et al. (1977), who made use of glass beads in a tubular membrane system for RO of a salt solution. The conclusion drawn from their work was that with a fluidized bed the same mass transfer coefficient can be reached as that in an empty tube, with only about 5% of the circulation energy. Damage to the membrane surface could not be observed when glass particles were used with a diameter of 0.7 mm or smaller. In addition to the literature review presented by Van der Waal et al. (1977), a short review of the effects of turbulence promoters in membrane processing of some food liquids is given here. Lowe and Durkee (1971) used beads of acrylic resin in a plate-and-frame RO equipment for the concentration of orange juice. A threefold flux improvement appeared to be possible, and a certain capability of self cleaning was also mentioned. Peri and Dunkley (1971) used static rods as turbulence promoters, which in the concentration of cottage cheese whey effected a threefold increase in permeate flux. However, they compared the effect of the turbulence promoters with results of empty-tube experiments at velocities of 10–40 cm/s, while in industrial installations velocities of $\geqslant$ 2 m/s are normal at the moment. In our study we tried to reduce the velocity and thus the energy consumption by using fluidized beds in a specially constructed tubular RO test equipment. Of three food liquids of different composition, sweet Gouda cheese whey, skim milk, and potato fruit water, numerical data about permeate flux and permeate quality were collected. These data were compared with the results obtained in the empty-tube experiments at flow rates normally used for industrial installations.

EXPERIMENTAL

Apparatus

The test equipment (supplied by Wafilin BV, Hardenberg, the Netherlands) consisted of 20 parallel tubes 1.5m in length, each followed by a transparent transport tube of 0.2m (Fig. 1). The tubes, in which the tubular membrane with a diameter of 14.8 mm was contained, were mounted in a vertical position. The cellulose-acetate membranes had a rejection for sodium chloride of 92.5%. The maximum membrane area of the equipment was 1.3 m^2, which made the test equipment less suitable for concentration experiments. The experiments were therefore carried out at constant volume reduction with recirculation of the permeate. For the comparison between fluidized-bed and empty-tube equipment the number of tubes in the installation was reduced to ten; due to the high velocity of the feed (2 m/s) required in the empty tube, only one of the tubes could be used as an empty tube. The fluidized bed particles used were glass, steel, and lead beads. The diameters of the glass beads (supplied by Tamson) were: 0.4, 0.7, 1.0, 1.3, 2.0, 3.0, and 4.0 mm, and the mass densities were 2890, 3500, 2670, 2870, 2500,

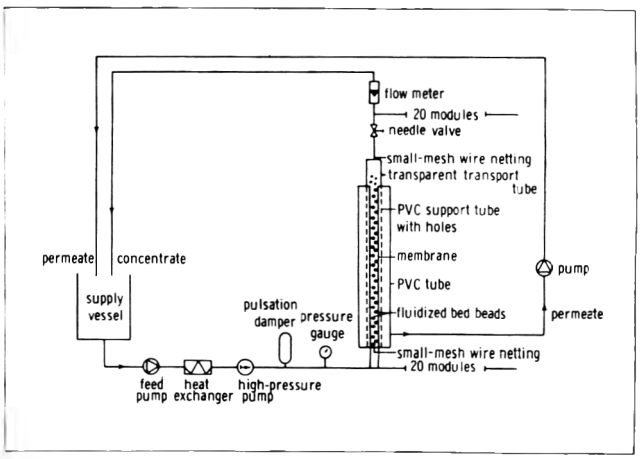

Fig. 1—Fluidized-bed reverse osmosis apparatus.

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2580, and 2610 kg/m^3, respectively. The steel beads had diameters of 1.0 and 2.5 mm, their mass density being 7640 and 7820 kg/m^3, respectively. The lead beads had a diameter of 3.0 mm and a mass density of 10 400 kg/m^3. Besides the diameter and type of material, the bed porosity (ϵ) also varied. It was defined as the volume of the empty tube minus the volume of the beads, divided by the volume of the empty tube.

A critical point in the use of fluidized beds is the starting-up procedure. Prior to each experiment the installation was rinsed with water. The flow rate and pressure were increased slowly, until all the fluidized beds were visible in the transparent transport tube at the desired pressure. During switching over to whey, skim milk or potato fruit water the fluidized beds had to remain visible; meanwhile the pressure (3–4 MPa) and the flow rates were adjusted. After completion of each experiment the installation was rinsed with water and subsequently cleaned with an enzymatic detergent (Tergazym, 0.5%, adjusted to pH 8 with citric acid, temperature 30°C, period 1½ hr). During cleaning the beds remained fluidized. Then the installation was rinsed again with water and finally disinfected with a sodium hypochlorite solution (50 mg/liter active chlorine, period ½ hr).

Feed solutions

Gouda cheese whey, skim milk, and potato fruit water were used as feed solutions. The composition of the cheese whey was roughly 5.5% total solids, 0.75% protein, 0.5% ash, 0.2% organic salts, and 4.0% lactose (pH 6.5). The skim milk contained 8.9% total solids, 3.4% protein, 0.7% ash, 0.2% organic salts, and 4.6% lactose, (pH 6.7). The potato fruit water consisted of 5.2% total solids, 2.9% protein, 1.2% ash, and 0.8% carbohydrates (pH 6.0). The potato fruit water was used directly after manufacture. The skim milk and cheese whey were first pasteurized (67°C/10s and 72°C/10s, respectively) and subsequently cooled to 4°C.

Membrane photographs

The membrane surfaces were studied by means of a scanning electron microscope. Photographs were taken after the membranes had been dried and carbon and gold had been deposited on the membrane surface.

Analytical methods

Total solids content by drying in an oven at 105°C.

Protein content by the Kjeldahl method (protein factor 6.38).

Ash concent by drying in an oven at 105°C and heating in an electric muffle furnace at 550°C for at least 8 hr.

Calcium, sodium and potassium contents by flame emission.

Chloride content according to IDF 17A (1972).

Lactose content according to the procedure of Luff-Schoorl (1929).

Bacterial count by the spiral plate method according to Gilchrist et al. (1977), counting medium plate count agar with 1% milk.

The number of population equivalents (PE) was calculated with the formula: $PE/m^3 = \frac{1}{180}(COD + 4.57\ N)$, where COD and N are both expressed as mg/liter, COD was estimated by a bichromate method according to NEN 3235 5.3.

RESULTS & DISCUSSION

Changes in the membrane skin caused by fluidized bed particles

One of the main problems concerned with the use of a fluidized bed in an RO system is the risk of the membrane becoming damaged due to the continuous bombardment by the beads. From the point of view of preventing such damage and of limiting energy consumption, beads with a small diameter and a low mass density are desirable. On the other hand, from the viewpoint of mechanical cleaning of the membrane by the fluidized bed, which may be more important with food liquids than with salt solutions, larger and heavier beads which increase the erosive action are to be preferred. These conflicting demands were the reason that the beads were varied as regards mass density and diameter. In addition to these points, the hardness of the beads and the bed porosity may also play a role in the damaging of the membrane. The number of collisions per unit membrane area per unit of time will be proportional to bed porosity. In Table 1 some characteristics of various fluidized beds are given. It is shown that the desired velocity for fluidization is different for glass, steel and lead. The higher the mass density and bed porosity, the higher is the flow velocity required for fluidization. A measure of the damage to the membrane is the total solids content of the permeate. The steel (d = 2.5 mm) and lead (d = 3 mm) beads were found to damage the cellulose acetate membranes to such an extent that the total solids content of the permeate was about ten times higher than when glass beads (d = 3 mm) were used. With the use of steel beads the total solids content of the permeate increased within 30 hr from 0.2 to 2.0%, whereas with glass beads the total solids content remained constant at 0.2% during a process time of 200 hr. The relatively good condition of the membrane after RO of concentrated whey when a fluidized bed of glass beads had been used may be explained by the lower momentum of the particles, which is the result of their low mass density and relatively low velocity (the velocity in Table 1 was calculated on the basis of an empty tube). Hardness and bed porosity seemed to be of less importance.

The damage to the membrane skin after exposure to lead beads is illustrated by the photographs (Fig. 2a). Figure 2b shows that even after chemical cleaning some deposits are left in the damaged parts. These deposits soon restrict the permeate flux, which in the beginning was higher than that obtained with glass beads. Deposition occurred particularly when heavily fouling feeds, such as skim milk and potato fruit water, were used. Steel beads had about the same effect as lead beads. Glass beads (d = 3 mm) appeared to cause no serious damage (Fig. 2c), but the skin of the membrane became corrugated. We have the impression that the corrugated skin was formed at the very beginning of the fluidized-bed experiments. This idea is supported by the fact that the total solids content of the permeate did not change under the same process conditions if the process time was increased. A corrugated membrane surface was not found when glass beads with a diameter below 3 mm were used. It is to be noted that the diameter at which

Table 1—Some characteristics of three fluidized beds used for reverse osmosis of Gouda whey

	Relative hardness[a] (Mohs)	Mass density (kg/m^3)	Bed porosity	Superficial flow velocity (m/s)	Total solids content of permeate[b] after a certain process time
glass ϕ 3 mm	4.5–6.5	2.5×10^3	0.65	0.06	0.2%–200 hr
steel ϕ 2.5 mm	5.0–8.0	7.8×10^3	0.85	0.32	2.0%–30 hr
lead ϕ 3 mm	1.5	10.4×10^3	0.95	0.40	2.2%–60 hr

[a] Hardness of cellulose acetate is 1.0.

[b] Permeate of concentrated whey (16% TS); initial TS permeate 0.2%.

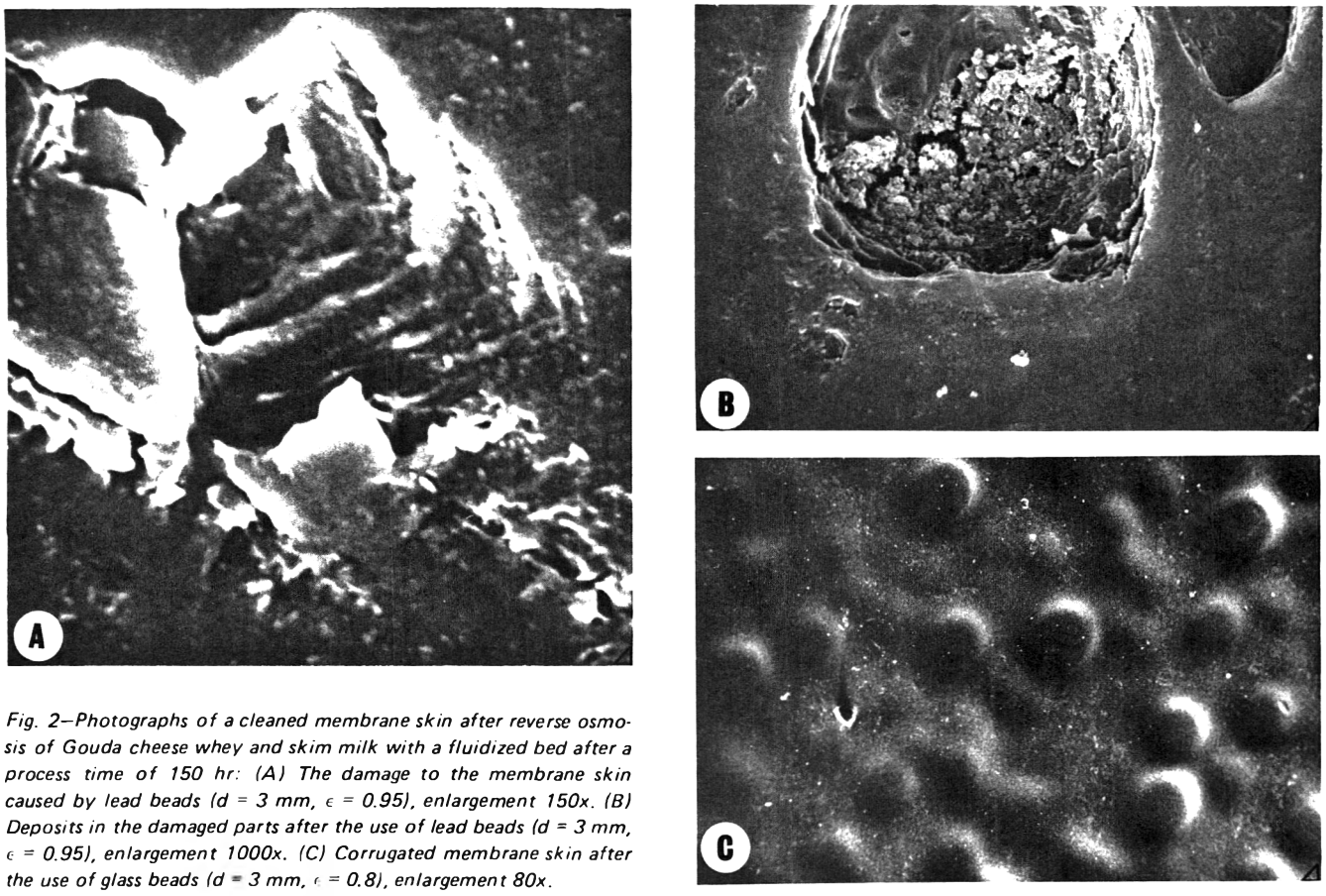

Fig. 2—Photographs of a cleaned membrane skin after reverse osmosis of Gouda cheese whey and skim milk with a fluidized bed after a process time of 150 hr: (A) The damage to the membrane skin caused by lead beads (d = 3 mm, ϵ = 0.95), enlargement 150x. (B) Deposits in the damaged parts after the use of lead beads (d = 3 mm, ϵ = 0.95), enlargement 1000x. (C) Corrugated membrane skin after the use of glass beads (d = 3 mm, ϵ = 0.8), enlargement 80x.

beads caused damage to the membrane is much larger than in the study of Van der Waal et al. (1977), which was ≤ 0.7 mm. Except for an improvement of the start-up procedure in the present study, it is probable that in the case of food liquids a gel layer acting as a secondary membrane, plays a role in decreasing membrane damage (Hiddink et al., 1980).

Composition of whey permeate in a fluidized bed consisting of glass beads

Because the exposure of the membrane to a fluidized bed of glass beads (d = 3 mm, ϵ = 0.8) may influence the separation efficiency, the composition of whey permeate in comparison with that of whey permeate in an empty tube (velocity 2 m/s) was examined (Table 2). At three levels of total solids content of the whey, the total solids content of the fluidized bed permeate was about twice as high as that obtained with the empty tube equipment. Three quarters of the total solids of both permeates are ash components such as sodium, potassium, and chloride. This difference in total solids may have been caused by the corrugated membrane skin (Fig. 2c), which suggests stretching of the membrane and, consequently, an adverse effect on the rejection. So, after a process time of 200 hr the glass beads were removed from the installation and the rejection was determined. The sodium chloride rejection of the membrane had diminished from 92.5% to 85%, which means that, due to the continuous bombardment of the glass beads, the membrane had become more open.

Additional desalting (mainly monovalent ions) may be expected when Gouda cheese whey is concentrated in a fluidized bed RO equipment, which is not normally considered a disadvantage. A drawback is, however, that the higher content of organic material in the permeate adversely influences the costs of discharging. In the Netherlands the "Population Equivalent" is used as a unit of pollution, which is calculated on the basis of COD and nitrogen content. The population equivalents of the two permeates differed less than the difference in total solids content. The difference of a factor of 2 is found again when the COD results of the fluidized-bed and empty-tube experiments are compared, the nitrogen content remaining the same in both cases. A similar effect was achieved with the open DDS 985 and the tight DDS 995 membrane, which had the same nitrogen loss (De Boer et al., 1977). This loss is caused by a

Table 2—The composition of Gouda whey permeate of a fluidized bed (glass beads ϕ 3 mm, ϵ = 0.65) in comparison with an empty tube system (V = 2 m/s) at three total solids levels of whey

		%		mg/100 l			
		TS	ash	Ca	K + Na	Cl	PE*/m³
whey —	5.6% TS						
	fluid bed	0.08	0.06	0.1	26	25	3.1
	empty tube	0.04	0.03	0.1	13	11	2.5
whey —	7.5% TS						
	fluid bed	0.09	0.07	0.1	33	33	3.1
	empty tube	0.05	0.03	0.1	16	16	2.5
whey —	21.9% TS						
	fluid bed	0.40	0.31	0.5	154	149	8.2
	empty tube	0.22	0.18	0.3	86	81	5.4

* PE = Population Equivalent

$PE/m^3 = \frac{1}{180}(COD + 4.57N)$; COD and N are both expressed as mg/liter.

part of the NPN fraction, the urea. Obviously the molecular structure of urea is such that it is capable of passing the open and the tight membrane.

Influence of bed porosity and glass bead diameter on the permeate flux

Since the permeate flux is an important factor from an economical point of view, the influence of glass beads on the permeate flux was studied under various conditions. Gouda cheese whey was used as feed solution, mostly at a process temperature of 10°C. In Figure 3 the permeate flux is given as a function of bed porosity. At a low value (0.52) the porosity of a packed bed is approached and at a high value (1.0) that of an empty tube. Below a bed porosity of 0.65 the bed is less stable, because then bridge-like particle agglomerates are formed, and also the risk of damage to the membrane may increase. The velocity varies according to the bed porosity; for 3 mm glass beads and at a porosity of 0.52, 0.65, and 0.90 the superficial flow velocity is 0.025, 0.06, and 0.16 m/s, respectively. Figure 3 shows that the permeate flux at a volume reduction of 0% is more favorable with 3 mm glass beads than with 0.7 and 2.0 mm beads. Obviously, the momentum of the beads plays an important role. With 4 mm beads the flux is less favorable than with 3 mm beads; here the ratio bead diameter/tube diameter seems too large to ensure good results. The graph with 0% volume reduction and 3 mm beads indicates that a bed porosity between 0.65 and 0.85 gave the highest flux figures. Comparison of these figures with those of a packed bed (ϵ = 0.52) gives a rough idea of the extent of the specific action of the fluidized bed. The increased permeate flux may be attributed to increased mass transfer and erosive action. A further understanding of the erosive action may be obtained when the results of fluidized-bed experiments are compared with those of empty-tube experiments at high velocity. Figure 4 shows the permeate flux as a function of the mass transfer coefficient for lactose. The mass transfer coefficient for the empty tube was calculated from the known mass transfer relationship in tubes under turbulent conditions (Rautenbach and Rauch, 1977), and the coefficient for the fluidized bed was calculated according to Van der Waal et al. (1977). For the fluidized bed the coefficient lies between narrow limits at about 8.5 x 10^{-6} m/s. For bed porosity 0.52 (approaching the packed bed) and 0.90 (approaching the empty tube) we find permeate fluxes almost equal to those in an empty tube at equal mass transfer coefficient, which can be understood from the fact that in both cases the erosive action will disappear. At a bed porosity of 0.65–0.75 the permeate flux is about the same as that at a mass transfer coefficient twice as high as that obtained in an empty tube at a velocity of about 2 m/s, which suggests the specific action mentioned. Thus, the erosive action doubles the permeate flux at the same mass transfer coefficient.

Apart from unconcentrated whey, Gouda whey was also used, at a volume reduction of 75%, in the fluidized-bed experiments with 3 mm glass beads. The curve for concentrated whey is flat, and there is no difference between results gained with the packed bed and those obtained at a bed porosity of 0.65–0.75 (Fig. 3). The effect of the erosive action has disappeared. During concentration the viscosity of the whey increases, at a temperature of 10°C, from 1.5 mPa·s at a volume reduction of 0% to 3.2 mPa·s at a volume reduction of 75%. This increase slows down the velocity of the beads proportionally and the lower velocity decreases in its turn the momentum of the glass beads. This lower momentum may explain the disappearance of the erosive action. In Figure 5 the flux figures of the fluidized bed are compared with those of an empty tube at three different volume reductions. At high volume reductions al-

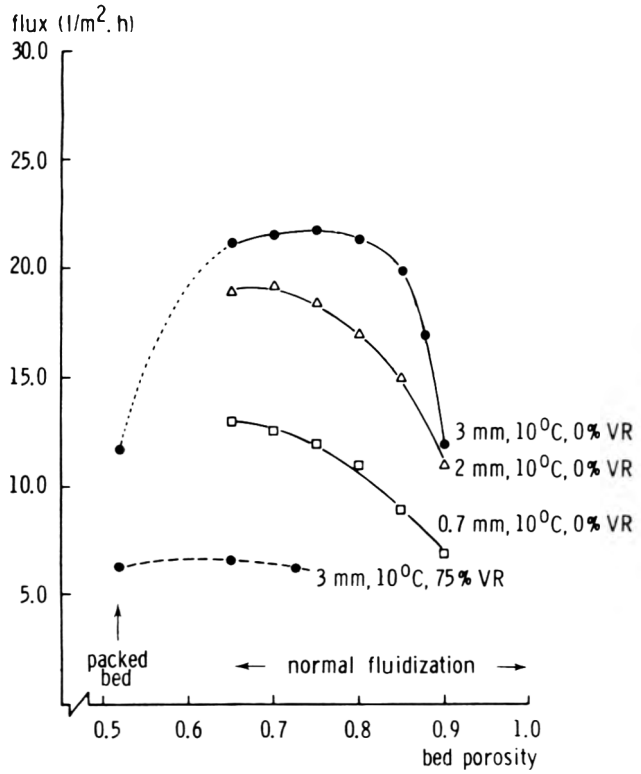

Fig. 3—Reverse osmosis of Gouda whey in a fluidized-bed system, carried out under recirculation of permeate and a pressure of 4 MPa. The influence of bed porosity and diameter of the glass beads on permeate flux is indicated, as well as the influence at two volume reductions for 3 mm glass beads.

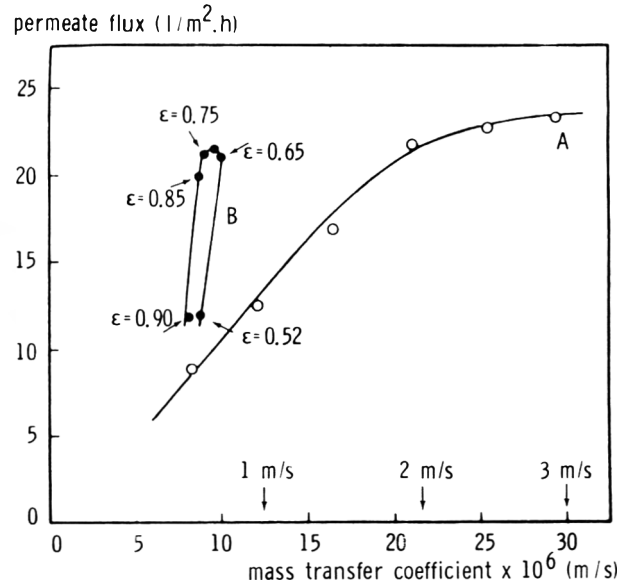

Fig. 4—Relationship between permeate flux and mass transfer coefficient (for lactose) for reverse osmosis of Gouda cheese whey at a temperature of 10°C and a pressure of 4 MPa. The velocities refer to those in an empty tube. (A) Experimental in an empty-tube, high-velocity system. (B) Experimental with a fluidized-bed system with glass beads 3 mm.

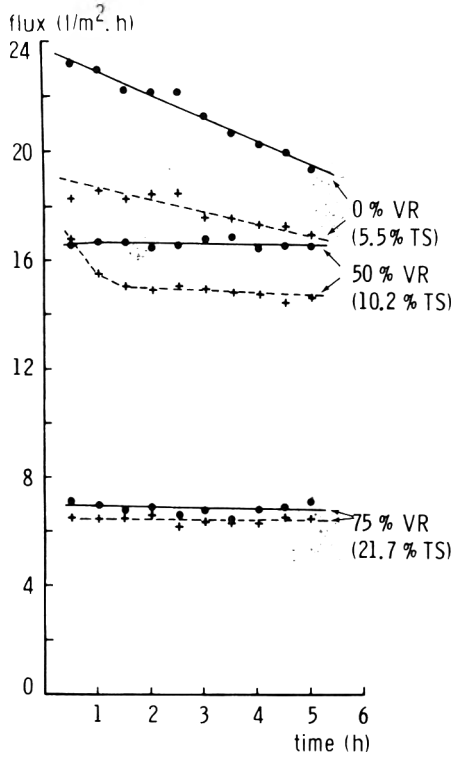

Fig. 5—Reverse osmosis of Gouda whey at a process temperature of 11°C and a pressure of 4 MPa in a fluidized bed (d glass beads = 3 mm, ϵ = 0.65) and an empty tube (v = 2 m/s) at various volume reductions (VR). Recirculation of permeate: ●——● fluidized bed, +-------+ empty tube.

most no difference is observed. In the concentrated whey with 21.7% total solids, both the erosive action of the beads and the high velocity along the membrane in an empty tube seem to be insufficient to control the fouling (see also Fig. 3, 75% volume reduction). At a volume reduction of 0% the flux obtained with the fluidized bed is high at first, but decreases gradually, so that the difference from that obtained with the empty tube becomes smaller. This indicates that, especially for the fluidized bed at 0% volume reduction, the fouling cannot be fully controlled during prolonged process time. It should be realized, however, that one of the advantages of a fluidized bed, the much lower superficial velocity and hence reduced energy consumption, still holds.

Permeate flux and bacterial counts in long-term experiments

Lowe and Durkee (1971) observed in their experiments with orange juice that turbulence promoters cause a certain amount of self cleaning of the membrane. This is of interest from the point of view of saving on cleaning agents and increasing the process time which is available for concentration. Long-term experiments were carried out with 3 mm glass beads and a bed porosity of 0.65, since preliminary experiments had shown that in the long run a porosity of 0.65 was more effective than 0.75. Furthermore, the control of the fluidized bed appeared to be very important, a collapse of the bed causing rapid fouling of the membrane due to the low velocity of the fluid. Tests were made with Gouda whey having total solids contents of 7.5% and 15.3%. In long-term experiments (up to 60 hr without cleaning) with whey of 7.5% total solids, the flux could be restored by releasing the pressure for a few minutes every 5 hr (Fig. 6). Furthermore, the equipment was rinsed and sterilized with a solution of 50 mg/liter active chlorine every 15 hr. The difference in permeate flux between the fluidized-bed and the empty-tube equipment which diminished during a 15-hr run, increased after this treatment in such a way that the permeate flux obtained with the fluidized bed became higher again. The restoration of the permeate flux could be observed only if rinsing and sterilization were carried out at low pressure. Probably in this way the fouling layer, or part of it, could be removed instead of being pressed on to the membrane. Figure 6 shows that by taking these measures the permeate flux is the same after 60 hr as it was in the beginning. In contrast to Figure 5, the results of the fluidized-bed experiments with whey of 15.3% total solids content were somewhat less favorable than those of the empty-tube tests. It may be that during starting-up the

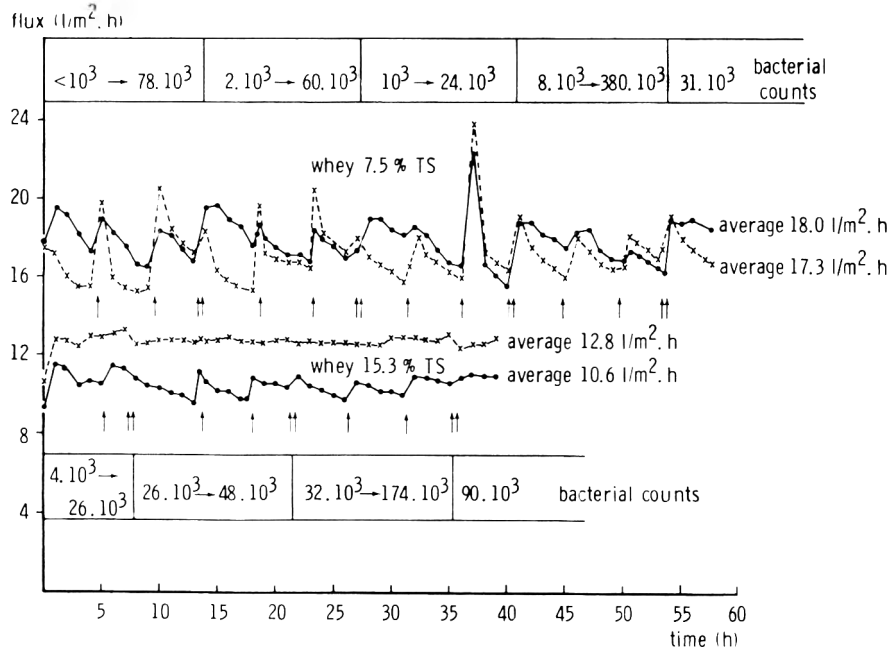

Fig. 6—The permeate flux of Gouda whey at two total solids levels (recirculation of permeate) in a fluidized-bed RO system compared with the flux of an empty tube during long-term experiments. In addition to the flux, bacterial counts are indicated. Process conditions: 11°C, 4 MPa (starting up at 3.5 MPa). ↑ without pressure for 5 min, ↑↑ rinsing and sterilizing at low pressure for 30 min. ●——● fluidized bed: (d glass beads = 3 mm, ϵ = 0.65); x-------x empty tube: v = 2 m/s.

Table 3—The permeate flux in a fluidized bed reverse osmosis system (glass beads ϕ 3 mm, ϵ = 0.65) of Gouda whey, skim milk and potato fruit water compared with the flux in an empty tube system ("Wafilin"). The flux was measured after a process time of 4 hr at a volume reduction of 50%

Raw materials	System	Permeate flux (liter/m²·hr)	Optimum process conditions V (m/s)	P (MPa)	T (°C)
whey (10.6% TS)	fluidized bed	16.6	0.1	4	10
	empty tube	15.5	2.6	4	10
	fluidized bed	17.2	0.1	4	30
	empty tube	15.2	2.6	4	30
skim milk (15.8% TS)	fluidized bed	9.6	0.1	3	30
	empty tube	12.5	2.6	3.5	30
potato fruit water (9.4% TS)	fluidized bed	7.1	0.1	3	25
	empty tube	12.5	2.6	4	25

conditions in the fluidized bed were not quite optimal. As the effects of pressure release and disinfecting rinse were less pronounced with the more concentrated whey, this difference could not be eliminated.

Another part of the investigation was concerned with the bacterial count of the whey, an important factor from the point of view of quality control. As the empty tube was part of the fluidized-bed installation the results could not be distinguished from those obtained with the tubes with 3 mm glass beads. The determinations indicate that there is a certain increase in bacterial count during every 15-hr run (Fig. 6). The counts are reasonably low, if it is taken into account that the sanitary aspects of the test equipment are not optimal and that the feed was circulated for 15 hr, which is much longer than in industrial plants.

Comparison of the permeate flux of whey, skim milk and potato fruit water

According to Hiddink et al. (1980), Gouda cheese whey at a process temperature of 10°C and a velocity $\geq$ 2 m/s is a low-fouling liquid. For this reason the fluidized-bed experiments included liquids which gave more serious fouling problems. As well as double-concentrated whey at a temperature of 30°C, skim milk (80% of the protein consists of casein micelles) and potato fruit water (50% of the protein is easily coagulable) were chosen. Due to limitations of the fluidized-bed test equipment, control experiments with the empty tube were carried out in a separate installation (Wafilin). In the empty-tube experiments optimum process conditions were chosen to restrict fouling as far as possible. The process conditions for skim milk given in Table 3 were taken from Hiddink et al. (1980) and those for potato fruit water from Meindertsma (1980). For the sake of comparison the whey experiments with the empty tube were also carried out at a velocity of 2.6 m/s.

As may be expected from previous experiments with whey and with twice concentrated whey, the fluidized bed had a somewhat higher permeate flux than the empty tube, both at 10°C and at 30°C (Table 3). Furthermore, there was no great difference between the flux at 10°C and that at 30°C. At a process temperature of 30°C, pretreatment of the whey is important to prevent fouling of the membrane, particularly at a volume reduction of 50%, as then the solubility limit of the calcium phosphate is exceeded and a deposit may be formed on the membrane. The pretreatment consisted in concentrating the whey to 50% volume reduction at 10°C and subsequently heating it to 30°C in a tank. This method is less effective than decalcification by ion exchange, by decreasing the pH from 6.5 to 6.1, or by a heat treatment at 65°C for 15 min (Hiddink and De Boer, 1979). For skim milk and potato fruit water, the fluidized bed (3 mm glass beads, bed porosity 0.65) had a considerably lower permeate flux than did the empty tube under optimum process conditions (Table 3). In the fluidized-bed system the pressure was reduced to 3 MPa, in order to restrict the permeate flux and to prevent serious fouling followed by rapid flux decline. Obviously, this fluidized bed is less suitable for preventing the fouling of the membrane than is the empty tube. Probably the momentum delivered by the glass beads is not high enough to reduce the fouling layer. For decreasing the fouling, a material with a somewhat higher mass density than glass may be more useful. It may be expected that with such a material the optimum pressure will be higher and the permeate flux will increase! The photograph (Fig. 7) shows an uncleaned membrane after RO of skim milk; the fouling layer with an imprint of a glass bead is very clear. In this type of fouling the protein may be of importance; skim milk and potato fruit water have a relatively high protein content of about 3%. Skudder et al. (1977) mentioned that in RO of skim milk the casein micelles are mainly responsible for the deposit on the membrane due to limited back diffusion especially at low flow velocities.

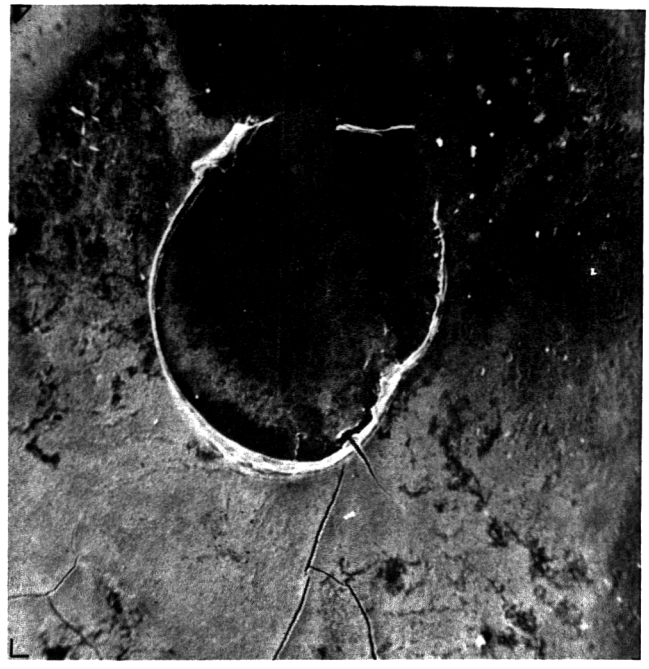

Fig. 7—Photograph of an uncleaned membrane skin after reverse osmosis of skim milk with a fluidized bed of glass beads (d = 3 mm, ϵ = 0.75), enlargement 25x.

PRACTICAL IMPLICATIONS & ENERGY CONSIDERATIONS

A TUBULAR fluidized-bed reverse-osmosis system is more suitable for the concentration of Gouda cheese whey than for that of more fouling liquids, such as skim milk and potato fruit water. Glass beads with a diameter of 3 mm and a fluidized bed with a porosity of 0.65 gave the highest permeate flux.

As contrasted with the normal system, the modules in a fluidized-bed system are framed in a vertical position. Due to the weight of the liquid column and of the glass beads (3.5 kg per m² membrane area) there is an additional pres-

sure drop of about 0.016 MPa/m tube length. This influences the system design, which means that more tubes have to be operated in parallel. One of the critical points in a fluidized-bed system is accurate control of the fluidization, which necessitates complicated control equipment. According to the energy efficiency relation of Van der Waal et al. (1977), for a fluidized-bed system a saving in circulation energy of about 90% can be achieved. In addition to a circulation pump, a high-pressure pump is necessary. In a normal system the high-pressure pump, which brings the liquid to a pressure of about 4 MPa, consumes about 45% of the total energy. So, in a fluidized-bed system a saving of approximately 50% on total electricity costs, in comparison with the costs of a normal system, can usually be reached. The fluidized-bed system, besides saving on energy, might also save on cleaning costs, as a result of the lower cleaning frequency and the smaller quantity of detergent (reduction about 30%) required per treatment. However, a lower cleaning frequency when whey is processed at 10°C is possible only if additional measures are taken, such as releasing the pressure for a few minutes every 5 hr, and rinsing and sterilizing at low pressure, for instance every 15 hr. A disadvantage is the somewhat higher pollution value of the permeate. This value—calculated on the basis of COD and nitrogen—rose to a factor 1.5. Further development will be necessary before the system has reached the industrial stage. In the present work, for example, more data will have to be provided about the useful life of the membranes.

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EFFECT OF MICRONIZING ON KERNEL CHARACTERISTICS OF SORGHUM VARIETIES WITH DIFFERENT ENDOSPERM TYPE

B. A. RUSNAK, CHOA-LIN CHOU, and L. W. ROONEY

ABSTRACT

Micronizing is accomplished by heating the grain with infra-red burners and crushing the heated kernels between corrugated steel rollers. A laboratory model Pierce micronizer was used to process five sorghum varieties with differing endosperm characteristics: two waxy, one heterowaxy, and two nonwaxy. The waxy grain had the greatest expansion followed by the heterowaxy and nonwaxy grains. The greatest alteration in endosperm structure occurred in the waxy sorghum varieties. The extent of starch gelatinization, measured by several methods, was in decreasing order: waxy, heterowaxy, and nonwaxy sorghum varieties. Waxy sorghum varieties are more extensively gelatinized than nonwaxy sorghums for a given increment of heat treatment. Thus, waxy sorghums can be processed under lower heat and pressure requirements than presently necessary for nonwaxy sorghums.

INTRODUCTION

NUMEROUS METHODS, including grinding, steam flaking, dry rolling, exploding, popping, and micronizing have been designed to improve the availability of the starch in cereal grains (Hale and Theurer, 1972). Sorghums, in particular, must be processed to achieve maximum utilization of its components. Micronizing is a dry heat process using gas fired infra-red generators to heat the grain. The infra-red rays penetrate the grain and excite the water molecules which then vibrate at a frequency of 600–1,200 million mega cycles per second (Micronizer Company, 1977). Rapid internal heating accompanies a rise in water vapor pressure. In effect, the grain is cooked from the inside out and each kernel subsequently expands almost to the point of eversion. Immediately prior to eversion, the heated kernels pass through corrugated rollers under pressure and are crushed into flakes.

Micronized grain has been used for livestock feeds because it improves efficiency of digestion (Hale and Theurer, 1972). Micronized grain is potentially useful for human foods, especially for breakfast cereals or snack products where processing with heat has previously been used to expand, puff, or pop cereal grains. It has been used to process grains for special granola types of products.

Therefore, the objectives of the research reported here were to utilize a laboratory scale micronizer to determine physical, chemical and structural changes in the kernels of several new, experimental sorghum varieties. This information would establish the potential usefulness of micronizing sorghum for human food products. In addition, it would determine how sorghum varieties with different kernel characteristics respond to micronizing and whether certain varieties are more easily processed.

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MATERIALS & METHODS

Samples

Five sorghum varieties with differing endosperm characteristics were selected: a white waxy (Tx 615 × NSA 954); a waxy redlan (B 413); a white heterowaxy (A 7504 × NSA 954); a white nonwaxy (CSH-5) and a nonwaxy redlan variety (B 378). Comparison of B 413 and B 378 data will best illustrate the effect of the waxy endosperm on the micronizing properties of sorghum. All samples were grown under similar conditions in the sorghum nursery at Lubbock, Texas in 1976.

Apparatus and processing procedure

A laboratory model Pierce micronizer was used to process the grain samples. A flow sheet for the micronizer is presented in Figure 1. The micronizer consists of five infra-red burners, each burner generating approximately 25,000 Btu of energy. The inner frame of each gas burner is 21 in. × 3.5 in. (53.34 cm × 8.89 cm). The total length of the channel under these five burners is 118 in. (299.72 cm). The quantity of grain and time required for it to pass down the

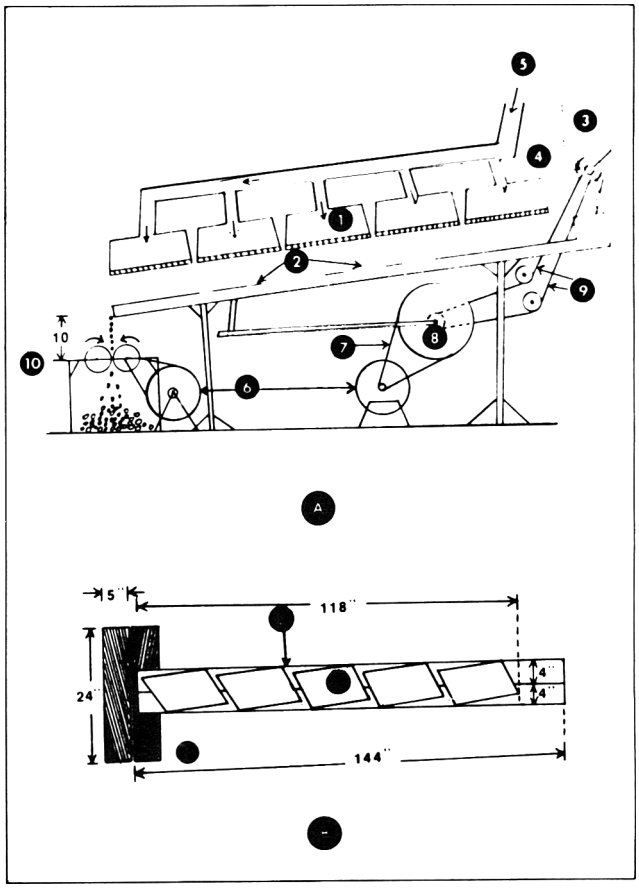

Fig. 1—Schematic diagram of the Pierce Laboratory micronizer and flow diagram of micronizing process for sorghum grains: (A) Side view; (B) Top view. The keys to the numbers are: (1) gas burners, (2) skillet, (3) grain hopper, (4) grain input regulator, (5) air-gas mixture, (6) 2 horse power motor, (7) belt to drive reciprocating pulley, (8) skillet reciprocating pulley, (9) belt to drive grain feed regulator, (1) rollers.

Table 1—Physical properties of whole grain (W.G.) and micronized (M.) sorghum

	Endosperm type	Test Weight (1b/bu) W.G.	M.	Test Weight (kg/hl) W.G.	M.	Density (g/cc) W.G.	M.
TX615xNSA954	waxy	60.7	22.9	78.3	29.5	1.34	0.97
B413	waxy	59.5	16.8	76.7	21.7	1.36	0.71
A7504xNSA954	heterowaxy	61.3	32.3	79.1	41.7	1.35	1.20
CSH-5	nonwaxy	58.2	35.9	75.1	46.3	1.35	1.29
B378	nonwaxy	60.2	32.1	77.6	41.4	1.35	1.27

skillet determine the total heat received by the grain during processing. The rate at which grain passes down the skillet is regulated by the slope of the skillet and the speed at which it reciprocates.

Grain was screened to remove foreign material, broken kernels and glumes before micronizing. Average moisture of the original kernels was 9.8% (range 9.3–10.1%). After micronizing the moisture was 9.1% (range 8.9–9.4%). The infra-red generators were lit 15 min before processing to equilibrate the burners and heat the skillet. Fifty to 100 pounds of a commercial hybrid grain sample were processed to warm the rollers and adjust the micronizer prior to processing experimental samples. The feed rate, slope, and reciprocating speed of the skillet were adjusted to obtain a flake density of 26.0 lb/bushel (33.5 kg/hl) for the reference sorghum sample. The rollers were set at 0.003 in. (762 mm) clearance with a pressure of 50 psi, a speed of 800 rpm, and no roll differential. The natural gas flow was maintained at 6 psi pressure. Then 50 lb of each of the five samples were micronized and collected for further analysis. Each time samples were micronized the micronizer was adjusted until the reference sorghum produced flakes with a bulk density of 26.0 lb/bushel (33.5 kg/hl).

Physical analysis

Test weight or bulk density was taken with a Winchester bushel meter according to official U.S. Grain Standard procedures (USDA, 1974). An air compression pycnometer was used to determine the specific gravity or density of the grain samples (Rooney and Sullins, 1970).

Gross morphology of the whole grain and micronized samples was observed and photographed with the Zeiss Tessovar Stereoscope equipped with a Pentax K-2 Automatic 35 mm camera. Black and white photography utilized Plus-X Panchromatic (ASA 125) film.

Scanning electron microscopy (SEM)

For SEM, the flakes were mounted on 9 mm aluminum stubs with silver conductive paint. Then, samples were coated with 200Å of gold palladium and viewed with the Joel JSM35 Scanning electron microscope with an accelerating voltage of 25KV. Photomicrographs were recorded on Tri-X Panchromatic film (ASA 400).

Starch gelatinization

Loss of birefringence. The micronized samples were soaked in excess 0.5% sulfur dioxide solution (0.742 g/L of $Na_2S_2O_5$) for 12 hr at 25°C. Starch was scratched from 5 steeped kernels and placed on a slide. Samples were examined under a binocular microscope with a polarizing lens at 450X. Two slides were prepared from each variety, and three fields viewed of each slide with 10–40 individual starch granules in each field.

Enzyme susceptibility. Digestion with amyloglucosidase was used to determine the extent of starch gelatinization. Twenty ml H_2O and 25 ml 0.1N Na acetate buffer were added to 200 mg ground grain sample. After addition of 1 ml amyloglucosidase Diazyme L100 (Miles Laboratories Inc., Elkhart, IN 46514) the sample was incubated 30 min at 60°C. Enzyme was inactivated with 3 ml (1:9 V/V H_2SO_4:H_2O) and 2 ml 12% Na tungstate. Samples were allowed to settle overnight. Two ml supernatant were diluted to 100 ml with distilled H_2O and this diluted volume analyzed by phenol-sulfuric acid technique for glucose determination (Dubois et al., 1956). Results were expressed as mg glucose per gram of sample dry weight.

Apparent viscosity. The micronized grain was gound with a Udy cyclone mill (1.0 mm screen). A Brookfield viscometer was used to determine the viscosity of ground micronized sorghums. All measurements were taken on 11.5% (dry wt.) suspensions of the ground samples in 420 ml of distilled water. Spindle No. 1 was inserted into the test material rotating at a speed of 20 rpm. For the high viscosity slurry or the waxy variety, a No. 2 spindle was used. Readings were taken at 1-min intervals until there was no increase in viscosity. Viscosity in centipoise units was obtained by multiplying the readings by a factor of 5 for spindle No. 1 and a factor of 20 for spindle No. 2. Values at 5 min were selected as representative sample illustrating differences in apparent viscosity.

Initial pasting viscosity. Starch dispersions of the ground and micronized and ground whole sorghum samples were examined using a Brabender visco/amylograph (Mazurs et al., 1957). An 11.5% (dry wt) suspension of each of the ground samples in 420 ml of distilled water was used to obtain the cooking curves.

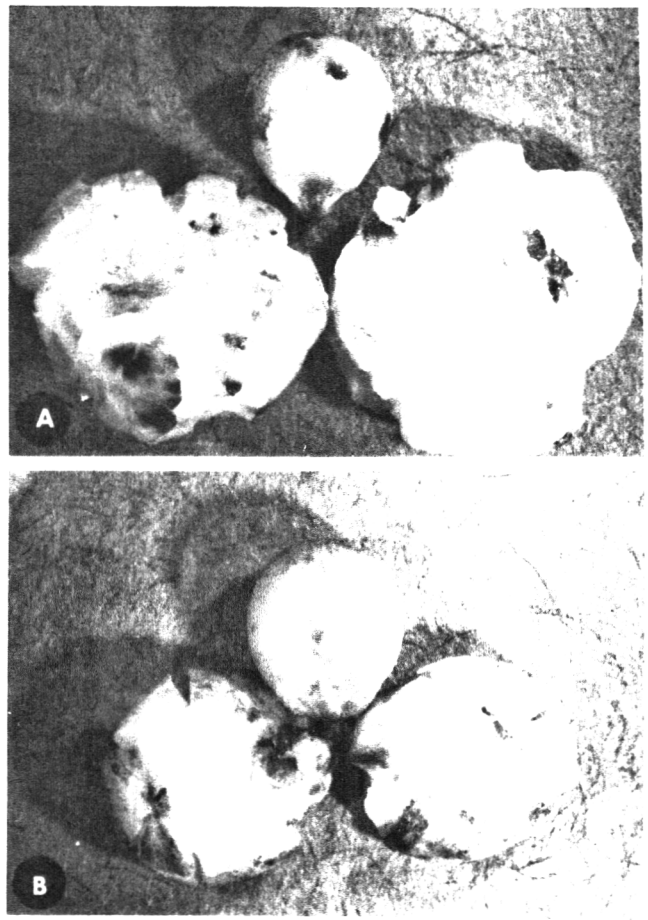

Fig. 2—Gross morphology of the whole grain and micronized sorghum. (a) White waxy sorghum (b) White nonwaxy sorghum.

RESULTS & DISCUSSIONS

THE MICRONIZING PROCESS decreased test weight and density of the whole grain (Table 1). The waxy variety showed the greatest percentage decrease in test weight and density, followed in order by the heterowaxy and nonwaxy grains. These values were used as an indication of expansion, i.e., a decrease in density corresponds to an increase in expansion. Kernel expansion during micronizing is a result of the swelling of individual gelatinized starch granules. When the infra-red energy vaporizes the moisture present in each starch granule, pressure develops and swelling occurs. The waxy sorghum variety (Fig. 2A), exhibiting a greater decrease in density, was more highly expanded than either the heterowaxy or nonwaxy varieties (Fig. 2B). This could be attributed to the more even distribution of protein bodies throughout the starchy endosperm and a lesser concentration of protein in the peripheral endosperm. In con-

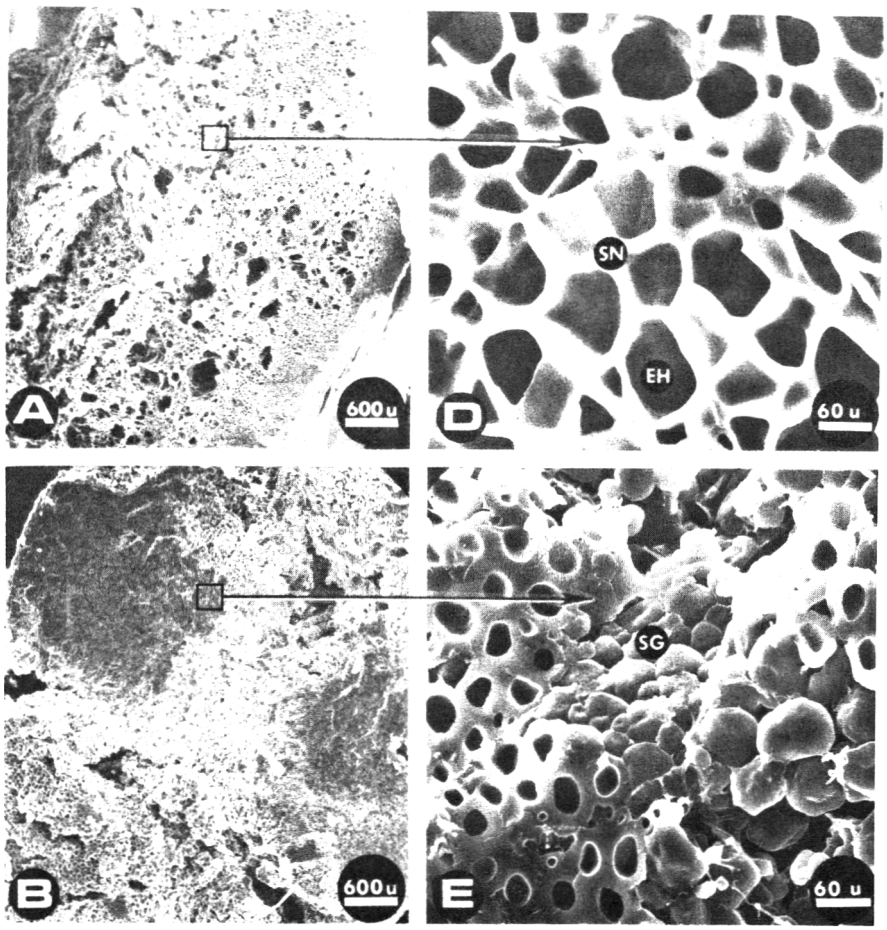

Fig. 3—Photomicrographs taken with the SEM of micronized sorghum flakes. Cross section (A) and (D) of waxy micronized flake, (B) and (E) Nonwaxy micronized flake. A, B = 38X, D, E = 380X. SG = starch granule, SN = starch network, EH = expansion hole.

trast, the normal nonwaxy sorghum has its protein concentrated in the peripheral endosperm which offers resistance to kernel expansion.

Scanning electron microscopy was used to examine more closely the ultrastructural changes to explain the expansion and decrease in density. Scanning electron photomicrographs of the micronized waxy flakes (Fig. 3A) illustrate that the integrity of the starch granule was lost and expansion occurred relatively uniformly throughout the entire starchy endosperm. The granules of the waxy sorghum greatly expanded into a starch network (Fig. 3D). However, in the nonwaxy flake (Fig. 3B) the majority of the starch granules were not expanded. Figure 3E confirms that the granules are not as thoroughly disrupted as they are in the waxy flake. The spherical integrity of the granules is still apparent.

Starch gelatinization

Gelatinization is the irreversible loss of organization of the starch granule. Changes in viscosity such as measured by the Brookfield Viscometer and amylograph indicate one method of evaluating gelatinization. Loss of birefringence (in native state, starch granules produce maltese cross under polarized light) denotes breakdown of the starch granule organization. Upon gelatinization the disorganized starch granules are more susceptible to enzyme attack.

The micronizing process caused starch gelatinization in all the varieties, but the extent of gelatinization was affected by the endosperm characteristics of the sorghum variety.

All measures of starch granule gelatinization indicated that the waxy sorghum was most completely gelatinized followed by the heterowaxy and the nonwaxy sorghums, respectively (Table 2). The most sensitive indices were the apparent initial viscosity measured with the Brookfield viscometer and the temperature of initial viscosity measured with the amylograph. The initial viscosities of the micronized waxy sorghums as measured with the Brookfield viscometer (Fig. 4) are much higher than the viscosities of the nonwaxy varieties. This would indicate more extensive gelatinization and starch granule breakdown in the waxy varieties due to the micronizing process.

Amylograph curves for waxy redlan (B 413) and nonwaxy redlan (B 378) before and after micronizing are presented in Figure 5. The waxy ground grain has a high peak viscosity that is drastically reduced by micronizing. For nonwaxy grain, the changes are not as drastic with only a slight decrease in peak viscosity. We have seen these differences among waxy sorghums consistently, but sometimes for unexplained reasons considerable unexpected variation occurs. The data indicate that within sorghums of a particular type, i.e. waxy endosperm, considerable variation exists. Additional experiments, in which sorghums grown under different conditions were processed by micronizing in a standard fashion, have confirmed that these observations are real (Rooney, 1976).

DISCUSSION & CONCLUSIONS

THE PROCESSING PROPERTIES of waxy sorghums are significantly different from those of nonwaxy sorghums. The waxy sorghums have all amylopectin starch and have a different kernel structure from that of nonwaxy sorghums. The combination of kernel structure and amylopectin

Table 2—Extent of starch gelatinization in the micronized grain

Sample	Endosperm type	Loss of birefringence (%)	Enzyme susceptibility (mg glucose/g sample)	Apparent viscosity (CP)[a]	Temperature of initial viscosity (°C)[b]
Tx 615 x NSA 954	waxy	57 ± 3%	520	1100	55.6 ± 1.1
B 413	waxy	47 ± 4%	546	1475	52.3 ± 1.9
A 7504 x NSA 954	heterowaxy	39 ± 4%	470	100	61.4 ± 0.4
CSH-5	nonwaxy	20 ± 3%	425	37	73.3 ± 1.1
B 378	nonwaxy	22 ± 3%	453	7	70.4 ± 0.5

[a] Determined with the Brookfield Viscometer
[b] Determined with the Brabender visco/amylo/graph

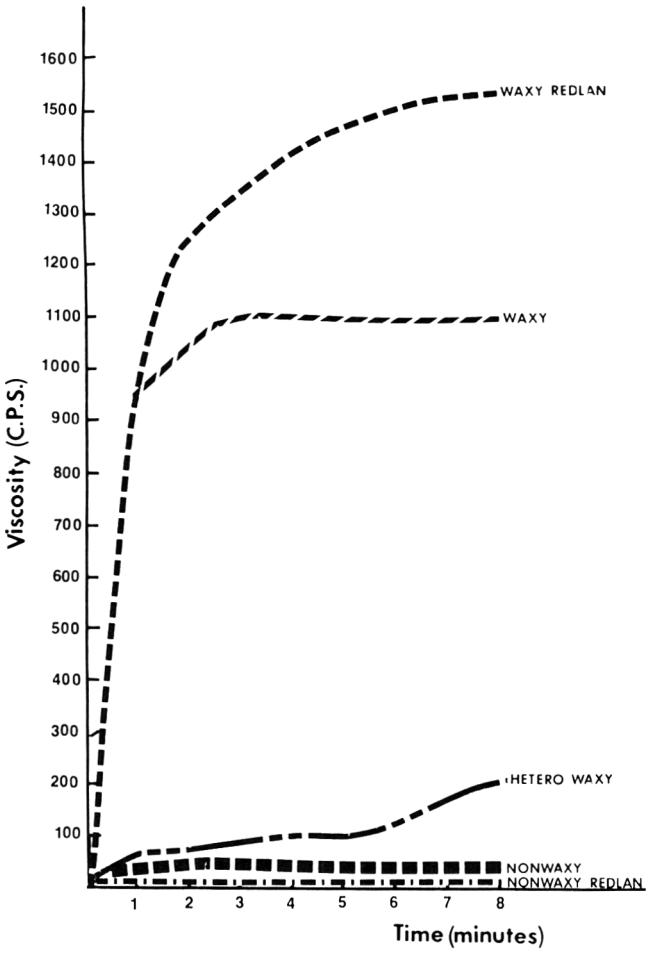

Fig. 4—Effect of time on initial viscosity of micronized sorghums.

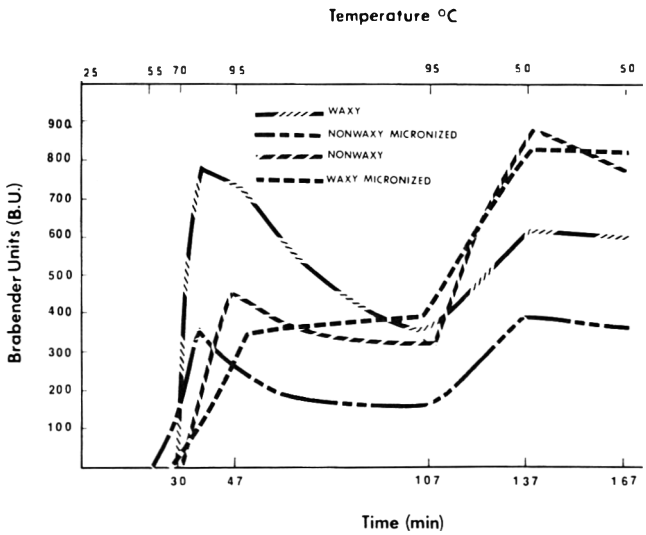

Fig. 5—Brabender/visco/amylograph cooking viscosities of 11.5% suspension of ground micronized grain from 2 sorghum varieties.

starch may explain why they are more thoroughly cooked. Previously, waxy sorghums have been shown to have improved digestibility when measured by in vitro and in vivo tests (Rooney and Sullins, 1974). The information presented here suggests that lower heat and pressure would be required to process waxy sorghum to the same degree as nonwaxy sorghums for feed purposes. Their greater expansion might suggest use in snacks and other processed products. At the present time, waxy sorghum hybrids produce lower grain yields than nonwaxy hybrids. Future development of waxy sorghums with white kernels and competitive yields of grain could provide sorghums with better nutritional and lower processing requirements for both food and feeds.

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EFFECTS OF PROCESSING ON THE DIETARY FIBER CONTENT OF WHEAT BRAN, PUREED GREEN BEANS, AND CARROTS

N. E. ANDERSON and F. M. CLYDESDALE

ABSTRACT

The dietary fiber (DF) components of green beans and carrots were fractionated and analyzed utilizing methodology developed by Anderson and Clydesdale (1980). Samples of each vegetable were examined as purchased, after boiling for 30 min, and after retorting for 60 min. Also, a standard wheat bran, whose DF profile was previously determined by Anderson and Clydesdale (1980), was analyzed after toasting for 30 and 60 min and after boiling for 30 min. Results indicated that toasting significantly increased the "lignin" content of the bran and had little effect on the other components. Wet heat processing tended to first solubilize and then destroy the pectin substances in the pureed green beans, carrots, and wheat bran.

INTRODUCTION

DUE TO THE INCREASING interest in dietary fiber (DF) much research has been conducted to determine the DF content of foodstuffs. Such methods include the neutral detergent (Van Soest and Wine, 1967), acid detergent (Van Soest, 1963), buffered acid detergent (Baker, 1977), enzymatic (Hellendoorn et al., 1975), and fractionation (Southgate, 1969) procedures. There are problems inherently associated with these methods which prompted Anderson and Clydesdale (1980) to develop methodology to fractionate and analyze the DF content of wheat bran. This methodology provided much more information about the nature of the components of DF and produced a better estimate for actual DF content than did existing methods. It was the first objective of this research to further utilize the methods proposed by Anderson and Clydesdale (1980) to analyze the DF content of carrots and green beans.

Secondly, most investigations have not been concerned with the effects of various processing conditions on the components of DF. Since most foods are not usually eaten in their raw state an examination of the effects of processing seemed worthwhile. Several studies have indicated that the components of DF may indeed be affected by processing. Van Soest (1963), utilizing the acid detergent (ADF) method, reported that exposure to dry heat above 50°C induced the formation of browning compounds which were measured as part of the ADF. Matthee and Appledorf (1978) examined the effects of boiling on the DF content of various vegetables. Using the neutral detergent (NDF) and ADF methods they found that both the ADF and NDF values increased with prolonged heating.

However, there are two problems associated with using the detergent methods. Firstly, those methods do not measure the individual components of DF, so no precise conclusion can be made as to which component(s) is being affected by the processing. Secondly, these methods do not measure the water-soluble components of DF which also may be affected by the processing. It was hoped that by using the methodology of Anderson and Clydesdale (1980) that the component or components of DF that are being affected will be measured directly. Therefore, pureed green beans and carrots were analyzed as purchased, after boiling for 30 min, and after retorting for 60 min. Wheat bran, whose DF profile was previously determined (Anderson and Clydesdale, 1980) was analyzed after toasting for 30 and 60 min and after boiling for 30 min.

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EXPERIMENTAL

Materials

Purified monosaccharides (Applied Science Laboratories) were used without further purification. Standard solutions (10 mg/ml) of rhamnose and myoinositol (Sigma Chemical Co.) were prepared with distilled water. A few thymol crystals were added as a preservative and the solutions were kept in a refrigerator until used. Rhamnose was used as the internal standard for the wheat bran while myo-inositol was used for the vegetables.

Galacturonic acid monohydrate (Sigma Chemical Co.) and carbozole (Eastman Kodak Co.) were utilized for uronic acid analyses.

A standardized, food-grade wheat bran (#R07-3691) was obtained from the American Association of Cereal Chemists and kept in a freezer until analyzed.

Carrots and green beans were purchased fresh from a local supermarket. They were refrigerated less than 24 hr prior to processing and/or analysis.

All other materials and methods may be found in a previous report by Anderson and Clydesdale (1980).

Sample preparation

Unprocessed samples. The wheat bran was freeze-dried and then ground in a micro-mill to insure sample homogeneity.

The carrots and green beans were mixed with water (100 ml/100g vegetable) and homogenized in a Waring Blendor. The purees were divided into three groups and then processed and/or frozen at −40°C. After freeze-drying for 36 hr the samples were ground in a micro-mill and kept frozen until analyzed.

Toasting process

Samples of the wheat bran were thinly spread out onto two aluminum cooking sheets. These were placed in a conventional oven preheated to approximately 177°C. One tray was removed after 30 min and the other after 60 min. These samples were cooled, ground in a micro-mill, and then placed in a freezer until analyzed.

Two hundred ml aliquots of the green bean and carrot purees as well as a wheat bran slurry (100 ml HOH/10g bran) were processed in a 500 ml round bottom flask equipped with a reflux condenser. The samples were brought rapidly to a boil and were maintained at a boil for 30 min. They were immediately frozen at −40°C, freeze-dried, ground in a micro-mill, and then stored in a freezer until analyzed.

Retorting process

Aliquots of the green bean and carrot purees were filled into 307 × 409 cans, sealed, and retorted in an agitating retort (Laboratory Sterilizer, Berlin Chapman, Inc.) at 121°C for 60 min. This process approximated that designed for commercial squash and pumpkin baby food (Lopez, 1975) and was meant to serve as a severe heat treatment. After processing the purees were cooled, frozen, freeze-dried, ground in a micro-mill and then kept frozen until analyzed.

Statistical analysis

Analysis of variance was performed on the data for effect of wet and dry heat processing on wheat bran and effect of wet heat on

carrots and green beans. One-way classifications were utilized for each fraction of the dietary fibers.

Apparatus

A Perkin-Elmer Model 881 gas chromatograph was utilized for the gas-liquid chromatography (GLC) analyses. It was equipped with a flame ionization detector and a Leeds and Northrop Speedomax recorder. Six-food glass columns (2 mm i.d.) were used with helium carrier gas at a flow rate of 40 ml/min. Supelco Sp-2340 on 100/120 Supelcoport (Supelco, Inc.) was the stationary phase. Operating conditions were: column temperature programmed from 180 to 220°C at 2°/min with an initial 5 min hold at 180°C, injection port at 230°C, and detector at 220°C.

Fractionation procedure

The fractionation and analytical procedures developed by Anderson and Clydesdale (1980) were utilized to analyze the samples. This method, which was used to analyze wheat bran, will be briefly described along with modifications used to accomodate the analysis of the vegetable samples.

The first step involves lipid extraction of the sample with ether. This is followed by several cold water extractions at 20°C (pH 7.0–7.5). The extracts are combined, soluble proteins are removed, and ethanol is added to precipitate the polysaccharides. The precipitates are then analyzed for neutral and acidic sugar content as described later.

Treatment of the cold water extract was modified for the samples subjected to the wet heat process. Preliminary studies on the wheat bran indicated that this process caused some of the starch to become soluble in cold water. Therefore the extracts were treated with an amylolytic enzyme, pancreatin, in the same manner as the hot water extracts are treated.

The cold water insoluble residue is extracted several times with hot (80°C) water containing 0.05M EDTA. The extracts are combined, treated with an amylolytic enzyme to remove starch, and ethanol is added to precipitate the polysaccharides.

The precipitated water-soluble polymers are solubilized with 0.3 ml 72% H_2SO_4 for 1 hr and then diluted with water (28 ml/ml acid). Several samples are then analyzed for uronic acid content by the carbazole method described later. Appropriate internal standard (50 µl) is added to several other samples, rhamnose for the wheat bran and myo-inositol for the vegetables. Aliquots are transferred to glass tubes which are then sealed and subjected to a secondary hydrolysis for 3 hr in a boiling water bath. The hydrolyzed samples are then analyzed for neutral sugar content by the GLC procedure.

The water-insoluble residue is treated with proteolytic and amylolytic enzymes to remove protein and starch. The washed residue is then hydrolyzed with 5% H_2SO_4 to remove the hemicellulose fraction, 10 ml were used to hydrolyze the vegetable samples and 15 ml for the bran samples. Internal standard was also added prior to analysis, 2 ml rhamnose for the bran and 0.5 ml myo-inositol for the vegetables. The hydrolyzed extracts are diluted, made to volume with water, 50 ml for the vegetables and 100 ml for the wheat bran, and analyzed for neutral and acidic sugar content. The variations in parameters is due to the much greater hemicellulose content of the bran samples compared to the vegetables.

The insoluble residue is treated with 72% H_2SO_4 to solublize the cellulose. The solublized extract is diluted to 5% H_2SO_4. Several samples are analyzed for acidic sugar content. Internal standard (1 ml) is added to several other samples. These are subjected to a secondary hydrolysis in sealed tubes in a boiling water bath and analyzed for neutral sugar content.

Finally, the insoluble residue is washed, dried, and weighed to determine the Klason lignin content.

Methods

Uronic acids were analyzed by the modified carbazole procedure of Bitter and Muir (1962) which was further modified by Anderson and Clydesdale (1980) to eliminate some of the interference caused by neutral sugars.

Values of the acidic sugars analyzed in the cold and hot water extracts plus those in the cellulose extract were added to provide an estimation of total pectic substances. The acidic sugars in the hemicellulose fraction were considered to be a true constituent of this extract since uronic acids are a natural component of hemicellulose. Since cellulose would not be expected to contain uronic acids, the acidic sugars analyzed in the fraction were expressed as part of the total pectic substances. Southgate (1969) also considered the uronic acids measured in the hemicellulose fraction as being derived principally from the hemicellulose and those measured in the cellulose fraction as being derived from pectic substances.

Neutral sugars were estimated by a GLC procedure. After hydrolysis samples are neutralized with $BaCO_3$, saponified with aqueous sodium carbonate to prevent uronic acids from becoming reduced, reduced with sodium borohydride to form the sugar alcohols, and finally acetylated with sodium acetate and acetic anhydride to form the respective alditol acetate derivatives. The final reaction mixture is injected directly on the GLC column.

RESULTS & DISCUSSION

THE FIRST PART of the study involved analysis of the DF content of green beans and carrots.

Analysis of green beans

Four samples of green beans were estimated to contain 27.53% DF (Table 1). This value is on a dry weight basis and is corrected for a fat content of 1.86 ± 0.07%. This is a much larger value than the 10.10% CF calculated from data in USDA Handbook #8 (Watt and Merrill, 1975). It does compare favorably with the NDF value of 17.6% (Table 2, Shipley, 1978). Since the NDF method does not measure the water-soluble components, which account for approximately 8% of the green beans, it does not produce as accurate an estimate of the total DF as does this methodology.

Table 1—Profile of dietary fiber constituents of various foodstuffs utilizing the proposed methodology

Fraction		Substrate[c] Wheat Bran	Green Beans (fresh)	Carrots (fresh)
Cold Water	N[a]	0.79 ± 0.09	0	C
	A[b]	0.13 ± 0.01	0	C
Hot Water	N	Tr	Tr	2.40 ± 0.49
	A	0.06 ± 0.02	5.64 ± 0.50	6.72 ± 0.51
Hemicellulose	N	25.14 ± 1.00	2.30 ± 0.13	1.36 ± 0.18
	A	3.31 ± 0.40	2.26 ± 0.11	1.00 ± 0.08
Cellulose	N	9.84 ± 0.75	11.39 ± 0.64	7.63 ± 0.33
	A	1.98 ± 0.09	4.48 ± 0.12	3.41 ± 0.15
Lignin		2.87 ± 0.21	1.46 ± 0.06	0.63 ± 0.06
% Dietary fiber		44.12	27.53	23.15

[a] N = Neutral fraction

[b] A = Acidic sugar fraction

[c] Substrate = values expressed as mean % recovery ± standard deviation on a % dry weight basis

Table 2—Comparisons of the dietary fiber contents of various foodstuffs using different methods on a % dry matter basis

Product	Dietary fiber[a]	ADF[b]	NDF[c]	Indigestible residue[d]	Crude fiber[e]
Wheat bran	44.12	11.19	40.2	46.0	8.91
Green beans	27.53	–	17.6	–	10.10
Carrots	23.15	–	9.2	13	9.09

[a] Dietary fiber = values are those obtained from proposed methology.

[b] ADF = Acid detergent fiber; value supplied with wheat bran.

[c] NDF = Neutral detergent fiber; value for wheat bran supplied with sample values for green beans and carrots from Shipley (1978).

[d] Indigestible residue = Values derived from enzymatic method of Hellendoorn et al. (1975); value for wheat bran this lab; value for carrots from Shipley (1978).

[e] Crude Fiber = values for carrots and green beans calculated from USDA Handbook #8 (Watt and Merrill, 1975); value for wheat bran obtained with sample.

Values for the DF constituents of green beans obtained by Southgate's method were compared in Table 3. Estimates of the major fractions agree fairly well with both methods. It should be noted that the green beans were probably not of the same variety or age and that these factors will affect the composition.

The hemicellulose fraction of the green beans differs significantly from the bran samples. The wheat bran sample contained approximately 25% neutral and 3% acidic sugars (Anderson and Clydesdale, 1980), whereas the green beans contained 2.3% neutral and 2.26% acidic sugars (Table 1). The profile of the neutral sugars was also quite different. The green beans contained 3.84 ± 0.22% rhamnose, 16.98 ± 3.31 arabinose, 34.82 ± 1.28% xylose, 5.54 ± 0.23% mannose, 26.79 ± 1.51% galactose, and 12.03 ± 2.53% glucose. Anderson and Clydesdale (1980) reported that wheat bran hemicellulose contained 31.3 ± 1.4% arabinose, 55.7 ± 1.6% xylose, 2.1 ± 0.2% galactose, and 11.0 ± 2.8% glucose. The major differences between the bran and green bean samples was the presence of rhamnose and large amounts of galactose in the green bean hemicellulose.

The cellulose fraction was very similar to the wheat bran sample. The green beans contained approximately the same amount of cellulose on a dry weight basis as did the bran sample (Table 1). Also, the presence and ratios of the neutral sugars were similar. The green bean cellulose contained 1.03 ± 0.83% arabinose, 4.30 ± 0.79% xylose, 7.39 ± 0.71% mannose, and 87.28 ± 2.02% glucose.

Analysis of carrots

Four samples of carrots were estimated to contain 23.15% DF (Table 1) and was corrected for a fat content of 1.48 ± 0.13%. This value is also much larger than the CF value of 9.09% estimated from USDA Handbook #8 (Watt and Merrill, 1975) the NDF value of 9.2%, and the Indigestible Residue of approximately 13% (Table 2). The carrots did contain about 9% hot water-soluble components which would not be measured by these other methods.

As with the green beans, the proposed values for the constituents of the DF of carrots agree fairly well with estimates derived from Southgate's method (Table 3). The relative amounts of each component are similar but the total amounts are less by the proposed methodology. Again, the carrots were probably of a different variety and age so the importance of these differences is not known.

Both the carrots and green beans were found to contain substantially more hot water-soluble, acidic sugar components (pectic materials) than the bran samples. This was to be expected. The green beans contained 5.64% and the carrots 6.72% such components. Even though these values seem to be a little low, this does point out the fact tl at the detergent and enzymatic methods do not measure a large constituent of the total DF, that being the water-soluble components.

Table 3—Comparison of values for dietary fiber and its major components obtained from proposed methodology, vs Southgate's method, on a % dry matter basis

Product		% Dietary fiber	Noncellulosic polysaccharides	Cellulose	Lignin
Wheat bran	A[a]	44.12	31.41	9.84	2.87
	B[b]	44.00	32.70	8.05	3.23
Green beans	A	27.53	14.68	11.39	1.46
	B	33.84	18.69	13.03	2.12
Carrots	A	23.15	14.89	7.63	0.63
	B	31.36	18.81	12.54	Tr

[a] A = values obtained from proposed methology.
[b] B = values obtained by Southgate's method from Shipley (1978).

The carrots were also found to contain a relatively large amount of a hot water-soluble, neutral sugar constituent (2.40 ± 0.49% of carrot on a dry weight basis). It was composed of 4.64 ± 0.50% rhamnose, 36.52 ± 0.72% arabinose, 1.14 ± 0.63% xylose, 55.40 ± 0.82% galactose, and 2.28 ± 0.80% glucose. The role of this gum-like material in the carrot was not known.

The hemicellulose fraction of the carrots resembled that of the green beans (Table 1). The overall content of hemicellulose was less but the results do agree with the 1.04% hemicellulose value obtained by Matthee and Appledorf (1978) using NDF and ADF methods. The neutral sugar profile was similar to the green beans except for a lesser amount of xylose and a greater amount of galactose in the carrots. The ratios of neutral sugars were 2.92 ± 0.46% rhamnose, 16.17 ± 1.32% arabinose, 23.63 ± 0.35% xylose, 3.35 ± 0.32% mannose, 45.90 ± 0.32% galactose, and 8.03 ± glucose.

The cellulose fraction resembled that of the brans and green bean fractions (Table 3) in content on a dry weight basis. The overall content was less but the profile of sugars was similar, 0.81 ± 0.38% arabinose, 2.70 ± 0.18% xylose, 4.53 ± 0.24% mannose, and 91.96 ± 0.39% glucose.

Effects of processing on dietary fiber

Having identified the DF profiles of these selected foods, the effects of processing on the DF constituents were evaluated.

Effect of toasting on wheat bran fiber. Table 4 displays the effects of dry heat on the constituents of DF in wheat bran. These values were corrected for fat contents of 3.60 ± 0.13%, 3.85 ± 0.10%, and 4.01 ± 0.06% for the samples with no processing, 30 min toasting, and 60 min toasting at 177°C, respectively. Visually, the samples were significantly brown after 30 min and dark brown after 60 min of toasting.

By far the largest change in the constituents was the increase in "lignin" from 2.87 ± 0.21% at time 0, to 4.28 ± 0.09% after 30 min and 12.02 ± 0.22% after 60 min of processing. This phenomonon agrees with the results obtained by Van Soest (1965). He found that heating above 50°C causes the formation of Maillard browning products which are insoluble in 72% H_2SO_4 and therefore isolated along with the true lignin fraction. The overall increase in DF shows that the increase in "lignin" content is due to fractions of the wheat bran other than the DF constituents.

—Continued on next page

Table 4—Effects of dry heat processing on the constituents of wheat bran fiber.

Fraction		Samples: Fresh	Toast (30 min)	Toast (60 min)	Stat. sign.[a]
Cold water	N[b]	0.79 ± 0.09[d]	0.49 ± 0.08	0.36 ± 0.01	HSD
	A[c]	0.13 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	SD
Hot water	N	Tr	Tr	Tr	–
	A	0.06 ± 0.02	0.05 ± 0.00	0.03 ± 0.01	NSD
Hemicellulose	N	25.14 ± 1.00	24.48 ± 0.41	25.61 ± 1.28	NSD
	A	3.31 ± 0.40	2.70 ± 0.12	2.50 ± 0.16	HSD
Cellulose	N	9.84 ± 0.75	8.68 ± 0.30	8.68 ± 0.66	HSD
	A	1.98 ± 0.09	1.84 ± 0.03	1.96 ± 0.10	NSD
Lignin		2.87 ± 0.21	4.28 ± 0.09	12.02 ± 0.22	HSD
Total dietary fiber %		44.12	42.61	51.27	–

[a] HSD = highly significant difference; SD = significant difference, NSD = no significant difference as determined by the analysis of variance.
[b] N = neutral sugar fraction.
[c] A = acidic sugar fraction.
[d] = mean % recovery ± standard deviation.

Table 5—Effects of boiling on the constituents of wheat bran fiber

Fraction		Samples: Fresh	Boil (30 min)	Stat. Sign.[a]
Cold water	N[b]	0.79 ± 0.09[d]	0.22 ± 0.14	HSD
	A[c]	0.13 ± 0.01	0.06 ± 0.02	HSD
Hot water	N	Tr	Tr	–
	A	0.06 ± 0.02	0.03 ± 0.01	SD
Hemicellulose	N	25.14 ± 1.00	24.22 ± 0.44	NSD
	A	3.31 ± 0.40	2.40 ± 0.22	HSD
Cellulose	N	9.84 ± 0.75	9.40 ± 0.55	NSD
	A	1.98 ± 0.09	1.89 ± 0.07	NSD
Lignin		2.87 ± 0.21	3.52 ± 0.24	HSD
Total dietary fiber (%)		44.12	41.74	

[a] Key, same as Table 4.
[b] N = neutral sugar fraction.
[c] A = acidic sugar fraction.
[d] Mean % recovery ± standard deviation.

There was no significant difference in the hemicellulose content with processing; however, there was a small but highly significant decrease in the cellulose content.

Evidently either the cellulose became slightly involved in the polymer formation, or the Maillard products interfered with the hydrolysis of the cellulose polymer. This was not investigated further.

There were also some small but significant decreases in both the neutral and acidic, cold water-soluble fractions and in the acidic fraction of the hemicellulose.

Effect of boiling on wheat bran fiber. Boiling wheat bran for 30 min had relatively little effect on the DF constituents (Table 5). There was no significant difference in the neutral sugar hemicellulose fraction, nor in the neutral or acidic fractions of the cellulose extract. There were small but highly significant losses in the cold and hot water-soluble fractions as well as a slightly larger loss in the acidic component of the hemicellulose extract. A small but significant increase in the lignin fraction was also realized.

Since these changes were similar to those found in the carrots and green beans, their significance will be discussed later.

Effect of wet heat processing on pureed green bean fiber. Boiling and retorting had significant effects primarily on the water soluble, acidic fractions of green beans (Table 6). There was no significant effect on the neutral sugar fraction of the hemicellulose, or cellulose extract.

The major effects appear to have been a dramatic decrease in the pectic substances. The acidic fraction of the HW extract went from 5.64 ± 0.50% with no processing, to 3.16 ± 0.21% with boiling, and to 0.21 ± 0.08% with retorting at 121°C for 60 min. There was a slight increase with boiling followed by a slight decrease with retorting in the acidic fraction of the CW extract. Since this increase in CW soluble, acidic components did not come close to equaling the loss in the HW soluble, acidic components it was concluded that the wet heat tended to first solubilize the pectic substances and then destroy them. In further support of this theory was the observation that there appeared to be much less precipitate in the centrifuge tubes containing the retorted samples than in the other samples.

Both of the acid fractions of the hemicellulose and cellulose extracts also experienced highly significant losses. This could mean that any pectic materials which were associated with these fractions in the raw state could have been solubilized and/or destroyed by the heat.

Effect of wet heat processing on pureed carrots. The carrots may have provided the clearest picture as to the effects of wet heat on DF (Table 7).

First of all there was an increase in the neutral sugar fraction of the CW extract which accounted for the loss in the neutral sugar extract of the HW fraction. This was true for both the boiled and retorted samples. Therefore, the wet heat tended to solubilize but not destroy those water-soluble, neutral sugar fractions.

After boiling there was a highly significant loss in the HW-soluble, acidic fraction (pectic substances) accompanied by an increase in the CW-soluble, acidic fraction. There was some loss in total pectic substances. The retorted sample contained almost no water-soluble pectic materials. Therefore, the hypothesis that wet heat first solubilizes and then destroys the pectic substances is supported.

There was an increased loss in the acidic components of the hemicellulose and cellulose fractions. This again may be due to the fact that these fractions contained some pectic materials which were affected by the processing.

Finally, there was no effect on the neutral sugar fractions of the hemicellulose or cellulose fractions, nor did the lignin value change.

Table 6—Effects of wet heat processing on the constituents of green bean fiber

Fraction		Sample: Fresh	Boil (30 min)	Retorted[a]	Stat. Sign.[b]
Cold water	N[c]	0	0.28 ± 0.05[e]	0.48 ± 0.05	HSD
	A[d]	0	0.28 ± 0.05	0.19 ± 0.03	HSD
Hot water	N	Tr	0.48 ± 0.31	0.34 ± 0.14	NSD
	A	5.64 + 0.50	3.16 ± 0.21	0.21 ± 0.08	HSD
Hemicellulose	N	2.30 ± 0.13	2.53 ± 0.09	2.44 ± 0.07	NSD
	A	2.26 ± 0.11	2.00 ± 0.06	0.69 ± 0.14	HSD
Cellulose	N	11.39 ± 0.64	11.02 ± 0.31	10.40 ± 0.63	NSD
	A	4.48 ± 0.12	3.78 ± 0.19	3.46 ± 0.15	HSD
Lignin		1.46 ± 0.06	1.11 ± 0.08	1.92 ± 0.06	HSD
Total dietary fiber (%)		27.53	24.64	20.13	

[a] Retorted = retorted purees at 121°C for 60 min.
[b] Key, same as Table 4.
[c] N = neutral sugar fraction.
[d] A = acidic sugar fraction.
[e] Mean % recovery ± standard deviation.

Table 7—Effects of wet heat processing on the constituents of carrot fiber

Fraction		Sample: Fresh	Boil (30 min)	Retorted[a]	Stat. Sign.[b]
Cold water	N[c]	0	1.03 ± 0.13[a]	2.19 ± 0.12	HSD
	A[d]	0	3.90 ± 0.38	0.53 ± 0.02	HSD
Hot water	N	2.40 ± 0.49	1.45 ± 0.49	0.28 ± 0.12	HSD
	A	6.72 ± 0.51	0.38 ± 0.12	0.55 ± 0.19	HSD
Hemicellulose	N	1.36 ± 0.18	1.44 ± 0.31	1.14 ± 0.08	NSD
	A	1.00 ± 0.08	1.03 ± 0.06	0.52 ± 0.06	HSD
Cellulose	N	7.63 ± 0.33	8.20 ± 0.37	7.65 ± 0.38	NSD
	A	3.41 ± 0.15	3.30 ± 0.13	3.05 ± 0.05	HSD
Lignin		0.63 ± 0.06	0.77 ± 0.09	0.77 ± 0.05	NSD
Total dietary fiber (%)		23.15	21.50	16.68	

[a] Retorted = retorted purees at 121°C for 60 min.
[b] Key, same as Table 4.
[c] N = neutral sugar fraction.
[d] A = acidic sugar fraction.
[e] Mean % recovery ± standard deviation.

Table 8—Effects of cooking on carrot fiber[a]

Fraction	Sample		
	Fresh	Short cook	Long cook
NDF	12.06 ± 0.98	14.02 ± 2.06	15.44 ± 2.81
ADF	11.02 ± 0.56	12.36 ± 0.66	13.95 ± 0.36
Lignin	1.32 ± 0.61	1.52 ± 0.39	1.36 ± 0.12
Hemicellulose	1.04 ± 1.13	1.66 ± 2.16	1.48 ± 2.82
Cellulose	9.70 ± 1.14	10.84 ± 1.04	12.59 ± 0.25
Crude fiber	5.22 ± 0.30	6.81 ± 0.35	7.52 ± 0.19

[a] Values obtained form Matthée and Appledorf (1978).

Comparison of processing results with other studies

When comparing the effects of wet heat processing on all three products, some definite trends become apparent. It may be hypothesized that wet heat processing primarily affects the pectic substances. It tends to first solubilize and then destroy them. Water-soluble, neutral sugar extracts are not affected to any great extent other than increased solubility. The hemicellulose and cellulose fractions remained virtually unchanged except for their acidic components which are solubilized and/or destroyed. Finally, the effects of wet heat on lignin content produced variable results: there was no significant difference in the carrots but there was a highly significant, but small, increase in the wheat bran and green beans.

Simpson and Halliday (1941) studied the disintegration of cell wall materials in carrots and parsnips during steaming. They reported that after steaming carrots, soluble pectic materials increased and protopectin decreased significantly. The decrease in protopectin was much larger than the increase in soluble pectin; therefore, they concluded that some pectin was being destroyed. They observed the same effects in parsnips. These changes agree closely with those found in this study.

McConnell et al. (1974) found that cooking did not affect the water-binding capacity of vegetable fibers but it did have a varied effect on the cation-exchange properties. They correlated water-binding capacity with ADF and not with lignin. This suggests that the polysaccharide content, in this case cellulose, of the plant material determines its ability to bind water. Since there was no change in the cellulose content. It is the acidic sugars which are primarily responsible for the cation-exchange capacities of these materials. Since processing did have some effect on this property, it also may be inferred that processing was affecting the pectin content of vegetable fibers. These conclusions again concur with the results obtained in this study.

Matthée and Appledorf (1978) examined the effects of cooking on vegetables. Since they measured ADF, NDF, and CF no conclusions could be made as to the effect of cooking on the water-soluble constituents of DF. They did get some interesting results as can be seen in Table 8. Their data indicated that there was no significant change in the hemicellulose and lignin content of carrots even though there was a significant increase in ADF, NDF, and CF. They concluded that this increase was due to an increase in cellulose content which might be the result of the liberation of cellulose, making it more available for analysis.

This conclusion seems highly unlikely and serves as an example of potantially erroneous conclusions which might be made as a result of not actually measuring the constituents of DF. Since they were measuring only residues (i.e. ADF, NDF, and CF), cellulose was measured indirectly. Any liberation of cellulose would not affect their values in these types of analyses. Furthermore, in this study an increase in cellulose was never observed.

Their results do support the findings of this study in that the hemicellulose fraction remained unchanged during cooking with wet heat. Their results also exhibited the same type of variation in the lignin values as did this study. Since the changes were also small it was difficult to decide if the statistically different values obtained were truly different.

SUMMARY & CONCLUSION

THE METHODOLOGY proposed by Anderson and Clydesdale (1980) was utilized to fractionate and analyze the DF content of green beans and carrots. Values obtained agree with existing estimates, most notably those provided by Southgate's method (Shipely, 1978) and appears to be superior to the other methods. An advantage of this method is that an estimate of the water-soluble components of DF is provided which is not measured by crude fiber, detergent, or enzymatic methods. This method also produces considerable information about the chemical composition of the various fiber fractions. This information may prove to be invaluable in helping to predict the physiochemical role(s) that DF may play in the human body.

The second part of this study examined the effects of various processing conditions on the DF constituents. Toasting of the wheat bran was shown to dramatically increase the apparent amount of "lignin" in the bran. Little is known about the nutritional implications of this phenomenon.

Wet heat processing exerted a deleterious effect primarily on the acidic sugar fractions (pectic substances) of the DF. There tended to be an increased solubilization of the pectic substances followed by their destruction and loss. This process was especially evident in the carrots.

It may be concluded that certain components of DF are affected by processing. These effects should be studied more closely in order that their nutritional implications might be evaluated.

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SCANNING ELECTRON MICROSCOPY OF THE Theobroma cacao SEED

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ABSTRACT

Scanning electron microscopy (SEM) of raw, roasted, unfermented and fermented cocoa beans (*Theobroma cacao* L.) revealed differences in morphology as a result of treatment. A 6-day microbial fermentation caused the testa to change from a leathery and continuous, closely adhering skin to a friable, more easily removable shell. Changes in the cotyledons were less obvious. When beans were roasted at 150°C for 20 min, the testa and endosperm became porous and brittle, and cellular contents of the germ and cotyledon became thermally coagulated. Also noted in the roasted shell were dissolution of the cutin layer accompanied by hairline fissures in the surface. Both these developments, as well as the increase in porosity, undoubtedly contribute to shell brittleness, and facilitate shell removal. Observations by SEM of the cocoa bean during various treatments may be considered useful in future quality control evaluations.

INTRODUCTION

THE CACAO SEED, *Theobroma cacao* L., is used principally in the manufacture of chocolate and its products. Although *T. cacao* is one of 22 species of the genus *Theobroma* of the predominantly tropical Sterculiaceae family, it is the one of economic importance. Despite its popularity, the description of this seed, commonly referred to as the bean, is not complete. Several researchers have investigaed the morphology of the cocoa bean utilizing the light microscope (del Boca, 1962; Hanssen, 1956), but their work has been limited to thin sections and isolated cell systems.

Scanning electron microscopy (SEM) is a technique particularly suited for the structural and topographical examination of seeds. The objective of this study was to define by SEM the morphology of raw and roasted, unfermented, and fermented cocoa beans. Study of the morphological aspects of the beans will aid in a better understanding of the complex physicochemical changes occurring during further processing, such as during conching and tempering of blended chocolate. Furthermore, observations made by SEM may lead to new or revised quality control procedures for cocoa beans and their processed products.

EXPERIMENTAL

COCOA BEANS of the botanical variety Forastero of the genus *Theobroma* were used in this study. The seeds were approximately 20–25 mm long, reddish-brown in color, and weighed approximately 1g each when dried. Beans from Brazil and the Ivory Coast were each subjected to a 6-day fermentation in their native countries. The source of raw unfermented beans was from Brazil. In addition, both unfermented and fermented Ivory Coast beans were roasted for 30 min in an air temperature of 150°C, and rapidly cooled to prevent over-roasting. All beans were sealed in plastic bags and stored at room temperature until analyzed. Beans were carefully dissected and sections were placed on double-sided adhesive tape affixed to specimen mounts. Small cotyledon fragments from all bean samples were soaked in 100% acetone for 1 hr before critical-point drying in carbon dioxide. A Polaron Critical Point Dryer, Model E3000, (Polaron Inst. Inc. Line Lexington, Pa.) was used and directions were followed according to the manual instructions. This step was necessary because the high fat content (>50%) of these cells obscured cellular structure. Approximately 450 Å of gold were deposited onto the mounted samples in a vacuum evaporator, International Scientific Instruments (ISI) Model PS-2. Specimens were viewed at an accelerating voltage of 15 kV in an ISI-60 scanning electron microscope. Photographs were made with Polaroid Type 55 PN film. The μm bar in the top left portion of each photograph represents 10 μm except where noted.

RESULTS & DISCUSSION

THE PROTECTIVE seed coat or testa of unfermented, raw beans was massive and was up to twenty cells thick. The outer epidermis of the outer integument was a single layer of cuticularized cells, previously described by Vaughan (1970), with each cell approximately 30-200 μm in length. A portion of the mucilage cell layer from raw fermented testa was removed to reveal thin-walled parenchyma cells which were packed with cellular contents (Fig. 1). In addition, large amounts of vascular tissue were observed to be composed of tracheary elements with helical secondary thickenings. A light microscopic count of these tracheary elements or "spiral vessels" is conducted as a quality measure to determine the percentage of shell in chocolate products such as liquor and cocoa powder (AOAC, 1975). This analysis will become increasingly important to manufacturers as more and more processed liquor is imported from bean-producing countries (Siebers, 1977). Since SEM requires minimum sample preparation time and greatly improves the depth of focus over light microscopy, this method may easily be adapted to spiral vessel counting. Furthermore, the realistic three-dimensional topography ob-

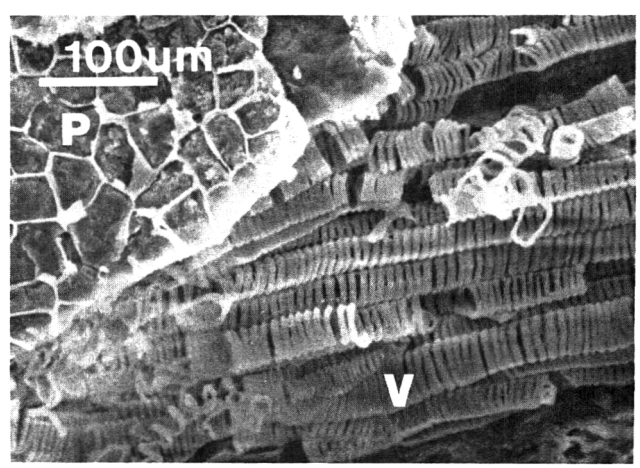

Fig. 1—Section through testa of raw fermented cocoa bean (P = parenchyma cells, V = vascular tissue).

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tained by SEM will permit easier and more accurate differentiation between fine cell wall structures and small, similarly-shaped spiral vessels, an apparent difficulty with the AOAC method.

Secretions of the outer epidermis of the outer integument were rough and contained wax-like protrusions (Fig. 2A). More commonly observed on the raw cocoa bean surfaces, however, were continuous was-like layers (Fig. 2B). It is thought that these protrusions clearly observed on the outer seed coat in Figure 2A represent sites where wax was initially deposited or extruded onto the surface. The continuous layer seen in Figure 2B is apparently a section of the established cutin layer, which is typically composed of long-chain polymerized alcohols and acids (Sargent, 1965). The cuticular layer, which imparts water impermeability to seed coats, is one of the important factors in the delayed germination of seeds (Esau, 1977). Leaves of aerial plants are also protected by this covering (Sargent, 1965). In contrast, the inner epidermis of the inner integument was devoid of such outgrowths and wax-like projections.

During fermentation of cocoa beans and the surrounding mucilaginous pulp, the beans swell, internal fissures appear in their structure, and they become brittle (Vaughan, 1970). Acetic and other acids produced by the fermenting bacteria leach through the testa layers and into the bean itself, eventually contributing to bean death and the associated changes in membrane permeability. Large numbers of rod-shaped bacteria were observed on the raw fermented bean testa (Fig. 3). In an earlier study, Ostovar and Keeney (1973) identified some of the fermentative microorganisms as lactic-, acetic-, and spore-forming bacilli. In addition to bacteria, yeasts, some in the budding stages, and unknown crystalline deposits were present on the outer seed coat. No microbial species were evident on unfermented bean surfaces, nor on the inner epidermis of fermented beans.

In practice, fermented dried and cleaned beans are roasted to reduce moisture content from approximately 7 to 1% less and develop flavor and brown color. This also permits easy removal of the shell in subsequent processing. The brittleness typically encountered as a result of roasting at high temperatures was identified by several physical changes to the seed coat surface. First, hairline cracks or fissures appeared on the outer roasted surface. These were accompanied by melting or dissolution of the cutin layer (Fig. 4A). Second, holes or pores exposing parenchyma cells developed on the surface closest to the cotyledon (Fig. 4B). The unroasted undersurface, by contrast, was smooth and uninterrupted (Fig. 4C). These developments, together with cotyledon shrinkage upon cooling, quite likely were responsible for shell brittleness. In cases of questionable roasting procedures, temperatures, etc., of processed beans, SEM analysis could be used to provide qualitative indicators such as the relative extent or degree of roasting as evidenced by the appearance and size of surface cracks and the melting of surface cutin.

The endosperm of the raw unfermented cocoa bean is a single layer of cells observed as a "silvery skin" around the embryo. These polygonal cells, measuring about 20 μm in width, also penetrated between the cotyledon folds. The endosperm layer appeared unchanged after all treatments except following roasting when fissures appeared. Of particular interest were crystalline clusters, presumed to be fat globules (Fig. 5A) and calcium oxalate (Fig. 5B), present on sections of the outer epidermis of the endosperm of all samples. Crystals of various chemical nature occur freely in and on plant cells. Calcium oxalate crystals are commonly found in a majority of plants (Lundegardh, 1966), and are thought to be insolubilized waste products of cellular metabolism. Fat globules, approximately 2–3 μm in diameter, were also present in abundance on this layer. Acetone-

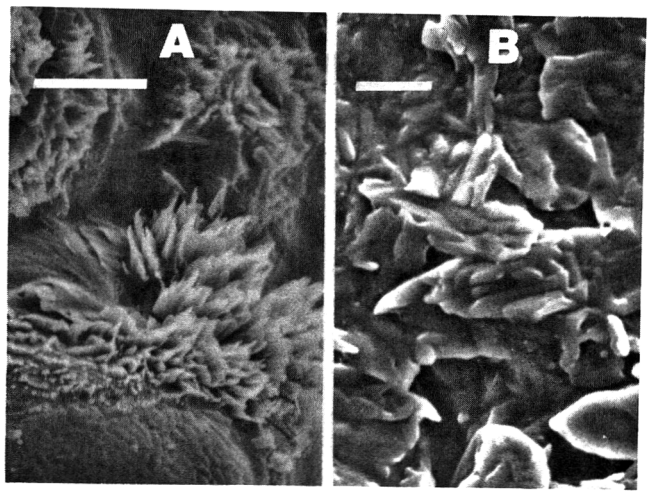

Fig. 2—Waxy discontinuous protrusions (A) and continuous layer of cutin (B) on the outer epidermis of raw unfermented cocoa bean testa.

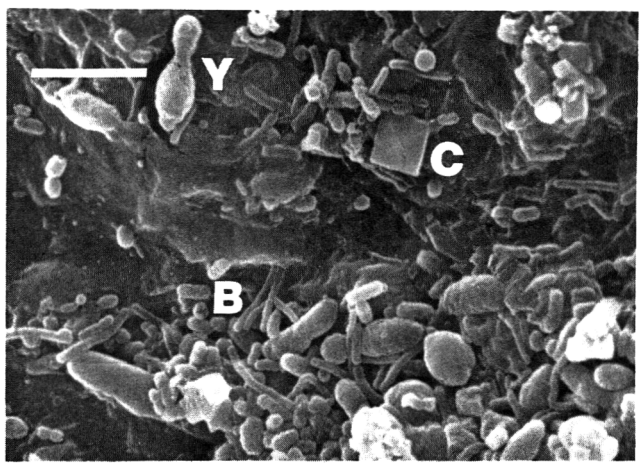

Fig. 3—Raw fermented cocoa bean testa showing bacilli, yeasts and unknown crystalline deposits (B = bacilli, Y = yeast, C = unknown crystalline deposit).

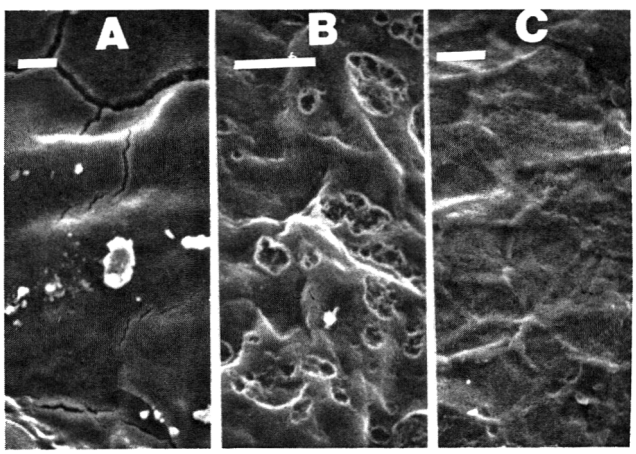

Fig. 4—Outer epidermis of roasted unfermented cocoa bean testa (A); Inner epidermis of roasted unfermented cocoa bean testa (B); Inner epidermis of raw unfermented cocoa bean testa (C).

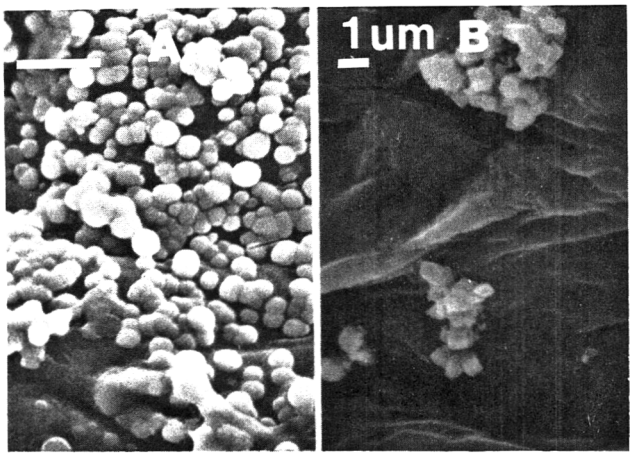

Fig. 5—Crystals presumed to be calcium oxalate on outer endosperm epidermis of raw unfermented cocoa bean (A); Fat globules on outer endosperm epidermis of raw unfermented cocoa bean (B).

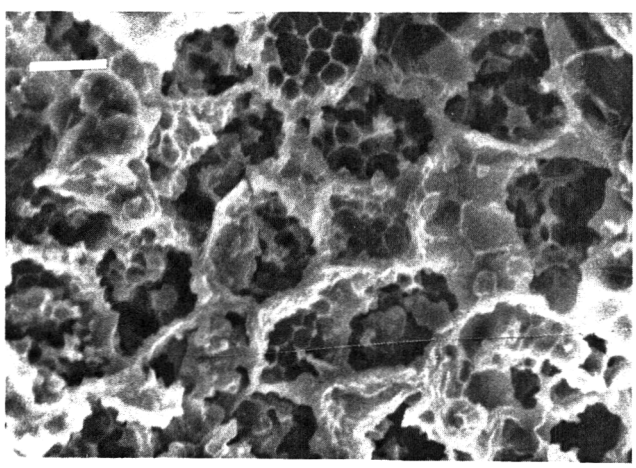

Fig. 6—Parenchyma cells in raw unfermented fat-extracted cotyledon.

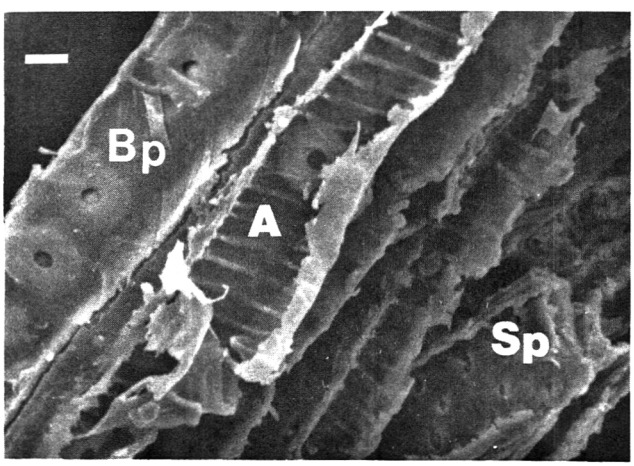

Fig. 7—Morphological diversity in the raw unfermented cotyledon (Bp = bordered pits, A = annular thickening, Sp = simple pits).

washed endosperm revealed no fat globules; however, the oxalate crystals were unaffected. Both components have been previously reported in cocoa bean endosperm (Vaughan, 1970).

The germ is often removed during chocolate processing because of its extreme hardness. Little difference in structure was observed in the germ after the various treatments. Cellular demarcation in the germ of roasted unfermented and fermented beans was, however, more defined than in the germ of unroasted beans. Since the germ contains some fat and starch (Esau, 1977), the thermal coagulation of these components within the cells is likely and the structures are stabilized by heat.

The greatest portion of the cotyledon, or "nibs", as they are commonly called, is comprised of storage or parenchyma cells which represent over 90% of the mass. The parenchyma cells contain cocoa butter, aleurone grains, and starch granules (del Boca, 1962). It appeared that fat within the parenchyma cells existed in a globular form, for when the acetone-treated cells were observed under the SEM. circular depressions within the cells indicated that the fat globules became dislodged (Fig. 6). The internal structure of the cotyledon parenchyma had a "honeycomb" appearance which are presumably the result of solubilization of fat globules embedded within the cell matrix. In addition, globular structures, approximately 2–5 μm in diameter, were not dissolved by the acetone and were likely aleurone and/or starch grains. Schmeider (1976) found cocoa starch granules to exist with a mean granule size of 4.6 μm, and a range from 2.1–12.5 μm. Light microscopy has also revealed these inclusions (del Boca, 1962).

Within the mass of the cotyledon parenchyma, simple and bordered pits, pit apertures and tracheary elements (Fig. 7) were evident. These pit structures function in the lateral, intercellular transport of water and dissolved materials. These various structures may be encountered in defatted beans and chocolate liquor and should not be mistaken for contaminants or unidentified structures during quality control evaluations.

Pigment cells, responsible for the color of cocoa beans, contain anthocyanins, theobromine, caffeine, polyphenols, catechin, and leucocyanins and are present in less than 10% of the cotyledon structure (del Boca, 1962). These cells could not be differentiated in this study, but have been extracted and studied elsewhere (del Boca, 1962). Hardy (1960) observed the diffusion and oxidation of colored pigments throughout the cotyledons as a result of changes in cell permeability in primarily a molecular phenomenon, the cell membranes of fermented samples remained intact and essentially unchanged from unfermented cotyledons.

The principal reason for roasting properly fermented and dried cocoa beans is to develop flavor from the chemical precursors formed in earlier steps. The formation of these roasted flavors is in part a result of thermal interations between soluble sugars and amino acids. A large number of volatile compounds are formed which give rise to characteristic chocolate flavors. Within the roasted cotyledons, therefore, the changes are primarily chemical (van Praag et al., 1968; Keeney, 1972).

CONCLUSIONS

THIS STUDY deals with some morphological aspects of the raw and processed cocoa bean. It also suggests some possible applications of SEM, for example, its use as a monitor of processing and/or quality control. Of particular interest are the changes observed during roasting, namely, melting or dissolution of the cutin layer, appearance of surface cracks and the increase in surface porosity. Certain plant structures, such as tracheary elements (spiral vessels), bordered and simple pits are easily detected by SEM.

—Continued on page 1545

OBSERVATIONS OF CHOCOLATE DURING CONCHING BY SCANNING ELECTRON MICROSCOPY AND VISCOMETRY

J. M. HOSKIN and P. S. DIMICK

ABSTRACT

Dark semi-sweet chocolate samples, obtained at various times during conching, were cooled and prepared for examination by scanning electron microscopy (SEM) in an attempt to observe structural changes during conching. In addition, ingredient mixtures containing known amounts of cocoa solids, microcrystalline cellulose, starch, sucrose and lecithin were studied to correctly identify the components of chocolate. Pulverized sucrose particles were easily characterized by their smooth surfaces and heterogeneous shapes. Defatted chocolate liquor was rougher in appearance and smaller in size than sucrose. Cellular detail of the cocoa bean was evident in liquor even after the disruptive effect of refining. Lecithin, when added to liquor-sugar and cocoa butter-sugar mixtures, had a remarkable and rapid effect in reducing three-dimensional structure. When processed chocolates from four different conches were examined by SEM, little or no differences were observed in the size or shape of the sugar or liquor particles as conching proceeded. Surface smoothening, however, was noted and attributed to the homogenization or spreading of cocoa butter during conching. When lecithin was added to chocolate during conching the surfaces were reduced and appeared to flatten. The effect of lecithin in reducing surface tension was also supported by viscometry. Viscometric measurements taken at this time showed that the increase in fluidity was accompanied by a sharp and rapid decrease in plastic viscosity.

INTRODUCTION

CONCHING in the manufacture of chocolate is a century-old process originally designed to mix a suspension of sugar and cocoa solids in a continuous fat phase so that the mixture becomes pourable. It is thought that pourability results from the relatively simple process of re-liquefaction of a newly-refined paste (Reade, 1978). Some researchers, however, believe that the physical effects brought about during conching encompass more than a thorough mixing (Sommer, 1974). Sommer states that the improvement of flow during the course of conching is initially caused by a change in structure which may be explained as a homogenizing effect on the material. Further liquefaction of the mass takes place without structural change as a consequence of reduced solid/solid interaction. Rounding of the sugar particles is also thought to occur during this mechanical mixing process. Several reports refute this claim but the visual evidence is still somewhat questionable (Hanssen, 1956; Wolf, 1961).

Many studies of the conche process are concerned with the rheological, viscometric measurements of melted chocolate. While the data generated are indeed useful, it also becomes desirable to visually observed the changes occurring during the conching process. The purpose of this study was to present by SEM some structural characteristics of chocolate and chocolate ingredients before, during and after this process. This was accomplished by studying ingredient mixtures as well as dark semi-sweet chocolate processed in four different conches.

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EXPERIMENTAL

IN ORDER TO identify the components of chocolate it was necessary to study model systems containing known amounts of cocoa solids, microcrystalline cellulose (MCC), (FMC Corp., Princeton, N.J.) corn starch, (Pfanstiehl Labs, Inc., Waukegan, Ill.), sucrose, and soybean lecithin (Table 1). Sucrose, pulverized to 35 μm, MCC and reagent-grade starch were sized with an 80-mesh sieve. Chocolate liquor (Main Lagos 45%, Ivory Coast 24%, Imported Bahian 31%) was defatted according to the *Methods of Analysis* (Office International du Cacao et du Chocolat, 1962). Acid hydrolysis, however, was not used in order to avoid cocoa starch hydrolysis. Liquor was grated and sized through an 80-mesh sieve, and all ingredients were combined in proportions listed in Table 1.

Dark semi-sweet chocolate (875 kg) was prepared with the ingredients shown in Table 2. For further details of the formulation and manufacturing procedures refer to Hoskin and Dimick (1979). The chocolate paste was refined to 35 μm, distributed and processed in four conches (Table 3). Some of the chocolate samples were defatted and all samples were grated and sieved. Samples were lightly dusted onto double-sided adhesive tape affixed to specimen mounts and the mounted samples were cooled with circulating cold water (15°C) for 15 min before coating. Approximately 450 Å of gold were deposited in an International Scientific Instruments (ISI)

Table 1—Ingredients and their percentages in model systems

Ingredients	Percent by weight
Microcrystalline cellulose	100
Pulverized sucrose	100
Defatted chocolate liquor	100
MCC-sucrose-starch	45–45–10
Sucrose-defatted liquor	50–50
Sucrose-liquor + lecithin[a]	50–50
Sucrose-cocoa butter + lecithin[a]	66–33

[a] Lecithin was addec at 0.2% of the final model system weight

Table 2—Formulation of dark semi-sweet chocolate

Ingredients	Percent by weight
Chocolate liquor	46.0
Pulverized sucrose	46.0
Cocoa butter	7.8
Lecithin[a]	0.2

[a] Added 1 hr prior to final conching time

Table 3—Conche type and processing times for dark semi-sweet chocolate

Conche type	Processing time (hr)
Longitudinal (four-pot)	73
Horizontal-rotary (Frisse)	11
Pug mill (McCarter)	19
Vertical (Petzholdt)	9

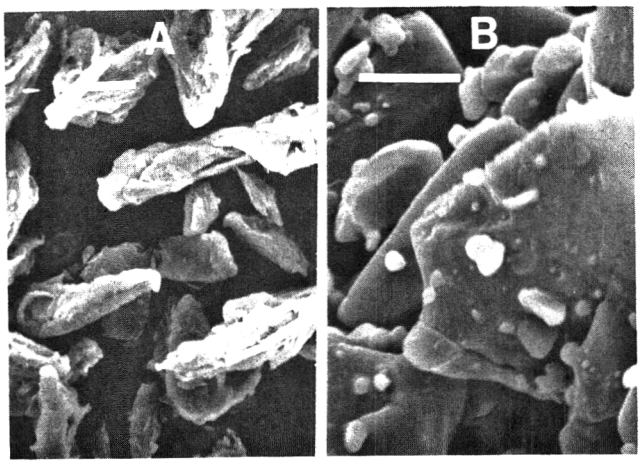

Fig. 1—Scanning electron micrograph of microcrystalline cellulose (A) and of pulverized sucrose refined to 35 μm (B).

Coating Unit, Model PS-2 (mA = 50, kV = 1.4, time = 1.2 min). Comparisons with samples prepared in this manner were made with samples prepared and gold coated at −30°C with a circulating dry ice and acetone bath. Differences in topography were so minimal that the less stringent, more convenient cooling method was used (15°C) with confidence. Specimens were viewed with an accelerating voltage of 15 kV in an ISI-60 scanning electron microscope. Micrographs were taken with Polaroid Type 55 PN film. The μm bar in the top left portion of each photograph represents 10 μm.

Plastic viscosities were measured on all dark semi-sweet chocolate samples at preselected conching times with a Haake Roto Visco RV2 viscometer (Haake, Inc., Saddle Brook, N.J.) according to the *Method of Analysis* (Office International du Cacao et du Chocolat, 1973).

RESULTS & DISCUSSION

THE MORPHOLOGY of the cocoa bean during fermentation and roasting has been described in the previous paper (Hoskin et al., 1980) and others (del Boca, 1962; Hanssen, 1956). In practice, roasted, cooled nibs pass through a breaker and winnower to separate the cotyledons, or nibs, from the hard shell and germ. When the nibs are ground, the cells release their stored lipid and the mass becomes liquid. The "liquor" consists therefore, of a suspension of solid cocoa particles in a continuous fat phase. A dark semi-sweet eating chocolate is processed by mixing liquor, cocoa butter and pulverized sugar, and refining this mixture until the particle area is so large that the refinings are dry and crumbly. In other words, the cocoa solid surfaces are incompletely surrounded by cocoa butter. Chocolate must first flow before it can be cast into molds or used as a couverture. One of the primary purposes of conching, therefore, is reestablishment of a homogenous, flowable suspension following the disruptive effect of refining. The mechanical or abrasive action during this process has led some researchers to believe that the edges of the sugar particles and cocoa solids become "polished" or rounded (Cook, 1976; Reade, 1978). The particles themselves are generally not believed to be reduced in size during conching (Wolf, 1961; Minifie, 1970).

To aid in the elaboration of changes by SEM that occur during the conching of chocolate, it was necessary to isolate and visually identify individual components in known model systems which approximated the composition of dark semi-sweet chocolate. Microcrystalline cellulose (Fig. 1A) and pulverized sucrose (Fig. 1B) were easily distinguished from each other. Microcrystalline cellulose was characterized by a rough, textured appearance in contrast to sucrose which was smoother, larger and much more heterogeneous in size and shape. When three components were mixed with starch (45 MCC-45 sucrose-10 starch) (Fig. 2), the system approximated defatted chocolate but was not as tightly compacted. The surfaces of the individual components, however, were readily distinguishable and comparisons with defatted liquor could be made (Fig. 3). Defatted chocolate liquor was composed of fibrous structures primarily derived from cell walls, and cellular inclusions such as protein bodies and what appeared to be starch granules. Schmieder (1976) identified starch granules in cocoa beans and most ranged from 2.1–12.5 μm, with an average diameter of 4.6 μm. Starch-like granules revealed in this study were approximately 5 μm. Sugar, which is present naturally in cocoa beans and liquor at a level of 1% on a dry weight basis, must be added during processing for sweetness. Usually the sugar added is sucrose. Pulverized sucrose was observed (Fig. 4A) as large, smooth-shaped structures dispersed among smaller, defatted cocoa particles. The size of many sucrose particles (30 μm and larger) and their smooth topography provided unmistakable clues for their identification in mixed systems. Cellular nib structure was evident even after refining. Minifie (1970) stated that it was not

Fig. 2—Scanning electron micrograph of model system composed of 45 MCC-45 sucrose-10 starch (C = sucrose, S = starch, M = MCC).

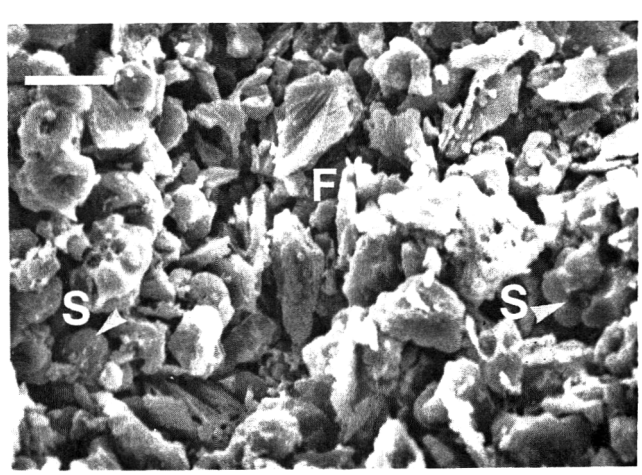

Fig. 3—Scanning electron micrograph of defatted chocolate liquor (S = starch, F = fibrous cellular components).

only the particle size which influenced mouthfeel but also the types of particles present. Sucrose particles, by nature of their large size, are not as numerous as cocoa solids but may give a sensation of occasional grittiness. Rough-edged cocoa particles, on the other hand, if near the critical size of 20–30 μm, may contribute to a persistent sensation of grittiness. When cocoa butter was present, however (Fig. 4B), detail was lost and it became impossible to distinguish between different types of structures.

Lecithin, a common surface-active agent used in chocolate products, caused an immediate reduction in surface projections when added to a 50–50, liquor-sucrose system at 0.2% of total chocolate weight (Fig. 4C). The effect of lecithin on a 66–33, sucrose-cocoa butter mixture was even more dramatic (Fig. 5). According to Harris (1968) and Minifie (1969), the effect of lecithin in reducing the viscosity of sugar and cocoa butter mixtures was greater than that in reducing the viscosity of liquor mass alone. Lecithin forms a monolayer on the hydrophilic sugar surfaces so that the friction and viscosity between the sugar particles and cocoa butter is reduced (Cook, 1948). In this study, SEM revealed a sharp loss of three-dimensional structure when lecithin was added to the sugar-cocoa butter model system. In light of these observations, it is not hard to visualize how lecithin might influence smoothness, flow and homogenity of processed chocolate.

Processed chocolate from four different conches was examined under SEM. Little or no difference was observed in texture between chocolates from the different conches; however, all samples at early conching times were characterized by a rough, coarse appearance (Fig. 6A). Apparently, cellular surfaces and fibrous structures became exposed following the disruptive effect of refining. By final conching time, however, all nonfat particles appeared to be covered by cocoa butter (Fig. 6B). This was shown by a flattening of the three-dimensional chocolate surface.

It was necessary to defat the chocolate in order to study the morphological details of sugar and cocoa solids during conching. No change in size or shape of sugar particles and cocoa solids was observed during conching in any of the processed chocolates (Fig. 7A, 0 hr and 7B, final hr). Sugar particles greater than 35 μm were not affected by the abrasive action of the conche rollers or paddles. Apparently, the clearance between the rollers or paddles in the conches was greater than the size of the largest sugar particles.

As mentioned, the textural changes observed in choco-

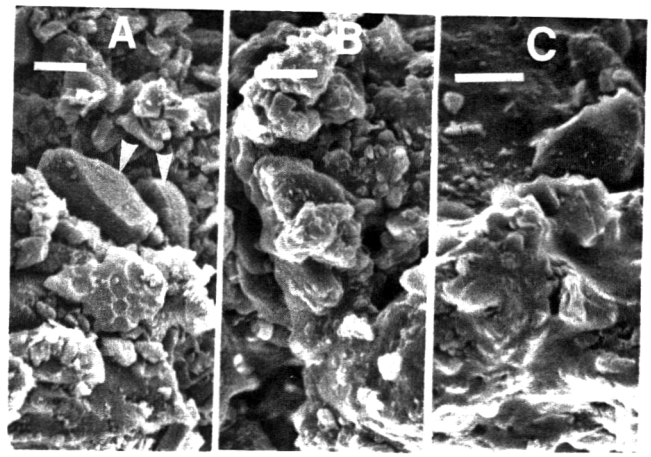

Fig. 4—Scanning electron micrograph of pulverized sucrose-defatted chocolate (A) and pulverized sucrose-chocolate liquor mixture 50/50 (B). In C, 0.2% lecithin was added to a pulverized sucrose-chocolate liquor mixture (50/50). (Arrows indicate sucrose particles in A).

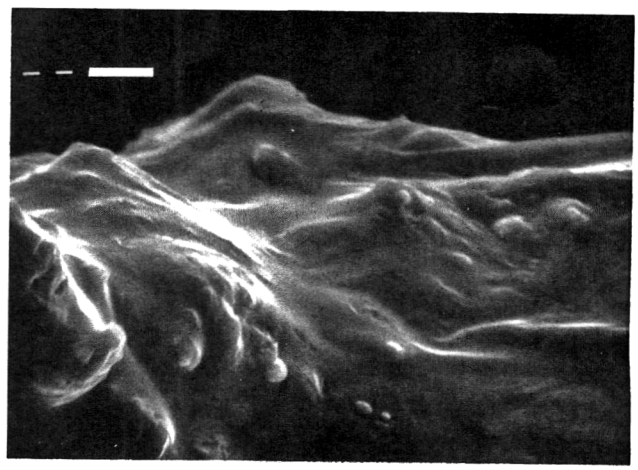

Fig. 5—Scanning electron micrograph of pulverized sucrose-cocoa butter mixture (66–33) to which 0.2% lecithin has been added.

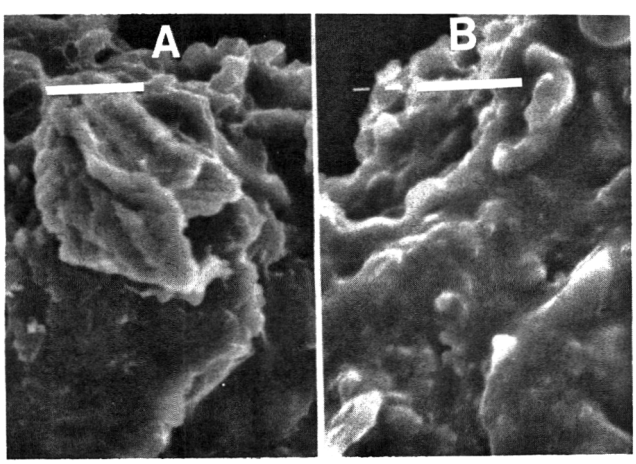

Fig. 6—Scanning electron micrograph of chocolate taken at zero hour (A) and at final hour (B) from the horizontal-rotary conche.

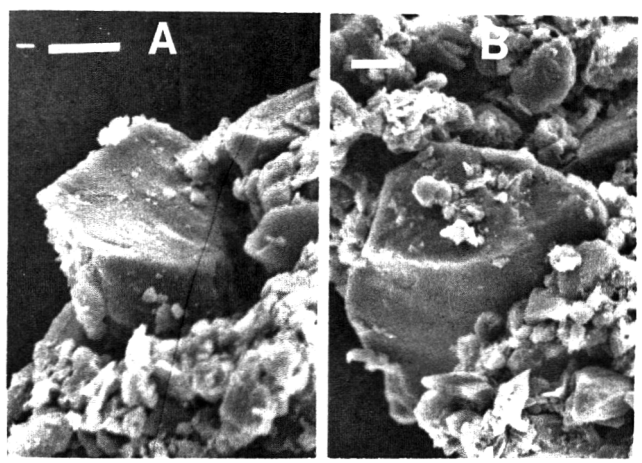

Fig. 7—Scanning electron micrograph of defatted chocolate taken at zero hour (A) and at final hour (B) from the horizontal-rotary conche.

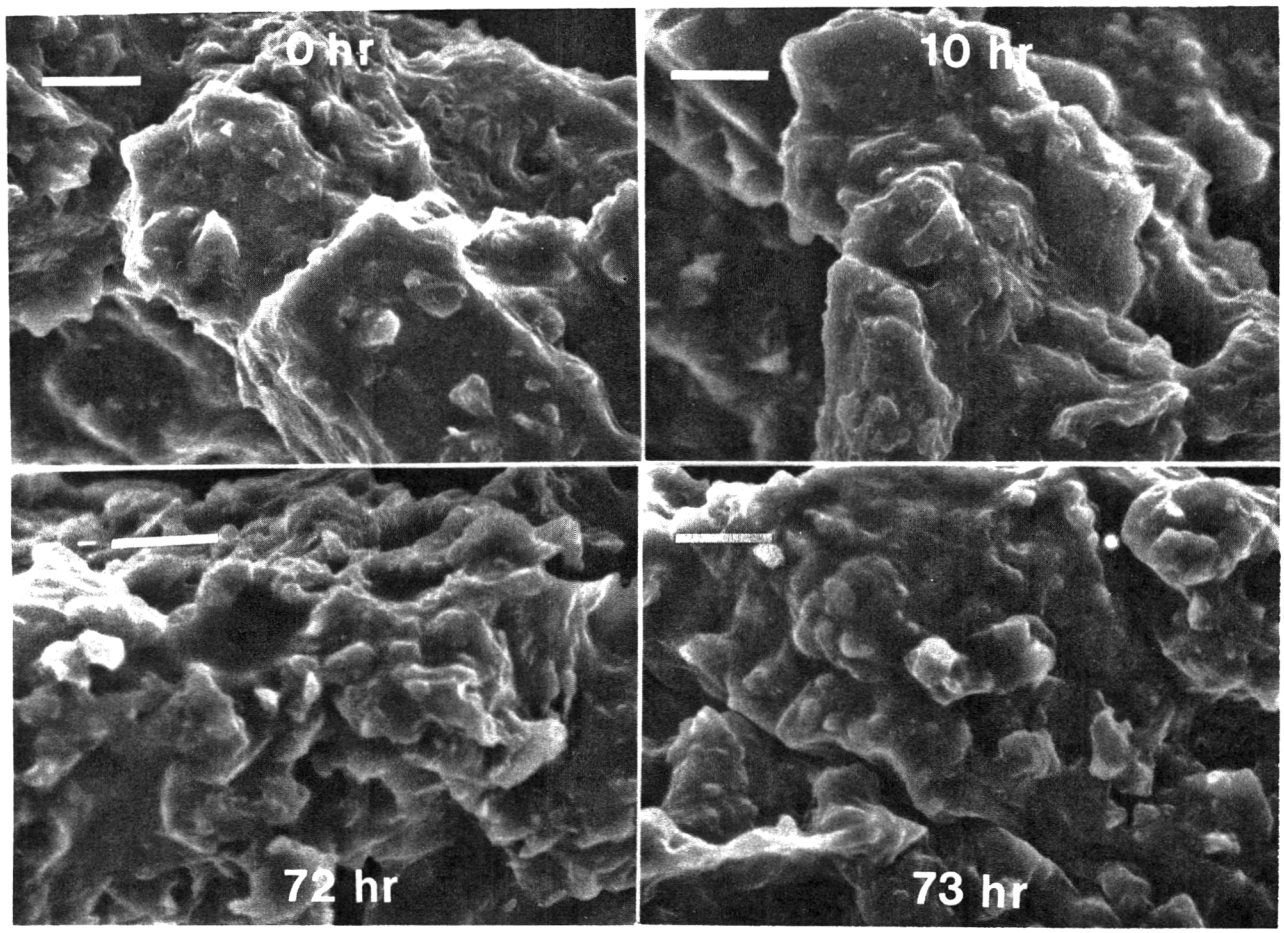

Fig. 8—Scanning electron micrographs of chocolate (not defatted) taken at 0, 10, 72 and 73 (final) hr from the longitudinal conche.

late (not defatted) processed in four conches were similar. For this reason, only micrographs from time-sequence studies of chocolate processed in the longitudinal conche are shown. Large agglomerates were evident at the early stages of conching (Fig. 8). These larger structures were composed of smaller particles held together by forces such as cocoa butter, since no diminution in particle size was noted. As conching proceeded (Fig. 8, 10–72 hr), the agglomerates dispersed and disintegrated and the mass became smoother in appearance. When lecithin was added (Fig. 8, 73 hr) surface smoothening was enhanced and was observed as a reduction in three-dimensional surface. A rapid decrease in plastic viscosity (Fig. 9) occurred during the initial stages of conching, decreasing from 142.16 poise in the 0-hr paste to 65–86 poise in the first sampling period. As conching progressed, the decrease in viscosity slowed and upon addition of lecithin decreased markedly in the final conche samples. The differences in viscosity between the chocolate processed in the four conche systems was due to factors other than fat content, particle size, sugar characteristics and lecithin addition because the original chocolate formulation was the same. The lower viscosities of the horizontal-rotary (Frisse) and vertical (Petzholdt) during conching may be attributed to the higher speed and temperatures compared to the longitudinal (4-pot) and pug mill (McCarter). In addition, the physical characteristics of the individual conches, i.e., shape, capacity and other design parameters undoubtedly contribute to factors which influence flow characteristics.

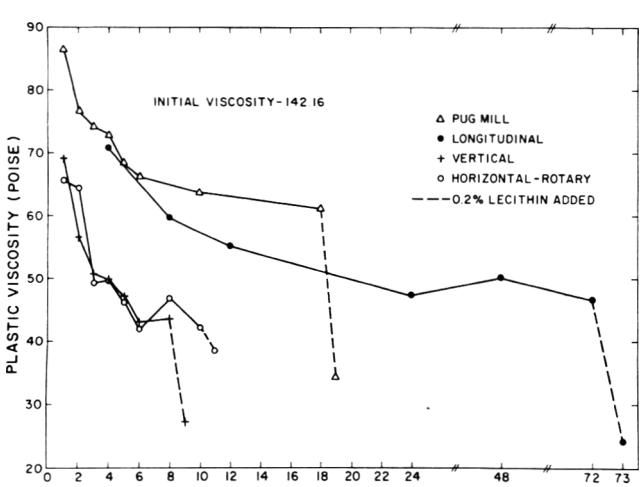

Fig. 9—Plastic viscosities of dark semi-sweet chocolate processed in four different conches.

CONCLUSIONS

SCANNING ELECTRON MICROSCOPY was used to show some physical changes occurring in dark semi-sweet chocolate during conching. This was accomplished by studying model systems of chocolate liquor, microcrystalline cellulose, sucrose, and lecithin, in addition to actual chocolate

samples processed in four different conches. Sugar and cocoa particles were identified by SEM as well as the effect of lecithin on these components. No changes in size or shape of sugar or cocoa particles took place during conching, yet a smoothening in topography was observed as conching proceeded. Conching, therefore, as a physical process appears to be one in which cooca butter lubricates or coats the nonfat portions. The effect of lecithin was dramatic in enhancing the spreadability of cocoa butter, and has been corroborated by viscometric measurements. Actual physical changes in structure between chocolates of different conches were not observed.

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AN IMPROVED PROCESS FOR CANNED SLICED TOMATOES

K. S. RYMAL, T. A. McCASKEY, and D. A. SMITH

ABSTRACT

A process utilizing the synergistic effects of minimal thermal processing in glass containers, acidification, calcium firming, and refrigerated storage to produce a superior quality canned sliced tomato has been developed. After 6 months of refrigerated storage these canned slices were judged to be equal in quality to peeled and sliced fresh winter-grown tomatoes when used in sandwiches and salads. Microbiological tests proved the efficacy of the process for all storage temperatures, and separately showed the additive preservative effect of each of the process variables.

INTRODUCTION

TOMATOES become a valuable convenience product when they are peserved in sliced form for use in sandwiches and salads. However, the conventional thermal processing of tomato products in metal containers, does not preserve enough of the fresh tomato qualities that are necessary if the canned slices are to be used for fresh tomato uses. Taste panelists found commercially canned tomato slices and wedges easily distinguishable from fresh slices (Rymal and McCaskey, 1979).

Acidified canned tomatoes have processing requirements similar to peaches and other acid fruits. Harris et al. (1967) showed that minimal heat processing in glass containers, low levels of preservatives or food acids, and refrigerated storage could be combined to preserve more of the fresh peach qualities than those found in the conventionally canned peach product. The acceptability of the "pasteurized-refrigerated peaches" was shown in good repeat sales during extensive market tests (Harris and Rymal, 1968). Food acids are permissible additives for tomatoes (Federal Register, 1966) and are desirable whenever minimal heat processing is applied (Lopez and Schoenemann, 1971). Prey and Powers (1966) found a significant effect on the flavor of tomato products from acidification. The flavor varied with the type of food acid used and in some instances the flavor of the acidified product was enhanced. However, metal container corrosion, which affects flavor, is accelerated by acidification (Powers, 1976).

Calcium salts may be added to sliced tomato products in amounts up to 0.1% of the weight of the finished product to compensate for the softening effect of heat processing (Federal Register, 1970). Bellucci et al. (1975) found no adverse effects on flavor or color from the addition of calcium chloride to canned whole tomatoes. The uptake of calcium in tomato products from added calcium salts varies with the cultivar and with the temperature and concentration of the calcium solutions used for dipping or peeling (Stephens et al., 1973; Brown et al., 1970).

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The objectives of this research were: (1) to apply the processing concepts of mild heat, acidification, calcium firming, and refrigeration to the production of canned, sliced tomatoes; (2) to determine if the product retained sufficient fresh tomato quality to compare favorably with fresh, winter-grown tomatoes; (3) to test and validate each processing operation microbiologically.

EXPERIMENTAL

Processing

Firm ripe Tropic variety tomatoes for this research were hand harvested at the Horticulture Farm, E.V. Smith Research Center, Shorter, Ala. Following harvest, tomatoes were placed in 4°C storage and processed within 48 hr. Fruit was washed in mild detergent solution, blanched for 15 sec in boiling water, water cooled, and peeled and trimmed by hand. Following peeling, tomatoes were sliced 9 mm thick with a hand slicing machine and immersed in a 1% W/V solution of calcium chloride at 25°C for 5 min with intermittent agitation. Excess $CaCl_2$ solution was drained off and slices were hand packed in wide mouth pint jars containing 60 ml hot water, 3g salt (½ tsp), 2.5g sugar (½ tsp), and 1.3g powdered citric acid U.S.P. (¼ tsp). Jars were filled to within 1 cm of the top with boiling water, closed at a center-jar temperature of 49°C, and processed in atmospheric steam for 10 min, followed by spray cooling and refrigeration at 4°C. Experimental variations in the above "standard pack" (SP) process included concentration of calcium chloride, type of acidulant, processing time, method of cooling, and storage temperature. Processing evaluations were made for flavor and mouthfeel, and texture was evaluated using a Model 1122 Instron equipped with a Kramer Food Cell. The first pack by the above SP process consisted of 50 cases with 12 pints per case of wide mouth glass jars (600 jars). Twenty-five cases were used for taste panel evaluation and 25 cases were inoculated for microbiological evaluation. The second and third packs were for microbiological tests only, and consisted of 3 cases (36 jars) and 12 cases (144 jars) respectively.

Taste panel tests

After 6 months storage at 4°C, samples were evaluated by taste panel. For the flavor-difference testing by triangular-comparison, a panel of 7 trained panelists were screened and selected according to the method of Kramer and Twigg (1966). Tomato samples were evaluated for the effect on flavor of type and level of acidulant, concentration and method of adding calcium chloride, time of processing, and temperature of storage. Fresh winter-grown tomatoes were purchased in local chainstores, peeled after immersing in boiling water for 15 sec, sliced, and compared with the canned experimental samples in salads and in sandwiches. The multiple-comparison ranking method (Kramer and Twigg, 1966) taste panel design was used with 19 semi-trained panelists.

Microbiological tests

Inoculants for the first pack of 300 jars consisted of cultures of *Bacillus stearothermophilus* and *B. coagulans* ATCC 7050. Organisms were propagated individually for 2 days in tomato juice, adjusted to pH 6.0 with sodium hydroxide and fortified with 0.1% peptone. Incubation temperatures were 55°C for *B. stearothermophilus* and 37°C *B. coagulans*. Five ml of each culture were added separately to 200 ml tomato juice medium and incubated for 2 days and pooled. Three ml of the pooled inoculum were added to each pint jar of packed tomato slices and jars were inverted three times. Following inoculation, jars were filled to within 1 cm of the top with boiling water, sealed, and processed according to the SP process. Following processing, 100 jars each were stored at 4°, 26°, and 32°C, observed daily for visible spoilage, and examined periodically up to 6 months for changes in pH, flavor, and odor.

Table 1—Detection of difference in flavor of canned sliced tomatoes due to type of acidulant[a]

Triangular comparison			
Panelist	Correct selections	Total comparisons	Difference significance level[b]
1	13	15	0.1 %
2	12	15	0.1 %
3	12	15	0.1 %
4	10	15	5 %
5	10	15	5 %
6	10	15	5 %
7	10	15	5 %
Overall	77	105	0.1 %

[a] Sliced tomatoes were canned with ¼ tsp powdered citric acid, or 2 tsp reconstituted frozen unsweetened lemon juice, or 2 tsp 50 grain distilled vinegar per pint jar.
[b] Significance levels from Appendix Table 89, Kramer and Twigg (1966).

Table 2—Preference for the flavor of acidulated canned-refrigerated sliced tomatoes

Triangular comparison		
Acidulant used	Number of times preferred[a]	Significance level[b]
Citric acid	19	N.S.
Lemon juice	38	1%
Vinegar	20	N.S.

[a] Combined judgements of seven panelists in 11 tests
[b] Significance levels from Appendix Table 89, Kramer and Twigg (1966)

Table 3—Effect of calcium chloride dip concentration and time of processing on firmness, calcium level, and temperature of canned sliced tomatoes

$CaCl_2$[a] (%)	Processing[b] (Min)	Firmness[c] (kg)	Calcium[d] (ppm)	Center-jar temp (°C)
1	10	37	58.6	82
1	15	28	58.7	89
1	20	24	68.8	92
1	25	24	59.1	95
2	10	35	92.2	85
2	15	29	96.9	90
2	20	31	77.7	93
2	25	27	81.9	96
3	10	39	116.2	84
3	15	34	122.3	89
3	20	36	133.0	92
3	25	33	121.9	96
5	10	41	250.6	82
5	15	43	253.3	89
5	20	37	255.0	92
5	25	38	250.1	96

[a] Slices dipped for 5 min in 1–5% W/V solutions of USP $CaCl_2$ at 25°C
[b] Processing in atmospheric steam
[c] Instron Model 1122 with Kramer Shear Cell
[d] Analyses by atomic absorption spectroscopy

For the second and third packs, soil from a tomato field was added to the pooled cultures at the rate of 0.1% W/V and a 3 ml combined inoculum was used for each jar as above. The soil inoculum was used to simulate the variety of organisms that might be encountered on the surface of field grown tomatoes.

For the second inoculated pack, 12 jars were prepared by the SP process except that no heat processing was used; 12 jars were prepared by the SP process except that cooling at room temperature was substituted for water spray cooling; 12 jars were prepared by the complete SP process. Four jars of each treatment were stored at 4°, 26°, and 32°C. Jars were observed daily for visible spoilage, and after 60 days storage, those jars which had not swelled, were opened and 4 ml of juice were plated on standard plate count (SPC) agar. Plates were incubated at 37° and 55°C.

For the third inoculated pack 12 jars were prepared by the SP process for each of the following process variations: 0 and 1% calcium chloride dip, 10 and 20 min heat treatments, and 4°, 26°, and 32°C storage temperatures. Jars were opened after 11, 40, and 99 day storage periods and were analyzed for the following groups of microorganisms: total aerobes, yeasts and molds, aerobic bacterial spores, and anaerobic bacterial spores. Total aerobic count was achieved by plating 4 ml of juice from each jar on standard plate count (SPC) agar and incubating at 26°, 37°, and 55°C for 2 days. Yeasts and molds were enumerated in 4 ml of juice by plating on potato dextrose agar (PDA) acidified to pH 3.5 with tartaric acid. The plates were incubated at 26°C for 5 days. Bacterial spores were enumerated by subjecting the juice of each jar to the spore-heat test (10 min at 80°C) and the juice was cooled and plated in quadruplicate on brain-heart infusion agar (BHI). One set of plates was incubated aerobically at 37° and 55°C and the other set was incubated anaerobically at 37° and 55°C for 3 days. Results were reported as total aerobic plate count (APC), yeasts and molds, and spores.

Microbiological-tests conducted in the third pack were duplicated on 10 cans of a similar commercial pack (Del Monte Tomato Wedges).

RESULTS & DISCUSSION

Taste panel tests

Panelists preferred the pH 3.9–4.0 level of acidity when the slices were sampled alone or in sandwiches or salads. When sampled alone, panelists were able to detect the effect on flavor of type of acidulant (Table 1). Lemon juice was preferred over citric acid or vinegar (Table 2).

When calcium chloride in amounts up to the 0.1% allowable by law, was added to the containers before processing, a bitter after taste was noted by most panelists. However, when the tomato slices were dipped in calcium chloride solutions and drained well before processing, no after taste was noted even when the highest concentration dip of 5% was used. Processing times and $CaCl_2$ concentrations which were tested are shown in Table 3. The 10 min processing in atmospheric steam produced a center-of-jar temperature of 82°C which was high enough for preservation, and resulted in the best flavored product. All $CaCl_2$ concentrations were acceptable in flavor, but the slices dipped in the 1% $CaCl_2$ were as firm after processing as fresh slices, and the slices dipped in the 5% were slightly rubbery when sampled alone.

Samples stored at 4°, 26°, and 32°C were evaluated periodically for changes in texture and flavor. Panelists were not able to discern any change in texture after storage for 6 months or longer regardless of storage temperature. However, samples stored at 32°C for 3 wk, or at 26°C for 6 wk, developed a typical cooked-tomato flavor. Samples stored at 4°C for 6 months or longer did not change in flavor from those freshly canned.

After 6 months storage at 4°C samples were compared in salads (Table 4) and sandwiches (Table 5) with fresh tomatoes purchased in local stores in February. There was no significant difference between any of the canned or fresh slices when sampled in salads. However, when served in sandwiches, the fresh slices and the citric acid and lemon juice acidulated slices were ranked significantly higher than the distilled vinegar acidulated slices.

Microbiologica tests

None of the first SP pack of 50 cases, 25 cases of which were inoculated with the pooled cultures as described,

Table 4—Comparison in salads[a] of sliced tomatoes canned with three different acidulants and fresh tomato slices

Rank[b]	Canned-refrigerated sliced tomatoes[c]			Fresh tomato slices
	Lemon juice	Citric acid	Vinegar	
First	32	32	12	32
Second	15	12	24	12
Third	10	4	12	6
Fourth	1	5	2	4
Rank sums	58[d]	53[d]	50[d]	54[d]

[a] Salads consisted of chopped lettuce and cut up tomato slices with a light application of oil and distilled vinegar.
[b] Samples were ranked as first, second, third, or fourth choice. Scores were assigned as 4, 3, 2, or 1 respectively.
[c] Sliced tomatoes were canned with ¼ tsp powdered citric acid, or 2 tsp reconstituted frozen unsweetened lemon juice or 2 tsp 50 grain distilled vinegar per pint jar.
[d] Not significantly different at 5% level

Table 5—Comparison in sandwiches[a] of sliced tomatoes canned with three different acidulants and fresh tomato slices

Rank[b]	Canned-refrigerated sliced tomatoes[c]			Fresh tomato slices
	Lemon juice	Citric acid	Vinegar	
First	8	40	8	36
Second	18	3	9	3
Third	12	2	4	4
Fourth	2	4	9	4
Rank sums	40[d]	49[d]	30[e]	47[d]

[a] Sandwiches were white bread with a light application of mayonnaise.
[b] Samples were ranked as first, second, third, or fourth choice. Scores were assigned as 4, 3, 2, or 1, respectively.
[c] Samples were canned with ¼ tsp powdered citric acid or 2 tsp reconstituted frozen unsweetened lemon juice or 2 tsp 50 grain distilled vinegar per pint jar.
[d] Not significantly different at 5% level
[e] Significantly lower at 5% level

Table 6—Effect of storage temperature and rate of cooling on spoilage[a] of inoculated canned sliced tomatoes[b]

Storage temp °C	4°				26°				32°			
Days of storage	1	10	40	60	1	10	40	60	1	10	40	60
No heat treatment	1	8	12	12	12	12	12	12	12	12	12	12
10 min heat slow cool[c]	1	1	1	1	1	2	2	2	0	4	4	4
10 min heat rapid cool[d]	0	0	0	0	0	0	0	0	1	4	4	4

[a] Number of containers visibly spoiled (swelled)
[b] Thirty-six pint jars of sliced tomatoes were prepared by the SP process for each treatment. All jars were inoculated with 3 ml of pooled inoculum (B. stearothermophilus, B. coagulans ATCC 7050, and infusion of soil from tomato plots).
[c] Slow cool = cooled in 26° air, 2 hr.
[d] Rapid cool = cooled in 26° air 30 min, then cooled in 13° water sprays.

showed any sign of visible spoilage regardless of storage temperature. Periodic examinations showed no changes in pH, flavor, odor, or appearance during the 6 months that observations were conducted. Apparently the 10 min processing which produced a center jar temperature of 82°C (Table 3) was sufficient when combined with acidification to pH 3.9–4.0, rapid cooling, and refrigeration for a minimum of 24 hr, to establish "commercial sterilization" even in jars inoculated with thermophilic spoilage organisms.

For the second microbiological test, in which soil from a tomato field was added to the pooled cultures as described above, samples were examined for visible spoilage (Table 6). After 60 days storage, those jars which had not shown visible spoilage were examined for viable organisms (Table 7). When no heat processing was applied, all the jars stored at 26°C or 32°C spoiled within 24 hr, Table 6. The spoilage inhibiting effect of 4°C refrigeration was shown by the fact that only one refrigerated jar spoiled the first day, and four still had not swelled after 10 days. The effect of rapid cooling on growth of thermophilic gas-producing organisms was illustrated by examining the number of jars which swelled at the various storage temperatures. With 10 min heat processing, but no rapid cooling, one jar swelled at 4°C storage, two at 26°C, and four at 32°C storage after 10 days. When rapid cooling was applied, no spoilage occurred at 4°C or 26°C storage, and four jars spoiled after 10 days at 32°C storage. The effect of storage temperature and rapid cooling on growth of thermophilic nongas-producing (flat sour) organisms was evident by examining those jars which had not swelled (Table 7). Twenty-three jars had not swelled after 60 days at 4°C. Those jars which had not been rapidly cooled averaged 76 organisms per ml but only six organisms per ml were present in similarly handled, rapidly cooled samples. Similar effects of slow versus rapid cooling were evident at the 26°C and 32°C storage temperatures.

The third inoculated pack showed the effect of processing time (Table 8) and calcium chloride dip (Table 9) on the number of viable organisms and spores present in sliced tomatoes canned by the SP process. There was essentially no additional reduction in numbers of organisms or spores when processing time was increased from 10 to 20 min in atmospheric steam (Table 8). The counts of organisms and spores found in this heavily inoculated pack after processing were no higher than those found in a similar commercial pack (Del Monte Tomato Wedges) (Table 10). The tests reported in Table 8 were conducted on samples stored at 26° and 32°C, also. However, the counts of organisms and spores were no higher than those found in the samples

Table 7—Effect of storage temperature and rate of cooling on numbers of flat sour organisms in inoculated canned sliced tomatoes[a]

Storage temperature		Incubation temperature	
		37°C	55°C
4°	slow cool[b]	76	1
	rapid cool[c]	6	1
26°C	slow cool	144	42
	rapid cool	37	1
32°C	slow cool	331	91
	rapid cool	3	1

[a] Jars had no visible spoilage (swell) after 60 days storage. Juice was plated on SPC agar. Organisms per 4 ml of juice are reported as averages.
[b] Jars cooled in 26°C air, 2 hr
[c] Jars cooled in 26°C air, 30 min followed by 13° water sprays

Table 8—Effect of processing time on microbial counts[a] of S.P. canned sliced tomatoes[b]

	Standard pack – 10 min processing					
	Incubation temperatures					
Days storage at 4°C	26°C		37°C		55°C	
	APC[c]	Y&M[d]	APC	Spores[e]	APC	Spores
11	1	1	1	3	0	0
40	1	1	0	4	0	1
99	1	1	1	1	0	0
Averages	1	1	0.7	2.7	0	0.3

	20 min processing					
	Incubation temperatures					
Days storage at 4°C	26°C		37°C		55°C	
	APC	Y&M	APC	Spores	APC	Spores
11	0	1	7	3	0	5
40	0	0	0	1	1	3
99	1	1	0	0	1	1
Averages	0.3	0.7	2.3	1.3	0.7	3.0

[a] Organisms in 4 ml sample
[b] Standard pack means: 1% $CaCl_2$ dip 5 min, acidulate to pH 3.9–4.0, process 10 min @ 100°, spray cool, store at 4°C.
[c] Aerobic plate count
[d] Yeast and molds
[e] Total aerobic and anaerobic spores

Table 9—Effect of calcium chloride dip on microbial counts[a] of S.P. canned sliced tomatoes[b]

	No $CaCl_2$					
	Incubation temperatures					
Days storage at 4°C	26°C		37°C		55°C	
	APC[c]	Y&M[d]	APC	Spores[e]	APC	Spores
11	27	1	30	15	2	9
40	4	2	7	6	3	6
99	40	37	20	14	5	1
Average	23.7	12.3	19.0	11.7	3.7	5.3

	1% $CaCl_2$ dip 5 min					
	Incubation temperatures					
Days storage at 4°C	26°C		37°C		55°C	
	APC	Y&M	APC	Spores	APC	Spores
11	1	1	1	3	0	0
40	1	1	0	4	0	1
99	1	1	1	1	0	0
Average	1	2	0.7	2.7	0	0.3

[a] Organisms in 4 ml sample
[b] Standard pack means: 1% $CaCl_2$ dip 5 min, acidulate to pH 3.9–4.0, process 10 min @ 100°, spray cool, store at 4°C.
[c] Aerobic plate count
[d] Yeasts and molds
[e] Total aerobic and anaerobic spores

stored at 4°C. Dipping in a 1% solution of $CaCl_2$ for 5 min reduced the number of organisms and spores (Table 9).

CONCLUSIONS

THE SP SLICED TOMATOES had better flavor, texture and color when compared with commercially processed, sliced tomatoes. They compared favorably with peeled and sliced fresh winter-grown tomatoes for use in salads and sandwiches. Microbiological testing showed the value of dipping in $CaCl_2$ solution, rapid cooling after heat processing, and refrigeration at 4°C. The 10 min heat treatment combined with rapid cooling was sufficient to preserve the product for any storage temperature, but low temperature storage was necessary to maintain fresh tomato flavor.

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Table 10—Microbial counts[a] of commercial canned tomato wedges – calcium firmed and acidulated

Sample no.[b]	Incubation temperatures					
	26°C		37°C		55°C	
	APC[c]	Y&M[d]	APC	Spores[e]	APC	Spores
1	2	0	1	8	6	5
2	6	0	2	5	3	5
3	5	0	1	5	4	5
4	2	0	1	1	3	4
5	1	0	2	6	1	1
6	0	0	12	3	3	5
7	3	1	0	7	1	6
8	3	0	2	2	3	6
9	2	1	3	7	0	4
10	0	0	4	6	2	6
Averages	2.4	0.2	2.8	5.0	2.6	4.7

[a] Organisms in 4 ml sample
[b] Each sample taken from freshly opened number 303 can
[c] Aerobic plate count
[d] Yeasts and molds
[e] Total aerobic and anaerobic spores

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CONTINUOUS EXPLOSION-PUFFING OF APPLES

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ABSTRACT

The explosion-puffing process produces excellent dehydrated apple products that can be used as crisp snacks, instant applesauce, and ingredients for pies, tarts, and cobblers. To make these products more competitive and facilitate commercialization of the process, a continuous explosion-puffing system (CEPS) has been developed. The CEPS process has been optimized for orchard run Rome Beauty apples. Leaching loss as well as SO_2 absorbance and processing losses were determined. Drying profiles from a continuous belt drier were made. Pressure, temperature, and feed moisture were used as control variables for CEPS optimization studies. Analytical tests for bulk density, rehydration, color differences, percent disintegration, and hydroxymethylfurfural were performed on each sample. From these responses a bias was discovered, necessitating a second study. The second optimization study of these responses produced an optimal region. Any point in this region gives a value of pressure, temperature, and moisture. When these conditions are used for CEPS an excellent apple product results. Orchard run Winesap apples were processed through CEPS at an optimal condition determined for Rome Beauty apples to evaluate varietal differences. While their response was significantly different, the product was acceptable.

INTRODUCTION

APPLES are commercially dried into two marketable forms: "dried apples" (24% moisture) and "dehydrated apples" (3.0% moisture or less). Washington and California have produced an average of 5.1 million bushels or 2.14 million hundred-weights of dried apples annually from 1960 to 1973 (Duymovic, 1975; Huehn, 1975). One-third of this production was subsequently converted into "dehydrated apples."

"Dehydrated apples" have all the advantages of "dried apples" and none of the drawbacks. "Dehydrated apples" below 3% moisture and at 25°C or below retain their original flavor, color, and odor and have retained these qualities for as long as 3 yr (Smook and Neubert, 1950). "Dried apples," however, deteriorate in color and flavor and lose ascorbic acid, carotene, and sulfur dioxide unless held below 3.3°C.

The production of "dehydrated apples" is limited by the costly commercial drying methods (vacuum and freeze-drying) (Borgstrom, 1968) that are required. The explosion-puffing process developed at the Eastern Regional Research Center is a fast, inexpensive method of obtaining "dehydrated apples."

Explosion-puffing of apples is carried out after apple pieces have been dried to about 18% moisture (all moistures are on wet basis) by conventional hot air drying. Explosion-puffing is carried out in a gun (Heiland and Eskew, 1965) or in the Continuous Explosion-Puffing System (Heiland et al., 1977) at an elevated pressure and in a stream of superheated steam. The water within the partially dried pieces is rapidly brought to a temperature above its atmospheric boiling point. When the pieces are instantly returned to atmospheric pressure, a fraction of the water flashes into steam, creating a porous structure (Eisenhardt et al., 1962; Sullivan et al., 1977a). After being puffed, the apple pieces are dried by conventional means to 3% moisture or less. Explosion-puffing is the critical step in the process and restores the partially dried, concave, case-hardened (Potter, 1968) apple pieces to their normal size and shape but with a porous structure. This study was made to determine the effect of process variables in continuous explosion puffing on dehydrated apple pieces using that operation. Optimal conditions were determined for one batch of Rome Beauty apples, and the conditions were tested on a batch of Winesap apples. Losses were measured in the other process steps. The "explosion-puffing" dehydration process is diagrammed in Figure 1.

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EXPERIMENTAL

ORCHARD RUN. Rome Beauty apples from Eastern Pennsylvania were used in all but one experiment. The apples were mechanically peeled, cored, sliced, and cut lengthwise into 16 wedge shaped pieces and were then cut crosswise to produce 32 half segments. The segments were immersed for 5 min in a circulating solution of sodium bisulfite (1%) to bring the residual SO_2 concentration to about 7,500 ppm (moisture free basis (MFB)). The pieces were thoroughly rinsed over a shaker washer to help prevent adherence to each other during drying. This washing reduces the residual SO_2 to about 3,200 ppm. The segments were then dried in a continuous belt drier to predetermined moistures as the experiments required.

After drying the apple segments were coated with 0.75% monoglyceride to help prevent sticking. The monoglyceride has no effect on color, taste, or texture of the apple product.

Raw material testing

One hundred five bushels (2,000 kg) of apples were processed, and 13 experimental runs were made to follow the variations in SO_2 and total reducing sugar (TRS). Sulfur dioxide pickup and losses were determined from the peeling step to the final dried product

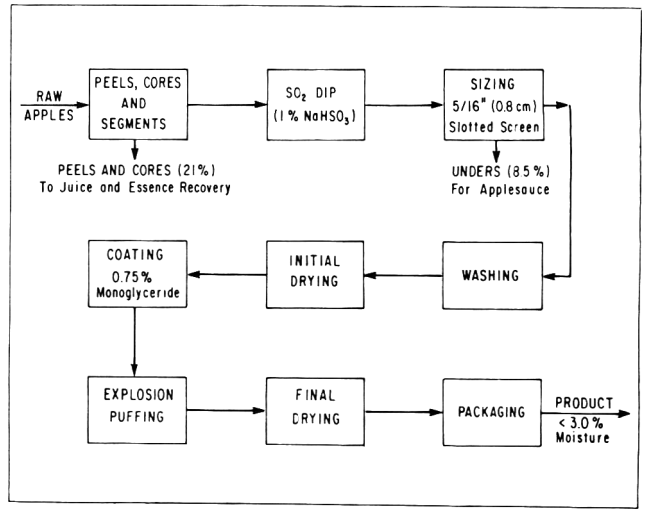

Fig. 1—Explosion-puffed "dehydrated apple" process.

but TRS values were obtained only through the initial drying step.

Drying studies

These studies report the difference in energy required to dry apple pieces by two processes (conventional and explosion-puffing) to 3% moisture. The study includes the determinations of moisture distribution and steam consumption in a two-stage multizone pilot scale continuous belt drier (Anon, 1963). This drier is a Sargent pilot plant research drier similar to their larger commercial driers. This drier was modified by adding doctor blades in the first and second zones to improve drying (Sullivan et al., 1977b). Figure 2 shows two curves (obtained with and without doctoring), both indicating apple piece temperature (thermocouple measurements) during drying in Stage 1. Curve 1 indicates the temperature of the apples when doctoring was used. The doctored apples dried faster and approached the dry bulb temperature of the drier sooner. Apple piece moistures were found to be more homogeneous after doctoring. For these reasons doctoring was used in all subsequent experiments.

Two moisture distribution drying experiments were made. In the first, apple slices were dried to explosion-puffing moisture ca. 18.5% and the second to a "dehydrated apple" moisture ca 3.5%. Samples were taken at the centerpoints of each zone, and before and after drying. Drying to these two different moistures required changing belt speeds. Other drying conditions were held constant for these experiments. They were: air velocity 3.0 m/s; direction of air flow downward; stage 1 temperature 82.2°C; stage 2 temperature 73.8°C, and bed depth entering stage 1, 5 cm.

Steam usage was determined for each stage by passing steam condensate through separate heat exchangers. The condensate was collected during a measured time period and weighed.

Testing the continuous explosion-puffing system (CEPS)

Capacity determination. Apple half segments, 15% moisture, were fed to CEPS (Heiland et al., 1977) at various rates. The segments were conveyed to the feed chamber of CEPS (volume 0.068 m^3) which was set to open every 20 sec. After each closing, the pressure in the feed chamber was brought up to the main chamber pressure within 2 sec, then the apple pieces in the feed chamber were discharged to the main chamber and onto the transfer belt. As the feed rate increased, the feed chamber and the transfer belt in the main chamber became vulnerable to overloading. Both areas were carefully monitored during the test.

Optimization

Optimization study #1. Over a 4-yr period, experiments were carried out to determine the effect of initial moisture (M), internal pressure (P), and temperature (T) on partially dried apple pieces in CEPS as reflected in the final product. These experiments were made to establish the optimum operating conditions of CEPS. Apples used were harvested during 1975, 1976, 1977, and 1978 (Table 1). The feed rate was 68 kg/hr for each test. After being explosion-puffed, all samples were dried to 2–3% moisture in a hot air tray drier and evaluated.

The chemical and physical attributes evaluated were: bulk density, rehydration, percent disintegration, color difference, and hydroxymethylfurfural (HMF). These properties were chosen because they most clearly reflected the quality of the explosion-puffed product. Fast rehydration or water pickup is a desired characteristic of explosion-puffed material. The greater the puff, the more porous the product, and the more readily the water is picked up, as reflected by rehydration values. Bulk density decreases during explosion-puffing, and should be between 160 and 128 kg/m^3; a value higher than this is indicative of an inadequate puff; lower, the product is overpuffed causing excessive breakage. Minimal disintegration is desired. HMF and color difference values relate to eye appeal and flavor. Generally, as these products brown they become less desirable. In color determinations, scales developed by Hunter (Hunter, 1942) were used. They are "R_d," "a," and "b." "R_d" measures the reflectance of the sample, "a" signifies redness if positive and greenness if negative, and "b" signifies yellowness if positive and blueness if negative. In the apple experimentations "a" but not "R_d" or "b" varied with operating conditions and correlated with them. Therefore, "a," the meaningful color difference measurement, was utilized. Methods used for these determinations are explained under analytical procedures.

Optimization study #2. A more limited study was made in optimum study #2 by the use of an experimental design of 15 conditions which included four levels of moisture (11.5–16.5%), five levels of pressure (62–124 kPa), and five levels of temperature (154–185°C). The apples used were from the batch harvested in 1978. The design was replicated three times. A bias present in the first optimal study from rewetted material was eliminated. The samples from this design were dried and evaluated as in the first study.

Effect of variety. The result of optimum study #2 (Rome Beauty Apples) was extended by testing it with Winesap apples. Winesaps were also compared in quality to Rome Beauties. The Winesaps were processed by (1) selecting a condition of pressure, temperature, and moisture within the optimal range for Rome Beauties, (2) drying apple pieces to the selected moisture (18.0%), and (3) processing these apples through CEPS at the selected internal pressure (95 kPa) and internal temperature (168°C). Values from the Rome Beauty design of bulk density, rehydration, percent disintegration, HMF, and "a" (color determination value) were calibrated for the specified conditions of pressure temperature and moisture. After being processed through CEPS, the Winesap apple pieces were dried, and the product characteristics were determined and compared to the predicted values of the Rome Beauties.

Steam usage. Steam consumed by CEPS during its operation was determined by flow meters. Flow rates were obtained by the use of steam turbine flow meters (Anon, 1976). The meters were located in the two steam inlets and in one of the two exhausts. Steam exhausting during explosion-puffing could not be measured but was calculated by difference. The steam turbines had been calibrated by collecting and weighing condensate prior to the experiments.

Final drying time determination

There was never a sufficient quantity of apples to determine the steam consumption during final drying in the continuous belt drier. An alternate method was used to simulate this processing step in this drier. To do this, a National tray drier was used in which smaller

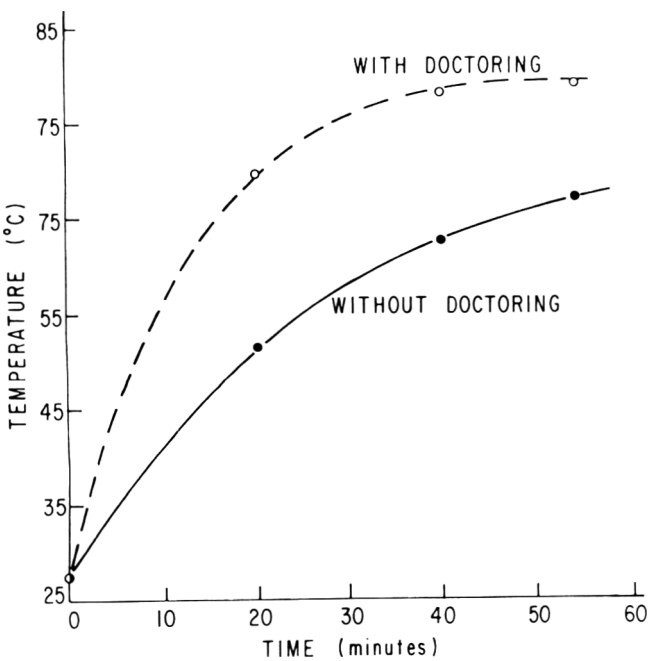

Fig. 2—Doctoring study in stage one of continuous belt dryer at 82.2°C.

Table 1—Experiments of optimization study #1

Year harvested	Pressure kPa Range	Pressure kPa No. of levels	Moisture % Range	Moisture % No. of levels	Temperature °C Range	Temperature °C No. of levels
1975	55–138	4	10.0–22.0	5	160	1
1976	55–103	3	10.0–21.0	4	160–190	3
1977	55–103	3	10.0–17.0	4	120–160	2
1978	62–124	5	11.5–18.0	6	155–185	6

drying lots could be compared. Apple pieces that had been dried to 13% moisture (wet basis) were used. Some of the same apples were explosion-puffed in CEPS. Their moisture increased to 17%. Equal weights of the explosion-puffed (17%) and conventional pieces (13%) were dried in the National drier to below 3% moisture. Weighings were made during the dryings at timed intervals until the products reached a constant weight. Drying curves were constructed based on the final moisture of each product and the difference in drying rates was determined. From this information, the simulated final drying time for the explosion-puffed apples in the continuous belt drier was determined.

Energy evaluation (Steam usage)

The total energy from steam consumption for the Explosion-Puffing Dehydrated Apple Process was determined from three steps: initial drying, CEPS, and final drying. Steam consumption while drying in the continuous drier was determined by weighing condensate for timed intervals. Steam used in CEPS was ascertained from the steam turbine flow meters.

Analytical procedure

Reconstituted apples at room temperature were used for determining hydroxymethylfurfural (HMF), color, and total reducing sugars (TRS). Moisture content, bulk density, rehydration, disintegration, and SO_2 analysis started with "dehydrated" apple slices.

Total reducing sugars (TRS). TRS were determined by the colorimetric method of Ting (1956).

Sample preparation was dependent on the moisture content of the apple. Fresh apples were peeled, cored, and pureed in a blender and the juice was squeezed through cheesecloth and then centrifuged. Dried or partially dried apples were reconstituted to fresh apple and the above procedures were followed.

An aliquot of the centrifuged juice was clarified with neutral lead acetate. Excess lead was precipitated by the addition of sodium oxalate. A portion of this clarified filtrate was used for the colorimetric analysis.

Hydroxymethylfurfural. HMF was determined by Method II of Winkler (1955) with modifications.

A 5.5-g sample of ground dried apple pieces was blended with 125 ml hot deionized H_2O for 5 min. With partially dried apples, 10.0g of diced apple pieces was blended with 125 ml hot deionized H_2O for 10 min. The blend was made to 250g and centrifuged for 10 min. The supernatant was filtered through filter paper and a 10.0 ml aliquot was analyzed by the Winkler Method II. Results are reported as mg HMF per 100g of fresh apple.

Color measurement. A Gardner Automatic Color Difference Meter was used for all color measurements. The reconstituted apples were placed in glass cells, and color values for each sample were obtained as Hunter a, b, and R_d units. The instrument was standardized each time with a standard color tile #CLY 0032 (R_d = 60.8; a = −1.8; b = +22.7).

Moisture. Moisture content was obtained by the standard vacuum oven method. All samples were dried at 70°C under vacuum for 16 hr. Results are expressed on a wet basis.

Sulfur dioxide (SO_2). Sulfur dioxide was determined by the method of Nury et al. (1959) with modifications.

A 15-g sample was extracted with a mixture containing 45 ml of a special buffer solution (Ross and Treadway, 1960) plus 240 ml of distilled water.

An aliquot of the blend was weighed into a tared 100 ml volumetric flask, then 2 ml of 0.5N NaOH was added. The solution was swirled 30 sec, permitted to stand an additional 1-1/2 min, then 2 ml of 0.5N HCl was added. Then 20 ml of sodium tetrachloromercurate solution containing sulfamic acid (0.60g/L) was added to prevent interference by nitrogen dioxide (West and Ordoveza, 1962). The mixture was diluted to 100 ml with water, then mixed and filtered. Two ml of the filtrate was used for the colorimetric analysis.

Disintegration. Percent disintegration was determined by manually separating disintegrated pieces in a 100-g sample and weighing this portion.

Rehydration. A 25-g sample was boiled in water 5 min, then drained and weighed. The amount of water picked up per gram of dry solids was determined by subtracting the original sample weight (25g) from the weight of the rehydration and dividing this weight by the solids in the 25-g sample.

Bulk density. Bulk density was determined by filling a tared crystallizing dish of known volume with dried apple pieces then weighing the apples and determining the weight per volume.

Table 2—Total reducing sugar (TRS) and SO_2 determinations for 13 runs

	% Moisture[c] (Wet basis)	g TRS/100g[c] raw apples	TRS loss %	SO_2 ppm
After peeling	87.22 ± 0.49	6.87 ± 0.56	—	—
After 5 min circulation (1% SO_2)	88.84 ± 0.40	6.08 ± 0.57	11.6	7,350
After wash	88.89 ± 0.38	5.93 ± 0.21	2.2	3,150
After drying	23.78[a] ± 4.04	5.47[b]	6.7	533[a]
Apple at 3% moisture	1.74[a]	—	—	47[a]

[a] Eight determinations
[b] Two determinations
[c] 95% Confidence limit

Table 3—Dryer steam usage

Final moisture	Steam rate		Drying time	
	Stage 1 kg/hr	Stage 2 kg/hr	Stage 1 hr	Stage 2 hr
ca 3.0%	350	150	2	14
ca 18.0%	377	161	1	1

RESULTS & DISCUSSION

Raw material experiments

Apple half segments (pieces) absorbed water during the pretreatment steps of sulfiting and washing and also lost sugar through leaching (Table 2). The sugar leaching losses of 11.6% are in agreement with the work of Mylne and Seegmiller (1950). A 2.2% loss of sugar resulted from washing and an additional 6.7% was lost during initial drying. Additional reducing sugar losses were prevented by the amount of SO_2 in the pieces (3,150 ppm).

Sulfur dioxide was readily absorbed by the apple pieces (Table 2), but 58% was lost during the washing step. However, the amount of SO_2 retained (3,150 ppm) suppressed the nonenzymatic browning (Resnik and Chirife, 1979) during the remaining processing, i.e., initial drying, explosion-puffing, and final drying. Forty to 60 ppm SO_2 was present in the "dehydrated apples" and was adequate to maintain color and flavor at 3.3°C.

Drying studies. Figure 3 depicts the two-stage, multizone drier. Sample points are indicated and moisture and their confidence limits (95%) are shown for eight experimental runs. Two hr and 25 min were required to dry 88 kg to 23.8 ± 4.6% moisture. Belt speeds to achieve this drying time were 5.1 cm/min first stage, and 4.2 cm/min second stage. Steam condensate was collected from the first and second stages and weighed during times intervals while the stages were under a full drying load. Steam usage rates were determined (Table 3).

Apple pieces were dried at slower belt speeds: 2.9 cm/min first stage and 2.7 cm/min second stage to simulate a "dehydrated apple" product without explosion-puffing to obtain steam usage rates. Steam condensate was collected and weighed during timed intervals and a usage rate was determined (Table 3). The apples for these tests were dried above the "critical" temperature (74°C) for apples (Jacobs, 1951); thus the product was off-color. Lower temperatures and much longer drying times would be required to dry apple pieces to approach 3.0% moisture with good color and flavor.

From Table 3 the amount of steam used for drying in

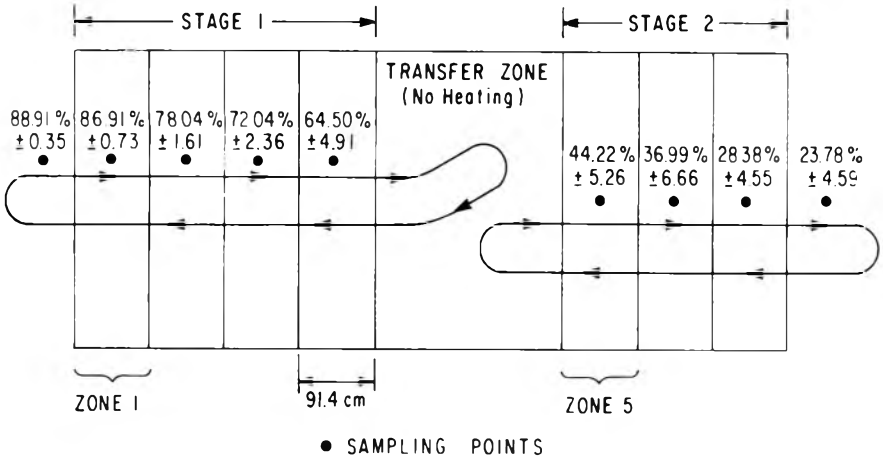

Fig. 3—Moisture distribution of apple pieces before, during, and after drying in continuous belt dryer.

each stage at the two drying conditions can be calculated. The total steam is determined by multiplying the drying time required for each stage by a steam rate for that stage. From the amount of steam and the line pressure, energy consumption was calculated.

Testing the continuous explosion-puffing system

Capacity determination. There are two low volume areas in CEPS that can restrict feed, namely the feed chamber and the channel above the transfer belt. Partially dried apple pieces were fed to CEPS at increasing rates from 140 kg/hr to 225 kg/hr. At the rate of 225 kg/hr the apples overfilled the transfer belt below the feed chamber and were not fully moved away befor the next discharge. This caused a blockage, and the apple pieces backed up through the feed chamber: however, CEPS functioned normally at feed rates of 195 kg/hr and lower.

Optimization study #1. The results of these experiments (responses) were related to the three controlled variables by simple quadratic equations. In most cases, 2-factor interactions were found significant, and thus were included. Approximately 500 data points, including within season replicates, were utilized. Table 4 shows the responses and results of the regressions.

An extensive analysis of residuals (predicted-observed) revealed a number of apparent biases which have not been explained. However, one critical experimental bias was uncovered. When drying to various moisture levels for testing, apples to be processed at a particular moisture sometimes were overdried. These moistures were corrected by the addition of water to that batch, and time was allowed for equilibration. On analysis of the residuals, it became evident that these samples formed a separate population. Consequently, the model developed was used solely to define an approximate optimal region. A new, definitive, experimental design was prepared with the criterion that the design would be near and around the estimated optimal region. All apples for this design were dried directly to the experimental moistures.

Optimization study #2. Results of regressions on this data are shown in Table 5. Quadratic equations, including 2-factor interactions, were fitted to data for each response. These equations were used to provide a set of constraints on the product. Table 5 shows the upper and lower limits for each response. On the application of an optimization routine, it was found that no constraint was "controlling," i.e., a feasible region of operation was found, wherein all constraints could be satisfied, according to the prediction equation. Figure 4 shows a typical case, at a fixed moisture level. Note that three different responses are constraining in different operating regions. No practical purpose would have been served by further narrowing the constraints, so the recommended operating conditions are offered as the center of the optimal region, generated by taking the centroids of successive constant moisture plots (Fig. 5). One advantage to this is that the operator need not dry to a given moisture exactly,

Table 4—Results of regressions optimum study =1

Y*	R^{2}**
Bulk density	0.840
Rehydration	0.741
"a"***	0.553
% Disintegration	0.674
HMF	0.825

* $Y_k = c_{Ok} + \sum_{i=1}^{3} c_{ik}X_i + \sum_{i=1}^{3}\sum_{j=1}^{3} c_{ijk}X_iX_j$

** (Multiple correlation coefficient)²

*** One of the color difference measurements

Table 5—Multiple correlations and coefficients of apple model for optimum study #2

Model form: $Y = c_0 + c_1P + c_2T + c_3M + c_4PT + c_5TM + c_6PM + c_7P^2 + c_8T^2 + c_9M^2$

Y	Bulk density	Rehydration	"a"	% Disintegration	HMF
R^2	0.851	0.852	0.759	0.591	0.737
Upper and lower limits	10.* (160.2 kg/m³) 8.* (128.1 kg/m³)	5 3.8	5 3	5 2	11 10
c_0**	61.080	−12.355	−20.466	38.051	387.611
c_1	−1.876	1.106	1.069	0.070	−4.937
c_2	−0.171	0.137	0.181	−0.378	−2.307
c_3	−0.672	−2.091	−1.522	3.582	3.660
c_4	−0.00180	−0.00239	−0.00463	0.00235	0.02043
c_5	0.00254	−0.00013	0.00486	−0.01107	−0.01879
c_6	−0.05292	0.00236	0.01806	−0.41986	−0.20153
c_7	0.10548	−0.00238	0.00311	0.20852	0.06954
c_8	0.00020	−0.00015	−0.00031	0.00075	0.00356
c_9	0.02194	0.06558	−0.00949	0.19397	0.16660

* Pounds per cubic foot was used in this correlation.

** Sufficient significant places are provided so that the reader can recalculate the response surface. No unexpected precision should be inferred.

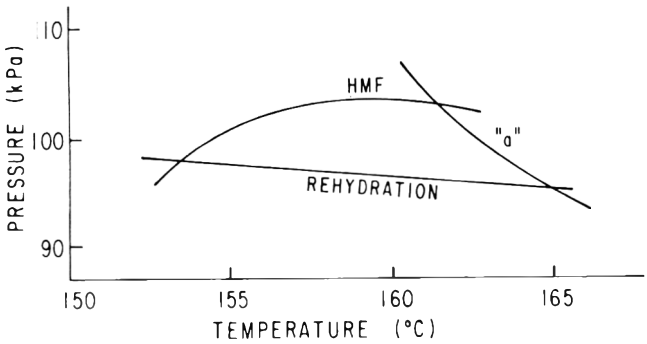

Fig. 4—Optimization area at 14.5% moisture.

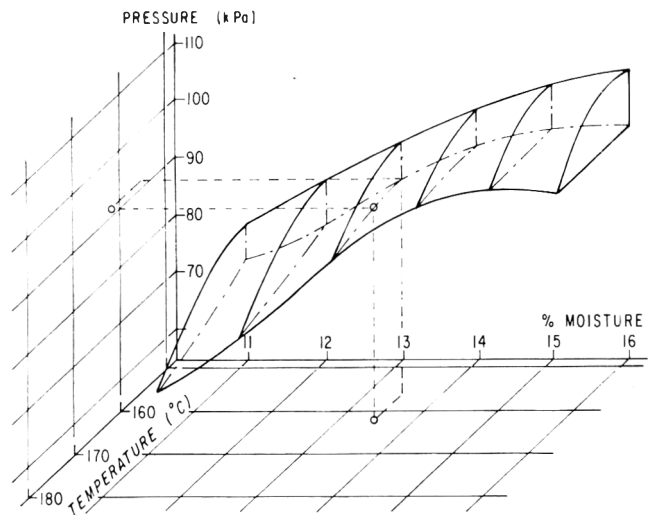

Fig. 5—Constraining response surfaces for optimal region.

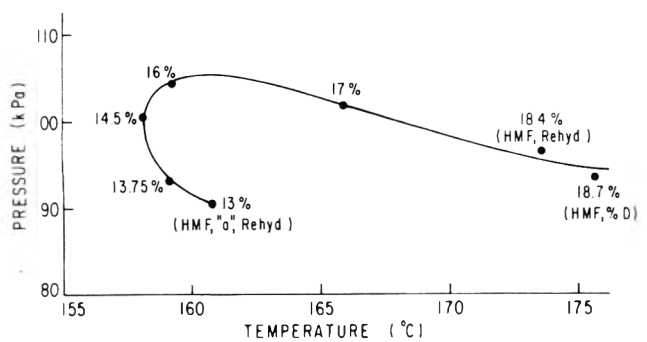

Fig. 6—Center points of centroids at fixed moistures.

Table 6—Chemical and physical property values

Property	Predicted (Rome Beauty)	Experimental (3 Runs) (Winesap)
Bulk Density kg/m^3	117.0	147.0
Disintegration %	3.8	5.3
"a"	− 3.1	− 3.2
Rehydration g H_2O pickup/g dry solids	3.89	4.84
HMF mg/100g	10.66	17.36

but can choose a point on the centroid line (Fig. 6) corresponding to his achieved moisture.

Effect of variety. The predicted responses for the Winesap apple (solids 15.1% fresh weight basis) from the Rome Beauty apple (solids 12.7% fresh weight basis) design and the Winesap experimental values are shown in Table 6. The bulk density reflects the amount of volumetric change effected in the piece during processing. However, the Winesap fresh solids content was higher than the Rome Beauty. While the bulk density experimental value does not appear to confirm the predicted value, if the Winesap value is put on the same solids basis as the Rome Beauty apple it would be 121 kg/m^3. The predicted and experimental values for percent disintegration and "a" are within experimental error.

The HMF value for Winesap apples was 10.30% before explosion-puffing, and the experimental value (Table 6) after explosion-puffing was 17.36%. The HMF value for Rome Beauty apples was 6.78% before explosion-puffing and the predicted value after explosion-puffing was 10.66%. The higher HMF values shown for the Winesap apples may have been due in part to the higher level of reducing sugars. The total reducing sugars (fresh weight basis) for the Winesap were 7.63% compared to 6.70% for the Rome Beauties.

The rehydration value of the Winesap was well out of the Rome Beauty range. The dehydrated Winesap apple pieces were excellent in texture, had the typical Winesap sweet tart taste, and possessed a slight yellow hue.

Steam usage. CEPS was operated at optimal conditions as determined from study #2 for three runs (replicates). The conditions used were: moisture 18%; pressure 95 kPa; and temperature 168°C. At these conditions, a total of 63.6–68.2 kg/hr of steam was used, 56.8 kg/hr to superheat the main chamber and 11.4 kg/hr to supply the energy to explosion-puff. For the three runs, 40.2 kg of partially dried apples were processed in total time of 22-1/3 min and used 25.3 kg of steam (27.7 x 10^6 Joules).

Final drying time determination. The drying time in the continuous belt drier to dry 64.7 kg of conventional apple pieces to 3% moisture was 16 hr (Fig. 7). The total drying time for the explosion puffing process had to be determined in two steps: (1) the time it took to dry to 13% moisture in the continuous belt drier (2 hr) plus (2) the final drying time which had to be estimated.

The final drying time was simulated by drying conventional and explosion puffed apples in a National tray drier and comparing drying rates. Equal weights of conventional apples (13.0%) and explosion-puffed apples (17.0%) were dried to below 3% (Fig. 8) and their drying times were compared (105.3 min to 41.3 min, respectively). The explosion-puffed apple pieces dried 2.55 times as fast as the con-

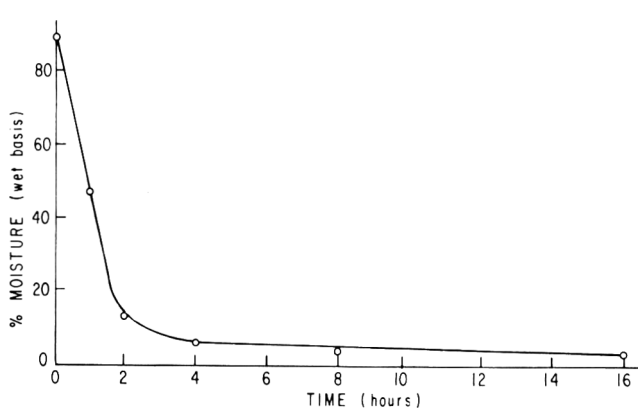

Fig. 7—Drying curve for conventional dried apple pieces.

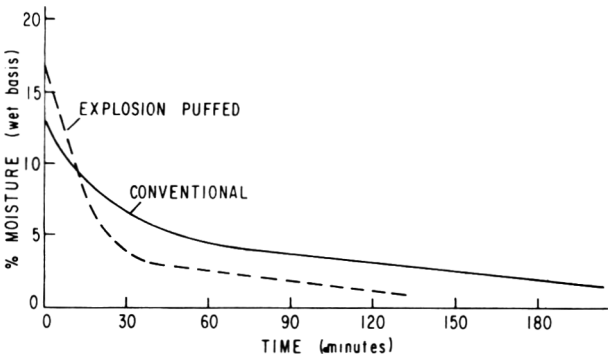

Fig. 8—Final drying curve of explosion-puffed apple pieces and corresponding drying curve for conventional apple pieces.

ventional pieces in the tray drier. We assumed this rate to hold for the continuous belt drier. Therefore, if the conventional apples take 14 hr to dry from 13% to 3%, then the explosion-puffed apple can be estimated to require 5.5 hr to dry from 17% to 3% in this drier. The total drying time for the explosion-puffed apples in the continuous belt drier was 2 hr plus 5.5 hr or 7.5 hr for 64.7 kg of apples (starting weight).

Energy evaluation (steam usage). The total amount of energy used in the explosion-puffing process was determined in three steps: (1) from steam used in initial drying, (2) from steam used in explosion-puffing, and (3) from steam used in final drying.

(1) *Energy used in initial drying.*
2 hr (initial drying time) × 376.8 kg/hr steam (Table 3)
or 753.6 kg of steam at 896.3 kPa 1.53×10^9 joules

(2) *Energy used in explosion-puffing*
(operational time 0.17 hr).
68.2 kg/hr × 0.17 hr equals 11.8 kg of steam:
11.8 kg of steam at 172.4 kPa (130.7°C) equals 0.025×10^9 joules
11.8 kg of steam at 130.7°C raised to 218.3°C 0.002×10^9 joules

(3) *Energy used in final drying.*
Final drying time (estimated 5-1/2 hr)
5-1/2 hr (final drying time) × 161 kg/hr steam (Table 3)
or 886.6 kg of steam at 896.3 kPa 1.79×10^9 joules

Total energy used for the explosion-puffing process (1 + 2 + 3) 3.35×10^9 joules

The steam used to dry 64.7 kg to a final weight (10 kg) by conventional air drying took 16 hr; 2 hr in the first stage and 14 hr in stage two.

(1) *Energy used in first stage.*
2 hr × 350 kg/hr steam (Table 3)
or 699.2 kg of steam @ 896.3 kPa 1.42×10^9 joules

(2) *Energy used in second stage.*
14 hr × 150 kg/hr (Table 3)
or 2097.2 kg of steam at 896.3 kPa 4.24×10^9 joules

Total energy used (1 + 2) 5.66×10^9 joules

CONCLUSIONS

AN EXCELLENT "dehydrated apple" piece (<3.0% moisture) can be made by the explosion-puffing process. The product retains characteristic taste and texture. In that regard, it is notable that there was an evident difference in flavor between products from Rome Beauty and Winesap apples. The product can be eaten dry, as a crisp snack, or can be rapidly reconstituted for use in pies and tarts.

An optimal operating region was determined for Rome Beauty apples. It appears to be insensitive to yearly variation in raw material and offers a range of operating condition combinations which will produce desirable products with minimal losses. While it should not be assumed that the optima specified are exactly correct for other varieties and growing areas, a test on Winesap was encouraging, as it is a substantially different apple variety. The product was acceptable, implying that optimal operating conditions for the Winesaps are near, and could be easily established. Obviously, processing other varieties would also require some modification of operating conditions, but the general interaction of process variables should hold. For Rome Beauties, at least, process control requirements were not stringent.

Sugar leaching losses were not excessive, but the SO_2 losses were high. Some of the SO_2 losses can be attributed to heat of volatilization but most losses result from Maillard reaction occurring during processing (McWeeny et al., 1964). High losses of vitamin C can be expected because of high processing temperatures.

Drying was made more efficient by pre-washing and doctoring because the apples were dried as individual segments rather than a solid slab. The washing step reduced the initial tendency of the apple pieces to cohere, and the mixing and scraping action during doctoring sped the drying of the surfaces sufficiently to prevent sticking to the drier belt and to themselves.

Drying efficiency can be increased by obtaining moisture profiles throughout the drier at various drying temperatures and belt speeds. Each drying zone can then be set to obtain maximum drying at the lowest temperature.

A 44% reduction in steam consumption was realized when the explosion-puffing process was used to dehydrate apple pieces rather than conventional dehydration. This reduction can be attributed to the time saved drying apple pieces from about 20% moisture (wet basis) to less than 3%. Because explosion-puffing imparted a porous structure to the apples, these pieces dried 2.1 times faster than apple pieces dried by the conventional hot air method.

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EFFECT OF ACID DIP ON THE SHELF LIFE OF COLESLAW

L. M. CHEN and A. C. PENG

ABSTRACT

The effect of acid treatments on coleslaw in relation to its shelf life was studied. The results indicated that citric acid, ascorbic acid, malic acid, and their combinations with 200 ppm potassium metabisulfite could slightly improve the keeping quality (mainly color) and concomitantly decrease polyphenol oxidase activity of coleslaw. There were no changes in pH and total acidity.

INTRODUCTION

DISCOLORATION is one of the factors which results in quality deterioration of coleslaw during storage. Traditional methods of preventing discoloration in this product were by dipping the cabbage shreds in acidic solutions (Kaufman and Lutz, 1954); in chemical solutions including potassium sorbate (Francis, 1960); sulfite and metabisulfite (Kaufman and Lutz, 1954). Most observations of treatments were objectively measured by the changes in color (Francis, 1960) and microbial populations (Shapiro and Holden, 1960); or subjective evaluations of appearance by trained panelists. This paper is concerned with the effects of organic acids and potassium metabisulfite on extending the shelf life of coleslaw. The changes of pH, total acidity, enzyme (polyphenol oxidase) activities and color in the treated coleslaw during the 3 wk of refrigerated storage have been studied.

MATERIAL & METHODS

CABBAGE HEADS obtained from the wholesale market were inspected, decored, and shredded. Shredded cabbage was then dipped in the test solutions at a proportion of 1,144g of shreds to 4L of solution for 2 min. After draining and drying with layers of cheesecloth, cabbage slaw (no dressing) and coleslaw (with dressing, Marzetti Co., Columbus, Oh.) were packed in rigid high-density polyethylene containers (1.8L) and stored in the refrigerator at 4°C for 3 wk.

Test solutions in this experiment were: 0.2% (w/v) citric acid, 0.2% ascorbic acid, and 0.2% malic acid. Each acid solution also contained 200 ppm potassium metabisulfite. Water-dipped samples were used as a control. Four replicates from each treatment were randomly selected at the 1 st, 7 th, 17 th, and 21 st day of storage period for the objective measurements.

pH and total acidity

The Glass Electrode Method (AOAC, 1975) was applied to measure pH and percent titratable acidity in a sample solution prepared by blending 5g of sample with 95 ml of distilled water. Total acidity was expressed as percent titratable citric acid in 50 ml of sample aliquot.

Color

Color of fresh coleslaw and acetone-extracted residues were measured by Hunter Color Difference Meter D25D3 against a yellow standard (L = 77.3, a = −2.0, b = 22.6). Samples were prepared by the method of Francis (1960).

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Polyphenol oxidase (PPO) activity

Acetone powder and enzyme extract were prepared and PPO activity was assayed as previously reported (Chen and Peng, 1978). The protein content of each enzyme extract was determined by the Lowry method (1951).

One unit of enzyme activity is defined as the change in absorbance of 0.001 per min at 410 nm. Specific activity is the units of activity per mg of protein.

Sensory evaluation

Sensory evaluations of coleslaw were performed by 6–10 untrained panel members on the basis of color, flavor, texture, and total acceptability at different intervals to monitor any biochemical changes during storage. Each attribute was scored on a hedonic scale of 1 to 10; 10 was considered as perfect, 5 was acceptable and 1 was off. A high score indicated better qualities. The Least Significance Difference (LSD) was calculated to evaluate differences among treatments (Larmond, 1977).

RESULTS & DISCUSSION

Effect of treatments and storage on pH and total acidity

The pH and percent acidity in coleslaw (with dressing) were in the range 4.10–4.32 and 0.0136–0.0216%, and in cabbage slaw (no dressing) ranged pH 5.9–6.1 and 0.0046%–0.0054% acidity (Table 1). Different acid dips as well as refrigerated storage did not significantly affect pH or total acidity in cabbage slaw at the 5% confidence level. However, an increase of acidity was observed in all the acid-dipped coleslaw during 3 wk of storage. It could possibly be the result of degradation of the slaw dressing.

Effect of treatments and storage on color values of coleslaw

The color parameter in this experiment was the L value. Since the lightness was the dominant aspect of color change occurring during browning, L value represented the degree of initial browning and was closely related to the visually observed color change (Golan and Sadovsk, 1977). The larger the L value, the lighter the color of the sample. Results showed a color correlation factor of 0.69 in coleslaw and its acetone residue; and 0.81 in cabbage slaw and its acetone residue. The lower correlation factor in the product with dressing could be the interference of slaw dressing. The L values of residues were therefore considered to represent the color values of coleslaw instead of the L values of cabbage slaw which could result in significant deviation.

Significant differences in color values between the control and treated samples were observed after 1 wk of storage with larger L values in the treated samples (Table 2). Among the different test solutions, no one appeared to be more effective in color retention than the others. Lighter color was also observed in coleslaw than cabbage slaw. Possibly the retardation of discoloration was attributed to the low pH and water activity (a_w) in coleslaw which provides unfavorable conditions for the enzymatic reaction to occur.

Effect of treatments and storage on specific activity of polyphenol oxidase

In general, specific activity decreased with time (Table 3). Assuming the enzyme concentration was constant, the decrease could be indicative of the occurrence of enzymatic browning in coleslaw. This is further demonstrated by the

Table 1—Means of pH and total acidity of slaw samples during storage (day)

Sample treatment[c]	Coleslaw[a]								Cabbage slaw[b]							
	1		7		17		21		1		7		17		21	
	pH	% Total acidity	pH	% Total acidity	pH	% Total acidity	pH	% Total acidity	pH	% Total acidity	pH	% Total acidity	pH	% Total acidity	pH	% Total acidity
1	4.30	0.0136	4.21	0.0167	4.20	0.0189	4.21	0.0192	5.9	0.0049	5.9	0.0046	6.0	0.0054	6.1	0.0053
2	4.30	0.0136	4.20	0.0167	4.31	0.0174	4.20	0.0192	6.0	0.0046	5.9	0.0053	5.9	0.0053	6.1	0.0053
3	4.30	0.0150	4.25	0.0163	4.20	0.0192	4.20	0.0192	5.9	0.0049	5.9	0.0046	6.1	0.0053	6.1	0.0053
4	4.10*	0.0153	4.20	0.0174	4.20	0.0192	4.30*	0.0189	6.0	0.0053	6.0	0.0053	6.1	0.0053	6.1	0.0053
5	4.29	0.0150	4.28	0.0160	4.30	0.0189	4.28	0.0174	6.0	0.0053	5.9	0.0053	6.1	0.0049	6.1	0.0053
6	4.20	0.0153	4.20	0.0160	4.25	0.0192	4.19	0.0208	5.9	0.0049	5.9	0.0049	6.1	0.0053	6.1	0.0053
7	4.30	0.0150	4.32*	0.0216	4.05	0.0179	4.20	0.0208	–	–	–	–	–	–	–	–

[a] With dressing
[b] No dressing
[c] 1—water dip; 2—0.2% citric acid; 3—0.2% citric acid/200 ppm potassium metabisulfite; 4—0.2% ascorbic acid; 5—0.2% ascorbic acid/200 ppm potassium metabisulfite; 6—0.2% malic acid; 7—0.2% malic acid/200 ppm potassium metabisulfite.
* Significantly different from sample 1 at 5% confidence level calculated from Duncan's Multiple Range Test

Table 2—Lightness (L) values of acetone-extract residue from slaw samples treated with acid and potassium metabisulfite and stored at 4°C

Sample treatment[c]	Coleslaw[a]				Cabbage slaw[b]			
	Storage (day)							
	1	7	17	21	1	7	17	21
1	65.86	61.22	61.20	59.34	70.69	67.49	58.32	54.63
2	65.38	63.08*	62.85	58.79	68.98	62.43*	60.85	56.32
3	66.31	65.63*	65.39*	63.05*	66.71	65.54*	62.76*	58.09
4	66.51	64.98*	63.29	63.38*	64.82*	63.42*	60.66*	58.90*
5	65.73	63.45*	60.41	61.73*	68.96	67.81	61.21*	60.53*
6	65.16	63.54*	61.79	63.39*	69.82	65.63*	62.04*	58.12*
7	67.55	65.34*	65.34*	62.23*	–	–	–	–

[a] With dressing
[b] No dressing
[c] 1—water dip; 2—0.2% citric acid; 3—0.2% citric acid/200 ppm potassium metabisulfite; 4—0.2% ascorbic acid; 5—0.2% ascorbic acid/200 ppm potassium metabisulfite; 6—0.2% malic acid; 7—0.2% malic acid/200 ppm potassium metabisulfite.
* Significantly different from sample 1 calculated from Duncan's multiple range test

Table 3—Specific activity[a] of polyphenoloxidase from slaw samples treated with acid and potassium metabisulfite and stored at 4°C

Sample treatment[d]	Coleslaw[b]				Cabbage slaw[c]			
	Storage (day)							
	1	7	17	21	1	7	17	21
1	28.45	29.03	27.14	25.72	39.30	38.05	38.00	32.50
2	28.57	30.33	28.57	25.86	35.90*	32.96*	32.14*	32.26
3	27.78	25.00*	25.16*	24.55	34.80*	32.69*	32.00*	31.25
4	29.04	26.43*	25.73	24.56	36.50*	32.69*	31.73*	30.83
5	30.17*	28.45	29.03	28.13*	34.60*	32.69*	30.76*	30.20
6	27.42	27.27*	25.00*	25.00	34.60*	32.69*	29.95*	29.62*
7	26.79*	30.45	28.32	30.17*	–	–	–	–

[a] Defined as units of enzyme activity/mg of protein in crude enzyme source
[b] With dressing
[c] No dressing
[d] 1—water dip; 2—0.2% citric acid; 3—0.2% citric acid/200 ppm potassium metabisulfite; 4—0.2% ascorbic acid; 5—0.2% ascorbic acid/200 ppm potassium metabisulfite; 6—0.2% malic acid; 7—0.2% malic acid/200 ppm potassium metabisulfite.
* Significantly different from sample 1 calculated from Duncan's multiple range test

fact that the extent of decrease was greater in the control samples than in the treated samples upon storage, and the former was also more discolored than the latter.

The specific activity of PPO in coleslaw was lower than the cabbage slaw. The pH and the ingredients of slaw dressing could influence the extraction of active PPO from coleslaw.

The tendency of PPO activity to decrease with storage was found in three cultivars of potato stored at 5°C for 60 days (Vamos Vigyazo et al., 1974) and in freeze-dried peaches and banana stored at 28°C for 60 days (Draudt and Huang, 1966). These results are in agreement with the findings in this study. However, PPO activity increased when dates were stored at 49°C for a month (Maier and Schiller, 1961). The increased activity was suggested as a result of tissue disintegration which released the soluble PPO originating in the particulate fraction, or in releasing the particulate fraction. Whether the contradiction is due to experi-

Table 4—Color scores of coleslaw given various chemical treatments as judged by panelists[a]

Treatment	Storage (days) 1	4	7
	Mean score		
Control (water dip)	5.80	6.80	4.50
0.2% Citric acid	6.40	7.00	4.67
0.2% Citric acid/200 ppm potassium metabisulfite	7.20	6.50	5.33
0.2% Ascorbic acid	7.00	6.50	5.00
0.2% Ascorbic acid/200 ppm potassium metabisulfite	6.40	6.70	4.67
0.2% Malic acid	7.20	6.70	5.70
0.2% Malic acid/200 ppm potassium metabisulfite	6.60	6.70	6.00
Grand mean	6.60	6.70	5.33
L.S.D. ($p < 0.01$) for judges*	1.82	0.41	0.92
L.S.D. ($p < 0.01$) for samples	ns**	ns	ns

[a] 10-point hedonic scales were used with 10 as perfect and 1 as off
* There is a significant difference among judges
** Not significant

mental errors, methodology or individual commodity, can only be answered by further research.

Sensory evaluations

Color scores of coleslaw judged by the panelists are shown in Table 4. There was no significant difference in the control and all treated samples; however, the scores were lower after storage. The grand mean at the beginning was 6.60 and 5.33 after 7 days of storage with the control sample at 5.80 and 4.50. Neither the treatment nor the storage resulted in different flavor and texture of coleslaw in this study and data are not presented. An acid-dip slightly improved the keeping quality of coleslaw regardless of the type of acid used in the dip solution.

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EFFECT OF COOKING ON THE CHEMICAL COMPOSITION OF WINGED BEANS (Psophocarpus tetragonolobus)

T. E. EKPENYONG and R. L. BORCHERS

ABSTRACT

The effect of soaking and cooking methods on the chemical composition, digestibility and antitryptic activity of winged beans was investigated. Water imbibition was rather slow with winged bean seeds. To obtain beans sufficiently tender for human consumption, prolonged soaking prior to cooking for 5 hr was found to be suitable. Short-cooking time was ineffective as the beans were very hard. Prolonged cooking after a prolonged soaking period improved the flavor, increased tenderness, and increased the weight of cooked beans. Also, increases in percent protein and in vitro digestibility and reduction in the activity of trypsin inhibitor were observed. Cooking influenced the mineral content of the beans leading to significant losses in potassium and magnesium.

INTRODUCTION

THE WINGED BEAN plant (*Psophocarpus tetragonolobus*) has been used as a source of food for centuries. It is indigenous to the humid tropics. It has been cultivated as a backyard vegetable crop in South-East Asia and Papua New Guinea where the ripe, mature seeds are roasted and eaten like peanuts (Anon, 1975a). Its exceptional agronomic and nutritional peculiarities have been well documented (Cerny et al., 1971; Pospisil et al., 1971; Masefield, 1973; Anon, 1975b; Claydon, 1975). All parts of the plant are consumed by man while the seed cake and plant residues are reportedly palatable to live-stock (Pospisil et al., 1971).

The dry, hard seed must be soaked in water and cooked to render the bean sufficiently tender for human consumption. The extensive time needed for soaking and cooking is a hindrance to the use of dry seeds. Some techniques have been used to render dry beans quick-cooking and edible. These include increasing the temperature of soaking water, mechanically increasing the rate of water imbibition by vacuum infiltration in a dilute solution of common inorganic salts and boiling of the beans in five volumes of water to which has been added 1% sodium bicarbonate (Rockland, 1972; Rockland and MEtzler, 1967; Rockland et al., 1975; Martins, 1978). Reports have shown that adequate cooking eliminates antinutritional factors and improves the biological value of cooked winged beans (Cerny et al., 1971; Jaffe and Korte, 1976).

The present study is aimed at establishing chemical changes that occur as a result of soaking and cooking of mature, dry winged beans.

MATERIALS & METHODS

Dry beans

The raw, dry winged beans (*P. tetragonolobus*) were obtained from the International Institute for Tropical Agriculture, Ibadan, Nigeria. Other edible legumes were purchased locally.

Sample preparation

Initially, the soaking process consisted of adding 40g of whole dry beans to 100 ml of deionized water for 5 min (Quick-cooking method) or 18 hr (long-cooking method) at 25°C. For proximate analysis, some of the beans were drained and made into a slurry with an equal volume of deionized water in a Waring Blendor. The rest was cooked for different lengths of time; 5 hr and for 30 min for long-cooking and for quick-cooking, respectively. It was found suitable to cook for 5 hr to render the beans tender. For analyses, the cooked beans were drained, made into a slurry, lyophilized, equilibrated in air, and milled to pass a 20 mesh sieve in a micro Wiley Mill. The pH of the soaked samples prior to and after cooking was taken. As cooking progressed, measured volumes of deionized water were added and care was taken to prevent the beans drying-out before they become tender.

Proximate analysis

Proximate composition was determined on all samples by standard methods of analysis (AOAC, 1970).

Trypsin inhibitor activity (TIU/mg)

The trypsin inhibitor activity was assayed using the method of Kakade et al. (1974). One trypsin unit is defined as an increase of 0.01 absorbance units at 410 nm/10 ml of reaction mixture under the conditions of the test.

—Continued on next page

Table 1—Composition of mature, raw dry winged bean seeds

Constituent (%)	Whole seeds	Literature values[a]
Moisture	10.4	7 – 25.0
Fat	15.8	15.0– 20.4
Crude protein	35.9	29.8– 37.4
Carbohydrates	23.9	21.6– 28.0
Fiber	9.2	5.0– 12.5
Ash	4.9	3.6– 4.0
Solids	89.6	n.a.[b]
Minerals (mg/100g)		
Calcium	275	204 –370
Phosphorus	310	276 –320
Potassium	117	110 –120
Iron	10	10 – 12
Sodium	35	20 – 56
Magnesium	178	120 –204

[a] Anon, 1975[b]
[b] not available

Table 2—Proximate composition of raw dry winged beans compared with other edible legumes

Constituents (%)	Winged bean	Soybean	Cowpea	Ground nut	Pinto
Moisture	10.4	8.2	11.3	7.8	10.4
Fat	15.8	16.1	1.7	46.2	1.9
Crude protein	35.9	39.0	22.9	21.2	21.0
Carbohydrates	23.9	25.4	57.1	20.8	54.6
Fiber	9.2	4.7	3.9	2.0	7.2
Ash	4.9	6.6	3.1	2.1	5.0

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Table 3—Proximate analysis of raw, long, and quick-cooking beans

Treatment	Moisture[a] (%)	Protein (N×6.25) (%)	Total carbohydrates (%)	Fat (%)	Ash (%)	Digestibility (%)
Raw	10.4	35.9	23.9	15.8	4.9	70.7
Dry heat[b]	3.65	35.6	41.5	15.2	4.1	71.6
Autoclaved[c]	15.45	35.0	38.1	7.1	4.2	84.3
Long-cooking[d]	5.40[a]	41.0	34.3	15.3	4.1	83.6
Quick-cooking[e]	2.74[a]	40.9	34.8	15.5	6.1	78.9

[a] Refers to moisture of dried sample
[b] Oven dry heat, 110°C, 30 min
[c] 110°C, 15 psi, 30 min
[d] Beans soaked for 18 hr and then cooked for 5 hr
[e] Beans soaked for 5 min and then cooked for 30 min

Table 4—Content of some minerals in raw, autoclaved, long-, and quick-cooking winged bean seeds (mg/100g)

Treatment	Ca	P	K	Fe	Na	Mg
Raw, dry seeds	275	310	117	10	35	178
Autoclaved	275	310	117	10	35	178
Long-cooking	278	201	551	10	31	173
Quick-cooking	275	261	530	10	20	69

Table 5—Comparison between long- and quick-cooking of winged bean seeds

Parameters	Long-cooking	Quick-cooking
Weight dry beans (g)	40.0	40.0
Total soaking time (min)	1080.0	5.0
Total cooking time (min)	300.0	30.0
Weight cooked beans (g)	92.0	60.0
Total H_2O added (ml)	1100.0	110.0
Total H_2O absorbed (ml)	1020.0	22.0
Cooked weight: dry weight	2.3	1.5
pH prior to cooking	6.6	6.9
pH after cooking	5.8	6.4
Moisture (%)	5.4	2.7
Fat (%)	15.3	15.5
Protein (%)	41.0	40.9
Total carbohydrates (%)	34.3	34.8
% Digestibility (in vitro)	83.6	78.9
Food energy	438.3	442.1
Antitrypsin activity (TIU/mg)	5.1	18.5

Digestibility

The in vitro digestiblity of the samples was assayed by the method of Hsu et al. (1977).

Heat treatment

Two types of heat treatment were used. The first was moist heat (autoclaving) which was applied at 110°C, 15 psi for 30 min. The second type of heat treatment was dry heat (roasting) in which the samples were placed in a hot oven at 110°C for 30 min.

Mineral analysis

The samples were wet-digested with perchloric acid and stock solutions were prepared according to AOAC method (1970). The atomic absorption spectroscopic method was used for analysis of calcium, potassium, iron, sodium, and magnesium using a Perkin-Elmer Atomic Absorption Spectrophotometer. Phosphorus was determined by the method of Fiske and Subbarow (1925) using a Beckman Model DU Spectrophotometer.

RESULTS & DISCUSSION

THE COMPOSITION of mature, raw dry winged bean seeds as compared to literature values is presented (Table 1). Protein, fat, carbohydrate, and moisture of the sample studied fall within the range of literature values. The ash content of the sample appears to be on the high side. With regard to the minerals, there is also general agreement with the values here falling within the range of published values.

A comparison was made between raw dry winged bean and some edible legumes (Table 2). The winged bean seeds contained about 36% protein, almost similar to that of soybean (39%). Besides groundnut (peanut), winged bean seeds contain a high level of oil. Compared to cowpea, groundnut, and pinto beans, the winged bean is better suited for human consumption and for livestock feed in terms of protein, fat (excepting groundnut), and ash.

It has been shown that it is expedient to subject dry legumes to a soaking process prior to cooking (Liener, 1962; Hintz et al., 1967). The proximate composition of raw dry bean seeds, long, and quick-cooking winged beans are compared (Table 3). The moisture content varies with the processing method. There is an increase in protein content, total carbohydrates, and digestibility in the samples subjected to soaking and cooking. The increase in protein is due to the loss of soluble solids during soaking and cooking. There is also an increase in ash of the quick-cooking beans. The low digestibility of raw, uncooked legumes is attributed to the presence of protease inhibitors which reduce the activity of the digestive enzymes. Soaking prior to cooking blocks the activity of the protease inhibitors thus exerting a beneficial effect on the digestibility. Results here show that like autoclaving, soaking and long-cooking improve the digestibility of winged beans.

Raw winged beans, like soybeans, are high in potassium (Table 4). However, potassium is lost during soaking and cooking due to leaching. This result agrees with that of Rockland et al. (1979). Autoclaving had no effect on K loss. Winged bean seeds contain the best sources of iron and processing has no effect on the iron content. The levels of sodium and magnesium were about identical in the raw, autoclaved, and long-cooking beans but the losses of both minerals varied between long- and quick-cooking beans.

Using different criteria, a comparison was made between long and quick-cooking winged bean seeds (Table 5). The total amount of water added to the beans to attain the level of tenderness suitable for human consumption was significantly higher for long- than for quick-cooking beans. The winged bean seeds absorb about their own weight in water as a consequence of soaking and cooking. However, the water absorption capacity of the beans is not uniform so that some beans are incompletely cooked. The weight of cooked beans is higher for the long-cooking than quick-cooking beans. Since more water was absorbed by the long-cooking beans, the weight and tenderness of the cooked beans were increased by hydration prior to cooking.

Trypsin inhibitors and hemagglutinins are the two most important antinutritional factors widely distributed in edi-

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ENERGY USE, COST, AND PRODUCT QUALITY IN PRESERVING VEGETABLES AT HOME BY CANNING, FREEZING, AND DEHYDRATION

FLOWAYNE DREW and KI SOON RHEE

ABSTRACT

Carrots, zucchini, and summer squash were processed by canning, freezing, and dehydration according to procedures used in the home. Energy used was measured and the cost of preservation was calculated with the packaging and amortized equipment cost included. Reduced ascorbic acid and carotene contents were determined within a week after processing and after 6 months of storage. Sensory quality of the processed vegetables was evaluated in less than a month after processing. The vegetables were cooked with salt added for sensory evaluation. Freezing required the shortest processing time and resulted in products with the highest sensory quality, with ratings well above minimum acceptability, and the most ascorbic acid (assuming the canned liquor is not used) and carotene. More energy (electricity) was required to process and store vegetables by freezing than by canning or dehydrating with a commercially available electric dehydrator; however, the overall cost for freezing preservation was estimated to be as low as or lower than the cost for the other two preservation methods if the freezer could be kept full. With the freezer only half-full freezing would cost more than canning or dehydration for a long term (>6 months) storage. Canning used much less electric energy and was slightly cheaper in overall preservation cost than dehydrating with the electric dehydrator, and the canned products retained much more ascorbic acid and carotene than the dehydrated products. Nearly all ascorbic acid was lost during dehydration. The canned and dehydrated products were rated low in sensory quality, with ratings mostly below minimum acceptability.

INTRODUCTION

INCREASED CONSUMER interest in home preservation of food has resulted in a need for recent information by people in extension, teaching, and other areas dealing with food and nutrition. The present study was designed to provide material useful in decision making when selecting a method for preserving food at home. Savings in money and energy are of increasing importance due to continuing inflation of prices and shortages of fuel. Yet, the consumer must also consider the palatability and nutritive value of the preserved food.

Surveys show that a major reason for preserving food at home is to save money (Goral, 1976; Kaitz, 1978; Phillips and Bass, 1976). Comparative studies were published in the forties and fifties of quality and nutritive value of vegetables as affected by canning, freezing, and dehydration (Bamford and Fenton, 1953; Dawson et al., 1949). These studies, however, did not include factors affecting energy consumption and preservation cost. More recently, additional studies have been made of different aspects of various methods of home food preservation, but few have compared different preservation methods in one study or included measurements of energy use. In a comparison of the effects of processing time and temperature on home-canned tomatoes, Zimmerman et al. (1978) reported a consumption of more electricity for processing in a water bath than in a pressure canner. Keith et al. (1979) compared the electrical costs of home canning and drying of peaches and sweet corn and found that drying was 1.2 to 9.9 times as expensive as canning depending on the method used.

Vegetables were found to be more palatable with better color when frozen than canned, and dehydrated vegetables were shown to be as good or better than the canned (Bamford and Fenton, 1953; Dawson et al., 1949). Porter et al. (1946) found that green beans preserved by commercial methods were most acceptable when frozen, but found no significant difference between those canned and dried. Alderman and Newcombe (1944) reported the palatability of dehydrated shredded green beans to be superior to those canned and equal to those frozen.

Vitamins are lost to some degree from vegetables during all types of processing (Harris and Karmas, 1975). Generally, losses are least when vegetables are frozen and greatest when they are dehydrated. Studies have frequently included the measurement of ascorbic acid and carotene retention.

In the present study, comparisons were made of canning, freezing and dehydration of vegetables at home in regard to energy consumption, total preservation cost, palatability of and vitamin retention in the processed products. Vegetables included were carrots, zucchini, and summer squash.

MATERIALS & METHODS

Processing

Fresh carrots were purchased at the retail market; zucchini and summer squash were obtained from the Dept. of Horticulture, Texas A&M Univ. Carrots were washed, peeled, and cut into slices of 0.5 cm thickness. Zucchini and summer squash were washed and cut unpeeled into 0.5 cm-thick slices for dehydration and 1.5 cm-thick slices for canning and freezing–slices of large diameter were halved or quartered. An electric home food slicer was used for slicing the vegetables. Slices of each vegetable were mixed well before being divided into three batches for canning, freezing, and dehydration. Processing procedures followed were those given in the USDA Home and Garden Bulletins (Miller et al., 1977; USDA, 1976, 1978). Care was taken to use methods and procedures that would generally be followed in a home situation.

Vegetables were steam-blanched in 480-g batches for freezing and dehydration. Additional batches of zucchini and summer squash were water-blanched.

For freezing, plastic freezer bags were each filled with an amount of blanched vegetable equivalent to 240g of the corresponding raw vegetable and frozen at −18°C in a 16-ft^3, cold-shelf, conventional freezer (Kenmore Freezer Model 28162, Sears Roebuck & Co.). Frozen storage was continued in the same freezer.

For dehydration, an electric dehydrator with 8 shelves (Harvest Maid Food Dehydrator, Model FD-300, 600 watts; Alternative Pioneering Systems, Inc., Minneapolis, Minn.) was used. Blanched vegetable in an amount equivalent to 480g of the raw product was spread in a single layer on each shelf and dried at 60°C. Dehydration was carried out with the dehydrator fully filled, i.e., with 8 shelves of vegetables (3,840g) in the dehydrator.

Vegetables were also dehydrated using a nonelectric dehydrator with 4 shelves (Sun Pantry Oven Fruit Dryer, Sun Fresh Products, Napa, Calif.) placed in the 30-inch oven of an electric or gas range for comparison of energy consumption or dehydration performance.

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The nonelectric dehydrator was fully filled with a total of 2,160g (as raw weight) vegetable, i.e., 480g/shelf for 4 shelves. Oven drying or oven-dried products were not included in estimating processing cost and in vitamin assay and sensory evaluation.

For canning, raw vegetable slices were packed into 9 glass Mason jars (1-pt size) in the amount of 240g/jar and processed for the prescribed 25 min at 116°C in a 16-qt pressure canner. (The canner was filled with 9 pt-size jars.) In order to ascertain accurate heat control, temperatures of one jar and the canner were monitored by copper-constantan thermocouples attached to a recorder. The thermocouple in the jar was located 2.5 cm from the bottom at the center, the area of slowest heating according to Thompson et al. (1979).

To measure the use of electricity, standard kilowatt-hour meters were modified, using a micro-switch photoelectric control designed and built by an electrical engineer in the College of Engineering, Texas A&M Univ. A digital counter recorded the consumption of electricity in readings that were precise to 0.1 watt-hour.

Cost estimations

The cost of preserving vegetables by canning, freezing, or dehydration was estimated with the inclusion of the energy (electricity) costs for actual processing and storage (frozen storage for freezing) and the costs of packaging materials and equipment.

The cost of electricity was calculated at the rate of 5 cents/kwh. Electricity used for blanching, canning, and dehydrating processes was actually measured. Estimations were made of the amounts of electrical energy needed to freeze and store vegetables using values of 0.1 kwh to freeze 1 lb of food and lower its temperature to −18°C (McCracken, 1964) and 0.20 kwh/ft^3 (freezer space) to maintain −18°C temperature for 24 hr with a 16-ft^3, cold-shelf, conventional freezer with 480-lb capacity (Heaton and Woodroof, 1962). No allowance was made for the cost of storing canned and dehydrated products because this expense is dependent on the storage facility of a house. It was simply assumed that a storage facility already available in the home would be utilized to store such products.

Packaging costs were calculated on the basis of $2.59/doz for pint-size glass Mason jars, amortized over 10 years (Haggard, 1976; Johnson, 1975); $0.35/doz for lids; $1.60/80 for freezer bags. The cost of equipment was $67.63 for canning, $132.45 for dehydration, and $381.75 for freezing. These costs were amortized over 20 years and a cost for maintenance, 2% of the purchase price per year, was added to the total annual cost for equipment (Haggard, 1976; Johnson, 1975; McCracken, 1964).

Vitamin assays

Vitamin analyses were made within 1 wk after processing. Reduced ascorbic acid content was determined in zucchini and summer squash by a 2,6-dichloroindophenol titration procedure described by Freed (1966) using metaphosphoric acid-acetic acid (3%/8%) as the extraction medium (AOAC, 1975) and an amount of each vegetable equivalent to 480g of the raw product to make the initial slurry. The drained solids and liquid of each canned product were separately analyzed.

Carotene content was determined in carrots and zucchini by column chromatography according to AOAC (1975) method with some modifications. A slurry was made of an amount of vegetable equivalent to 480g of the raw product and distilled water by blending. Total carotenoids were extracted from an aliquot of the slurry by stirring for 30 min in hexane-acetone (3:2). Chromatograms were developed with 4% acetone in hexane to separate carotenes from other carotenoids.

Sensory evaluation

Sensory evaluation was conducted in less than a month after processing. The timing of sensory evaluation (<1 month) was dictated by such experimental circumstances as completion of sequential processing of the three vegetables by the three methods and availability of the sensory judges. The processed products were first cooked before presenting them to the sensory panel. Salt was added in the amount of 0.25 tsp/240g as raw. Canned vegetables (solids + liquid) were boiled 10 min as generally recommended for home-canned vegetables for safety reasons. Dehydrated vegetables (240g as raw) were first soaked to rehydrate and then cooked in 150 ml water (including the soaking water) until tender. Frozen vegetables were partially thawed to break pieces apart and cooked in water (150 ml/240g as raw) until just tender.

Cooked frozen, canned, and dehydrated vegetables were evaluated in 3 sessions (replications) by a panel of 7–9 experienced judges using a 9-point scale (9=like extremely; 1=dislike extremely). A Latin square design (Cochran and Cox, 1964) was used for assigning the three treatments to serving order and sessions. Attributes judged were general appearance, color, flavor, texture, and overall acceptability.

Statistical analysis

Statistical significance of the data obtained was determined by analysis of variance. The Student-Newman-Keuls' test (Steel and Torrie, 1960) was used for mean separation.

RESULTS & DISCUSSION

Processing time

Regardless of the processing method used, a large part of the time required for home processing of vegetables is that spent in preparation, i.e., washing, peeling, slicing, etc. Slicing vegetables more thinly for dehydration required some additional time over that needed to prepare thicker slices or chunky cuts used for freezing or canning. Blanching in steam required slightly longer than in water. Packaging the vegetable required about the same length of time for all three preservation methods when an equivalent amount was packaged per container. For dehydration, additional short periods of time were required every 2–3 hr during drying to turn and rotate the trays in the dehydrator or oven.

The person processing a 2,160g (4.78 lb) batch of a vegetable was involved 52 min to steam-blanch and package for freezing, 72 min to steam-blanch and process in a dehydrator excluding the drying time proper, and 80 min to pack the jars and can in a pressure canner. The reason for basing the processing time on 2,160g of vegetable is that this amount filled 9 pt-size Mason jars which in turn filled the canner and also was the amount that could be dehydrated at one time in the nonelectric dehydrator.

Energy use

Actual measurements were made of the amounts of energy used during canning and dehydrating the vegetables as described under "MATERIALS & METHODS" and the amounts to be required to process 50 lb were calculated to present a more realistic situation of home food processing and preservation (Table 1). Steam-blanching and dehydrating in an electric dehydrator required 1.6–2.1 times as much as canning in a pressure canner. Using an electric oven for dehydration used about five times as much electricity as canning.

The electric oven was an unsatisfactory appliance to use for dehydrating food since it was impossible to obtain acceptable control of the low oven temperature. The tempera-

Table 1—Energy use (kwh) for processing and storing 50 lb of vegetables

Processing method		Carrots	Zucchini	Summer squash
Canning		19.01	24.15	22.26
Dehydration				
Dehydrator,	steam-blanched	40.10	38.45	42.37
	water-blanched	–	40.63	44.27
	unblanched	–	31.42	32.70
Range oven,	steam blanched	101.96	–	–
Freezing				
To blanch, freeze and lower to −18°C (to process)		13.44	13.65	13.13
To maintain at −18°C (to store)				
	1 mo	10.19	10.19	10.19
	3 mo	30.45	30.45	30.45
	6 mo	60.80	60.80	60.80
	12 mo	121.59	121.59	121.59

ture control had to be operated manually according to the thermocouple readings on a potentiometer. Even then, the fluctuations in temperature were quite extensive. Low level temperatures were maintained rather well by the thermostat in a gas oven.

The difference in energy use between steam- and water-blanching was small. Water-blanching consumed about 4% more electricity than steam-blanching.

Various conditions affect the amount of energy used when foods are frozen. Major factors affecting the energy use are size and type of freezer, freezer load, and the length of storage. The electrical energy required to maintain or store food at frozen state varies depending upon the size of the freezer and whether it is a conventional or frostless type. A frostless freezer will use about 1.5 times as much energy as a conventional type of the same size and the amount of energy to operate increases with the size of the unit (McCracken, 1964).

It was estimated that 50 lb of vegetable could be blanched, frozen, and held at −18°C for about 1 month before energy consumption would exceed the amount of electricity used for canning the same quantity of vegetable and about 3 months before exceeding that used for dehydrating, when the 50 lb were considered as a fraction of the full load of the freezer (Table 1). The energy use per pound of vegetable, however, would increase drastically with reduced freezer load, as can be seen in Table 2.

Cost

One of the major aims in home food preservation is to save money on food. There are several sources of expense for processing vegetables. In addition to the cost of the produce, there are costs for packaging, equipment, and energy used. The cost of produce is variable depending on whether it is purchased (also where it is purchased), is a gift, or is home-grown. And, produce is cheaper when in season.

The cost of packaging depends on the type of container used. Foil or plastic containers, either disposable or reusable, are most frequently used for freezing. Glass Mason jars usually used for canning are sometimes used for freezing, and are good for storing dehydrated foods. The jars are reusable, but new lids must be purchased each time the jar is used for canning and a safe seal is needed.

The cost of preserving vegetables in the home is dependent on the long term use of supplies and equipment. Amortization of the purchase price should be made for an estimate of the annual cost of owning a pressure canner, electric dehydrator, and freezer. Costs of annual repairs for the equipment should be included. An amortization of the cost of small equipment (e.g., a blancher) should also be included in the calculation of the fixed annual overhead cost.

A general estimate of the cost for preserving vegetables by canning, dehydration, or freezing is shown in Table 3. The details and bases for the cost estimations have already been described in the "MATERIALS & METHODS" section. It is emphasized that the figures shown in the table are estimates using equipment and packaging of only one kind and size for each type of preservation. Costs will vary with the use of different or alternative equipment, packaging, fuel and storage. The information given in Table 3 is, however, indicative of the relative costs of processing and preserving vegetables in the home.

Based on the estimates in Table 3, canning appears to be more economical than dehydrating with an electrical dehydrator. Dehydrating cost may be reduced by utilizing cans or jars saved from other container-packaged foods, and by using a home-made dehydrator which may be built at a cost lower than the price of a commercially available dehydrator. Variations in the size of canner and dehydrator used and food load per processing period will affect the fuel cost per pound of food being processed. The cost per pound would be more if less than maximum amounts of food were processed at one time. Dehydrating with a half-full load used about 30% more fuel per pound of vegetable than dehydrating with a full load (data not shown).

In estimating the cost of preservation by canning or dehydration, no allowance was made for the cost of storing the processed products. Canned and dehydrated products were assumed to be stored in pantry or other appropriate storage facility already available in the home. It is recommended in the USDA Home and Garden Bulletins for home canning and dehydration of foods (Miller et al., 1977; USDA, 1976) that canned and dehydrated products be stored in a cool, dry, dark place for maximum maintenance of quality. If it were necessary to provide such a facility, there could be considerable added cost involved.

The cost for freezing versus other preservation methods is highly dependent on how much the freezer is used. By making maximum use of a freezer, i.e., by keeping the freezer full as much as possible, the cost/pound of preserving vegetables by freezing can be kept as low as or lower than the cost for other preservation methods (Table 3). For 6-month storage, the cost/pound would be lowest for freezing with the freezer kept full and highest for dehydration.

—Continued on next page

Table 2—Estimated energy use per pound of vegetable to maintain at −18° C as affected by food load in the freezer

Extent of fill in 16-ft³ freezer	Food load (lb)	Energy use/lb to maintain at −18°C (X10⁻² kwh)			
		24 hr	1 mo	6 mo	12 mo
Full	480	0.67	20.45	122.69	245.38
3/4 Full	360	0.89	27.07	162.43	324.85
1/2 Full	240	1.33	40.55	243.33	486.67
1/4 Full	120	2.67	81.15	486.92	973.84

Table 3—Estimated cost ($) for home preservation of 50 lb of vegetables

			Freezing[a]		
	Canning	Dehydration	Length of storage at −18°C	With full freezer	With 1/2 full freezer
Processing and storing					
Processing[b]	1.09	2.02		0.63	0.63
Storing	—	—	1 mo	0.50	1.00
			6 mo	3.00	6.00
			12 mo	6.00	12.00
Packaging					
Jars	1.98	0.44		—	—
Lids	2.74	0.61		—	—
Freezer bags	—	—		1.89	1.89
Amortized cost for equipment	4.73	9.27		2.78	2.78
Total cost/50 lb	10.50	12.34	1 mo	5.80	6.30
			6 mo	8.30	11.30
			12 mo	10.67	17.30
Total cost/lb	0.21	0.25	1 mo	0.12	0.13
			6 mo	0.17	0.23
			12 mo	0.21	0.35

[a] Estimated under the assumption that 50 lb were a portion of the food load of a full or half-full freezer and under the assumption of no decrease in the food load during the storage periods.

[b] The processing cost is the cost of electricity used for actual processing, i.e., canning process proper for canning, steam-blanching plus drying process for dehydration, and steam-blanching plus freezing to −18°C for freezing. The values are the average costs for the three vegetables used in this study.

For 12-month storage, however, freezing with a full freezer would cost as much as canning, with dehydration still costing most. With the freezer only half-full throughout storage, freezing would cost more than canning or dehydration for storage periods longer than 6 months.

Sensory quality

Frozen products were rated highest, well above minimum acceptability, for all the sensory attributes evaluated with each of the vegetables (Table 4). Canned and dehydrated products were rated just barely acceptable (canned and dehydrated carrots and dehydrated zucchini) or unacceptable (canned zucchini and canned and dehydrated summer squash). The acceptability of the canned and dehydrated products may improve if they are prepared with other seasonings and/or incorporated in mixed dishes such as soups, casseroles, etc.

Preference between canned and dehydrated products varied according to the vegetable judged. For carrots and zucchini, the dehydrated was rated higher than the canned; for summer squash, the canned generally had better scores than the dehydrated.

The sensory quality of differently processed vegetables will depend to a great extent on the kind of vegetables and thus no generalization or prediction can be made of the sensory quality, as affected by the processing methods, of different vegetables to be processed in the home.

Table 4—Mean sensory quality scores[a] of processed vegetables

Product	General appearance	Color	Flavor	Texture	Overall acceptability
Carrots					
Canned	5.78b	5.67c	5.19c	5.07b	5.15c
Dehydrated (blanched)	6.04b	6.55b	6.04b	5.81a	5.96b
Frozen (blanched)	8.19a	8.55a	7.22a	6.56a	7.22a
Zucchini					
Canned	2.81c	2.62c	3.62c	2.33c	2.62c
Dehydrated (blanched)	5.38b	5.90b	5.71b	5.43b	5.33b
Frozen (blanched)	8.24a	8.29a	7.71a	6.71a	7.57a
Summer squash					
Canned	5.22b	5.39b	4.65b	4.70b	4.70b
Dehydrated (blanched)	3.61c	3.83c	4.09b	4.35b	3.65c
Frozen (blanched)	8.39a	8.48a	7.61a	7.30a	7.78a

[a] Based on a 9-point scale (9=like extremely; 1=dislike extremely) with a score below 5 indicating unacceptable product. Means in the same column, within a vegetable, which are not followed by the same letter are significantly different ($p<0.05$).

Table 5—Reduced ascorbic acid content following processing and after 6 months of storage

	mg Reduced ascorbic acid/100g vegetable as raw			
	Zucchini		Summer squash	
Product	Before storage	After storage	Before storage	After storage
Canned				
Solids	5.54	3.78	9.66	5.08
Liquid	4.53	2.11	6.49	3.85
Dehydrated (blanched)	0.45	0.22	0.25	0.11
Frozen				
Unblanched	2.44	–	3.96	–
Steam-blanched	8.40	3.73	5.05	5.22
Water-blanched	8.40	3.58	10.86	9.55

Table 6—Carotene content before and after 6 months of storage

	mg Carotene/100g vegetable as raw			
	Carrots		Zucchini	
Product	Before storage	After storage	Before storage	After storage
Canned (solids)	37.63	37.01	0.77	1.17
Dehydrated (blanched)	37.60	19.57	0.79	0.40
Frozen (blanched)	34.88	31.01	1.14	1.00
Raw	39.20	–	1.12	–

Vitamin content

Reduced ascorbic acid. The ascorbic acid content of the vegetables was greatly affected by processing methods (Table 5). The differences between canned and frozen products were dependent on blanching procedure before freezing and inclusion or exclusion of the canned liquid. About 40% of the vitamin in canned products was found in the liquid protion. Other studies also found a relatively large percentage of ascorbic acid in the liquid contents of canned vegetables (Farrel and Fellers, 1942; Kramer, 1979). For freezing, ascorbic acid content was much lower in the unblanched than blanched product, as expected. When steam- and water-blanching were compared in freezing, no difference was shown between the blanching methods with zucchini; however, ascorbic acid content was higher when summer squash was water-blanched than when it was steam-blanched. Uneven scalding appeared to have occurred during steam-blanching of summer squash even though the same procedure was followed for both vegetables.

In general, more ascorbic acid was found in the canned than frozen product when the values of the drained solids and liquid were combined. However, the drained solids alone contained less of the vitamin than the adequately blanched-frozen product. Nearly all ascorbic acid was lost in dehydration. Other researchers also demonstrated high loss of the vitamin during dehydration (Farrel and Fellers, 1942; Homes et al., 1979; Mahoney et al., 1946; Moyer, 1943). The ascorbic acid content of frozen and canned products decreased further during 6 months of storage.

Freezing may be the best preservation method for ascorbic acid content since the liquid portion of canned vegetables is often discarded in actual food preparation and serving.

Carotene. As shown in Table 6, the differences in carotene content among the canned, dehydrated, and frozen products were much smaller in comparison with the differences in ascorbic acid content, especially for carrots which contained an extremely high amount of carotene. There was less than 10% difference in carotene content of variously processed carrots. Zucchini processed by freezing contained more carotene than that processed by canning or dehydration.

After 6 months of storage, frozen and canned vegetables had good retention of carotene. In contrast, nearly half of the carotene in dehydrated vegetables was lost during the storage.

CONCLUSIONS

FROM THE PRESENT comparative study of home canning, freezing and dehydration (with a commercially available electric dehydrator) of carrots, zucchini, and summer squash, it is concluded that freezing requires the shortest processing time and results in products with the best eating quality and the most reduced ascorbic acid (assuming canned liquor not used) and carotene. Although more electric energy is required to process and store vegetables by freezing than by canning or dehydration, the overall cost, includ-

ing packaging cost and amortized cost for equipment, for freezing preservation can be kept as low as or lower than the cost for other preservation methods, providing that the freezer is kept full as much as possible. With the freezer kept only half-full, freezing would cost more than canning or dehydration for a long term (>6 months) storage.

When canning is compared with dehydration, canning uses much less electric energy and may be slightly cheaper in overall preservation cost; also, canned products retain much more reduced ascorbic acid and carotene than dehydrated products. A vegetable should not be dehydrated if it is expected to supply ascorbic acid in the diet since most of the vitamin is lost during the dehydration process. The sensory quality of canned and dehydrated products is rather low for the three vegetables tested in the study.

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CHEMICAL COMPOSITION OF WINGED BEANS. . .From page 1560

ble legumes. Results obtained here show that soaking beans prior to cooking for a sufficiently long time causes a leaching-out of the trypsin factors. Once these factors are eliminated or substantially reduced, the digestibility is improved due to an inhibition on the activity of the antinutritional factors.

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PROTEIN DISPLACEMENT DURING CLASSIFICATION OF GLANDED COTTONSEED

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ABSTRACT

The effects of classification of glanded cottonseed flour on protein displacement were studied. Media were liquid (nonpolar solvent) and air. The fines and coarse fractions had different ratios of water-soluble functional and storage proteins than the starting material. The difference in protein distribution was reflected in the contents of essential amino acids of each fraction. Fines or "overs" fractions had lower amounts of some limiting essential amino acids such as lysine, threonine and leucine as compared with the coarse fraction. Degossypolised cottonseed flour therefore had lower protein quality than the starting material. Although the fines fractions have lower amounts of essential amino acids as compared with the coarse fractions, they did not differ in the type of proteins as shown by gel electrophoresis.

INTRODUCTION

COTTONSEED is an important oilseed in many parts of the world (Harden, 1975) and is used primarily to produce edible oil and in animal feeds. In recent years, processes have been developed to produce edible flours and concentrates (Gardner et al., 1976; Kadan et al., 1979). These protein products are reported to have good protein quality (Martinez et al., 1970; Cherry et al., 1978).

To recover edible protein from glanded cottonseed, one must remove intact pigment glands which contain gossypol, a toxic substance to monogastric animals. All the gossypol is contained in pigment glands, which are about 50–400 μm in diameter and are scattered throughout the kernel (Boatner, 1948). Two processing approaches have been developed for this purpose. Both employ the differences in density, size and shape of the pigment glands from other components of the seed material to obtain gland-free, low-gossypol, edible protein products. These approaches employ classification of either milled full-fat kernel in nonpolar solvents (Gardner et al., 1976) or of defatted milled seed material in an air medium (Meinke and Reiser, 1964; Kadan et al., 1979).

There are two kinds of proteins in cottonseed, i.e. low molecular weight, water soluble, functional (nonstorage) proteins, and high molecular weight storage proteins. The water-soluble proteins are 8–10 in number (gel electrophoresis), and represent about 25% of the total nitrogen. The storage proteins, which are water insoluble, are 2–3 in number, and represent the majority of proteins in cottonseed. The storage proteins are localized in aleurone grain bodies. These bodies range in size from 1–20 μm in diameter and average around 4–6 μm (Yatsu, 1965). The storage and water-soluble proteins have significantly different amino acids composition and functional properties (Martinez et al., 1970). Some of the essential amino acids found in cottonseed have also been reported as "limiting" (Fisher, 1965), even though the protein efficiency ratio (PER) of the degossypolized cottonseed flour is reported to be about 2.5 (Ridlehuber and Gardner, 1974).

Only limited information is available on protein displacement during classification of cottonseed. Cater et al. (1977) concluded that there is no significant protein fractionation during air-classification of glandless defatted cottonseed and that no significant protein shift occurs during centrifugal processing of both glandless and glanded seed. On the other hand, Martinez (1969) reported small but significant differences between the basic amino acid composition of air-classified protein concentrate and the original defatted glandless flour, suggesting a protein shift during air-classification. In another paper Martinez et al. (1970) said that there is no appreciable difference between the protein characteristics of air-classified protein concentrate from those of the proteins of the parent flour.

A clear understanding of the nature of protein displacement is important since it can affect the quality of the final degossypolized product. This study was undertaken to elucidate the extent of protein displacement during classification by centrifugal methods and by differential settling. The latter method produces deglanded material by gravity settling of pigment glands rather than by centrifugal separation and was precursor of classification of glanded cottonseed in both liquid and air medium.

MATERIALS & METHODS

Preparation of full-fat flour

Texas High Plains glanded cottonseed, containing 1.1% free fatty acids and 8.5% moisture, was dehulled, and the whole and partially broken kernels used for classification studies. Portions of the kernels were dried to 1.8% moisture. The dried kernels were ground in a Kolloplex mill at 11,500 rpm to produce milled, full-fat flour which was used for differential settling (DS) and liquid cyclone processing (LCP).

DS and LCP of flour

The technique of Vix et al. (1949) for DS was modified to fractionate comminuted cottonseed kernels. One hundred grams of milled material was suspended in 900 ml of hexane at ambient temperature in a 1-L glass-stoppered graduated cylinder. The slurry was vigorously shaken for 2 min and allowed to settle for 20 min. The supernatant (fines) layer was separated by siphoning to about 0.5 in. above the settled "coarse" fraction. Five hundred milliliters of fresh hexane was added to the coarse fraction and the procedure repeated for removing the fines portion. The coarse fraction was once again suspended and the "fines" fraction removed. The combination of the three fines fractions was filtered on a Whatman #4 filter paper and the resulting cake washed with about 50 ml of hexane to remove the residual lipids. The remaining coarse fraction was also filtered and washed as described above. The filtered fines and coarse fractions were desolventized at room temperature.

LCP was carried out following the procedure of Gardner et al. (1976). The "overs" and "unders" fractions (fines and coarse, respectively) were collected separately to determine the yields of each fraction. An aliquot of each fraction was filtered, and desolventized as described above.

Air-classification Process

Another portion of dehulled kernels was flaked to 0.015 in. thickness on a smooth Ross rolls and dried to about 2.0% moisture in a Proctor and Schwartz oven at 83°C. The dried flakes were extracted in a basket extractor with hexane at ambient temperature and desolventized for 2 hr in a vacuum oven at 83°C. The desolventized flakes were milled at 10,600 rpm with a Rietz mill equipped with a 0.016 in. opening sizing screen to produce defatted flour which was air-classified by the methods of Martinez (1969) and Kadan et al. (1979), except that a Donaldson AcuCut A-12 air-clas-

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Table 1—Protein displacement during classification in liquid and air media—Defatted cottonseed flour

Fractions	Original	Differentially Settled (DS)		Liquid Cyclone Process (LCP)		Air-Classification (AC-I)		Air-Classification (AC-II)	
		Fines	Coarse	Fines	Coarse	Fines	Coarse	Fines	Coarse
Weight fraction, %	100	46	54	42	58	40	60	56	44
Protein, % (N×6.25)	58.8	64.8	53.4	66.9	46.6	68.4	45.5	65.3	49.6
Water-soluble proteins, %	24.6	19.4	29.1	19.1	29.1	20.6	27.7	22.9	26.7
Storage proteins, %	75.4	80.6	70.9	80.9	70.9	79.4	72.3	77.1	73.3
Essential amino acids g/16gN									
Isoleucine	2.51	2.62	2.57	2.63	2.20	1.99	2.40	2.40	2.32
Leucine	5.42	5.36	5.65	5.12	5.66	5.12	6.22	5.44	5.50
Lysine	4.23	4.08	4.42	4.03	4.46	3.92	4.99	4.07	4.33
Methionine	1.23	1.21	1.50	1.29	1.18	1.11	1.49	1.06	1.39
Phenylalanine	5.05	5.18	5.27	5.19	5.13	5.06	5.36	5.27	4.88
Threonine	3.15	2.77	3.44	2.87	3.22	2.87	3.87	3.06	3.20
Tryptophan	1.38	1.23	1.46	1.19	1.69	1.17	1.56	1.52	1.27
Valine	3.59	3.84	3.66	3.46	3.45	2.93	4.13	3.61	3.45

sifier was used in each case. In the Martinez (1969) method of air-classification (AC-I), the milled material is first air-classified to obtain a relatively small "fines" fraction. The larger coarse fraction was then classified and the procedure repeated to produce a total of three fines fractions and one coarse fraction. Rotor speeds of the air-classifier were 1100, 950, and 750 rpm respectively for 1st, 2nd, and 3rd air-classification steps. The Kadan et al. (1979) method of classification (AC-II) is a reverse of the former in that a relatively small course fraction is removed first and the fines fractions are reclassified. Rotor speeds of 750, 850, and 950 rpms were used for 1st, 2nd and 3rd classification steps. The airflow for all air-classification steps was 70 SCFM.

Sample analysis

Various fractions thus obtained were analyzed for proximate analysis by the official AOCS methods (1976). The water soluble proteins were measured as follows: a 5-g sample was suspended in 50 ml of deionized water; the mixture was stirred for 30 min; an aliquot was centrifuged for 15 min at 1080 × G; the supernatant was filtered through Whatman #4 filter paper; protein was determined on an aliquot of clear filtrate; and the amount of soluble (functional) proteins was determined. The storage proteins were determined by subtracting the amount of water soluble proteins from the total proteins. Amino acids contents were determined by the gas liquid chromatographic method of Kaiser et al. (1974) at a commercial laboratory. Spies and Chambers (1948) calorimetric procedure was used to determine tryptophan. Water-soluble and storage proteins were gel electrophoresed by the procedure of Cherry et al. (1970) and Canalco (1973).

Scanning Electron Microscopic Examination

Specimen planchets for the scanning electron microscope (SEM) were covered with an adhesive, which was allowed to dry until it became tacky. They were then touched to the surface of the cottonseed flours to permit particles to adhere. The planchets were tapped to remove excess flour and then coated with gold-palladium by sputtering, to prevent charging while examining in the SEM. Micrographs were made using Polaroid 55 P/N film at 400 × magnification.

RESULTS & DISCUSSIONS

THE PROTEIN distribution and the essential amino acid contents of liquid (DS and LCP) and air-classified (AC-I and AC-II) fractions are shown in Table 1. In all four classifications, the fines fractions had higher amounts of total proteins and lower amounts of water-soluble proteins than the starting defatted cottonseed flour. On the other hand, the coarse fractions had lower amounts of total proteins and higher amounts of water-soluble proteins than the starting material. The amounts of water-soluble proteins in the coarse fractions of liquid classified (DS and LCP) samples were approximately one and a half times than that of fines the fractions. The amount of water-soluble proteins in the fines of the air-classified samples was less than that in the liquid-classified samples. The percentage of water-soluble protein was greater with the higher yield of the fines fraction.

The change in water-soluble proteins distribution during classification was reflected in the essential amino acids composition of the various fractions. Some of the limiting amino acids of cottonseed proteins (Fisher, 1965) such as lysine, threonine and leucine were lower in the fines fractions than in the coarse fraction. Another essential amino acid, tryptophan, was also lower in the fines fractions from DS, LCP, and AC-I produced materials than in the coarse fraction. The proportion of tryptophan reversed in the fractions produced by AC-II method.

In fact, the amounts of other essential amino acids, except for methionine and isoleucine were higher in the fines of AC-II fraction than in the fines fractions produced by the other three methods. The main reason was that the AC-II fines fraction, becuase of its relatively high yield, had some coarse material that would have been part of the coarse fraction. Valine was slightly higher in the fines fraction than in the coarse fraction. These results indicated that during milling of seed material, components that contained water-soluble protein were not sufficiently pulverized. Any undermilled seed material would end up in the coarse fraction. Because the storage proteins are localized in well-defined aleurone grain bodies (Yatsu, 1965), they are probably easier to separate from other cellular material during milling. Thus the majority of these grain bodies were apparently disintegrated into smaller particles and accumulated in the fines fraction. The remaining aleurone grain bodies, which did not break loose from the lumps of kernel tissue, aggregate in the coarse fraction along with the loose pigment glands. Obviously, there would be less protein displacement if proper milling techniques can be developed. Such techniques would preferably pulverize all the glanded cottonseed material, except the pigment glands, to less than 20 μm. The cottonseed pigment glands are fairly resilient under certain processing conditions (Gardner et al. 1976) and efforts to develop better milling methods should therefore prove successful.

The comparative particle size and shape of the starting material and of the various air-classified fractions, prepared by the AC-II method are shown in the SEM photomicrographs (Fig. 1) all of which were taken at 400 × magnification. Components of the starting material are of varying size and are randomly distributed (Fig. 1a). Figure 1b shows the typical particles from the 1st coarse fraction. The large circular particle is a pigment gland and the others are large lumps of cellular mass and a few aleurone grain bod-

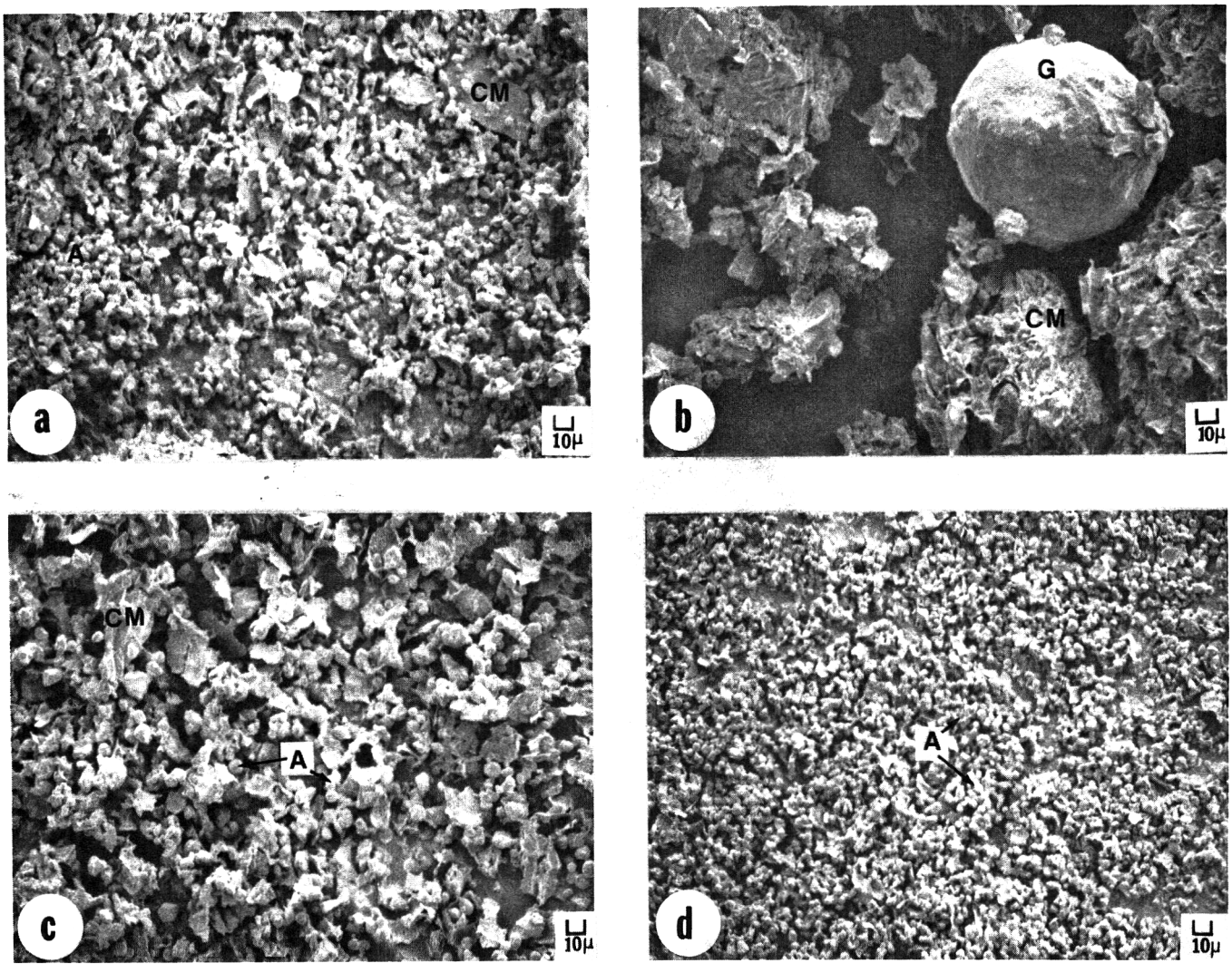

Fig. 1—Scanning electron micrographs of cottonseed flours prepared by AC-II method. Aleurone grain bodies (A), cellular mass fragments (CM), and pigment gland (G). Taken at a magnification of 285 (a) Original defatted flour (starting material), (b) Fraction 1; (c) Fraction 2; (d) Fraction 4 (Fraction 3, not shown).

ies. The average particle size of 2nd coarse fraction (Fig. 1c) is smaller than the 1st coarse fraction. Lumps of cellular mass, aleurone grain bodies and few pigment glands made up this fraction. The 3rd coarse fraction (not shown) had even smaller average particle size, otherwise the same kinds of particles were found as in the 2nd coarse fraction. Figure 1d shows the 4th fraction, i.e., final 'fines' fraction. The average particle size is about 4–6 μm and it consists primarily of aleurone grain bodies.

In Table 2 are presented the protein and amino acids

Table 2—Protein displacement during air-classification—Defatted cottonseed flour

Fraction ¤	Original	Air-classification I Coarse fraction reclassified				Air-classification II Fines fraction reclassified			
		1	2	3	4	1	2	3	4
Weight Fraction, %	100	40	13	12	35	44	8	9	39
Protein, % (N×6.25)	58.8	68.4	62.2	56.6	46.8	49.6	57.9	61.5	68.0
Water-soluble Proteins, %	24.6	20.6	22.0	25.2	27.6	26.7	23.9	22.6	20.8
Storage Proteins, %	75.4	79.4	78.0	74.8	72.4	73.3	76.1	77.4	79.2
Essential amino acids, g/16gN									
Isoleucine	2.51	1.99	2.46	2.79	3.25	2.32	2.47	2.70	2.74
Leucine	5.42	5.12	5.47	5.80	6.80	5.50	5.59	5.63	5.39
Lysine	4.23	3.92	4.13	4.33	4.36	4.33	4.25	4.21	3.96
Methionine	1.23	1.11	1.50	1.56	1.71	1.19	1.28	0.81	1.26
Phenylalanine	5.05	5.06	5.23	5.22	6.01	4.88	5.23	5.45	5.21
Threonine	3.15	2.87	3.07	3.09	3.57	3.20	3.16	3.14	2.75
Tryptophan	1.38	1.17	1.40	1.27	1.18	1.27	1.52	1.54	1.46
Valine	3.59	2.93	3.48	3.94	4.60	3.45	3.64	3.89	3.93

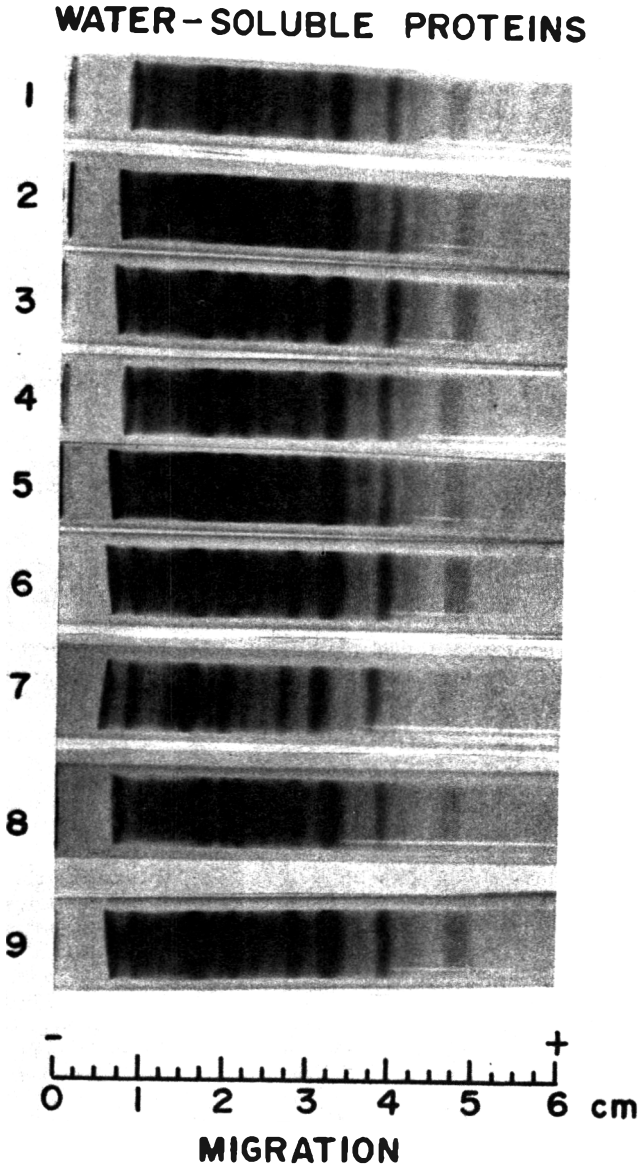

Fig. 2—Polyacrylamide disc-gel electrophoretic patterns of water-soluble proteins from cottonseed protein fractions obtained by classification in liquid and air media: 1. Whole cottonseed proteins (as is). 2. Fines fraction from differential settling process. 3. Coarse fraction from differential settling process. 4. Fines fraction from liquid cyclone process. 5. Coarse fraction from liquid cyclone process. 6. Fines fraction from AC-I process. 7. Coarse fraction from AC-I process. 8. Fines fraction from AC-II process. 9. Coarse fraction from AC-II process.

contents of fractions obtained by the two air-classification methods. For the AC-I method, when the coarse fraction was reclassified, total protein content decreased, water-soluble proteins increased and the essential amino acids also increased. The results again showed that increase in protein during centrifugal classification is due largely to the preferential concentration of storage proteins, that is, loose aleurone grain bodies in the fines fraction. As the fraction increased in particle size, it has fewer aleurone grain bodies. This relation is reflected in the protein quality of subsequent coarse fractions. All essential amino acids except tryptophan progressively increased in each coarse fraction. Removal of pigment glands by this method of air-classification is done by sacrificing the quality of final low-gossypol product.

The data from AC-II method of classification are the reverse of AC-I method except for the content of essential amino acids. The amino acids from AC-II fractions did not follow any pattern, perhaps because of breakage of large lumps into small particles during subsequent air-classification. The fraction 4 of AC-II is comparable to fraction 1 of AC-I, both are fines fractions and have comparable yields and protein contents. However, the essential amino acids of fines fraction from AC-II are higher than the fines fraction from AC-I, indicating that re-air-classification of fines, produces material not only with lower gossypol (Kadan et al., 1979) but also with higher amounts of essential amino acids and hence better quality protein than the single air-classification method such as AC-I.

The gel electrophoresis of the water-soluble proteins from coarse and fines fractions from air- and liquid-classified samples in Table 1 is shown in Figure 1. A comparison of water-soluble protein patterns showed that there is no significant variation in the gel electrophoretic pattern of their water-soluble proteins (Fig. 2). Similar proteins were present in all samples. There seem to be some differences in the amounts of proteins in the sample applied to the gel because the concentration of proteins in each band is not similar. This variation may result from the differences in the amount of water soluble proteins in each fraction. The electrophoretic patterns show that the same type of protein bands are distributed in each fraction. Water-insoluble (storage protein) fractions (not shown) also did not show differences.

There is a significant protein displacement during centrifugal classification of glanded cottonseed proteins. However, there is no apparent difference between classification in liquid and air medium. The storage proteins, localized in aleurone grain, concentrate in the fines fraction, thus making it rich in total protein content but lower in essential amino acids than the coarse fraction. Gel electrophoretic patterns showed that the same types of proteins are distributed in the fines and coarse fractions.

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QUALITY CHANGES IN BULK STORED CITRUS CONCENTRATE MADE FROM FREEZE-DAMAGED FRUIT

D. L. HENDRIX and R. C. GHEGAN

ABSTRACT

Four batches of frozen concentrated orange juice were prepared using commercial equipment. The juice from three batches of the "Hamlin-Pineapple" cultivar and one batch of a "Valencia" cultivar was mechanically extracted and concentrated on four different occasions. The control was prepared from fruit harvested prior to the January 18–20, 1977 freeze. The remaining three batches were prepared from fruit harvested at 5, 28, and 180 days after the freeze. Samples from each batch were stored at −20.5°C and −7.7°C. Samples were analyzed every 2 wk to compare the changes in the quality of concentrate made from freeze damaged fruit to the control. Three quality characteristics, Brix/acid ratio, bottom pulp, and apparent viscosity, showed distinct differences between the control and freeze-damaged samples. In the freeze-damaged samples the Brix/acid ratio were initially found to be higher and continued to increase with storage. Bottom pulp content increased with storage in the freeze-damaged samples as did apparent viscosity.

INTRODUCTION

THE STORAGE of frozen concentrated orange juice (FCOJ) in large tanks is a relatively new approach in the citrus industry. Tank farms consist of large stainless steel tanks usually situated in a large refrigerated room (Riley, 1972) and range in size from 568,000 liters to over 18,000,000 liters.

The use of a tank farm for bulk storage has risks. No information is available from actual commercial scale investigations on quality changes during storage. Risks are increased during a freeze year because of the unknown effects the freezing temperatures can have on the fruit prior to processing. With the possibility of a severe freeze during any winter, knowledge is needed to determine the effects a freeze can have on quality characteristics of juice concentrates made from freeze-damaged fruit.

Investigators have previously looked at the effects a freeze has on concentrate. These studies provide an insight into the processing and packaging of FCOJ prepared from freeze-damaged fruit. However, there is no information on the effects of a freeze on FCOJ that is going into bulk storage.

Rouse et al. (1958), reported a decrease in acidity, pectinesterease activity, and percentage light transmittance in "Pineapple" type oranges along with an increase in the Brix/acid ratio, pH, and viscosity.

Westbrook (1957), also revealed an increase in Brix/acid ratio, apparent viscosity, and pulp content along with a decrease in acidity.

Studies by other investigators have not necessarily substantiated these results. This does not indicate that the individual studies are of no importance. This probably indicates the effects of each freeze have been somewhat different.

In 1972, Wardowski and Grierson investigated freeze patterns in oranges. Their work revealed the part of the orange that was damaged by freezing temperatures will vary, depending on whether the freeze is a radiation or convection type freeze. Varying freeze patterns in the orange affect the fruit membranes differently which results in varying effects on the FCOJ made from freeze-damaged fruit. Thus, because of the changing freeze patterns, the results of experiments on freeze-damaged fruit have not necessarily been the same from freeze to freeze.

In addition to difficulties caused by differences among freezes, the use of data from research done under laboratory conditions is sometimes difficult to use in manufacturing practice. Therefore, the purpose of this study was to determine, under commercial conditions, the quality changes in FCOJ made from freeze-damaged fruit during storage in a tank farm.

MATERIALS & METHODS

FOUR BATCHES of FCOJ, three of the "Hamlin-Pineapple" cultivars and one "Valencia," were processed and concentrated on four different processing dates. All four batches were 10,500 liters each. The number one sample of juice was processed and concentrated on January 11th, prior to the January 18–20, 1977 freeze. Juice sample number two was processed 5 days after the January 18–20 freeze on January 25th. Juice sample number three was processed 28 days after the January freeze on March 3rd. Juice sample number four, the "Valencia" sample, was similarly processed 180 days after the January freeze on June 3rd. For each batch, the juice was extracted from fruit using FMC Corporation's in-line extractors. The juice was finished with FMC Corporation's screw-type finishers (Model 75), equipped with 0.050–0.058 cm screens. Finished juice was concentrated by using a 36,287 kg H_2O/hr thermal accelerated short-time (TASTE) evaporator. The concentrated orange juice was pumped from the evaporator to a tank mounted on a load cell for weighing then into the tank farm.

Samples from each batch were taken at the load cell tank. Forty 6-oz cans (177.5 ml) were sterilized, filled with concentrate, and closed. Twenty cans from each batch were stored in the bulk storage tank farm at −7.7°C. The remaining twenty cans from each batch were stored at −20.5°C. One can representing each temperature and batch was analyzed every 2 wk for a period of 6 months.

Sample evaluations

Standard citrus quality control evaluation methods (Hendrix and Jefferson, 1966; Hendrix et al., 1977), were used to analyze 15 quality control parameters. Brix/acid ratio, color/flavor/defects, bottom pulp, pH, pectinesterease activity, gelation, cloud stability, recoverable oil, diacetyl, microbiological, and apparent viscosity were determined.

Microbiological examination

One can representing each storage temperature was thawed under running tap water. The can was aseptically opened and sampled for plating. One ml of concentrate was diluted to 1:1000 and 1 ml of the dilution was plated on Difco orange serum agar incubated at 32°C for 48 hr and Difco malt agar incubated at room temperature for five days using a pour plate technique (Hendrix and Jefferson, 1966).

Viscosity measurements

Apparent viscosity was determined on each can using a Brookfield synchrolectric viscometer (Model LVT) with a number four

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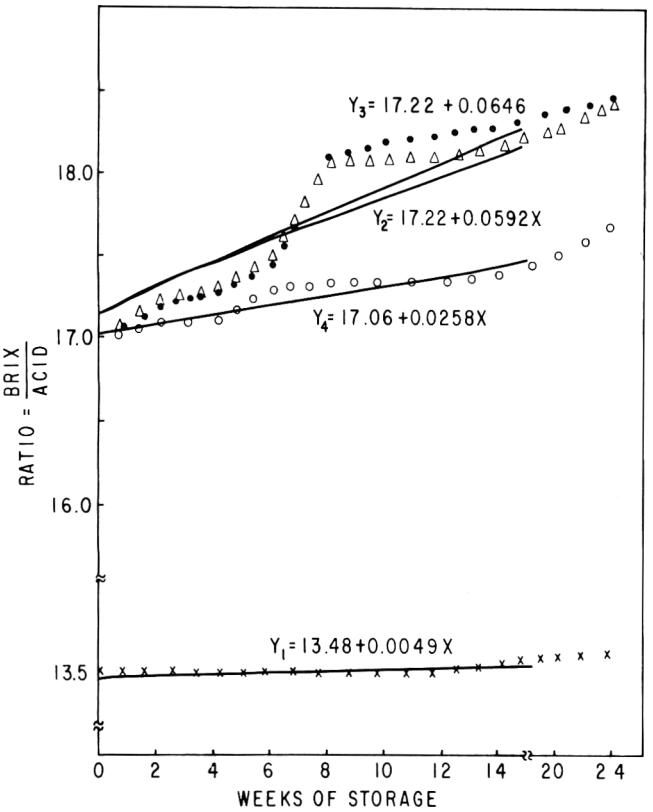

Fig. 1—Comparison of the changes in Brix/acid ratio of the four samples during the 6 months of storage, with linear regression analysis. Sample 1: X X X (Control); Sample 2: Δ Δ Δ (Freeze damaged-5 days after freeze); Sample 3: ● ● ● (28 days after freeze); and Sample 4: ○ ○ ○ (180 days after freeze).

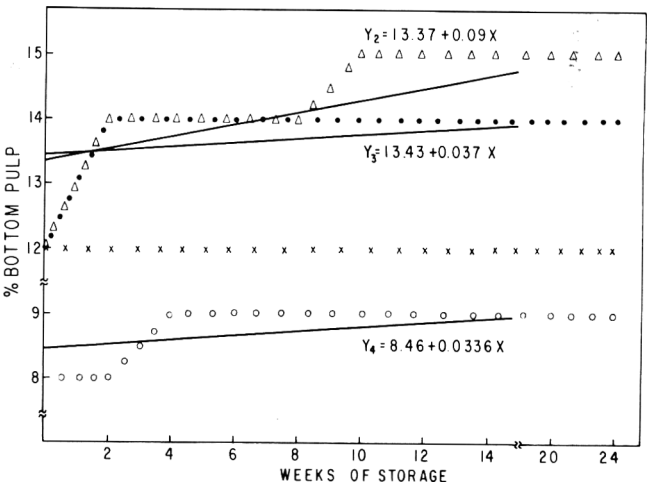

Fig. 2—Comparison of the changes in bottom pulp in the four samples during the 6 months of storage, with linear regression analysis. (Symbols: Same as Fig. 1.)

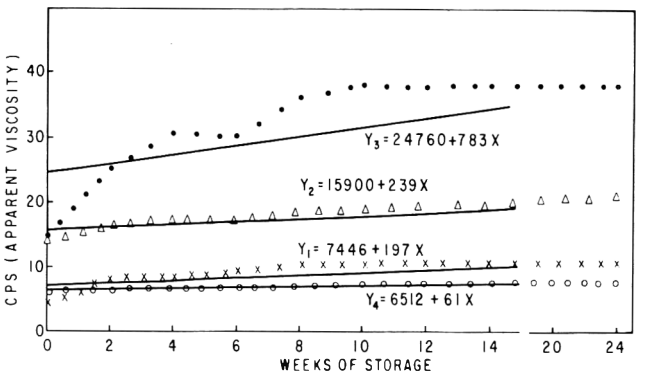

Fig. 3—Comparison of the changes in apparent viscosity of the four samples during the 6 months of storage, with linear regression analysis. (Symbols: Same as Fig. 1.)

spindle operated at 12 rpm. Each can was diluted to 45° Brix and warmed to 15.5°C before viscosity determination was made.

RESULTS

COMPARISON of the three freeze-damaged samples with the control revealed that certain qualities of FCOJ are effected when fruit has been exposed to temperatures below freezing. Three of the 15 parameters analyzed (Brix/acid ratio, bottom pulp, and apparent viscosity) showed definite differences between the control sample and those from the freeze-damaged fruit.

The remaining parameters showed no changes. This was probably because processing parameters were adjusted in order to insure the production of Grade A concentrate (such as changes in settings of the extractors, finishers, and evaporators). Studies by Veldhuis and Kew (1962), Kew and Veldhuis (1962), DuBois and Kew (1951), Cotton et al. (1947), and McCulloch et al. (1956), showed how increased evaporator temperatures can stabilize certain qualities such as cloud stability, gelation, pectinesterease and diacetyl.

No microbiological problems were encountered probably because of good grading of fruit along with higher than normal evaporation temperatures.

Brix/acid ratio determination revealed a decrease in acid during 6 months of storage. This decrease in acid resulted in an increase in the ratio (Fig. 1). This finding is consistent with previous findings (Rouse et al., 1958; Westbrook, 1957). Initial Brix/acid ratios were higher in the freeze-damaged samples as compared with the control.

During the 6-month study, bottom pulp content showed an increase in the freeze-damaged samples with no increase in the control (Fig. 2). The increase in bottom pulp was attributed to membrane degradation typical of freeze-damaged fruit (Wardowski and Grierson, 1972).

Measurements of apparent viscosity revealed the greatest increase of all 15 parameters tested. Figure 3 shows the comparison of the control with the freeze-damaged samples. The control had the lowest initial apparent viscosity which increased slightly till the 12th wk of storage. Sample #2, processed 5 days after the freeze, had an initial apparent viscosity higher than that of the control and increased until the 20th wk of storage. In Sample #3, processed 28 days after the freeze, the initial apparent viscosity was comparable with that of Sample #2. However, the increase shown in Sample #3 was at a much higher rate. Sample #4, the "Valencia" sample, processed 180 days after the freeze, had an initial low apparent viscosity comparable with that of the control. This low apparent viscosity in "Valencia" type fruit, regardless of the amount of freeze damage, was also reported by Westbrook (1957). Huggart et al. (1973) reported that the heat treatment used in the processing of FCOJ protects the water-soluble pectin from deesterification caused by pectinesterease. This precludes more of the pectin fraction from being converted into insoluble pectinates and pectates which can contribute to high viscosity in concentrates. All four samples were subjected to the same

temperature for the same length of time in the evaporators. So, apparently there was initially more water-soluble pectin in the freeze-damaged samples.

DISCUSSION

COMPARISON OF DATA from the control and the three freeze-damaged samples revealed quality changes do occur in FCOJ made from freeze-damaged fruit while stored in a bulk storage tank farm.

Comparison of data from the two storage temperatures revealed no significant differences. However, with the current high cost of energy, this finding could have possible economic implications. An additional more specific study on storage temperatures might reveal that the quality characteristics achieved with current temperatures for bulk storage could be met at higher, more economical temperatures.

Test data indicated possible viscosity problems during freeze years. Sizing of pumps and lines for a bulk storage tank farm therefore becomes critical. Without adequate planning, thousands of gallons of concentrate could be pumped into a tank farm only to find out at a later date the concentrate has become too viscous to pump out of the tanks.

For manufacturing of chilled juice in glass containers and manufacturing of concentrate for dispensers, bottom pulp could cause minor problems. These include excess sediment in glass containers making the product esthetically unpleasing and could cause plugging of juice dispensers making marketing of the product difficult. With the amount of bottom pulp in the concentrate increasing during storage, processors may find it necessary to centrifuge the product before use, causing loss of product.

Substandard product due to a high Brix/acid ratio is usually of little concern because the situation does not normally arise. However, this could become a real problem in a freeze year. Not only did the freeze-damaged samples have an initially higher Brix/acid ratio, the ratio continued to increase throughout the 6 month study.

Finally, although it was evident that certain quality factors change, it was also evident that most quality factors can be maintained by good manufacturing practices. Changes in manufacturing processes can compensate to insure a final product with Grade A quality.

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EFFECT OF MICROWAVE BLANCHING ON THE COLOR AND COMPOSITION OF STRAWBERRY CONCENTRATE

R. E. WROLSTAD, D. D. LEE, and M. S. POEI

ABSTRACT

The influence of native enzymes on the color and composition of strawberry juice and concentrate was investigated through analyses of juice and concentrate which had been made from microwave-blanched and from unheated fruit. Changes in anthocyanin (ACN) pigment, leucoanthocyanins, flavanols, total phenolics, ascorbic acid, and dehydroascorbic acid were monitored during 8 wk storage at 20°C; Hunter color parameters, polymeric color, and browning were also measured. Blanching results in improved color stability and has a protective effect on ACN pigments, reactive phenolics, and ascorbic acid. Blanched strawberry concentrate browns slower than the control. Monomeric ACN pigment undergoes rapid degradation to form polymeric pigments which are resistant to bisulfite bleaching.

INTRODUCTION

VARIABILITY in color and lack of color stability are problems of major concern to manufacturers of strawberry juice concentrate. A previous study (Abers and Wrolstad, 1979) compared the composition and color quality of strawberry preserves made from two different varieties. Results showed that the variety higher in leucoanthocyanins, flavanols, and total phenolics yielded a preserve with less color stability during storage. The authors proposed that reactive phenolics were involved in the color deterioration and the degradation reactions could be enzymic and/or chemical. Numerous workers have demonstrated that various enzyme systems—polyphenoloxidase (Peng and Markakis, 1963; Pifferi and Cultrera, 1974; Cash et al., 1976), glycosidase (Huang, 1955), peroxidase (Grommeck and Markakis, 1964)—accelerate the destruction of ACN pigment. Pallavicinni (1969) and Cash and Sistrunk (1971) have reported the presence of polyphenoloxidase in strawberries.

Nebesky et al. (1949) and Lukton et al. (1956) have reported that oxygen accelerates pigment degradation in strawberry juice. Several workers (Meschter, 1953; Markakis et al., 1957; Sondheimer and Kertesz, 1953) have reported that ascorbic acid degradation products accelerate pigment degradation in strawberry products. Therefore ascorbic acid and dehydroascorbic along with reactive phenolics—ACN, leucoanthocyanins, and flavanols—were monitored during storage. The influence of nitrogen saturation on juice color was also studied. The major purpose of this investigation was to examine the effect of blanching on the color and composition of strawberry concentrate.

EXPERIMENTAL

Preparation of juice and concentrate

Strawberries (mixture of Hood and Benton varieties) were obtained from Clermont West Co., Forest Grove, Ore. The fruit was washed, destemmed, decapped, individually quick frozen (IQF) at −40°C, and stored at −30°C.

A 4-kg lot of IQF fruit was blanched by heating to an internal temperature of 82–88°C in a Litton System 70/50 microwave oven for 3–4 min; (fruit was heated statically). Loss of polyphenolase activity was indicated by irrigating the surface of halved fruit samples with 10 mM catechol solution. Another 4-kg lot of fruit was thawed at room temperature. Both blanched and unblanched fruit were made into juice using an Ostic automatic juice extractor. The juice was depectinized by treating with 0.2% (v/v) Pectinol-L (Rohm and Haas Co.) and incubating in a 37°C water bath for 4 hr. The juice was strained through a nylon cloth and vacuum filtered through Whatman No. 1 filter paper coated with a 1 cm layer of celite in a Buchner funnel. Approximately 3L of 9° Brix blanched and unblanched juice were obtained.

Blanched and unblanched juice was concentrated (58° Brix) in a Buchi rotary evaporator (water bath = 37°C; pressure = 29 in. Hg).

Juice and concentrate preparations were transferred to 500 ml glass stoppered Erlenmeyer flasks; potassium sorbate (0.1% w/v) was added to prevent microbial growth. Some blanched and unblanched juice samples were sparged with nitrogen; other samples remained exposed to air. All samples were stored in the dark at 20°C.

Compositional and color measurements were made periodically over an 8-wk storage period.

Compositional analyses

Monomeric anthocyanin (ACN) pigment content was determined using a pH differential method (Wrolstad, 1976). ACN concentration is reported as mg of pelargonidin-3-glucoside per g fruit using a molecular absorbtivity of 22,400.

Flavanols and leucoanthocyanins were determined by the methods of Swain and Hillis (1959). Results are reported as absorbance units per g fruit.

Total phenolics were determined by the method of Singleton and Rossi (1965). Results are reported as mg of gallic acid equivalents per g fruit.

Ascorbic acid was determined by the indophenol method of Loeffler and Ponting (1942) and dehydroascorbic acid content was assayed by the method described by Pearson (1976). Results are reported as mg/kg fruit.

All analyses were performed in duplicate; mean values are reported.

Color analyses

Color density (sum of the absorbance at 420 and 500 nm of strawberry juice), polymeric color (sum of the absorbance at 420 and 500 nm of bisulfite-treated juice) and % polymeric color (polymeric color/color density × 100) were determined from spectral analyses as described by Somers (1972) and Wrolstad (1976). Browning was measured as the absorbance at 420 nm of the bisulfite-treated sample. Dilution factor corrections were made to express results as single-strength juice equivalents.

Color was determined on a Hunter model D25P-2 color difference meter using the transmission mode with the light source pivoted and the specular component excluded (arrangement III). The instrument was standardized against a white tile (DC 122, L = 94.0, a = −0.9, b = +1.2). Samples were stored in lucite 5 cm × 5 cm × 1 cm cells and measured at weekly intervals during the 8-wk storage study.

Gel-filtration

One ml of juice or concentrate diluted to single strength was applied to a 1 × 50 cm solvent resistant column filled with Sephadex G-25 fine previously swelled overnight in 3% aqueous acetic acid. The column was developed with 3% aqueous acetic acid for 3½ hr after which the residue was eluted with 50% aqueous acetone-HCl (1.5 ml conc HCl/L of 50% acetone-water). The absorbance of the

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Table 1—Changes in anthocyanin pigment[a] in strawberry juice and concentrate during storage

Sample day	0	3	6	9	13	20	34	55	% loss @ 55 days
Blanched juice	0.224	0.204	0.135	0.119	0.090	0.077	0.021	0.011	95.0
Blanched juice-N_2	0.237	0.210	0.160	0.141	0.121	0.077	0.032	0.017	92.8
Blanched conc	0.374	0.228	0.163	0.133	0.111	0.069	0.032	0.014	96.2
Control juice	0.127	0.098	0.068	0.055	0.042	0.027	0.011	0.006	95.3
Control juice-N_2	0.139	0.110	0.082	0.073	0.053	0.029	0.015	0.006	95.6
Control conc	0.245	0.148	0.103	0.083	0.063	0.056	0.020	0.014	94.3

[a] Expressed as mg/g fruit. Values are the means of replicate determinations which varied ± 1% or less.

effluent was monitored at 500 nm with a Beckman DB-G spectrophotometer equipped with a flow cell assembly and strip chart recorder. After passing through the spectrophotometer the effluent was collected in 10 ml quantities in a fraction collector (ISCO-Model 272) coupled with an ISCO Model 400 Volumeter. The response of the fractions to bleaching with bisulfite was determined as described by Somers (1972). Peak areas were determined by triangulation.

Statistical methods

Analysis of variance was applied to changes of ascorbic acid and dehydroascorbic acid in the samples. F values were tabulated for each treatment and because of the very small sample size, the level of significance was determined by comparing F values for all treatments within the same analysis. Any treatment with a markedly high F value within the particular analysis was considered statistically significant.

RESULTS & DISCUSSION

Compositional changes

Quantitative ACN changes for the juice and concentrate samples are shown in Table 1. Initial ACN content is substantially higher in the blanched samples than in the controls which can be attributed to enzyme action in the control samples during depectinizing. Nitrogen sparging had a sparing effect on ACN. All samples showed very rapid destruction of ACN during storage: over 50% degradation occurred during 2-wk storage. Over 90% of the monomeric anthocyanin was degraded during the 8-wk experiment.

Table 2—Changes in leucoanthocyanins, flavanols and total phenolics in strawberry juice and concentrate during storage

Sample day	0	13	55	% loss at 55 days
Leucoanthocyanins[a]				
Blanched juice	1.88 ± 0.2	1.26 ± 2.5	0.78 ± 0.1	58.5
Blanched juice-N_2	1.92 ± 0.2	1.87 ± 3.4	0.91 ± 0.1	52.6
Blanched conc	1.47 ± 0.3	1.12 ± 2.4	1.03 ± 0.1	30.0
Control juice	1.42 ± 0.2	0.69 ± 3.5	0.50 ± 0.1	64.8
Control juice-N_2	1.53 ± 0.2	1.27 ± 4.1	0.59 ± 0.2	61.4
Control conc	1.12 ± 0.2	0.98 ± 0.2	0.64 ± 0.2	42.7
Flavanols[a]				
Blanched juice	6.78 ± 1.5	5.72 ± 4.2	3.27 ± 0.7	51.3
Blanched juice-N_2	7.05 ± 0.2	6.13 ± 1.3	4.45 ± 0.9	36.9
Blanched conc	4.73 ± 6.2	3.85 ± 0.1	3.82 ± 1.0	19.2
Control juice	5.38 ± 5.7	4.17 ± 4.0	2.34 ± 0.1	56.5
Control juice-N_2	5.64 ± 0.8	4.34 ± 0.2	2.96 ± 1.1	47.1
Control conc	3.25 ± 0.4	3.19 ± 0.2	2.72 ± 0.1	16.1
Total phenolics[b]				
Blanched juice	1.35 ± 2.2	1.34 ± 0.8	0.97 ± 0.2	28.2
Blanched juice-N_2	1.49 ± 1.2	1.16 ± 0.2	1.04 ± 0.2	30.2
Blanched conc	1.30 ± 1.7	1.18 ± 0.5	1.00 ± 0.6	23.1
Control juice	1.05 ± 0.5	1.78 ± 0.7	0.67 ± 0.4	36.2
Control juice-N_2	1.09 ± 0.5	0.82 ± 0.3	0.73 ± 0.2	33.0
Control conc	0.90 ± 1.2	0.85 ± 0.2	0.74 ± 0.2	17.8

[a] Expressed as absorbance units per g fruit ± % variation of the means.
[b] Expressed as mg gallic acid per g fruit ± % variation of the means.

Changes in leucoanthocyanin, flavanols, and total phenolics are shown in Table 2. The blanched samples contained significantly higher levels of leucoanthocyanins than the controls. Manufacture into concentrate resulted in lower leucoanthocyanin concentration and nitrogen sparging had a small protective effect on these reactive phenolics in the juice samples. All samples showed considerable leucoanthocyanin loss during storage, the controls losing more on a percentage basis than the blanched samples. Flavanol and total phenolic losses follow patterns similar to the leucoanthocyanins.

The change of ascorbic acid content in the juice and concentrate samples during storage is shown in Figure 1.

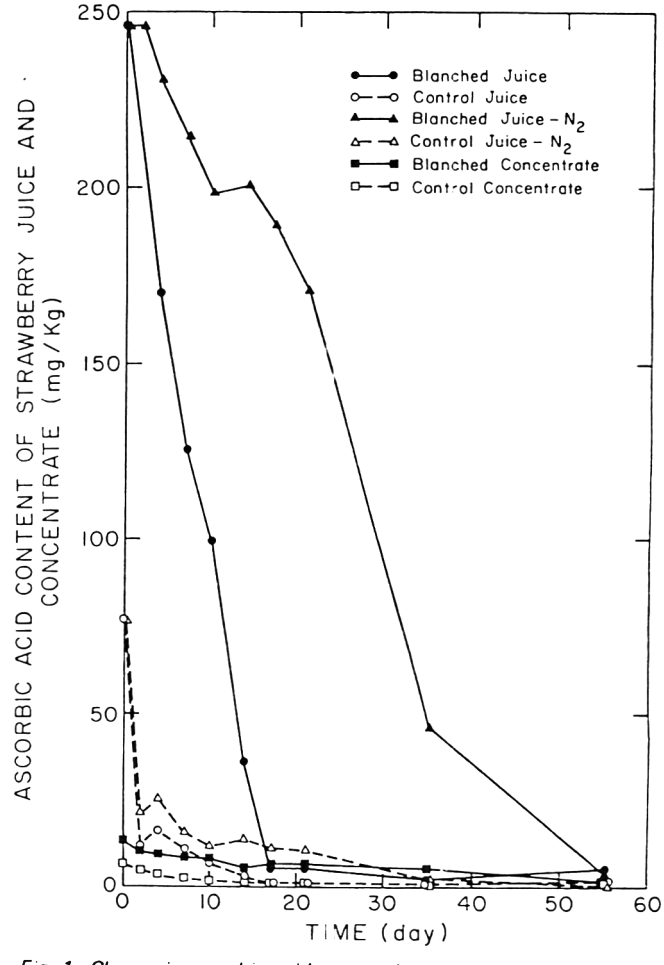

Fig. 1—Change in ascorbic acid content in strawberry juice and concentrate during storage. Average variation of the means: control conc = 11.77% control juice = 6.15%; control juice—N_2 = 9.26%; blanched conc = 3.09%; blanched juice = 1.64%; blanched juice—N_2 = 2.48%.

Table 3—F value of the statistical analyses of ascorbic and dehydroascorbic

Treatment	Parameter Measured: Change of ascorbic acid content	Change of dehydroascorbic acid content	Change of dehydroascorbic + ascorbic acid content
Blanching	5.729*	3.248	5.947*
Nitrogen	1.275	1.428	1.262
Blanching + Nitrogen	1.0	1.0	1.0

* Statistically significant result

Initially, the ascorbic acid content of the blanched juice was more than three times that of the unblanched samples. The larger F value for the blanching treatment (Table 3) indicates that blanching has a statistically significant effect on the loss of ascorbic acid during storage. The mechanism for enzymic degradation could be direct by ascorbic acid oxidase or indirect through oxidase enzymes such as polyphenoloxidase, cytochrome oxidase, or perioxidase (Ponting and Joslyn, 1948). Green (1971) reported that in soft fruits such as berries and currants, ascorbic acid loss is due mainly to ascorbic acid oxidase. The ascorbic acid content of the concentrate samples was very low (< 15 mg/kg) indicating ascorbic acid destruction during concentration; concentrate from blanched fruit had slightly higher ascorbic levels than concentrate from unblanched fruit during the earlier stages of storage. While enzymes accelerated ascorbic acid destruction, all samples contained negligible ascorbic acid at the end of the experiment. Ascorbic acid retention was somewhat improved by replacing air with nitrogen. The protective effect of nitrogen seemed more pronounced in blanched juice than in the control. Ascorbic acid destruction appears to proceed anaerobically as well as aerobically; Kirata and Sakurai (1967a, b) have proposed nonenzymic mechanisms for oxidative and nonoxidative degradation of ascorbic acid.

Change of dehydroascorbic acid content in juice and concentrate samples during storage is shown in Figure 2 and the results are what one would expect from degradation of ascorbic acid via dehydroascorbic acid. Dehydroascorbic acid levels are higher in the control juice samples during the early stages of the experiment indicating enzymic formation of dehydroascorbic acid. Nitrogen treatment appeared to delay dehydroascorbic formation, however the F value (Table 3) is quite low and considered insignificant. Dehydroascorbic acid levels were extremely low in the concentrates indicating degradation during concentration. Dehydroascorbic acid does not persist after its formation, but undergoes further degradative reactions. While blanching and nitrogen treatment appear to delay ascorbic and dehydroascorbic acid losses, all samples contain negligible quantities at the end of the experiment.

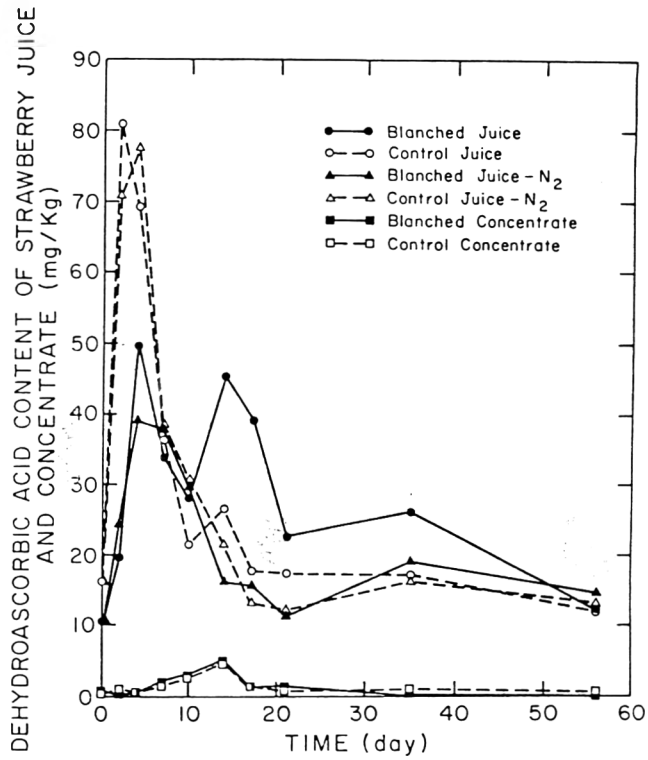

Fig. 2—Change of dehydroascorbic acid content of strawberry juice and concentrate during storage. Average variation of the means: control conc = 12.05%; control juice = 2.83%; control juice—N_2 = 4.20%; blanched conc = 12.07%; blanched juice = 9.57%; blanched juice—N_2 = 9.27%.

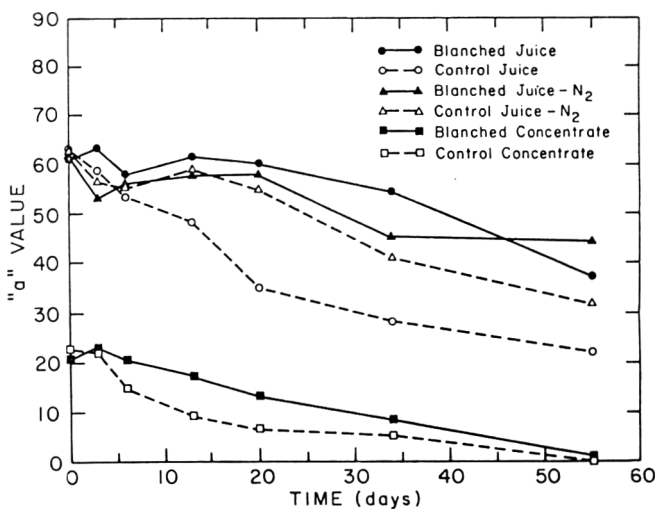

Fig. 3—Change of Hunter a value (transmittance) in strawberry juice and concentrate during storage.

Color changes

While over 90% of the anthocyanin pigment was degraded by the end of the experiment (Table 1), the samples were still reddish in color. Figure 3 shows the change in Hunter "a" value for the juice and concentrate samples during storage. All samples showed a decline in Hunter "a" value, blanched juice samples maintaining higher "a" values than the controls. Concentrate samples had lower "a" values, blanched concentrate samples retained slightly higher "a" values than the controls. The change in Hunter L value for the samples during storage is shown in Figure 4. Concentrate samples decreased in L value during storage (became darker) and juice samples increased (became lighter). The control sample which was exposed to air showed the most marked change. The pronounced lightening is believed to be due to accelerated oxidation, polymerization, and precipitation of pigment complexes.

Figure 5 shows the change in "percent polymeric color" in the juice and concentrate samples during storage. This value is derived from spectral measurements and is based on the resistance of polymeric pigments to decolorization by bisulfite. Concentrate samples possessed more polymeric

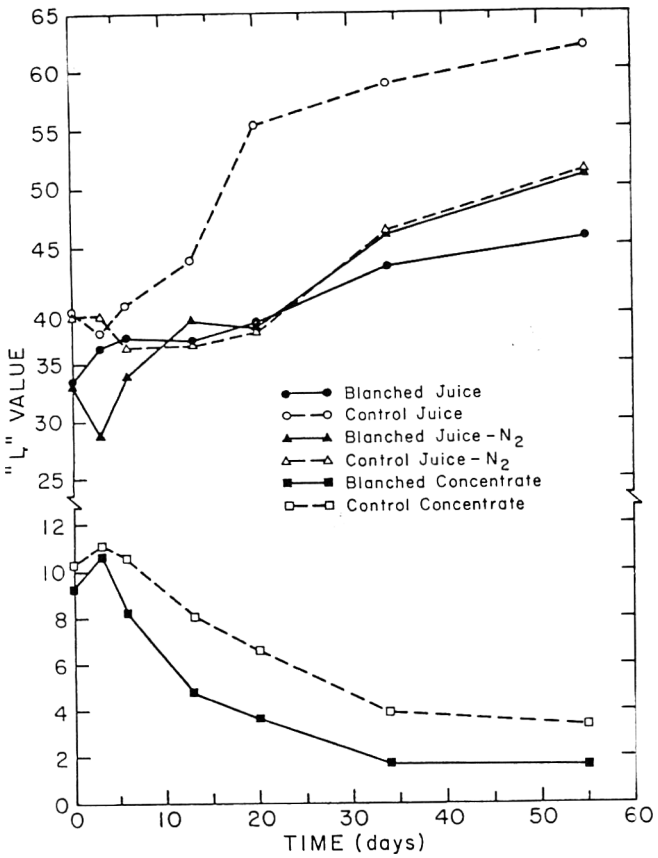

Fig. 4—Change of Hunter L value (transmittance) in strawberry juice and concentrate during storage.

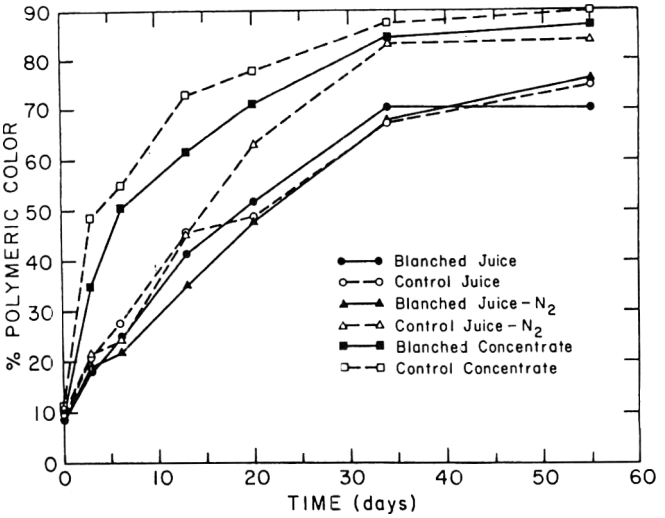

Fig. 5—Formation of percent polymeric color in strawberry juice and concentrate during storage.

color, the control sample containing slightly higher amounts than the blanched concentrate. The juice samples showed similar rates of polymer development, except for the nitrogen-treated control sample which had a higher level of polymer color. Strawberry concentrate pigments were also separated by gel filtration (Fig. 6). Pure pelargonidin-3-glucoside, the major ACN of strawberries (Sondheimer and Kertesz, 1948), had an elution volume which corresponded to the first fraction of strawberry concentrate which was eluted with 3% aqueous acetic acid. This fraction was bright red in color and readily decolorized with bisulfite. The second peak which was eluted with 50% aqueous acetone-HCl was resistant to bleaching by bisulfite, a characteristic similar to that of the polymeric wine pigments described by Somers (1971). As the monomeric pigment was eluted first, the mechanism for pigment separation was presumably by the absorptive property of the gel rather than by molecular sieving. Peak area measurements indithat the monomeric pigment decreased to 12% of the original monomeric pigment after 55 days of storage; the polymeric peak increased by 70%. This compares to an increase in polymeric color by 80% in this same sample as measured by spectral methods. These results indicate that anthocyanin pigments in strawberry juice and concentrate are following degradative patterns similar to those described by Somers (1971) for wine pigments, i.e. monomeric ACN pigment is displaced progressively and irreversibly by polymeric pigments which are resistant to bleaching by bisulfite.

In an earlier investigation on the causes of color deterioration in strawberry preserves (Abers and Wrolstad, 1979), it was concluded that the more rapid color deterioration in preserves made from the Tioga variety was due to their more rapid rate of browning. Figure 7 shows the change in browning for the juice and concentrate samples. Blanched juice samples show more browning than control juice samples; presumably this difference was induced by heat during blanching. The juice samples exhibited similar patterns for increase of browning during storage. The concentrate samples have a much higher browning index as would be expected from the conditions of temperature and concentra-

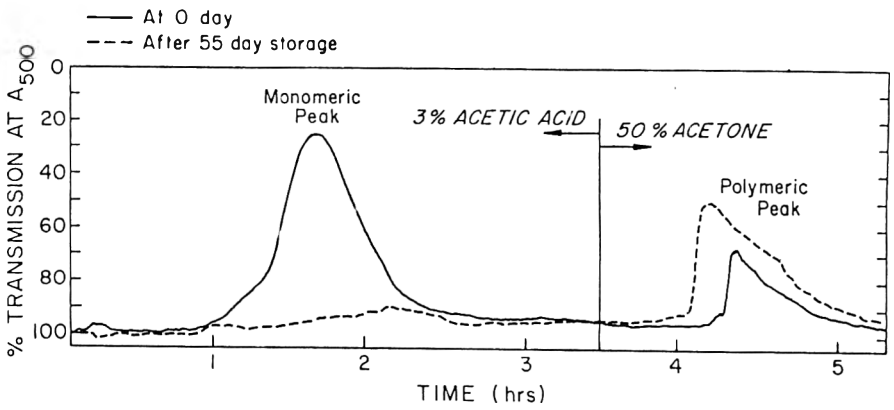

Fig. 6—Gel filtration curve of strawberry concentrate on Sephadex G-25 Fine in 3% aqueous acetic acid and 50% aqueous acetone-HCl solvents.

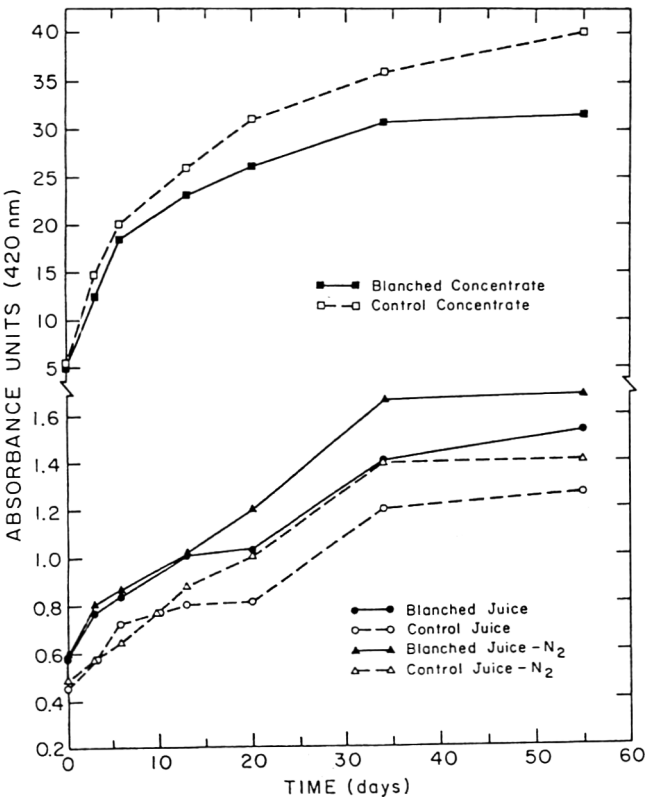

Fig. 7—Formation of browning (absorbance at 420 nm of bisulfite-treated samples) in strawberry juice and concentrate during storage.

tion of reactants inherent in the concentration process. The initial browning values for the blanched and control concentrate samples are the same. The control concentrate sample exhibits a higher rate of browning during storage. This difference in browning could be attributed to polymerization of enzymically formed colorless compounds. Other reactions that can be contributing to browning are the Maillard reaction and browning of ascorbic acid.

Important findings in this investigation are that blanching has a protective effect on ACN pigments, leucoanthocyanins, flavanols, total phenolics, and ascorbic acid and also results in improved color stability as measured by Hunter L and "a" values. Blanched strawberry concentrate browns slower than the control concentrate. Monomeric ACN pigment undergoes rapid degradation in strawberry juice and concentrate during storage to form polymeric pigments which are resistant to bisulfite bleaching. Maillard browning, enzymic browning, ascorbic acid degradation, and polymerization of reactive phenolics can all contribute to browning in strawberry juice. Additional research is necessary to more accurately assess the relative contribution of these different browning reactions to color deterioration.

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BRINING PROPERTIES OF CUCUMBERS EXPOSED TO PURE OXYGEN BEFORE BRINING

H. P. FLEMING, D. M. PHARR, and R. L. THOMPSON

ABSTRACT

Replacement of the internal gas atmosphere of pickling cucumbers with O_2 (O_2-exchanged) greatly altered their brining properties. Oxygen-exchanged cucumbers absorbed brine rapidly, apparently as a result of reduced pressure inside the cucumbers due to respiratory conversion of O_2 to CO_2. The brine uptake reached a maximum and approximated the gas volume reported for fresh cucumbers (4–5%) within 24 hr. Upon brining, the O_2-exchanged cucumbers acquired a translucent internal appearance of fully-cured, brine-stock cucumbers within a few days, as compared to several months for untreated brine stock. The O_2-exchanged cucumbers were less susceptible to bloater damage than control cucumbers during brine fermentation. The density of O_2-exchanged cucumbers was significantly greater than that of control cucumbers within a day after brining, due to the greater brine absorption. No important effects of O_2 treatment were noted regarding rate and extent of fermentation.

INTRODUCTION

BLOATER DAMAGE in brined cucumbers is associated with a build-up of CO_2 in the brine surrounding the cucumbers (Fleming et al., 1973a). The CO_2 may originate from microbial activity in the brine or from the cucumber tissue (Fleming et al., 1973b). Etchells et al. (1968) proposed that bloater formation occurs when the brine becomes *supersaturated* with CO_2. However, Fleming et al. (1978) found that bloater formation can occur at *subsaturation* levels of CO_2. Fleming and Pharr (1980) hypothesized that bloater formation occurs because of differences in rate of transfer of CO_2 and N_2 in the brined cucumbers. This difference in transfer rate was suggested to be related to liquid clogging of the intercellular air space in the outer layer of mesocarp tissue, with the liquid-clogged tissue functioning as a differentially permeable barrier to CO_2 and N_2. They proposed that CO_2 diffuses into the cucumber from the surrounding brine faster than N_2 diffuses out, resulting in a net increase in total pressure inside the cucumber, with resultant bloater formation.

Replacement of the internal gas of fresh cucumbers with CO_2 or O_2 prior to brining resulted in reduced susceptibility of the cucumbers to bloater damage and served as a partial basis for the above hypothesis (Fleming and Pharr, 1980). The O_2-exchanged cucumbers absorbed brine rapidly, and the cucumbers acquired a cured appearance within a day or so after brining; CO_2-exchanged cucumbers did not consume brine and acquire the appearance of cure as rapidly.

The purpose of this study was to learn the rate of O_2 exchange in fresh cucumbers, the rate and extent of brine uptake in O_2-exchanged cucumbers, and the brining properties (rate of fermentation, bloater formation, cure, firmness) of O_2-exchanged as compared to unexchanged cucumbers during brine storage.

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MATERIALS & METHODS

Cucumbers

Pickling cucumbers of size nos. 1, 2, 3, and 4 (1.9–2.7 cm, 2.7–3.8 cm, 3.8–5.1 cm, and 5.1–5.8 cm diam, respectively) were obtained from a nearby pickle company. Only cucumbers free of obvious physical damage and disease were used. Brined cucumbers were evaluated for bloater damage according to Etchells et al. (1974), and bloater indexes were calculated according to Fleming et al. (1977). Firmness of the brined cucumbers was determined with a USDA Fruit Pressure Tester, using a 5/16 inch diam plunger, according to Bell and Etchells (1961). Appearance of cure in brined cucumbers was estimated as the percentage of the surface area of longitudinally cut cucumbers which had a translucent appearance. Uncured flesh was white and opaque. Also estimated from examination of the longitudinally cut cucumbers was the percentage of the fruit with soft centers, i.e., soft and/or liquified seed area.

Gas exchange of cucumbers prior to brining

Cucumbers (1.9 kg) were packed into 3.8-liter (1-gal) glass jars. Each jar was fitted with an expansion reservoir and a gas inlet tube, as described previously (Fleming and Pharr, 1980). Before addition of the brine, O_2 was metered into the jars continuously or intermittently, as designated in individual experiments.

Brining

After gas exchange, brine was added through the reservoir and into the jar, while O_2 flow was continued in order to exclude air from the jar. Brines used for fermentation contained 10.6% (w/v) NaCl and 0.32% (v/v) glacial acetic acid. Sodium acetate (3 H_2O), 0.5% (w/v) at equalization, was added 24 hr after brining and just prior to inoculation. Brines were inoculated with *Lactobacillus plantarum* WSO (ca. 10^9 cells/3.8 liter brined cucumbers). This is the general procedure described in the controlled fermentation process of Etchells et al. (1973), except that chlorine was not added to sanitize the cucumbers.

Brines that were not intended for fermentation contained 10.6% NaCl, 0.32% acetic acid, and 0.2% sodium benzoate.

Brine uptake

Brine level in the expansion reservoir of O_2-exchanged cucumbers receded soon after addition of the brine. The drop in brine level was monitored in the graduated expansion reservoir. Brine drop was calculated as a percentage of the volume of the cucumbers.

Expansion volume

Expansion volume of the brined cucumbers was determined from the rise in the brine level in the reservoir (Fleming et al., 1973a) and was expressed as a percentage of the volume of the cucumbers in the jar.

Brine analyses

Brines were sampled with sterile, 10-ml plastic syringes through a rubber serum stopper placed in the cap of each jar. Titratable acidity (calculated as lactic acid), pH, NaCl, and reducing sugars were determined by methods described or cited earlier (Fleming et al., 1973a). Dissolved CO_2 was determined by microdiffusion and entrapment in standardized NaOH according to Fleming et al. (1974).

Density of brined cucumbers

Cucumbers (five per determination) were removed from brines, blotted dry, and then weighed in a 2-liter graduated cylinder. The weight of water (25°C) needed to bring the volume to 2 liters in the cylinder was determined. The cucumbers were wedged in the cylinder so that they could not float when covered with water. The volume of cucumbers was determined as the difference in the volume of water added (assuming a density of one for water at 25°C) and the 2-liter total volume of the cylinder. Cucumber density was calculated as the cucumber mass divided by the cucumber volume at 25°C.

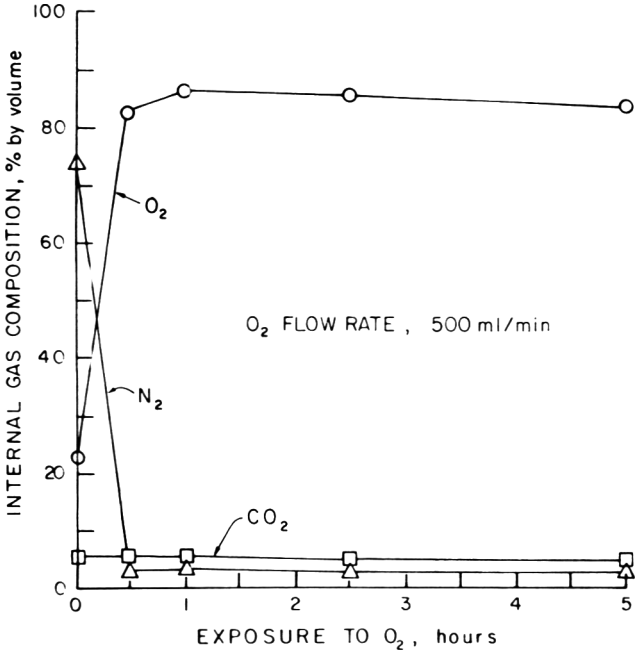

Fig. 1—Internal gas composition of fresh cucumbers exposed to O_2 in 1-gal jers.

Gas extraction and analysis

Gases were extracted from cucumbers and analyzed as described previously (Fleming and Pharr, 1980).

RESULTS

Oxygen exchange in fresh cucumbers

When fresh cucumbers were exposed to pure O_2 at a flow rate of 500 ml/min/gal of cucumbers, the internal gas composition of the fruit reached nearly 90% O_2 in 1 hr (Fig. 1). The exchange was about 95% complete in 0.5 hr, and this exchange rate is similar to the rate of CO_2 exchange in fresh cucumbers (Fleming and Pharr, 1980).

Brine uptake of O_2-exchanged cucumbers

When brine was added to O_2-exchanged cucumbers, the brine level began to recede within a few minutes and reached a plateau within ca. 20 hr for all four sizes of cucumbers tested (Fig. 2). The brine level of unexchanged cucumbers receded only slightly, and then rose slightly during the 20-hr brine storage period.

Cucumbers were exchanged continuously (Fig. 3A) or intermittently (Fig. 3B) with O_2. The maximum brine drop for the no. 3 size cucumbers was about 4.5% of the volume of the cucumbers by each method of exchange. Continuous O_2 at 100 ml/min/gal for ca. 1 hr or two intermittent introductions of O_2 at 300 ml/min/gal for 6 min. at 15 min intervals was sufficient to produce the maximum brine drop.

Fermentation and bloater damage of O_2-exchanged cucumbers

Cucumbers exposed to four intermittent exchanges of O_2 bloated only slightly, whereas unexchanged cucumbers had significant bloater damage, as evidenced by bloater index and brine expansion volume (Table 1; Fig. 4). Two O_2 exchanges resulted in intermediate bloater damage. The rate and extent of acid production was not altered appreciably by the O_2 treatments ($P > 0.05$). CO_2 levels in the fermentation brine (Table 1) after 7 days were not significantly affected by the O_2 treatments ($P > 0.05$).

Physical properties of brined cucumbers

There was a direct relationship between the time of exposure of fresh cucumbers to continuous O_2 flow prior to brining and the appearance of cure of the internal flesh 24 hr after brine addition (Fig. 5). Also, maximum brine uptake and the appearance of cure in the cucumbers were directly related, as may be seen by a comparison of Figures

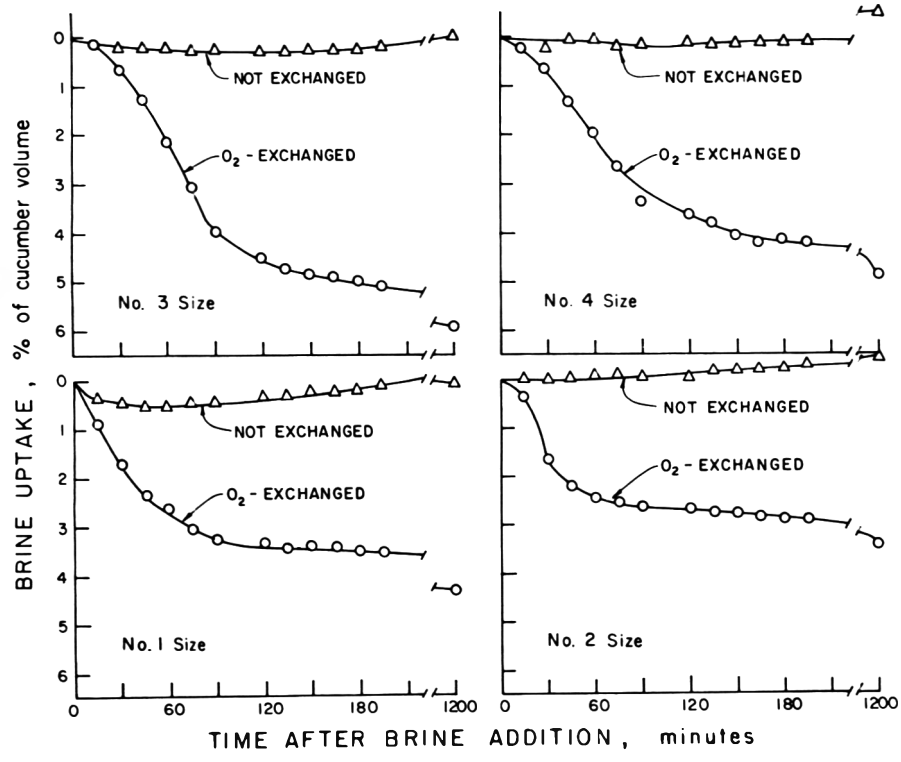

Fig. 2—Brine uptake by O_2-exchanged and untreated cucumbers of four sizes. The O_2-exchanged cucumbers had been exposed to O_2 at 50 ml/min/gal cucumbers for 1 hr before brining.

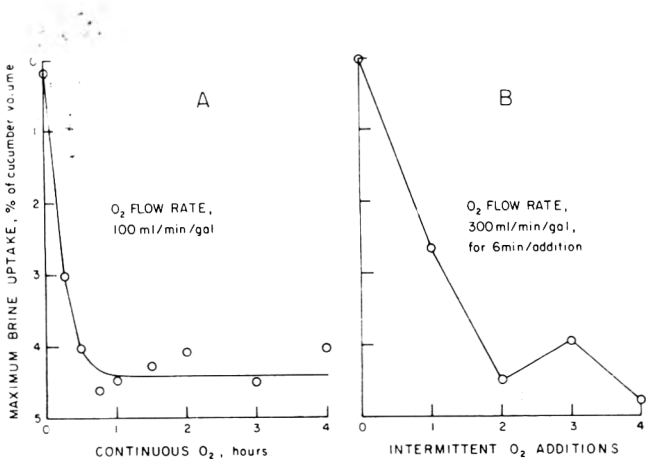

Fig. 3—Effect of continuous or intermittent additions of O_2 to no. 3 size cucumbers prior to brining on subsequent brine uptake by the cucumbers. For intermittent additions, there was a 15-min interval between additions. See Fig. 4 and 5 for related information.

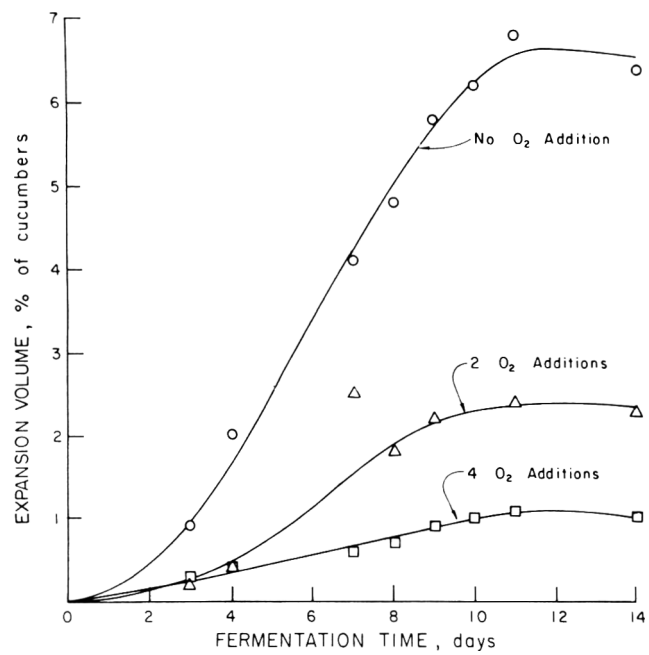

Fig. 4—Effect of O_2 additions to freah cucumbers upon subsequent bloater damage of the brined, fermented cucumbers. Expansion volume of the brine was used as an indicator of bloater formation. See Fig. 3 for explanation of intermittent O_2 additions.

Table 1—Effect of O_2 exchange in fresh cucumbers on bloater damage and fermentation of the brined cucumbers[a]

Intermittent O_2 exposures[b]	Bloater index	Expansion volume (%)	Brine analyses[c] pH	Acid (%)	CO_2 (mg/100 ml brine)
0	20.2	6.8	3.55	0.74	66.4
2	9.5	2.4	3.57	0.76	75.3
4	1.5	1.1	3.64	0.70	74.4
LSD 0.05	12.3	3.8	0.18	0.11	13.0

[a] See Figures 3 and 4 and Table 2 for related information.
[b] O_2 at 500 ml/min for 6 min per exposure. O_2 added two or four times, intermittently, with 15-min intervals between additions.
[c] Determined after fermentation for 7 days.

3 and 5. Likewise, O_2-exchanged cucumbers, obtained by intermittent exposures of fresh cucumbers to O_2, acquired an almost fully-cured appearance after being in brine for only 4 days (Table 2). The white, opaque appearance, typical of fresh cucumbers, was absent. The cucumbers were translucent and light olive green inside, typical of fully-cured, brine-stock pickles. Cucumbers that were not O_2 exchanged did not acquire a fully-cured appearance even after 90 days of brine storage. Fermented and unfermented cucumbers exhibited the above behavior (Table 2).

Firmness of the brined cucumbers was not significantly affected by the O_2-exchange treatments due to fermentation, number of O_2 exchanges, and storage time ($P > 0.05$ by ANOVA). A preliminary experiment suggested that the occurrence of soft centers may be more prominent in O_2-exchanged cucumbers which were subsequently brined. A more extensive experiment (Table 2) did not support that suggestion.

Density of brined cucumbers

Early brining tests showed that control cucumbers were more buoyant than O_2-exchanged cucumbers, after they had been in brine for a few days. The control cucumbers were pressed tightly against the jar lid; whereas, O_2-exchanged cucumbers floated freely or settled to the bottom of the container. Further experimentation confirmed that O_2-exchanged cucumbers were more dense than unexchanged cucumbers ($P < 0.01$) within the first 24 hr after brining (Fig. 6). Water uptake by the O_2-exchanged cucumbers accounted for an increase of 0.038 g/ml in cucumber density, as shown by density intercepts (0% NaCl in the cover brine) in Figure 6. The density of the cucumbers increased as the salt concentration in the cover brine was increased, the rate of increase being greater for O_2-exchanged than unexchanged cucumbers ($P < 0.01$). Although liquid uptake of the cucumbers maximized within the 24-hr period, NaCl within the brine and cucumbers did not reach equilibrium for several days. Therefore, cucumber densities continued to increase beyond those shown in Figure 6, until salt equilibrium was attained.

DISCUSSION

OXYGEN REPLACEMENT of the gases within fresh pickling cucumbers apparently lowered the gas pressure inside the cucumbers when they were placed in brine, and, thereby, caused liquid to be taken into the tissue. We think that O_2 was quickly converted to CO_2 by respiratory activity within the cucumbers when the fruit were brined. As O_2 was metabolized to CO_2, the resulting gas pressure due to CO_2 was less than that formerly due to O_2 because of the greater solubility of CO_2 (CO_2 is 80 times more soluble than O_2 in water at 27°C, Hodgman et al., 1955). Carbon dioxide dissolves in tissue fluids much more than O_2 does. The lowered gas pressure inside the cucumbers caused brine to move into the cucumbers, thus accounting for the brine drop. Forward (1959) noted that the concentration of dissolved CO_2 parallels the concentration of CO_2 *in* the gaseous phase of fruit, and concluded that CO_2 must equilibrate rapidly between liquid and gas phases inside the fruit. Her reasoning is consistent with out hypothesis that pressure drops inside the O_2-exchanged and brined cucumbers.

We attributed the rapid appearance of cure in the O_2-exchanged cucumbers to the rapid uptake of brine and its replacement of gas inside the fruit. The white opaqueness of fresh cucumbers has been attributed to the presence of gas within the tissue (Veldhuis and Etchells, 1939). Brined cucumbers normally acquire a cured appearance gradually over a period of months. In unexchanged, brined cucumbers, N_2 is the primary internal gas (Fleming and Pharr, 1980), and its displacement from the interior of the fruit

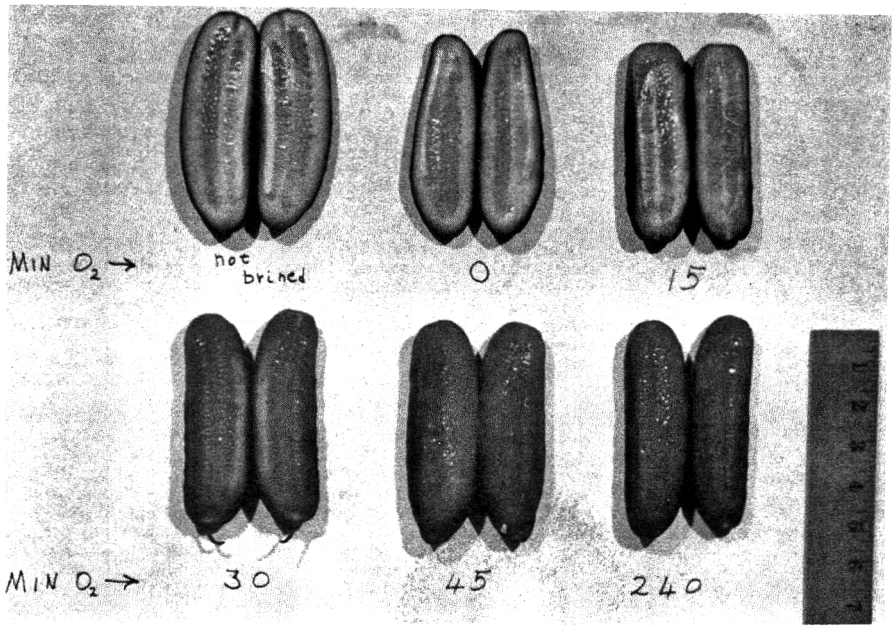

Fig. 5—Effect of exposure time of fresh cucumbers to a continuous O_2 flow on visual cure of the internal flesh 24 hr after addition of brine. Note the raw (white, opaque) appearance in the "not brined" cucumbers as compared to the progressive increase in cured appearance with increasing exposure time to O_2. See Fig. 3 for related information.

apparently is slow, at least partly because of its low solubility.

The reduced susceptibility of O_2-exchanged cucumbers to bloater damage was probably due to the removal of N_2 from the interior of the fruit, as reasoned earlier (Fleming and Pharr, 1980), and to brine filling the intercellular air spaces. In unexchanged cucumbers, N_2, because it is less water soluble than CO_2, diffused out of the brined fruit slower than CO_2 diffused in from the fermenting brine; hence, the internal gas pressure of the fruit, due to the combined partial pressures of N_2 and CO_2, increased. Brine uptake by O_2-exchanged cucumbers eliminated intercellular gas spaces that probably serve as nuclei for bloater formation. Marshall et al. (1973) reported that balloon bloater formation in brined cucumbers was inversely related to density of the green cucumbers. Low density cucumbers apparently have a greater volume of intercellular air space than high density cucumbers, and this difference may account for lower bloating susceptibility of high density fruit.

Alteration in the brining properties of O_2-exchanged cucumbers may offer several practical advantages. Greater tolerance of O_2-exchanged cucumbers to brine CO_2 would lower purging costs, since less N_2 would be required to maintain CO_2 at levels higher than those currently sug-

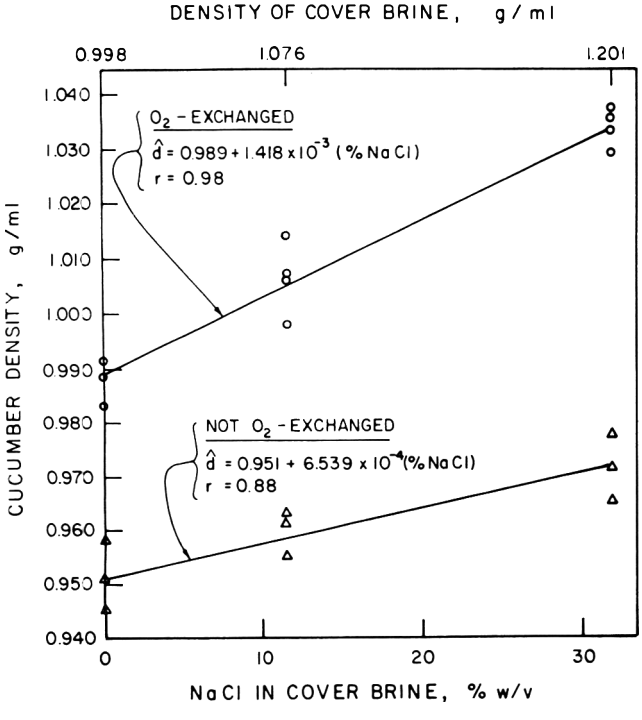

Fig. 6—Effect of O_2 exchange in cucumbers upon density of the brined cucumbers. Densities (d) were determined after the cucumbers had been in brine for 24 hr at 25° C.

Table 2—Effect of O_2 exchange in fresh cucumbers on physical properties of the brined cucumbers[a]

No. of intermittent O_2 exposures[b]	Days of brine storage	Soft centers, (%)	Pressure test (lb)	Cure, (%)
Fermented				
0	4	4	16.7	2
	14	0	18.0	30
	90	15	18.2	80
2	4	0	16.8	92
	14	4	17.9	96
	90	4	17.9	100
4	4	4	17.4	100
	14	4	17.9	97
	90	19	18.3	100
Not fermented				
0	4	0	17.2	5
	14	12	16.4	12
	90	8	17.6	65
2	4	8	15.3	98
	14	27	17.8	100
	90	23	16.6	100
4	4	0	17.6	100
	14	12	18.6	100
	90	23	16.6	100

[a] Values are averages from duplicate 1-gal jars, each containing 13 no. 3 size cucumbers.

[b] See footnote "a" for Table 1 for explanation of intermittent exposures.

gested. Originally, it was recommended that brine CO_2 be maintained below 20 mg/100 ml brine (Etchells et al., 1973). More recently, concentrations of 30–60 mg CO_2/100 ml brine have been suggested, depending on the brine temperature (Fleming, 1979).

The rapid appearance of cure in O_2-exchanged cucumbers may be of importance in certain applications. For example, some picklers state that their customers insist upon hamburger dill chips that appear fully cured. The controlled fermentation of cucumbers (Etchells et al., 1973) results in fully fermented cucumbers within about 10 days. The cucumbers have no residual sugar and are, therefore, stable to secondary fermentation; nevertheless, certain customers apparently object to them because they have a raw, uncured appearance.

Nitrogen-purged cucumbers acquire the cured appearance more slowly than unpurged cucumbers (Fleming, 1979). In contrast, air-purged cucumbers acquire a cured appearance faster than unpurged cucumbers. Reasons for the rapid cure in air-purged, brined cucumbers are unknown.

Cucumbers are buoyant when first brined, due largely to the gas trapped inside the fruit and to the high salt level of the cover brine. Marshall (1975) reported that the specific gravities of fresh cucumbers depended on cultivar and ranged from 0.958–0.982. Fresh cucumbers may be placed in brines of up to 40° salometer (density, 1.076) commercially. Buoyancy causes the fruit to be forced upward against the headboards, which are inserted into the brining tank to keep the cucumbers submerged. This buoyancy pressure can cause physical damage to the cucumbers, particularly those in the upper levels of the tank. It is possible to increase the density and thereby reduce the buoyancy of cucumbers rapidly by first treating them with O_2. The uptake of brine increases the desnity of the cucumbers and, thereby, reduces buoyancy of the fruit during early stages of brine storage.

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LIPIDS IN FAST FOODS

H. T. SLOVER, E. LANZA, and R. H. THOMPSON JR.

ABSTRACT

The lipid composition of food items from three hamburger-type fast food chains has been determined. Samples obtained over-the-counter were extracted with chloroform/methanol and analyzed for total fat, fatty acids, cholesterol, plant sterols, and tocopherols. Fatty acids were determined by an internally standardized gas chromatographic procedure on glass capillary columns coated with SP2340. Cholesterol, tocopherols and plant sterols were also determined by gas chromatography as trimethylsilyl ether derivatives. Condiments were analyzed separately. Limited data on the variability of French fries and plain hamburgers is also given.

INTRODUCTION

SCIENTISTS in the United States Department of Agriculture study the nutrient composition of foods, to improve the available analytical methodology and extend our knowledge of the food we eat. Lipid nutrients are emphasized because of the general interest in the need for data on food lipids. The relationship of dietary lipids to normal human health and to heart disease, obesity, and cancer has been the subject of much research and public concern, and specific recommendations have been made regarding desirable changes in the kinds and amounts of dietary lipids consumed (Select Committee on Nutrition and Human Needs, 1977). In order to evaluate current dietary habits, plan and execute the necessary nutritional research, and implement dietary recommendations, we need detailed information on the amounts of fat, specific fatty acids, including *trans*-unsaturated and polyunsaturated fatty acids (PUFA), vitamin E, cholesterol and plant sterols in foods as eaten.

It is difficult to relate the data for nutrients in foods to actual diets as eaten. Problems in identifying specific foods, the natural variability of foods, and the influence of processing and food preparation contribute to the difficulties. Eating habits and portions of meals rejected vary among individuals.

The so-called "fast foods" offer a unique opportunity for the collection of data on foods as eaten. The large chains try to standardize their products, controlling amounts and ingredients as much as possible. In the hamburger-type fast food chains the entire serving is edible, minimizing the problems caused by variation in eating habits.

We collected samples from three hamburger-type fast food chains: Burger Chef (BC), Burger King (BK), and McDonald's (MD), that were among the top 100 restaurant chains in dollar sales in 1978 (Anon., 1978) and accounted for $5 billion of the total of $87 billion spent for all food service. Since it has been estimated (Anon., 1977) that food service sales were 36% of all food sales in 1978, these chains accounted for ($5 billion/$87 billion) × 36 = 2.1% of all 1978 food sales. Isom (1976) found that fast food meals cost about twice as much as similar meals prepared at home. Therefore, the contribution of the chains to the total food consumption in the U.S. may be crudely estimated at 1% and the data reported here describe roughly 1% of the lipid consumed.

We designed this pilot study of the lipid nutrients in fast food items to investigate the analytical problems presented by the range of foods sold, the lipid content of these foods, and the variability among a few local outlets.

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METHODS

Sampling

Samples were bought over-the-counter at outlets in the Maryland suburbs of the District of Columbia and either analyzed immediately or stored in the refrigerator at approximately 4°C for 1–4 days before analysis. The entire serving was placed in a 200-ml stainless steel cup (Sorval Omnimixer), a calculated amount of degassed distilled water containing 2% pyrogallol added to produce a suitable emulsion, and the charge homogenized under nitrogen at 16000 rpm for 3 min. Samples of this homogenate were taken for lipid extraction and moisture determination.

Lipid extraction

Subsamples (about 10g) of the homogenate were extracted with chloroform/methanol (2/1, V/V) essentially as described by Folch et al. (1957), but with a few necessary modifications. Butylatedhydroxyanisole (5 mg) and pyrogallol (200 mg) were added to each sample before extraction, to minimize losses of tocopherols and unsaturated fatty acids. We routinely extracted twice with chloroform/methanol, since 2–3% of lipid could be removed from the residue remaining after one extraction by hydrolyzing with HCl and extracting with petroleum ether. The combined extracts were washed with 0.58% NaCl in degassed H_2O, diluted to volume, and aliquots dispensed for fatty acid and tocopherol-sterol determinations.

Moisture

Moisture (plus volatiles) was determined gravimetrically by AOAC (1975) Method 24.003.

Fatty acids

Fatty acids were determined by gas chromatography on a 100-m × 0.25-mm glass capillary column coated with SP2340 (Quadrex Corp.), using methyl heneicosanoate as an internal standard (Slover and Lanza, 1979). Fatty acids were identified by comparison of retention times with those of known reference compounds, semi-log plots of retention ratios vs. chainlength of specific homologous series, GLC of hydrogenated mixtures, and gas chromatography/mass spectrometry. Not all peaks were specifically identified, but all peaks reported were shown to be fatty acids by a thin-layer chromatographic separation followed by gas chromatographic analysis.

Tocopherols and sterols

The Folch extractable lipid from 2–5g of sample was mixed with 50-μg of 5,7-dimethyltocol (tocopherol internal standard), 200-μg of betulin (sterol internal standard), and 0.25-g pyrogallol.

After saponification the nonsaponifiable fraction was extracted into cyclohexane, freed of solvent, then separated into tocopherol and sterol fractions by thin layer chromatography on silica gel G plates with cyclohexane/ethyl acetate (80/20) as the developing solvent. The tocopherol and sterol bands were scraped from the plate and extracted with ethanol. Individual tocopherols and sterols were identified and quantitated by gas chromatography (Slover et al., 1979).

Energy

Energy was calculated from fat and moisture data using the following equation: Energy (Kcal) = [Weight(g) − Moisture(g) − Fat(g)] × 4 + Fat(g) × 9.

–Continued on next page

Table 1—Composition of commercial French fries (per item) from fast food chains

	Burger Chef Location				Burger King Location				McDonald's Location				Total variation		Range of Individual values	
	1		2		1		2		1		2					
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Low	High
Weight (g)	82.2	7.0	65.8	9.1	80.4	15.1	75.7	12.8	69.5	15.4	64.6	14.0	73.3	14.9	52.6	97.9
Moisture (g)	31.2	14.1	21.6	18.7	31.6	14.2	30.2	18.3	24.3	15.1	22.9	24.7	27.2	21.9	15.8	37.7
Fat (g)	15.24	5.3	11.61	13.4	13.81	15.3	12.42	7.8	13.76	15.1	11.94	13.8	13.18	14.8	9.39	16.47
Saturated fatty acids (g)	6.71	4.9	5.00	13.1	6.08	15.0	5.33	9.3	6.59[a]	14.6	5.36	15.0	5.85	15.8	4.02	7.27
PUFA (cis) (g)	0.46	18.3	0.34	15.8	0.55	29.6	0.43	10.2	0.49[a]	17.5	0.39	12.4	0.45	23.9	0.27	0.71
P/S	0.07	18.6	0.07	7.1	0.09	24.3	0.08	10.5	0.08[a]	7.2	0.07	7.8	0.08	17.9	0.06	0.12
Transunsaturation (%)	4.12	4.0	3.96	10.5	3.90	10.6	3.85	3.5	4.12[a]	2.3	4.15	7.3	4.01	7.1	3.54	4.59
Cholesterol (mg)	13.9	13.7	11.3	24.8	12.3	18.6	10.7	8.4	12.5	6.0	11.5	13.6	12.1	16.1	7.2	16.4
Alpha-tocopherol (mg)	0.62[a]	148.1	0.14	27.0	0.18	46.5	0.10	54.0	0.08	81.7	0.08	36.0	0.12	58.4	0.02	1.99
Energy (Kcal)	280	8.6	235	8.9	246	19.1	244	9.8	249	16.2	226	10.3	250	13.7	186	323
Calories from fat (%)	49.0	4.2	44.5	7.4	47.2	4.8	45.9	7.8	49.7	1.6	47.4	6.6	47.4	6.3	40.3	51.4
No. samples	5		4		5		5		5		5					

[a] N = 4

RESULTS & DISCUSSION

Variability

We evaluated variability of French fried potatoes (French fries) and hamburgers from each of the three chains by collecting samples at 1-wk intervals from two locations. The data (amount per item) for the French fries appear in Table 1. Weights of "regular" or "small" servings ranged from 52.6–97.9g and averaged 73.3g. For individual outlets the coefficient of variation (CV) for weight of servings ranged from 7.0–15.4%, and was lowest for Burger Chef. Servings from McDonald's weighed least. The wide range of weights is only partially responsible for the variations in other values. The composition of the fat in French fries was similar for Burger King-1 and McDonalds-1, although the weight of the former was greater. Amounts of *cis*-PUFA were low and highly variable. Ratios of *cis*-PUFA to saturated fatty acids (P/S) were uniformly low (mean, 0.08) and rarely above 0.1. The *trans*-unsaturated fatty acid content (as a percentage of all fatty acids) was remarkably constant, with a CV of 7.1%. Cholesterol was found in all French fries and averaged 12.1-mg per serving (CV = 16.1%). This corresponds to a mean of 92-mg cholesterol/100-g of fat, which is consistent with the cholesterol content of animal fat (lard or beef tallow). Small, highly variable (CV = 58.4%) amounts of alpha-tocopherol were present in all samples; this variability reflects both differences in the original oils and destruction during frying. The percent of calories from fat (47.4%, CV = 6.3%) was the most consistent characteristic compared.

Weights varied much less for hamburgers (mean = 80.1-g, Table 2) than for French fries (CV = 7.6% for hamburgers, 14.9% for French fries). Differences and CV between locations were small. Hamburger moisture was variable (CV = 11.8%) and was slightly higher in McDonald's hamburgers than in the others. Fat and saturated fatty acids also varied with CV = 16.2% and 16.7%, respectively. Levels of *cis*-PUFA were 0.45–1.17 g/item, and were highly variable (CV = 32.1%). The mean PUFA value represented 2.8% of total hamburger calories, which is more than adequate to meet the suggested daily intake of 1–2% of total calories (NAS, 1974). P/S ratios were uniformly low, and were lowest and least variable in McDonald's hamburgers. The percentage of fatty acids with *trans*-unsaturation varied from 2.42–4.73% (mean = 3.67%, CV = 19.7%). This amount could come from the beef fat itself, with some contribution from hydrogenated fat in the bun or in the fat used for frying. The cholesterol content was 34.9 mg/item (CV = 15.5%). Small amounts of alpha-tocopherol were found in all hamburgers, probably from the bun or the fat used in frying.

Three food items were chosen at random to illustrate the detailed information about fatty acids that was obtained using capillary gas chromatography (Table 3). Many minor acids were found, including branch-chain acids, positional isomers and geometric isomers. A few unknown components, shown to be fatty acids by thin-layer chromatography, were also found. No aldehydes were found; if present they may have been destroyed during methylation. The summations at the end of the table were helpful in interpreting the data. The ratio ALL FA/% FAT provides a

Table 2—Composition of commercial hamburgers (per item)[c] from fast food chains

	Burger Chef Location				Burger King Location				McDonald's Location				Total variation		Range of individual values	
	1		2		1		2		1		2					
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Low	High
Weight (g)	73.1	5.9	76.2	4.6	83.4	5.0	80.2	8.9	84.3	6.1	84.2	2.5	80.1	7.6	67.5	89.1
Moisture (g)	28.5	7.2	29.3[b]	5.4	31.3[a]	10.7	28.6	16.5	33.2	14.3	33.4	5.7	30.8	11.8	23.0	38.1
Fat (g)	9.43	11.0	10.97[a]	23.6	11.64	6.8	12.09	12.4	9.48	9.8	9.19	3.9	10.42	16.2	8.29	15.31
Saturated Fatty Acids (g)	4.23	14.1	4.86	26.5	4.51	9.8	4.84	16.8	4.08	8.3	3.84	5.1	4.38	16.7	3.16	5.76
PUFA (cis) (g)	0.76	28.3	0.96	14.6	0.97	13.7	0.97	8.7	0.50	9.7	0.47	3.0	0.77	32.1	0.45	1.17
P/S	0.19	36.3	0.21	31.2	0.22	16.2	0.21	22.7	0.12	14.9	0.12	7.0	0.18	33.3	0.10	0.30
Transunsaturation (%)	2.87	14.9	3.18	12.3	4.16	13.4	4.35	6.2	3.62	26.3	3.83	11.5	3.67	19.7	2.42	4.73
Cholesterol (mg)	31.3[a]	10.1	33.5	13.8	38.5[a]	16.7	41.4	8.8	31.9[b]	12.8	33.6	10.6	34.9	15.5	26.5	48.3
Alpha-Tocopherol (mg)	0.34[b]	49.0	0.33	25.2	0.48	40.8	0.71	59.1	0.26[b]	40.7	0.32	37.1	0.40	58.5	0.16	1.07
Energy (Kcal)	226	4.9	248[b]	8.9	272	2.6	267	6.2	252	2.4	249	1.4	251	7.7	210	288
Calories from Fat (%)	37.7	9.7	41.2	14.2	39.4	2.3	40.7	7.4	33.8	7.8	33.2	3.0	37.4	11.3	30.8	49.3
No. Samples	6		5		6		4		5		5					

[a] N = 5
[b] N = 4
[c] Hamburgers analyzed did not include condiments. See Table 6 and Table 7

Table 3—Fatty acid composition of representative items from fast food chains

Fatty acid[a]	Vanilla shake (Burger Chef)	Apple pie (McDonald's)	Cheeseburger[b] (Burger King)
	(grams/100 grams, wet weight)[c]		
8:0	0.053	0.017	0.060
10.0	0.113	0.011	0.134
11.0	—[d]	—	0.014
12.0	0.135	0.013	0.155
A13:0	0.001	—	—
13:0	0.007	—	0.007
U[a]	0.003	—	—
U	0.004	0.005	0.009
14:0	0.387	0.528	0.763
I15:0	0.008	0.021	0.025
A15:0	0.017	0.029	0.038
14:1ω5c	0.035	0.082	0.126
15:0	0.037	0.079	0.091
I16:0	0.009	0.023	0.029
16:0	1.034	4.212	3.768
U	—	0.008	—
U	0.013	0.050	0.047
U	0.008	0.032	0.027
U	—[d]	0.052	—
16:1ω7c	0.070	0.018	0.504
U	0.004	—	0.019
U	—	0.006	0.012
17:0	0.006	0.238	0.133
U	—	—	0.007
17:1ω?c	0.007	0.108	0.089
18:0	0.385	3.235	2.022
18:1ω12t	0.010	0.092	0.043
18:1ω9t	0.008	0.110	0.049
18:1ω?t	0.016	0.439	0.119
18:1ω7t	0.040	0.289	0.227
18:1ω?t	—	0.068	—
18:1ω9c	0.708	5.440	4.928
18:1ω7c	0.030	0.255	0.215
18:1ω?c	0.007	0.048	0.046
U	0.018	0.051	0.037
19:0	0.018	0.050	0.040
18:2ω6tt	0.012	0.054	0.059
18:1ω6ct	—[d]	0.021	0.046
18:2ω6tc	—	0.015	0.027
U	—	0.031	0.026
18:2ω6cc	0.091	0.659	1.008
19:1ω9c	—	0.017	0.020
U	—	0.010	0.013
U	—	0.021	0.034
20:0	0.006	0.030	0.052
U	—	—	0.004
U	0.003	0.010	0.016
18:3ω3c	0.013	0.042	0.091
20:1ω9c	—[d]	0.037	0.026
U	0.007	0.036	0.026
U	—	—	0.006
U	0.009	0.048	0.034
20:2ω6c	—	0.015	0.006
I22:0	—	0.010	0.017
U	—	—	0.006
U	—	—	0.006
22:0	0.005	—	0.013
20:3ω6c	0.004	0.008	0.033
20:4ω6c	0.006	—	0.031
U	—	—	0.004
20:5ω3	—	—	0.006
24:0	—	—	0.004
	Summations		
% Fat	3.770	20.250	16.260
All F.A.	3.334	17.186	15.513
All F.A./% Fat	0.936	0.849	0.932
All Known F.A.	3.264	16.860	15.158
Saturated	2.194	8.496	7.365
Cis Monoene	0.864	6.444	5.959
Trans Monoene	0.074	0.930	0.438
Total Monoene	0.945	7.550	6.486
Cis PUFA[e]	0.114	0.724	1.175
Trans PUFA	0.012	0.090	0.132
Total PUFA	0.126	0.814	1.307
Trans (%)[f]	2.579	5.935	2.823
P/S[g]	0.052	0.085	0.160

[a] I—iso; A—anteiso; ?—exact position of the double bond is not known; U—unknown fatty acid (shown to be a fatty acid methyl ester by TLC, hydrogenation (GLC, and/or GC/MS).
[b] Does not include condiments (See Table 6).
[c] Calculated as the triglyceride.
[d] Amounts not measurable, or total absence, indicated by "—".
[e] PUFA = Polyunsaturated fatty acids.
[f] $\text{Trans (\%)} = \frac{\text{Trans monoene + Trans PUFA}}{\text{All Fatty Acids}} \times 100.$
[g] $\text{P/S} = \frac{\text{cis-PUFA}}{\text{Saturated fatty acids}}.$

measure of the contribution of fatty acids (calculated as triglycerides) to the total extractable lipid. For pure triglycerides the ratio should be 1.0. Lower values indicate the presence either of other lipid species, such as phospholipids, or of extractable material containing no fatty acids. Weihrauch et al. (1977) calculated similar ratios for a variety of food fats. The calculated ratio also serves, by comparison with expected ratios from each particular food, as a check on the validity of the fatty acid analysis. The *trans*-unsaturation, in these and other samples, is due principally to the presence of *trans*-monoene fatty acids, but small amounts of *trans*-PUFA also occur. These *trans*-acids are summed and their percentage of the total fatty acids calculated as % TRANS. This value agrees well with the total *trans*-unsaturation determined by infrared spectroscopy. *Cis*-PUFA is the sum of PUFA containing only *cis*-unsaturation, and agrees well with amounts of *cis,cis*-methylene interrupted-diene determined enzymatically by the lipoxidase procedure. The P/S ratio is calculated as the sum of *cis*-PUFA divided by the sum of all saturated fatty acids. Other ratios (as Σ cis-PUFA/Σ (16:0 + 18:0)) may be easily calculated from the data provided.

Representative data for the amounts of tocopherols and sterols for the same three food items are given in Table 4.

—Text continued on page 1590

Table 4—Tocopherol and sterol composition of representative items from fast food chains

Tocopherols and Sterols: mg/100g, wet weight	Vanilla shake (Burger Chef)	Apple pie (McDonald's)	Cheeseburger (Burger King)
Alpha tocopherol	0.08	0.16	0.56
Gamma Tocopherol	—[a]	0.25	0.89
Delta tocopherol	—	0.09	0.24
Cholesterol	13.2	25.2	57.5
Campesterol	—	2.6	3.6
Stigmasterol	—	—	1.8
Sitosterol	—	15.0	12.5
	SUMMATIONS		
Plant sterols[b] (mg/100g)	—	17.6	17.8
Vitamin E[c] (International Units)	0.12	0.28	0.97
Alpha tocopherol/cis PUFA ($\frac{\text{mg/100g}}{\text{g/100g}}$)	0.70	0.22	0.48

[a] Amounts not measurable, or total absence, shown by "—".
[b] Plant sterols (mg/100g) = Campesterol + Stigmasterol + Sitosterol
[c] Vitamin E (International Units) = 1.49 [(mg of alpha-tocopherol) + 0.1 (mg of gamma-tocopherol)].

Table 5—Lipid composition (per item) of items from fast food chains

	Sandwiches[f] beef							Sandwiches[f] Beef with Cheese					
	Ham-burger	Ham-burger	Ham-burger	Big Shef	Super Shef	Double Beef Hamburger	Whopper	Whopper Jr.	Double Beef Whopper	Quarter Pounder	Cheese-burger	Cheese-burger	Cheese-burger
Company[a]	BC	BK	MD	BC	BC	BK	BK	BK	BK	MD	BC	BK	MD
Weight (g)	74.5	82.1	84.2	146.2	173.7	117.4	152.2	84.8	214.3	136.2	85.9	99.4	98.2
Fat (g)	10.13	11.82	9.34	24.62	27.92	20.48	23.93	13.07	37.91	21.05	13.34	16.50	14.65
Moisture (g)	28.8	30.1	33.3	59.4	76.3	48.9	61.3	30.4	96.3	57.5	31.6	38.3	36.1
Fatty acids (g)													
14:0	0.39	0.29	0.25	1.11	1.22	0.66	0.65	0.33	1.09	0.65	0.76	0.75	0.71
16:0	2.13	2.51	2.15	5.58	6.69	5.15	5.54	2.85	8.65	5.25	3.09	3.75	3.45
16:1ω7	0.32	0.41	0.32	0.76	0.93	0.86	0.94	0.46	1.54	0.76	0.35	0.49	0.42
18:0	1.24	1.56	1.34	3.06	3.72	3.22	3.20	1.68	5.13	3.16	1.61	2.08	1.87
18:1ω9c	3.11	3.88	3.03	7.46	8.88	7.62	8.76	4.41	13.21	7.19	3.68	4.84	4.21
18:2ω6	0.74	0.84	0.42	1.37	1.33	1.04	1.48	0.84	1.68	0.86	0.61	0.96	0.55
18:3ω3	0.08	0.08	0.04	0.11	0.07	0.09	0.12	0.06	0.17	0.22	0.06	0.08	0.10
20:4ω6	0.02	0.03	0.01	0.03	0.04	0.04	0.04	0.02	0.07	0.04	0.01	0.03	0.03
Other	1.58	1.49	1.01	4.11	3.97	2.89	2.86	1.48	4.80	2.79	2.17	2.41	1.50
Saturated	4.52	4.64	3.96	11.44	13.27	9.63	10.03	5.15	15.83	9.59	6.56	7.38	6.64
Monoene	4.05	5.14	4.01	9.83	11.60	10.08	11.29	5.77	17.46	9.67	4.75	6.42	5.08
cis PUFA	0.85	0.97	0.49	1.58	1.54	1.20	1.69	0.93	1.95	1.03	0.71	1.11	0.64
Trans (%)[b]	3.00	4.24	3.72	3.78	3.26	4.07	3.04	3.27	3.73	5.16	3.15	3.90	3.56
P/S	0.15	0.21	0.12	0.14	0.12	0.13	0.17	0.19	0.13	0.11	0.11	0.15	0.10
Tocopherols (mg)													
alpha	0.3	0.6	0.3	0.8	1.4	1.2	0.4	0.6	1.0	0.6	0.3	0.8	0.3
gamma	0.3	0.9	—[g]	0.5	0.9	0.8	0.5	0.6	0.8	0.2	0.6	0.9	0.0[h]
delta	0.1	0.2	0.0[h]	0.3	0.2	0.2	0.1	0.2	0.3	—[g]	0.0	0.3	0.0
vitamin E (IU)[c]	0.5	1.0	0.4	1.2	2.2	1.8	0.7	1.0	1.7	0.9	0.5	1.3	0.5
Sterols (mg)													
Cholesterol	32.4	39.8	32.8	74.5	92.6	91.3	87.6	41.5	175.3	76.6	43.8	59.6	49.5
Campesterol	2.0	2.8	1.7	3.4	3.4	3.9	5.8	2.7	6.2	2.3	1.4	3.5	1.3
Stigmasterol	1.0	1.6	0.9	3.7	3.8	1.4	1.5	1.0	1.6	0.5	0.6	1.7	0.8
Sitosterol	8.4	10.7	7.6	15.0	14.3	13.2	20.0	11.4	22.3	10.4	7.0	12.8	7.0
Plant Sterols[d]	11.4	15.2	10.2	22.1	21.8	18.4	27.3	15.2	29.3	13.2	9.0	16.0	9.1
Energy (Kcal)[e]	235	270	250	470	529	377	483	283	662	420	285	327	321
Fat Calories (%)	39.1	40.0	33.5	47.2	47.4	48.8	44.4	41.5	51.5	45.1	42.1	45.4	41.0
No. Samples	11	10	10	2	2	2	2	2	2	2	2	2	2

	Breakfast Items					Desserts					
	Scrambled Eggs	Hot Cakes w/Butter[i]	English muffin w/Butter	Egg McMuffin	Sausage Sandwich	Apple Pie	Apple Pie	Cherry Pie	Apple Turnover	Lemon Turnover	McDonald-land Cookies
Company[a]	MD	MD	MD	MD	MD	BK	MD	MD	BC	BC	MD
Weight (g)	97.5	140.8	53.8	130.19	111.5	81.0	87.9	83.1	85.8	84.1	60.7
Fat (g)	19.40	9.45	5.39	15.13	18.72	11.27	16.72	14.49	13.43	14.45	8.95
Moisture (g)	63.8	74.1	16.0	67.0	47.1	35.7	37.1	35.8	35.3	31.7	2.29
Fatty Acids (g)											
14:0	0.90	0.43	0.43	0.64	0.27	0.10	0.46	0.38	0.22	0.20	0.25
16:0	4.84	2.13	1.35	3.63	4.59	1.83	3.69	3.13	2.48	2.77	2.00
16:1ω7	0.43	0.11	0.09	0.38	0.52	0.07	0.41	0.35	0.21	0.19	0.28
18:0	1.80	0.89	0.51	1.38	2.29	1.39	2.81	2.35	1.91	1.91	1.31
18:1ω9c	5.54	2.49	1.11	4.36	7.58	3.02	4.67	3.98	3.49	4.13	2.99
18:2ω6	1.71	0.83	0.35	1.32	1.92	1.09	0.33	0.40	1.55	1.17	0.48
18:3ω3	0.06	0.07	0.04	0.09	0.07	0.05	0.03	0.04	0.11	0.05	0.04
20:4ω6	0.18	0.03	0.01	0.13	0.06	0.00	0.00	0.00	0.00	0.03	0.00
Other	1.79	1.82	0.71	1.82	1.15	3.34	2.34	1.56	3.00	3.36	0.94
Saturated	8.51	4.02	2.72	6.28	7.33	3.47	7.47	6.15	4.90	5.22	3.82
Monoene	6.46	3.64	1.40	5.24	8.91	5.67	6.52	5.42	5.80	6.74	3.85
cis PUFA	2.02	0.96	0.38	1.58	2.14	1.14	0.37	0.43	1.76	1.25	0.52
Trans (%)[b]	1.16	8.02	2.27	0.87	0.27	16.63	6.33	5.56	11.57	12.30	3.17
P/S	0.25	0.24	0.15	0.26	0.30	0.36	0.05	0.07	0.37	0.24	0.14
Tocopherols (mg)											
alpha	1.9	1.7	0.0[h]	0.3	0.1	0.6	0.1	0.1	0.5	0.4	0.3
gamma	0.4	0.7	0.3	0.6	0.1	2.3	0.4	0.5	1.6	1.7	0.3
delta	—[g]	0.3	—	—	—	0.8	0.1	0.2	0.3	0.4	0.0[h]
vitamin E (IU)[c]	1.5	1.4	0.1	0.5	0.1	1.3	0.2	0.3	1.0	0.8	0.4
Sterols (mg)											
Cholesterol	365.9	47.2	11.7	247.9	54.2	2.5	17.2	12.7	4.9	7.8	11.3
Campesterol	2.9	4.5	1.4	2.3	1.5	5.5	1.6	1.3	2.8	5.8	1.9
Stigmasterol	—	1.9	1.1	0.54	0.1	4.7	0.9	0.5	2.1	5.1	0.6
Sitosterol	0.7	17.2	6.8	7.5	7.1	21.4	9.4	6.7	15.1	21.1	7.4
Plant Sterols[d]	3.6	23.5	9.4	10.3	8.7	31.8	11.4	8.4	20.0	32.1	9.8
Energy (Kcal)[e]	232	314	178	332	351	238	287	261	269	282	278
Fat Calories (%)	75.0	27.6	27.3	41.2	48.0	42.7	52.4	49.8	44.9	46.2	28.9
No. Samples	2	2	2	2	2	2	2	2	2	2	2

[a] BC = Burger Chef; BK = Burger King; MD = McDonald's.

[b] Trans (%) = $\frac{\text{Trans monoene + Trans PUFA}}{\text{All Fatty Acids}} \times 100$

[c] Vitamin E (IU) = 1.49 [(mg of α-tocopherol) + 0.1 (mg of γ-tocopherol)]

[d] Plant sterols = mg Campesterol + mg Stigmasterol + mg Sitosterol

[e] Energy (Kcal) = 4 [wt (g) − Moisture (g) − Fat (g)] + 9 [Fat (g)]

[f] Sandwiches analyzed did not include condiments. See Table 6 and Table 7.

[g] Amounts not measurable, or total absence, shown by "—"

[h] Small amount detected, becoming "0.0" through rounding operation

[i] Syrup not included

Table 5—Continued

Sandwiches[f] Beef With Cheese						Sandwiches[f]						
						Fish		Fish w/Cheese			Platters	
Double Cheese-burger	Double Beef Cheese-burger	Whopper with Cheese	Double Beef Whopper w/Cheese	Big Mac	Quarter Pounder w/Cheese	Skipper's Treat	Whaler	Whaler w/Cheese	Filet O-Fish	Ham w/ Cheese Yumbo	Rancher (Beef)	Mariner (Fish)
BC	BK	BK	BK	MD	MD	BC	BK	BK	MD	BK	BC	BC
128.8	144.9	175.2	244.5	144.8	165.4	121.7	170.4	202.7	129.3	137.2	210.6	210.1
25.63	26.98	32.36	47.64	19.66	30.25	15.31	23.86	33.65	23.59	17.96	42.86	39 00
54.2	59.2	68.3	108.0	62.9	63.3	51.7	82.8	94.3	47.2	64.5	85.3	93.0
1.45	1.43	1.59	2.06	0.71	1.36	0.77	0.20	1.27	0.47	0.25	1.40	1.11
6.33	6.34	7.52	10.93	4.58	6.61	3.31	3.14	6.32	3.63	4.04	10.27	7.81
0.79	0.84	1.01	1.68	0.66	0.82	0.32	0.19	0.42	0.26	0.06	1.57	1.13
3.32	3.28	3.90	6.04	2.73	3.66	1.81	1.72	3.14	2.01	1.82	6.30	5.77
7.60	7.87	9.81	14.86	6.29	8.60	3.92	5.41	7.68	5.21	4.91	14.07	11.25
0.87	1.11	1.49	1.88	0.76	0.90	0.95	7.05	7.24	6.26	1.43	1.36	1.52
0.15	0.16	0.18	0.21	0.06	0.13	0.05	0.78	0.86	0.87	0.08	0.13	0.05
0.04	0.06	0.05	0.08	0.03	0.03	0.01	0.15	0.03	0.02	0.07	0.05	0.00
4.65	3.98	4.15	6.58	2.84	3.40	2.18	1.97	3.97	1.45	2.11	5.48	4.43
13.51	12.55	14.55	20.91	8.90	12.85	6.94	5.34	12.22	6.58	7.89	19.68	17 15
9.96	10.29	12.67	19.61	8.35	11.15	5.12	6.73	9.63	6.15	6.10	18.39	15.38
1.09	1.41	1.77	2.26	0.89	1.10	1.04	7.91	8.23	7.25	1.62	1.59	1.69
3.39	3.22	2.66	3.52	4.32	4.38	3.24	3.58	2.79	1.39	1.62	3.54	4.59
0.08	0.12	0.12	0.11	0.11	0.09	0.15	1.50	0.67	1.10	0.21	0.08	0.10
1.2	1.3	0.6	1.3	0.5	0.8	0.5	1.3	1.7	1.4	0.2	0.7	0.7
0.4	0.7	0.5	0.7	0.2	0.0[h]	0.4	5.2	7.1	8.6	0.4	0.2	0.7
0.1	0.2	0.2	0.2	0.0	0.2	0.1	2.0	1.8	2.0	0.2	0.0[h]	0 2
1.9	2.1	1.0	2.0	0.8	1.2	0.8	2.7	3.6	3.4	0.4	1.1	1.1
93.9	111.3[i]	121.8	222.8	72.3	109.6	57.5	91.7	121.6	61.2	68.5	118.0	94 4
2.4	3.3	5.4	8.2	2.4	2.1	2.1	13.2	14.2	10.6	2.9	1.5	3 2
0.9	2.4	1.8	1.5	1.8	2.1	2.0	11.9	12.2	11.0	3.4	0.6	2 0
9.9	12.3	19.5	26.3	11.8	9.5	10.4	44.0	44.0	35.4	11.0	10.7	17 7
13.2	18.1	26.6	36.0	16.0	13.7	14.6	69.0	70.3	56.9	17.3	12.8	22 9
427	478	589	785	425	559	356	470	602	447	381	716	663
54.1	50.8	49.5	54.7	41.4	48.6	38.7	45.7	50.3	47.6	42.5	54.0	53.0
2	2	2	2	2	2	2	2	1	2	2	2	2

Shakes									Miscellaneous			
Vanilla	Vanilla	Vanilla	Choco-late	Choco-late	Choco-late	Straw-berry	Straw-berry	Straw-berry	French Fries	French Fries	French Fries	Onion Rings
BC	BK	MD	BC	BK	MD	BC	BK	MD	BC	BK	MD	BK
248.1	251.8	253.1	309.7	249.6	253.9	267.6	260.5	254.8	74.9	78.1	67.0	43.0
9.59	8.29	7.92	12.03	9.06	7.88	8.55	7.34	8.66	13.63	13.11	12.85	7.15
195.2	194.6	187.0	236.3	186.4	178.9	192.5	200.3	184.0	27.0	30.9	23.6	17.0
0.97	0.86	0.83	1 12	0.91	0.77	0.85	0.79	0.84	0.37	0.37	0.36	0.23
2.51	2.20	2.17	3.09	2.41	2.08	2.22	2.03	2.23	2.99	2.92	2.95	1.23
0.17	0.14	0.14	0.18	0.15	0.13	0.14	0.13	0.14	0.31	0.33	0.33	0.08
0.90	0.79	0.74	1.23	0.92	0.85	0.78	0.73	0.75	2.31	2.20	2.29	0.74
1.74	1.47	1.45	1.85	1.66	1.51	1.53	1.38	1.48	4.04	3.82	3.84	1.81
0.21	0.18	0.19	0.22	0.20	0.18	0.22	0.19	0.22	0.35	0.43	0.38	1.13
0.03	0.03	0.02	0.03	0.01	0.03	0.03	0.03	0.03	0.06	0.06	0.05	0.09
0.17	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
1.67	1.49	1.44	1.88	1.44	1.33	1.39	1.26	1.52	1.35	1.24	1.30	1.21
5.42	4.81	4.13	6.52	5.22	4.61	4.76	4.39	4.81	5.95	5.71	5.91	2.19
2.32	1.95	1.96	2.61	2.16	1.93	2.02	1.81	1.98	5.28	4.97	5.02	2.62
0.26	0.23	0.23	0.31	0.25	0.24	0.27	0.24	0.27	0.41	0.49	0.44	1.21
2.43	2.13	2.57	3.10	1.76	1.77	2.22	1.84	1.98	4.05	3.87	4.13	3.78
0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.09	0.07	0.29
0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.2
0.0[h]	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.4	0.4
0.0	0.0	0.0	0.0	0.0	0.0[h]	0.0	0.0	0.0	0.2	0.2	0.1	0.3
0.3	0.2	0.2	0.3	0.3	0.3	0.1	0.3	0.3	0.2	0.2	0.2	0.3
33.7	30.8	25.3	36.5	38.0	27.6	36.3	29.0	28.3	12.8	11.5	11.9	4.4
0.1	0.2	0.2	0.0	0.2	0.0	0.1	0.0	0.2	0.3	0.4	0.3	3.1
0.0	0.0	0.0	0.0	0.6	0.5	0,0	0.5	0.7	0.5	0.4	0.4	2.7
0.1	0.2	0.2	0.4	0.7	1.4	0.1	2.2	3.3	1.7	2.1	2.0	11.6
0.3	0.3	0.5	0.4	1.5	1.9	0.3	2.7	4.2	2.5	2.9	2.6	17.4
259	271	305	352	298	340	343	277	327	260	254	238	140
33.2	27.7	23.6	30.8	27.4	20.9	22.5	23.9	23.9	47.0	46.6	48.5	46.0
2	2	2	2	1	2	2	2	2	9	10	10	2

Table 6—Lipid composition (per 100-g wet weight) of items from fast food chains

	Sandwiches[f] Beef							Sandwiches[f] Beef With Cheese							
	Ham-burger	Ham-burger	Ham-burger	Big Shef	Super Shef	Double Beef Ham-burger	Whopper	Whopper Jr.	Double Beef Whopper	Quarter Pounder	Cheese-burger	Cheese-burger	Cheese-burger	Double Cheese-burger	Double Beef Cheese-burger
Company[a]	BC	BK	MD	BC	BC	BK	EK	BK	BK	BK	BC	BK	MD	BC	BK
Weight (g)	74.5	82.1	84.2	146.2	173.7	117.4	152.2	84.8	214.3	136.2	85.9	99.4	98.2	128.8	144.9
Fat (%)	13.99	14.40	11.08	16.85	16.03	17.38	15.66	15.35	17.67	15.45	15.52	16.61	14.92	19.90	18.66
Moisture (%)	38.6	36.3	39.4	40.7	43.9	41.5	40.2	35.9	45.0	42.2	36.6	38.5	36.8	42.1	40.8
Fatty acids (g)															
14:0	0.53	0.36	0.30	0.76	0.70	0.53	0.45	0.39	0.51	0.48	0.88	0.76	0.72	1.13	0.99
16:0	2.86	3.06	2.55	3.82	3.86	4.11	3.63	3.36	4.03	3.86	3.59	3.77	3.51	4.93	4.39
16:1ω7	0.42	0.50	0.38	0.53	0.54	0.69	0.61	0.55	0.72	0.56	0.42	0.49	0.43	0.62	0.59
18:0	1.66	1.90	1.59	2.10	2.14	2.57	2.09	1.97	2.39	2.33	1.87	2.09	1.91	2.58	2.27
18:1ω9c	4.18	4.72	3.60	5.11	5.12	6.09	5.74	5.21	6.16	5.28	4.29	4.87	4.28	5.91	5.44
18:2ω6	1.00	1.02	0.50	0.94	0.77	0.83	0.97	0.99	0.78	0.63	0.71	0.96	0.56	0.67	0.77
18:3ω3	0.10	0.09	0.04	0.08	0.08	0.07	0.08	0.07	0.08	0.09	0.08	0.09	0.09	0.12	0.11
20:4ω6	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.04
Other	2.13	1.81	1.20	2.81	2.27	2.32	1.87	1.48	2.24	2.04	2.53	2.42	1.52	4.63	2.76
Saturated	6.06	5.64	4.71	7.83	7.63	7.69	6.56	6.05	7.38	7.05	7.64	7.43	6.76	10.51	8.68
Monoene	5.44	6.26	4.76	6.73	6.68	8.05	7.39	6.79	8.14	7.10	5.53	6.46	5.18	7.75	7.12
cis PUFA	1.14	1.19	0.58	1.08	0.89	0.96	1.11	1.10	0.91	0.76	0.82	1.12	0.65	0.85	0.98
Trans (%)[b]	3.00	4.24	3.72	3.73	3.26	4.07	3.04	3.27	3.73	5.16	3.15	3.90	3.56	3.39	3.22
P/S	0.20	0.21	0.12	0.14	0.12	0.13	0.17	0.19	0.13	0.11	0.11	0.15	0.10	0.08	0.12
Tocopherols (mg)															
alpha	0.4	0.7	0.4	0.5	0.8	1.0	0.3	0.7	0.5	0.4	0.3	0.8	0.3	0.9	0.9
gamma	0.5	1.1	0.0[h]	0.4	0.5	0.7	0.3	0.7	0.4	0.2	0.7	0.9	0.0[h]	0.3	0.5
de ta	0.1	0.3	0.0	0.2	0.1	0.2	0.1	0.2	0.1	0.0[h]	0.1	0.3	0.0	0.1	0.1
vitamin E (IU)[c]	0.7	1.3	0.5	0.8	1.2	1.6	0.5	1.1	0.8	0.7	0.6	1.4	0.5	1.4	1.5
Sterols (mg)															
Cholesterol	43.6	49.1	39.2	51.0	51.1	77.8	57.6	48.9	81.7	56.3	51.0	60.0	50.4	70.2	76.9
Campesterol	2.7	3.5	2.1	2.3	1.9	3.4	3.8	3.2	2.9	1.7	1.7	3.5	1.4	1.8	2.3
Stigmasterol	1.4	2.0	1.0	2.6	2.1	1.2	1.0	1.2	0.8	0.4	0.7	1.7	0.8	0.7	1.7
Sitosterol	11.3	13.3	9.0	10.3	7.9	11.3	13.2	13.5	10.4	7.7	8.2	12.9	7.2	7.4	8.6
Plant Sterols[d]	15.3	18.8	12.2	15.1	12.0	15.7	18.0	18.0	13.7	9.7	10.5	18.0	9.3	9.9	12.6
Energy (Kcal)[e]	316	327	298	322	305	321	318	333	309	308	331	329	327	331	330
Calories From Fat	39.9	39.7	33.5	47.2	47.4	48.8	44.4	41.4	51.5	45.1	42.2	45.4	41.0	54.1	50.9

	Desserts						Shakes								
	Apple Pie	Apple Pie	Cherry Pie	Apple Turnover	Lemon Turnover	McDonald-land Cookies	Vanilla	Vanilla	Vanilla	Choco-late	Choco-late	Choco-late	Straw-berry	Straw-berry	Straw-berry
Company[a]	BK	MD	MD	BC	BC	MD	BC	BK	MD	BC	BK	MD	BC	BK	MD
Weight (g)	81.0	87.9	83.1	85.8	84.1	60.7	248.1	251.8	253.1	309.7	249.6	253.9	267.6	260.5	254.8
Fat(%)	13.91	18.99	17.47	15.70	17.20	14.70	3.86	3.30	3.13	3.89	3.73	3.10	3.20	2.83	3.44
Moisture (%)	44.1	42.2	43.1	41.1	37.7	3.9	78.7	77.3	74.0	76.4	74.7	70.3	72.0	76.9	72.2
Fatty acids (g)															
14:0	0.12	0.52	0.46	0.26	0.24	0.42	0.39	0.35	0.33	0.37	0.36	0.31	0.32	0.30	0.33
16:0	2.24	4.19	3.77	2.89	3.30	3.32	1.01	0.88	0.85	1.00	0.97	0.81	0.83	0.78	0.87
16:1ω7	0.09	0.46	0.42	0.26	0.23	0.45	0.07	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0.06
18:0	1.71	3.19	2.84	2.23	2.27	2.19	0.36	0.32	0.29	0.40	0.37	0.34	0.29	0.28	0.30
18:1ω9c	3.72	5.31	4.80	4.07	4.91	4.95	0.70	0.59	0.57	0.61	0.67	0.59	0.57	0.53	0.58
18:2ω6	1.36	0.37	0.48	1.81	1.39	0.78	0.09	0.07	0.08	0.07	0.08	0.07	0.08	0.07	0.09
18:3ω3	0.07	0.04	0.05	0.14	0.06	0.06	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
20:4ω6	0.00[h]	0.00	0.00	0.00	0.03	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01
Other	4.11	2.66	1.88	3.49	3.99	1.59	0.67	0.58	0.57	0.61	0.58	0.53	0.52	0.49	0.60
Saturated	4.26	8.50	7.42	5.71	6.21	6.32	2.19	1.91	1.82	2.11	2.09	1.81	1.78	1.69	1.89
Monoene	6.98	7.42	6.53	6.77	8.03	6.39	0.94	0.78	0.77	0.85	0.87	0.76	0.76	0.70	0.78
cis PUFA	1.42	0.42	0.52	2.06	1.48	0.85	0.11	0.09	0.09	0.10	0.10	0.10	0.10	0.09	0.11
Trans (%)[b]	16.63	6.33	5.56	11.57	12.30	3.64	2.43	1.91	2.57	3.10	1.76	1.77	2.22	1.84	2.48
P/S	0.36	0.05	0.07	0.37	0.24	0.14	0.05	0.05	0.05	0.04	0.05	0.06	0.06	0.06	0.06
Tocopherols (mg)															
alpha	0.8	0.1	0.2	0.6	0.5	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.0[h]	0.1	0.1
gamma	2.9	0.4	0.6	1.8	2.0	0.6	0.0[h]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
delta	1.0	0.2	0.2	0.4	0.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
vitamin E (IU)[c]	1.6	0.2	0.3	1.1	1.0	0.7	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
Sterols (mg)															
Cholesterol	3.1	19.5	15.3	5.7	9.2	13.6	13.6	12.2	11.1	11.7	15.2	10.7	13.6	11.1	12.1
Campesterol	6.9	1.8	1.5	3.2	6.9	2.9	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1
Stigmasterol	6.1	0.5	0.7	2.4	6.0	1.1	0.0	0.0	0.0	0.1	0.3	0.2	0.0	0.2	0.3
Sitosterol	26.7	10.6	8.0	17.5	25.0	11.7	0.1	0.1	0.1	0.2	0.3	0.6	0.1	0.9	1.4
Plant Sterols[d]	39.7	12.9	10.2	23.1	37.9	15.6	0.1	0.2	0.1	0.3	0.7	0.7	0.1	1.1	1.9
Energy (Kcal)[e]	293	326	315	314	335	453	105	107	120	114	120	134	128	106	129
Calories From Fat	42.7	52.4	49.9	45.0	46.2	23.9	33.2	27.7	23.5	30.8	28.0	20.8	22.5	23.9	24.1

[a] BC = Burger Chef; BK = Burger King; MD = McDonald's.

[b] Trans (%) = $\frac{\text{Trans Monoene + Trans PUFA}}{\text{All Fatty Acids}} \times 100$

[c] V tamin E (IU) = 1.49 [mg of α-Tocopherol) + 0.1 (mg of γ-tocopherol)]

[d] Plant sterols = mg Campesterol + mg Stigmasterol + mg Sitosterol

[e] Energy (Kcal) = 4 [wt (g) − Moisture (g) − Fat (g)] + 9 [Fat (g)]

[f] Sandwiches analyzed did not include condiments. See Table 7 and data in this table.

[g] Amounts not measurable, or total absence, shown by "—".

[h] Small amount detected, becoming "0.0" through rounding operation.

[i] Syrup not included

Table 6—Continued

Sandwiches[f] Beef With Cheese				Sandwiches[f]					Platters		Breakfast Items				
Whopper with Cheese	Double Beef Whopper w/Cheese	Big Mac	Quarter Pounder w/Cheese	Fish Skipper's Treat	Fish Whaler	Fish w/Cheese Whaler w/Cheese	Fish w/Cheese Filet O-Fish	Ham with Cheese Yumbo	Rancher (Beef)	Mariner (Fish)	Scrambled Eggs	Hot Cakes w/Butter[i]	English Muffin w/Butter	Egg McMuffin	Sausage Sandwich
BK	BK	MD	MD	BC	BK	BK	MD	BK	BC	BC	MD	MD	MD	MD	MD
175.2	244.5	144.8	165.4	121.7	170.4	202.7	129.3	137.2	210.6	213.1	97.5	140.8	53.8	130.9	111.5
18.47	19.46	13.51	18.26	12.58	14.0	16.60	18.26	13.11	20.38	13.57	19.9	6.83	10.03	11.57	16.80
39.0	44.2	43.4	38.3	42.5	48.6	46.5	36.5	47.0	40.5	44.4	65.4	52.8	29.8	51.2	42.2
0.91	0.83	0.48	0.82	0.64	0.12	0.63	0.36	0.75	0.67	0.53	0.93	0.34	0.79	0.50	0.25
4.29	4.47	3.13	3.99	2.73	1.84	3.12	2.81	2.95	4.89	4.32	4.98	1.56	2.51	2.79	4.12
0.58	0.69	0.45	0.50	0.26	0.11	0.21	0.20	0.25	0.75	0.54	0.44	0.12	0.18	0.29	0.47
2.23	2.47	1.87	2.21	1.49	1.01	1.55	1.55	1.33	3.00	2.83	1.85	0.66	0.95	1.06	2.05
5.60	6.07	2.30	5.19	3.22	3.18	3.79	4.03	3.60	6.70	5.51	5.68	1.84	2.07	3.34	6.80
0.85	0.77	0.53	0.55	0.78	4.13	3.57	4.85	1.04	0.65	0.74	1.75	0.61	0.66	1.01	1.72
0.10	0.09	0.06	0.09	0.05	0.46	0.43	0.68	0.06	0.07	0.02	0.07	0.05	0.08	0.07	0.07
0.05	0.04	0.03	0.02	0.01	0.01	0.01	0.02	0.05	0.03	0.00	0.19	0.02	0.01	0.10	0.06
2.37	2.69	1.90	2.06	1.79	1.15	1.95	1.13	1.53	2.61	2.11	1.85	1.31	1.35	0.98	1.03
8.31	8.55	6.05	7.77	5.70	3.13	6.03	5.09	5.76	9.37	8.15	8.75	2.94	5.08	4.82	6.57
7.23	8.01	5.71	6.73	4.21	3.95	4.75	4.76	4.48	8.77	7.31	6.63	2.66	2.63	4.02	7.99
1.01	0.93	0.62	0.67	0.85	4.64	4.06	5.61	1.18	0.76	0.80	2.07	0.71	0.75	1.21	1.92
2.66	3.52	4.32	4.38	3.24	3.58	2.79	1.39	1.57	3.59	4.68	1.16	8.02	2.27	0.87	0.27
0.12	0.11	0.11	0.09	0.15	1.50	0.67	1.10	0.21	0.09	0.10	0.25	0.24	0.15	0.26	0.30
0.4	0.5	0.4	0.5	0.4	0.7	0.8	1.1	0.2	0.4	0.3	1.0	0.6	0.1	0.2	0.1
0.3	0.3	0.1	0.0	0.3	3.0	3.5	6.7	0.3	0.1	0.3	0.5	0.5	0.5	0.5	0.1
0.1	0.1	0.0[h]	0.1	0.1	1.2	0.9	1.6	0.1	0.0[h]	0.1	0.0[h]	0.2	0.0	0.0	0.0
0.6	0.8	0.5	0.7	0.6	1.5	1.8	2.6	0.3	0.5	0.5	1.5	0.9	0.2	0.4	0.1
69.6	90.8	50.3	66.5	47.2	53.3	60.0	47.5	49.8	55.9	44.8	352.2	33.9	21.9	189.0	48.7
3.1	3.4	1.7	1.3	1.8	7.7	7.0	8.2	2.1	0.7	1.5	3.0	3.1	2.7	1.7	1.4
1.0	0.7	1.3	1.3	1.6	6.9	6.0	8.5	2.5	0.3	1.0	0.0	1.4	2.1	0.4	0.1
11.2	10.8	8.2	5.8	8.6	25.6	21.7	27.5	8.0	5.1	8.4	0.7	12.3	12.6	5.7	6.4
15.2	14.7	11.1	8.4	12.0	40.1	34.7	44.2	12.6	6.1	10.9	3.7	16.7	17.3	7.8	7.8
336	321	293	338	293	276	297	345	278	340	315	238	223	331	253	315
49.4	54.6	41.4	48.6	38.7	45.7	50.3	47.6	42.5	53.9	53.0	75.4	27.6	27.3	41.1	48.0

Miscellaneous					Condiments							Salad Dressings	
French Fries	French Fries	French Fries	Onion Rings	Salad	Big Mac Special Sauce	Catsup	Mustard	Big Shef Sauce	Mayonnaise	Tartar Sauce	Super Shef Salad Dressing	Thousand Island	Blue Cheese
BC	BK	MD	BK	BC	MD	MD	MD	BC	BK	BC	BC	BC	BC
74.9	78.1	67.0	43.0										
18.18	16.82	19.22	16.68	0.14	51.27	0.33	3.07	34.96	82.50	34.00	31.45	34.99	40.52
35.6	39.6	35.1	39.0	95.51	33.4	70.4	85.3	46.9	13.9	46.21	47.77	44.65	32.06
0.49	0.46	0.55	0.12	0.00	0.03	0.00	0.00	0.02	0.05	0.01	0.02	0.02	0.58
3.98	3.72	4.40	2.92	0.02	4.88	0.02	0.09	3.38	8.19	3.33	3.20	3.49	6.45
0.47	0.43	0.50	0.20	0.00	0.05	0.00	0.01	0.03	0.07	0.03	0.02	0.04	0.15
3.08	2.82	3.44	1.78	0.00	1.86	0.01	0.03	1.37	3.31	1.33	1.19	1.34	2.58
5.39	4.91	5.72	4.25	0.02	10.27	0.01	0.46	7.09	17.45	6.93	6.47	7.36	11.54
0.46	0.54	0.58	2.53	0.04	23.66	0.04	0.37	15.31	39.50	15.80	13.86	13.65	22.25
0.08	0.07	0.08	0.16	0.01	3.18	0.01	0.29	1.67	4.61	1.92	1.49	1.50	2.61
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.06	0.05	0.06	0.05	0.06
1.79	1.59	1.95	2.76	0.01	1.41	0.01	1.01	0.74	1.86	2.08	0.82	1.12	0.60
7.93	7.33	8.88	5.25	0.03	7.22	0.03	0.16	4.90	11.93	4.79	4.53	5.01	10.26
7.03	6.39	7.65	6.10	0.02	11.01	0.01	1.23	7.54	18.64	7.36	6.91	7.84	12.44
0.54	0.62	0.66	2.71	0.05	27.01	0.05	0.66	17.15	44.27	17.89	15.53	15.31	25.12
4.05	3.87	4.13	8.55	0.00	0.17	0.00	0.00	0.11	0.34	0.37	0.00	0.22	0.26
0.07	0.09	0.07	0.64	1.86	3.74	1.45	4.11	3.50	3.71	3.73	3.43	3.06	2.45
0.2	0.2	0.1	0.4	0.5	2.2	2.0	2.6	0.0[h]	0.0	0.0[h]	0.0	0.0	2.7
0.1	0.1	0.5	1.1	0.2	32.6	0.2	2.2	12.1	16.7	13.2	9.1	11.4	18.9
0.2	0.2	0.2	0.8	0.0[h]	11.5	0.0	0.0	6.2	7.3	6.6	4.8	4.4	4.7
0.3	0.3	0.2	0.8	0.8	8.2	3.0	4.2	1.8	2.5	3.9	2.7	1.7	6.9
17.1	14.8	18.1	10.0	0.0	46.1	0.2	1.4	53.3	63.1	49.3	46.2	53.6	42.6
0.4	0.5	0.4	7.5	0.2	35.2	0.6	13.6	23.0	36.0	18.9	17.5	16.2	11.8
0.6	0.5	0.6	6.8	1.4	36.2	1.0	0.1	22.1	36.3	20.1	17.5	16.1	14.7
2.2	2.7	3.0	28.4	3.4	96.6	0.7	21.9	60.5	99.9	51.6	51.2	46.1	41.7
3.3	3.7	3.9	42.7	5.0	167.9	2.3	35.6	105.5	172.2	90.6	86.0	78.4	65.8
348	326	356	327	18.7	553	120	74	389	757	385	366	396	475
47.0	46.5	48.6	45.9	6.8	88.2	2.5	37.2	81.3	98.1	79.4	77.3	79.5	76.9

Only alpha, gamma, and delta tocopherols were found; the other tocopherols, if present, were below the threshold of detection. The sterol fraction was composed principally of cholesterol, campesterol, stigmasterol, and sitosterol, although other unidentified materials were usually present in lower amounts. Plant sterols indicate the presence of vegetable lipid, and cholesterol that of animal lipid, but not in a quantitative way. By this criterion only the vanilla shake, of the three items shown in Table 4, is devoid of vegetable fat, and contains only animal fat; the other two contain fat from both sources.

It has been customary to ascribe Vitamin E activity only to alpha tocopherol (1-mg [d]-alpha tocopherol = 1.49 International Units). Other tocopherol forms have some activity and may contribute as much as 20% of the total vitamin E activity (NAS, 1974). Gamma-tocopherol reported to have from 1–83% of the activity of alpha tocopherol (Bunyan et al., 1961) depending on the assay technique, is the major form in many foods (Slover, 1971). We have attributed to gamma-tocopherol 10% of the activity of alpha-tocopherol (Table 4) in an effort to arrive at a more valid estimate of vitamin E activity. Other estimates can be calculated from the complete tocopherol data presented.

The ratio of alpha-tocopherol to *cis*-PUFA has been used as a measure of vitamin E adequacy, since the requirement for vitamin E is related to the amounts of dietary PUFA acids. A lower limit of 0.6 mg alpha-tocopherol per gram of PUFA has been recommended (Harris and Embree, 1963), although more recent work has suggested that lower ratios of about 0.4 (Bieri and Evarts, 1973) are adequate.

Data on lipid composition (Tables 3 and 4), plus additional data on moisture, sample weight, and source, for all individual samples are available on request. Tables 5 and 6 summarize the average data for each fast food sampled, omitting some of the fatty acids. The data are reported as amounts per item in Table 5 and as amounts per 100g in Table 6.

Simple hamburgers, shakes, and servings of French fries weighed about the same among the three chains, but specialty sandwiches varied considerably; the largest was the Double Beef Whopper with cheese from Burger King at 244.5g. This sandwich also contained more fat than any other item–47.6g, representing 428 calories. The amounts of 18:2ω6, as well as of *cis*-PUFA, were highest in the fish sandwiches, specifically the Whaler, Whaler with cheese, and Filet-o-Fish, doubtless contributed by the vegetable fat used in preparing them as well as by the fish, since tocopherols and plant sterols were also high.

The *trans*-unsaturated fatty acids, which were found in all items, usually accounted for less than 5% of the total fatty acids, but were higher in some desserts (Apple Pie (BK), % TRANS = 16.6%). *trans*-Unsaturated fatty acids may come from hydrogenated fat used for frying or baking, or from beef fat, that contains small amounts, usually 2–5%, as a result of biohydrogenation by bovine rumen bacteria.

Two items, the Whaler (BK) and Filet-o-Fish (MD) had P/S ratios above 1.0. P/S ratios for the other 48 items ranged from 0.05–0.67.

Alpha-tocopherol occurred in all the foods, in amounts varying from 0.04 mg in English Muffin with butter (MD) to 1.86 mg in scrambled eggs (MD). Gamma and delta-tocopherols, which also were present in most samples, are major forms in soybean oil, which is the most common vegetable oil in the U.S.

Vitamin E activity from alpha and gamma tocopherols was present in all items examined and was highest in the Whaler (BK), Whaler with cheese (BK) and Filet-o-fish (MD). Most items contained less than 1.0 IU., representing a small proportion of the Recommended Daily Allowance, currently set at 15 IU per day (NAS, 1974).

All items contained cholesterol, which is derived almost wholly from animal sources. In most items the content was less than 100 mg, except for eggs, which as expected contained much more, at 366 mg. One 50-g egg contains about 274 mg (USDA, 1976). Plant sterols were also present in most samples, but not in large amounts.

The method of calculating energy assigns factors of 9 Kcal/g to fat and 4 Kcal/g to everything that is neither fat nor moisture–principally carbohydrate and protein. Fiber and ash are also included in this latter fraction, and the estimate is therefore slightly high. If a maximum of 4% is assumed for the sum of fiber and ash, the energy values reported here are 4–6% high (BK Hamburger, 4.9%; MD Big Mac 5.5%; and BC Rancher 4.7%). The discrepancy for most items will be lower. The values given for fat calories would be slightly higher if fiber and ash were subtracted.

Table 7 –Amounts of condiments normally added to sandwiches by fast food chains

Food Item	Chain[a]	Condiment	Amount/Item
Hamburger	BC	catsup	7g
Hamburger	BC	mustard	2.8g
Hamburger	BC	pickle	1 large slice
Cheeseburger	BC	catsup	7g
Cheeseburger	BC	mustard	2.8g
Cheeseburger	BC	pickle	1 large slice
Double Cheeseburger	BC	catsup	7g
Double Cheeseburger	BC	mustard	2.8g
Double Cheeseburger	BC	pickle	1 large slice
Super Shef	BC	catsup	14g
Super Shef	BC	salad dressing	14g
Super Shef	BC	lettuce	28g
Super Shef	BC	tomato	1 slice
Super Shef	BC	onion	4 raw onion rings
Big Shef	BC	tartar sauce	21g
Big Shef	BC	lettuce	21g
Skipper's Treat	BC	tartar sauce	21g
Skipper's Treat	BC	lettuce	14g
Hamburger	MD	catsup	7g
Hamburger	MD	mustard	14g
Cheeseburger	MD	catsup	7g
Cheeseburger	MD	mustard	14g
Quarter Pounder	MD	catsup	21g
Quarter Pounder	MD	mustard	14g
Quarter Pounder	MD	onion	not available
Big Mac	MD	special sauce	19g
Big Mac	MD	onion	not available
Big Mac	MD	lettuce	not available
Filet-O-Fish	MD	tartar sauce	21g
Hamburger	BK	catsup	not available
Hamburger	BK	mustard	not available
Hamburger	BK	pickles	not available
Cheeseburger	BK	catsup	not available
Cheeseburger	BK	mustard	not available
Cheeseburger	BK	pickles	not available
Double Beef Cheeseburger	BK	catsup	not available
Double Beef Cheeseburger	BK	mustard	not available
Double Beef Cheeseburger	BK	pickles	not available
Whopper	BK	mayonnaise	21g
Whopper	BK	catsup	not available
Whopper	BK	pickles	not available
Whopper	BK	onions	not available
Whopper	BK	lettuce	not available
Whopper	BK	tomatoes	not available
Whopper, Jr.	BK	mayonnaise	7g
Whopper, Jr.	BK	catsup	not available
Whopper, Jr.	BK	pickles	not available
Whopper, Jr.	BK	lettuce	not available
Whopper, Jr.	BK	tomatoes	not available
Whaler	BK	tartar sauce	not available
Whaler	BK	lettuce	not available

[a] BC = Burger Chef; BK = Burger King; MD = McDonald's

Recommendations for desirable changes in the American diet have included a restriction of the fraction of calories derived from fat to 30% of the total intake (Select Committee on Nutrition and Human Needs, 1977). When energy was estimated as described above, calculations of calories from fat were 40% or less for plain hamburgers, but for specialty sandwiches ranged from 41.5% (Whopper, Jr.) to 51.5% (Double Beef Whopper) with an average of 46.6%. Plain cheeseburgers averaged 42.8%; other specialty sandwiches containing cheese ranged from 41.4% (Big Mac) to 54.7% (Double Beef Whopper with cheese) and averaged 49.9%. Sandwiches containing fish ranged from 38.7–50.3% (average 45.6%).

Table 6 gives the lipid composition per 100 g of the items in Table 5 and of the condiments that may be added to sandwiches. Condiments were omitted from the sandwiches analyzed to permit realistic comparisons among chains and locations, since some chains add condiments to their sandwiches while others leave this to the customer; most serve sandwiches without condiments if requested. The lipid contribution is high from some condiments, particularly mayonnaise and the special sauces, and some customers may choose to omit them. Table 7 gives the identities and amounts of condiments that normally are added to the sandwiches by the chains.

Other data on these foods have been reported. McDonald's recently published (WARF, 1977) information on the composition of a composite sampling of their products, including data on total fat, moisture, vitamin E and cholesterol. Our values tended to be slightly higher for fat and cholesterol, and slightly lower for moisture than those found by McDonald's. Vitamin E data were not directly comparable, since we included the contribution from gamma tocopherol, but our values for sandwiches (corrected by the additions of the contribution of the condiments) were higher than McDonald's except for Filet-O-Fish, for which values were equal (3.0 mg/100g). For all other items our values were equal or lower than McDonald's. Appledorf (1974) reported the weight, fat, moisture, and other nutrients of a few representative items sold by these three chains–Burger Chef, Burger King, and McDonald's. In general his data agree well with ours, although he found less variation among chains than we did, both per item and per 100g.

The data reported here permit evaluations of 1% of the lipid consumed in the U.S. in 1978, and provide information for estimating the contribution of specific items to the diet. Complete data are available from the Nutrient Composition Laboratory.

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This research conducted by the Science and Education Administration, USDA, on the commercial foods as reported in this manuscript was limited to analyses of their lipid compositions. The data are reported solely as factual information and are limited to the samples analyzed. No warranty or guarantee is made or implied that other samples of these products will have the same or similar composition. It is the policy of the USDA not to endorse those commercial products used in research over those which were not included in the research.

FLEXIBLE POUCH PROCESS DEVELOPMENT AND EVALUATION OF PASTEURIZED-REFRIGERATED MANGO SLICES

J. B. GOMEZ, R. P. BATES, and E. M. AHMED

ABSTRACT

A continuous pasteurized-refrigerated process was developed for mango slices in aluminum laminate and transparent boil in bag pouches (17 × 15 × 2.5 cm) and compared to slices in ½ pint jars. The effect of pack composition, geometry, and process time at 98°C upon enzyme and microbial activity was determined. A 24-wk storage study monitored by periodic instrumental and sensory evaluations indicated better quality retention at 2 and 10°C than at 20°C. Product packed in aluminum laminate pouches or glass jars stored better than slices in transparent pouches. The retortable pouch served well even at abuse temperature, whereas the boil-in-bag has only short term (<5 months) refrigerated storage capability.

INTRODUCTION

MANGO is one of the world's most popular tropical fruits, possessing both flavor and nutritional appeal, as the annual 10 million metric ton consumption attests (Bhatmagar and Subramanyan, 1973). However, it has a limited shelf life as a fresh fruit and its availability is restricted to a relatively limited harvest season. Mango preservation has been hampered by the economics and logistics of processing in many tropical regions and by quality reduction induced by thermal processing.

An alternative process to partially overcome the quality limitation is suggested in the pasteurized-refrigerated (P-R) process developed for peaches (Harris, 1963), later adapted to mango slices (Dougherty and Koburger, 1972), and refined to a continuous operation (Falcone et al., 1975). In this latter study, P-R mango slices in ½ pint jars demonstrated an acceptable shelf life of over 9, 6, and 4½ months at 2, 6, and 10°C storage temperatures, respectively. Subsequently, excessive softening, darkening, and off-flavor development occurred. Residual enzyme activity because of the mild heat treatment was hypothesized to be storage limiting.

A partial answer to processing logistics is suggested by flexible pouch processing (aluminum laminate retortable pouches) (Mermelstein, 1976; 1978) and the potentially applicable transparent boil-in-bag pouch (Bates et al., 1978). Unlike glass jars, pouches may be rapidly heated and cooled without the hazard of thermal shock. The storage characteristics of the transparent pouch needed to be determined, since they were not designed for such applications.

This study was undertaken to improve the P-R mango slice process, study the processing and storage characteristics of available pouch materials, and determine the storage tolerance of P-R mango slices at refrigeration and abuse temperatures.

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MATERIALS & METHODS

Fruit

A total of 510 kg of 'Tommy Atkins' and 280 kg of 'Keitt' mangos were donated by a commercial grower in Dade County, Fla., and harvested on July 10 and August 10, 1978, respectively. Fruit were obtained at the green-mature stage, immediately transported to the Food Science & Human Nutrition Dept., Gainesville, Fla., washed, and sorted by maturity stages into hard-green, firm ripe green, and approaching firm-ripe. Fruit were stored at 13°C and even ripening was accomplished by freqeunt sorting and storing the less ripe mangos at 25°C. Several randomized samples of 'Tommy Atkins' and 'Keitt' mango varieties at the firm-ripe stage of maturity (when the fruit was ready to be processed) were analyzed for Brix, pH, titratable acidity (as % citric acid). Spear-shaped slices about 7 cm long, 1 cm thick, weighing 7–12g were prepared from firm-ripe mangos.

Packaging

Slices were packaged in: (1) half-pint glass canning jars with a capacity of 240 ml; (2) Aluminum pouch laminate (Reynolds Metals Co.) 0.098 mm thick with exterior polyester layer, middle aluminum foil, and an interior polypropylene layer (Mermelstein, 1976); (3) Transparent pouches, (Cryovac boil-in-bag), pouch stock 0.15 mm thick. Flexible pouches were pre-formed using roll stock, 40 cm wide available in the two types of material. The final size of the pouch was 17 × 15 cm and about 2.5 cm thick when filled to a capacity of approximately 240 ml.

Process

The standardized process was: Containers were packed with 120 ± 5g of mango slices. Pouches were held vertically using 2.5 × 18.0 × 16.0 cm wire racks. About 120g of boiling syrup (104°C) 17° Brix with 0.5% citric acid was poured over the slices. Immediately, jars were capped and pouches were sealed using an impulse heat sealer. A major consideration during the pouch sealing was the minimization of the head space by sealing the pouch while still hot as close as possible to the liquid level.

Immediately after sealing, pouches and glass jars were placed on the speed adjustable belt of a laboratory fabricated blancher/washer and subjected to a steam atmosphere (98°C) for 5, 8, or 10 min. After steaming, containers were cooled in tapwater spray for about 3 min, chilled in iced water, dried, placed in cartons or plastic boxes, and stored at 2°C for 2 wk.

Heat penetration

Thirty gauge thermocouple junctions were inserted through mango slices to the outer surface or embedded at the geometric center of slices in order to obtain temperature profiles of the process. Wire long enough to permit monitoring pouch and glass jar temperatures throughout the length of the blancher belt, from filling the containers and sealing, to the final cooling step, were sealed in the containers by a gasketed packing gland and connected to a temperature recorder. Temperature profiles were obtained in duplicate for each of the nine different treatments.

Evaluation of samples

Microbiological. Assays followed the *Bacteriological Analytical Manual* (HEW, 1978). Total aerobic plate counts were conducted during the preparation and processing steps and after 60 and 120 days of storage.

Process comparison. After 2 wk equilibration at 2°C, a multiple comparison difference analysis test was conducted employing 14 persons familiar with mangos. The panelists were each presented 2 of the 9 (container and/or steaming time) treatments randomly chosen and asked to compare them with a 'reference' sample. The reference consisted of slices in aluminum pouches and processed 8 min at 98°C. Because no differences were found between 5, 8, and 10 min processing times, the three types of containers stored at the

three different temperatures (2, 10, and 20°C) were the only storage treatments tested. Samples from 5, 8, and 10 min process times were pooled and randomly divided for the subsequent 24 wk storage study.

Sensory evaluation. Preference tests were conducted throughout the storage period. Panelists were asked to evaluate the color, texture, flavor, and overall acceptability of 6 randomly presented samples chilled to about 10°C. Ratings were based on a nine-point hedonic scale, without a reference or control sample.

Color. A Hunter Color Difference Meter, Model D25D2, was used to measure color. The Standard Plate was #C2-2719 (L_L = 78.1; a_L = −2.4; b_L = +21.7. Mango slices were diluted 1:2 with distilled water and blended for 2 min. Three replications of each treatment were analyzed.

Texture. Mango slices were subjected to a punch penetration test using the Instron Universal Testing Instrument Model TM. Crosshead and recorder chart speeds were set at 2.0 cm/min; mango slices were punched with a 0.16 cm diameter probe, which penetrated 0.5 cm into the slice. The force was measured in grams at the peak point of the penetration curve. Three slices from each treatment were randomly selected, and each slice was punched at three different locations, giving nine values per treatment.

Statistics. The treatment effects in terms of container types, storage temperatures, and storage times for sensory and instrumental analysis were studied using analysis of variance. Simple correlation coefficients were calculated between the instrumental and sensory evaluation for both color and texture. Significant differences among certain treatment means were assessed using Duncan's New Multiple Range test or Scheffe's procedure. Unless otherwise noted, the 0.05 level of probability was used to define statistical significance.

RESULTS & DISCUSSION

Fruit and pack composition

Fresh 'Tommy Atkins' mangos averaged 4.7 pH (4.5–4.8 range), 13.4° Brix and 0.34% acid. Values for 'Keitt' were 4.5 (4.3–4.8), 14.7° and 0.48%, respectively. The 0.5% citric acid added to the 17° Brix packing syrup resulted in a pack pH of 3.5 when the syrup was added 1:1 to the mango slices at hot filling. This step reduced the danger of microbial spoilage and eliminated the need for overnight soaking (Falcone et al., 1975). A pH of 3.5 was selected because this level of acidification combined with the syrup sweetness produced a balanced, characteristic mango flavor. Citrus juices may be used to acidify the syrup. However, citrus masks the typical mango flavor, although a pleasant citrus-mango blend results. The slice: acidified syrup proportion 1:1 was chosen to provide sufficient fluid for convection heat transfer and adequate mango slices for a well proportioned, attractive pack which retained the typical mango flavor. By allowing the slices to equilibrate for 2 wk in the acidified syrup, a uniformly low pH (3.4) was obtained in both slices and syrup.

Process effects

Processing runs were conducted to determine minimum time to achieve enzyme inactivation. After a 4-min process at 98°C, peroxidase enzyme activity was observed, mainly in the fibers of the mango slices and at the slice center. No residual enzyme activity was detected in containers subjected to 5 min at 98°C. The 5-min process was chosen as the minimum, 8 min intermediate, and 10 min as an upper limit.

Both transparent and aluminum laminated pouches showed identical heat penetration profiles at both surface and slice center (Fig. 1). Heat penetration to higher temperatures at the slice surface and center was faster in pouches than in glass jars. The temperature of the slice surface in pouches and glass jars, rose immediately to about 74–75°C upon filling with 104°C syrup. After 4 min of steaming, slice surface temperature reached 91°C and 87°C in pouches and glass jars, respectively.

Microbiology

With such mild thermal processes as conducted here, microbial survival is of prime concern. Total aerobic plate counts during slice processing are shown in Table 1. Counts as high as 7.5×10^5 organisms/g were observed for the 'Keitt' mangos after washing. Adding 104°C boiling syrup to the slices dramatically reduced the microbial population. After processing, the population was reduced almost to zero after 60 days storage. Transparent pouches proved to be satisfactory up to 5 months storage without microbiological problems at 2, 10, and 20°C. Bates et al. (1978) reported low microbial loads of P-R peach halves, packaged in transparent pouches.

Sensory

Sensory ratings at time zero (2 wk equilibration) indicated no significant preference among processing times or packaging combinations, and the "reference" sample, aluminum pouch, 8 min. Thus, all processing times (5, 8, 10 min) were randomly divided into three groups for storage at 2°C, 10°C, and 20°C. Because of limited fruit quantity, 'Keitt' packs were stored at 2°C and 10°C only.

Storage

Data analysis. When the data collected over 24-wk storage was subjected to statistical analysis, it became evident that the anticipated effect of storage on many of the mango

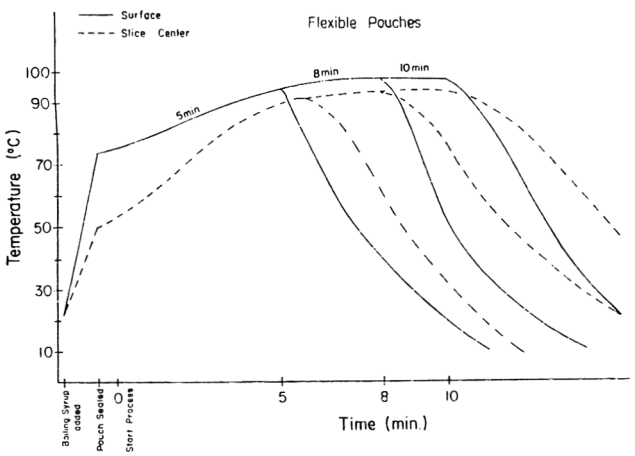

Fig. 1—Temperature profile of pasteurized-refrigerated mango slices in transparent and aluminum 17 X 15 X 2 cm flexible pouches.

Table 1—Total aerobic plate counts during mango processing (organisms/g).

	'Tommy Atkins'		'Keitt'	
Incubation temp	25°C/3 days	35°C/2 days	25°C/3 days	35°C/2 days
Fresh-whole washed fruits	3.4×10^5	–	7.5×10^5	–
Slices with peel	1.2×10^5	–	1.5×10^6	–
Slices without peel	2.9×10^4	–	9.8×10^5	–
Slices in boiling syrup	2.5×10	–	3.0×10	–
Processed products[a]				
Transparent pouches	2.0×10	2.0×10	2.0×10	1.0×10
Aluminum pouches	0.7×10	1.0×10	2.0×10	2.0×10
Glass jars	0.0×10	0.0×10	1.0×10	1.5×10

[a] Analyzed within 2 hr after processing

Table 2—Effect of container type and storage temperature on sensory color ratings of 'Tommy Atkins' mango slices over 5 months

Container[a]	Storage temp °C	Means
Transparent pouches	2	6.68a
	10	6.59a
	20	6.25b
Aluminum pouches	2	6.85c
	10	6.57c
	20	6.78c
Glass jars	2	6.41d
	10	6.53de
	20	6.88e

[a] Temperature means within each container type followed by the same letter are not different at the 0.05 level.

Table 3—Effect of container type and storage temperature on the sensory flavor and overall acceptance of 'Tommy Atkins' mango slices over 5 months

	Sensory means[a]	
Container	Flavor	Overall acceptance
Transparent pouches	5.75b	5.75w
Aluminum pouches	6.38a	6.34x
Glass jars	6.32a	6.13x
Temperature		
2°C	6.41c	6.38y
10°C	6.12d	6.02z
20°C	6.08d	6.00z

[a] Column means followed by the same letter are not different at the 0.05 level.

slice evaluation parameters was not significant. For example, it was expected that a periodic decrease in mango slice quality would be demonstrated due to changes in sensory or instrumental values, particularly in the less protective container (transparent pouches) and/or at the highest storage temperature (20°C). Such a trend was observed only for several quality parameters. However, the statistical analysis clearly indicated the anticipated quality reduction over the entire study, although individual data points (i.e., any specific storage temperature or container treatment at biweekly sampling intervals) were too variable. Longer storage time intervals between sampling would probably have reduced this variability.

Instrumental. Slice color as measured by lightness, hue angle, and saturation index demonstrated significant changes associated with 'Tommy Atkins' mangos packed in transparent pouches stored at 20°C. This relationship was not observed in 'Keitt', due to greater initial fruit color variability and deletion of the 20°C storage regime (insufficient fruit). Over 5 months, slices became darker (L value 49.0 to 47.0), redder (hue angle 92.2 to 88.9) and less intense (saturation index 26.6 to 25.2). This darkening was also significant for transparent pouches at 10°C (L value and hue angle only) but not observed in glass or aluminum pouch packs at any temperature or transparent pouches stored at 2°C.

Puncture tests were performed on all treatments throughout the storage study. No significant changes were observed for the 'Tommy Atkins' mangos as related to the type of container, storage temperature, or storage time and/or interactions. Significant differences, however, were found when the two varieties were compared. 'Keitt' mangos showed a softer texture (puncture force, 83.8g) compared to 'Tommy Atkins' mangos (137.2g).

Sensory

Color. No significant differences were found between the container mean values or between storage temperatures for the 'Tommy Atkins' mangos. Significant differences were found due to the container and storage temperature interaction for the same variety for transparent pouches and glass jars (Table 2). 'Tommy Atkins' slices packaged in aluminum pouches were rated highest in both varieties, indicating better color retention throughout storage.

Texture. 'Tommy Atkins' mangos rated significantly higher (5.91) than 'Keitt' mangos (5.53), though variability in the texture of individual slices resulted in a wide variation of panelists ratings. When the storage temperature was analyzed, those products stored at 2°C (both varieties) received a rating of 6.47, significantly higher than those stored at 10°C which rated 6.30. No significant changes were observed as related to container type, storage times or their interactions.

Flavor and overall acceptance. Panelists significantly preferred either aluminum pouch or glass jar packed 'Tommy Atkins' compared to transparent pouches (Table 3). All products stored at 2°C were given significantly better ratings by the panelists. 'Tommy Atkins' mangos were also significantly preferred (6.19) to 'Keitt' (5.64) in overall acceptance.

CONCLUSION

IT IS particularly encouraging that the packs stored at 20°C held up comparatively well in glass jars and aluminum laminate pouches. The possibility of eliminating or reducing the need for refrigerated storage, while still maintaining acceptable quality would greatly extend the utility of this process. It is suspected, although not monitored in this study, that oxygen permeability is the shelf-life limiting factor in boil-in-bag pouch. This material does not approach the retortable aluminum laminate in storage inertness or process versatility. It would, therefore, be practical to study a wider range of commercially and experimentally available nonretortable transparent and metallic laminate pouch materials as potential inexpensive packaging that could be used in P-R food processing. The P-R process is an effective, simple and inexpensive process which could be adapted to other acid foods in which traditional thermal processing results in reduced quality or atypical processed characteristics.

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MECHANISM FOR BLOATER FORMATION IN BRINED CUCUMBERS

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ABSTRACT

The susceptibility of pickling cucumbers to bloater damage during storage in CO_2-charged brine depended upon the internal gas composition of the cucumbers before brining. Gas extracted from fresh cucumbers consisted of about 75% N_2, 20% O_2, and 6.0% CO_2. Replacement of that gas with CO_2 or O_2 reduced the susceptibility of the fruit to bloater damage upon subsequent storage in carbonated brine. Bloater damage was related directly to % N_2 and inversely to % CO_2 in the internal gas of the cucumbers when they were brined. These and other findings are the basis of the following mechanism we propose to explain bloater formation of cucumbers in brine containing CO_2. When cucumbers are brined, liquid clogs the intercellular gas spaces of the tissues that normally permit rapid diffusion of gases in fresh cucumbers. The liquid-clogged layer encloses the internal gases within the fruit and functions as a differentially permeable barrier to N_2 and CO_2. Since N_2 is the predominant gas within the fresh fruit, and since CO_2 concentration in the brine is high, a diffusion gradient for CO_2 exists toward the fruit interior. CO_2, which is much more water soluble than N_2, diffuses from the brine into the fruit faster than N_2 can diffuse from the fruit. Ultimately, the transfer of CO_2 to the fruit interior results in sufficient internal gas pressure due to CO_2 plus N_2 to rupture the flesh, causing a gas pocket (bloater formation).

INTRODUCTION

PURGING of CO_2 from the brine of fermenting cucumbers with N_2 or air has been shown to greatly reduce bloater damage in the brine-stock cucumbers (Etchells et al., 1973; Fleming et al., 1973, 1975; Costilow et al., 1977). Many pickle companies now purge their brines to reduce losses. Although N_2 has been generally recommended as the purging gas, some briners are either experimenting with or are using air because of its relatively low cost. The use of air presents possible problems with softening, off colors and flavors, and lowering of brine acidity (Fleming et al., 1975; Potts and Fleming, 1979).

Economically, it would be desirable to minimize the amount of N_2 required for purging. For this reason, Fleming et al. (1978) tested the susceptibility of cucumbers to various levels of CO_2 in the brine in order to establish a critical level of CO_2 for bloater damage. Concentration of CO_2 in the brine was controlled by bubbling various mixtures of CO_2 in N_2 through the cucumber brines. Bloater damage resulted from this artificial means of carbonation in heated and unheated cucumbers and in fermented and unfermented cucumbers. They concluded, therefore, that bloater damage can occur by a physicochemical mechanism, independent of metabolism and CO_2 production from microbial action or cucumber tissue. Etchells et al. (1968) earlier suggested a physicochemical mechanism for bloater damage, and indicated that *supersaturated* levels of CO_2 were needed for bloater formation to occur. Fleming et al. (1978) showed, however, that bloater damage can occur at *subsaturation* levels of CO_2 in the brine. The critical level of CO_2 for bloater damage depended on numerous variables, including brine temperature, salt concentration, and the time when CO_2 was added to the brine.

If carbonation was begun immediately after brining, the cucumbers did not bloat extensively, even after extended carbonation. Bloater damage was extensive, however, if carbonation was begun about 1 day after brining. After about a month or longer in brine storage, the cucumbers were no longer susceptible to bloater damage by artificial carbonation, even when the brine was saturated with CO_2. These cucumbers did bloat, however, if subjected to supersaturated levels of CO_2 due to yeast fermentation.

The objective of our study was to gather sufficient data on the gas composition and rates of gas exchange of fresh and brined cucumbers so that we might develop a working hypothesis for the mechanism of bloater formation due to carbonation of brined cucumbers. Specifically, we: (a) determined rates of exchange of N_2, CO_2, and O_2 in fresh and brined cucumbers, and (b) studied the relation between internal gas composition of cucumbers before brining and bloater damage of the cucumbers in artificially carbonated brine.

MATERIALS & METHODS

Cucumbers

Size no. 3 pickling cucumbers (3.8–5.1 cm diam) were obtained from nearby pickle companies. Only fruit free of obvious physical damage and disease were used. Brined cucumbers were evaluated for bloater damage according to Etchells et al. (1974), and bloater indexes were calculated according to Fleming et al. (1977).

Gas exchange of fresh cucumbers

Cucumbers (1.9 kg) were packed into 3.8-liter (1-gal) glass jars. Each jar was then sealed with a screw-on cap fitted with a 250-ml expansion reservoir calibrated to measure brine expansion volume during bloater formation (Fleming et al., 1973). The jar cap also contained portholes through which were inserted a glass gas dispersion tube and a 0.8 cm diam glass rod to support the reservoir (Fig. 1). Air, CO_2, O_2, or N_2 was introduced through a flowmeter into the jar of cucumbers.

Brining

After gas exchange treatment, brine was poured through the reservoir and into the jar. During brine addition, the gas flow was allowed to continue so that air was excluded from the jar. The brine contained 9.8% (w/v) NaCl, 0.32% (v/v) glacial acetic acid, and 0.2% sodium benzoate. When the jar was filled, the gas flow was discontinued, and brine added to the 50 ml mark in the reservoir. Brines were artificially carbonated by bubbling pure CO_2 (50 ml/min) through the brines. as described earlier (Fleming et al., 1978). Expansion volume of the brined cucumbers was determined from the rise in brine level in the reservoir (Fleming et al., 1973) and was expressed as percent of the volume of cucumbers in the jar. Expansion volume is directly related to bloater formation (Fleming et al., 1973; 1978).

Gas extraction and analysis

Fruit were immersed in a 2M $MgSO_4$ solution which had been adjusted to pH 2.5 with HCl and which was contained in an inverted bell jar. An inverted, plastic funnel was placed over the fruit within the bell jar and pushed into the solution until all gas was displaced from the funnel; then, the funnel was sealed with a rubber serum cap. A vacuum desiccator lid connected to a vacuum pump was then placed on the bell jar, and the system was subjected to a vacuum of 0.1 atmosphere for about 15 sec. Gases in the interior of the fruit

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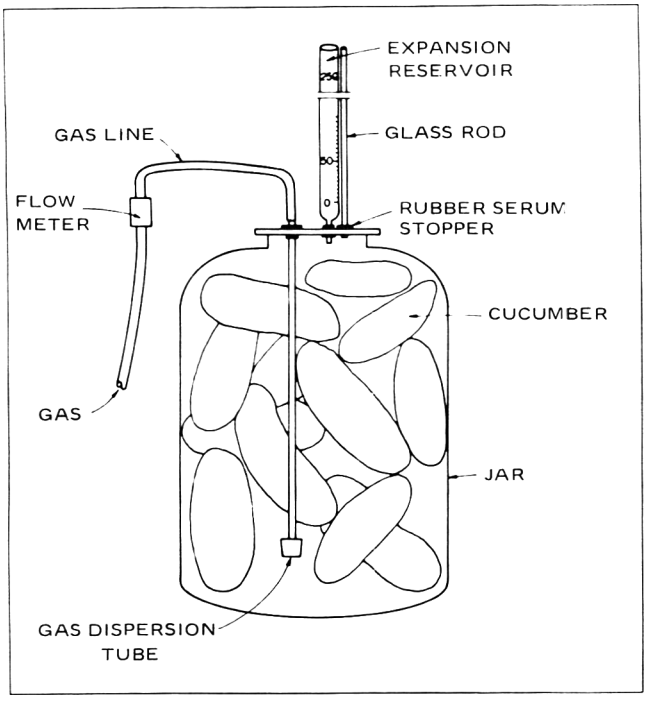

Fig. 1—Jar-reservoir assembly used to expose cucumbers to gases prior to brining and to subsequently brine the cucumbers.

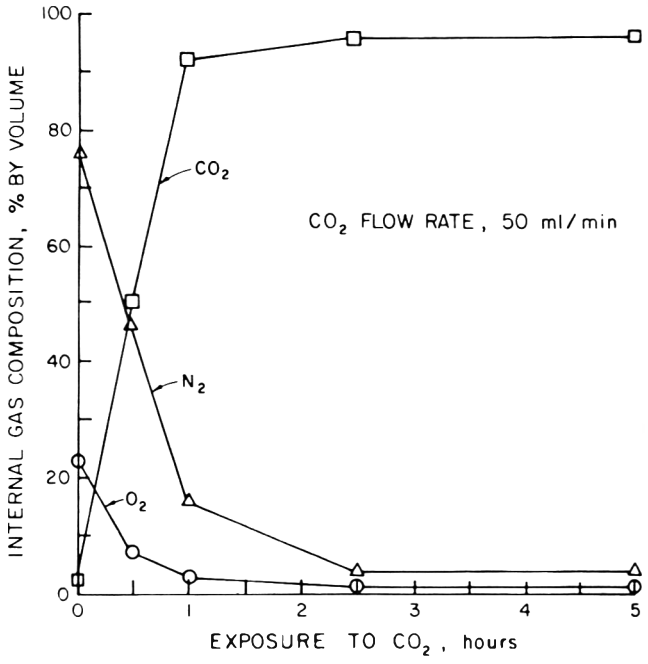

Fig. 2—Internal gas composition of cucumbers exposed to CO_2.

expanded, escaped from the fruit surface as streaming bubbles, and were collected in the funnel. For fruit which had been under brine for several days, it was necessary to puncture their surface with a hypodermic needle immediately prior to gas extraction, otherwise they exploded at the low pressure. After return to atmospheric pressure, a 0.5-cc sample of the gas was removed with a syringe through the rubber serum cap and its composition analyzed with a Hamilton-Fisher Gas Partitioner, model no. 29. Gas analyses are reported as percentage composition by volume of the extracted gas sample, using pure O_2, N_2, or CO_2 as calibration standards. Failure of composition to total 100% represents analytical variations. The high aqueous solubility of CO_2 can present serious problems in such a procedure. However, we found that by using acidified $MgSO_4$ according to Jorge (1978), the composition of the headspace gas was highly stable.

Rubber balloon model

Gas-filled balloons were used to demonstrate principles involved in bloater formation. An apparatus identical to that shown in Figure 1 was used, except that the cucumbers were replaced by a submerged, deflated balloon attached through its opening to a capillary tube fitted to a rubber serum stopper in the jar lid (balloons were inflated and deflated several times to increase their elasticity prior to use in the experiments). Jars were filled with water and carbonated overnight to saturate the water with CO_2. Gas mixtures of 200 ml volume at room temperature were introduced by syringe into the balloons through the serum stoppers and capillary tube to initiate the experiment. The initial gases placed inside the balloons within three jars were 100% N_2, 100% CO_2, and a 1:1 (v/v) mixture of CO_2 and N_2. Expansion of the balloons was determined from the rise in the water in the expansion reservoir upon continued carbonation of the water within the jars at 50 ml of CO_2/min.

Exchange rates of fresh versus brined cucumbers

The rates at which N_2 and CO_2 would exchange in fresh cucumbers and in cucumbers that had been brined for 3 days were measured. Fresh cucumbers were placed in a series of jars, and the jars were then flushed with CO_2 at a flow rate of 500 ml/min for 1 hr so that their internal atmosphere was 100% CO_2. One-half of these jars were filled with CO_2-saturated brine and CO_2 was bubbled through the brine at 50 ml/min CO_2 for 3 days. The remaining jars were filled with carbonated brine to wet the surfaces of the fresh fruit and the brine was immediately removed in a manner such that CO_2 continued to flow into the jar during this surface wetting procedure. These wetted, fresh fruit were then exposed within the jars to pure N_2 at a flow rate of 50 ml/min. Immediately prior to introduction of N_2 into the jar and after 15, 30, 45, and 60 min of N_2 exposure, the internal gas of the cucumbers was extracted and analyzed as described above. The fruit brined for 3 days were drained and the procedure described above for introducing N_2 into the jars and periodically analyzing the internal atmospheres of the fruit was carried out.

RESULTS

Gas exchange in fresh cucumbers

The natural internal atmospheres of four separate harvests of pickling cucumbers averaged 5.7% CO_2 (SD 2.8%), 20.5% O_2 (SD 1.3%), and 75.2% N_2 (SD 1.5%). These values are consistent with findings of Jorge (1978).

When cucumbers were exposed to a CO_2 atmosphere, their internal gas composition changed from ca 2% CO_2 to over 90% within 1 hr, at a CO_2 flow rate of 50 ml/min (Fig. 2). Cucumbers treated in this manner are hereinafter referred to as "CO_2-exchanged." The percentages of N_2 and O_2 in the fruit interior decreased correspondingly. Changes in internal gas composition were even more rapid at a flow rate of 500 ml CO_2/min, being ca 90% complete within 0.5 hr. Thus, the internal gas of fresh pickling cucumbers apparently can exchange rapidly with the external atmosphere. When CO_2 was bubbled (50 ml/min for 5 hr) through freshly brined cucumbers, before the cucumbers became susceptible to bloater damage by brine carbonation, the internal gas composition of the fruit was 89% CO_2, 1% O_2, and 12% N_2. Thus, cucumbers also are capable of rapid gas exchange immediately after brining.

Bloating susceptibility of CO_2-exchanged cucumbers

The magnitude and rate of expansion volume changes in CO_2-exchanged cucumbers, which were subsequently brined and carbonated, varied inversely with the time the cucumbers had been exposed to a CO_2 atmosphere prior to brining (Fig. 3). The bloater index of CO_2-exchanged cucumbers was directly related to the % N_2 and inversely

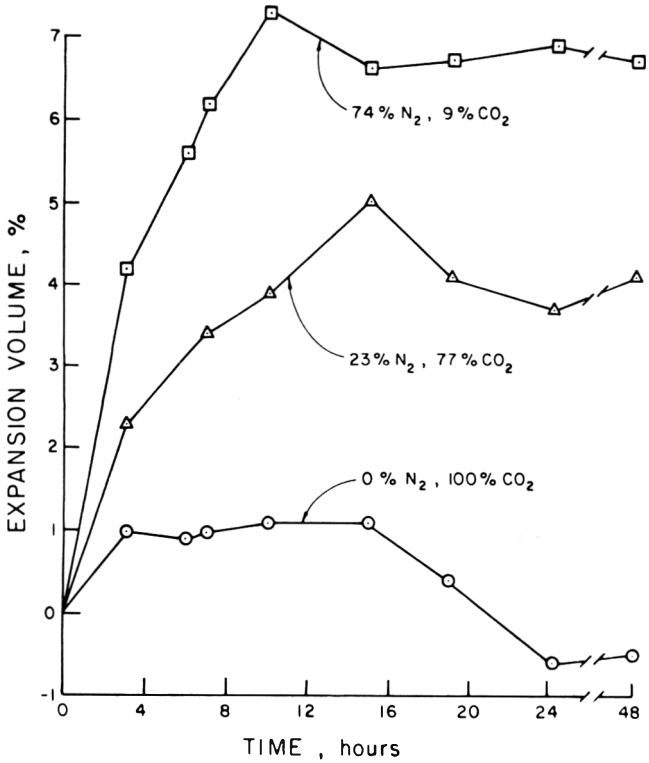

Fig. 3—Expansion volume changes in CO_2-exchanged cucumbers upon brining and carbonation. Cucumbers contained gases of the indicated compositions prior to brining. See Table 1 and Fig. 4 for related data.

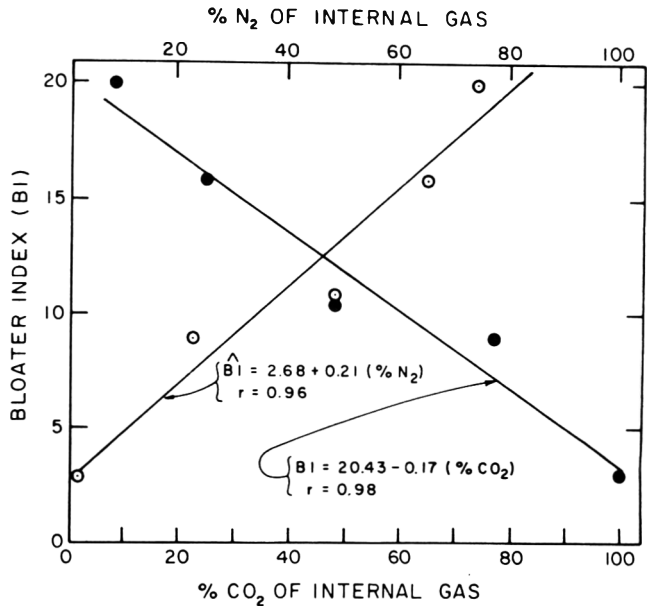

Fig. 4—Effects of % N_2 and % CO_2 in the internal gas of fresh pickling cucumbers on bloater damage upon subsequent brining and carbonation.

related to the % CO_2 composition of the internal gas of the cucumbers prior to brining (Fig. 4). Bloater damage and the maximum % expansion volume were directly related, as has been shown previously (Fleming et al., 1978). Bloater damage consisted of balloon, lens, and honeycomb types (Table 1) and was typical of damage incurred in brine-fermented cucumbers.

Immediately after cucumbers were exchanged with CO_2 or O_2, their internal gas consisted mostly of the exchange gas (Table 2). Exchange of the internal gas of cucumbers with CO_2 or O_2 reduced bloater damage upon subsequent brining and carbonation (Table 2). Cucumbers without gas exchange served as controls, showing the effects of normal internal gas composition on bloater damage.

Effects of CO_2 and O_2 exchange gases on internal gas composition of cucumbers after a 2-day storage in brine were determined (Table 3) for comparison with internal composition immediately after gas exchange (Table 2). The internal gas of both CO_2- and O_2-exchanged cucumbers contained mostly CO_2 after the 2-day brine storage. The 5.1% O_2 in the O_2-exchanged and brined cucumbers (Table 3) contrasted greatly with the 86% O_2, which the fruit contained immediately after O_2 exchange (Table 2). Again, bloater damage was significantly lower in CO_2- or O_2-exchanged cucumbers than in cucumbers that were not exchanged with pure gases.

Particularly interesting in the O_2-exchanged cucumbers was the visual observation of complete cure (translucent as compared to white, opaque flesh in fresh cucumbers) when the fruit were cut and examined 2 days after brining (Fig.

Table 1—Bloating susceptibility of CO_2-exchanged cucumbers to carbonation when brined

CO_2-exchange treatment[a]		Bloater damage after carbonation			
(min)	(flow, ml/min)	Balloon[b] (%)	Lens (%)	Honeycomb[b] (%)	Bloater index
0	50	35 SM	5 S	60 SM	20.0
15	50	25 SA	15 SM	45 S	14.0
30	50	15 SM	10 SM	40 SM	13.5
45	50	15 SM	10 S	40 SM	11.5
60	500	10 S	0	0	2.0

[a] CO_2 was allowed to flow into 1-gal jars of cucumbers at the times and rates indicated before introducing the cover brine.

[b] Letters following numerals indicate the severity of bloater damage: S = slight; M = moderate; A = advanced. When two letters are shown, the first indicates the degree of damage in the major ty of the cucumbers. See Etchells et al. (1974).

Table 2—Effects of exchange gas on internal gas composition of cucumbers prior to brining and on bloater damage upon subsequent carbonation of the brined cucumbers

	Internal gas comp.[a] (%)			Bloater damage after carbonation	
Exchange gas	CO_2	O_2	N_2	Max expansion volume (%)	Bloater index
None	8.9	21.9	74.1	9.2	20.0
CO_2	101.5	0.9	2.4	4.5	3.0
O_2	5.0	86.4	4.0	3.2	6.2
LSD 0.05				1.6	9.2

[a] Cucumbers exposed to exchange gas, 500 ml/min, for 1 hr before internal gas composition was analyzed.

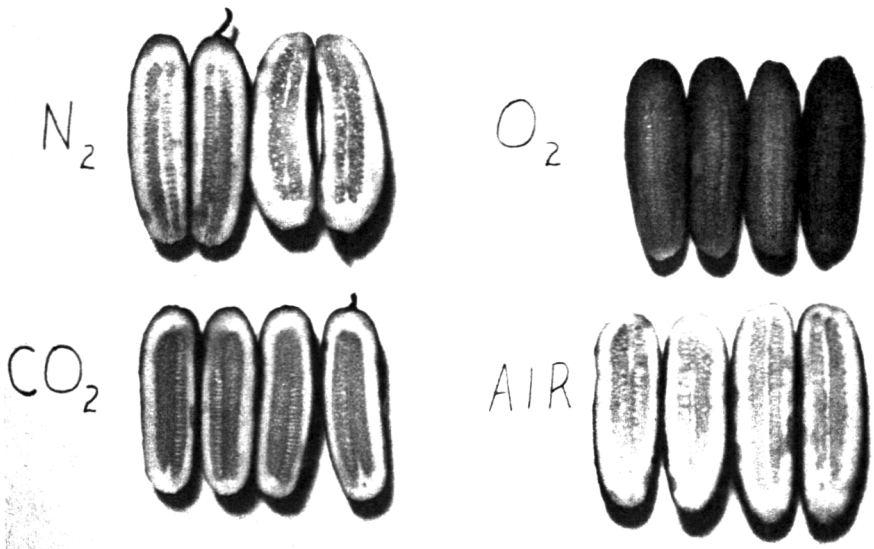

Fig. 5—Effect of O_2 exchange of fresh cucumbers on internal appearance of the flesh 2 days after brining. Note the fully-cured appearance of the O_2-exchanged cucumbers as compared to the raw (white, opaque) or partially cured appearance of the CO_2-exchanged, air (not exchanged) and N_2-purged cucumbers. See Table 3 and footnotes for details.

5). This rapid visual cure did not result with the other treatments detailed in Table 3. Also, we noted that O_2-exchanged cucumbers absorbed brine rapidly immediately upon immersion in brine. Apparently, the brine filled the air spaces within the tissue, and thereby, resulted in the cured appearance.

Comparison of rates at which N_2 exchanged with CO_2 in fresh and brined cucumbers

A comparison of the rates of CO_2 removal from fresh and brined cucumbers was determined by exposing CO_2-exchanged fruit to a flow of N_2. CO_2 removal was more rapid from fresh than from brined cucumbers upon exposing the surface-dried cucumbers to N_2. Also, the internal % N_2 increased more rapidly in fresh than brined fruit (Fig. 6).

Rubber balloon model for bloater formation

Upon exposure of gas-filled balloons to carbonated water, the water steadily rose in the expansion reservoirs a net total of 265 ml after 2 hr when the balloon initially contained 100% N_2, a net total of 128 ml when the balloon initially contained 1:1 (v/v) CO_2 and N_2, and 0 ml when the balloon initially contained only CO_2 (Fig. 7). We subsequently demonstrated that water displaced into the expansion reservoir was almost quantitatively related to measured volumes of air added to the balloons by a syringe through the rubber serum stoppers. Thus, hydrostatic pressure effects and physical resistance of the balloon to expansion were negligible in the apparatus.

Periodic analyses of the composition of gas contained within the balloons during the course of volume changes revealed a constantly increasing % CO_2 and decreasing % N_2 in both balloons which contained N_2 gas (data not shown). However, within the limits of experimental error, no appreciable net loss in N_2 from the balloons occurred during the 2-hr experiment, when volume changes were

Table 3—Effects of exchange gas on internal gas composition of cucumbers after 2-day brine storage and on bloater damage upon subsequent carbonation of the brined cucumbers

Exchange gas	N_2 purged after brining and before carbonation	Internal gas comp. (%)[c]			Visual cure[c] (%)	Bloater index[d]
		CO_2	O_2	N_2		
None	Yes[b]	2.1	3.7	93.3	20	13.9
None	No	49.7	4.8	47.5	20	17.8
CO_2[a]	No	97.7	1.3	5.9	45	3.0
O_2[a]	No	83.3	5.1	17.3	100	0.0
LSD 0.05		12.0	5.2	12.4	–	12.4

[a] Fresh cucumbers were exposed to a gas flow of 500 ml/min for 1 hr prior to addition of brine.

[b] Purged continuously with nitrogen at 50 ml/min after addition of brine.

[c] Determined 2 days after brining. See Figure 5 for visual appearance of the cut cucumbers.

[d] A duplicate lot of cucumbers was carbonated at 50 ml CO_2/min, starting 3 days after brining and continuing for 2 days for the purpose of determining bloating susceptibility of the variously treated cucumbers.

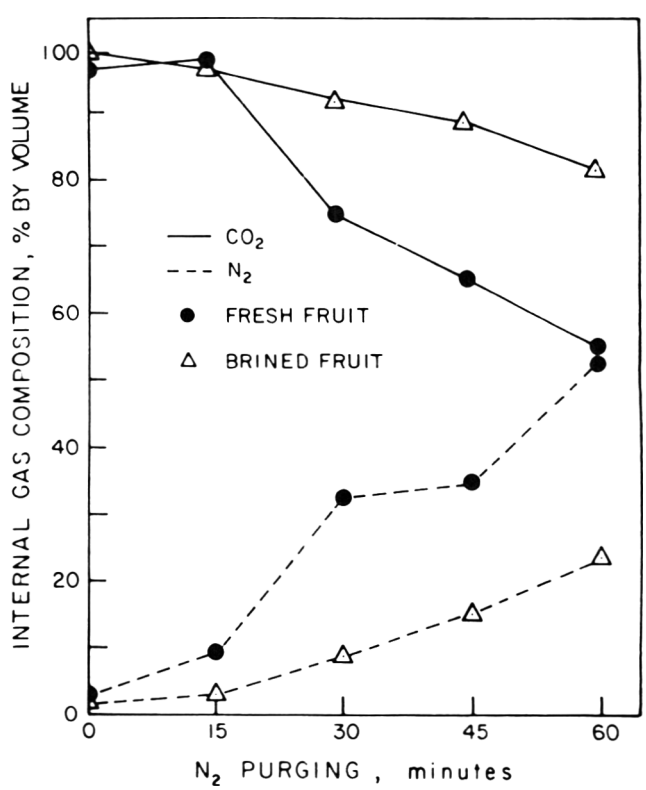

Fig. 6—Changes in the internal gas composition of fresh and brined cucumbers during N_2 purging.

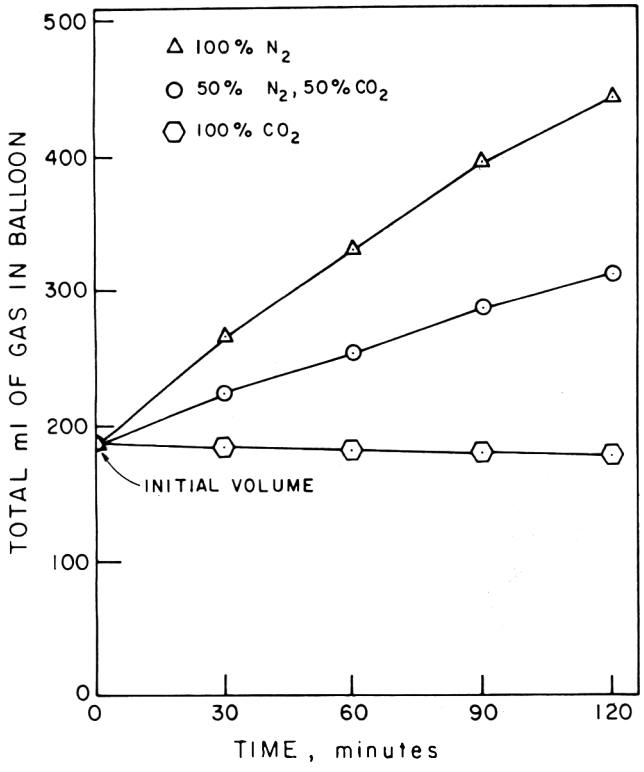

Fig. 7—Increase in volumes of gas-filled rubber balloons placed in CO_2-saturated water. The initial composition of gases in the balloons is given.

taken into account. Thus, volume changes were caused primarily by movement of CO_2 from the brine into the balloons.

DISCUSSION

ETCHELLS et al. (1968) studied the gaseous fermentations of brined cucumbers produced by pure cultures of lactic acid bacteria and proposed the following mechanism for bloater formation: "The fermentation gas, which is produced solely in the cover brine, diffuses into the cucumber via the brine in a supersaturated state, and is released and accumulates at a location of structural weakness inside the cucumber fruit,..." They confirmed their hypothesis by showing that brine supersaturated with CO_2 from sodium carbonate caused bloater formation typical of that caused by microbially-produced CO_2.

Their hypothesis fails to explain, however, how bloater damage can occur at subsaturation levels of CO_2. Serious bloater damage occurred in artificially carbonated, brined cucumbers at 52 and 66% CO_2 saturation, e.g., depending on brine temperature (Fleming et al., 1978). The results of our study show that the internal gas composition of cucumbers at the time of brining can greatly influence their susceptibility to bloater damage upon brining.

Table 4—Properties of gases in water and in air

Variable	Gas[c] CO_2	O_2	N_2
Diffusion coefficient			
In air ($cm^2 \cdot S^{-1}$)[a]	0.139	0.178	—[d]
In water ($cm^2 \cdot S^{-1} \times 10^5$)[b]	1.71^{18}	1.98^{18}	2.02^{22}
Solubility in water[a]			
g/100g water, 27°C	0.1366	0.0038	0.0017

[a] From Hodgman et al. (1955).
[b] From International Critical Tables (1929).
[c] Superscript numbers indicate temperature (°C).
[d] Data not given.

Mechanism of bloater formation at subsaturation levels of CO_2

The following hypothesis may explain how bloater formation occurs when brined cucumbers are exposed to subsaturation levels of CO_2 in the surrounding brine. We propose that CO_2 diffuses into the brined fruit more rapidly than N_2 diffuses out, such that, eventually, gas pressure within the fruit exceeds the external pressure. Tissues then rupture, and pockets of gas accumulate (bloater formation) inside the fruit. Differential rates of gas diffusion in brined cucumbers are due to the formation of a continuous, liquid-clogged outer layer of tissue which is not present in fresh fruit, and the lower water solubility of N_2 than CO_2. Physical constants for gas diffusion and solubility (Table 4), and the following further evidence support this hypothesis.

The fleshy mesocarp of plant tissue contains intercellular air spaces (Esau, 1976). Fresh pickling cucumbers contain about 5–9%, by volume, of gases (Veldhuis et al., 1939; Jorge, 1978). These gases can rapidly exchange with the ambient gas atmosphere, as shown by our study and as illustrated in Figure 8A. The internal gas composition in Figure 8A is typical of that in fresh cucumbers, although we found the composition to vary. Air composition is that reported by Weast (1973).

Stomata are present in the cucumber epidermis (Smith et al., 1979) and may be an avenue through which gases pass. The rate of exchange between internal and external gases was much slower for brined than for fresh cucumbers (Fig. 6). We think that the intrusion of liquid into the skin and air spaces of the outer mesocarp tissue of brined cucumbers clogs the normal avenues for diffusion (Fig. 8B).

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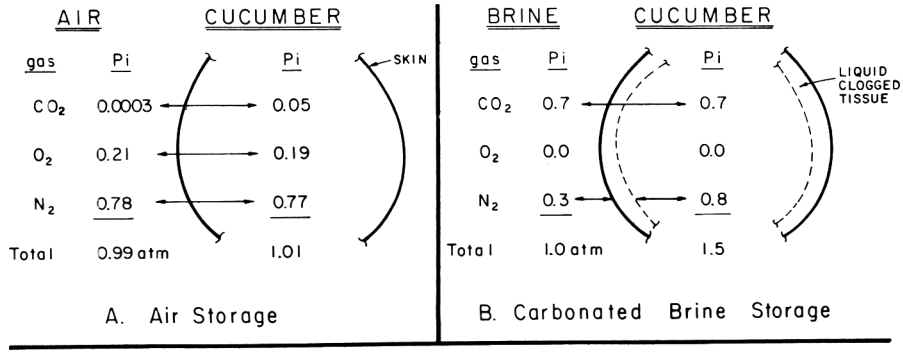

Fig. 8—Hypothetical exchange of gases of cucumbers held in air and in carbonated brine. Pi is the partial pressure, in atmospheres. See text for discussion.

Liquid intrusion into the tissue could occur soon after brining as a consequence of O_2 from the internal atmosphere of the cucumber being converted to CO_2 with a resultant drop in internal gas pressure due to dissolution of CO_2 in the tissue fluids as proposed by Fleming et al. (1980). Additional support for the formation of a liquid-clogged layer is the fact that brined fruit, but not fresh fruit, exploded when exposed to 0.1 atmosphere in the gas extraction apparatus, unless the fruit surface was intentionally punctured prior to gas extraction. Increased resistance to gas diffusion in avocado fruit, as a consequence of fruit softening, has been attributed to liquid clogging of the air spaces by cell exudates (Ben-Yehoshue et al., 1963).

Liquid clogging of air spaces in outer mesocarp and skin tissue (Fig. 8B) would cause the enclosure of internal gases, which consist primarily of N_2 in nonexchanged cucumbers. We propose that in the brined cucumbers, the liquid layer functions as a differentially permeable barrier to CO_2 and N_2. The much greater water solubility of CO_2 than N_2 (Table 4) would allow a greater diffusion rate of CO_2 than N_2 through this aqueous barrier. Diffusion coefficients of these gases in water are not greatly different (Table 4). It is the difference in solubility of gases, therefore, that largely accounts for differences in relative diffusion rates in water for O_2, CO_2, and N_2 (1.0, 23.1, and 0.53, respectively; Krogh, 1919) and in body fluids (1.0, 20.3, and 0.53, respectively; Guyton, 1971).

Bloater damage results, therefore, from the entrapment of N_2 inside of brined cucumbers by outer, liquid-clogged tissue and a subsequent increase in the internal, partial pressure of CO_2 (Fig. 8B). The CO_2 may originate within the tissue, or from carbonation of the brine surrounding the cucumbers. Ultimately, when the internal total gas pressure exceeds one atmosphere, the tissues give way, and gas pockets form (bloated areas). The onset of bloater formation occurs when the internal pressure, due to CO_2 plus N_2, exceeds resistance by the tissue. The brine may contain a subsaturation level of CO_2, therefore, for bloater formation. Oxygen-exchanged cucumbers did not bloat appreciably, apparently because O_2 was converted to CO_2 by tissue respiration as suggested by Fleming et al. (1980).

If cucumbers are carbonated immediately upon brining, bloater damage is minimal, even with extended brine carbonation (Fleming et al., 1978). Apparently in this case, N_2 is displaced from the cucumbers by CO_2 before intercellular gas spaces become clogged and restrict the rate of N_2 removal from the tissue.

Bloating of brined cucumbers is analogous to the expansion of the rubber balloons, as shown in Figure 7. Rubber is over 10 times more permeable to CO_2 than the N_2 (International Critical Tables, 1929). CO_2 diffused from the surrounding carbonated liquid into the balloon in proportion to the CO_2 diffusion gradient. The rubber, therefore, acted like a differentially permeable membrane, as did the hydrated layer which forms in brined cucumbers.

Although microbial fermentation was excluded in all cucumber brining experiments reported herein, we believe that the above hypothesis for bloater formation also applies when the source of CO_2 is from microbial growth.

Implications

The internal gas composition of pickling cucumbers before brining influences their bloating susceptibility during brine storage. Thus, factors which influence the gas composition of cucumbers prior to or during brining should be considered in relation to brining properties, especially in relation to bloater damage. We and others have observed unexplained variations in bloater damage of pickling cucumbers. Physiological state of the fruit, as it influences gas composition, would be a prime factor for further study.

The possibility of intentionally altering the gas composition of fresh cucumbers prior to brining, in order to reduce susceptibility to bloater damage of the fruit during subsequent brining, has been explored (Fleming et al., 1980). That study showed that exchange of the internal gas with CO_2 or O_2 will greatly reduce susceptibility of the cucumbers to bloater damage by carbonation. The study also showed that the use of O_2 as the exchange gas offers unique possibilities because O_2-exchanged cucumbers took up brine much more rapidly, and thus, acquired a more rapid appearance of cure than cucumbers with a normal internal atmosphere.

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EFFECT OF VARIOUS TREATMENTS OF PULSES ON IN VITRO GAS PRODUCTION BY SELECTED INTESTINAL CLOSTRIDIA

S. K. GARG, A. C. BANERJEA, J. VERMA, and M. J. ABRAHAM

ABSTRACT

Studies were performed to find out the effect of different treatments of pulses, like cooking, fermentation, incorporation of spices like raw garlic, and ginger powder on in vitro gas production by selected intestinal Clostridia. All treatments had profound influence on gas production; the amount of gas was reduced between 30–60%. Maximum reduction was noticed at 1.0% concentration of spices. Lag phase of growth was prolonged considerably, suggestive of temporary suppression of gas production. *C. perfringens* produced the highest amount of gas in all control samples and all treatments had maximum inhibitory effects on this organism.

INTRODUCTION

LEGUMES AND PULSES are primary sources of proteins in India. Pulses are potential producers of flatulence (Murphy, 1964; Steggerda and Dimmick, 1968; Hellendoorn, 1969). In man, chickpea reportedly produces more intestinal gas than do mature pea, soybean, and lentil. One of the main causes of intestinal gas production is fermentation of various substrates by the indigenous microflora. Most investigators ascribe flatulence to the action of intestinal anaerobic bacteria on the oligosaccharides present in mature legumes and pulses which can be degraded by specific enzymes present in bacteria but not mammalian enzymes (Rackis et al., 1970; Hymowitz et al., 1972). Clostridia play an important role in in vitro gas production (Richards et al., 1968). Following the ingestion the oligosaccharides remain unhydrolyzed by gastric secretions and hence not readily absorbed, continue down the intestine into the presence of a variety of microbes common to the colon which ferment them with the production of gases i.e. flatus.

Some traditional and experimental food processing methods might be expected to alter bean flatulence factor (FF) or its behavior. The Indonesian soybean product, tempeh made by mold fermentation from soybean grits caused a significant delay in the time of gas formation suggestive of temporary suppression of intestinal bacteria (Hesseltine, 1965). Similarly certain spices like ginger, garlic, and asafoetida are also known to inhibit the growth of indigenous microflora, though published data are scanty.

In the present investigation, the reduction in the gas forming properties of legumes by various treatments like cooking, fermentation, and addition of spices like raw garlic and ginger powder on in vitro gas production by selected Clostridia are reported.

MATERIALS & METHODS

Microorganisms

Three different species, namely *Clostridium perfringenes*, *C. sporogenes*, and *C. butyricum* isolated and characterized in our laboratory were used for the study.

Preparation of substrate

For the present study two pulses were tested viz. chick pea (*Cicer arietinum*) and dried pea (*Pisum sativum*). The pulses were powdered with the help of an electric grinder. The moisture content was determined by drying in an oven until concordant values were obtained. The powder was made into a paste with water 60:40 (v/w) with minimum incorporation of air bubbles to maintain anaerobic conditions. The paste materials were cooked in a pressure cooker for 15 min.

In vitro gas production

The method of Richards et al. (1968) was followed for in vitro gas production. The test samples (10 ml) were filled in sterile injection syringes, at each stage of filling a little amount of sodium thioglycollate was added to make the conditions anaerobic. The clostridial cultures, grown in sodium thioglycollate broth at 37°C for 24–48 hr, were inoculated subsequently with the help of a 2 ml sterilized syringe through the nozzle end (Mossel et al., 1956).

Fermentation studies

Tempeh was prepared by the method of Djoin and Hesseltine (1961). Chick pea and dried pea grains were soaked in water for 1 hr, washed thoroughly in tap water, sun-dried and dehulled in a hand grinder. Hundred grams each of the above grains were washed repeatedly to remove dirt and seed coats. The cleaned grains (100g each) were autoclaved with three times its volume of water (300 ml) at 15 lb pressure for 3–4 min. Cooked grains were inoculated with mold spores and packed tightly in sterile petridishes. The plates were incubated at 30 ± 1°C for 3–4 days. Fermentation was completed when a compact white cake was formed. *Rhizopus oligosporus* was used for inoculating the substrates. The organism was grown on potato dextrose agar for 7–10 days at 30°C, then washed from the cultures with 2 ml sterile 0.5% Tween-20 to prepare inoculum. A 1-ml inoculum containing approximately 10^5 spores was used for fermentation.

Preparation of spices

Raw garlic and ginger powder were selected for the study. Garlic was thoroughly cleaned, cut into pieces and a homogeneous paste was prepared with the help of pestle and mortar under aseptic conditions. Edible concentrations (0.25, 0.50, 0.75, and 1.0%) of garlic paste and ginger powder were used for inhibitory studies. The pulse/spice mixture was incubated in a water bath at 37°C for 24 hr after inoculation with Clostridia.

RESULTS & DISCUSSION

Effect of cooking

Cooking of the substrates caused a decrease in gas production compared to uncooked pulses but the pattern remained the same (Table 1). The decrease in gas production is due to change in the levels of oligosaccharides present in pulses. Considerable reduction in galacto-oligosaccharides (raffinose, stachyose and verbascose) content of pulses by autoclaving was reported by Kawamura (1968). After heat treatment only less amount of oligosaccharides might be available for fermentation by Clostridia and hence the reduction in gas production. Among the substrates tested, Chick pea induced maximum gas production followed by dried pea (*Pisum sativum*) in both cooked and uncooked substrates. This is attributed to the higher contents of galacto-oligosaccharides in chick pea (*Cicer arietinum*) than dried pea (*Pisum sativum*) as reported by Rackis et al. (1970). The enzymes responsible for the hydrolysis of these

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Table 1—Gas produced with cooked substrates (per 10g dry wt)[a]

Substrate	ml gas produced in 24 hr using Clostridial cultures				
	A	B	C	D	E
Uncooked (Control)					
Chick pea	12.62	16.26	7.52	14.08	23.06
Dried pea	10.61	15.27	5.69	11.64	20.70
Cooked					
Chick pea	8.74	10.92	4.37	9.47	15.29
Dried pea	6.47	9.31	3.10	7.24	12.94

[a] Figures are averages of three trials; A = *C. butyricum*; C = *C. sporogenes*; B, D, and E = *C. perfringens*.

Table 2—Effect of fermentation on gas production (per 10g dry wt)[a]

Substrats		ml gas produced in 24 hr using Clostridial cultures				
		A	B	C	D	E
Chick pea						
a.	Control (Cooked)	8.98	10.44	5.34	9.95	14.32
b.	Tempeh	3.16	3.88	1.94	3.40	4.37
Dried pea						
a.	Control (Cooked)	6.32	9.41	3.26	7.20	12.91
b.	Tempeh	2.68	4.10	1.07	2.92	3.85

[a] Figures are averages of three trials. A = *C. butyricum*; B, D, & E = *C. perfringens*; C = *C. sporogenes*.

Table 3—Effect of edible concentrations of dry ginger on gas production (per 10g dry wt of cooked substrate)[a]

Substrate	Conc dry ginger (%)	ml gas produced in 24 hr using Clostridial cultures				
		A	B	C	D	E
Chick pea						
a	0.00	8.74	10.68	3.88	9.22	15.05
b	0.25	7.04	9.22	3.16	7.28	13.11
c	0.50	5.58	7.52	2.59	6.07	9.22
d	0.75	2.91	3.16	1.70	3.88	7.28
e	1.00	1.94	2.18	1.21	2.67	5.15
Dried pea						
a	0.00	6.47	9.31	3.88	6.73	7.76
b	0.25	5.17	8.02	3.10	5.43	6.21
c	0.50	3.62	5.95	2.85	4.14	5.17
d	0.75	2.59	4.14	1.55	3.35	3.88
e	1.00	1.81	2.85	1.03	2.07	2.85

[a] Figures are average of three trials. A = *C. butyricum*; B, D, & E = *C. perfringens*; C = *C. sperogenes*.

tri, tetra and penta oligosaccharides are absent in the human intestine and they remain unabsorbed. These galactooligosaccharides are acted upon by specific enzymes α-D-galactosidases and β-D-fructosidases of Clostridia and the monosaccharides thus formed are utilized for gas production. *C. perfringens* produced the highest amount of gas, 14.08–23.06 with uncooked chick pea and 9.47–15.29 ml with cooked. The gas production property of three *C. perfringens* strains were different and might be due to strain variation. *C. sporogenes* produced the lowest amount of gas by both substrates before and after cooking.

Table 4—Effect of edible concentrations of garlic on gas production (per 10g dry wt of cooked substrate)[a]

Substrate	Conc of garlic (%)	ml gas produced in 24 hr using Clostridial cultures				
		A	B	C	D	E
Chick pea						
a	0.00	8.50	10.19	4.85	8.98	13.80
b	0.25	7.04	8.74	3.88	7.28	11.17
c	0.50	5.10	6.07	3.16	5.34	8.50
d	0.75	3.64	4.61	2.67	2.91	5.58
e	1.00	2.18	3.16	1.21	3.43	3.40
Dried pea						
a	0.00	6.21	9.57	3.88	6.99	7.50
b	0.25	5.69	7.50	3.10	5.95	6.73
c	0.50	3.88	5.43	2.59	4.40	4.92
d	0.75	2.07	3.88	1.55	2.07	2.85
e	1.00	1.03	1.81	0.78	1.29	1.55

[a] Figures are average of three trials. A = *C. butyricum*; B, D, & E = *C. perfringens*; C = *C. sporogenes*.

Effect of fermentation

Tempeh, made from chick pea (*Cicer arietinum*) and dried pea (*Pisum sativum*) by fermentation with *Rhizopus oligosporus* decreased the amount of gas produced to a great extent and caused a significant delay in the time of gas formation (Table 2).

This might be expected due to several reasons. During fermentation oligosaccharides content is diminished (Calloway et al., 1971) and hence the product became essentially more nonflatulent. The time of peak gas production with tempeh was significantly delayed as compared with control samples. Also this delay could well be due to the production of an antibacterial substance by the Rhizopus which might be inhibitory to Clostridial growth (Wang et al., 1969). Although this compound does not exhibit a broad spectrum activity, it is said to be very active against many gram positive bacteria including some of the typical gas-forming inhabitants of the intestinal tract.

Effect of spices

The role of spices as inhibitory agents is well known though the literature available is limited. The inhibition of gas production was experimentally observed by using ginger and garlic. Increasing concentrations of both spices were tried and a parallel decrease in gas production was noticed with both substrates by all Clostridial species. The decrease was more pronounced with *C. perfringens* compared with the other two (Table 3 and 4). The reduction in the gas production is understandable considering the antibacterial properties of spices used. The gas production was delayed by over 9–11 hr and after that there was an exponential increase in production which reached maximum at 24 hr and remained almost constant afterwards. This type of gas production is in agreement with the typical bacterial growth curve. The stationary phase after 24 hr may be due to low pH (4.4–4.8) due to acid production.

This investigation indicates that various treatments of pulses like cooking, fermentation, incorporation of spices like raw garlic and ginger powder minimize the gas production by prolonging the lag phase of growth of Clostridia. The data are of importance in the use of traditional and experimental processing in the preparation of pulses which form a major dietary source of protein among vegetarians.

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INHIBITION OF TWO PSYCHROTROPHIC Pseudomonas SPECIES BY BUTYLATED HYDROXYANISOLE

P. M. DAVIDSON and A. L. BRANEN

ABSTRACT

The antimicrobial activity of the phenolic antioxidant, butylated hydroxyanisole (BHA), was evaluated against *Pseudomonas fluorescens* and *Pseudomonas fragi*. *Pseudomonas fluorescens* ATCC 15456 was extremely susceptible to the antimicrobial effects of BHA. In Trypticase Soy Broth (TSB) 100 ppm BHA delayed the growth of *P. fluorescens* at 22°C and totally inhibited growth at 7°C. In phosphate-peptone buffer, 100 or 200 ppm BHA was lethal to *P. fluorescens*. The extent of lethality was dependent upon BHA concentration, temperature and prior exposure to sub-inhibitory levels of BHA. In contrast to *P. fluorescens*, growth occurred with *P. fragi* ATCC 4973 in TSB even at 400 ppm BHA at 7° and 22°C. *Pseudomonas fragi* was also more resistant to the lethal effects of BHA in phosphate-peptone buffer.

INTRODUCTION

STORAGE OF MEATS and dairy products at refrigeration temperatures is a primary method of preserving these foods from the effects of microorganisms. These temperatures prevent the spoilage of foods by thermophilic and mesophilic microorganisms; however, they do not substantially inhibit the growth of psychrotrophic bacteria (Gill and Newton, 1977). Spoilage of refrigerated products by psychrotrophic *Pseudomonas* species is one of the main factors which limit the shelf-life of these foods (Jay, 1967; Jones and Langlois, 1977; Lerke et al., 1965; Nagel et al., 1960).

Little work has been done on investigating preservative compounds for use in refrigerated food products. Some work has been done using various organic chemicals to inhibit the growth of psychrotrophs (Islam et al., 1978). These studies have met with little success. Greater success has been achieved using naturally occurring antimicrobials produced by lactic acid bacteria (Gilliland and Speck, 1975; Branen et al., 1975). Growth of natural psychrotrophs in refrigerated ground beef was inhibited by the spent fermentation medium of a lactic organism (Abo-gnah, 1978). The primary problem with these compounds is that they are not yet allowed in foods. It is logical to test available additives for their effectiveness as inhibitors of psychrotrophic growth.

Phenolic antioxidants have commonly been used to prevent autoxidation of the lipids in a variety of food products. Several of these phenolic antioxidants have been found to possess dual functions as antioxidants and antimicrobials. The antimicrobial activity of these compounds extends to a variety of bacteria, fungi and viruses. One phenolic antioxidant in particular, butylated hydroxyanisole (BHA), has been found to have good antimicrobial activity towards a variety of bacteria (Branen et al., 1980).

The objective of this study was to determine the antimicrobial activity of BHA against two psychrotrophic food spoilage microorganisms, *Pseudomonas fluorescens* and *Pseudomonas fragi*. Growth inhibition and lethality were measured in the presence of BHA. The purpose of these experiments was to evaluate the possible effectiveness of BHA as an antimicrobial in refrigerated foods.

MATERIALS & METHODS

Culture treatment

Stock *Pseudomonas fluorescens* ATCC 15456 culture was obtained from the Department of Food Science & Technology culture collection. *Pseudomonas fragi* ATCC 4973 was obtained from the American Type Culture Collection, Rockville, MD. Both organisms were grown on Trypticase Soy Agar (TSA) slants (BBL, Cockeysville, Md.) at 22°C or 26°C for 24–48 hr. These slants were stored at 4°C and transferred every 2 wk to maintain viability. Unless otherwise stated, all cultures used were grown in Trypticase Soy Broth (TSB) (BBL) for 24 hr before being inoculated into the test media.

Preparation of BHA

Butylated hydroxyanisole (BHA) was obtained from Eastman Kodak Co., Kingsport, Tenn. A powder of BHA was made by grinding the original tablets in a Sorvall Omnimixer (Newton, Conn.). The powder was then weighed and dissolved in 95% ethanol at concentrations of 1–10% (w/v) to prepare the stock solutions. Appropriate quantities of the stock solutions were added to TSB prior to autoclaving to obtain the desired concentration of BHA.

Growth Inhibition by BHA

For this study, 100 ml of TSB was prepared and the pH adjusted to 6.0 or 7.0 with concentrated HCl. Appropriate aliquots of BHA from stock ethanolic solutions were added to the TSB and 5 ml of the medium was then dispensed into 13 × 100 mm screw-capped culture tubes. The medium was autoclaved at 121°C for 15 min. Duplicate or triplicate tubes of sterilized medium were inoculated with 0.1 ml of a 1:100 dilution of a 24 hr TSB culture of the appropriate microorganism. The inoculum corresponded to approximately 2.0×10^4 *P. fragi*/ml or 8.5×10^4 *P. fluorescens*/ml. Growth was monitored by measuring the absorbance increase at 600 nm on a Bausch and Lomb Spectronic 20 Spectrophotometer (Bausch and Lomb, Inc., Rochester, N.Y.).

Percentage inhibition (PI) was calculated using the following formula:

$$PI = \frac{(\text{Control O.D.} - \text{Control blank O.D.}) - (\text{BHA treatment O.D.} - \text{BHA blank O.D.})}{(\text{Control O.D.} - \text{Control blank O.D.})} \times 100$$

Control blanks were the absorbance reading at time 0 of the incubation period. Butylated hydroxyanisole blanks were uninoculated tubes of TSB with the appropriate amount of BHA.

Both *P. fluorescens* and *P. fragi* were initially grown in the presence of 0, 50, 100, 150, 200, and 400 ppm BHA. Later, *P. fragi* was also grown with 300 ppm BHA. The tubes were incubated at 7° and 22°C for up to 9 days. Controls for ethanol were made by adding the amount of 95% ethanol present in the highest concentration of BHA.

Lethality of BHA

Effect of concentration. A 0.05M phosphate-0.1% peptone buffer, pH 7.0, was prepared in 100 ml aliquots and autoclaved at 121°C for 15 min. One milliliter of a 20-hr culture of either *P. fragi*

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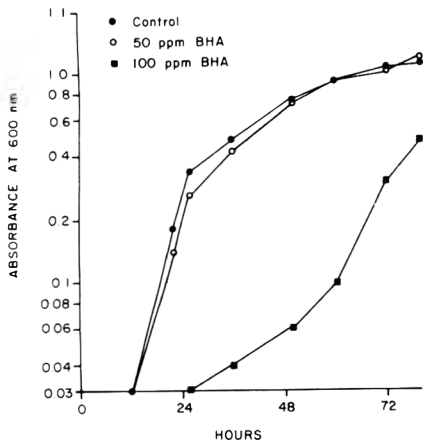

Fig. 1—Inhibition of Pseudomonas fluorescens by butylated hydroxyanisole in Trypticase Soy Broth at 22°C, pH 7.0.

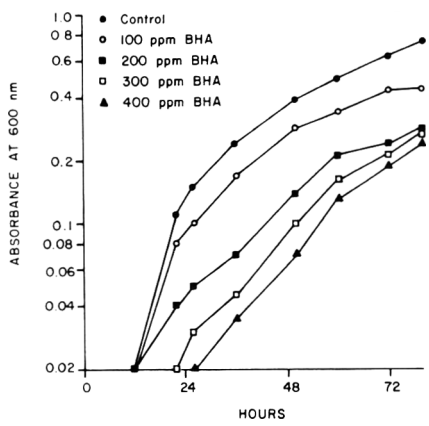

Fig. 2—Inhibition of Pseudomonas fragi by butylated hydroxyanisole in Trypticase Soy Broth at 22°C, pH 7.0.

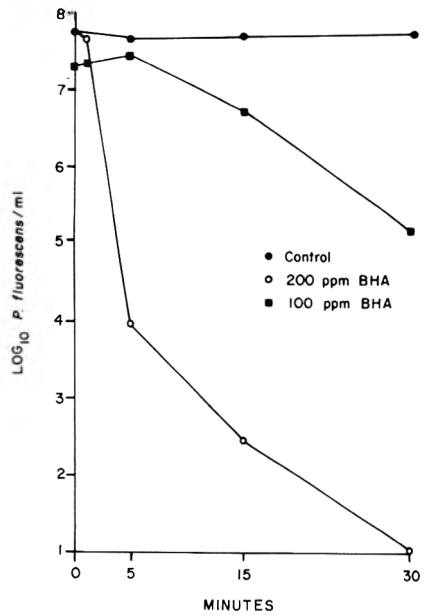

Fig. 3—Lethality rate of butylated hydroxyanisole on Pseudomonas fluorescens in 0.05M phosphate-0.1% peptone buffer, pH 7.0 at 26°C as determined on Trypticase Soy Agar.

or *P. fluorescens*, grown at 26°C in TSB, was added to each flask. Butylated hydroxyanisole in ethanol or 95% ethanol alone (control) was then added to the desired concentration. *Pseudomonas fragi* was treated with 200 ppm BHA while *P. fluorescens* was exposed to 100 and 200 ppm BHA. All tests were done at 26°C. Samples (1 ml) were aseptically removed from the control flasks after 0, 5, 15 and 30 min and from the flasks with BHA after 0, 1, 5, 15, and 30 min. The samples were serially diluted in 99 ml phosphate buffered dilution blanks (APHA, 1978) and pour plates were made using Trypticase Soy Agar. The plates were incubated 48 hr at 22°C.

Effect of temperature. *Pseudomonas fluorescens* was grown as before and suspended in the sterile phosphate-peptone buffer. The inoculated buffer was incubated at either 1C or 26C during the experiment. The cells were then treated with 100 ppm BHA for 30 min. Samples (1 ml) were removed after 0, 5, 15, and 30 min from the control flasks and after 0, 1, 5, 15, and 30 min from the BHA treated samples. Plate counts were made as described previously.

Effect of prior exposure to BHA. *Pseudomonas fluorescens* was grown in the presence of 0, 50, and 100 ppm BHA in TSB for 24–48 hr. The cells were suspended in phosphate-peptone buffer and lethality testing was carried out as described above.

Table 1—Percentage inhibition of Pseudomonas fluorescens and Pseudomonas fragi by butylated hydroxyanisole

	Percentage Inhibition[a]					
	Pseudomonas fluorescens			Pseudomonas fragi		
BHA ppm	22°C pH 7.0	22°C pH 6.0	7°C pH 6.0	22°C pH 7.0	22°C pH 6.0	7°C pH 6.0
50	0	0	65.0	0	0	0
100	56.7	45.5	100.0	40.5	5.2	0
150	–	71.3	100.0	–[b]	–	–
200	–	–	–	63.5	56.2	44.4
300	–	–	–	67.6	49.0	–
400	–	–	–	64.9	–	52.7

[a] Tested in Trypticase Soy Broth, inhibition determined at 3 days for 22°C and 8 days for 7°C. Determined using:

$$\text{Percentage inhibition} = \frac{\text{Control O.D.} - \text{BHA treatment O.D.}}{\text{Control O.D.}} \times 100$$

[b] Not determined

RESULTS & DISCUSSION

THE EFFECTS of temperature and pH on growth inhibition by BHA in Trypticase Soy Broth are compiled in Table 1. A pH of 6.0 was used to simulate common food environments of *Pseudomonas* species. Incubation at 7°C simulated refrigerated storage of foods while 22°C is a commonly used incubation temperature for psychrotrophic organisms. BHA had significant but contrasting antimicrobial activity against the two *Pseudomonas* species in Trypticase Soy Broth (Fig. 1 and 2; Table 1). Under optimal growth conditions of 22°C and pH 7.0, growth of *P. fluorescens* ATCC 15456 and *P. fragi* ATCC 4973 were unaffected by 50 ppm BHA. Growth of both organisms was inhibited by 100 ppm; however, initiation of growth by *P. fluorescens* was significantly delayed in comparison to *P. fragi*. Initiation of growth by *P. fragi* was delayed only at 300 ppm BHA and above. *P. fragi* was also more resistant to BHA than *P. fluorescens* at a pH of 6.0 and a temperature of 7°C (Table 1). One hundred ppm and above totally inhibited growth of *P. fluorescens* and 50 ppm resulted in 65% inhibition at pH 6.0 and 7°C, which is significantly higher than the inhibition at pH 6.0 or 7.0 at 22°C. In contrast the percentage inhibition of *P. fragi* at pH 6.0 and 7°C was lower at all concentrations as compared to the same concentrations at pH 6.0 or 7.0 at 22°C.

Since *P. fluorescens* and *P. fragi* are from the same genus and have similar characteristics, it might be expected that they would have similar susceptibilities to BHA. In most cases however, *P. fragi* was more resistant than *P. fluorescens* to growth inhibition by BHA. The difference in susceptibility to BHA of these closely related microorganisms is probably related to metabolic or structural differences. Klindworth et al. (1979) found that culture age played an important role in the susceptibility of *Clostridium perfringens* to BHA. They concluded that young or old cultures had increased susceptibility to BHA. The susceptibility was independent of cell number and was related to metabolic rate and toxic end product formation. These findings may partially explain the increased susceptibility of *P. fluorescens* compared to *P. fragi*. Twenty-four hour

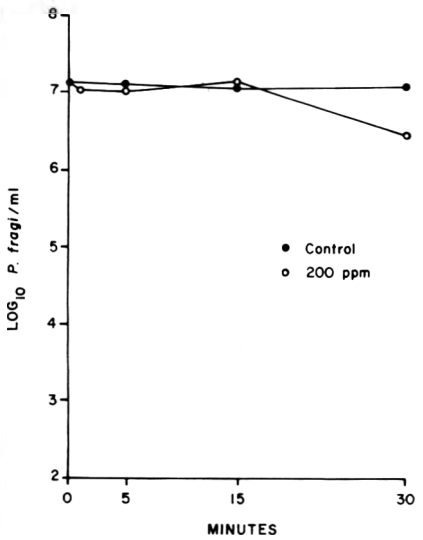

Fig. 4—Lethality rate of 200 ppm butylated hydroxyanisole on Pseudomonas fragi in 0.05M phosphate-0.1% peptone buffer, pH 7.0 at 26°C as determined on Trypticase Soy Agar.

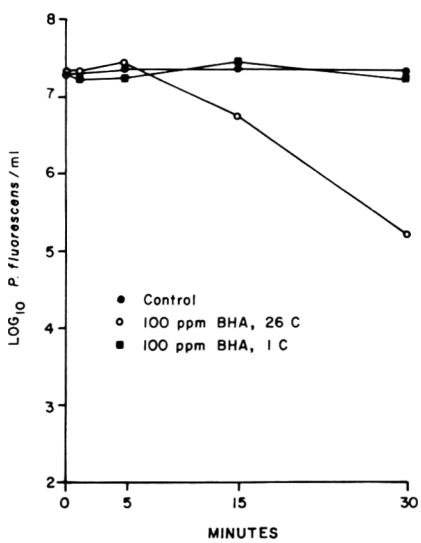

Fig. 5—Lethality rate of 100 ppm butylated hydroxyanisole on Pseudomonas fluorescens in 0.05M phosphate-0.1% peptone buffer, pH 7.0, at 1° and 26°C as determined on Trypticase Soy Agar.

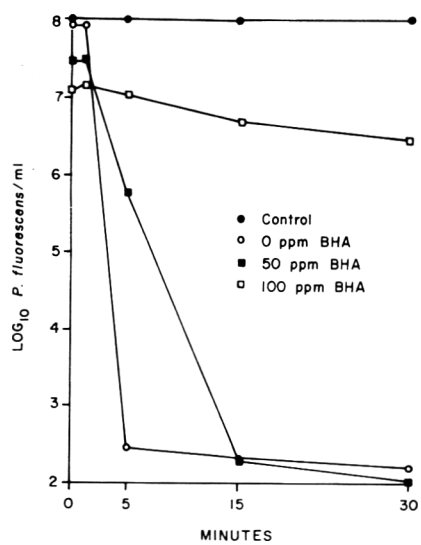

Fig. 6—Lethality rate of 200 ppm butlyated hydroxyanisole on Pseudomonas fluorescens cells grown in the presence of 0, 50, and 100 ppm BHA. Cells were treated in 0.05M phosphate-0.1% peptone buffer, pH 7.0, at 26°C and plated on Trypticase Soy Agar.

cultures containing similar numbers of cells were used in all inhibition tests which minimized the effect of cell number. Inspection of the growth curves of each organism shows however, that *P. fluorescens* had a faster growth rate than *P. fragi* in TSB. This meant that cultures of *P. fluorescens* could have been in a more susceptible growth stage when inoculated into the test medium. At the same time, *P. fragi* could also have been harvested at its peak of resistance or susceptibility. A difference in membrane structure could also be responsible for difference in susceptibility. Since the basic structure of *Pseudomonas* species is similar, these differences would probably be subtle. For example, phenolic compounds such as BHA have been reported to act on the cytoplasmic membrane (Judis, 1963; Juven et al., 1972). The organization of the proteins or lipids in the cytoplasmic membranes of *P. fragi* or *P. fluorescens* could confer more or less resistance to the effects of BHA.

In phosphate-peptone buffer at pH 7.0, viable *P. fluorescens* cells treated with 200 ppm BHA decreased approximately 3.5 log cycles after 5 min of exposure (Fig. 3). After 30 min an estimated 10 cells/ml remained of the original 7.8×10^7 *P. fluorescens* per ml. In contrast, with 100 ppm BHA a two log cycle decrease in viable cells occurred after 30 min. The control count remained constant throughout the test period. *Pseudomonas fragi* was fairly resistant to 200 ppm BHA in the buffer (Fig. 4). After 15 min, no decrease in viable cells was demonstrated and after 30 min the number had decreased less than one log cycle.

The above results are generally in agreement with the data observed in the growth inhibition studies. Growth of *Pseudomonas fluorescens* was partially inhibited by 100 ppm BHA and totally inhibited by 200 ppm BHA at 22°C. In contrast, *P. fragi* growth was only partially inhibited by 200 ppm BHA which was reflected in the lethality study.

Another test was run to compare the effects of different temperatures on lethality. At 26°C, viable *P. fluorescens* cells decreased approximately 70% in 15 min and 99% in 30 min in the presence of 100 ppm BHA (Fig. 5). At 1C, the cell numbers did not decrease during the 30 min test period with 100 ppm BHA. The lack of inhibition at 1°C indicated that the lower temperature had a protective effect on the cells or possibly that the solubility of the BHA was less at this temperature.

The data on *P. fluorescens* inactivation at 1° and 26°C by 100 ppm BHA are in agreement with an earlier study by Wanda et al. (1976). They found a significant decrease in the inactivation of a bacteriophage by butylated hydroxytoluene (BHT) when the exposure temperature was decreased from 25° to 0°C. They postulated that there was a decrease in the partitioning of BHT into the viral membrane which was thought to be the site of inhibition by BHT. The lower partitioning was due to the increased order of lipid alkyl chains of the membrane at lower temperatures preventing the entrance of the BHT. Therefore, the results demonstrating decreased antimicrobial activity of BHA at lower temperatures indicate that BHA possibly acts on the cell membrane.

In growth inhibition tests, *P. fluorescens* was more sensitive to BHA at 7°C than 22°C. This is obviously contradictory to the lethality results. The growth of *P. fluorescens* at 7°C however, was very limited even in the control. Therefore, the temperature could have stressed the organism and caused it to be more susceptible to BHA.

Pseudomonas fluorescens was grown in the presence of various concentrations of BHA to determine if it could become resistant to the antimicrobial effects of BHA. The organism grown in TSB with no BHA present was extremely susceptible to 200 ppm BHA (Fig. 6). In phosphate-peptone buffer at 26C the cell number decreased from 8.5×10^7 per ml to an estimated 300 per ml in 5 min. When *P. fluorescens* was grown in the presence of 50 ppm BHA, the cells showed slightly more resistance to 200 ppm BHA. At 5 min, the number of cells had declined only 1.5 log cycles although by 15 min the number had also decreased to an estimated 200 per ml. *Pseudomonas fluorescens* cells grown in TSB in the presence of 100 ppm BHA showed the most dramatic resistance. After 30 min in the presence of a normally lethal dose of 200 ppm BHA, the number of cells had declined less than one log cycle. The cells had developed approximately the same resistance to 200 ppm BHA as was

demonstrated by *P. fragi*.

The ability of *P. fluorescens* to become resistant to normally lethal concentrations of BHA when grown in the presence of subinhibitory levels is probably related to changes in metabolism or structure of the microorganism. It is possible that the microorganism was able to develop enzymes capable of degrading BHA. However, because of the normally rapid lethal effect produced by BHA, it seems more likely that the organism was changed structurally.

Both *P. fluorescens* and *P. fragi* appear to be more resistant to BHA than other microorganisms which have been tested. Both organisms were able to grow under certain conditions in the presence of 150 ppm BHA. Other studies, however, have shown that the Gram-positive organisms *Clostridium perfringens* and *Staphylococcus aureus* both are totally inhibited by 150 ppm BHA (Klindworth et al., 1979; Chang and Branen, 1975). *Pseudomonas fragi* and *P. fluorescens* are also more resistant than other Gram-negative organisms such as *Vibrio parahaemolyticus* (Robach et al., 1977) and *Salmonella typhimurium* (Davidson et al., 1979). *Pseudomonas fragi* is similar in resistance to BHA as that reported for enteropathogenic *Escherichia coli* (Chang and Branen, 1975). Both *P. fluorescens* and *P. fragi* also showed greater resistance to the lethal effects of BHA in a buffer system compared to *Clostridium perfringens*. *Clostridium perfringens* NCTC 10239 cells were found to be totally destroyed in 10 min by 100 ppm BHA at 37°C in a 0.1% peptone-0.1% cysteine-0.05% sodium thioglycollate-0.9% NaCl buffer at pH 7.0 (Klindworth et al., 1979).

Further studies are needed to determine the usefulness of BHA against *Pseudomonas* species in foods. Work by Robach et al. (1977) and Klindworth et al. (1979) has indicated that high lipid content of foods decreases the activity of BHA toward other microorganisms. Although this hasn't been shown with *Pseudomonas* species it is likely that the same problem would occur. The observed increased resistance to BHA by *Pseudomonas* species could also lead to problems in foods. If BHA were used to inhibit microorganisms there could be selective inhibition of the microorganisms with which *Pseudomonas* normally competes. This would lead to increased growth of *Pseudomonas* and resultant food spoilage. It is also likely, however, that environmental factors in a food system which result in stress on the organism could result in decreased resistance to BHA. Factors such as pH, water activity, oxygen availability, substrate availability, thermal processing and other chemical preservatives appear to influence the activity of BHA and other antimicrobials (Branen et al., 1980).

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ANTIMICROBIAL MECHANISMS OF BUTYLATED HYDROXYANISOLE AGAINST TWO Pseudomonas SPECIES

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ABSTRACT

Exposure of *Pseudomonas fluorescens* and *Pseudomonas fragi* cells to butylated hydroxyanisole (BHA) resulted in a rapid loss of UV absorbing material and previously incorporated ^{14}C-labeled compounds from the cells. *Pseudomonas fluorescens* lost a maximum of 15.3% of the incorporated ^{14}C-label when incubated with 200 ppm BHA and 8.0% when incubated with 100 ppm BHA. *Pseudomonas fragi* was more resistant to BHA losing only 6.4% of the label at 200 ppm and 2.1% at 100 ppm. It was determined that lethality was at least partially due to leakage. The relative percentages of phospholipids in *P. fragi* and major fatty acids in both *P. fragi* and *P. fluorescens* were altered by growth in the presence of various concentrations of BHA.

INTRODUCTION

BUTYLATED HYDROXYANISOLE (BHA) is a phenolic antioxidant which has been commonly used to prevent the autoxidation of lipids in a variety of food products. It has also been found to possess antimicrobial activity against a variety of bacteria. Concentrations of from 50 to 400 ppm have been found to inhibit the growth of *Staphylococcus aureus, Escherichia coli, Vibrio parahaemolyticus, Clostridium perfringens,* and some species of *Salmonella* and *Pseudomonas* (Chang and Branen, 1975; Davidson and Branen, 1980; Davidson et al., 1979; Klindworth et al., 1979; Robach et al., 1977).

Little previous work has been done on elucidating the mechanism of action of BHA as an antimicrobial. Therefore, the relationship of BHA to other antimicrobials must be examined. Since BHA is a phenolic compound, its mechanism of bacterial inhibition is probably, at least partly, related to phenol and its derivatives.

Fogg and Lodge (1945) first proposed that the mechanism of antimicrobial activity of phenols was the inactivation of cellular enzymes. They recognized that the rate limiting step in inhibition was the penetration of the compound into the cell. Vas (1953), however, proposed that phenol attacked the cytoplasmic membrane releasing intracellular constituents. Judis (1963) found that up to 50% of the Na glutamate-3,4-^{14}C and 12% of the $NaH_2{}^{32}PO_4$ were lost by *E. coli* in the presence of the phenolics. It was concluded that the loss was due to a weakening or destruction of the permeability barrier of the cell membrane. Juven et al. (1972) showed that the phenolic glycoside, oleuropein, from olives caused leakage of radioactively labeled glutamate, potassium, and phosphorus from *Lactobacillus plantarum*. They found no effect on the glycolysis rate of the microorganism. This led to the conclusion that oleuropein caused membrane permeability changes. Bernheim (1972) proposed that phenol reacted primarily with the phospholipid component of the cell membrane of *Pseudonomas aeruginosa* subsequently causing an increase in the permeability of the cell membrane. Phenol was later shown to cause rapid swelling of *P. aeruginosa* cells, which further substantiates this hypothesis (Bernheim, 1974). Kaye and Proudfoot (1971) investigated the effect of phenolics on phosphatidyl ethanolamine monolayers. They found that various phenolics caused disruption of the integrity of the monolayers in direct correlation with their antimicrobial activites.

The mechanism of microbial inhibition by BHA may also be related to its aromatic character. For example, toluene has been shown to cause considerable cytoplasmic membrane damage in *E. coli* (Woldringh, 1973; DeSmet et al., 1978). DeSmet et al. (1978) also found that the outer membrane of *E. coli* remained relatively intact. It would seem logical then that microbial inhibition by BHA is at least partially due to the action of the compound on cytoplasmic membranes.

The purpose of these experiments was to provide possible explanations as to why BHA inhibits bacteria. This included measuring the effects of BHA on the leakage and the lipid composition of two *Pseudomonas* species.

MATERIALS & METHODS

Cultures and media

Pseudomonas fluorescens ATCC 15456 and *Pseudomonas fragi* ATCC 4973 were grown on Trypticase Soy Agar (TSA) slants (BBL, Cockeysville, Md.) at 26°C for 24–48 hr. The slants were stored at 4°C and transferred every 2 wk to maintain viability.

A glucose salts (GS) medium first described by Kay and Gronlund (1969) was used in some of the experiments. The medium contained the following ingredients: ammonium phosphate, 3.0 g/L; dipotassium phosphate, 2.0 g/L; ferric sulfate, 0.5 mg/L; glucose, 2.0 g/L; and magnesium suflate, 0.5 g/L. The first three ingredients were added and dissolved. The medium was then adjusted to pH 7.0 with HCl and KOH prior to autoclaving at 121°C for 15 min. Appropriate amounts of autoclaved stock solutions of glucose and magnesium sulfate were added to complete the medium.

Preparation of BHA

Stock solutions of BHA were prepared as described previously (Davidson and Branen, 1980). When necessary the BHA was filter-sterilized using 0.45 μm nitrocellulose membrane filters (Schleicher and Scheull, Keene, N.H.) before adding to the previously sterilized media.

Effect of BHA on leakage

Leakage of UV absorbing material. *Pseudomonas fragi* or *P. fluorescens* cells were grown 24 hr at 22°C in Trypticase Soy Broth (TSB) (BBL). The cells were centrifuged at 8000 × G for 20 min at 5°C in a Beckman Model J-21C Centrifuge (Beckman Instruments, Inc.) and washed twice with a 0.1M phosphate-0.9% (w/v) NaCl buffer, pH 7.0. The cells were suspended to an O.D. at 600 nm of 0.04–0.06 in the phosphate-NaCl buffer. Butylated hydroxyanisole in ethanol was added to a final concentration of 100 ppm to the cells while 95% ethanol was added to the controls. Five milliliter samples were removed at various times and filtered using 0.45 μm pore size membrane filters in Swinnex filter holders (Millipore Corp., Bedford, Mass.) to remove the cells. Absorbance of the filtrates was determined at 260 and 280 nm using a Beckman DU Spectrophotometer (Beckman Instruments, Inc.).

Lethality in conjunction with leakage of UV absorbing material

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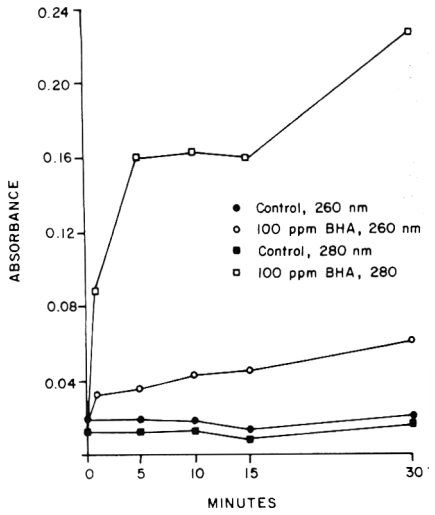

Fig. 1—Ultraviolet absorbance of membrane filtrates of Pseudomonas fluorescens cells treated with 100 ppm butylated hydroxyanisole in 0.1M phosphate-0.9% NaCl buffer, pH 7.0.

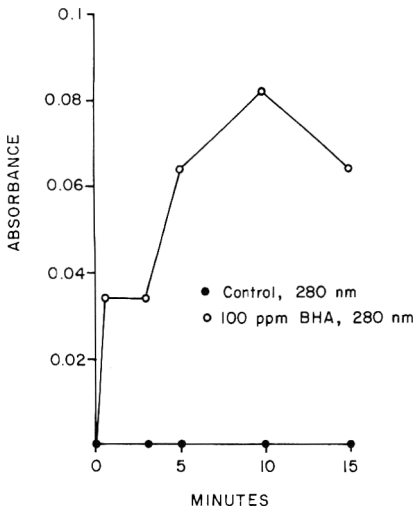

Fig. 2—Ultraviolet absorbance of membrane filtrates of Pseudomonas fragi cells treated with 100 ppm butylated hydroxyanisole in 0.1M phosphate-0.9% NaCl buffer, pH 7.0. The ultraviolet absorbance of the control and treated samples at 260 nm was 0 for the test period.

was measured as described previously (Davidson and Branen, 1980).

Leakage of radioactive compounds. *Pseudomonas fluorescens* and *P. fragi* cells were grown 48 hr at 26°C in 100 ml GS medium containing 3 μg/ml of an amino acid mixture containing the following amino acids at 0.3 mg/L of each: L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine. The cells were transferred to 100 ml of fresh GS medium containing 2.5 μCi of [^{14}C(U)]-L-amino acid mixture (New England Nuclear, Boston, Mass.). The final concentration of ^{14}C-amino acids was 0.19 μg/ml of GS medium. The same amino acids used in the cold sample were present in the radioactive sample. The flasks were incubated 24–30 hr at 26°C. The cells were removed by centrifugation (8000–9000 × G at 5°C) and washed three times with 0.05M phosphate-0.1% peptone buffer, pH 7.0. The cells were then diluted 1:10 in the buffer and distributed in 15–20 ml aliquots in centrifuge tubes. Either 95% ethanol (control) or BHA in ethanol (100 or 200 ppm) was added to the tubes. Tubes were taken at 0, 5, 10, and 15 min intervals and centrifuged at 12,000–13,000 × G to remove the cells. Samples (0.5 ml) of the resulting supernatant liquid were placed in scintillation vials. Aquasol (New England Nuclear) or Scintiverse (Fischer Scientific Co., Fairlawn, N.J.) scintillation fluids were added to the samples before counting them on a Packard Tri Carb Liquid Scintillation Spectrometer Model 3330 (Packard Instruments Co., Inc., Downers Grove, Ill.). Disintigrations per minute per milliliter (Dpm/ml) were calculated using the channels ratio method (Kobayashi and Maudsley, 1974).

Effect of BHA on microbial lipids

Lipid extraction. *Pseudomonas fragi* and *P. fluorescens* were grown 48 hr at 26°C in 500 ml GS medium containing 0, 50, and 100 (*P. fragi* only) ppm BHA. Controls contained an equal amount of 95% ethanol to that used as the BHA carrier. The cells were removed by centrifugation at 8000 × G for 20 min and suspended in 100 ml of distilled water. Lipids were extracted using modifications of Kanfer and Kennedy (1963) and Folch et al. (1951). Trichloroacetic acid (5% w/v) was added to the suspended cells to precipitate the proteins. The suspension was centrifuged and the precipitate mixed with approximately 17 ml/g of chloroform:methanol (2:1). The mixture was homogenized 3 min at high speed in a Waring Blendor and filtered through glass wool and a sintered glass filter. The remainder of the procedure followed that of Folch et al. (1951) without modification. These and all of the following extracts were stored under nitrogen at −20°C.

Phospholipids. Polar and nonpolar lipid fractions were separated on a silica gel column. Silica gel powder (5g, 60–200 mesh) was added to chloroform and placed in a 3 cm × 2m glass column. The column was washed successively with 100 ml of chloroform, 100 ml of methanol, and 50 ml of chloroform. The sample was then placed on the column and the neutral (nonpolar) lipids were eluted with chloroform while the polar lipids were eluted with methanol. The fractions were then evaporated to dryness on a rotary evaporator and redissolved in approximately 5 ml of chloroform.

Thin-layer chromatography (TLC) was used to separate, identify, and quantitate the phospholipid fractions. The TLC system used was a modification of Parsons and Patton (1967). The solid support was pre-activated 250 μm silica gel G on 5 × 20 cm glass plates (Whatman, Inc., Clifton, N.J.). The plates were developed in standard TLC tanks lined with solvent-saturated filter paper. A one-dimensional TLC was run using chloroform-acetone-methanol-acetic acid-water (100:40:20:20:10). Identification of phospholipids was confirmed by running a one-dimensional TLC in a second solvent: chloroform-methanol-water-28% aqueous ammonia (130:70:8:0.5). Phospholipids were visualized by spraying with sulfuric acid-potassium dichromate (1.4g potassium dichormate in 100 ml of 75% concentrated sulfuric acid) and charring at 160°C for 5–20 min. Phospholipid spots were identified by comparing sample R_f values with those obtained for known phospholipid standards (Supelco, Inc., Bellefonte, Pa. and Sigma, St. Louis, Mo.). Percentages of each phospholipid were determined by scanning the developed TLC plates with a Beckman CDS-100 Computing Densitometer (Beckman Instruments, Inc.).

Fatty acids

Fatty acids of total and polar lipid fractions were determined using gas-liquid chromatography (GLC). Methyl esters were prepared by adding 500–1000 μg of the lipid in chloroform into a glass test tube with a teflon-lined screw cap. The chloroform was evaporated with nitrogen and 1 ml of benzene, 2 ml of methanol, and 1 ml of boron trifluoride in methanol were added to the tube. The contents were heated in a boiling water bath for 30 min then cooled under running water. Two to 3 ml of a saturated NaCl solution were added and the methyl esters were extracted twice with 2 to 3 ml petroleum ether. The solvent was evaporated under nitrogen.

Fatty acid composition was determined by GLC of the methyl esters in a Varian Aerograph Series 1700 Gas Chromatograph (Varian Aerograph, Walnut Creek, Calif.) equipped with a flame ionization detector. The 0.3 cm × 1.8m stainless steel column was packed with 15% DEGS on Chromosorb W. The injector, column, and detector temperatures were 220, 190 (isothermal), and 250°C, respectively. Nitrogen was used as the carrier gas at a flow rate of approximately 60 ml/min. Identification of the major fatty acids was done by comparing retention times with those of pure fatty acid methyl ester standards obtained from Nu-Chek Prep, Inc. (Elysian, Minn.).

RESULTS & DISCUSSION

THE DATA PRESENTED in Figures 1 and 2 show increase of UV absorbing material in the filtrates of both *P. fluorescens* and *P. fragi* cells treated with BHA. The largest increase occurred at 280 nm in both samples suggesting that the cells were leaking intracellular (cytoplasmic or periplasmic) proteins. The results agree with previous work which reported that phenolics cause leakage of intracellular constituents (Judis, 1963; Juven et al., 1972). There was also a large difference in the magnitude of the UV absorbance

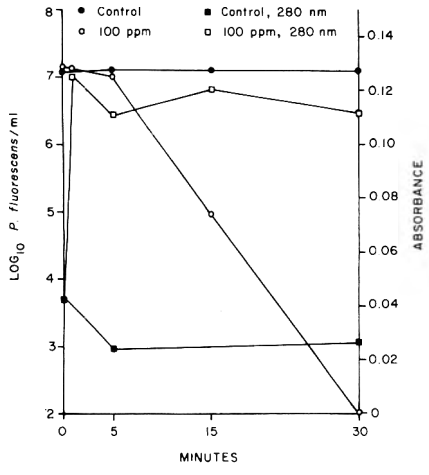

Fig. 3—Lethality and leakage of UV absorbable material of Pseudomonas fluorescens samples in 0.05M phosphate-0.9% NaCl, pH 7.0, treated with 100 ppm butylated hydroxyanisole. Lethality was determined on Trypticase Soy Agar.

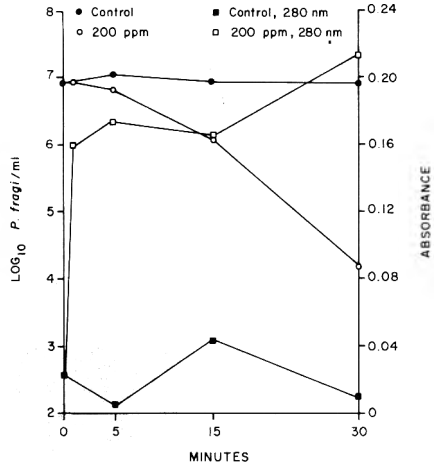

Fig. 4—Lethality and leakage of UV absorbable material of Pseudomonas fragi samples in 0.05M phosphate-0.9% NaCl, pH 7.0, treated with 200 ppm butylated hydroxyanisole. Lethality was determined on Trypticase Soy Agar.

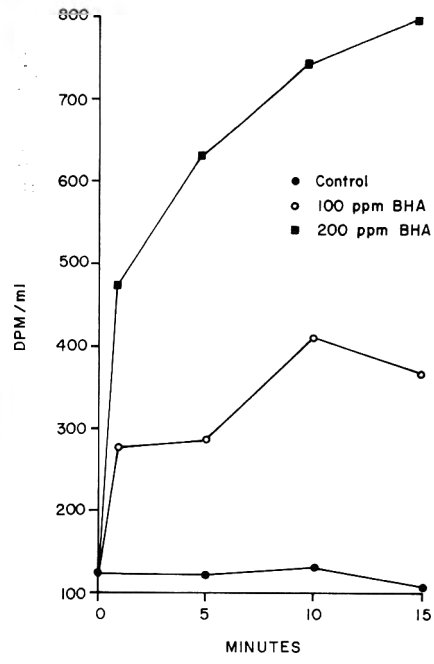

Fig. 5—Leakage of ^{14}C-labeled compounds from Pseudomonas fluorescens treated with butylated hydroxyanisole in 0.05M phosphate, 0.1% peptone, pH 7.0, at 26°C.

increases between *P. fluorescens* and *P. fragi*. *Pseudomonas fluorescens* filtrates reached a maximum of approximately 0.22 O.D. while the *P. fragi* filtrates peaked at 0.08 O.D. This is consistent with the susceptibility of the two organisms to BHA as determined in previous inhibition and lethality testing (Davidson and Branen, 1980).

The leakage of UV absorbable materials appeared to be directly correlated with loss of viability of both *P. fluorescens* and *P. fragi* (Fig. 3 and 4). The increase at 280 nm occurred in the first minute and remained relatively constant after the first sampling. The number of survivors of both microorganisms decreased dramatically after 5 min. *Pseudomonas fluorescens* decreased two log cycles by 15 min and five log cycles by 30 min. *Pseudomonas fragi* decreased one log cycle by 15 min and approximately three log cycles by 30 min. In both cases the increase in UV absorbance was detected before any appreciable lethality took place. This suggests that lethality is partially a result of leakage and not vice versa. This does not mean that lethality is the total result of leakage. Since leakage is the result of disruption of the cell membrane, it is possible that some membrane-bound enzymes of the microorganism could be affected by the BHA which also contribute to the death of the organism.

BHA resulted in increased loss of ^{14}C labelled materials in both *P. fluorescens* and *P. fragi* cells which were previously grown in ^{14}C-amino acids (Fig. 5 and 6). Generally, the major loss in incorporated ^{14}C came at the first sample period (assumed to be 1 min due to the come up time of the centrifuge). In the first minute, the counts of *P. fluorescens* filtrates were 223 and 382% of the control at 100 and 200 ppm BHA, respectively. The counts reached a maximum of 312% at 100 ppm and 742% at 200 ppm. At 200 ppm BHA, *P. fragi* samples were 378% of the control at 1 min and reached a maximum of 561% at 15 min. At 100 ppm, the loss of ^{14}C was much lower from *P. fragi* as compared to *P. fluorescens*. It reached a maximum at 15 min of 261% of the control.

A plot of the loss of ^{14}C as a percentage of the original dpm/ml of the whole cells further indicated that *P. fragi* is more resistant to BHA than is *P. fluorescens* (Fig. 7). Even at 200 ppm, the *P. fragi* leakage did not reach that of *P. fluorescens* at 100 ppm. Additional experiments indicated that both microorganisms reached approximately maximum ^{14}C loss at 400 ppm BHA after a 15 min exposure (Fig. 8). Although leakage of ^{14}C appears higher from *P. fragi* as compared to *P. fluorescens*, the total loss of ^{14}C expressed as a percentage of cpm/ml of the whole cells indicates that *P. fragi* lost only 11.5% of the total count as compared to 24.8% loss by *P. fluorescens* at 400 ppm. The fact that the organisms reached a maximum percentage leakage indicates that some of the ^{14}C may have been incorporated into portions of the cell which can be centrifuged out, such as the cell wall. It is also conceivable that the loss of intracellular constituents reached an equilibrium with the surrounding medium as previously reported by Lamanna et al. (1973). In any event the studies with ^{14}C leakage appear to confirm that BHA causes leakage of proteinaceous materials from both *P. fragi* and *P. fluorescens*. The studies also indicate that the leakage of these materials is greater in BHA-treated *P. fluorescens* cells than BHA-treated *P. fragi* cells, which supports the contention that *P. fragi* is more resistant to BHA than *P. fluorescens*.

The phospholipids of *P. fluorescens* and *P. fragi* were separated and identified using thin-layer chromatography (TLC). Three spots were detected for each of the organisms in all the treatments. Phosphatidyl inositol, phosphatidyl ethanolamine, and phosphatidyl glycerol standards were consistent with the spots detected from the polar lipid fractions of *P. fragi* and *P. fluorescens* grown in the presence of 0 and 50 ppm BHA (Table 1). *Pseudomonas fragi* grown with 100 ppm BHA had consistently higher R_f values for

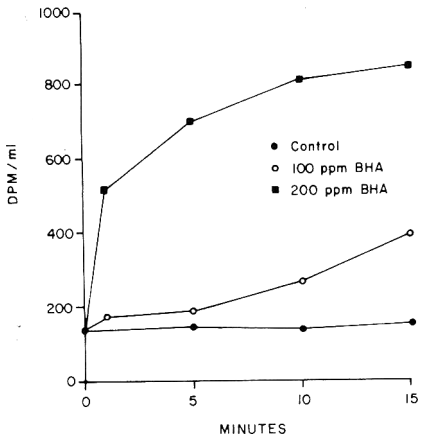

Fig. 6—Leakage of ^{14}C-labeled compounds from Pseudomonas fragi treated with butylated hydroxyanisole in 0.05M phosphate-0.1% peptone, pH 7.0, at 26° C.

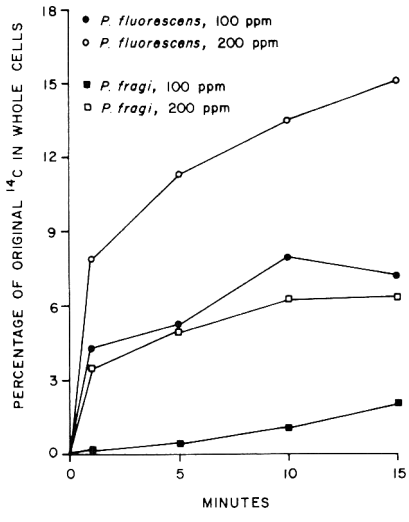

Fig. 7—Leakage of ^{14}C-labeled compounds from Pseudomonas fluorescens and Pseudomonas fragi treated with butylated hydroxyanisole expressed as a percentage of the dpm/ml of the whole cells.

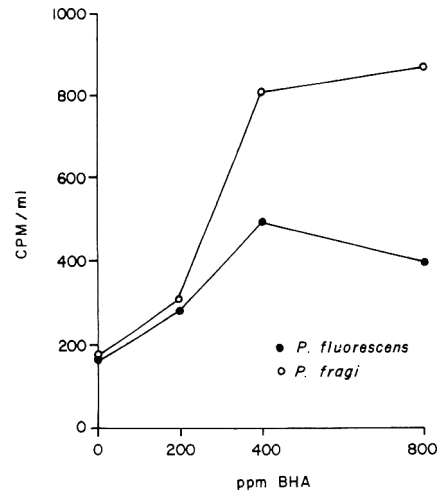

Fig. 8—Leakage of ^{14}C-labeled compounds from Pseudomonas fluorescens and Pseudomonas fragi treated with various concentrations of butylated hydroxyanisole in 0.05M phosphate-0.1% peptone, pH 7.0, at 26° C for 15 min. The cpm/ml in the whole cells were 1364 cpm/ml for P. fluorescens and 5523 cpm/ml for P. fragi.

the second and third spots than the other two *P. fragi* samples. If the two phospholipid spots were phosphatidylethanolamine and phosphatidyl glycerol, their polarities must have been altered slightly. The altered polarity could have resulted from a response by the organism to growth in the presence of BHA. It is also possible that the mobility of the phospholipids was affected by BHA in the lipid extracts. These two spots could also have been entirely different phospholipids than those in the control. None of the phospholipid standards used, however, had similar mobilities in the solvent system used.

The relative percent of each of the phospholipid fractions was determined by scanning the TLC plates on a densitometer (Table 2). Phosphatidyl inositol remained constant in all treatments of *P. fragi*. There also was no significant difference ($P < 0.05$) in the mean percentages of phosphatidyl ethanolamine and phosphatidyl glycerol between the control and 50 ppm treatment. *Pseudomonas fragi* grown at 100 ppm, however, did have a significantly higher ($P < 0.05$) amount of phosphatidyl glycerol and lower amount of phosphatidyl ethanolamine than the control. There was no significant difference ($P < 0.05$) between the 50 ppm treatment and the 100 ppm treatment. *Pseudomonas fluorescens* did not show any significant differences ($P < 0.05$) between the control and 50 ppm treatments in any of the phospholipid fractions.

The types and amounts of phospholipids detected in this study are generally in agreement with previously published data on bacterial phospholipids. Ingram (1977) found *Escherichia coli* to contain approximately 15% phosphatidyl glycerol and 80% phophatidyl ethanolamine with 3% cardiolipin and 1% lysophosphatidylethanolamine. Meadow (1975) stated that the majority of phospholipid in *Pseudomonas* spp. was in the form of phosphatidyl ethanolamine with smaller amounts of phosphatidyl glycerol.

The difference in the amount of phosphatidyl ethanolamine and phosphatidyl glycerol between the *P. fragi* control and 100 ppm treatment indicates that BHA may have

Table 1—Thin-layer chromatographic separation of phospholipids from Pseudomonas fragi and Psuedomonas fluorescens grown in the presence of various concentrations of butylated hydroxyanisole[a]

Sample	R_f[b] 1	2	3
P. fragi, control	0.20	0.45	0.51
P. fragi, 50 ppm	0.20	0.46	0.52
P. fragi, 100 ppm	0.19	0.52	0.57
P. fluorescens, control	0.21	0.45	0.51
P. fluorescens, 50 ppm	0.21	0.45	0.51
Phosphatidyl inositol standard	0.21	–	–
Phosphatidyl ethanolamine standard	–	0.44	–
Phosphatidyl glycerol standard	–	–	0.49

[a] Solvent used was chloroform:acetone:methanol:acetic acid: water, 100:40:20:20:10.
[b] R_f—Relative to the solvent front.

Table 2—Relative percentages of the phospholipid fractions of Pseudomonas fragi and Pseudomonas fluorescens grown in the presence of various concentrations of butylated hydroxyanisole

Sample extract	Relative percentage Phosphatidyl inositol	Phosphatidyl ethanolamine	Phosphatidyl glycerol
Pseudomonas fragi			
Control	7.0	78.6	14.4
50 ppm BHA	6.7	74.5	18.7
100 ppm BHA	7.1	69.3[a]	23.6[a]
Pseudomonas fluorescens			
Control	9.0	70.4	20.6
50 ppm BHA	5.5	71.4	23.1

[a] Significantly different ($P < 0.05$) than the control.

Table 3—Fatty acid composition of the total lipid fraction from Pseudomonas fluorescens grown in Glucose Salts Medium at 26°C containing 0 and 50 ppm butylated hydroxyanisole

Fatty acid[a]	Relative percent peak area	
	Control	50 ppm BHA
12:0[b]	1.83	1.91
14:0	1.94	2.17
15:0[b]	0.96	0.99
16:0	34.01	36.31
16:1	35.08	36.86
Un 1[c]	2.37	3.81
Un 2[c]	5.09	4.18
18:1	16.18	12.54
Others	2.44	1.22
Unsaturated/Saturated	1.32	1.19

[a] First number denotes number of carbon atoms, second number is number of double bonds.
[b] Identified by plotting log of relative retention time vs carbon number.
[c] Unidentified fatty acids.

Table 5—Fatty acid composition of the total lipid fraction from Pseudomonas fragi grown in Glucose Salts Medium at 26°C containing various concentrations of butylated hydroxyanisole

Fatty acid[a]	Relative percent peak area		
	Control	50 ppm BHA	100 ppm BHA
12:0[b]	2.04	1.73	2.02
14:0	3.46	2.43	3.05
15:0[b]	1.80	0.94	1.66
16:0	36.34	38.93	36.03
16:1	22.67	31.90	28.04
Un 1[c]	13.93	7.18	8.53
Un 2[c]	7.12	2.80	6.94
18:1	12.44	12.39	13.21
Others	0.18	1.72	0.52
Unsaturated/Saturated	0.80	1.01	0.96

[a] First number denotes number of carbon atoms, second number is number of double bonds.
[b] Identified by plotting log of relative retention time vs carbon number.
[c] Unidentified fatty acids.

caused an alteration in the amount of synthesis of these phospholipids. Ingram (1977) studied the effect of various lipophilic compounds on phospholipid components of *E. coli*. No significant effect on the individual components was found when the organism was grown in the presence of benzene, toluene, or methyl paraben. Butylated hydroxyanisole then, would appear to have a more dramatic effect on the phospholipid components than these aromatic compounds although the effect could be related to concentration. The result of even a small change in the amount of individual phospholipid components could change the charge distribution of the membrane and effect protein-lipid interactions (Ingram, 1977). Therefore, the change in the phospholipid percentages of *P. fragi* grown in 100 ppm BHA may have resulted in membrane alterations. The organism could have responded in this manner as a protection mechanism. The reason that the phospholipid percentages of *P. fragi* and *P. fluorescens* grown at 50 ppm BHA were not altered is not clear. The concentration of BHA might have been too low to significantly affect the microorganisms.

The types and relative percentages of each fatty acid in the total and polar lipid fractions of *P. fluorescens* and *P. fragi* are shown in Tables 3 to 6. There was little qualitative difference between the fatty acids identified in the polar and total lipid extracts of *P. fluorescens*. A small amount of saturated 15 carbon fatty acid was present in the total but not in the polar lipid fractions.

The largest percentages of the fatty acids detected in *P. fluorescens* lipids were palmitic (16:0), palmitoleic (16:1), and oleic (18:1). The sum of the relative percentages of these three fatty acids ranged from 85–90% of the total fatty acids extracted. In the total lipid extract, only slight differences were found between the amount of palmitic and palmitoleic in the control and 50 ppm extracts. The amount of oleic in the control, however, was significantly higher ($P < 0.05$) than in the extract from 50 ppm. The ratio of unsaturated to saturated fatty acids declined, due primarily to this decrease in oleic acid. Significant decreases ($P < 0.05$) in both palmitic and palmitoleic acids were found in the polar fraction when the organism was grown in the presence of 50 ppm BHA. A corresponding significant increase in the percentage of oleic acid occurred in the same fraction. As a result the unsaturated to saturated fatty acid ratio remained constant for the control and 50 ppm polar extracts.

The qualitative and quantitative data presented are in general agreement with literature on lipids in *P. fluorescens*

Table 4—Fatty acid composition of the polar lipid fraction from Pseudomonas fluorescens grown in Glucose Salts Medium at 26°C containing 0 and 50 ppm butylated hydroxyanisole

Fatty acid[a]	Relative percent peak area	
	Control	50 ppm BHA
12:0[b]	1.42	1.18
14:0	1.47	1.69
16:0	36.67	34.66
16:1	36.58	34.20
Un 1[c]	1.69	3.26
Un 2[c]	3.91	4.64
18:1	16.10	17.59
Others	2.17	2.78
Unsaturated/Saturated	1.33	1.38

[a] First number denotes number of carbon atoms, second number is number of double bonds.
[b] Identified by plotting log of relative retention time vs carbon number.
[c] Unidentified fatty acids.

Table 6—Fatty acid composition of the polar lipid fraction from Pseudomonas fragi grown in Glucose Salts Medium at 26°C containing various concentrations of butylated hydroxyanisole

Fatty acid[a]	Relative percent peak area		
	Control	50 ppm BHA	100 ppm BHA
12:0[b]	0.47	1.50	1.03
14:0	1.62	2.62	2.52
15:0[b]	0.64	1.54	1.66
16:0	36.84	34.36	34.17
16:1	20.85	24.25	26.38
Un 1[c]	16.23	4.97	5.31
Un 2[c]	7.00	10.44	9.64
18:1	13.08	17.39	17.16
Others	3.28	2.93	2.15
Unsaturated/Saturated	0.86	1.04	1.11

[a] First number denotes number of carbon atoms, second number is number of double bonds.
[b] Identified by plotting log of relative retention time vs carbon number.
[c] Unidentified fatty acids.

(Brian and Gardner, 1968). The retention time data reported by these workers suggest that one of the two unidentified peaks in our study was cyc 17:0. The major fatty acids of the *P. fluorescens* used in their study were palmitic (29.3%), palmitoleic (12.3%), and oleic (42.3%). The quantitative differences in this study and those of Brian and Gardner were probably due to incubation temperature, growth medium, or bacterial strain.

There were no qualitative differences in the identifiable fatty acids from *P. fragi* for any of the treatments. The largest percentage of fatty acids detected were palmitic, palmitoleic, and oleic with the sum of the relative percentages of these three fatty acids ranging from 71–83% of the total extracted. A larger percentage of the fatty acids were present in the two unidentified fractions than existed for *P. fluorescens*. There also was much greater variation among the identified fatty acids than was found in *P. fluorescens*. In the total lipid fraction, palmitoleic was found to be in significantly higher ($P < 0.05$) amounts in both cultures grown in the presence of BHA. There was also an approximate 50% decrease in the amount of unidentified fatty acid No. 1 from the organisms grown in BHA. The increase in palmitoleic acid was primarily responsible for shifting the unsaturated to saturated fatty acid ratio upwards from the control. In the polar extracts, palmitoleic acid was again significantly higher ($P < 0.05$) than the control, but only in the organism grown at 100 ppm BHA. It was found that the percentage of oleic acid was significantly higher in the cultures treated with 50 ppm BHA ($P < 0.05$) and 100 ppm ($P < 0.1$). Unidentified fatty acid No. 1 was also detected in much lower amounts in both organisms grown with BHA present than in the control. The increases in oleic and palmitoleic acids contributed to an overall increase in the unsaturated to saturated fatty acid ratio over the control.

Miller et al. (1971) studied the fatty acid composition of *P. fragi* and their qualitative results were consistent with those of our study. Miller et al. also reported unidentified fatty acids which they postulated to be primarily cyc 17:0. The relative retention times of Brian and Gardner (1968) suggest, as they did with *P. fluorescens*, that one of the unidentified peaks of *P. fragi* was probably cyc 17:0. The quantitative results of Miller et al. (1971) are also in agreement with the present study in relation to the types of major fatty acids detected. The actual percentages were different however, which was probably related to environmental growth conditions.

Previous evidence has shown that BHA and BHT cause structural modifications in biomembranes (Sgaragli et al., 1977). These modifications are the result of perturbations of the lipid alkyl chains in the membranes caused by the compounds. When the lipid alkyl chains are perturbed, they lose their ordered state and become more fluid (Eletr et al., 1974; Singer and Wan, 1977). Therefore it might be expected that microorganisms in the presence of BHA would synthesize and add increased amounts of saturated fatty acids to their membranes to maintain the proper fluid state. The extract of total fatty acids from *P. fluorescens* grown with 50 ppm BHA was the only sample to demonstrate a decrease in the unsaturated to saturated fatty acid ratio from the control. Conversely, *P. fragi* cultures grown in BHA actually showed increased unsaturated to saturated fatty acid ratios over the control. Eletr et al. (1974) studied the effect of adamantane, a saturated hydrocarbon of "quasi-spherical" shape, on the fatty acid content of *Saccharomyces cerevisiae*. Adamantane was found to perturb membranes and cause increased fluidity but there was no change in the fatty acid composition of yeast cells grown with and without the compound. It is probable then that, at the concentrations of BHA used, *P. fragi* and *P. fluorescens* do not necessarily respond to BHA simply by synthesizing increased amounts of saturated fatty acids.

Ingram (1977) concluded that small changes in the fatty acid composition coupled with changes in the phospholipid content of membranes could alter membrane organization. It is possible that in the case of *P. fragi* the changes in phospholipid content discussed in the previous section coupled with the changes in fatty acids altered the organization of its membranes. A change in membrane organization is consistent with findings of Eletr et al. (1974). These workers found that compounds which perturb the lipids of membranes cause decreased activity of membrane-bound enzymes. Therefore, *P. fragi* may have changed the organization of its membranes to minimize the effect of BHA on membrane-bound enzymes. This does not explain why *P. fluorescens* was not affected in the same way.

The data on fatty acids also indicated a possible reason for the difference in susceptibility to BHA between *P. fragi* and *P. fluorescens*. The control *P. fragi* culture had a much higher ratio of saturated to unsaturated fatty acids than *P. fluorescens*. This suggests that membranes high in saturated fatty acids are more resistant to the initial effects of BHA. This is probably due to a higher degree of organization or packing of the lipid alkyl chains within the membrane.

In summary, the effects shown by BHA against *P. fluorescens* and *P. fragi* suggest that disruption of the cytoplasmic membrane is at least partially responsible for the inhibitory power of this compound. Evidence for this conclusion comes from the fact that both microorganisms were found to leak intracellular UV absorbing material and ^{14}C labeled compounds in the presence of BHA. The leakage of UV absorbing material was also found to occur before any appreciable lethality of the microorganisms. This indicated that lethality was at least partially a result of leakage. However, leakage of intracellular compounds probably does not totally account for the inhibitory powers of BHA. Eletr et al. (1977) theorized that compounds which disrupt membranes also decrease the activity of membrane-bound enzyme systems. Research is needed to further define the possible effect of BHA on those enzyme systems.

Growth of *P. fluorescens* and *P. fragi* in the presence of BHA caused some significant changes in the fatty acid composition and phospholipid content of the organisms. This suggested that the presence of BHA could have caused changes in the organization of the membrane or outer wall to protect the organism from the effects of the compound. It could also be suggested that these changes were the result of the toxicological effect of BHA.

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EFFECTS OF pH, ENZYMES, AND STORAGE TIME ON THE RHEOLOGY OF SWEET POTATO PUREE

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ABSTRACT

Sweet potato purees were pH adjusted (some also heat treated) and stored up to 9 months in sealed containers at room temperature. Some purees were treated with amylolytic enzymes before or after storage. Rheology and carbohydrates were evaluated after 0, 3, 7, and 9 months. Nonenzyme treated purees at 4.2 pH were semi-solids after 3 months storage, but those at 1.5 and 11.5 pH had lower viscosities. Amylolytic enzyme treatments lowered molecular weights and viscosities. Pre-storage treatments were more effective than post storage in molecular weight reduction. Post storage treatments were more effective in viscosity reduction.

INTRODUCTION

SWEET POTATOES are potentially a good source of carbohydrates, proteins, and vitamins suitable for food and industrial uses (Purcell et al., 1972). However, length of growing season and storage problems, involving energy use and decay, prevent realization of the potential in this crop. Techniques presently used for storage are inadequate to extend the operating season of processing plants (Ice, 1978); therefore, this, and related studies were initiated to explore methods of increasing the usability of sweet potatoes through processing.

One such method is to store sweet potatoes as a microbiologically stabilized puree. Pureeing has been a method of preparing several crops for storage, and the purees—either cooked or raw—are stored in large containers for further processing into various products (Anon., 1976; Wilson, 1976). In previous applications of this type of technology, materials of low starch and high water content were used; thus, concern about flow characteristics of the puree was reduced. Use of materials high in acid content also reduces the need for extensive thermal processing (NCA, 1973).

Plant tissue purees generally have pseudoplastic flow characteristics and a yield stress (Muller, 1973). Under certain conditions, such as heat or NaOH treatment, starch or pectin gels can form and increase both the yield stress and the apparent viscosity of the puree such that it will act more like a solid. If a commodity is to be pureed, flow characteristics of the puree should be investigated so that it might be prepared and handled expeditiously. These characteristics would not only determine the kinds and amounts of chemicals to be included in the puree, but also the penetration of heat into the puree.

The technology used before storage should be simple and rapid to minimize handling. If the sweet potato puree were fluid and had a relatively low yield stress, it could be pumped into and out of storage tanks.

The viscosity of sweet potato puree would be expected to depend on the carbohydrate fraction (Heckman, 1977). If the starch gelatinizes the puree will increase in viscosity and may even solidify. Heat treatment or solubilization of starch with NaOH would change the form of the starch from granular to diffuse and the change would markedly increase the viscosity of the puree (Heckman, 1977). Hydrolysis of starch would be expected to reduce viscosity by reducing the sizes of the starch molecules (Whitaker, 1972); but, pectin could play a role (Nelson et al., 1977) and thus, complicate the problem.

The present study was to develop data on the flow characteristics of sweet potato purees prepared and preserved by different methods.

EXPERIMENTAL

THE VARIABLES in this study were microbiological stabilization treatments, amylolytic enzyme treatments to hydrolyze starch, and storage times (Ice, 1978). Microbiological stabilization involved pH adjustment with or without pasteurization. The enzyme treatments included no enzyme addition, prestorage enzyme treatment and post storage treatment. Storage times were for 0, 3, 6, 7, and 9 months at room temperature (18–25°C).

Freshly harvested Jewel cultivar sweet potatoes were comminuted with a Fitz Mill (Fitzpatrick Co., Chicago, Ill.) to particles of 0.03 inches or smaller in diameter. Five percent by weight water and 3% by weight 0.25M $CaCl_2$ solution in water were added. Water was added to improve flowability of the raw puree, and $CaCl_2$ was added to increase Ca^{++} concentration of the puree to stabilize an α-amylase enzyme used later in processing some of the puree. The puree was divided into two lots. One lot was subdivided into three parts, and these were brought to pH 1.5, 4.2, and 11.5 with either concentrated H_3PO_4 or 10N NaOH. The portion acidified to pH 4.2 was also pasteurized at 90°C for at least 5 min. The three portions were canned and stored for different periods. Samples of each of these canned portions were evaluated and analyzed at specified times as described below. For the post storage treatment other samples from each of the three portions were brought to pH 5 with NaOH or H_3PO_4, treated with amylolytic enzymes, and analyzed. The second lot was enzymatically hydrolyzed and then divided into three parts. These were microbiologically stabilized like the first lot and then canned. Organization of the procedure is given in Tables 1

Table 1—Codes for identifying samples

Three-part codes that begin with a number

A. The number at the start of the code indicates the pH of the samples (1.5, 4.2, and 11.5). Samples that were adjusted to pH 4.2 were also pasteurized for storage.

B. The designations E0, E1, and E2 indicate no enzyme treatment, treatment before storage, and treatment after storage, respectively.

C. The last part of the code indicates storage time in months. When all storage periods are meant, "X" is used.

Two-part codes

PRO:E0 indicates the raw puree with only the addition of water.
PRO:E0 + Ca indicates PRO:E0 with the $CaCl_2$ solution added.
GEL:E0 indicates PRO:E0 + Ca with heat gelatinization.
GEL:E1 indicates GEL:E0 which has undergone the enzyme treatment.

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and 2. Dilution effects are listed in Table 2.

The canning procedure included filling clean glass (1 qt) containers with puree, filling the headspace with toluene, and sealing containers with enamel-lined lids. Only the samples at pH 4.2, which were pasteurized before canning, were packed hot.

For enzyme treatment, the puree was heated to 90°C by steam injection heating to gelatinize the starch. Next, an α-amylase preparation [Tenase® (Miles Laboratories, Inc.) used at a level of 1 ml per kg puree] was added to the puree to reduce its viscosity by randomly hydrolyzing bonds within the starch molecules. The puree was then allowed to cool slowly at room temperature. This attack on starch increased the number of carbohydrate molecules and, hence, the number of carbohydrate end-groups, which were hydrolyzable by β-amylase and amyloglucosidase. Without inactivating either the natural or supplementary α-amylase, we added β-amylase [Clarase® (Miles Laboratories, Inc.)] and amyloglucosidase [Diazyme® (Miles Laboratories, Inc.)] at levels of 0.06g and 0.01 ml per kg of puree, respectively, and then incubated the puree for about 48 hr at 55°C (Ice, 1978).

At completion of the specified storage periods, samples (E0 and E1) were examined as to general physical character. Containers were checked for etching, and closures were checked for corrosion and other changes. Vacuum or pressure inside the containers was measured by a penetrating gauge, and the pH of the contents was determined. Color, texture, and odor of the samples were evaluated subjectively. Viscometric tests and carbohydrate analyses were made for all samples, including those in the E2 series. Some of the 4.2:E0:X samples were treated with a pectinase enzyme to observe if gelation was at least in part due to pectin.

A collateral study was made with sweet potatoes which had cured naturally incubating at about 29°C and 90% RH for 15 days. These cured sweet potatoes were processed and tested like the freshly harvested ones, except that only one storage time (7 months) was tested. The results were compared with the corresponding results for the uncured sweet potatoes to determine whether curing affected the quality of the stored puree.

Instrumentation

Three different instruments were used to measure, in duplicate, the rheological properties of the purees. A Brookfield Synchro-Lectric Viscometer, Model RVT (Brookfield Engineering Laboratory, Inc.) was used during processing to determine the effect of α-amylase hydrolysis on the viscosity of the starch gel. This instrument was also used to obtain static yield stress values and stress values at 5 rpm for purees after storage treatment. We used a Rotovisco, Model RVI (Haake, Inc.) to obtain viscosity data at different shear rates and then developed flow characteristic curves from those data. An Instron Universal Testing Instrument, Table Model 1130 (Instron Corp.) was used with extrusion devices when the samples were too viscous to be tested with the Haake instrument.

The Brookfield instrument was used with a spindle consisting of a fluted metal cylinder 2 cm in height and 0.8 cm diameter. The surface of the cylinder had 12 flutes 0.05 cm deep and 0.05 cm wide oriented parallel to the axis of the rotation to prevent formation of a water layer which would allow the puree to slip. The rotational speed of the Brookfield instrument could not be converted to shear strain rate, because this test was run under wide rotational gap conditions. The width of the gap was variable because it depended on the yield stress. Yield shear stress was obtained from the same test that gave the steady state shear stress at 5 rpm. It was calculated from the maximum reading on the torque scale before rotation started.

The Haake Rotovisco instrument was used with three different rotors–Rotor SVIIP (height 1.96 cm, diameter 2.02 cm), rotor MVI (height 6.00 cm, diameter 4.01 cm), and rotor MVII (height 6.00 cm, diameter 3.68 cm). Different cups were used with the rotors, and the gaps between rotor and cup surfaces differed. Narrow gap conditions were maintained, so the gap was constant for a given rotor-cup combination; thus, shear strain rates could be calculated. The SVIIP rotor was fluted when manufactured, and the other rotors were fluted later to prevent separation of a water layer and, therefore, misleading readings. The data were converted to shear stress, shear rate, and dynamic yield stress (Rao, 1974). These data formed the basis of flow characteristic curves, which were plots of shear stress versus strain rate.

The Instron Machine was used with two extrusion rheometers, each of which was equipped with a piston that fitted closely into a cup. Forcing the piston into the cup when it was filled with puree caused the puree to flow through a small discharge cylinder attached to the cup. The diameter of the cup in one rheometer was 5.72 cm, and the attached discharge cylinder was 10.00 cm long and 0.476 cm in diameter. The cup diameter of the other rheometer was 3.80 cm; and the discharge cylinder was 10.60 cm long and 0.3173 cm in diameter. Force required to extrude the puree was recorded. Piston velocities varied from 3.33×10^{-4} to 3.33×10^{-3} m/s. These data were converted to shear stress and shear strain rate, and were plotted the same way that data from the rotational viscometers were plotted.

In all viscometry, except where specifically stated, the samples were tested at room temperature, i.e., 16–25°C. The rheological properties of the samples were not temperature dependent within this range.

Treatment of viscosity data

In general, plots of shear stress versus shear strain rate fit a curve represented by the following equation:

$$\tau = \tau_o + k\dot{\gamma}^n \quad (1)$$

where τ is shear stress; τ_o is yield stress; $\dot{\gamma}$ is shear strain rate; and k and n are constants. We calculated the constants n, k and τ_o from rotational viscometer data, using appropriate equations from standard references (i.e. Van Wazer et al., 1963). Details are given in Ice (1978).

Because of our inability to measure the extent of plug flow (solid behavior at the center), data from extrusion rheometers were fit to the following simple power (equation) which does not include the yield stress:

$$\tau = k\dot{\gamma}^n \quad (2)$$

For statistical analysis of combined data from both the Haake and extrusion instruments, the latter, simpler equation was used.

Because the values of n and k varied inversely, and because very similar curves could be produced by different pairings of n and k, the product of these terms was calculated when Eq (2) was the model used. If Eq (2) is differentiated, the result is

Table 2–Samples and dilutions caused by additions of reagents during treatments

Sample	Starting material[a]	Additions	% Sweet potato material, by wt
PRO:E0	Sweet Potato	Water	95
PRO:E0 + Ca	PRO:E0	$CaCl_2$ solution	92
1.5:E0:X	PRO:E0 + Ca	H_3PO_4 to lower pH to 1.5	68
1.5:E2:X	1.5:E0:X	NaOH to raise pH to 5 after storage, enzyme preparations	50
4.2:E0:X	PRO:E0 + Ca	H_3PO_4 to lower pH to 4.2, pasteurize	91
4.2:E2:X	4.2:E0:X	NaOH to raise pH to 5 after storage, enzyme preparations	91
11.5:E0:X	PRO:E0 + Ca	NaOH to raise pH to 11.5	86
11.5:E2:X	11.5:E0:X	H_3PO_4 to lower pH to 5 after storage, enzyme preparations	84
GEL:E0	PRO:E0 + Ca	Steam injection	92
GEL:E1	GEL:E0	Enzyme preparations	92
1.5:E1:X	GEL:E1	H_3PO_4 to lower pH to 1.5	70
4.2:E1:X	GEL:E1	H_3PO_4 to lower pH to 4.2, pasteurize	
11.5:E1:X	GEL:E1	NaOH to raise pH to 11.5	88

[a] Material in this column plus material in additions column are constituents of sample column, i.e., 1.5:E0:X is composed of PRO:E0 + Ca plus H_3PO_4.

$$\frac{d\tau}{d\dot{\gamma}} = nk\dot{\gamma}^{(n-1)} \quad (3)$$

If $\dot{\gamma}$ is set equal to 1, the term $\dot{\gamma}^{(n-1)}$ is equal to 1; and therefore, can be dropped from Eq (3). The result is

$$\left.\frac{d\tau}{d\dot{\gamma}}\right|_{\dot{\gamma}=1} = nk \quad (4)$$

The product nk is then the slope of τ versus $\dot{\gamma}$ at a strain rate of unity. We used nk to compare fluids since it was a good approximation of the initial slope of the τ versus $\dot{\gamma}$ curve for the data collected, assuming no yield stress (Fig. 1).

An apparent viscosity of the puree was determined at a shear strain rate of 200 s^{-1} by interpolation. This shear strain rate was chosen because it represented a point on the nearly linear part of the shear stress versus shear strain rate curve (Fig. 1).

Yield stress (Fig. 1) was obtained by two methods. Static yield stress was derived from the torque required to induce shearing in the puree with the Brookfield instrument. We obtained dynamic yield stress from a curve that could be expressed by Eq (1) (Rao, 1974). In most instances, the curve of best fit intercepted the shear stress axis, and the intercept equalled the yield stress.

Carbohydrate data

Of the various carbohydrates in raw sweet potato, starch is the predominant one. Therefore, the state of the starch would be expected to strongly affect the flow character of the puree. We measured the amounts of total carbohydrate that could be extracted from the purees with 80% ethanol (Dubois et al., 1956) and used the values as indicators of the amount of starch that had degraded. All carbohydrate measurements were made in duplicate.

Statistical analysis

A three-way analysis of variance was used to indicate the relationships of the independent variables (enzyme treatment, storage time, storage pH) and dependent variables (apparent viscosity at a shear rate of 200 s^{-1}, yield stress, and nk). The nk values were from curves expressing Eq (2). The values for carbohydrate soluble in 80% ethanol were compared with the rheological data.

The design of this experiment (Tables 1 and 2) called for comparisons between the post storage enzyme treatment and the prestorage enzyme treatment and between the prestorage enzyme treatment and no enzyme treatment.

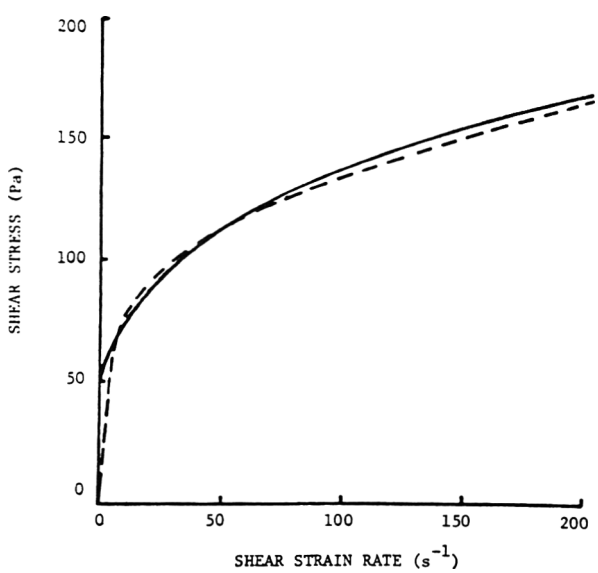

Fig. 1—Representation of the flow character of a specific puree by Eq. (2) (– – –): n = 0.253, k = 43.6; and Eq. (1) (——): n = 0.454, k = 11.0, and τ_o = 50.2.

The analysis of data for comparisons at 7 months' storage of purees made from cured and uncured roots was made with Student's t-test (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

General evaluation of containers, closures, and purees

The containers showed no damage from any of the treatments. The caps were sound in all instances; however, in samples at pH 11.5 stored 9 months without enzyme treatment the enamel lining had begun to turn green. Because toluene filled the headspace, this was not due to direct contact between cap and puree.

Measurement of internal pressure made before opening containers showed a vacuum in all instances, indicating a lack of growth of gas-producing microorganisms. The vacuum readings could be separated into two general groupings. First were those samples which were pasteurized and hot filled into containers, both with and without enzyme treatment (the pH 4.2 samples). Vacuums in these series of samples ranged from 4.7×10^4 to 6.1×10^4 Pa (14–18 inches of mercury). The second grouping of vacuums included all other samples. The vacuums of these samples varied somewhat—3.4×10^3 to 3.0×10^4 Pa (1–9 inches of mercury)—because the containers were filled and sealed before the puree had equilibrated to ambient temperature.

Samples at pH 1.5 stored 6 months without enzyme treatment gelled and could be rotated as a unit within the container. The static yield stress averaged 325 Pa and was higher than the average for this series (Table 3). The puree did not flow easily with gravity. The sample series at pH 1.5 pretreated with enzymes had a smoother appearance than the series without enzyme treatment.

Graininess was apparent in the sample series at pH 11.5 without enzyme treatment and the static yield stress was higher than could be measured with the Brookfield RVT instrument (Table 3). Some of the cellular structures of the tissue were retained intact. The series at pH 11.5 with an enzyme pretreatment was smoother and much less viscous than the series without enzyme treatment.

All samples at pH 4.2 had a smoother appearance because of the heat treatment involved in pasteurization or gelatinization. Samples adjusted to pH 4.2 before storage were highly viscous when being filled into containers at 80–90°C; and over a period of 3 months those without added enzymes—4.2:E0:X—became the strongest gels in the experiment. Their static yield stresses were much greater than could be measured with the Brookfield RVT instrument. The sample series at pH 4.2 with enzyme pretreatment poured easily during filling, but these samples were also gelled by the end of 3 months' storage. These gels were much weaker than the gels in series at pH 4.2 without

Table 3—Average static yield stress values and standard deviations for the three series

Treatment series	Average static yield stress (Pa)	
1.5:E0:X	249	(80)
1.5:E1:X	258	(140)
1.5:E2:X	31	(12)
4.2:E0:X	Much greater than could be evaluated[a]	
4.2:E1:X	225	(21)
4.2:E2:X	169	(88)
11.5:E0:X	Greater than could be evaluated (approx. 400)	
11.5:E1:X	38	(17)
11.5:E2:X	110	(54)

[a] The maximum static yield stress that could be measured accurately with the apparatus used was 358 Pa.

enzyme treatment. The series at pH 4.2 without enzyme treatment and with pretreatment discolored at the interface between the puree and toluene filling the headspace of the containers. This was caused by pigments leaching into the toluene. This phenomenon was not noted in the other series of samples.

Generally, addition of a large amount of acid darkened the puree noticeably. The NaOH addition also caused darkening. These color changes were reversible; for when the purees were brought to pH 5 for post storage treatment, they assumed a more nearly normal color.

At the end of storage, the pH of the most highly acidified series of samples varied somewhat (1.2–1.8), possibly because of the extreme acidity and its effect on the pH meter. Variations may also have been caused by changes in temperature during the processing of the puree. There was no noticeable variation in pH in the other series of samples.

Lack of appreciable variation between samples in a series was taken as an indication that acid producing microorganisms were not active in the puree. The presence of vacuum and lack of visual evidence of mold growth or other microbiological activity also indicated that the purees were stable through the storage periods. No off odors, which might have indicated spoilage, were noted. Additional support for the indication that these purees were microbiologically stable was the fact that the amount of carbohydrate did not change significantly with storage time (Ice, 1978).

Shear stress changes caused by α-amylase hydrolysis

Typical effects of the α-amylase hydrolysis over a period of 15 min are shown in Figure 2. Both static yield stress and stress at 5 rpm indicated that the enzyme preparation containing α-amylase effectively hydrolyzed gelatinized starch. The plots show that these parameters dropped dramatically over the first 5 min and then appeared to stabilize.

Material flow representations

When data from the Haake instrument were represented by Eq (1), which included the dynamic yield stress, correlation coefficients derived from curve fitting were generally in excess of 0.99. Specific yield stresses were reported by Ice (1978). When Eq (2) was used (no yield stress value), the correlation coefficients were somewhat reduced, but were generally in excess of 0.97. In only three instances were the r values between 0.90 and 0.94, and no correlation coefficient was lower than 0.90.

The apparent viscosity at $\dot{\gamma} = 200\ s^{-1}$ and nk [from Eq (2)] varied directly and almost identically (Fig. 3).

Effects of independent variables on flow characteristics

The enzyme treatments significantly reduced nk and apparent viscosity (Table 4), especially in the pH 4.2 samples. At that pH, the viscosity measurements of the E1 and E2 samples were less than one-third those of the E0 samples (Fig. 3). When gels from the sample series at pH 4.2 without enzyme treatment were subsequently exposed to enzymes which act on pectins, the purees became less solid. When these samples were treated with α-amylase to form the post storage enzyme-treated samples the gels were also broken. These observations indicate that both starch and pectin contributed to gel formation. The higher average viscosity for samples at pH 4.2 with enzyme pretreatment over the series with enzyme post treatment (Fig. 3) may have been due to pectin gel formation. The post storage enzyme-treated series was measured soon after enzyme hydrolysis and may not have had time to form a pectin gel.

The nk average and apparent viscosity data show that only in the 11.5 pH series was the pre-storage enzyme treatment more effective in reducing viscosity than the post storage enzyme treatment (Fig. 3). This lower viscosity with pre-storage enzyme treatment may be indicative of the effects of the enzymes in the absence of pectic effects. The pH was too high for pectin gel formation (Nelson et al., 1977), and the purees were only moderately diluted with reagents.

Statistically, storage pH had a highly significant effect on nk and apparent viscosity. The high level of significance was largely due to the high nk and apparent viscosity values for the sample series stored at pH 4.2 without enzyme

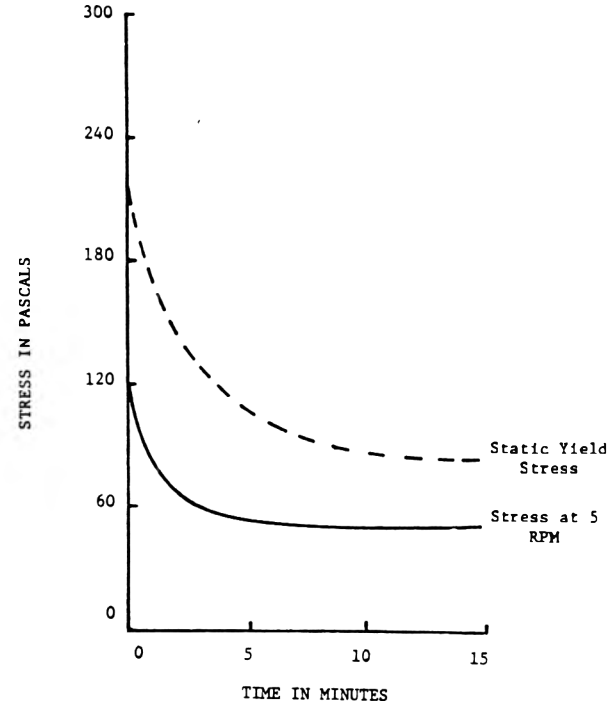

Fig. 2—Effect of α-amylase on the strength of starch gel at 90° C as measured with the Brookfield viscometer.

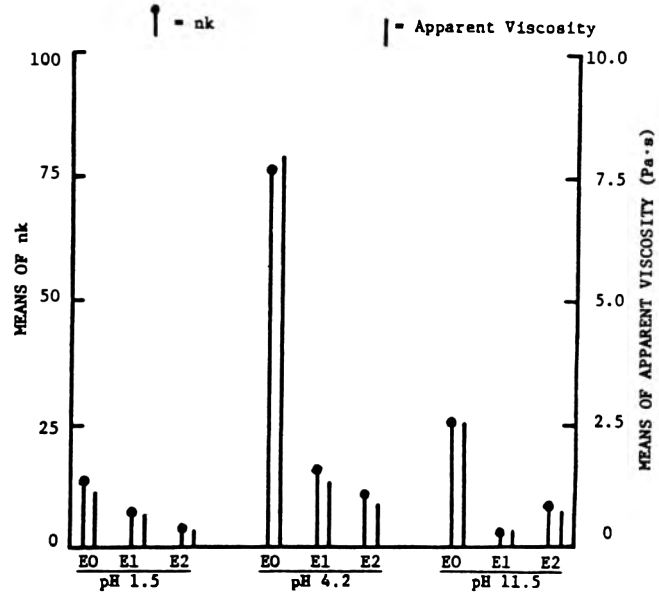

Fig. 3—Means of nk and apparent viscosity for all storage times for each enzyme and pH treatment.

Table 4—Statistical significance level of enzyme effects

E0 vs E1	Level of Significance
Ethanol Soluble Carbohydrate (lower in E0 than E1)	0.01
Apparent Viscosity (lower in E1 than E0)	0.05
nk (lower in E1 than E0)	0.01
E1 vs E2	**Level of Significance**
Ethanol Soluble Carbohydrate (lower in E2 than E1)	0.05
Apparent Viscosity (lower in E1 than E2)	NS[a]
nk (lower in E1 than E2)	NS[a]

[a] Note from Fig. 3 that at pH 1.5 and 4.2 E2<E1 but this is reversed at pH 11.5.

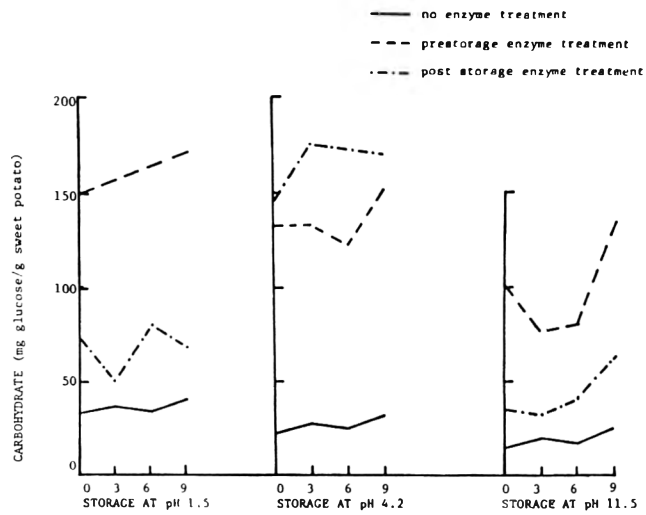

Fig. 4—Changes in carbohydrate soluble in 80% ethanol with months of storage.

treatment which had formed gels. Other contributing causes were the dilution effect of the reagents and the effects of the reagents on the starch. Storage time had no statistically significant effect on any of the dependent variables.

Effects of independent variables on carbohydrates

To better understand the cause of rheological changes, we evaluated our data on carbohydrates extracted in 80% ethanol. The concentration of total soluble carbohydrates (expressed in terms of undiluted puree) tended to increase with storage time and was greater for the hydrolyzed than for the unhydrolyzed samples (Fig. 4). However, the increase in concentration with time was not statistically significant. Pretreatment was more effective than post storage treatment with enzymes in increasing the concentration of the ethanol-soluble carbohydrates (Table 4). Probably the ion concentration of the post storage enzyme-treated samples was high enough to slightly depress the activity of the amylolytic enzymes. Data for the enzyme-treated samples show that, with one exception, apparent viscosity and soluble carbohydrate concentration were inversely related (cf. Fig. 3 and 4). The direct relation between these two parameters in the samples at pH 1.5 was probably due to the greater dilution in the post storage enzyme treatment than prestorage treatment.

The values for total carbohydrate extracted with 80% ethanol are consistent with the fact that viscosity could be reduced by enzymatic hydrolysis. The enzymes may either reduce the amount of starch by converting it into sugars, or they may lower the average molecular weight of starch by breaking off polymeric units from the molecules as indicated by Muller (1971) and Heckman (1977).

Comparison between purees made with cured and uncured roots

Comparison on data on purees stored for 7 months showed that purees made from cured roots were slightly, but not significantly, lower in viscosity and starch content than those made from uncured sweet potatoes.

CONCLUSIONS

SWEET POTATO PUREE can be stored for 9 months at pH 1.5, 4.2 (+ pasteurization), or 11.5. The storage pH and/or enzyme treatment could determine the end use of the puree, for it affects the extent of starch hydrolysis.

We believe that purees like those we prepared could be pumped into storage tanks, but that purees stored at pH 4.2 without enzyme treatment might not be pumped out of the storage tanks unless stirred with a device to overcome yield stress.

The enzymatic hydrolysis was effective in reducing viscosity. If pectin gel formation is likely, the enzyme system should include enzymes that act on pectin.

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POLYPHENOL OXIDASE OF MANGO
(Mangifera indica var. Haden)

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ABSTRACT

The characteristics of mango (*Mangifera indica* var. Haden) polyphenol oxidase (PPO) extract were investigated in order to use it in mango processing. The optimum pH of the enzyme was 5.6–6.0, which is unusual as compared to other fruits. For heat inactivation, half-activity of the enzyme was lost after 2.1 and 4.0 min of treatment at 85 and 80°C, respectively. Regarding the effect of inhibitors on the enzyme, sodium metabisulfite was the most effective, while ascorbic acid was the least. Polyacrylamide gel electrophoresis revealed two isoenzymes which moved toward the anode at pH 8.6 as detected with catechol as substrate; no bands were observed when L-tyrosine was used as substrate. It was found that electrophoretic patterns of mango PPO were quite different, compared to other plant PPO systems.

INTRODUCTION

BROWNING REACTION occurs in many fruits when the tissue is injured, peeled, or diseased. This reaction is due to the conversion of phenolic compounds to quinones by polyphenol oxidase (E.C. 1.10.3.1) (PPO), then undergoing polymerization to impart brown discoloration.

Enzymatic browning of many fruits such as banana (Palmer, 1963), avocado (Knapp, 1965), peaches (Wong et al., 1971), apples (Stelzig et al., 1972), pears (Rivas and Whitaker, 1973), cherries (Benjamin and Montgomery, 1973), and d'Anjou pears (Halim and Montgomery, 1978) have been reported previously.

Recently, we have faced serious problems of discoloration of mango during processing. Although enzymatic browning in fruit systems has been extensively investigated, no work has been published regarding mango PPO. It is necessary to have an understanding of the mango PPO in order to control it effectively in food processing. Accordingly, this work was directed towards isolation of PPO and investigation of some characterization, mainly with respect to heat and chemical inactivation of the enzyme.

MATERIALS & METHODS

Mango sample

The variety of mangos used was *Mangifera indica* var. Haden, grown in Campinas, Brazil, and obtained from a local farm.

Fresh ripe mangos were washed with deionized water and cut quickly into thin slices. Then 500g of sliced mango were immediately blended with 1 liter of anhydrous ethyl alcohol in a Waring Blendor for 1 min and the homogenates were filtered through a Büchner filter funnel with suction. The insoluble solid materials were reblended with anhydrous ethyl alcohol twice and finally, with acetone. After filtration and drying under vacuum, a white powder was obtained. The mango powder was stored at −20°C.

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Preparation of mango PPO

The mango powder was placed in a beaker and deionized water added (1:20 W/V). After agitating for 1 hr, the mixture was centrifuged at 20,000 × G for 15 min. The clear supernatant was decanted and the PPO precipitated by acetone fractionation (1:2 by vol). The precipitate obtained by centrifugation was dissolved in 30 ml of deionized water. This solution was then dialyzed for 48 hr against frequent changes of 0.05M acetone buffer of pH 5.6.

Assay of PPO activity

The enzyme activity was determined by measuring initial rates of the increase in absorbance at 410 nm with a Coleman model 124 D double beam spectrophotometer with direct digital readout at 30°C. The sample cuvette contained 1 ml 0.2M sodium acetate buffer pH 5.6, 1 ml 10 mM catechol, 0.8 ml deionized water, and 0.2 ml of enzyme solution. The reference cuvette contained 1 ml of the same buffer, 1 ml 10 mM catechol, and 1 ml deionized water. Under these conditions, initial rate of enzyme catalyzed reaction was a linear function of time for 2 min. One unit of PPO activity was defined as the potency of enzyme that increased in absorbance by 0.001/min.

Thermal inactivation of the PPO

The thermal denaturation of the enzyme was investigated using the following procedures: Five ml portions of PPO solution were sealed in test tubes and heated in a water bath at 75, 80, and 85°C, respectively. After various heating intervals, 0.5 ml of samples were withdrawn and immediately cooled by immersion in ice-water. The initial rates of PPO activity for the samples were measured as described in the enzyme assay.

pH optimum

pH optimum of mango PPO sample was examined by measuring initial rates of the enzyme activity as described in the enzyme assay, using 0.2M sodium acetate buffer for pH 3.6–5.6, 0.2M sodium phosphate buffer for pH 5.7–8.0, and 0.2M boric acid-borax buffer for pH 8.2–9.2.

Investigation of the enzyme inhibitors

The effect of inhibitors of the enzyme was determined by measuring initial rates of the enzyme activity of mixture of 1 ml 10 mM catechol, 1 ml of various concentrations of inhibitors, 0.8 ml 0.2M sodium acetate buffer pH 5.6, and 0.2 ml PPO solution.

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis was performed as described by Davis (1964). The gel was made of 10g of Cyanogum (95% acrylamide and 5% N,N'-methylene-bisacrylamide), 0.1g of ammonium persulfate and 20 μl of N,N,N',N'-tetramethylethylenediamine in 100 ml of 0.05M phosphate buffer, pH 8.6. Enzyme sample (40 μl) was applied on top of the gel by mixing with 50 μl of 40% sucrose solution and 10 μl of 0.1% bromophenol blue. Electrophoresis was performed at a constant current of 4 mA per gel at 4°C for 2 hr and the electrode buffer was the same phosphate buffer used in gel preparation. After the run, the gels were removed from the tubes and placed in 15 ml of 10 mM catechol solution containing 0.05% p-phenylenediamine for 1 hr.

RESULTS & DISCUSSION

Substrate specificity

As shown in Table 1, various o-diphenolic compounds and L-tyrosine as monophenol were used to determine the substrate specificity of mango PPO. The enzyme was active towards the o-diphenolic compounds but not with monophenol.

pH optimum

Figure 1 illustrates pH optimum of mango PPO activity. Maximum PPO activity occurred at pH 5.6–6.0. The pH

Table 1—Substrate specificity of mango PPO extract

Substrate	Conc (mM)	PPO activity (units/min/ml)
Catechol	5	0.100
Caffeic acid	5	0.050
Catechin	5	0.035
Chlorogenic acid	5	0.090
Pyrogaliol	5	0.070
L-tyrosine	2.5	0.000

curve is characterized by a rapid decrease in enzyme activity at both alkaline and acidic pH values. It is reported that PPO systems in fruits such as d'Anjou pears (Halim and Montgomery, 1978), apple (Betrosian et al., 1960), cranberry (Chan and Yang, 1971), apricot and prunes (Joslyn and Ponting, 1951), clingstone peaches (Wong et al., 1971), are most active at neutral pH. Tate et al. (1964) reported that the pH optimum of Bartlett pear is 6.2. It is clear that mango PPO is most active at a more acidic pH than other fruits.

Thermal inactivation of the enzyme

Figure 2 shows the results of heat inactivation of mango PPO at 75, 80, and 85°C. When the logarithm of the relative activities is plotted against the time interval of heat treatment, straight lines are obtained with slope dependent on temperature. From the resulting graph, the rate of thermal inactivation was proportional to increasing temperature and followed first order kinetics. Thus, half-activity of the mango PPO was inactivated after heating 2.1 and 4.0 min at 85 and 80°C, respectively.

Effect of inhibitors

The results of the effect of inhibitors on mango PPO, using catechol as substrate, are shown in Table 2. Initial rates of the increase in absorbance at 410 nm decreased significantly when ascorbic acid, mercaptoethanol, L-cysteine, and sodium metabisulfite were used at a 0.01 mM level, and no increase in absorbance was observed at 0.05 mM, while diethyldithiocarbamate inhibited 68% at 0.05 mM. Although initial rates of increase in absorbance did not occur at 0.05 mM concentration of inhibitors, visible browning reactions appeared after prolonged time even at high concentrations of inhibitors, except sodium metabisulfite, as shown in Table 3. It is apparent that sodium metabisulfite is the most effective inhibitor of mango PPO, while ascorbic acid was the least potent inhibitor.

Table 2—Effect of inhibitors on mango PPO[a]

Inhibitor	Conc (mM)	Percent inhibition
Ascorbic acid	0.01	17
	0.05	100
L-cysteine	0.01	27
	0.05	100
Diethyldithiocarbamate	0.01	0
	0.05	68
2-Mercaptoethanol	0.01	40
	0.05	100
Sodium metabisulfite	0.01	67
	0.05	100

[a] Method is described in the text.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of mango PPO, as shown in Figure 3, revealed two bands which moved toward the anode at pH 8.6 as detected with catechol as substrate

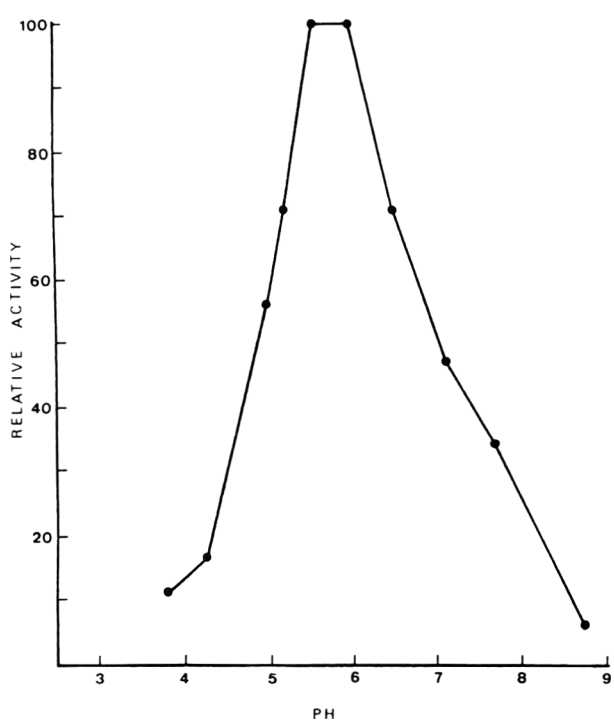

Fig. 1—pH optimum of mango PPO extract.

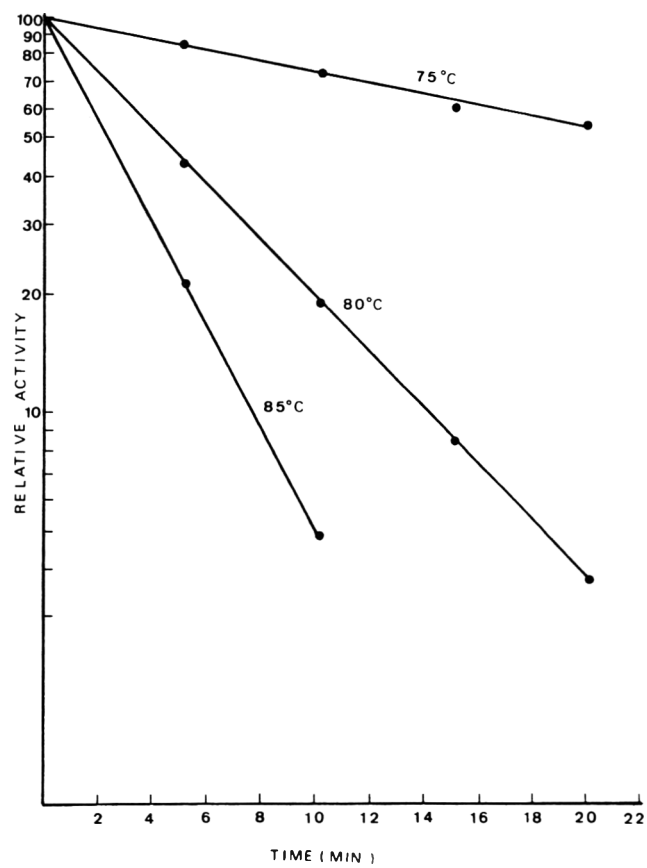

Fig. 2—Thermal inactivation of mango PPO.

Table 3—Relationship between concentration of inhibitors and degree of enzymatic browning

	Holding time (hr)	Concentration of inhibitors (mM)[a]						
		1	2	3	4	5	6	7
Ascorbic acid	24	+++	++	+	±	0	0	0
	72	++++	++++	++++	+++	+++	++	+
Diethyldithiocarbamate	24	+++	++	+	±	0	0	0
	72	+++	++	+	±	0	0	0
L-cysteine	24	++++	+++	0	0	0	0	0
	72	+++	++	+	0	0	0	0
Mercaptoethanol	24	0	0	0	0	0	0	0
	72	++++	+	0	0	0	0	0
Sodium metabisulfite	24	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0

[a] ++++ Deep brown; +++ Brown; ++ Moderate brown; + Weak brown; ± trace.

resembling isoenzymes; no bands were observed when L-tyrosine was used as substrate. This would indicate that isoenzymes of mango PPO were specific for o-diphenol. It was also found that patterns of isoenzymes of the mango PPO system are different as compared to electrophoretic patterns of Royal Ann cherries (Benjamin and Montgomery, 1973), and d'Anjou pears (Halim and Montgomery, 1978).

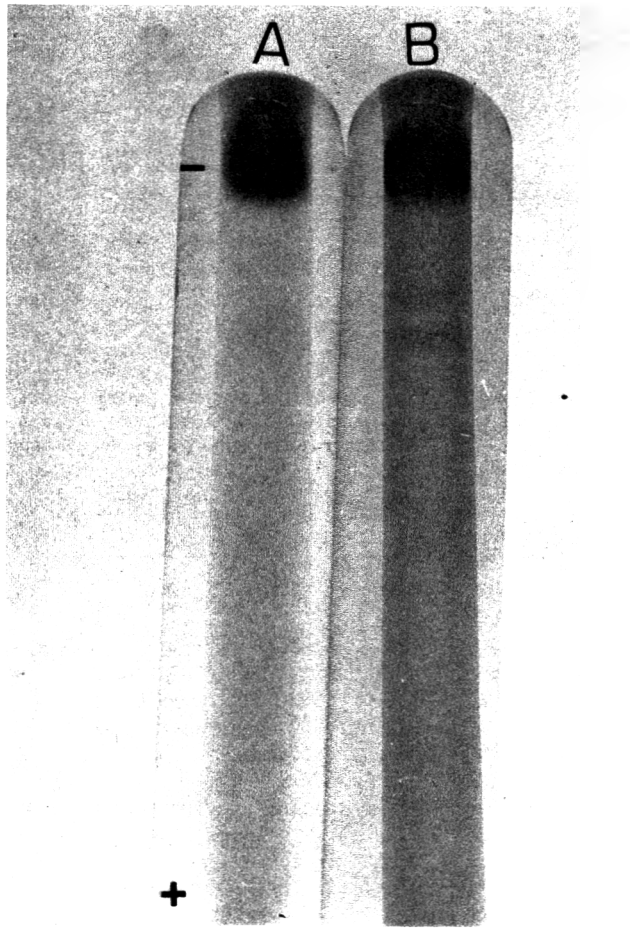

Fig. 3—Electrophoretic patterns of isoenzymes of mango PPO system: (A) L-tyrosine; (B) Catechol.

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HYDROPHOBIC ADSORPTION CHROMATOGRAPHY OF PEACH POLYPHENOL OXIDASE

WILLIAM H. FLURKEY and JOSEPH J. JEN

ABSTRACT

Peach fruit polyphenol oxidase, both crude and pure preparations, were found to adsorb to Phenyl Sepharose, 4-phenylbutylamine Sepharose, CBZ-phenylalanine-TETA-alkyl Sepharose, octyl- and decyl-agarose columns. The enzyme showed no affinity to amino-alkyl agaroses, hydrophobic media with terminal phenol, amino, or carboxyl groups on the ligands. The enzyme is weakly hydrophobic and can be purified and characterized with hydrophobic chromatography. Phenyl Sepharose CL-4B columns gave the best separation of isoenzyme forms of the enzyme probably due to biospecific effect in addition to the hydrophobic interactions.

INTRODUCTION

HYDROPHOBIC adsorption chromatography is a relatively recent technique that has received considerable attention (Hjerten, 1976; Hofstee, 1973; Shaltiel, 1974). This technique allows the separation of proteins by making use of the differences in their size and distribution of available hydrophobic pockets or regions on their surface. The resolution potential of hydrophobic adsorption chromatography depends on choosing the appropriate ligand and/or spacer arm, pH, temperature, salt concentration, and the polarity of the solvents (Shaltiel, 1974). Previously, peach polyphenol oxidase (PPO) was found to preferentially bind to Phenyl Sepharose CL-4B under the high salt condition (Flurkey and Jen, 1978). Adsorption of peach PPO to the hydrophobic media was dependent upon several factors including the salt concentration (Flurkey, 1979). The enzyme could be eluted from the media by decreasing the salt concentration. The advantages of hydrophobic adsorption chromatography on Phenyl Sepharose CL-4B were reported earlier for peach PPO (Jen and Flurkey, 1979), soybean lipoxygenase (Flurkey et al., 1978), and for tomato peroxidase (Jen et al., 1980).

In this study, the characteristics and adsorption of peach PPO to various hydrophobic media under conditions which promote hydrophobic adsorption are reported. The advantages and potential uses of hydrophobic adsoprtion chromatography for PPO are discussed.

EXPERIMENTAL

Plant materials

Peach fruits (*Prunus persica* Batsch cv. Redhaven and Redskin) were harvested and acetone powders were prepared, extracted and assayed for PPO activity as described earlier (Jen and Graham, 1975; Flurkey and Jen, 1978). The Redhaven PPO crude extracts were made to 1M in ammonium sulfate before application onto the hydrophobic columns. The Redskin PPO was purified to apparent homogeneity and dissolved in the column equilibration buffer before application onto the hydrophobic columns (Flurkey, 1979).

Assays

Polyphenol oxidase activity was assayed as described previously using catechol as substrate (Flurkey and Jen, 1978). One unit of PPO activity was defined as a change of 0.1 absorbancy unit per min at 420 nm at 30°C. Column eluates were monitored at 280 nm for approximate protein contents and by a quick PPO activity assay method which involved the incubation of 50 μL of the elutes with 5 ml of 10 mM catechol for 15 min and measuring the absorbance at 420 nm to locate the fractions with PPO activity. Exact protein contents were determined by the Lowry method (Lowry et al., 1951) with BSA as standards.

Adsorbants

Phenyl Sepharose CL-4B (PS) was obtained from Pharmacia Fine Chemicals, Piscataway, N.J. L-leucine agarose (L-leu), L-phenylalanine agarose (L-phe), L-tyrosine agarose (L-tyr), and N-(p-aminophenyl) oxamic acid agarose (APOA) were obtained from Sigma Chemical Co., St. Louis, Mo. Iminoacetic acid Sepharose CL-4B (IAA), aminocaproyl-p-aminobenzamidine Sepharose 4B (ACAB), diaminopropylamine Sepharose CL-4B (DADPA), CBZ-D-phenylalanine-TETA Sepharose 4B (CBZ-phe-TETA), 6-aminocaproic acid Sepharose CL-4B (6-ACA) and 4-phenylbutylamine Sepharose 4B (PBA) were purchased from Pierce Chemical Co., Rockford, Ill. Phenoxyacetyl cellulose (PAC) was obtained from Regis Chemical Co., Morton Grove, Ill. Shaltiel hydrophobic kits I and II consisting of alkyl and amino alkyl agarose (C_n and CN_n) columns, were obtained from Miles Laboratory Inc., Elkhart, Ind.

Chromatography

All resins were deaerated, poured into glass columns, and thoroughly equilibrated with 50 mM phosphate buffer, pH 6.2, containing 1M potassium chloride and 1M ammonium sulfate. This buffer was designated as the equilibration buffer (EB). After sample application, the columns were developed with decreasing step gradients by diluting the EB (i.e., 0.8 × EB, 0.6 × EB etc.). The columns were finally washed with 5 mM phosphate buffer, pH 6.2, and with water. In some cases, the columns were washed with 25% ethyleneglycol (v/v) to remove tightly bound material.

RESULTS

REDHAVEN PPO was chromatographed on Shaltiel alkyl agarose (C_n) columns. The enzyme was completely adsorbed to octyl-(C_8) and decyl-(C_{10}) agarose. Polyphenol oxidase showed minimal adsorption to butyl-(C_4) and slight retention on hexyl-(C_6) agarose under similar chromatographic conditions (Fig. 1). The enzyme was also adsorbed onto Phenyl Sepharose and was retained on this column to a much greater extent as compared to C_{10} column even though their elution patterns appeared similar.

The enzyme showed little adsorption on any of the Shaltiel amino alkyl agarose (CN_n) columns. Slight retention of the enzyme was observed on amino octyl alkyl (CN_8) and decyl alkyl (CN_{10}) agarose (Fig. 2). No adsorption of PPO occurred when chromatographed on the PAC column, a medium designed specifically for hydrophobic adsorption.

Purified Redskin PPO was chromatographed on hydrophobic media under conditions which would promote hydrophobic adsorption (Table 1). The enzyme showed no adsorption to any of the amino alkyl agarose (CN_n) columns. Similarly, little adsorption of Redskin PPO was ob-

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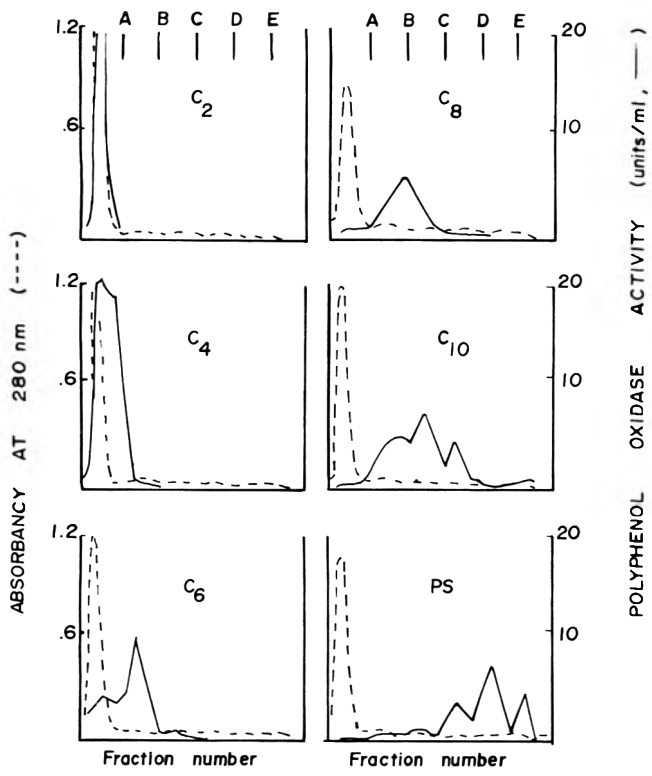

Fig. 1—Hydrophobic adsorption chromatography of Redhaven peach PPO on alkyl agarose and Phenyl Sepharose columns. All column bed volumes were 1 ml. Two ml of crude extract were applied to each column. The columns were eluted with 10 ml each of equilibration buffer (EB), 0.8 X EB, 0.6 X EB, 0.4 X EB, 0.2 X EB, and 5 mM phosphate buffer, all at pH 6.2. The letters ABCDE refer to the change of elution buffers. Three ml fractions were collected and PPO activities were assayed as described in text. C denotes alkyl agarose with x number of carbon atoms in the spacer arm. PS denotes Phenyl Sepharose CL-4B.

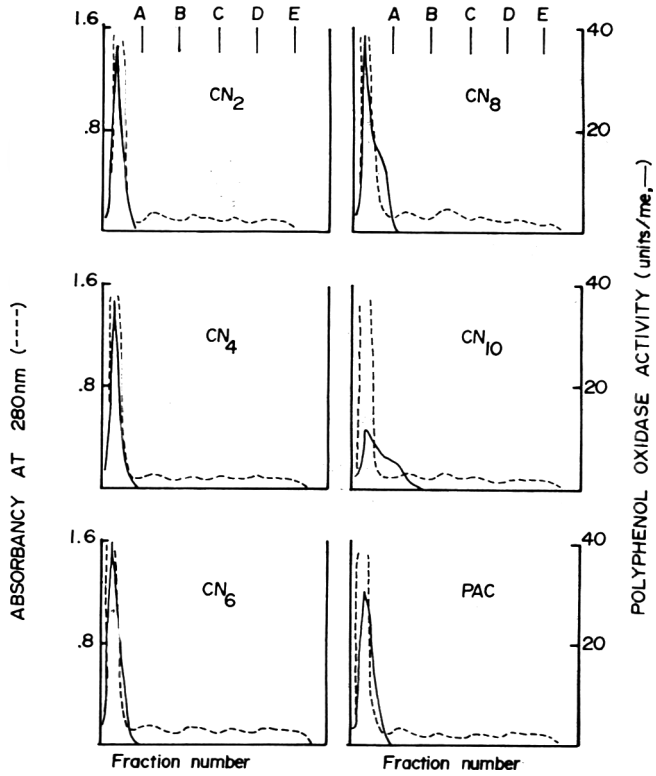

Fig. 2—Hydrophobic adsorption chromatography of Redhaven peach PPO on aminoalkyl agarose and phenoxyacetyl cellulose columns. All conditions were the same as in Fig. 1. CN denotes amino alkyl agarose with x number of carbon atoms in the spacer arm. PAC denotes phenoxyacetyl cellulose.

served on the alkyl agarose columns until an eight carbon alkyl chain length was reached. The enzyme did not adsorb to those media whose ligand has a terminal $-NH_2$, -COOH, or a $HN{=}C{-}NH_2$ groups. Peach PPO was adsorbed to three of the seven media containing a terminal phenyl or phenol group on the ligand or spacer arm (PS, 4-PBA, CBZ-phe-TETA). The enzyme was not absorbed to two of the phenyl type media, PAC and L-phe agarose columns. In addition, PPO did not show any affinity toward those media with a terminal phenol group, such as L-tyr agarose. The enzyme did not adsorb to unsubstituted agarose (C_0) column as did rabbit monamine oxidase under high salt and low pH conditions (Lin and Casteel, 1978).

DISCUSSION

PHENYL SEPHAROSE was shown to be suitable for the purification and characterization of peach PPO (Flurkey and Jen, 1978; Jen and Flurkey, 1979). The medium is equivalent to a straight chain alkyl carbon length between 4 and 5 carbons long (Hjerten, 1976). Since the extent of adsorption of peach PPO to Phenyl Sepharose was greater than a C_8 or C_{10} agarose, forces other than hydrophobic interaction were probably involved in the adsorption. These forces may be biospecific in nature due to the complementarity of molecular contours of the biospecific center of the enzyme (phenol binding) and of the phenyl group in the hydrophobic media (Hofstee, 1976).

Polyphenol oxidase would also adsorb to C_8 and C_{10} agarose columns, but not to the corresponding amino alkyl agarose columns or to other media containing a terminal amino group. Electrostatic adsorption effects are usually eliminated at high salt concentrations (Hofstee, 1976). Under the salt conditions employed in this study, the electrostatic effects should have been minimized. The data suggested that either the electrostatic effects have not been completely eliminated or that the presence of a terminal amino group prevented adsorption of the enzyme by some other means. Increasing the alkyl chain length from a C_8 to a C_{10} did appear to increase retention of peach PPO slightly, presumably by increasing the overall hydrophobicity of the ligand. These results are in agreement with the observation that the binding of amino acyl t-RNA synthetase to aminoalkyl Sepharose was dependent on the alkyl chain length (Jakubowski and Pawelkiewicz, 1973). Similar interference by amino group in the binding of threonine-sensitive aspartokinase homoserine dehydrogenase complex to amino alkyl Sepharose was reported by Broussard et al. (1976). Recently, Hofstee and Otillio (1978) surveyed the hydrophobicity of 12 proteins and found that some proteins were preferentially bound by the aliphatic, others by the aromatic (terminal phenyl group) adsorbants of about the same hydrophobicity and ligand content.

Peach PPO showed no affinity toward many other media listed in Table 1. Many of these media contain a potentially charged group in the ligand. The results presented here suggest that these groups may have prevented the hydrophobic adsorption of PPO. Therefore, the hydrophobicity of peach PPO is probably relatively weak in nature.

Affinity chromatography has been used to purify mush-

Table 1—Hydrophobic adsorption chromatography of purified Redskin peach PPO[a]

Alkyl agarose media		Amino and carboxyl media	
C_0	–	ACAB	–
C_2	–	IAA	–
C_4	–	DADPA	–
C_6	–	6-ACA	–
C_8	+	Phenol media	
C_{10}	+	L-tyr	–
L-leu	–	APOA	–
Amino alkyl agarose media		Phenyl media	
CN_0	–	PS	+
CN_2	–	4-PBA	+
CN_4	–	CBZ-phe-TETA	+
CN_6	–	PAC	–
CN_8	–	L-Phe	–
CN_{10}	–		

[a] All columns (1 ml bed volume) were equilibrated with 40 ml of equilibration buffer (EB). Purified enzyme (20 μg) was added to 1 ml EB before applied onto each column. The columns were eluted with 20 ml of EB, 20 ml of 5 mM phosphate buffer and 5 ml of 25% ethylene glycol. Two ml fractions were collected. Qualitative binding to the columns was indicated by release of PPO with 5 mM phosphate buffer. A + sign indicates adsorption, while a – sign indicates nonadsorption. For abbreviations of the media, see Experimental procedure section.

room tyrosinase (Gutteridge and Robb, 1973; O'Neil et al., 1973) and phenol oxidase in the larvae of housefly (Shimoda et al., 1975). Hydrophobic adsorption chromatography of PPO may be more advantageous than affinity techniques because (1) a ligand attached to a spacer arm is not really necessary for adsorption as in the case of alkyl agarose columns, and (2) the spacer arms of straight chain alkyl media would not be oxidizable by the enzyme or by high pH as were the case in some affinity columns (O'Neil et al., 1973). It is very possible that hydrophobic columns are very useful in the purification and characterization of PPO from other species than peaches and other food enzymes in general.

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AVOCADO CELLULASE: EXTRACTION AND PURIFICATION

MARCEL AWAD and LOWELL N. LEWIS

ABSTRACT

Optimum conditions for the extraction and purification of avocado cellulase have been established. The best quantiative recovery of the cellulase from avocado tissue was obtained with a 0.04M acetate buffer, pH 5.0, containing 0.4M NaCl and 0.25% (v/v) Triton X-100. The isoelectric point of the enzyme was 4.7 and the molecular weight, based on SDS gel electrophoresis, was 49,000. The enzyme was purified on a cellulose column to a single protein band on SDS gel electrophoresis. Rabbit antibody produced to the cellulase gave further proof of the enzyme's purity.

INTRODUCTION

THE AVOCADO FRUIT starts to soften only after being removed from the tree. After harvest, the mesocarp goes through a rapid transition from a hard, leathery texture to a soft, buttery consistency which eventually leads to a loss in all structural integrity. The relationship between this softening of the avocado fruit and the activity of enzymes which degrade the cell wall has been investigated only recently. Reymond and Phaff (1965) showed a positive relationship between polygalacturonase activity and softening of the avocado fruit. Zauberman and Schiffmann-Nadel (1972) demonstrated a postharvest decline in avocado pectinmethylesterase (PME); softening occurred when PME reached its lowest level.

The presence of cellulase in avocado fruit was first mentioned by Lewis et al. (1974), who noted that the cellulase content of ripe avocado mesocarp was more than 100 times higher than that in ripe tomato and peach fruits. They also showed that the enzyme had an isoelectric point of 4.7 and a molecular weight of 50,000–55,000 based on a molecular sieve column. In virtually simultaneous articles, Awad (1977) and Pesis et al. (1977, 1978), reported a close relationship among (1) the rapid increase in cellulase content after harvest, (2) ethylene production, (3) the climacteric rise in respiration, and (4) softening of the fruit. They also showed that fruit softness occurred before maximum cellulase levels were reached.

The large quantity of cellulase found in the avocado mesocarp and the changes in this enzyme's activity during ripening suggest that cellulase plays a critical role in the ripening process. The work described in this paper was done to establish the optimum extraction and purification procedures for the enzyme as a preliminary basis for conducting further studies on the regulation of cellulase synthesis and its mechanism of action in the ripening avocado fruit.

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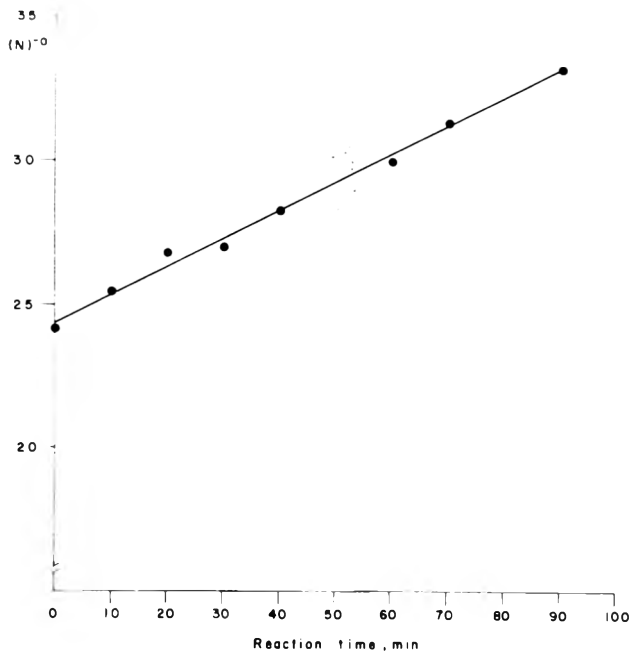

Fig. 1—Effect of time on the intrinsic viscosity (N) to the power -a where a = 3.66 of a mixture of 1% (w/v) carboxymethylcellulose solution in 0.02M Na phosphate buffer, pH 6, and a cellulase extract from lyophilized Fuerte avocado mesocarp. (Ripening stage: 1 day after the climacteric. Extracted with 0.04M K acetate buffer, pH 5 + 0.4M NaCl + 0.25% (v/v) Triton X-100).

MATERIALS & METHODS

Fruit samples

Immediately after harvest, mature avocado fruits were placed in 2000-ml glass jars at constant temperature (20°C) and flushed continuously with water-saturated ethylene-free air (100 ml/min).

At 4-hr intervals, the outflow from the jars was directed to a Beckman 215A Infrared Gas Analyzer for the determination of CO_2 evolution. Data obtained were used to draw the respiration curve for each fruit and served to identify the ripening stage of each fruit used in relation to the climacteric peak of respiration. When fruits were used before the climacteric peak of respiration, the ripening stage was estimated in relation to the climacteric peak of comparable fruits which were not used for the determination of cellulase.

Sliced wedges of mesocarp tissue were frozen in liquid N_2, lyophilized, and ground to a fine powder with a porcelain mortar and pestle. This powder was stored in a desiccator at room temperature and used for cellulase determinations over a period of 1 month. For long-term storage, the material was kept at −20°C. In both cases, cellulase activity showed excellent stability.

Enzyme extraction

Unless otherwise specified, hard tissue was homogenized with 10 ml of cold (2°C) extracting solution (0.04M acetate buffer pH 5.0, 0.4M NaCl, 0.25% v/v Triton X-100) in a Polytron (Brinkmann Instruments). Soft or lyophilized tissue was homogenized in a ground glass mortar and pestle. Usually less than 1g of fresh tissue was taken whereas 100 mg of lyophilized tissue was needed. After a 15-min desorption period, the mixture was centrifuged at 6,000–12,000 × G for 10 min. The supernatant solution below the oily layer was used for the cellulase assay.

—Continued on next page

Table 1—Cellulase extraction from fresh and lyophilized Fuerte avocado mesocarp[a]

Ripening stage[b]	Cellulase units/g fresh wt: Ground in polytron, Fresh mesocarp	Ground in polytron, Lyophilized mesocarp	Ground in mortar, Lyophilized mesocarp
Climacteric	15,800	5,800	8,500
2 days ac	55,200	23,600	33,100
4 days ac	68,300	32,100	39,000

[a] Extracted with 0.04M K acetate buffer, pH 5 + 0.4M NaCl
[b] ac: after the climacteric

Table 2—Effect of pH on cellulase extraction from lyophilized Fuerte avocado mesocarp[a]

pH	Cellulase units/g fresh wt: Ripening stage[b], 2 days bc	1 day bc	1 day ac
4.0	900	3,300	21,800
4.5	1,100	6,500	27,400
5.0	1,500	10,000	35,600
5.5	1,200	9.700	27,400
6.0	1,600	7,800	26,300
7.0	1,100	6,900	24,500
8.0	900	4,800	19,000

[a] Extracted with 0.04M buffer + 0.4M NaCl + 1% (v/v) Triton X-100; K acetate buffer, pH 4–5.5; MES buffer, pH 6; Na phosphate buffer, pH 7; TRIS buffer, pH 8
[b] bc: before the climacteric; ac: after the climacteric.

Table 3—Effect of NaCl concentration on cellulase extraction from lyophilized Fuerte avocado mesocarp[a]

NaCl concn	Cellulase units/g fresh wt: Ripening stage[b], 2 days bc	1 day bc	1 day ac
0.1 M	1,200	6,800	32,200
0.2 M	1,500	6,300	33,800
0.4 M	1,800	8,000	37,400
0.8 M	1,700	7,600	36,400
1.6 M	1,300	6,400	22,800
3.2 M	1,000	3,800	17,900

[a] Extracted with 0.04M K acetate buffer, pH 5 + NaCl + 1% (v/v) Triton X-100
[b] bc: before the climacteric; ac: after the climacteric

Table 4—Effect of Triton X-100 on cellulase extraction from fresh and lyophilized Fuerte avocado mesocarp

Ripening stage[b]	Cellulase units/g fresh wt: Fresh mesocarp, ground in mortar: Extracted with buffer + salt[a]	Extracted with buffer + salt + Triton X-100[a]
2 days ac	32,400	64,800
4 days ac	70,600	127,600
	Lyophilized mesocarp, ground in mortar	
Climacteric	8,500	19,500
2 days ac	33,100	80,000
4 days ac	39,000	84,800

[a] 0.04M K acetate buffer, pH 5; 0.4M NaCl; 1% (v/v) Triton X-100
[b] bc: before the climacteric; ac: after the climacteric

Viscometric assay

The very high cellulase activity present in fruit a few days after harvest necessitated diluting of the supernatant for the viscometric assay. The dilution was made with the same solution used in the extraction.

The assay mixture consisted of 200 μl of the enzyme solution and 400 μl of either 1% (w/v) carboxymethylcellulose (CMC) Type 7H3SF (Hercules Incorporated) in 0.02M Na phosphate buffer, pH 6 or 1.5% (w/v) CMC in 0.04M Na acetate buffer, pH 5. The mixture's drainage time through a calibrated upper portion of a 0.1 ml pipette at 24°C was used to measure viscosity. Drainage times were converted to relative units of cellulase activity using the method of Almin et al. (1967) after demonstrating experimentally that the effect of time on the intrinsic viscosity (N^{-a} where a = 3.66) of a mixture of avocado cellulase and carboxymethylcellulose showed good linearity with time (Fig. 1). All assays were run in duplicate and the results presented are the average of two assays.

Isoelectric focusing (IEF)

IEF was carried out on 2 ml of the extract (equivalent to 200 mg fresh wt) from a lyophilized mesocarp sample obtained 3 days after the fruit's climacteric. The procedure of Haglund (1967) was followed using an ampholine buffer, pH 3–10, and a sucrose-ampholine gradient. The enzyme was focused in a LKB 8101 column at 300V and 4°C for 40 hr. The IEF column was drained and 2.7 ml fractions were collected. The pH of each fraction was measured and then brought to pH 5 for the viscometric assay of cellulase.

Enzyme purification

A lyophilized mesocarp sample obtained 3 days after the climacteric of the fruit was extracted with a solution of 0.04M Na acetate buffer, pH 5 + 0.1M NaCl. The cellulase activity of the supernatant was assayed viscometrically. Ten ml of the supernatant solution were placed at the top of a column (2 × 18 cm) of Cellulose Powder CF-11 (Whatman) equilibrated with 0.02M Na acetate buffer, pH 5.5. This pH was found to result in excellent avocado cellulase retention. The column was eluted with a solution of 0.02M Na acetate buffer, pH 5.5 + 0.1M NaCl + 0.1M cellobiose. Fractions (2.7 ml) were collected and assayed viscometrically for cellulase activity.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis

The enzyme fraction obtained from column purification was subject to SDS-polyacrylamide gel electrophoresis to detect the presence of isoenzymes and to estimate their molecular weight by co-electrophoresis with BSA (MW = 68,000) and ovalbumin (MW = 43,000). Electrophoresis was carried on gel slabs at 240V, 40 mamps and pH 8.

Rabbit antibody (Ab) production

Five hundred μg of purified avocado cellulase stored in the form of an 85% ammonium sulfate pellet was centrifuged at 7000 × G and the supernatant solution was discarded. The pelleted protein was dissolved in 100 λ 0.15M NaCl, and 150 λ Fruend's complete adjuvant.

A young female rabbit was used to produce the antibodies. The 0.5 mg was injected subcutaneously at six sites around the region of the lymph nodes on the hind legs. After 30 days, the rabbit was boosted with 3 mg of cellulase in Freund's incomplete adjuvant over a 3-day period. All injections were subcutaneous.

The rabbit was bled for serum 10 days after the last injection. The Ab titer was checked via Ouchterlony double diffusion plates.

Table 5—Effect of desorption time on the extraction of cellulase from lyophilized Fuerte avocado mesocarp[a]

Desorption time, min	Cellulase units/g fresh wt: Ripening stage[b], 2 days bc	1 day ac
15	1,240	39,500
30	740	42,000
60	860	40,000
120	660	37,900

[a] Extracted with 0.04M K acetate buffer, pH 5 + 0.4M NaCl + 0.25% (v/v) Triton X-100
[b] bc: before the climacteric; ac: after the climacteric

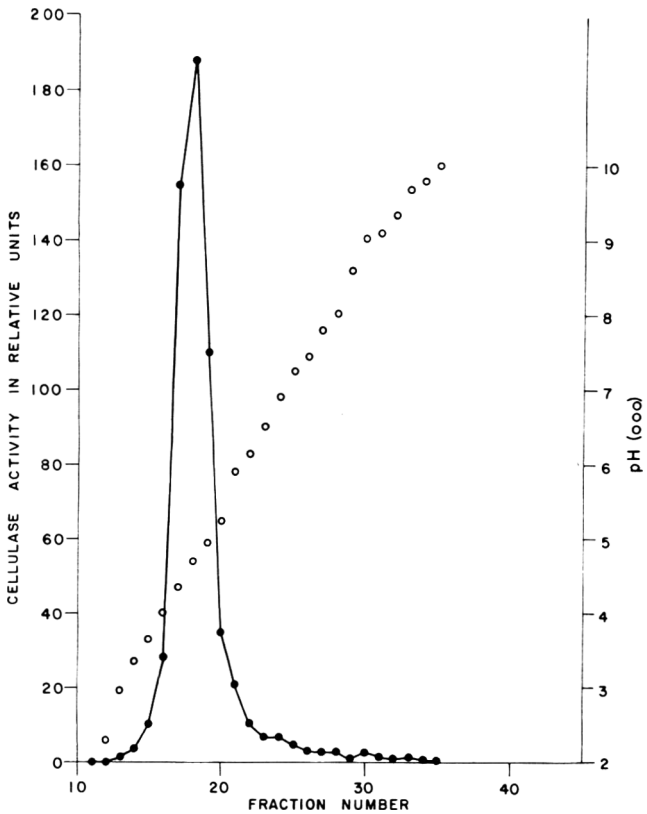

Fig. 2—Isoelectric characterization of cellulase extracted from lyophilized Fuerte avocado mesocarp. Isoelectric focusing in ampholine buffer, pH 3–10. Ripening stage: 3 days after the climacteric. Extraction with 0.4M Na acetate buffer, pH 5 + 0.4M NaCl.

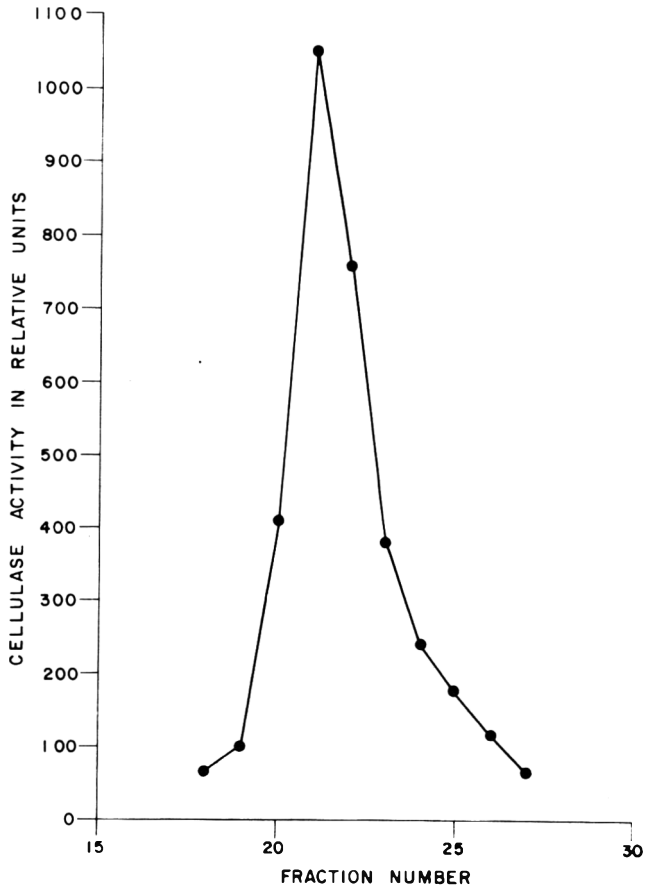

Fig. 3—Purification of cellulase by affinity chromatography on a cellulose powder column equilibrated with 0.02M Na acetate buffer, pH 5.5. Elution with 0.02M Na acetate buffer pH 5.5 + 0.1M NaCl + 0.1M cellobiose.

The titer was found to be low enough to warrant a second booster injection. Forty-five days after the initial injection, 500 μg were injected via the tow pads over a two-day period. Beginning on day 50, the rabbit was bled for serum (≈50 ml each time) twice a week for 1 month until the titer dropped off. The serum from each bleeding was checked on an Ouchterlony double diffusion plate for purity of both the Ab and titer of the serum. When the bleedings were finished, all of the serum was pooled.

The titer of the Ab was determined by running a series of dilutions of the enzyme with a constant amount of anti-serum. The concentration of cellulase at which the Ab no longer removed most of the activity from the supernatant was taken to be the serum titer.

RESULTS & DISCUSSION

AVOCADO CELLULASE activity was consistently higher in fresh tissue than in lyophilized tissue (Table 1); however, the relative levels of activity at various stages of ripening were similar under both conditions. Lyophilized mesocarp has the advantage of uniformity and stability over long storage periods.

The optimum pH for extraction of the enzyme was 5.0 for most pre- and post-climacteric fruit (Table 2). The acetate concentration in the buffer over a range 0.04–0.35M seemed to have little effect on the efficiency of extraction. For all ripening stages studied, cellulase extraction was most efficient when 0.4M NaCl was added to the buffer (Table 3); higher concentrations decreased activity by about 50%. Pesis et al. (1978) found no effect of 1M NaCl on cellulase extraction from acetone powders of avocado fruit pulp. A concentration of 0.25% Triton X-100 (Table 4) generally doubled the amount of cellulase extracted. Adding polyvinylpyrrolidone, diethyldithiocarbamic acid, dithioerithritol, or $CaCl_2$ had no significant effect on the ability of cellulase to be extracted from pre- or post-climacteric fruit. Hence, it would appear that avocado fruit does not contain compounds which interfere with cellulase activity.

The optimum time to solubilize cellulase in the tissue was 15 min for preclimacteric fruit (Table 5); longer times resulted in a decrease in enzyme activity. In post-climacteric fruit, however, the length of the extraction time did not seem to be critical. At least 90% of the enzyme was recovered in the initial extraction.

The isoelectric focusing (IEF) of an extract from lyophilized fruit (Fig. 2) showed that cellulase has an isoelectric point of 4.7 (Lewis et al., 1974). The assay of avocado polygalacturonase (PG) in the fractions obtained from the IEF column showed that the peak of PG activity was also present in the fraction with the highest cellulase activity. The cellulose affinity column is an effective way to purify avocado cellulase. Of the cellulase applied to the column, 97% was retained and 65% was released with the cellobiose-fortified buffer (Fig. 3). Two passes through the cellulose column resulted in a highly purifed sample of cellulase as shown by a single protein-staining band on SDS-acrylamide gel electrophoresis (Fig. 4). Co-electrophoresis of the purified cellulase with protein standards showed that the molecular weight of cellulase was 49,000. This confirms the molecular weight previously established by using bio gel molecular sieving (Lewis et al., 1974).

Rabbit antibodies were produced to the purified avo-

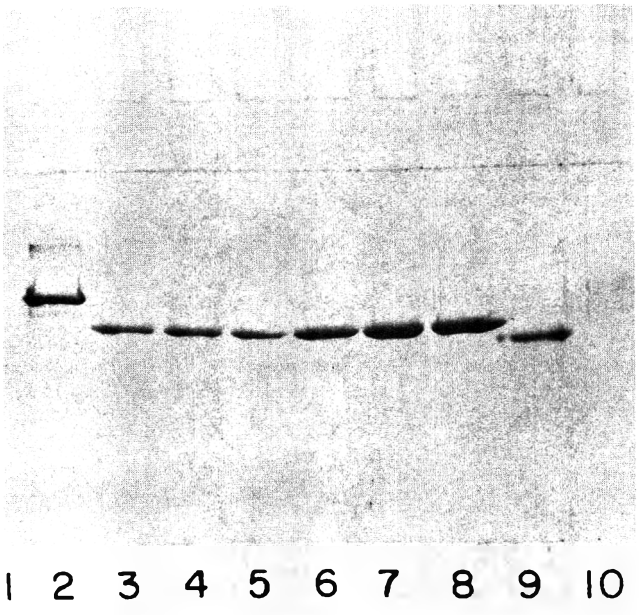

Fig. 4—SDS-PAGE of avocado cellulase; 1 and 10, buffer blanks; 2, 2 μg BSA standard; 3–5, 5 μg cellulase; 6, 7, and 8, 10, 16 and 20 μg cellulase, respectively; 9, 2 μg ovalbumin.

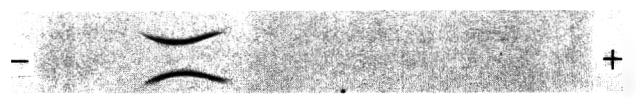

Fig. 5—Immunoelectrophoresis of 5 μg of avocado cellulase against rabbit anticellulase run at pH 7.4.

cado cellulase. The antibody showed a single precipitation line on Ouchterlony gel diffusion plates and on immunodiffusion electrophoresis (Fig. 5) against crude and purified avocado extracts. This is further evidence that the cellulase was purified primarily to a single protein.

Previous experiments in our laboratory showed that a rabbit Ab to Red Kidney bean pI 9.5 cellulase showed slight cross reactivity with avocado cellulase. The Ab to avocado cellulase does not cross react with the bean pI 9.5 cellulase.

There is little known about the role, mechanism of action, or cellular control of synthesis and transport of cellulase in plant tissue development. The avocado fruit is a system which provides an opportunity for further investigations in this area.

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Pisum sativum AND Vicia faba CARBOHYDRATES: STUDIES OF FRACTIONS OBTAINED AFTER DRY AND WET PROTEIN EXTRACTION PROCESSES

P. COLONNA, D. GALLANT, and C. MERCIER

ABSTRACT

The distribution and the composition of the oligosaccharides, starch, and cell-wall polysaccharides were determined on the lower protein air-classified flours of broadbeans, smooth peas, and wrinkled peas and the starchy by-products obtained after the wet process. α-Galactosides are still present in the air-classified fractions whereas they are almost extracted by wet process. In contrast, the fiber content was high in the starchy by-product and in both cases, the sugar constituents of hemicelluloses material indicate that the fraction still present is not representative of the initial hemicelluloses. Starches were isolated in a high yield (60%) from air-classified flours whereas the isolation was not possible from the starchy by-product, probably due to the high hemicelluloses content preventing their extraction. The laboratory scale purification has led to refined starches with no effect of the process on the granules as viewed by light and electron microscopies.

INTRODUCTION

CROPS such as broad beans (*Vicia faba minor*), peas (*Pisum sativum*) and lupines (*Lupinus*) are seen as an agronomically viable alternative to the cereal grains. Protein production will be economic only if the starchy by-products are made profitable. The potential for commercial extraction of leguminous starches has been investigated by Vose (1977, 1978) and Vose et al., (1976) because of the large amount of starch in the flour, except for lupines which contain only traces (0.4%) of starch (Cerning-Beroard and Filiatre, 1976). Two varieties of peas are grown in France: smooth-seeded and wrinkled-seeded peas. Broadbeans and smooth-seeded peas are grown for feeding and protein extraction; wrinkled-seeded peas are used for canning and not for protein extraction due to the difficulty of dehulling. The studies on their starches have been recently reviewed by Colonna and Mercier (1979).

Laboratory and pilot-plant facilities have been developed for the production of protein and starch concentrates from broadbeans and peas. The dry process involved successively pin-milling, air-classification, and refining techniques; the wet concentration method used water solubilization of proteins and removal of the by-products by centrifugation.

The objectives of the present study were to concentrate, isolate, and purify starch, to identify and quantify the free mono- and oligosaccharides and the constituent sugars in polysaccharides of the different fractions—flour, concentrate, refined starch. The chemical composition, the α-amylase susceptibility of starch, and the microscopic appearance of the starch granules were investigated.

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MATERIALS & METHODS

Sample preparation

Mature seeds (moisture content about 13% on wet basis) of *Ascott* broadbeans (*Vicia faba minor*), *Frimas* smooth seeded peas (*Pisum sativum*), *Frogel* wrinkled seeded peas (*Pisum sativum*) were kindly supplied by the Station d'Amélioration des Plantes, I.N.R.A., Dijon (France). The seeds were first cracked between Socam rolls (space 2.8 mm) and then dehulled by a HEF dehuller (Hydromécanique et Frottement, Saint-Etienne), germs remaining with the cotyledons.

In the dry process, the dehulled cotyledons were ground into a flour in an Alpine Kolloplex 1602 grinder, homogenized in a Nauta mixer and then fractionated into "light" and "heavy" fractions using Walther 150 air classifier. This operation was repeated twice for wrinkled peas and broadbeans and three times for smooth peas. The entire process operated at 30 kg/hr. Figure 1 shows the schematic diagram used for the preparation of air-classified samples. Starches were purified from the heavy air-classified fractions by treating with toluene, according to the method of Banks and Greenwood (1975); a previous treatment with alkali was necessary only for wrinkled seeded peas (0.05N NaOH during 2 hr at + 4°C). Prime starch, air-dried in a thin layer at room temperature, was powdered to pass through a 160μ sieve.

In the wet process, the dehulled cotyledons were ground into a flour of 100μ mean particle size, dispersed in water (pH 7.0 adjusted with 2N NaOH) in the ratio 1/10: flour/water during 30 min at 20°C and then fractionated into residual and supernatant fractions using a Westphalia horizontal centrifuge; the speed was 600 L/hr.

Reagents

Aspergillus niger amyloglucosidase (50 UI/mg) (Merck); *Bacillus subtilis* α-amylase (4500 SKB units/g) (Koch Light Lab. Ltd.); Spherisorb-NH_2 (5μ) (Phase Separations Ltd.); Gas chromosorb Q with 3% ECNSS-M (Supelco Inc.); all other reagents were of analytical grade.

Chemical analysis

Moisture content of the sample was determined by drying at 130°C for 2 hr; yield and proximate analysis were expressed on a moisture free basis. Fat was determined according to Drapron (1975), protein by the Kjeldahl method (N × 5.8) (Derbyshire et al., 1976), mineral content by ashing in a platinium dish at 550°C according to the AOAC procedure (1976), and starch by the amyloglucosidase method (Thivend et al., 1972). After 80% ethanol extraction, total free sugars were measured by anthrone method (glucose as standard) (Hood and Mercier, 1978), identified and quantitated by high-pressure liquid chromatography (HPLC) on a column of Spherisorb-NH_2 (Quemener and Mercier, 1980). Furfural generators (Pentosan) content was measured according to Cerning and Guilbot (1973) (arabinose/xylose reactivity, 0.9); results were expressed as anhydroxylose. Acid detergent fiber, cellulose and lignin were determined by the Van Soest (1963) and Van Soest and Wine (1968) methods.

Samples were hydrolyzed according to Saeman et al. (1954) (25.5N sulphuric acid; 30°C; 0.5 hr then dilution to 1N; 100°C; 6 hr) and hydrolyzates were neutralized with barium carbonate. Neutral sugars were converted to their alditol acetates (Sawardeker et al., 1965) and analyzed by gas-liquid chromatography on a Girdel 3000 chromatograph fitted up with a column (180 × 0.2 mm) of 3% ECNSS-M on Gas Chromosorb Q (100–120 mesh) at 185°C (nitrogen: 20 $ml.min^{-1}$). Sugars were detected by flame ionization and quantitated by electronic integration; inositol was used as the internal standard. By this method, analytical data on hemicelluloses and on their chemical composition were obtained. The pentosan content, determined by gas-chromatography, was identical with the furfural generators content; the glucose amount was not taken into account since it comes from starch, cellulose, and hemicelluloses as well.

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Characterization of starches

The susceptibility to *Bacillus subtilis* α-amylase of the sample was carried out as described by Tollier and Guilbot (1971). The initial rate of α-amylolysis Vi corresponds to the amount of starch hydrolyzed during the first 5 min (expressed in gram per cent of total starch). F is the easily degradable starch fraction, represented by extrapolation to zero of the linear part of the α-amylolysis curve.

Particle size analysis of the starch granules was carried out using a Coulter Counter (Model TA II), fitted up with the population accessory attachment. Two different aperture tubes of 100 μm and 140 μm size were used for precise measurement in the high as well as the low diameter range. About 100 mg of the sample were dispersed thoroughly in 100 ml of 0.85% (w:v) NaCl solution (Isoton II), using magnetic stirring. Each sample was analyzed at least four times; the size distribution and the number of particles in each channel were printed using population accessory. The plots were recorded for differential and cumulative size distributions. Particle size graphs are histograms of fractions of total volume of particles as a function of diameter, with the assumption of an uniform spherical shape factor.

Amylose content of the refined starches were determined by amperometric method (Larson et al., 1953) at 25°C, assuming that "pure" amylose has an iodine-binding capacity of 19.0%.

Photomicrographs of the starch granules in aqueous suspensions were taken on an Olympus light microscope (model Vanox) able to work in the polarization mode.

SEM examination. Samples were fixed with double coated tape on a copper horizontal specimen holder. Gold coating was done using the F C 1100 Ion Sputtering Device (Jeol). The thickness of gold deposition was 300 Angstroms. The starch granules were observed under a scanning electron microscope JSM 50A (Jeol), under 20 KeV and very low intensity (8.0×10^{-13} A) to avoid beam damage and charging. To perform observations of starch surface, image signals were treated by a special differential amplifier (VCA).

Microanalysis. Starch ashes were deposited into a small cavity in the surface of cylinders of pure graphite using the method of Gallant and Federowicz-Fortuna (1979). These samples were observed under a scanning electron microscope JSM 50A (Jeol) without metal deposit, under 18 KeV. Microanalytical spectra were obtained by scanning a surface of homogenous material (1050 microns square) of the ashes under absorbed current of 2.0×10^{-11} A. X-ray emissions were analyzed during 200 sec by an EDAX F-System (nondispersive microanalyzer) with a detector Si (Li) installed at 45°, the resolution being 145 eV for Mn Kα under 18 KeV. Calculations were performed with a computer NOVA 3/4 General Data (16K) with two floppy disks. Analyses were obtained between 0–16 KeV on the Kα emission X-rays for the following elements: sodium (1.040 KeV), magnesium (1.253 KeV), aluminium (1.490 KeV), silicon (1.739 KeV), phosphorus (2.013 KeV), potassium (3.310 KeV), calcium (3.690 KeV), and iron (6.400 KeV). Relative concentrations were calculated using Z.A.F. corrections for three characteristics: V = 18 KeV, sample tilting S = 0 and X-rays emergency angle value T = 31.5°.

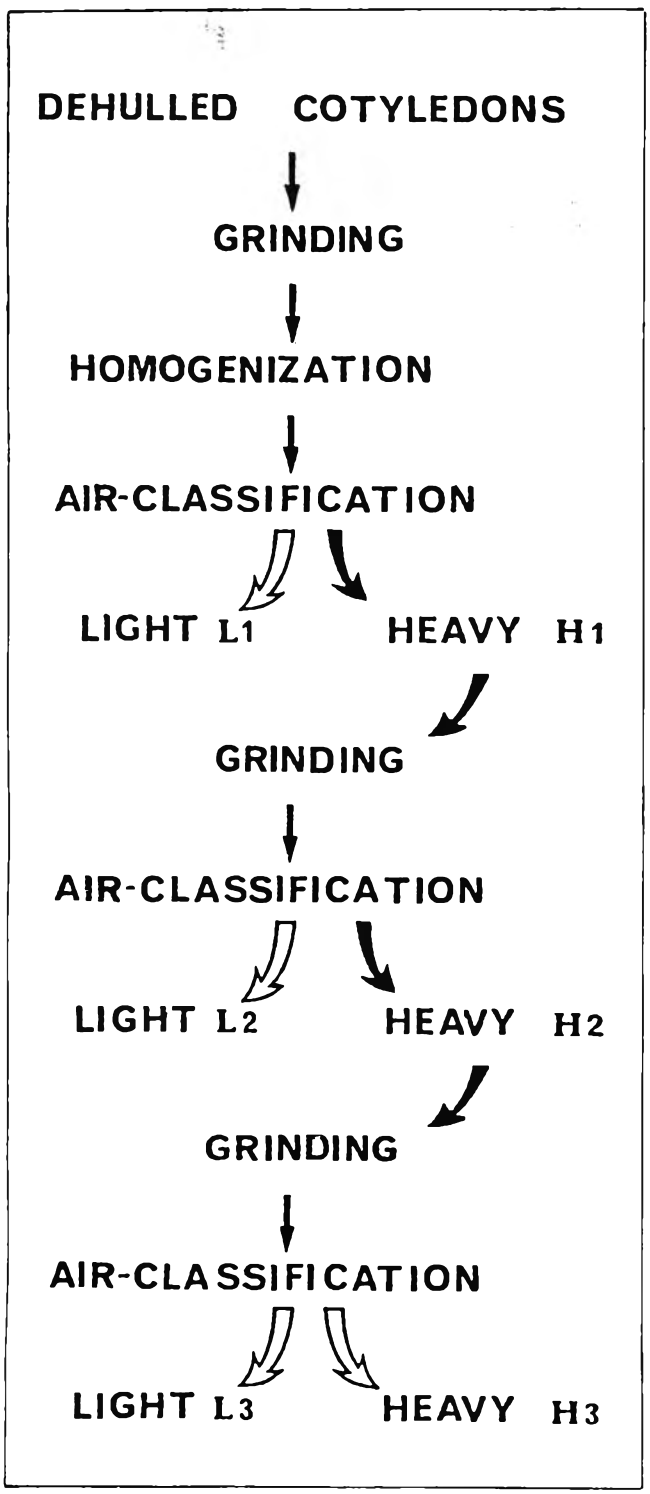

Fig. 1—Schematic flowsheet for dry processing of legume flours.

RESULTS

Air classification

The fractionation procedure of the legumes, including fine milling and air classification, is represented in Table 1. For smooth peas, the protein content of the light fractions L_1 and L_2 increases to 53.7 and 35.7%, respectively, while the dense material H_2 contains only 3.4% protein after a remilling and a second air classification. A third run gives only slight impoverishment of protein with low yield of light fraction, indicating the impossibility to obtain a better separation of proteins from starch by this way. The protein shift is better in the case of broadbeans with the production of two light fractions L_1 and L_2 of 61.7% and 56.4% protein content, respectively, and a coarse fraction of 4.0% protein content. By combining the two light fractions L_1 and L_2, broadbeans give a 55.2% yield of protein concentrate (59.1% protein content) and smooth peas produce a fraction of 49.8% of the initial material. Air classification of wrinkled peas leads to a poor enrichment of protein in the light fractions L_1 and L_2, respectively, 43.0 and 28.5% with much more of proteins remaining in the dense materials H_1 and H_2, respectively, 19.4% and 13.5%.

For further purification of starch on a laboratory scale, particular air classified fractions with lowest protein content were prepared by varying the cut point. They all corresponded to H_2, except for wrinkled peas where it was H_1; in this case, a simple run was preferred to avoid damaging of the compound starch granules.

Composition of starchy fractions, compared to those of initial flours, shows an increase of starch content: 83.5% for broadbeans (Table 2), 89.8% for smooth peas (Table 3),

and 45.5% for wrinkled peas (Table 4). Lipids and ash decrease for the three legumes. No general figure can be given for the variation of structural polysaccharides, including lignin. With broadbeans, cellulose, lignin and pentosan decrease to 0.26%, 0.04%, and 0.35%, respectively; with smooth peas, the cellulose content increases to 1.7% whereas the lignin and pentosan contents decrease to 0.2% and 0.6%, respectively. In the case of wrinkled peas, cellulose decreases to 0.3%, pentosan to 3.0%, and lignin to 0.1%.

The hemicellulosic fractions were studied quantitatively (Table 5). Among the pentoses, arabinose and xylose are the most important, with traces of ribose, fucose, and rhamnose present. The ratios arabinose/xylose are different for the three legume flours and vary specifically by air-classification, indicating that the hemicelluloses, still present in the air-classified fractions, are not chemically identical with the total initial hemicelluloses. Mannose and mainly galactose have been detected; galactose/xylose ratio follows the same variation as arabinose/xylose ratio.

The partition of low molecular weight carbohydrates is different for each legume (Table 6). No variation is observed for wrinkled peas. The total content of mono- and oligosaccharides decrease to 5.7% in smooth peas and 3.7% in broadbeans. Relative to the total oligosaccharides, sucrose content increases whereas stachyose and verbascose decrease without any detection of monosaccharides (sensitivity: 0.1 mg . ml^{-1}). Starch granules in broadbeans and smooth pea flours are equally susceptible to the α-amylase

Table 1—Yield and efficiency of protein fractionation by air-classification of legume flours

	Broadbeans		Smooth peas		Wrinkled peas	
	Yield (% initial material)	Protein (%)	Yield (% initial material)	Protein (%)	Yield (% initial material)	Protein (%)
Flour	100	34.1	100	26.2	100	27.1
Fractions						
L_1	28.0	61.7	38.1	53.7	29.7	43.0
H_1	72.0	23.6	61.9	10.4	70.3	19.4
L_2	27.2	56.4	11.7	35.7	28.1	28.5
H_2	44.8	4.0	50.2	3.4	42.2	13.5
L_3			1.3	36.5		
H_3			48.9	2.5		
$L_1 + L_2$	55.2	59.1	49.8	49.5	57.8	35.9

Table 2—Composition of initial flours and fractions obtained after dry and wet processes of broadbeans (results expressed in % of dry matter)

	Dry process			Wet process	
	Flour	Starchy fraction	Refined starch	Flour	Starchy by-product
Protein (N × 5.8)	30.7	1.5	0.25	32.2	5.7
Lipid	2.1	0.2	0.1	2.5	0.6
Ash	3.1	0.8	0.02	4.4	1.2
Starch	46.6	83.5	98.7	45.3	78.5
Ethanol soluble sugars[a]	5.2	4.1	ND[c]	4.8	0.05
Furfural generators	2.0	0.35	0.19	2.5	4.1
Cellulose	1.0	0.26	ND	1.8	1.5
Lignin	0.7	0.04	ND	0.2	0.3
α-amylolysis[b]					
V_i	5.5	5.5	3.5	12.5	12.5
F	9.0	12.0	6.5	6.0	3.5

[a] Anthrone method
[b] Expressed in percent of starch of the dry product
[c] ND = not determined

Table 3—Composition of initial flours and fractions obtained after dry and wet processes of smooth peas (results expressed in % of dry matter)

	Dry process			Wet process	
	Flour	Starchy fraction	Refined starch	Flour	Starchy by-product
Protein (N × 5.8)	22.7	1.9	0.24	24.0	4.2
Lipid	2.0	0.3	0.01	2.3	0.5
Ash	3.4	0.8	0.02	3.4	0.7
Starch	48.6	89.8	98.2	50.8	78.7
Ethanol soluble sugars[a]	7.6	5.6	ND[c]	7.6	0.6
Furfural generators	2.3	0.6	0.13	2.9	3.9
Cellulose	0.9	1.7	ND	1.7	1.8
Lignin	0.5	0.2	ND	0.2	0.3
α-amylolysis[b]					
V_i	4.0	5.0	5.0	11.0	3.0
F	15.0	15.5	15.5	23.5	12.0

[a] Anthrone method
[b] Expressed in percent of starch of the dry product
[c] ND = not determined

with Vi of 5 and F ranging from 12 to 15.5 (Tables 2 and 3), whereas those from wrinkled peas are four times more degradable (Vi = 22, F = 53) (Table 4). No effect of the dry process has been observed with the three legumes as shown by no change in α-amylolysis parameters. However, scanning electron micrographs of the starch granules (Fig. 2A, C, and E) seem to indicate that milling and air-classification resulted in both shattering of the granules and a superficial exfoliation of the granule surface. It is also possible to see that the granules of wrinkled peas have retained a lot of material, being probably proteinaceous (Fig. 2E).

Wet process

In the aqueous process of legume seed slurries, starch is a by-product of the protein extraction, obtained in the residual fraction after centrifugation. 64.1% of initial dry material is recovered in the broadbeans by-product and 63.1% in the smooth peas one; their proximate analyses are presented in Tables 2 and 3, respectively. Starch contents increase to 78.5% for broadbeans and 78.7% for smooth peas, leading to recovered starch yields of 111.1% for broadbeans and 97.7% for smooth peas. The remaining protein contents are 4.2% for broadbeans and 5.7% for smooth peas.

Free sugars decrease greatly to 0.05% for broadbeans and 0.6% for smooth peas (anthrone method); the remaining sugars (Table 6) are traces of stachyose and raffinose for broadbeans and sucrose, raffinose, and stachyose for smooth peas. Lipids and ashes decrease, respectively, to 0.5% and 0.7% for smooth peas, to 0.6% and 1.2% for broadbeans. Based upon recoveries of the starchy by-products, lignin is in both legumes completely recovered; furfural generators are lost only during the smooth peas processing: 85% of initial pentosans are recovered; cellulose is partly recovered in smooth peas (67% of initial cellulose) and broadbeans (53% of initial cellulose). These results are confirmed by the variations of molar ratio of polysaccharides component sugars of smooth peas and broadbeans, during this process (Table 5): for broadbeans, the ratios arabinose/xylose, rhamnose/xylose, and ribose/xylose remain constant whereas mannose/xylose and galactose/xylose decrease; for smooth peas, the pentoses and hexoses ratios vary, showing that the remaining furfural generators are not chemically identical to the flours ones. The α-amylase susceptibility test indicates that the starch is more resistant to α-amylase after the protein extraction than before (Tables 2 and 3), as shown by the lower values of V_i and F. This result is independent of the way to dry the samples, freeze or air-drying. By the scanning electron microscopy observation, starch granules do not seem enchased in a matrix, therefore the decrease in α-amylase susceptibility cannot be explained by restriction of attack surfaces.

Table 4—Composition of initial flour and fractions obtained after dry process of wrinkled peas (results expressed in % of dry matter)

	Flour	Starchy fraction	Refined starch
Protein (N × 5.8)	26.5	21.9	0.2
Lipid	4.5	3.2	0.1
Ash	3.1	2.5	0.05
Starch	36.6	45.5	97.6
Ethanol soluble sugars[a]	15.1	15.0	ND[c]
Furfural generators	3.3	3.0	0.17
Cellulose	1.2	0.3	ND
Lignin	0.3	0.1	ND
α-amylolysis[b]			
V_i	22.0	22.0	22.0
F	50.0	53.0	48.0

[a] Anthrone method
[b] Expressed in percent of starch of the dry product
[c] ND = not determinated.

Table 5—Molar ratio of polysaccharides constituent sugars of peas and broadbeans. (molar ratio relative to xylose = 1)

	Broadbeans					Smooth peas					Wrinkled peas		
	Dry process			Wet process		Dry process			Wet process		Dry process		
	Flour	Starchy fraction	Refined starch	Flour	Starchy by-product	Flour	Starchy fraction	Refined starch	Flour	Starchy by-product	Flour	Starchy fraction	Refined starch
Rhamnose	0.39	0.22	Nil	0.29	0.30	0.22	0.18	Nil	0.27	0.40	0.35	0.85	0.18
Fucose	0.38	Traces	Nil	0.54	0.04	0.91	Traces	Nil	1.08	Nil	0.54	Traces	Nil
Ribose	0.08	0.39	0.92	0.17	0.13	0.50	0.62	3.11	0.61	0.30	0.93	1.13	2.87
Arabinose	2.14	9.00	7.42	6.85	7.52	12.31	3.32	9.44	8.52	11.40	9.82	9.96	11.45
Xylose	1	1	1	1	1	1	1	1	1	1	1	1	1
Mannose	0.66	2.10	Nil	0.80	0.27	1.15	0.08	0.57	1.25	0.20	0.59	0.63	1.01
Galactose	1.88	8.50	Nil	2.13	0.44	14.09	6.66	1.61	13.76	2.90	13.44	18.08	5.73

Table 6—Partition of low molecular weight carbohydrates in the starchy fractions obtained by dry and wet processes (HPLC method; results expressed in % of dry matter)

	Broadbeans				Smooth peas				Wrinkled peas	
	Dry process		Wet process		Dry process		Wet process		Dry process	
	Flour	Starchy fraction	Flour	Starchy by-product	Flour	Starchy fraction	Flour	Starchy by-product	Flour	Starchy fraction
TOTAL	5.2	3.7	5.2	traces	7.5	5.7	7.9	0.6	14.9	15.2
Monosaccharide	Nil	Nil	Nil	Nil	traces	Nil	traces	Nil	traces	traces
Sucrose	2.7	2.6	2.1	Nil	2.3	3.5	2.9	0.2	4.2	4.3
Raffinose	0.1	0.1	0.1	traces	0.7	0.4	0.4	0.1	1.2	1.2
Stachyose	0.5	0.2	0.6	traces	2.2	0.9	1.4	0.3	5.5	5.9
Verbascose	1.9	0.8	2.4	Nil	2.3	0.9	3.2	Nil	4.0	3.8

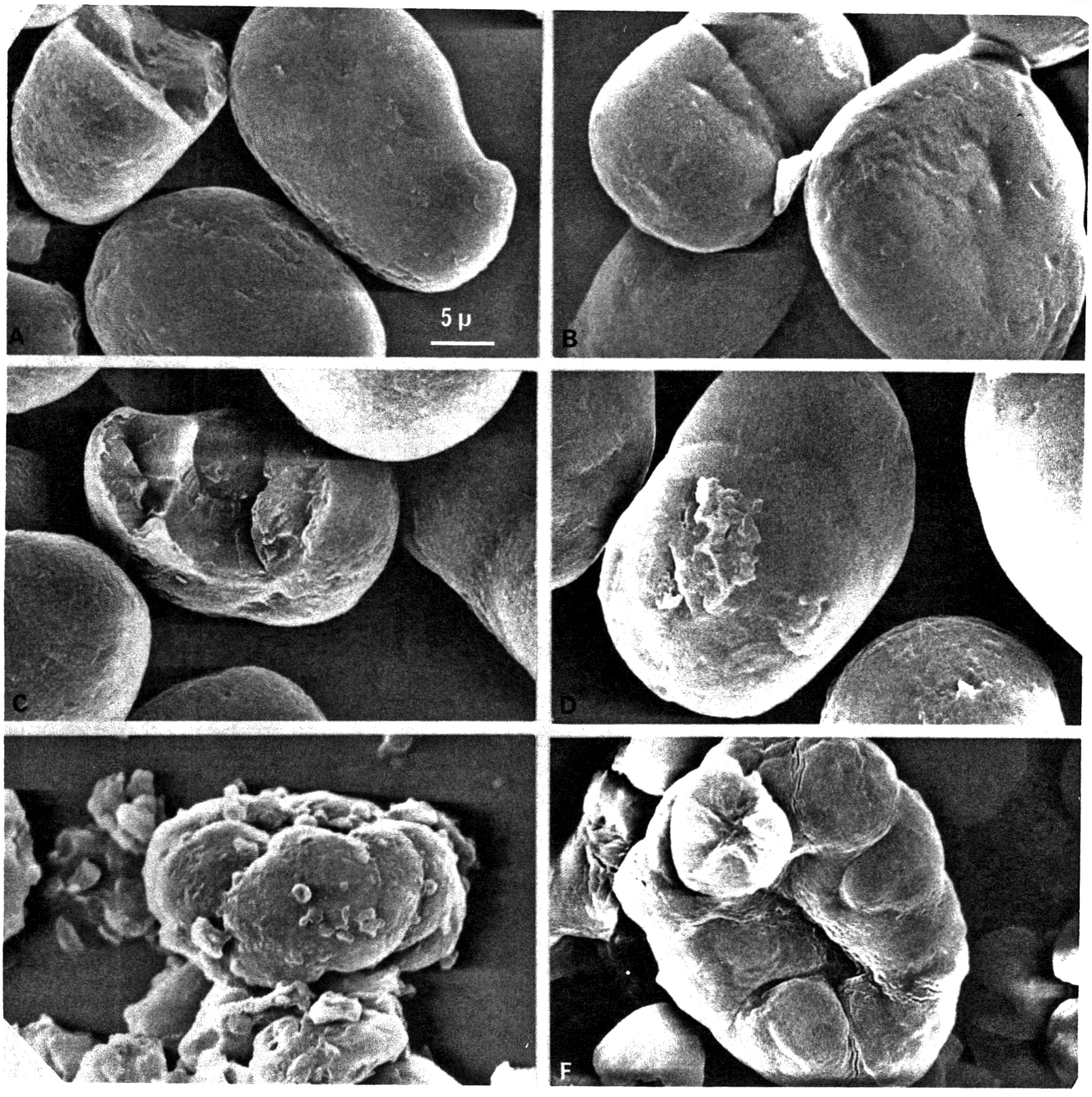

Fig. 2—Scanning electron micrographs of legume starches (magnification 2100X): (A) smooth peas, air-classified fraction; (B) smooth peas, purified starch; (C) broadbeans, air-classified fraction; (D) broadbeans, purified starch; (E) wrinkled peas, air-classified fraction; (F) wrinkled peas, purified starch.

Refined starches

Isolation. Purification of the starches was carried out on particular air-classified fractions of flour with a protein content of 1.5%, 1.9%, and 21.9% for broadbeans, smooth peas, and wrinkled peas, respectively. The yields of refined starch on a laboratory scale were 62.3% for broadbeans, 76.1% for smooth peas, and 45.7% for wrinkled peas.

In contrast, the diluted slurries (ratio water/dry matter: 50/1), remaining from the wet protein extraction, could not pass through a 160μ sieve because the suspensions clogged up the sieve, preventing material to be removed by adding more water.

Analytical data. The chemical analysis, including starch, proteins, lipids, furfural generators and ash determinations, leads to a total of 99.3% for broadbeans starch (Table 2), 99.6% for smooth peas starch (Table 3), and 98.1% for wrinkled peas starch (Table 4). The starches are white. The content of proteins is less than 0.25% and in lipids less than 0.1%. The furfural generators content ranged from 0.13–0.19%. These hemicellulosic fractions are characterized by high values for arabinose/xylose ratio (Table 5): 7.4, 9.4, and 11.5 for broadbeans, smooth peas, and wrinkled peas, respectively. Others sugars are observed: ribose for broadbeans; ribose, mannose, and galactose for smooth peas; rhamnose, ribose, mannose, and galactose for wrinkled peas. The ash contents are lower than 0.05% (Tables 2, 3 and 4). For the three legumes, phosphorus (Table 7) is the most important mineral: 0.009% for broadbeans, 0.007% for smooth peas, and 0.019% for wrinkled peas. Partition of minerals (Table 7) for smooth peas and broad-

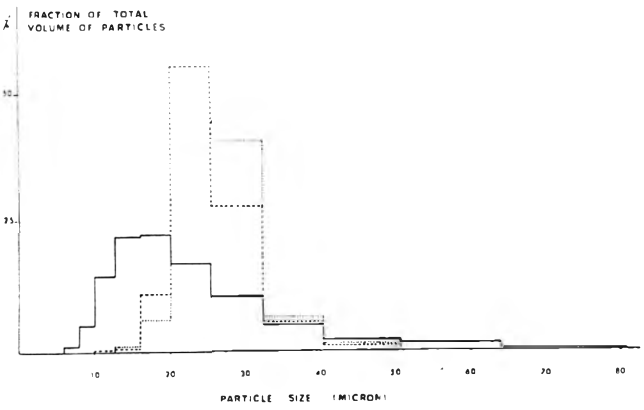

Fig. 3—Particle size distribution of purified legume starches: (.....) broadbeans; (-----) smooth peas, (——) wrinkled peas.

beans are similar except for silicon and potassium for which contents are approximately three times higher in smooth peas than in broadbeans. For wrinkled peas (Table 7), sodium and potassium occur at higher levels than for broadbeans and smooth peas; in contrast, calcium content is lower. Other minerals (titanium, chromium, copper, and zinc) are identified in the three starches, but could not be quantified as they were present at less than 0.1% of minerals content.

The amylose contents are 32.9% for broadbeans, 32.5% for smooth peas, and 62.8% for wrinkled peas.

Granule size and microscopic appearance. The distribution in granule size of the isolated legume starches is shown in Figure 3. Granule sizes means are 26.5μ and 23.75μ for the broadbeans and smooth peas, respectively. The granules of wrinkled peas range in diameter from 6–80μ, with a mean value of 18μ.

The observations of wrinkled peas starch, under the light microscope show a mixture of simple and compound granules (Fig. 4A and 4B). The last ones (Fig. 2F) are composed of a number (3–10) of individual sub-units joined together; they appear to have a central hole and unusual birefringence patterns, which in no way resembles simple crosses; the mean characteristic is their weakness, which is also observed for the simple granules (Fig. 4A and 4B).

Granules of smooth peas and broadbeans are simple and present as oval or spherical or irregular shaped as well (Fig. 2B and 2D). Under the light microscope, their observations show internal fissures which cannot be seen with a scanning electron microscope. Nevertheless, all the granules are strongly birefringent (Fig. 4D and 4F).

α-amylase susceptibility. The α-amylase susceptibility of purified starches for the three legumes are not significantly different from those of the original flours. The initial rates of α-amylolysis (Vi) are in the same range (3.5–5) for broadbeans and smooth peas; however, the easily degradable fraction is higher for smooth peas (F = 15.5) than for broadbeans (F = 6.5). With wrinkled peas, the susceptibility to the enzyme is much higher than with the two others legumes (Vi = 22 and F = 48). Under the light microscope, the observation of the residual starch shows mainly the disappearance of the compound granules by splitting in their sub-units.

DISCUSSION

FOR BROADBEANS and smooth peas, two different flours from the same variety were used in the dry and the wet processes and their proximate analyses are similar. The results are in good agreement with previous works for broadbeans (Pritchard et al., 1973; Cerning-Beroard and Filiatre, 1976; Naivikul and D'Appolonia, 1978, 1979a, b) and for peas (Cerning-Beroard and Filiatre-Verel, 1979). Although the starch contents of the flours are the same, the α-amylase susceptibilities of the wet processed flours are two times higher than the ones of the dry processed flours.

Table 7—Distribution of minerals in starches (% of ash content)

Mineral	Broadbeans	Smooth peas	Wrinkled peas
Sodium	2.9	2.4	15.5
Magnesium	9.8	6.3	2.8
Aluminum	4.4	4.3	2.8
Silicon	6.2	21.9	8.7
Phosphorus	44.1	35.6	39.0
Potassium	1.1	3.8	22.2
Calcium	21.9	18.1	2.3
Iron	9.8	7.6	6.7

Results after air-classification, which confirm those of Vose et al. (1976), show that broadbeans are more easily fractionated into starch and proteins than smooth peas. During this process, proteins are separated from starch by density and particle size and in the case of broadbeans and smooth peas, high protein (approx 60%) and low protein (approx 5%) flours were produced. This efficiency is due to the content of uniform large-sized starch granules (15–30μ) compared to the population of small (0–5μ) and medium (10–20μ) diameter granules in cereal grains. For wrinkled peas, the rather low efficiency of the air-classification could be due to the higher lipid content of the initial flour, preventing a good separation of particles in the air-stream, and to the broad distribution of the granule size. The preparation, by air-classification, of high starch content fractions is a way to concentrate starch with only a slight damage of the granules, a reduction of the fiber, and low molecular weight carbohydrates contents.

During the wet process of smooth peas and broadbeans, starch is completely recovered in the by-product (97.7% and 111.1%, respectively) which contains only traces of α-galactosides, but a higher fiber content; ashes and lipids are concentrated in the protein fraction. The yield of 111.1% obtained from broadbeans is explained by the difficulty to have a representative sample of the wet by-product containing about 60% water. The protein content is higher than in the case of air-classification. However, preliminary assays carried out on smooth peas at pH 9 has led to a lower protein of 1.5%. The starch granules in this fraction are more resistant to the α-amylase than the ones of the original flours. Knowing that hemicellulosic material has the property to swell in aqueous medium and that their composition in the by-products are different from those of the initial flours, this fraction could be responsible for both the impossibility to sieve starch and the higher resistance of their granules to α-amylase. For this last observation, another assumption is that damaged granules, present in a small amount in the original flour, could be lost in the light fraction during centrifugation, accounting for less damaged granules in the by-product.

Extraction and purification of starches from air-classified fractions were easily made; the recovery yields are higher than those obtained by Schoch and Maywald (1968), Lineback and Ke (1975), Naivikul and D'Appolonia (1979a) working on legume flours. It can be explained by the successive treatment of fine milling and air-classification which disrupt probably all the cells of the flour, liberating the starch granules from the cell and the protein matrix.

Vose (1978), using a wet technology to fractionate starch and proteins, reported a starch recovery yield of only

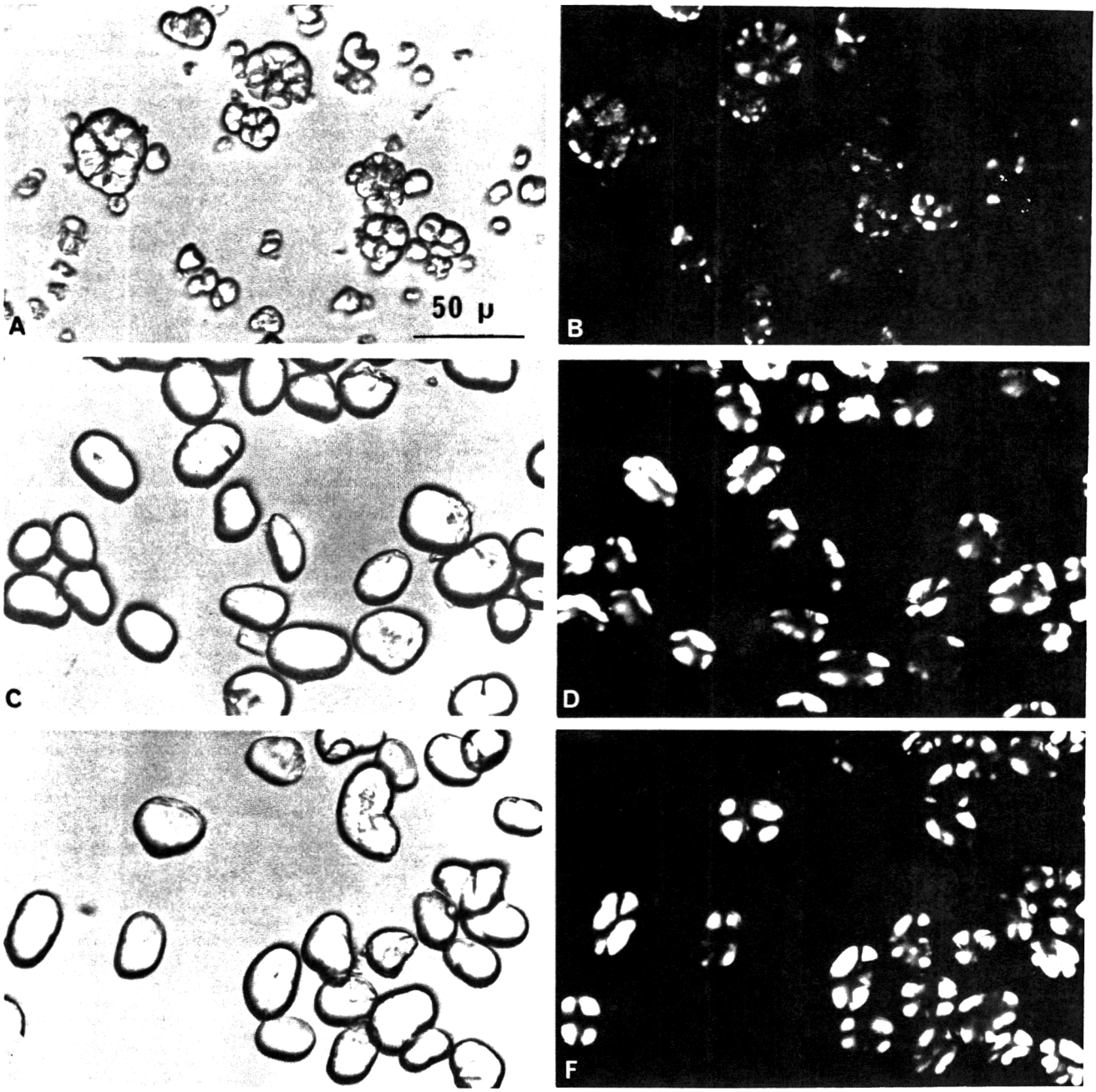

Fig. 4—Light micrographs of purified legume starches (magnification 350X): (A) wrinkled peas, regular light; (B) same under polarized light; (C) smooth peas, regular light; (D) same under polarized light; (E) broadbeans, regular light; (F) same under polarized light.

38–40%, without any explanation for this low value. With a similar technology, Biliaderis et al. (1979) have obtained starches with final purity much lower than the starches purified from the air-classified fractions in the present study. The protein removal by toluene is more efficient than by 95% ethanol or water, since the levels of protein in our studies (0.2–0.25%) are lower than the protein contents (0.5%) of legume starches purified by Biliaderis et al. (1979) and Lorenz (1979). The lipid content is very low and of the same order as those for tuber starches (0.01–0.1%); however, they are higher than those of Biliaderis et al. (1979) due probably to the method used, liberating the total lipids. The furfural generator contents (0.1–0.2%), corresponding to the pentosan content, are lower than those of wheat starch (Baker et al., 1943). Naivikul and D'Appolonia (1979a), working on broadbeans starch, showed an acid detergent fiber content of 0.06% whereas Biliaderis et al. (1979) claimed values ranging from 0.01–0.11% for the three starches, expressed by the crude fiber determination. The study of the constituent sugars of polysaccharides impurities, after hydrolysis, shows the predominance of arabinose, xylose, and ribose among pentoses, mannose, and galactose. Ribose would be coming from nucleic acids; the others pentoses could be either components of the starch granules or present as contaminants.

The mineral content (0.02–0.05%) is lower than the results of Biliaderis et al. (1979), phosphorus being at the same level as in manioc starch (Posternak, 1935), but at a lower value than the ones described by Greenwood and Thomson (1962) for the same legumes.

—Continued on next page

The partition of minerals for smooth peas and broadbeans is near from those obtained by El Gindy et al. (1957) on wheat starches and different from the one of wrinkled peas. Minerals occuring at the lowest levels were not quantified because the Edax method is not sensitive enough, compared to the spectrographic means used by El Gindy et al. (1957). The amylose contents, about 30–33% for smooth peas and broadbeans and 60–65% for wrinkled peas, are comparable with previous works (Greenwood and Thomson, 1962; Schoch and Maywald, 1968; Banks and Greenwood, 1975; Biliaderis et al., 1979; Lorenz, 1979). The α-amylase susceptibility of broadbeans and smooth peas starch granules is of the same order as the one of native cereal starches such as wheat and maize (containing 20–25% of amylose) (Mercier, 1968). Wrinkled peas starch granules, with as high an amylose content as the amylomaize (60–65%), are highly susceptible to α-amylase, whereas amylomaize starch is very resistant. This feature could be explained by a preferential splitting of the compound granules in their sub-units, which increase the attack surface for the enzyme, leading therefore to a higher degradation. The microscopic appearance indicates no effect during the purification on the shape, size, and birefringence of the starch granules, as it has been shown previously by Greenwood and Thomson (1962).

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LYSINOALANINE DETERMINATION IN PROTEINS USING HIGH-PRESSURE LIQUID CHROMATOGRAPHY

J. C. WOOD-RETHWILL and J. J. WARTHESEN

ABSTRACT

The use of high pressure liquid chromatography (HPLC) to determine the lysinoalanine (LAL) content in protein hydrolysates was investigated. The dansyl derivative of LAL was formed by reacting protein hydrolysates with dansyl chloride and HPLC was used to separate and quantify nanogram quantities of LAL. An excess level of dansyl chloride was necessary to form a consistent derivative peak for chromatographic analysis. Mass spectrometry was used to analyze the LAL derivative in a casein hydrolysate but a definitive spectrum was not obtained. The HPLC procedure for LAL was found to be less variable than a thin-layer chromatographic technique and has potential for use as a rapid method for the analysis of LAL in food proteins.

INTRODUCTION

ALKALINE PROCESSING of food proteins is used in the production of protein concentrates and isolates, in protein texturizing methods such as spun fiber production (Anon, 1967) and in the production of proteins with specific functional properties such as foaming (Aymard et al., 1978). Alkaline treatment of food proteins can cause chemical modifications of both essential and nonessential amino acids (Whitabker and Feeney, 1977). Adverse effects on nutritional quality can be due to amino acid destruction, racemization, or crosslink formation (Provensal et al., 1975; Tannenbaum et al., 1970; de Groot and Slump, 1969). In addition to nutritional losses, the formation of crosslinked amino acids such as lysinoalanine (LAL) has raised a concern about the possible toxic effects of alkaline processing.

LAL is formed by the nucleophilic addition of the ϵ-amino group of a lysyl residue to the double bond of a dehydroalanyl residue during alkali treatment of proteins (Bohak, 1964; Nashef et al., 1977). Under alkaline conditions, dehydroalanine may form from the β-elimination of a variety of compounds including cysteine (Asquith et al., 1974), cystine (Asquith and Carthew, 1972), serine (Asquith et al., 1969), o-phosphoryl serine (Manson and Carolan, 1972; Sen et al., 1977), or o-glycosyl serine (Whiting, 1971). LAL has been implicated as a toxic amino acid since it induces cytomegalic lesions in the renal tubular cells of the rat (Woodard and Short, 1973; 1977). The nephrocytomegaly induced by LAL may be specific to the rat species (de Groot et al., 1976) and appears to be reversible if the amino acid is removed from the diet (Struthers et al., 1978). Leegwater (1978) suggested that investigations of the metabolic pathway of LAL may be important in ascertaining if the nephrotoxicity of LAL applies to humans or if toxicity in the rat is due to species-specific metabolism.

Sternberg et al. (1975a) studied the LAL content in a variety of food ingredients, home-cooked foods and commercial food preparations. The occurrence of LAL was found to be widespread not only in foods and ingredients subjected to alkaline treatment but also when heated in nonalkaline conditions.

LAL has been estimated in protein hydrolysates by several chromatographic methods. Automated amino acid analysis (AAA) which utilizes ion exchange chromatography and colorimetric detection with ninhydrin was reported by Slump (1977) to require 2 hr per sample with a sensitivity down to 200 ng LAL. Raymond (1980) suggested some procedural improvements that decreased the AAA time to 1 hr. Difficulties have been noted in AAA separation of LAL from other compounds such as galactosamine (Raymond and Viviano, 1977), lysinomethylalanine (Slump, 1977) and ornithinoalanine (Finley and Friedman, 1977). Paper chromatographic techniques using high voltage electrophoresis and color development with ninhydrin have been applied to separate and detect LAL in approximately 20 hr (Asquith et al., 1969). A thin-layer chromatography (TLC) method for the separation and densitometric detection of LAL in protein hydrolysates was used by Sternberg et al. (1975b). The minimum detectability of the method was reported to be 3 ng. However, approximately 12 hr was required for this type of analysis (Haagsma and Gortemaker, 1979) and ornithinoalanine apparently interferes with the LAL spot. Extensive comparisons of the TLC and AAA methods of LAL determination in foods and food proteins revealed that TLC-densitometric analysis showed comparatively large standard deviations especially in the low LAL concentration range (Haagsma and Slump, 1978). Aymard et al. (1978) modified the TLC method of Sternberg et al. (1975b) to obtain rapid separation of LAL in about 4 hr. However, parallel analyses were necessary to differentiate between LAL and lanthionine by the preparation of lanthionine-sulfoxide with a hydrogen peroxide treatment.

High pressure liquid chromatography (HPLC) has been successfully used by several investigators for the separation and detection of polyamines such as putrescine, spermidine, and spermine (Newton et al., 1976; Seiler et al., 1978). After formation of the dansyl derivatives, picamole quantities of several polyamines can be measured by reverse phase chromatography within 12–20 min (Vandemark et al., 1978). The formation of dansyl derivatives followed by HPLC has also been used for the analysis of common amino acids (Bayer et al., 1976; Peterson and Warthesen, 1979). The objective of the present study was to evaluate the use of HPLC for the measurement of LAL in food protein hydrolysates.

MATERIALS & METHODS

Reaction of protein hydrolysates with dansyl chloride

A sample containing approximately 0.5g protein was accurately weighed and placed in a 250 ml boiling flask. One hundred milliliters 6.0N HCl were added along with boiling chips and the mixture was refluxed at 100–110°C for 22 hr. These conditions were reported to be optimal for hydrolysis of LAL from protein (Sternberg et al., 1975b; Slump, 1977). After refluxing, the hydrolysate was quantitatively transferred to a 200 ml volumetric flask and the pH was adjusted to 9.0 with 12N NaOH. The flask was allowed to cool

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and the volume adjusted with distilled water. The formation of the dansyl derivative of LAL was accomplished by combining 0.5 ml of the protein hydrolysate with 0.5 ml of 0.5M borate buffer (pH 9.0) in a 2 dram vial. One half milliliter of 180 mM dansyl chloride (Sigma Chemical Co.) in acetone was added and vial contents were vigorously mixed. The reaction mixture was incubated for 40 min in a 40°C water bath which was covered to exclude light. The sample was then filtered through a 0.2 μm filter and 10 μl was injected on the high pressure liquid chromatograph.

HPLC of dansylated hydrolysates

HPLC of dansylated protein hydrolysates was performed on a Waters Associates High Pressure Liquid Chromatograph (Model U6K injector, Model 6000A solvent delivery system, Model 660 solvent programmer, Model 440 absorbance detector). The detector monitored absorbance at 254 nm. The data were recorded on a Hewlett Packard 3380A recorder-integrator.

Separation of the dansyl derivative of LAL was accomplished on a μBondapack C_{18} column (30 cm × 3.9 mm i.d. Waters Associates) with a mobile phase of 35% nanograde acetonitrile (Mallinckrodt) and 65% phosphate buffer (0.01M) pH 7.0. The analysis was carried out under isocratic conditions with a flow rate of 1.5 ml/min. After LAL eluted, a gradient elution was routinely used to decrease the retention time of late-eluting compounds in the protein hydrolysate. The following program conditions were used: pump A, 90% phosphate buffer (0.01M) pH 7.0, 10% acetonitrile; pump B, 50% phosphate buffer (0.01M) pH 7.0, 50% acetonitrile. After the isocratic elution of LAL (8 min at 62% pump B), pump B was programmed from 62% to 85%. A 3-min reverse program was used to return the system to initial conditions.

Quantitation was accomplished using an external standard of the dansyl derivative of LAL prepared by combining 0.5 ml of 0.1 mg LAL·2HCl/ml H_2O, 0.5 ml 0.1M borate buffer (pH 9.0) and 0.5 ml 10 mM dansyl chloride in acetone. Lysinoalanine was obtained from Miles-Yeda, Ltd. (Israel) and from P.A. Finot, Nestlé Products Technical Assistance Co. (Switzerland). The external standard was freshly prepared in duplicate by the same procedure and at the same time as the protein samples. Five microliters of the external standard were injected on the chromatograph. The peak area response gave a linear relationship to concentration of the LAL dansyl derivative in the range of LAL concentrations used in this study. Increasing amounts representing up to 800 ng of LAL were injected and peak area increases were proportional to LAL concentration.

Recovery of LAL from a hydrolysate was estimated by spiking a hydrolysate from an untreated casein sample with 4000 ppm of LAL. Recovery was calculated based on the increase in peak area due to the added LAL.

To determine if lanthionine would interfere with the LAL peak elution, a lanthionine standard (Sigma Chemical Co.) was reacted with dansyl chloride and analyzed by HPLC. Elution of the lanthionine derivative was several minutes earlier than the LAL derivative.

LAL dansyl derivative formation

To investigate the dansyl derivative formation of LAL, dansyl chloride was reacted with LAL standard (0.1 mg LAL·2HCl/ml H_2O) at molar ratios (moles DNS-Cl/moles LAL) of 1.8, 10.0, 18.4, and 34.0. The reaction of dansyl chloride with the LAL standard was carried out as previously described. However, appropriate concentrations of dansyl chloride solution were prepared in acetonitrile instead of acetone because acetone gave a peak that eluted close to one of the LAL derivatives. The reactions were incubated at 40°C for 60 min rather than 40 min to insure a more complete reaction at low dansyl chloride concentrations. Samples reacted with dansyl chloride at each molar ratio were analyzed in triplicate by HPLC.

Analysis of LAL derivative by mass spectrometry

Solid sampling mass spectrometry was used to verify the presence of the dansyl derivative of LAL in a chromatogram of an alkali-treated casein hydrolysate. Casein (Sigma Chemical Co.) was prepared as a 20% w/v slurry in water which was then adjusted to pH 12 with 8.0N NaOH. After heating at 100°C for 90 min, an aliquot was removed and adjusted to pH 7 with 1.0N HCl to eliminate the influence of strong base on the acid hydrolysis conditions. The sample was then hydrolyzed in 6.0N HCl as described earlier. Samples of the casein hydrolysate and a LAL standard, which was analyzed for comparison, were reacted with dansyl chloride and subjected to HPLC. Eluting fractions corresponding to the LAL dansyl derivative peak from the standard and casein were collected as they eluted. The fractions from multiple injections were evaporated to dryness in 50 ml pear-shaped flasks on a rotary evaporator in a 40°C water bath. The flasks were placed in a vacuum desiccator over Drierite for approximately 3 hr for completion of drying. Acetonitrile (100 μl) was added to each flask to redissolve the collected fractions. Five microliters of the acetonitrile solution were analyzed by HPLC to verify the purity and determine the quantity of LAL in the collected fractions. The remaining acetonitrile solution was drawn off by syringe, transferred directly into mass spectrometer direct-inlet sample tubes and evaporated to dryness in a vacuum desiccator. The estimated quantities of LAL derivative used for mass spectrometry were 1.80 and 0.84 μg for the standard and the casein, respectively.

The collected fractions from the standard and the treated casein were analyzed separately on an L.K.B. 9000 Mass Spectrometer using the direct inlet probe. The direct inlet probe was heated from ambient temperature to 250°C and the spectra scanned at an ionization voltage of 20 eV from mass 5 to mass 957.

LAL determination by TLC

The LAL content in alkali-treated casein (pH 12, 100°C for 90 min) was determined by TLC and compared to results obtained by HPLC analysis. TLC separation and color development with ninhydrin were accomplished on Eastman cellulose sheets according to the method of Sternberg et al. (1975b). One milliliter of the LAL standard solution (0.1 mg LAL·2HCL/ml H_2O) was mixed with 1.0 ml 0.1N HCl to obtain a standard concentration of 0.05 mg LAL·2HCl/ml dilute HCl for analysis by TLC. The LAL standard was spotted on a line 1.5 cm from the bottom edge of each of three replicate TLC plates in 10, 20, and 30 μl amounts. Five microliters of the treated casein hydrolysate were spotted in four different positions on each plate. Sample applications were made in 5 μl amounts using volumetric capillary pipettes. Each plate required approximately 7.5 hr in the solvent chamber.

The TLC-separated LAL spots were quantified by a photographic method presented by Stahl (1965). The developed spots from each chromatogram were photocopied and the areas were measured by planimetry. The mean area of each spot was calculated from six consecutive planimetry measurements. A calibration curve for each TLC plate was prepared from the areas of the three LAL standard spots. The absolute quantity of LAL in the casein sample was obtained from the calibration curve using the area of the casein LAL spots.

RESULTS & DISCUSSION

Chromatography

A reverse-phase HPLC method utilizing dansyl chloride was developed to measure LAL in protein hydrolysates. Isocratic conditions were used to separate the LAL dansyl derivative from the other amino acid derivatives in the protein hydrolysates. A chromatogram of the external LAL standard is shown in Figure 1. The peak eluting at 7 min is the tridansyl derivative of LAL. The first of two peaks near 9 min is due to a small amount of lysine present in the LAL standard. Other peaks on the chromatogram are associated with the dansyl chloride reagent. The lower limit of detectability for the HPLC method with the LAL standard was 3 ng LAL per injection. Recovery of LAL spiked in a casein hydrolysate was 88.7%. In Figure 2, a chromatogram of an alkaline-treated casein hydrolysate which contains LAL is shown when analyzed under isocratic conditions for the entire chromatogram. When a gradient elution was used after the elution of LAL, the HPLC analysis time per injection was 12 min plus a 4 min equilibration period for a total analysis time of 16 min. While lanthionine was shown to not interfere with LAL elution, there still remains a possibility that other crosslinked amino acids that might form during alkaline treatment of protein could elute near or with the dansyl derivative of LAL. The elution of these other uncommon amino acids should be investigated as standards become available.

Dansyl derivative formation

Dansyl chloride has been shown to react with primary

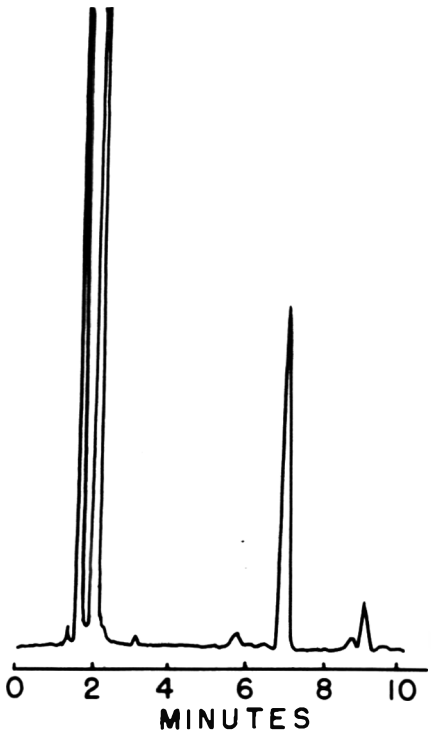

Fig. 1—Chromatogram of the LAL standard after reaction with dansyl chloride.

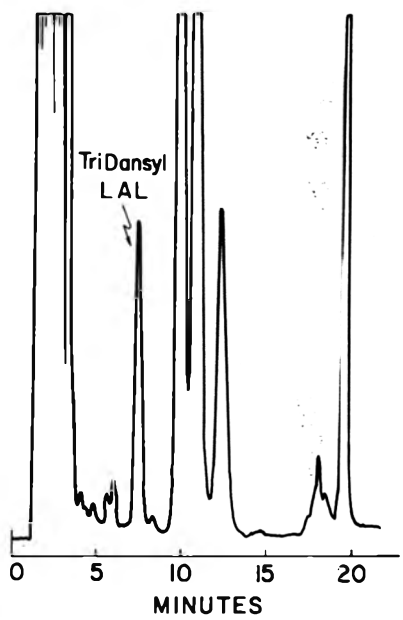

Fig. 2—Chromatogram of a hydrolysate from alkali-treated casein after reaction with dansyl chloride.

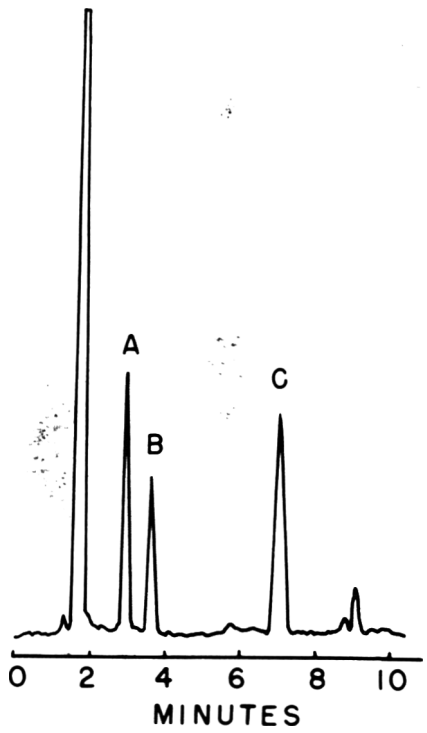

Fig. 3—Peaks A, B, and C are dansyl derivatives formed from the reaction of LAL standard and dansyl chloride at a dansyl chloride to LAL molar ratio of 10.

and secondary amino groups (Seiler et al., 1978). Various concentrations of the tagging agent were reacted with LAL to investigate derivative formation. Because LAL is a polyamine with one secondary and two primary amino groups, there are three sites for reaction with the dansyl tagging agent. At high concentrations of dansyl chloride, reaction with all three amino groups would be expected, while at lower molar ratios of dansyl chloride, several types of derivatives might form. A chromatogram with the three types of dansyl derivatives formed at a molar ratio of ten is shown in Figure 3. Peak C corresponded to the peak obtained earlier with LAL standard using larger amounts of dansyl chloride. This peak was tentatively identified as tridansyl LAL. When increasing concentrations of dansyl chloride were used, dramatic changes in peak areas were noted (Fig. 4). The changes in peak areas associated with increases in dansyl chloride concentration suggest the presence of mono-, di-, and tridansyl LAL derivatives. As the molar ratio of dansyl chloride increased, the first peak to disappear (peak B) was probably the monoderivative. The second peak to decrease (peak A) was probably the diderivative. At the highest molar ratio, peak C was the only LAL derivative, providing additional evidence that this was the tridansyl derivative. The results shown in Figure 4 also point out the need for using a sufficient excess of dansyl chloride to allow complete derivatization so that the fully dansylated LAL will be the only LAL peak eluting in a chromatogram of a protein hydrolysate.

Analysis of LAL derivative by mass spectrometry

In an attempt to further verify the presence of the dansyl derivative of LAL, the peak corresponding to the LAL derivative from a casein hydrolysate was collected off the liquid chromatograph and analyzed by solid sampling mass spectrometry. Although the molecular ion of tridansyl LAL (m/e 932) was not observed in the spectrum from LAL standard or from the casein sample, the presence of the LAL dansyl derivative in the casein hydrolysate was suggested by similar ion fragments in both spectra. Fragments at 170 and 171 m/e, corresponding to dimethylaminonaphthalene, were observed in highest abundance which is characteristic of dansyl derivatives (Seiler, 1977). A related fragment ion appearing in both spectra was 250 m/e, probably corresponding to dimethylaminonaphthalene sulfonyl amide. Other fragment ions observed in both spectra which were indicative of the dansyl derivative of LAL were m/e 331 and 369. These two could be fragments containing one dansyl group attached to the carbon chain of lysine or a dansyl group attached to the carbon chain of LAL. The mass spectral data was not definitive enough for positive identification of the LAL dansyl derivative due perhaps in part to the nature of the compound being studied. The analysis, however, when coupled with retention time data and information on the dansylation reaction provide support for the formation of the tridansyl derivative of LAL from the casein hydrolysate.

LAL determination by TLC

The LAL content of the alkali-treated casein hydrolysate determined by the TLC method of Sternberg et al. (1975b) was 18,545 ± 6234 ppm LAL (mean ± standard deviation) for twelve analyses. HPLC analysis of twelve replicates resulted in a LAL content of 19,976 ± 1070 ppm LAL (mean ± standard deviation). This level of LAL is in the range of reported values for casein treated under similar conditions (de Groot and Slump, 1969; de Groot et al., 1976).

In addition to being a more rapid method, the HPLC procedure also gave a much lower coefficient of variation (5.4%) compared to the TLC method (33.6%). While LAL analysis by the HPLC method is more rapid than with the amino acid analyzer method of Slump (1977), a comparison of the HPLC analysis and ion exchange amino acid

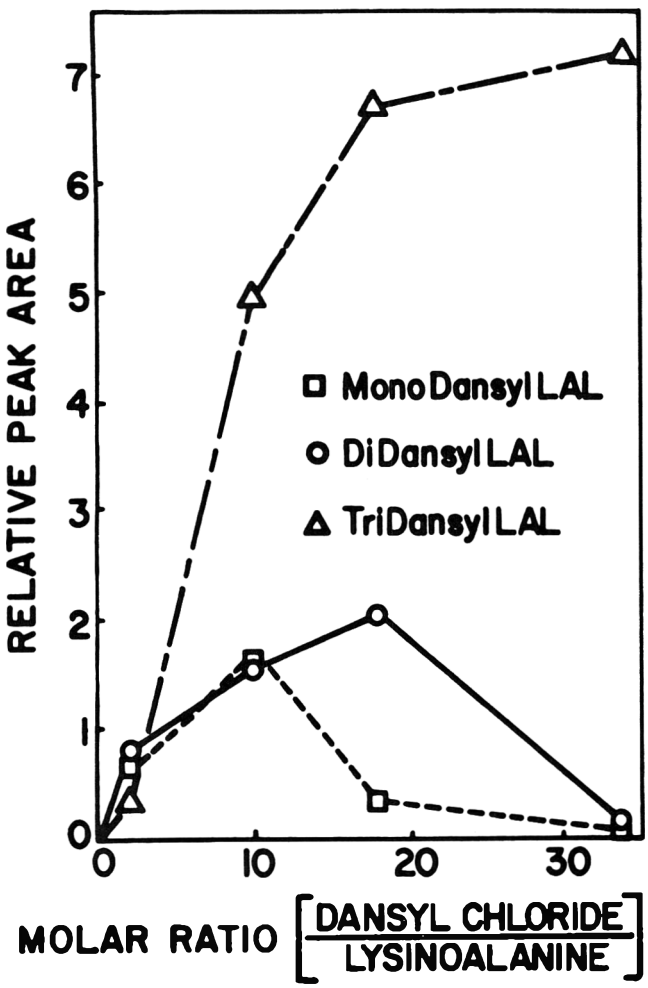

Fig. 4—Changes in relative peak area brought about by changing ratios of dansyl chloride to LAL. Peaks A, B, and C in Fig. 3 probably correspond to di-, mono-, and tridansyl LAL, respectively, based on changes in areas with increasing dansyl chloride concentrations. The relative peak area was obtained by multiplying the arbitrary integrator area units by 10^{-5}.

analysis would be an area for further study. The formation of the dansyl derivative of LAL followed by quantitation using HPLC should be a useful method for studying the factors influencing the formation of LAL in food proteins.

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CONVERSION OF CYSTINE OR CYSTEINE TO THE ANTIBACTERIAL COMPOUND BIS-(2-AMINO-2-CARBOXYETHYL) TRISULFIDE (BACTIN) ENHANCED BY FERRIC CHLORIDE AND SODIUM NITRITE

S. JÓNSSON and J. RAA

ABSTRACT

Heating of cystine at pH 6 or in hydrochloric acid, yielded Bis-(2-amino-2-carboxyethyl) trisulfide (Bactin), which had a bacteriostatic action on *Lactobacillus plantarum*. Heating of cysteine did not yield this compound, unless ferric chloride was present and the pH was low. In the presence of nitrite and ferric chloride, cysteine was efficiently converted to Bactin, also at pH 6. The bacteriostatic action was abolished by cysteine. We suggest that the bacteriostatic action of nitrite is due to facilitated conversion of cysteine to Bactin and a concommitant removal of thiol compounds, which otherwise abolish the bacteriostatic action.

INTRODUCTION

IT IS KNOWN among microbiologists that cystine rich nutrient media become antibacterial after autoclaving, and that thiol containing compounds usually abolish this inhibition. This inhibition has been described with several species of bacteria and suggested to be due to collodial sulfur (S°), formed by thermal degradation of cystine (Schuhardt et al., 1952; Rose et al., 1952; Konowalchuk et al., 1954a, b; Woiwod, 1954). The inhibition could be reversed by cysteine, thioglycollate, thiomalate, and by cystine in mixture with ascorbic acid, but not by the two latter compounds separately (Rose et al., 1952).

Heating did not yield inhibitory products from cysteine, unless iron was present and the pH was below 6.8 (Konowalchuk et al., 1954a). The authors suggested that S° was the inhibitory principle, also in this case.

There are striking similarities between the inhibition by heated cystine and the heat induced inhibitory effect of nitrite in nutrient media (Perigo et al., 1967; Riha and Solberg, 1975). The nature of the inhibitor formed in the presence of nitrite is not yet known, but it has been suggested that it is formed in a reaction which involves cysteine, iron, and nitrite (Asan and Solberg, 1976; Huhtanen and Wasserman, 1975). Thiol compounds could reverse also the heat induced inhibition by nitrite in nutrient media, whereas other reducing substances were without effect on the inhibition, which increased with decreasing pH (Castellani and Niven, 1955).

Bis-(2-amino-2-carboxyethyl) trisulfide is a well characterized compound (Fletcher and Robson, 1962; 1963), which can be formed from cystine or cysteine, and it is stable at low pH. But it has apparently not previously been examined whether this compound could be responsible for the heat induced inhibition described in the foregoing.

In the present paper we show that bis-(2-amino-2-carboxyethyl) trisulfide indeed has a bacteriostatic action, and is formed by heating of cystine. Moreover, nitrite and ferric chloride facilitate the conversion of cysteine to this compound, which we have designated Bactin because it is a functionalistic abbreviation of its chemical name.

The *Lactobacillus plantarum* strain was used in this study because it is under extensive studies in our laboratory, in particular with regard to fish preservation (Schroeder et al., 1979).

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MATERIALS & METHODS

Organism

Lactobacillus plantarum (Univ. of Tromsø culture collection UTC 101 – 11 saithe) was used (Schroeder et al., 1979). Growth of *L. plantarum* was measured spectrophotometrically at 600 nm, using a Spectronic 20. The bacteria were grown at 20°C on a reciproke shaker (100 ml Bactotryptone medium), and samples (5 ml) withdrawn to measure A_{600}.

Bactotryptone medium

Bactotryptone medium contained per liter: Bactotryptone (Difco) (5g), glucose (5g), $MgCl_2 \cdot 6H_2O$ (0.2g), $CaCl_2$ (50 mg), $FeCl_3 \cdot 6H_2O$ (5 mg) $ZnSO_4 \cdot 7H_2O$ (5 mg), $MnSO_4 \cdot H_2O$ (5 mg), $CuSO_4 \cdot 5H_2O$ (2.5 mg), $Na_2MoO_4 \cdot 2H_2O$ (2.5 mg), $CoCl_2 \cdot 6H_2O$ (2.5 mg), pyridoxine-HCl (2 mg), nicotinic acid (1 mg), Ca-pantothenate (1 mg), thiamin-HCl (1 mg), p-aminobenzoic acid (0.5 mg), riboflavine (0.5 mg), folic acid (0.25 mg), biotine (0.01 mg), cobalamine (0.001 mg), adenine (5 mg), guanine-HCl (5 mg), uracil (5 mg), and distilled water.

The pH was adjusted before autoclaving (20 min 120°C) so that the pH after was 6.5 ± 0.1.

Tryptone Soya Agar (TSA) (Oxoid) was used to determine viable plate count.

Bactin

Bis-(2-amino-2-carboxyethyl) trisulfide was synthesized either from cysteine and S°, as described by Fletcher and Robson (1963), or by autoclaving of cystine. In the latter case, 1g cystine was dissolved in 100 ml 0.1M HCl and the solution autoclaved for 60 min at 120°C. The yellow solution was concentrated on rotavapor (40°C) and the residue suspended in 20 ml 2.5M HCl. The filtered solution was applied to a column (17 × 400 mm) of Zerolit 225 (BDH Chemicals Ltd, Poole, England) and eluted with 2.5M HCl at a rate of 0.6 ml/min, and fractions of 14.4 ml collected. The fraction numbers 45 to 70 (which were completely separated from cystine) gave ninhydrin positive reaction and also a blue color reaction with phosphotungstic acid after reaction with sodium bisulfite (Fletcher and Robson, 1963; Blackburn, 1968). The fractions between 45 and 70 were pooled, the content dried in rotavapor (40°C), dissolved in 2.5M HCl and rechromatographed on the same column. The corresponding fractions were pooled, dried, and stored in a vacuum desiccator over solid KOH. The compound was identified as described by Fletcher and Robson (1963).

Preparations of Bactin were added to the Bactotryptone medium after autoclaving.

Chemicals

Chemicals for the nutrient media were of analytical grade. L-Cysteine (Merck), L-cystine (Sigma), iron (III) chloride (6 H_2O) (Merck), sodium nitrite (Merck), and HCl (Merck) were used in experiments on the conversions of cystine and cysteine. Further details are given in legends of the figures.

Amino acid analyzer. Jeol, JLC-6AH.

RESULTS

IF CYSTINE were added to the Bactotryptone medium before autoclaving, it became inhibitory to *L. plantarum* (Fig. 1) The ag-phase of growth was longer, but the maximum growth rate was not affected. Addition of nonheated

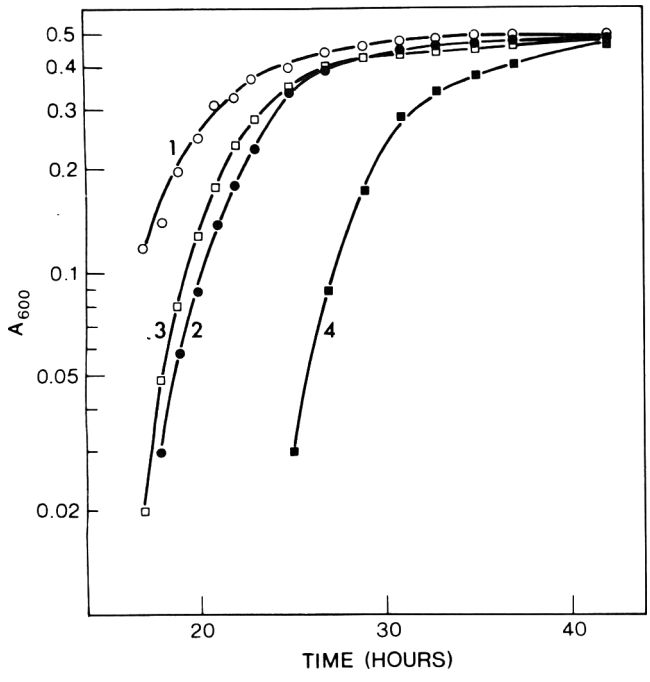


Fig. 1—Growth at 20°C of Lactobacillus plantarum in Bactotryptone medium without additional cystine (curve 1 and curve 3) and with 0.2 g/L of cystine added prior to autoclaving (curve 2 and curve 4). Curves 1 and 2 show growth when the initial number of bacteria was $2.5 \cdot 10^4$ per ml, and curves 3 and 4 with $2.5 \cdot 10^3$ bacteria per ml. Abscissa: Time (hr) after inoculating. Ordinate: Cell density measured as A_{600} nm.

cystine had no effect on growth, but cystine autoclaved separately in hydrochloric acid, caused the same characteristic growth delay (Fig. 2). The presence of ferric chloride during heating apparently increased the conversion of cystine to the inhibitory principle. The inhibition was completely abolished by the addition of cysteine. Cysteine reduced the lag-phase of growth, even in the Bactotryptone medium. Cysteine did not yield inhibitory products unless ferric chloride was present and the pH was low during heating (Fig. 3). The bacteria in the maximum stationary phase were more sensitive to the inhibiting products from cystine or cysteine than bacteria in the logarithmic growth phase.

Since cystine, or cysteine, yielded bis-(2-amino-2-carboxyethyl) trisulfide (Bactin) at the same conditions which render the nutrient media inhibitory, the effect of this compound on growth of *L. plantarum* was examined (Fig. 4). Bactin caused the same characteristic growth delay as heated cystine, or cysteine heated in the presence of acid ferric chloride. Twenty mg purified Bactin per liter nutrient medium delayed growth 11–12 hr; a fivefold higher concentration prolonged the lag-phase about five times more.

The results shown in Figure 4 were obtained with Bactin which was purified from heated acid cystine. The results were qualitatively the same with Bactin synthesized from S^0 and cysteine, but the growth delaying effect was slightly less. The elution profiles in the amino acid analyzer of Bactin preparations, synthesized by autoclaving cystine, or from S^0 and cysteine, and purified according to Fletcher and Robson (1963), are shown in Figure 5(a, b). Minor impurities were present in the Bactin preparation isolated from heated acid cystine, one chromatographing in front of Bactin, the other after tyrosin (probably the tetrasulfide). Figure 5 shows moreover the formation of Bactin by auto-

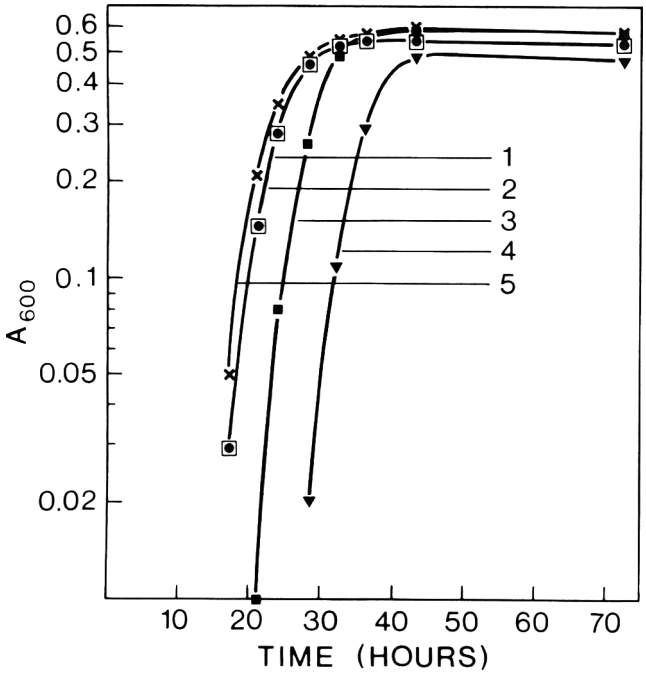


Fig. 2—Growth at 20°C of Lactobacillus plantarum in Bactotryptone medium (curve 1 = control), and the effect on growth in this medium of 0.2 g/L sterile filtered (0.45μ Millipore) cystine (curve 2), of cystine (0.2 g/L) autoclaved for 20 min in 0.1M HCl (curve 3) of cystine (0.2 g/L) and ferric chloride (0.44 g/L) autoclaved together in 0.1M HCl (curve 4) and of autoclaved cystine mixed with 0.2 g/L of sterile filtered cysteine (curve 5). Abscissa: Time (hr) after inoculation. Ordinate: Cell density measured as A_{600} nm.

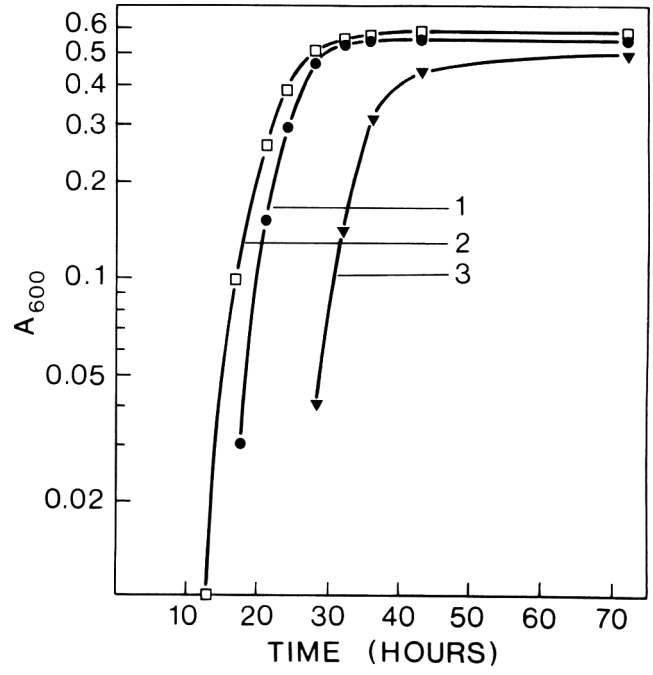


Fig. 3—Growth at 20°C of Lactobacillus plantarum in Bactotryptone medium (curve 1 = control), and the effect on growth in this medium of cysteine (0.2 g/L) autoclaved for 20 min in 0.1M HCl (curve 2), and of cysteine (0.2 g/L) and ferric chloride (0.44 g/L) autoclaved together in 0.1M HCl (curve 3). Abscissa: Time (hr) after inoculation. Ordinate: Cell density measured as A_{600} nm.

claving cystine, or cysteine, and the effect of ferric chloride and/or sodium nitrite on this conversion. Cystine yielded Bactin (5c), ferric chloride or nitrite increased the yield slightly (5d, 5e). Cysteine did not yield Bactin, but some cystine was formed (5f), ferric chloride stimulated the conversion of cysteine to cystine, and very little Bactin was formed (5g). Nitrite caused an almost complete conversion of cysteine, and Bactin and cystine were reaction products (5h). The yield of Bactin was highest if cysteine were heated in the presence of both ferric chloride and sodium nitrite (5i).

Similar results were obtained in acid solution, but the

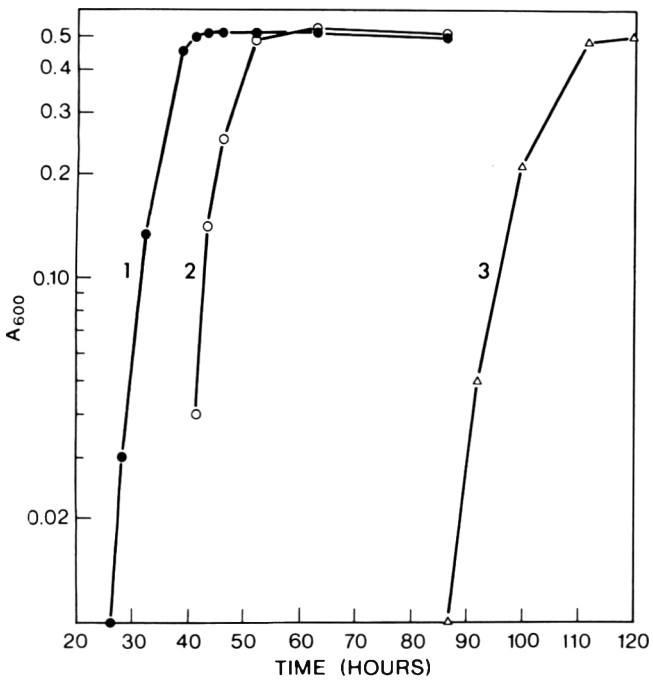
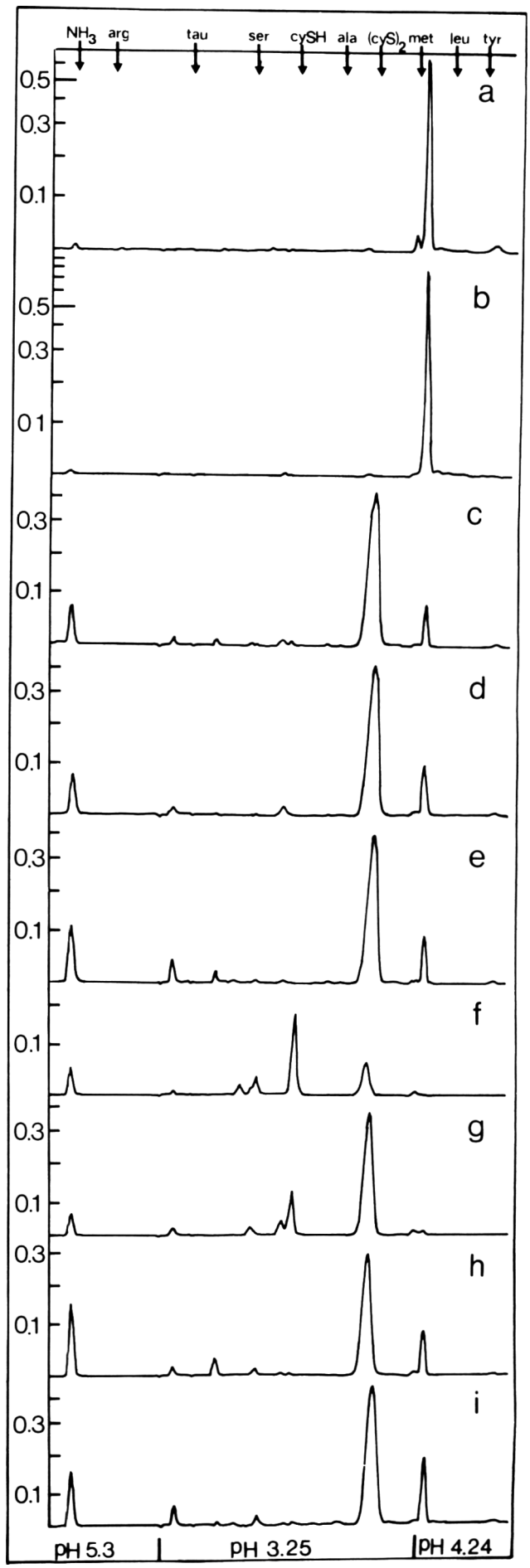


Fig. 4—Effect of bis-(2-amino-2-carboxyethyl)trisulfide (=Bactin) on growth of Lactobacillus plantarum at 20°C in Bactotryptone medium. Curve 1: control; curve 2: 20 mg Bactin/L medium; curve 3: 100 mg Bactin/L medium. The Bactin used was synthesized by autoclaving cystine in 0.1M HCl and purified as described in Materials and Methods. Abscissa: Time (hr) after inoculation. Ordinate: Cell density measured as A_{600} nm.

Fig. 5—The formation of Bactin by heating of cystine, or cysteine, and the effect of ferric chloride and/or sodium nitrite on this reaction. The curves (redrawn) show the elution of ninhydrin positive compounds when the following samples were subjected to automatic amino acid analyses: a: 0.345 mg/ml Bactin (as HCl salt) synthesized by heating cystine in HCl and purified by cation exchange chromotography on Zerolit 225; b: as a, but synthesized from S^0 and cysteine; c: cystine (2.4 mg/ml) autoclaved for 20 min in 0.1M sodium phosphate at pH 6; d: as c, but in the presence during autoclaving of 2.7 mg/ml $FeCl_3 \cdot 6H_2O$; e: as c, but in the presence of 0.7 mg/ml $NaNO_2$; f: cysteine (2.4 mg/ml) autoclaved for 20 min in 0.1M sodium phosphate at pH 6; g: as f, but in the presence during autoclaving of 2.7 mg/ml $FeCl_3 \cdot 6H_2O$; h: as f, but in the presence during autoclaving of 0.7 mg/ml $NaNO_2$; i: as f, but in the presence during autoclaving of 2.7 mg/ml $FeCl_3 \cdot 6H_2O$ and 0.7 mg/ml $NaNO_2$. The samples c to i were diluted 1:10 before chromatography. Arrows indicate elution volumes of some amino acids. Buffer pH and shifts are indicated at the bottom of the graph. Ordinate shows the A_{570} of the eluate from the amino acid analyzer.



catalytic effects of ferric chloride on the conversion of cysteine to Bactin was more pronounced, thus in accordance with the observation that heating of cysteine and ferric chloride in acid solution yielded inhibiting products (Fig. 3). The catalytic conversion of cysteine by the mixture of ferric chloride and sodium nitrite was fast also at room temperature (concentrations as in Fig. 5). After 5 hr at 20°C and pH 6, all the cysteine had disappeared, but the level of Bactin was then still low. In a mixture of cysteine, cystine, ferric chloride, and sodium nitrite some Bactin was found after 24 hr at 20°C and pH 6.

The growth delay might result if Bactin killed a certain fraction of the bacteria in the inoculum. However, the viable count on TSA plates was unchanged after 1 hr exposure of $2.7 \cdot 10^2$ bacteria/ml to the growth inhibitory autoclaved Bactotryptone medium (same solution as in Fig. 2, curve 3). Purified Bactin (100 ppm) did neither cause a significant change in the number of viable bacteria ($5 \cdot 10^7$ per ml) after 4 hr at 20°C, in 0.1M potassium phosphate at pH 6, or in Bactotryptone medium. Other bacteria were affected by Bactin in qualitatively the same way as *L. plantarum*, and some were more sensitive. Even fungi were inhibited, particularly the germination of conidia.

DISCUSSION

Bis-(2-Amino-2-Carboxyethyl) Trisulfide (=Bactin) has been shown to prolong the lag-phase of growth of *L. plantarum* without affecting its maximum growth rate. The degree of inhibition, expressed as a delay in the increase of turbidity of the liquid culture, increased with increasing concentration of the compound. Such a delay of growth would result if a certain fraction of the bacteria in the inoculum lost their viability by exposure to Bactin, while the survivors retained their normal growth potential. This explanation seemed plausible from Figure 1, which shows that the growth in a heated cystine medium followed closely the growth in a control medium that had been inoculated with 1/10 the number of bacteria. However, we have shown that the bacteria retained their viability during the prolonged lag-phase, in heated nutrient media, and that 100 mg/liter Bactin did not act bactericidally at pH 6. It is premature to speculate on the mode of action of Bactin, but it seems to induce a state of rest in the bacteria.

Bactin was formed from cystine, or cysteine, at the same conditions which yielded growth inhibitory products from these amino acids. The inhibition of heated cystine, or cysteine was qualitatively the same as with purified Bactin. We could not find, however, a strict quantitative relationship between the content of Bactin in nonpurified mixtures and the duration of the growth delay, maybe because other components modify the activity of Bactin. This assumption is supported by studies on the catalytic effect of trisulfides on the reduction of cytochrome c (Massey et al., 1971), showing that the disulfide anion also affected this process. We have not yet examined whether there is a similar reason for the slightly higher activity of the Bactin preparation produced by heating of cystine (Fig. 5a), than of that synthesized from S° and cysteine (Fig. 5b), but the former preparation contained minor contaminants, one probably being the tetrasulfide.

The conversion of cystine, or cysteine, to Bactin was examined also at pH 6, in order to be able to compare conditions for Bactin formation to literature data on the heat induced formation of antibacterial principles in nutrient media. Nutrient media containing cystine become inhibitory after heating, but not if cystine is replaced by cysteine (Moran et al., 1975). Cysteine may become inhibitory, however, if ferric chloride is present during heating (Konowalchuk et al., 1954a), and the pH is acid. These conditions for inhibitor formation coincide with the conditions which yield Bactin, and moreover, with the fact that Bactin is stable at acid pH values (Fletcher and Robson, 1963).

There is no accepted explanation of the heat induced antibacterial effect of nitrite in nutrient media, or in food (Larkworthy et al., 1977; Ashworth and Spencer, 1972; Lee et al., 1978). Since the concentration of free nitrite is reduced during heat treatment, and iron and cysteine/cystine seem necessary for the generation of antibacterial activity, it has been logical to assume that the inhibitor was formed in a reaction which involved all these compounds (Asan and Solberg, 1976; Huhtanen and Wasserman, 1975; Kubberød et al., 1974; Mirna and Hofman, 1969). S-Nitrosocysteine, Roussin red and Roussin black are plausible reaction products, but Moran et al. (1975) were unable to attribute the nitrite inhibition to any of these compounds. It remains of course to be proven that Bactin has been the antibacterial principle formed in these cases, but the present data are in favor of this suggestion. Bactin acts bacteriostatically and it is formed as a major product (ca 33%) during heating for 20 min of cysteine in the presence of nitrite and ferric chloride. Ferric chloride catalyzed the oxidation of cysteine to cystine, but Bactin was then formed in small quantities, unless the pH was very low. Nitrite and ferric chloride in mixture not only caused formation of the bacteriostatic compound Bactin, but also depletion of cysteine, which otherwise abolished the inhibition due to Bactin.

Other authors suggesting that S° may be the antibacterial degradation product of heated cystine, or of cysteine plus iron, have not mentioned the possibility that polysulfides might have been the active molecules, and the chemical tests used to identify S° (Konowalchuk et al., 1954b) coincide with those for trisulfides (Fletcher and Robson, 1963). Polysulfides, including Bactin, are intermediates in the sulfur metabolism. Polysulfides can be formed enzymatically from mercaptopyruvate (Hylin and Wood, 1959). Liver cystathionase converts cystine to Bactin, and rhodanese transfers the sulfur further to cyanide, sulfite, or other acceptors (Szczepkowski and Wood, 1967; Abdolrasulnia and Wood, 1979). The trisulfide is also involved in regulation of the activity of 5-aminolaevulinate synthetase (Davies and Neuberger, 1979).

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WHEAT BRAN'S EFFECT ON DIGESTIVE ENZYME ACTIVITY AND BILE ACID LEVELS IN RATS

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ABSTRACT

Rats were fed either a fiber-free control diet or a diet containing 5% wheat bran. After 10 days the total activity of lipase, amylase, trypsin, and chymotrypsin, and total protein were elevated in pancreatic tissue. Activity per mg protein of lipase and amylase was also elevated in the pancreas. However, there were no differences in activity of these enzymes nor in bile acid levels in the small intestine after consuming a meal. Wheat bran may alter pancreatic secretion or absorption of digestion products leading to adaptive changes in the pancreas.

INTRODUCTION

DIETARY FIBER can increase the fecal excretion of fat and protein (Cummings, 1978), perhaps by affecting digestion and absorption in the small intestine. Studies in vitro with several dietary fiber sources have shown that the activity of pancreatic lipase, trypsin, and chymotrypsin can be lower after incubation with certain fiber sources (Schneeman, 1978). Rats fed a cellulose-containing diet had lower levels of trypsin, chymotrypsin, amylase, and lipase per mg intestinal contents (Schneeman and Gallaher, 1980). These studies have indicated that fiber is not innocuous as it passes through the small intestine, but can alter the availability of enzyme activity, perhaps leading to an effect on the rate of digestion. The present study was undertaken to determine whether a nonpurified fiber source, wheat bran, could affect digestive enzyme activity and bile acid levels in the alimentary tract.

EXPERIMENTAL

THE PROTOCOL for the experiment was similar to that described previously (Schneeman and Gallaher, 1980). Briefly, the procedure was as follows: 100g rats were fed either the control diet (C) or 5% wheat bran (WB) diet for 10 days (Table 1). Animals had diet available between 1700 hr and 0800 hr daily and water ad libitum. One part of each diet group was killed 2-1/2 hr after being offered 3.0g of their diet (fed group). The rest of the group was killed without consuming any diet for 12 hr (unfed group). The pancreas, small intestinal contents, and intestinal mucosa were collected, frozen immediately, lyophilized and stored dessicated under vacuum. The intestinal and pancreatic activity of amylase, lipase, trypsin, chymotrypsin, and protein and the intestinal level of bile acids were determined. Statistical evaluation of the results was by one-way analysis of variance with orthogonal contrasts to determine treatment differences and with Student's t-test (Nie et al., 1975). Within the fed group or the unfed group the dietary treatment and the overall effect of feeding was determined.

RESULTS

TABLE 2 contains the food intake, final body weight, weight of the intestinal contents and mucosa and pancreas. Consumption of wheat bran did not significantly affect any of these parameters; however, feeding did increase the weight of the intestinal contents and decreased pancreatic weight.

Total protein (Table 3) was elevated in the intestinal contents of the fed group. In the intestinal mucosa, total protein was lower in the WB group for both the fed and unfed animals. Feeding lowered the pancreatic content of protein ($P \leq 0.10$) and in the unfed group total pancreatic protein was slightly elevated in animals consuming wheat bran.

–Continued on next page

Table 1–Diet composition

Ingredient	Control	Wheat bran
Cerelose	60%	55%
Casein	24%	24%
Corn oil	8%	8%
Salt mix[a]	6%	6%
Wheat bran[b]	–	5%
Vitamin mix[c]	2%	2%
BHT	0.02%	0.02%

[a] Salt content of the diet (g/kg-diet) $CaCO_3$, 18.0; K_2HPO_4, 19.5; $CaHPO_4$, 3.6; NaCl, 10.08; $FeSO_4 \cdot 7H_2O$, 1.5; $MgSO_4 \cdot 7H_2O$, 3.88; KI, 0.015; $ZnCO_3$, 0.048; $CuSO_4 \cdot 5H_2O$, 0.018; $MnSO_4 \cdot H_2O$, 0.138.

[b] AACC certified Food Grade Wheat Bran, R07-3691: 8.9% crude fiber, 14.3% protein, 10.4% moisture, 5.22% fat, 5.12% ash, 11.9% acid detergent fiber, 40.2% neutral detergent fiber, 3.2% lignin, 3.0% pectin, 9.5 g/g water-holding capacity, 0% cutin, mixed particle size.

[c] Composition per kg of vitamin mix (g) choline chloride, 50.0; Inositol, 25.0; Ascorbic Acid, 5.0; Calcium pantothenate, 2.5; Thiamin hydrochloride, 1.5; Pyridoxine, 1.5; Nicotinic acid, 1.5; Menadione, 1.25; Riboflavin, 0.5; Para-amino benzoic acid, 0.5; Folic Acid, 0.03; Biotin, 0.125; Retinyl palmitate, 2.1 (325,000 IU/g); Cholecalciferol, 0.23 (325,000 IU/g); B_{12} plus Mannitol, 1.5; alpha-tocopheryl acetate, 21.4 (25,000 IU/g); and Cerelose, 885.0.

Table 2–Food intake, body and tissue weights of rats adapted to diets with (WB) or without (C) wheat bran

Total food intake (g)	WB	157.4 ± 9.9[a]		
	C	167.5 ± 6.6		
Final body weight (g)	WB	172.5 ± 7.6		
	C	179.0 ± 4.9		
		Unfed	Fed	Significant main effects[b]
Intestinal contents (mg)	WB	25.2 ± 4.7	72.2 ± 15.8	Feeding
	C	39.8 ± 11.2	54.6 ± 11.8	
Mucosa (mg)	WB	502.2 ± 31.2	512.1 ± 41.8	
	C	516.2 ± 37.2	584.4 ± 27.3	
Pancreas (mg)	WB	192.6 ± 20.3	130.7 ± 16.0	Feeding
	C	160.3 ± 11.6	143.0 ± 11.8	

[a] Each value is the mean ± SEM WB n = 12 (5 unfed, 7 fed), C n = 12 (5 unfed, 7 fed).

[b] $P \leq 0.05$

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Amylase activity (Table 4) per mg intestinal content of the fed groups was about half of the unfed groups. Total activity in the contents was similar in all groups; however, in the mucosa of the fed groups, amylase activity of the WB group was 60% of the controls. In the pancreatic tissue feeding decreased amylase activity expressed per mg protein or total activity. In the unfed group amylase activity per mg protein was almost 40% higher in the WB group, and total activity was double that of controls.

Total lipase activity (Table 5) was elevated in the intestinal contents after feeding ($P < 0.10$). The enzyme activity associated with the mucosa was higher in the WB group in both fed and unfed animals and feeding elevated the amount of enzyme activity present. Both the dietary treatment and feeding had a significant effect on the lipase activity in the pancreas. In the unfed group, activity per mg protein if fourfold greater in the WB group and total activity is sixfold greater. In the fed group both these values are still twofold greater in the WB group. Feeding led to lower pancreatic lipase values, primarily in the WB group.

Trypsin activity (Table 6) per mg intestinal contents in the WB group was almost half of the activity of the C group. Total trypsin in the contents was elevated at least twofold by feeding, likewise the activity associated with the mucosal scraping was elevated almost fivefold by feeding. Mucosal trypsin activity was slightly lower ($P < 0.10$) in the WB group. Total trypsin activity in the pancreas of unfed animals was 1.5 times greater in the WB group. Feeding resulted in lower pancreatic enzyme levels, primarily in the WB group, for which the activity decreased by 50%. The specific activity of trypsin was slightly lower in the fed WB group ($P < 0.10$) and also lower due to feeding.

Chymotrypsin activity (Table 7) per mg intestinal contents was lower, and total intestinal content and mucosal activity was elevated in the fed groups. Feeding led to lower specific activity and total activity of chymotrypsin in the pancreas. Total pancreatic chymotrypsin activity in the unfed group was about 25% higher in the WB group than in controls.

Micromoles of bile acids (Table 8) per mg intestinal contents and total bile acids in the intestinal contents were similar in both groups of the fed animals. Bile acids in the mucosal scraping were slightly lower in the WB group.

Table 3—Protein content of the intestine and pancreas of rats fed diets with (WB) or without (C) wheat bran

		Unfed	Fed	Significant main effects[c]
		Intestine		
		mg	mg	
Protein/mg intestinal contents	WB	0.5 ± 0.02[a]	0.3 ± 0.02[b]	Diet in fed group Feeding
	C	0.5 ± 0.02	0.5 ± 0.03	
Total intestinal protein	WB	10.3 ± 1.9	22.5 ± 4.4	Feeding
	C	14.0 ± 2.7	25.9 ± 6.8	
Total mucosal protein	WB	320.8 ± 11.4	390.8 ± 36.9	Diet in fed and unfed groups
	C	453.8 ± 40.8	503.8 ± 28.3	
		Pancreas		
		mg	mg	
Protein/mg pancreas	WB	1.2 ± 0.09	1.2 ± 0.06	
	C	1.1 ± 0.05	1.1 ± 0.10	
Total pancreatic protein	WB	230.7 ± 17.8	161.5 ± 19.2	Diet in unfed group[d]
	C	170.3 ± 11.0	160.5 ± 21.0	Feeding[d]

[a] Mean ± SEM of 5 animals for each diet
[b] Mean ± SEM of 7 animals for each diet
[c] $P \leq 0.05$
[d] $P < 0.10$

Table 4—Amylase activity[a] in the intestine and pancreas of rats fed diets with (WB) or without (C) wheat bran

		Unfed	Fed	Significant main effects[d]
		Intestine		
Activity/mg intestinal contents	WB	10.9 ± 1.6[b]	4.7 ± 0.9[c]	Feeding
	C	9.6 ± 1.2	6.9 ± 1.2	
Total intestinal activity	WB	240.9 ± 67.6	323.0 ± 95.0	
	C	297.4 ± 70.7	372.0 ± 106.0	
Total mucosal activity	WB	886.9 ± 70.4	585.8 ± 121.6	Diet in fed group
	C	769.2 ± 109.0	938.2 ± 83.9	
		Pancreas		
Activity/mg protein	WB	85.0 ± 10.2	56.7 ± 3.8	Diet in unfed group Feeding
	C	53.8 ± 8.7	50.3 ± 6.8	
Activity × 10^3/ total pancreas	WB	19.8 ± 3.4	8.9 ± 0.9	Diet in unfed group Feeding
	C	8.9 ± 1.2	7.4 ± 0.9	

[a] One unit of activity = 1 micromole maltose formed
[b] Mean ± SEM of 5 animals for each diet
[c] Mean ± SEM of 7 animals for each diet
[d] $P \leq 0.05$

Table 5—Lipase activity[a] in the intestine and pancreas of rats fed diets with (WB) or without (C) wheat bran

		Unfed	Fed	Significant main effects[d]
		Intestine		
Activity/mg intestinal contents	WB	0.7 ± 0.1[b]	0.6 ± 0.06[c]	
	C	0.8 ± 0.2	0.7 ± 0.10	
Total intestinal activity	WB	16.7 ± 5.2	44.0 ± 10.5	Feeding[e]
	C	24.7 ± 6.9	36.2 ± 11.2	
Total mucosal activity	WB	52.3 ± 6.4	78.7 ± 7.7	Diet in fed and unfed group
	C	27.1 ± 2.6	49.0 ± 4.1	Feeding
		Pancreas		
Activity/mg protein	WB	52.3 ± 6.1	29.6 ± 1.6	Diet in fed and unfed group
	C	13.6 ± 1.9	14.3 ± 1.5	Feeding
Activity × 10^3/total pancreas	WB	12.3 ± 2.0	4.9 ± 0.8	Diet in fed and unfed group
	C	2.3 ± 0.3	2.1 ± 0.2	Feeding

[a] One unit of activity = 1 microequivalent of hydroxyl ion titrated per minute
[b] Mean ± SEM of 5 animals for each diet
[c] Mean ± SEM of 7 animals for each diet
[d] $P \leq 0.05$
[e] $P < 0.10$

DISCUSSION

WHEAT BRAN, as a nonpurified food source of dietary

fiber, has been reported to increase fecal weight and may decrease intestinal transit time (Kelsay, 1978). Several reports have focused on the effects of wheat bran on the functioning of the large intestine, but there has been very limited information on the effects of dietary fiber on conditions in the upper digestive tract. The results of the present study indicate that enzyme activity in the intestine was not altered to great extent, but within the pancreatic tissue several changes occurred due to consuming wheat bran.

The enzyme activity within the pancreatic tissue of the unfed groups reflects adaptation of the composition to the dietary treatment. The total amount of all the enzymes measured, amylase, lipase, trypsin, chymotrypsin, as well as total protein, was elevated in the wheat bran group. This increase was greatest for lipase and amylase, additionally, the specific activity (units per mg protein) was elevated for these two enzymes.

The mechanisms by which pancreatic enzyme adaptation to diet composition occurs is not clearly understood. In general, an increase in the dietary content of carbohydrate, lipid or protein leads to an elevation of amylolytic, lipolytic or proteolytic activity, respectively (Corring, 1977; Johnson et al., 1977). For amylase and lipase the products of carbohydrate and lipid digestion may be involved in the adaptive changes that have been observed, and adaptation of proteolytic enzymes appears to be related to protein levels in the intestine. The pancreatic stimulatory ability of the diet also seems to influence adaptive changes (Schneeman et al., 1977). Five percent of the carbohydrate in the diet was substituted with WB, however, this minor change in diet composition is not sufficient to lead to the differences in pancreatic enzyme composition observed (i.e. addition of about 0.7% protein and 0.3% fat). Since changes in diet composition per se cannot be used to explain the elevation of enzyme activities, perhaps there were differences in secretion from the pancreas or in absorption of products of digestion. The difference between the total enzyme level of the unfed and fed groups was generally greater in the WB treated group, implying a greater pancreatic secretion. It is of interest that a non-digestible dietary ingredient, such as wheat bran, can also lead to changes in the composition of pancreatic enzymes, and further work is needed to determine the mechanisms involved.

The differences in the enzyme content of the pancreas were not reflected in the intestinal contents. Enzyme activity in the small intestine of fed animals indicates activity which is available for digestion of a meal. This activity at any one time represents the sum of enzyme secretion and turnover. The total enzyme activity present, that is the sum of activity in the contents and the mucosa, was similar in the C and WB groups. Perhaps the changes in the pancreas reflect a mechanism attempting to maintain levels of enzyme activity in the small intestine. An adaptation of this type does occur when trypsin inhibitors are fed in the diet (Green and Lyman, 1972). Additionally, we observed that intestinal enzyme levels were highly variable and perhaps feeding higher levels of WB could lead to some measurable differences.

In the small intestinal mucosa, protein content was lower in the WB groups. Such a difference might reflect differences in cell sloughing between groups. Several investi-

Table 6—Trypsin activity[a] in the intestine and pancreas of rats fed diets with (WB) or without (C) wheat bran

		Unfed	Fed	Significant main effects[d]
		Intestine		
Activity/mg intestinal contents	WB	1.1 ± 0.1[b]	0.9 ± 0.1[c]	Diet in fed group
	C	1.5 ± 0.2	1.7 ± 0.5	
Total intestinal activity	WB	25.8 ± 6.5	74.9 ± 23.2	Feeding
	C	42.6 ± 7.0	86.3 ± 18.0	
Total mucosal activity	WB	49.0 ± 8.1	236.4 ± 46.7	Diet in fed group[e] Feeding
	C	80.4 ± 14.1	384.3 ± 93.3	
		Pancreas		
Activity/mg protein	WB	2.2 ± 0.2	1.6 ± 0.1	Diet in fed group[e] Feeding[e]
	C	2.2 ± 0.2	2.0 ± 0.3	
Activity/ total pancreas	WB	507 ± 64	252 ± 30	Diet in unfed group Feeding
	C	361 ± 31	300 ± 28	

[a] One unit of activity = 1 micromole TAME hydrolyzed per minute
[b] Mean ± SEM of 5 animals for each diet
[c] Mean ± SEM of 7 animals for each diet
[d] $P \leq 0.05$
[e] $P < 0.10$

Table 7—Chymotrypsin activity[a] in the intestine and pancreas of rats fed diets with (WB) or without (C) wheat bran

		Unfed	Fed	Significant main effects[d]
		Intestine		
Activity/mg intestinal contents	WB	1.4 ± 0.1[b]	1.0 ± 0.1[c]	Diet in fed group[e] Feeding
	C	1.6 ± 0.1	1.2 ± 0.3	
Total intestinal activity	WB	31.2 ± 6.6	69.7 ± 17.9	Feeding[e]
	C	47.9 ± 7.3	62.6 ± 11.9	
Total mucosal activity	WB	108.9 ± 18.1	181.0 ± 17.9	Feeding[e]
	C	150.3 ± 20.3	155.3 ± 18.9	
		Pancreas		
Activity/mg protein	WB	2.7 ± 0.2	2.1 ± 0.1	Feeding
	C	2.8 ± 0.2	2.4 ± 0.3	
Activity/ total pancreas	WB	615 ± 53	343 ± 44	Diet in unfed group Feeding
	C	469 ± 28	359 ± 39	

[a] One unit of activity = 1 micromole BTEE hydrolyzed per minute
[b] Mean ± SEM of 5 animals for each diet
[c] Mean ± SEM of 7 animals for each diet
[d] $P \leq 0.05$
[e] $P < 0.10$

Table 8—Bile acid[a] content in the intestine of rats fed diets with (WB) or without (C) wheat bran (fed group)

		μ moles
Bile acids/mg intestinal contents	WB	0.02 ± 0.04[b]
	C	0.02 ± 0.02
Total intestinal bile acids	WB	13.4 ± 2.2
	C	11.4 ± 2.2
Total mucosal bile acids	WB	24.3 ± 3.0
	C	37.5 ± 3.2*

[a] μmoles taurocholate based on α-steroid dehydrogenase activity
[b] Mean ± SEM of 7 animals for each diet
* C group is significantly greater than the WB group, $P \leq 0.05$

gators have speculated that dietary fiber could accelerate sloughing and this area should be investigated further (Cummings, 1978).

When fed to human subjects, wheat bran leads to a lower concentration of fecal bile acids, but because total fecal weight is greater, total fecal bile acid excretion is elevated (Kritchevsky, 1978; Kay and Truswell, 1977). In our study, the total amount of bile acids in the small intestine was similar for both groups (Table 8). At this level of WB any increase in fecal bile acid excretion does not appear to be related to changes in bile acid levels in the small intestine.

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SOME FACTORS CONTROLLING THE KINETICS OF MOISTURE MOVEMENT DURING AVOCADO DEHYDRATION

STELLA M. ALZAMORA and JORGE CHIRIFE

ABSTRACT

The effect of some factors such as initial oil content, blanching, and freezing before drying, on the rate of moisture movement during avocado dehydration was studied. It was found that the diffusion coefficients of water in avocado were strongly affected by its oil content.

INTRODUCTION

WORK carried out at this laboratory in recent years aimed to study the kinetics and mechanism of moisture movement during the early stages of the falling rate period (or first falling rate period) of vegetable dehydration. Research was conducted on sugar beet root and avocado and aspects studied included the application of Fick's law of diffusion to predict average drying times and internal moisture distribution (Vaccarezza et al., 1974a; Alzamora et al., 1978a), heat transfer effects on drying rate and on the application of Fick's law (Vaccarezza et al., 1974b, Vaccarezza and Chirife, 1975a; Alzamora et al., 1978a), the nature of the mechanism of moisture transport (Vaccarezza and Chirife, 1975b) and the prediction of temperature evolution during air drying (Alzamora et al., 1978b).

Recently, Alzamora et al. (1978a) studied the initial phase of the falling rate period of avocado air drying. They found an exponential moisture-time relationship in the first falling rate period and calculated diffusion coefficients which were correlated with temperature in accordance with an Arrhenius type relationship. The empirically found drying behavior was then used to solve the energy balance equation yielding an expression which was able to predict satisfactorily the temperature evolution of the sample during drying.

It is the purpose of the present work to study the effect of avocado oil content and pretreatments such as freezing and blanching, on the kinetics of moisture movement during the first falling rate period. These studies were aimed at acquiring a better understanding of the complex nature of moisture movement during the early stages of drying. In this regard, avocado represents an interesting raw material because of the presence of a relatively large amount of oil emulsified with water. It is also interesting to note that avocado drying is sometimes a preliminary step to oil extraction (Bertoni et al., 1967). By this reason the knowledge of factors which affect the rate of drying may be of practical interest.

EXPERIMENTAL

Laboratory dryer

The drying equipment has been described in detail in a previous paper (Vaccarezza et al., 1974a). It consists of a centrifugal fan which blows the air through a heating section and then upwards through a vertical duct at the end of which the sample is suspended from a precision balance. A high constant air velocity (13 m/sec) was used in all experiments in order to eliminate external resistances to the moisture loss (Vaccarezza et al., 1974a).

Materials

Vegetables studied in this work included, in addition to avocado pulp, potato and beets. Foods were sliced in a food cutter and cut in approximately square pieces with thickness varying from about 0.33 cm to 0.50 cm. Various measurements of the thickness were made at different points with a dial micrometer; only slices that fell within a 6% range of the average thickness were used. In all experiments reported the thickness was approximately equal or smaller than one-tenth of the size; in this condition drying took place only from the two major faces of the slab.

Medium size avocados of uniform size and maturity were kept in a refrigerator until use. Each set of experiments performed to study the effect of a given variable (except when studying the influence of oil content) was always done using samples obtained from a same fruit. This was done in order to reduce as much as possible the influence of biological variations (mainly oil content) of the raw material.

Model systems

Oil-based model systems were prepared as follows. Slabs of sugar beet root were leached with a large excess of water over prolongued periods in order to remove its soluble solids.

Soluble solids of sugar beet root (mainly sucrose) constitute over 90% of its dry matter (Vaccarezza and Chirife, 1975b). The extracted material—consisting in a matrix of insoluble polysaccharides—was then rapidly frozen and freeze-dried at a pressure less than 100μ and at room temperature to minimize structural changes. Freeze-dried slabs were then rehydrated by soaking in water-unrefined soybean oil emulsions containing various oil proportions. The lecithin content of crude oil permitted obtaining relatively stable emulsions. Percent oil in the slabs was calculated gravimetrically from the weight difference between the initially freeze-dried slab and the dry weight obtained after soaking in the emulsions. The surface tension at 25°C of the soybean oil-water emulsions was measured with the Du Nouy tensiometer and was found to be 32.7 dynes/cm.

Pretreatments

Water and steam blanching. Samples subjected to water blanching were immersed in boiling water for 4 min and then cooled at room temperature.

Samples subjected to steam blanching were wrapped in a moisture-proof plastic film (Saran), exposed for 4 min to steam at atmospheric pressure and then allowed to cool at room temperature. The film prevented any small leaching of soluble solids which could occur due to vapor condensed on the food's surfaces.

Freezing. Slabs of avocado were frozen by placing in a freezer at −30°C for a period of 18 hr. The frozen samples were then allowed to thaw at room temperature.

Determination of oil content in avocado pulp

The method described by Bertoni et al. (1967) was used for the determination of oil content.

RESULTS & DISCUSSION

THE EFFECT of blanching (steam) and freezing-thawing on the rate of drying at 45°C of avocado slabs is shown in Figure 1, in which values of log $\bar{W}/W_o$ vs time/(thickness)$^{1.69}$ were plotted for untreated (control) and treated samples. The factor (thickness)$^{1.69}$ was introduced for purposes of comparison to allow for small differences (maxi-

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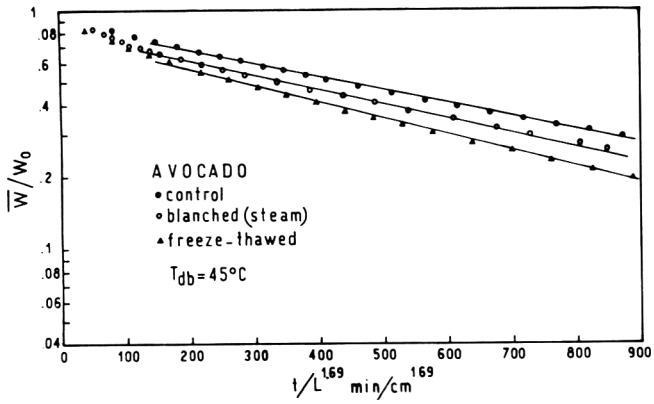

Fig. 1—Effect of blanching and freezing on drying curve of avocado.

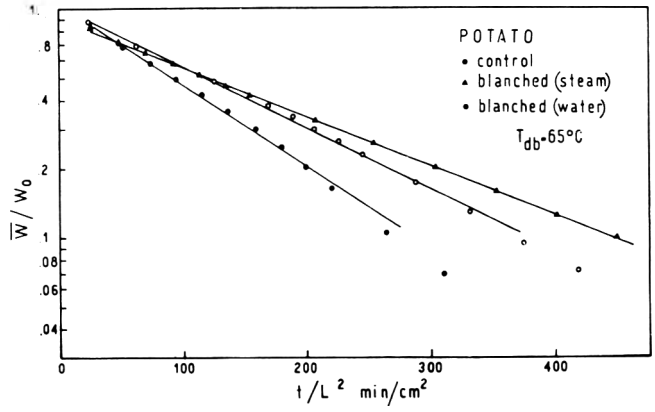

Fig. 2—Effect of steam and water blanching on drying curve of potato.

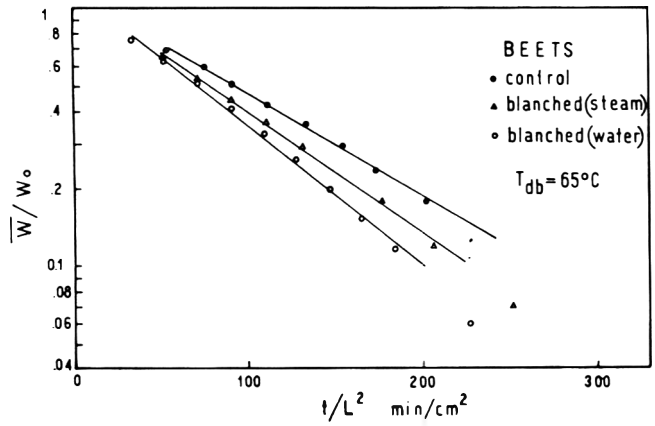

Fig. 3—Effect of steam and water blanching on drying curve of beets.

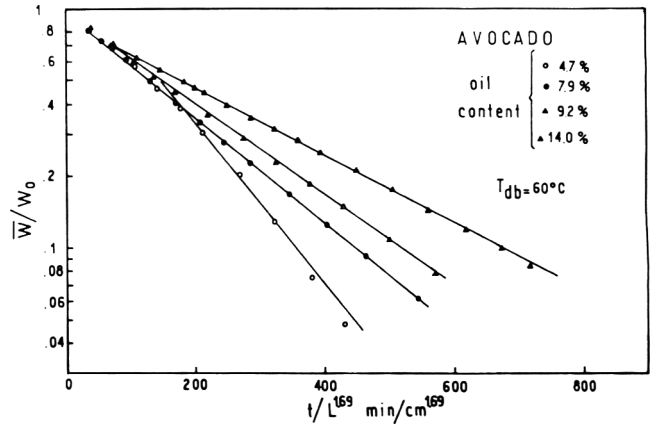

Fig. 4—Effect of initial oil content on drying curve of avocado.

mum 6.8%) in the thickness of samples (Alzamora et al., 1978a). The slopes of the lines in Figure 1 can be related to the moisture diffusivity in this period. Although both pretreatments somewhat increase the slopes of the straight lines, freezing appears to have a more significant influence than blanching on the rate of moisture transport in avocado. Blanching is considered in the literature to affect the rate of vegetable drying although there is little experimental evidence. Saravacos and Charm (1962) found that steam blanching of potatoes had no significant effect on drying rate when compared with the control sample dried at 66°C. Vaccarezza et al. (1974a) found that water blanching (using wrapped samples to avoid soluble solid loss) had no significant effect on drying rate at 60°C, but at 47°C it somewhat increased the rate. It is believed that various aspects should be considered in order to explain the effect of blanching on fruit and vegetable rate of drying. These aspects may be summarized as follows: (a) heating leads to changes in the physical properties of tissue, among them it destroys the semipermeability of the cell membranes and with it the osmotic properties of the cell (Gane and Wager, 1958); (b) Rotstein and Cornish (1978), however, have recently studied the influence of cellular membrane permeability on drying behavior and concluded that at least for apples, the drying phenomenon is not controlled by the cellular permeability; (c) heating of starchy foods, like potato, may also lead to gelatinization of starch; (d) drying at elevated temperatures may also modify the physical characteristics of food masking the effect of blanching; and (e) water blanching may lead to loss of soluble solids, which, as shown by Vaccarezza and Chirife (1975b), affects the rate of drying.

The above considerations are useful to understand why steam blanching had only little effect on rate of drying of avocado.

Freezing-thawing was shown to have a more significant effect on rate of drying of avocado. Although freezing, particularly slow freezing, may alter physical characteristics of tissue, it is believed that the observed increase in the rate of drying is mainly due to the effect of freezing-thawing on the stability of the oil-water emulsion which constitutes the major part of the avocado pulp. Chen (1975) found that freezing-thawing of oil in water emulsions causes coalescence of the drops and thus increases the average droplet diameter of the emulsions. He also discussed various literature results which indicate that frozen emulsions break down or can be observed to have an increase in droplet diameter after freezing.

Therefore it may be assumed that the increase in drying rate of avocado upon a freezing-thawing cycle is due to coalescence of the oil droplets emulsified with water.

In order to further clarify the influence of blanching on the rate of drying of vegetables, experiments were carried out with potato (high starch content) and beets (relatively high soluble solids content). Samples of potato and beets (about 0.43–0.46 cm thick) were steam or water blanched and then dried at 65°C and compared to unblanched (control) samples. The results are presented in Figures 2 and 3,

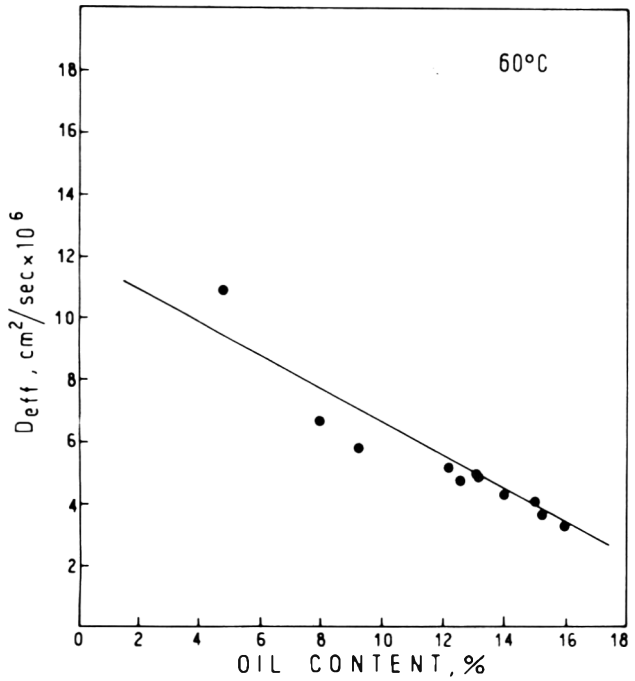

Fig. 5—Effect of initial oil content of avocado on moisture diffusion coefficients at 60° C.

in which values of log $\bar{W}/W_o$ are plotted vs time/(thickness)^2. The factor 2 was assumed for purposes of comparison to allow for small differences between thickness of control and blanched samples. Figure 2 shows that blanching, either steam or water, decreased the rate of drying of potato; this effect may be ascribed to changes caused by gelatinization of the starch. In beets, however, blanching increases the rate of moisture movement (Fig. 3), the effect being more noticeable in the case of water blanching. This may be attributed to leaching of the soluble solids of beets.

The effect of oil content in avocado pulp on drying rate was also studied. El-Zeftawi (1978) studied physical and chemical changes in fruit of seven different avocado cultivars. He found that for all varieties, moisture content was negatively correlated with oil content. El-Zeftawi (1978) provided the following correlation to calculate the oil content from the knowledge of the moisture content of pulp,

$$Y = 72.47 - 0.77\,X$$

where Y is the oil content (%) and X is the percentage of moisture. Various samples of avocado from different varieties and maturity were analyzed for oil and moisture content and shown to obey fairly well the above equation. Figure 4 shows the drying rate curves at 60°C for avocado pulp of different initial oil content ranged between 4.7–14.0%. It can be seen that increasing the initial oil content decreases the rate of drying. Moisture diffusion coefficients, D_{eff}, were calculated from the slopes of experimental straight lines as shown in Figure 4, according to Fick's law for one-dimensional transport:

$$\frac{\bar{w} - w_e}{w_o - w_e} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left\{-(2n+1)^2 \pi^2 \frac{D_{eff}\, t}{L^2}\right\}$$

where, L = thickness of the slab; $\bar{w}$ = moisture content dry basis; w_o = initial moisture content, dry basis; w_e = equilibrium moisture content, dry basis; and t = drying time. Linear regression analysis was employed to obtain values of

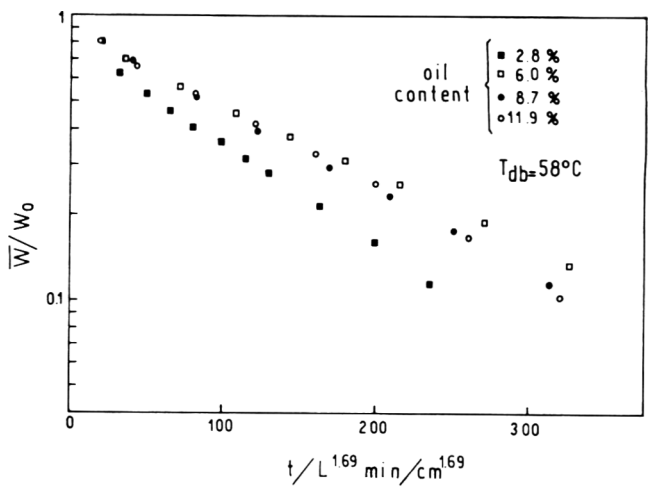

Fig. 6—Drying curves of model systems with different oil contents.

diffusion coefficients for different initial oil contents, which were plotted in Figure 5 as D_{eff} vs oil content (%). A negatively sloped straight line was obtained, whose equation is:

$$D_{eff\ (at\ 60°C)}\ (cm^2/sec) = 1.20 \times 10^{-5} - 5.41 \times 10^{-7}\ Y$$

It is interesting to note that the moisture diffusion coefficient at very low oil contents (e.g. Y→0) is in the same order of magnitude as the coefficient of self-diffusion for water which is about 4.5 × 10^{-5} cm^2/sec at 60°C (Lawrence and Scott, 1966).

The effect of oil content on avocado drying rate was confirmed with experiments using model systems containing different contents of emulsified soybean oil. Figure 6 shows the drying behavior at 58°C of the different models. It can be seen that increasing oil content also decreases the rate of drying; above a certain oil content level, however, no further reduction in drying rate appears to occur. Other authors also found that the drying behavior of foods was influenced by its fat content. Palumbo et al. (1977) found that the drying rate constants (directly related to moisture diffusivity) of pepperoni decreased as the fat content increased. Jason (1965) also found that the diffusion coefficients of water in fish muscle were inversely correlated with the fat content. These effects were attributed to the hydrophobic character of fat which imposes a resistance to the flow of water

In conclusion it may be said that, among factors here studied, oil content was found to be the one which most influenced the rate of avocado drying, the water diffusivity being inversely correlated with oil content of the pulp. Blanching and freezing showed relatively little influence on rate of drying, with the effect of freezing being somewhat more noticeable.

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CHANGES IN MICROSTRUCTURE, STARCH GRANULES AND SUGARS OF GERMINATING BROAD BEANS

NAHED M. EL-SHIMI, B. S. LUH, and AHMED EL-TABEY SHEHATA

ABSTRACT

Scanning electron microscopy was used to study changes in microstructure of broad beans during the germination process. Photomicrographs showed only slight differences in the appearance of cotyledon cell structure between the dry and soaked broad beans, but the size of the starch granules increased after soaking. Also scanning electron micrographs of the cotyledons and the starch and protein fractions of broad beans revealed changes in the surface of the cells during germination of the seed. The starch granules appeared to be very fragile and proteins began to break into small fragments after 8 days of germination. There was a decrease in amylose, amylopectin, and raffinose during germination. Sucrose and fructose increased during the 4-day germination period, but decreased thereafter. Raffinose decreased gradually during the 0–6 day germination period, and was not detectable after 8 days.

INTRODUCTION

LEGUME SEEDS have been used as human foods for more than 80 centuries and are still one of the most important sources of proteins for a large segment of the world population (Rockland and Jones, 1974).

Broad bean (*Vicia faba* L.) is one of the important legumes in the middle east countries. The microscopic structure of broad beans has been studied by McEwen et al. (1974) using scanning electron microscope (SEM). They examined the cells in the cotyledon and found granules of about 25–40 μm in diameter, plus amorphous material. SEM has revealed considerable variation in shape and surface structure among starch granules from wheat endosperm. Evers (1969) confirmed the presence of an equatorial groove in large granules and its absence in small granules. The complex granules of wrinkle seeded pea starch were examined by Banks et al. (1974). Their structure was related to the changes in starch during growth.

In a SEM investigation of the cotyledons of water-soaked soybeans, protein bodies of 1–10 μm in diameter exhibited a covering spongy network (Wolf and Baker, 1972). Protein bodies and spherosomes of soybean cotyledons were observed in SEM studies of commercial soy flours, protein concentrates, and protein isolates (Wolf and Baker, 1975). Hahn et al. (1977) employed scanning electron microscopy to characterize configurational changes of starch granules during gelatinization. When viewed under the SEM microscope, it was shown that each stage of gelatinization was characterized by distribution of granule configuration. The changes in conformation of the granules was observed during extracellular gelatinization. Also in water-soaked beans, birefringence of starch granules was completely eliminated under the same conditions (Hahn et al., 1977). Silva and Luh (1978), through scanning electron microscopic examination of cotyledons from red kidney beans (*Phaseolus vulgaris L.*), revealed changes on the surface of starch granules during germination of the seeds. There was a distinct transformation of the surface of the granules after 8 days of germination.

In the present study SEM was used to show surface characteristics of the starch granules and protein matrix in dry broad beans, and after soaking and germination.

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MATERIALS & METHODS

Broad beans

Dry broad beans were obtained from a market in Oakland, Calif. The beans were stored in hermatically sealed can at 2°C for 3 months.

Preparation of sample for SEM

Dry broad beans were fractured with a razor blade to provide a cross section near the plumule.

Cotyledons after soaking

After soaking the beans in distilled water for 14 hr, the seed coat was removed. The cotyledons were fractured with a sharp blade to provide a cross section near the plumule. The fractured cotyledons were placed in 4% glutaraldehyde-0.2M phosphate buffer at pH 7.1, for 120 min, dehydrated stepwise in increasing concentrations of 25, 50, and 75% ethanol for 60 min each, and then in 100% ethanol for 120 min. This was followed by immersion of the fractured cotyledons in mixtures of ethanol and amylacetate with increasing concentrations of the latter (25, 50, and 75%) for 60 min each, and then overnight in 100% amylacetate. The fractured cotyledons were then dried by the critical point method (Anderson, 1951).

Sprouted samples

Broad bean sprouts were harvested at 4 and 8 days after germination. The hypocotyl and seed coats were removed. The cotyledons were treated by the same procedure as dry and soaked broad bean cotyledons and dried by the critical point method (Anderson, 1951).

Preparation of flour

Dry, soaked, 4-day and 8-day sprouts of germinated and freeze-dried broad beans were milled in a Mikro sample mill (Micropul) to pass through a 40-mesh sieve, and then kept in tightly covered bottles.

Starch preparation

Starch samples for SEM study were prepared from the beans and sprouts by the procedure of Evers (1971) with some modification. The sample was extracted with 80% ethanol (1/20 w/v) to remove sugars. The sample was repeatedly treated with 80% ethanol after centrifugation. The supernatant was discarded and the starch was then freeze-dried in a Stoker Model 2004L freezer-drier for 24 hr.

Protein preparation

One part of the flour was dispersed in 10 parts of 0.16N NaOH at pH 8.5 to extract the proteins (McEwen et al., 1974). The proteins were precipitated with 0.1 NHCl, pH 4.5, washed with water, and then freeze-dried.

Scanning electron microscope

The protein and starch specimens were mounted separately on circular stubs using double-sided tape and coated with gold to thickness of 40 nm. The fractured cotyledon specimens were mounted on aluminum stubs with silver paint, sputtered with gold to a thickness of 40 nm (Silva and Luh, 1979). All specimen were examined on a Cambridge Steroscan Mark II scanning electron microscope at an

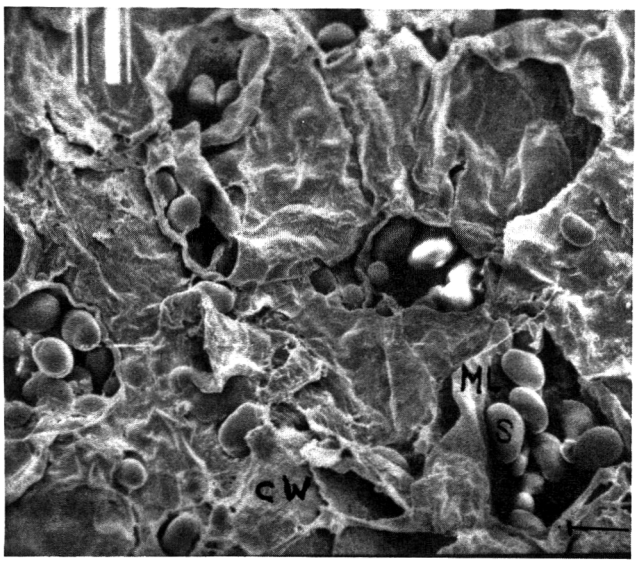


Fig. 1—Cross-section of dry broad bean cotyledons. Note cell walls with attached cytoplasmic residue and starch grains with some attached cytoplasmic materials (840X). S: Starch granule, ML: Middle lamela; CW: Cell wall. ⊢——⊣ 11.7μ.

accelerating potential of 10KV. Selected areas were photographed on panatomic X film.

Determination of starch

Total starch content of the freeze-dried sample (40 mesh) was determined by the anthrone procedure and the amylose content by the I_2 + KI reagent as described by McCready et al. (1950). The amylopectin content was calculated by substracting the amylose value from total starch.

Analyses of oligosaccharides by HPLC. Oligosaccharides in the freeze-dried samples were identified and quantified by High Pressure Liquid Chromatography (HPLC). A 10-g sample (40 mesh) was refluxed for 1.5 hr with 75 ml of 80% ethanol in the presence of a small quantity of $CaCO_3$. The alcoholic extract was cooled and filtered through Whatman #1 paper. The residue was washed three times with 50 ml each of 80% ethanol. The combined washings were evaporated to dryness in a rotary vacuum evaporator at 50°C. The residue was washed, brought to volume with deionized water and filtered through a 0.22 μm pore diameter Celotate filter (Millipore Corp.). The aqueous solution was assayed for sugars using HPLC (Palmer and Brandes, 1974).

A Waters Associates chromatograph equipped with a model-6000A solvent delivery system, a Model R401 refractometer detector, a U6K universal injector and a variable speed omniscribe recorder was used. The column was 30 cm × 4 mm i.d. stainless steel μ-Bondapack-Carbohydrate (Waters Associates).

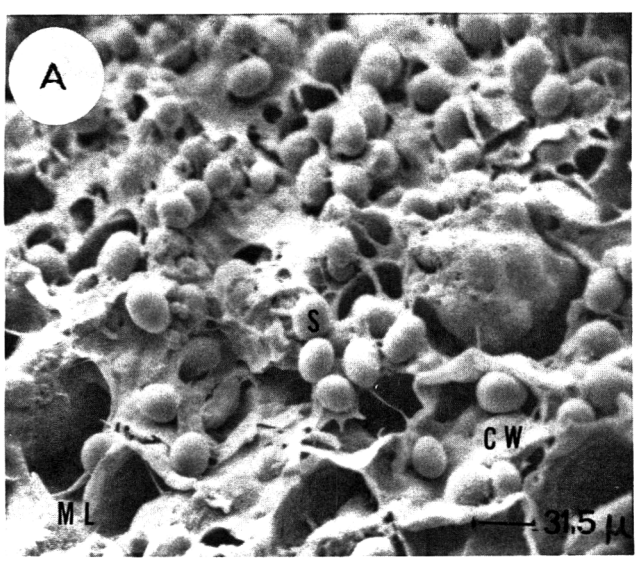
The eluant was acetonitrile (Burdick and Jackson Lab. Inc., High purity grade) and distilled water (85:15 v/v). Acetonitrile was filtered through a 0.5 μm Millipore filter. Distilled water was purified through Millipore Milli-Q system. Samples of 25 μl each was injected with a syringe. The detector was operated at an attenuation setting of 8X, 22°C. Standard curves were determined from peak areas generated by injecting standard solutions of fructose, sucrose, raffinose and stachyose. Sugars were identified by comparison of retention times of peaks with those of known sugars run under the same conditions. The quantity of sugars present were determined from the peak area as compared with the standard curves.

RESULTS & DISCUSSION

Dry and soaked broad beans

Fracturing the dry and soaked cotyledons of broad beans normally ruptures the cell walls and exposes the cell content for SEM study. Figure 1 shows the fractured surface of the cotyledons of the dry broad beans, and Figure 2 the fractured surface of cotyledons of soaked beans. It appears that the starch granules were attached to cell wall and cytoplasmic matrix. There were distinct differences in appearance and size of starch granules of broad beans and that of lima beans according to Rockland et al. (1977) who studied the extracellular gelatinization of lima bean starch in water and in a mixed salt solution by scanning electron microscopy. The starch granules in dry lima beans were different in shape and size than those observed in broad beans.

There were irregular matrix particles, persumably proteinaceous, occupying the spaces among the starch granules and on the surface of some starch granules. Some cytoplasmic residues were observed on the surface of some starch grains. This was also observed in the cotyledons of soaked beans (Fig. 2-A). The primary cell wall consists of cellulose microfibrils which are loosely woven together in a pattern and embedded in an amorphous matrix (Sterling, 1963). The middle lamella which cements the individual cells together, consists principally of calcium salts of polymers of



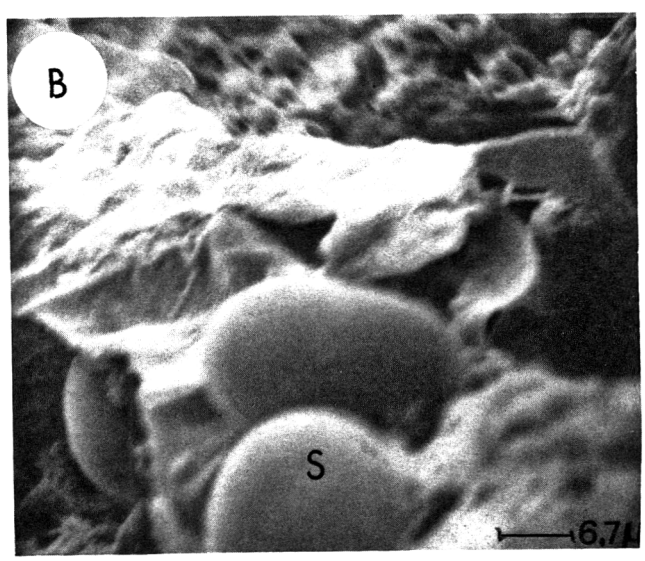


Fig. 2—Microstructure of soaked broad bean cotyledon: A. 320X, B. 1,500X; S represents the starch granules.

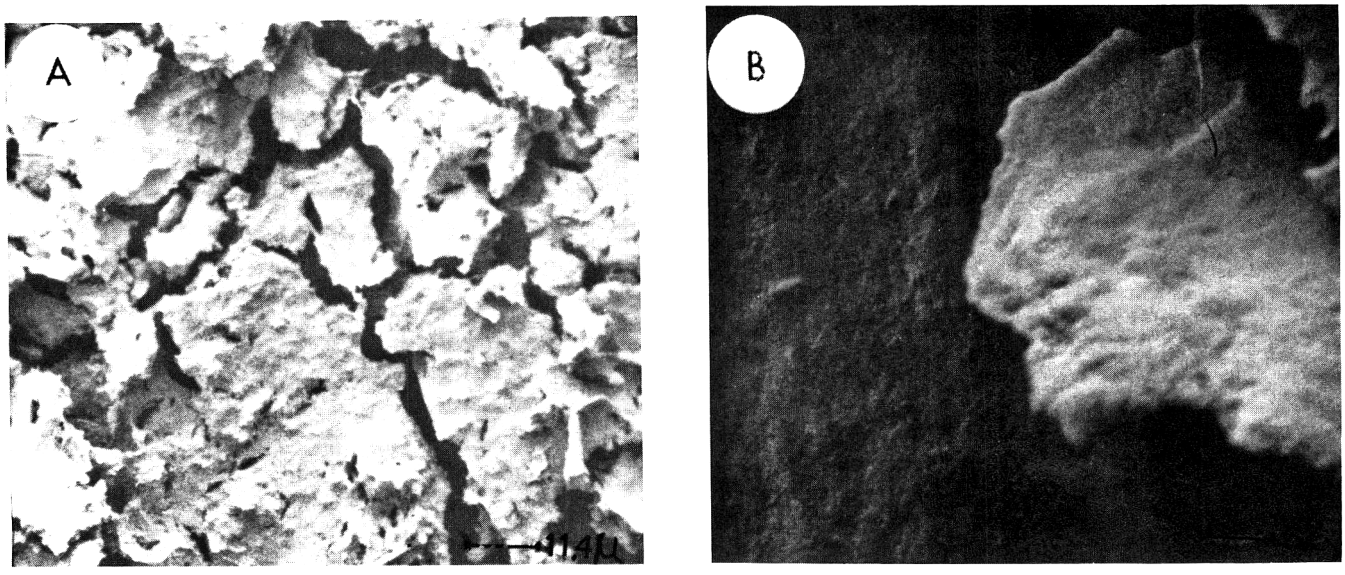


Fig. 3—Scanning electron micrograph of protein matrix isolated from dry broad beans: A. Structure of protein at 70X; Surface of protein particle at 1,500X.

galacturonic acid that have been esterified with methanol (Sefa-Dedeh et al., 1978). Figures 3A, B, and 4A, B illustrate protein materials prepared through alkali extraction followed by acid precipitation at the isoelectric point and then viewed under SEM. The protein matrix seems to have a spongy structure on the surface. The results are in agreement with those reported by McEwen et al. (1974). The swelling of the protein matrix after soaking in water demonstrates the ability of proteins to absorb water.

Starch granules isolated from dry and soaked broad bean flours are shown in Figures 5 and 6. In the present work, it was observed that the starch granules of broad beans are quite different from those of lima beans (Rockland et al., 1974; 1977). The size of the starch grains increased after soaking in water. The length of dry broad bean starch grains ranged from 17.9–22.8 μm. After soaking, the grains averaged 18.5–26.8 μm in length. The width of the dry bean starch granules ranged from 8.3–16.8 μm. After soaking, they averaged 12.5–20.1 μm in width.

Germinated broad beans

Figure 7A, B shows the cut surface of broad bean cotyledons after 4 days of germination. The starch granules were surrounded by proteins. Loss of cytoplasmic matrix in the fractured cotyledon was also seen. The membrane-like materials covering the starch granules in the dry and soaked cotyledons began to show rupture after germination for 4 days. The rupture may be caused by some biochemical changes as a result of enzymatic reactions which were activated during germination. McEwen et al. (1974) stated that probably the membrane-like materials are proteinaceous in nature. At 8 days of germination the cell contents were loosely attached and began to lose its structure and shape. In Figure 8 a starch grain is shown with the cytoplasmic

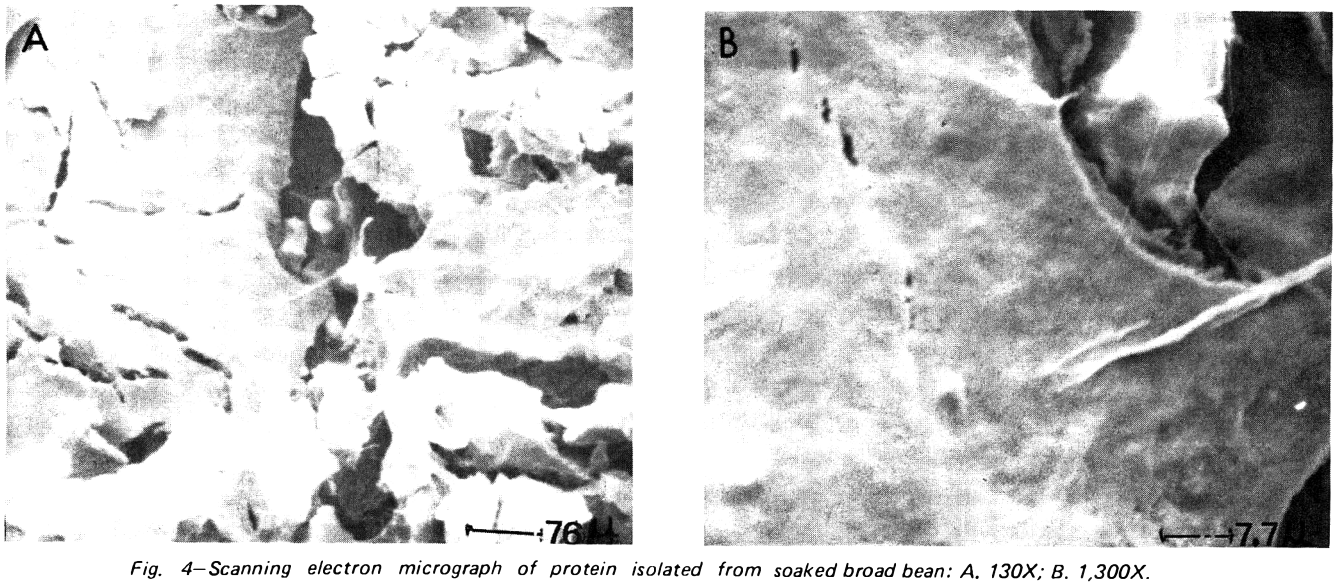


Fig. 4—Scanning electron micrograph of protein isolated from soaked broad bean: A. 130X; B. 1,300X.

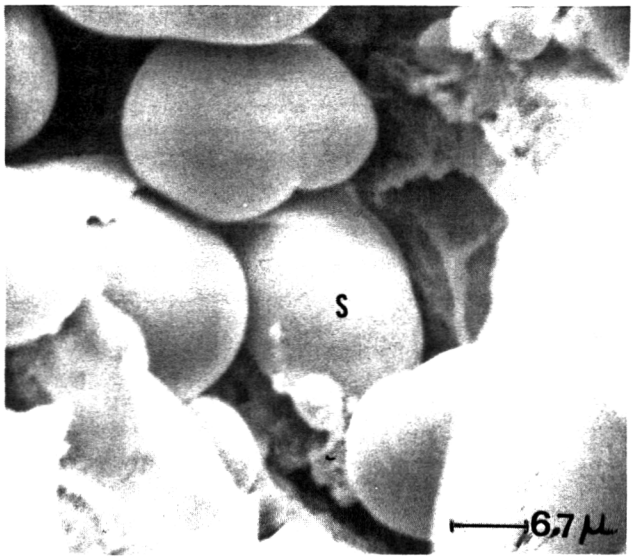


Fig. 5—Starch granules in dry broad beans (1,500X).

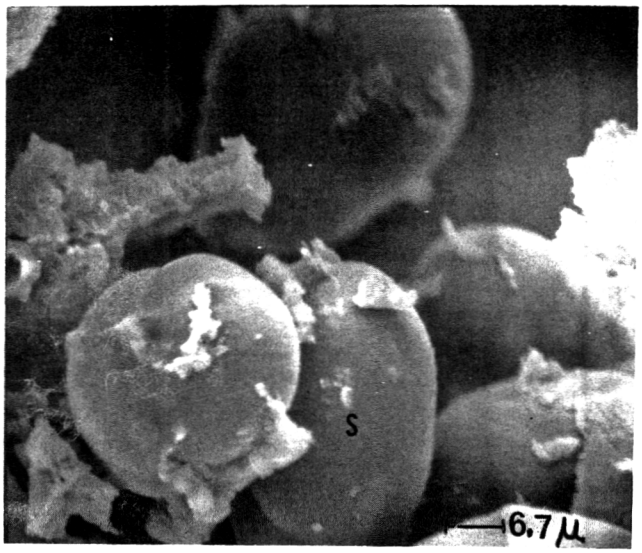


Fig. 6—Scanning electron micrograph of starch (1,500X) isolated from soaked broad bean flour.

residue observable on the exposed cell wall surface. The cytoplasmic residues were smaller at 8 days of germination in comparison with those of the fractured cotyledons of the dry broad beans. In Figure 9 it is shown in high magnification (3,200X) of the cell content of broad beans after germination for 8 days. The surface of starch was attached to a rough cytoplasmic residue. Silva and Luh (1978) revealed changes on the surface of starch granules during germination of red kidney beans, and found that there were distinct transformation of the surface of the granules after 8 days of germination. The starch isolated from the 4-day and 8-day germinated broad bean flour was investigated with SEM. The results are shown in Figures 10 and 11. The starch granules were keeping their integrity in some, while in others they were broken after 8 days of germination. In Figure 11, the irregular fragments were frequently seen with some modifications appeared on the surface of the starch granules after germination for 8 days. At this stage the cell content were easily detached during fracture of the cotyledon (Silva and Luh, 1978).

In summary, starch granules in the cotyledons of broad beans showed visible signs of deterioration when germinated for 8 days. After that, the granules began to fragment. Starch degradation started along specific sites of the granules. After germination for four days the protein matrix began to break up with the loss of cytoplasmic matrix in the fractured cotyledons. The proteins began to break into smaller fragments after germination for 8 days.

Starch

The changes in total starch, amylose and amylopectin in the broad beans during germination are presented in Table 1. There was no significant change in amylose and amylopectin after soaking, but a gradual decrease of of the two

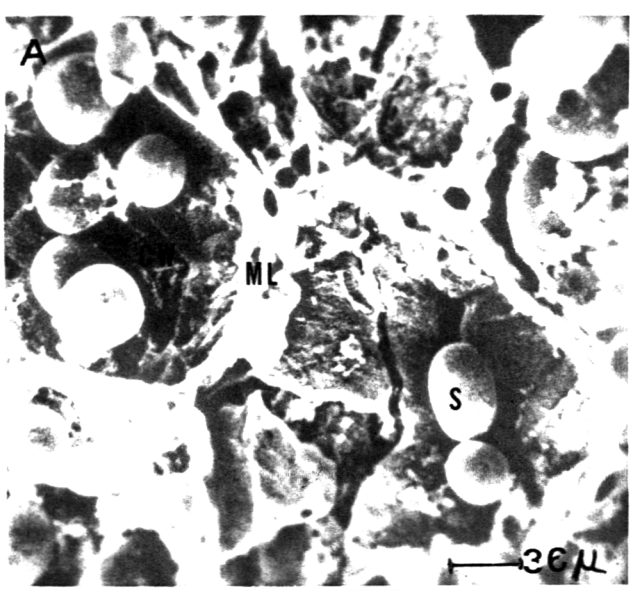


Fig. 7—Cross-section of cotyledon of 4-day germinated broad beans: A. 280X; B. 500X.

starch forms during germination. The ratio of amylopectin to amylose was maintained in a narrow range of 4.76–4.81 during the first two days but increased during the 4–6 day germination period. A more pronounced decrease of amylopectin occurred there after.

Table 1—Changes of starch, amylose, and amylopectin in broad beans during germination at 22° C

Bean samples	Total starch %	Amylose %	Amylopectin %	Amylopectin to amylose ratio
Dry	50.0	8.60	41.4	4.81
Soaked	49.3	8.60	40.7	4.73
2-days	38.0	6.60	31.4	4.76
4-days	34.0	5.40	28.6	5.30
6-days	31.5	3.70	27.8	7.51
8-days	18.8	3.20	15.6	4.88

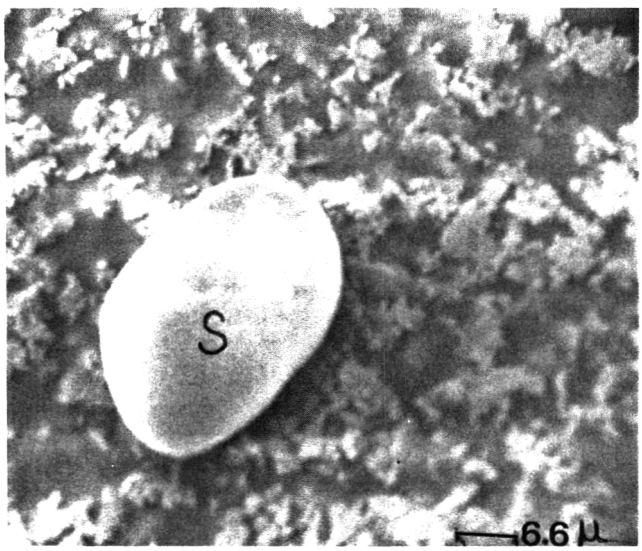


Fig. 8—Broad bean starch granule after 8 days of germination. Note: The cytoplasmic residue in the background (1,500X).

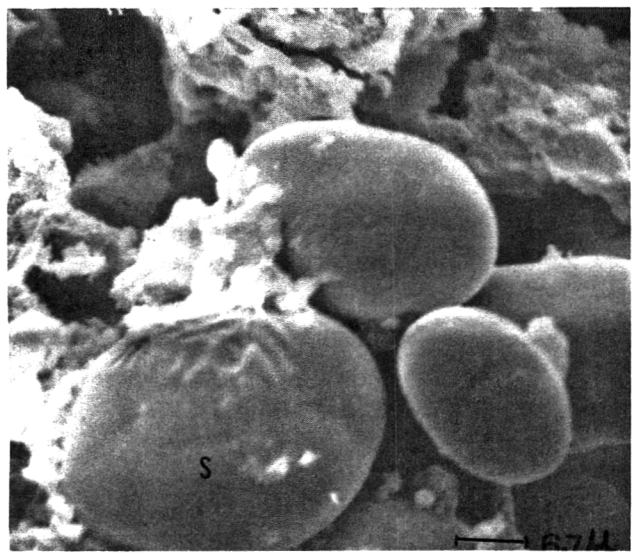


Fig. 10—Scanning electron micrograph for starch granules isolated from broad beans after germination for 4 days (1,500X)

Oligosaccharides

The presence of sucrose and raffinose in dry broad beans was demonstrated (Table 2). There was some loss of sucrose and raffinose after soaking. During the 2–4 day germination period, there was an increase in both sucrose and fruc-

Table 2—Changes of oligosaccharides in broad beans during germination

Bean samples	Sucrose %	Fructose %	Raffinose %
Dry	3.70	0	0.500
Soaked	3.22	0.37	0.375
2-days	6.50	0.05	0.370
4-days	5.00	0.85	0.250
6-days	3.00	0.25	0.125
8-days	1.50	0	0

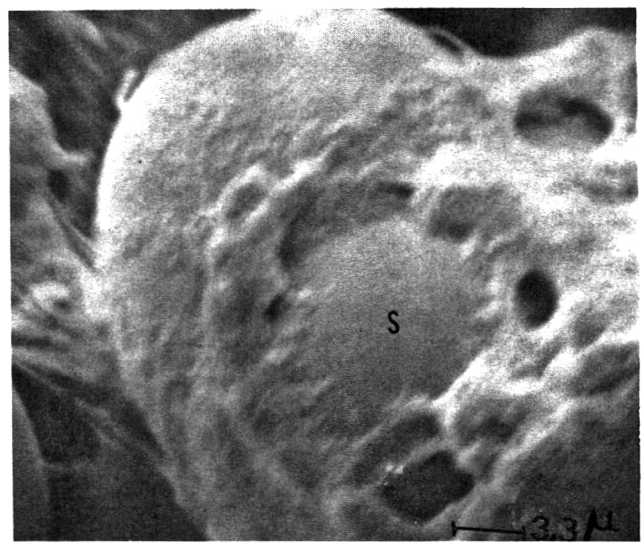


Fig. 9—Broad bean cotyledons after germination for 8 days showing a starch granule with attached cytoplasmic matrix (3,200X).

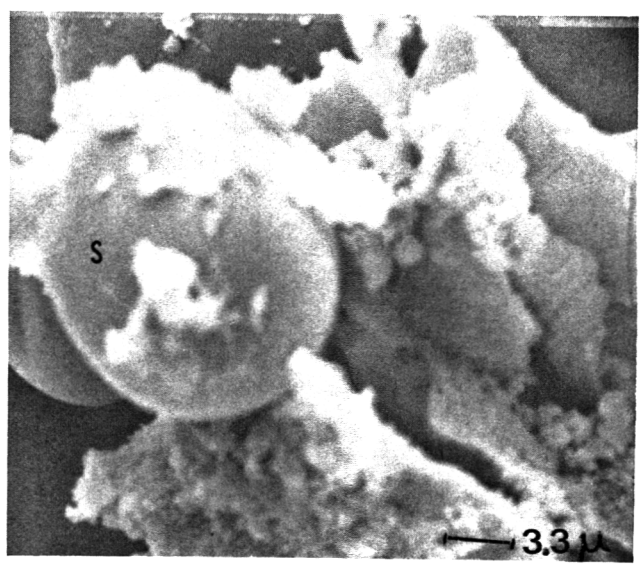


Fig. 11—Scanning electron micrograph for starch granules isolated from broad beans after germination for 8 days (3,000X).

tose. But they decreased thereafter. Raffinose gradually decreased during germination, and was not detectable after germination for 8 days. The disappearance of raffinose from the broad beans after germination was beneficial because it will cause flatulance in humans consuming cooked dry beans (Chen and Luh, 1976; Chen et al., 1975).

The results obtained in this investigation are in agreement with those obtained on black eye and pink beans (Silva and Luh, 1979) although stachyose was not found in broad beans. Young and Varner (1959) stated that during germination, the activity of amylase increased many folds. The amylase activity caused the decrease in starch content during germination. The starch in the storage cells undergo changes to form sucrose and fructose through the action of starch phosphorylase, phosphoglucomutase, and phosphohexoisomerase (Eskin et al., 1971).

Scanning electron microscopy showed alterations affecting the surface and the entire granule of starch in cotyledons of the broad beans germinated for 8 days.

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AMYLASE ACTIVITY IN STERILE SPOILED PUDDING

S. F. BAREFOOT and D. M. ADAMS

ABSTRACT

Ultrahigh temperature sterilized canned puddings occasionally exhibit spoilage in the form of a dramatic loss of viscosity or thinning. Studies on a concentrated fraction of spoiled pudding indicate that thinning was due to a heat-resistant amylase. The concentrate was obtained by centrifugation and ultrafiltration of the thinned pudding. Ultrafiltration characteristics of the active agent indicate a molecular weight of $1-30 \times 10^4$ daltons. The agent attacked starch with a decrease in iodine-binding capability and an increase in reducing units. Treatment of the concentrate with proteolytic enzymes decreased enzymatic activity by 50%; treatment with sodium ethylene diaminetetraacetate decreased activity by 80%. The levels of starch and pH of the spoiled puddings were suitable for action of the amylase, and the enzyme was quite active at normal and elevated room temperatures.

INTRODUCTION

ULTRAHIGH TEMPERATURE (UHT) sterilization processes have been utilized for a variety of food products, including starch-based puddings. These puddings may be exposed to heat treatments of 142–149°C for 10 or more seconds and aseptically filled into sterile containers. These products should have long shelf-life at room temperature. However, some batches of UHT sterilized puddings have been reported to undergo a dramatic loss of viscosity within 2 wk to 6 months. The spoiled puddings were confirmed to be sterile (Denny, 1978) which suggested that they contained an amylase which survived UHT treatment and attacked the modified food starch used as the primary thickening agent.

The existence of extremely heat-stable enzymes has become increasingly apparent in the last several years. Very heat resistant proteases and lipases have been studied in the United States (Adams et al., 1975; Meyerhofer et al., 1973; Malik and Swanson, 1974; Speck and Adams, 1976), Sweden (Bengtsson et al., 1973; Andersson et al., 1979), the Netherlands (Driessen and Stadhouders, 1974), and New Zealand (Richardson and TeWhaiti, 1978). These enzymes have been associated with the spoilage of a variety of UHT sterilized dairy products (West et al., 1978; Richter et al., 1979; Bengtsson et al., 1973; Biryukova et al., 1975). The enzymes are present in the raw products, survive UHT sterilization, and during subsequent storage, spoil the products by attacking the protein and fat components.

Although the heat resistance of amylases at UHT has not been studied, they are recognized as quite heat resistant enzymes (Madson et al., 1973; Miller et al., 1953; Fullbrook, 1976). The objective of this study was to determine if the spoilage or thinning of sterile pudding could have been mediated by amylases that survived the UHT sterilization process.

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MATERIALS & METHODS

Action of amylase on pudding

To determine the suitability of the pudding for amylase action, a sterile culture filtrate of *Bacillus stearothermophilus* was added to sterile unspoiled starch-based pudding and incubated at 37°C. Periodically the enzyme-pudding mixture was examined for visible changes in viscosity and portions were removed for extraction and analysis for reducing sugars. Reducing sugars were extracted according to a method suggested for the separation of starch from confectionary products (AOAC, 1975) except that the original supernatant and washings were retained rather than discarded. Supernatants and washings were pooled, evaporated to dryness on a rotary evaporator, redissolved in water, and analyzed for reducing power by the method of Bernfeld (1955).

Extraction and concentration of amylase in spoiled pudding

Spoiled pudding was aseptically removed from the cans and centrifuged at 15,000 × G for 30 min to remove solids (Fig. 1). Xanthan gum was separated from the supernatant by thin-channel ultrafiltration through a membrane with a nominal exclusion limit of 3×10^5 daltons (Amicon Corp., Lexington, Mass.). The retained xanthan gum was washed by filtration with 1–3 volumes of sterile distilled water. The filtrate and washings were pooled and concentrated to approximately one-tenth the original volume of the pudding by thin-channel ultrafiltration using a membrane with a 1×10^4 dalton nominal exclusion limit. The concentrated extract was sterilized by filtration through a 0.45 μm pore size membrane (Gelman Instrument Co., Ann Arbor, Mich.), aseptically dispensed into sterile tubes, and stored at 0°C. All ultrafiltration procedures were carried out at refrigeration temperatures.

Amylase assay

To measure amylase activity, spoiled pudding extract was combined with an equal volume of starch substrate and incubated at 37°C. Starch substrate was 8% Lintner modified starch (Fisher Scientific Co., Raleigh, N. Car. or Sigma Chemical Co., St. Louis, Mo.)

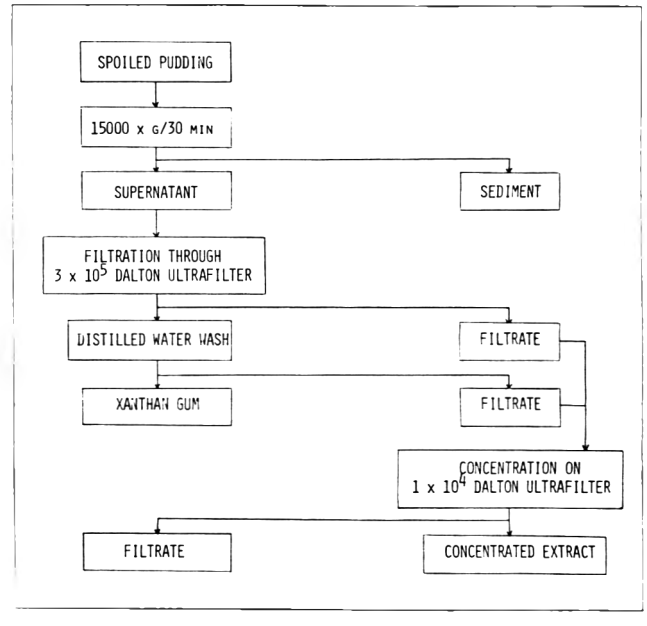

Fig. 1—Preparation of concentrated extract from spoiled pudding.

in 0.08M acetate buffer, pH 7, with 20 mM $CaCl_2$. Amylase activity was measured as a loss of starch reactivity with iodine (dextrinizing power) by the method of Fuwa (1954) as modified by Manning and Campbell (1961) or as an increase in reducing power by the method of Bernfeld (1955). Unless indicated otherwise, loss of starch reactivity with iodine was calculated as the percent decrease in the iodine-binding capacity of the starch substrate during 2 days at 37°C, i.e.,

$$\%\ \text{decrease in } I_2 \text{ binding capacity} = \left[1 - \frac{\text{OD (620 nm) at day 2}}{\text{OD (620 nm) at day 0}}\right] \times 100\%$$

Increase in reducing power was calculated as

$$\frac{\text{OD (540 nm) at day of analysis} - \text{OD (540 nm) at day 0}}{\text{OD (540 nm) at day 0}} \times 100\%$$

Factors influencing the amylolytic activity of the pudding concentrate

Standard assay conditions were 4% starch, 10 mM calcium chloride, and 0.04M acetate buffer, pH 7. To evaluate the requirement for cations, calcium chloride was deleted from the standard assay medium and tetra sodium ethylenediamine tetraacetate (EDTA), pH 7, was incorporated to a final concentration of 100 mM. To evaluate the influence of pH, temperature, and starch concentration on amylase activity, each factor was varied with the others held standard.

In evaluating the effect of starch on activity, the extract was combined with assay substrate containing 0.5–7% starch. Due to the wide range of starch concentrations, amylase activity had to be expressed directly as the decrease in iodine-binding capacity rather than as the percent decrease in iodine-binding capacity, i.e., decrease in OD_{620} = OD_{620} on Day 2 – OD_{620} on Day 0. All OD's were corrected for dilution during assay.

Effect of proteolytic enzymes

To evaluate the effect of proteolytic enzymes on amylase activity, pudding concentrate was pre-incubated 14 hr at 37°C with 11 units Pronase P from *Streptomyces grieseus* (Sigma Chemical Co., St. Louis, Mo.) per milliliter of concentrate. Concentrate was then combined with substrate, incubated at 37°C, and assayed as usual. Activity was compared with that of pudding concentrate preincubated without protease.

RESULTS

THE SUITABILITY of pudding starch and the pudding environment for amylase action was evaluated by adding *Bacillus stearothermophilus* amylase to sterile, unspoiled starch-based vanilla pudding. The pudding exhibited a rapid loss of viscosity and almost a doubling of reducing units in 6 days (Table 1). A measure of dextrinizing power could not be made because of the food colors and the difficulty in separating the modified food starch from the remaining pudding ingredients.

The concentrate obtained by centrifugation and ultrafiltration of spoiled pudding exhibited amylase activity. When mixed with the starch substrate, a complete loss of iodine-binding capacity and more than a doubling in reducing units were observed in 8 days at 37°C (Table 2). The change in iodine-binding capacity or reducing power of the substrate was negligible when sterile water was substituted for pudding extract.

The active agent in the spoiled pudding appeared to be proteinaceous. When crude pudding concentrate was incubated with proteolytic enzymes prior to addition of the assay substrate, amylolytic activity, measured as dextrinizing power, was reduced by about 50% (Table 3).

The optimum temperature for amylolytic activity of the concentrate was 45–55°C (Fig. 2). Activity was 42% of maximum at normal room temperature and below (15–25°C) and 72% of maximum at elevated room temperature (40°C). The concentrate exhibited amylase activity over a pH range 4–9 (Fig. 3). Amylase activity was greatest

Table 1—Increase in reducing power of starch-based pudding inoculated with Bacillus stearothermophilus amylase

Days	% Increase in reducing sugars
0	0
2	37.5
6	96.7

Table 2—Effect of concentrated spoiled-pudding extract on starch

	% Decrease in iodine binding capacity		% Increase in reducing units	
Days	Starch + Extract	Starch + Water	Starch + Extract	Starch + Water
0	0	0	0	0
2	42.5	7.5	39.6	0
4	73.0	0.9	70.0	0
6	90.7	1.2	113.4	0
8	99.7	0.4	114.6	0

Table 3—Effect of proteolytic enzymes on amylase activity of concentrated extract of spoiled pudding

	% Decrease in iodine-binding capacity	
Days	Extract + Starch – Protease	Extract + Starch + Protease
0	0	0
2	80.9	31.7
4	99.8	53.3

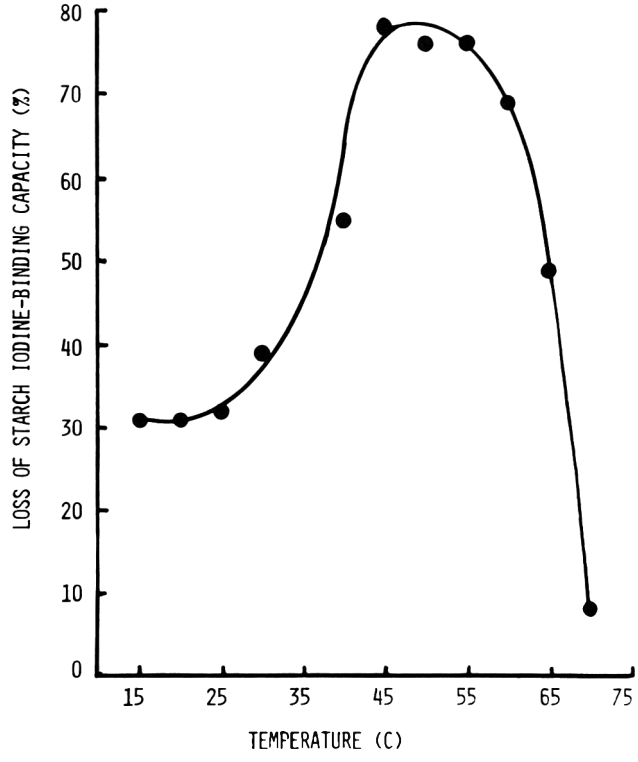

Fig. 2—Effect of temperature on amylase activity in concentrated extract of spoiled pudding.

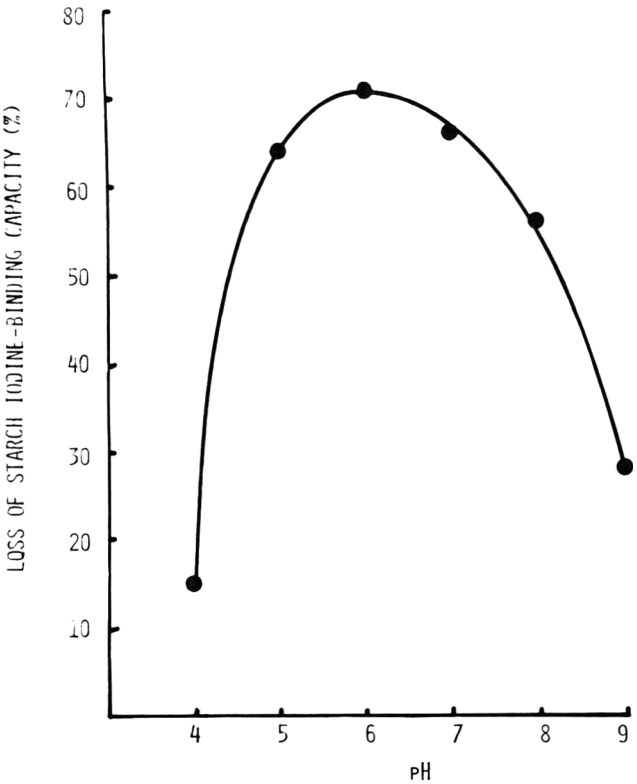

Fig. 3—Effect of pH on amylase activity in concentrated extract of spoiled pudding.

at pH 5–7 with an optimum of pH 6. Activity at pH 4 was low but detectable. Activity of the concentrate appeared to be unaffected by starch concentrations between 1–7% (Table 4).

The amylase in spoiled pudding appears to be a metalloenzyme. The presence of 100 mM EDTA reduced amylolytic activity by about 80% (Table 5). EDTA itself had no effect on the measurement of iodine-binding capacity.

DISCUSSION

THE RESULTS indicate that the spoilage of sterile, starch-based UHT treated puddings was mediated by an amylase. First, the active agent passed through a 3×10^5 dalton ultrafilter and was retained by a 1×10^4 dalton ultrafilter suggesting a molecular weight of between 1×10^4 and 3×10^5 daltons. All amylases studied to date have molecular weights within these limits. α-Amylases have molecular weights of approximately 5×10^4 daltons (Whitaker, 1972; Pfueller and Elliott, 1969; Robty and Whelan, 1968a; Fischer and Stein, 1960). β-Amylases have somewhat higher molecular weights; e.g. 197,000 for sweet potato β-amylase (Spradlin and Thoma, 1970). The molecular weights of amyloglucosidases are also within the $1 \times 10^4 - 3 \times 10^5$ dalton range (Pazur et al., 1962; Pazur, 1964).

Second, the active agent in the pudding affects starch in the manner of an amylase. The action of amylases on starch results in a decrease in the viscosity of their pastes, a reduction of their ability to form a blue color with iodine, i.e. their iodine-binding capacity, an appearance of reducing groups, and an increase in maltose, glucose, or dextrins (Whitaker, 1972). The active agent in the pudding caused the production of reducing power in the starch substrate and the reduction of the iodine-binding capacity of the substrate.

Table 4—Effect of starch concentration on amylase activity in concentrated extract of spoiled pudding

Conc of starch (%)	Decrease in OD_{620} of starch substrate (× 0.05)
0.50	0.365
0.75	0.46
1.0	0.585
2.0	0.585
3.0	0.46
4.0	0.79
5.0	0.45
6.0	0.72
7.0	0.98

Table 5—Effect of EDTA on amylase activity in concentrated extract of spoiled pudding

	% Decrease in iodine-binding capacity	
Days	Extract + Starch − EDTA	Extract + Starch + EDTA
0	0	0
2	98.1	21.6

Third, the active agent in the pudding was proteinaceous. Treatment of the concentrated extract with proteolytic enzymes resulted in a 50% reduction in its activity. The lack of complete loss of amylase activity may be explained by the resistance of native α-amylases to proteolytic attack. Stein and Fischer (1958) found native α-amylases with a full complement of Ca^{++} were stable in the presence of proteolytic enzymes. The effect of EDTA on amylase activity suggests that cations, possibly Ca^{++}, are associated with the active agent.

The pudding provides a good environment for the activity of amylases surviving UHT sterilization. The decrease in viscosity and increase in reducing groups brought about by *B. stearothermophilus* amylase added to unspoiled, starch-based pudding indicated that the modified food starch was susceptible to amylase attack leading to the thinning reported for spoiled, sterile pudding. The optimum temperature of the amylase extracted from the pudding was 45–55°C. At normal room temperature, its activity was 42% of maximum. Sterile pudding is stored unrefrigerated and could be exposed to temperatures more closely approaching 40°C (104°F) at which its activity was 72% of maximum. Moreover, the long storage period of the pudding allows for the activity of even low concentrations of surviving amylases. The amylase had an optimum pH of 6; many of the puddings have pH's of 6–7 (Merritt, 1979). Little enzymatic activity was observed at pH 4. This is consistent with a role for the amylase in the spoilage of UHT puddings. Little or no liquefaction has been reported for UHT-sterilized starch-based lemon puddings which have a pH of 4.2–4.5 (Merritt, 1979). The pudding contains skim milk and nonfat dry milk as major ingredients and magnesium carbonate, magnesium sulfate, and zinc oxide (Table 6) as minor ingredients. The thinning of the puddings suggests that the ingredients provide part or all of the metal ions needed for the activity of the metallo-enzyme. Undoubtedly, the levels of starch in the pudding (approximately 4%) supply adequate substrate for the enzyme. Amylase activity appeared to be independent of starch concentration for those levels that might be used in a pudding.

Table 6—Ingredients in sterile spoiled UHT-treated pudding

(IN ORDER OF APPEARANCE ON LABEL)

FRESH SKIM MILK
SUCROSE
FOOD STARCH-MODIFIED
NONFAT DRY MILK
SODIUM CASEINATE
VEGETABLE FAT
ARTIFICIAL FLAVORS
SODIUM CITRATE
MAGNESIUM CARBONATE
XANTHAN GUM
SALT
MONO AND DIGLYCERIDES
MAGNESIUM SULFATE
SODIUM STEAROYL-2-LACTYLATE
CARRAGEENAN
SODIUM ASCORBATE
ARTIFICIAL COLOR
FERRIC ORTHOPHOSPHATE
α-TOCOPHERYL ACETATE
SODIUM HEXAMETAPHOSPHATE
NIACINAMIDE
ZINC OXIDE
COPPER GLUCONATE
CALCIUM PANTOTHENATE
ASCORBIC ACID
VITAMIN A PALMITATE
PYRIDOXINE HYDROCHLORIDE
RIBOFLAVIN PHOSPHATE
THIAMINE HYDROCHLORIDE
FOLIC ACID
BIOTIN
POTASSIUM IODIDE
VITAMIN D_2
VITAMIN B_{12}

Consequently, making adjustments in the levels of starch does not appear to be a feasible method for controlling amylases in sterile pudding.

The amylase in spoiled pudding appears to be an α-amylase. β-Amylases occur almost exclusively in higher plants, whereas α-amylases are much more widespread (Kulp, 1975; Robyt and Whelan, 1968a).

The amylase was inhibited by EDTA indicating that it is a metallo-enzyme. All α-amylases with the possible exception of *B. lichenformis* amylase (Fullbrook, 1976), contain at least 1 gram-atom of Ca^{++} per mole of enzyme (Fischer and Stein, 1960; Whitaker, 1972). The metal appears to stabilize the enzyme molecule, maintain it in an active conformation (Hsiu et al., 1964; Vallee et al., 1959), and probably is the basis for its heat stability (Stein and Fischer, 1959; Heinen and Lauwers, 1976; Yutani, 1976; Robyt and Whelan, 1968a; Ogasahara et al., 1970). The β-amylases do not require either organic or inorganic cofactors (Kulp, 1975), and neither the β-amylases nor the amyloglucosidases are protected by calcium against heat (Fischer and Stein, 1960; Robyt and Whelan, 1968b).

A pH optimum of 6 also is consistent with the enzyme being an α-amylase. The pH optima reported for α-amylases range from 4.5–7 with the shape of the pH profile unique to the source of the amylase (Robyt and Whelan, 1968a; Greenwood and Milne, 1968). Generally, the pH optima for β-amylases are between 5 and 6 (Robyt and Whelan, 1968b; French, 1960). The pH optima for amyloglucosidases are lower, between 4.0 and 4.5 (Fleming, 1968).

The findings presented here indicate that an extremely heat-stable enzyme(s) is the probable cause of thinning of sterile UHT treated puddings. The source of the enzyme is unknown and is under current investigation. Bacterial α-amylases are more heat-stable than those from other sources (Wallerstein, 1939; Hartman et al., 1955; Wiseman, 1975) suggesting that the enzyme(s) is of bacterial origin. The ability of the enzyme to survive UHT treatments poses a considerable problem to the manufacturers of the puddings or similar products containing starch as the primary thickening agent. Some means for controlling the enzyme(s) must be found—either by keeping the enzyme out of the product or by inactivating the enzyme in the product.

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FUNCTIONAL CHARACTERISTICS OF PROTEIN STABILIZED EMULSIONS: EMULSIFYING BEHAVIOR OF PROTEINS IN A SONIFIER

E. TORNBERG

ABSTRACT

Protein stabilized emulsions made up of 40% soybean oil by weight and protein dispersions of 2.5% (w/w) protein content have been prepared in an ultrasonic device. The emulsifying apparatus was incorporated into a recirculating system, where power input and number of passes were varied. The food proteins studied were a soy bean protein isolate, a whey protein concentrate (WPC), and a sodium caseinate. The emulsions obtained were characterized in terms of droplet size distribution and amount of protein adsorbed per unit fat surface area (protein load). The results were compared to the fat surface area and the protein load of emulsions made in different ways in a valve homogenizer. Flocculated emulsions, such as the types stabilized with soy protein and WPC (0.2–7), produced either in a sonifier or in a valve homogenizer, have larger fat surface areas and broader size distributions than nonflocculating systems. In the sonifier, overprocessing in terms of fat surface area occurs at high power inputs for all protein stabilized emulsions. Sonified emulsions have smaller droplet size and a broader spectrum of globule size than valve homogenized emulsions made with the same power input respectively, and number of passes. The protein load is largely determined by the protein/fat surface area ratio and by the type of protein used as an emulsifier, irrespective of the emulsifying conditions (emulsifying apparatus, intensity, and time). But the latter are dominating with regard to the final droplet size distribution of the emulsions, where the choice of protein is of minor importance. The relation between percentage protein adsorbed from the bulk phase as a function of the fat surface area suggests that the caseinates are mainly adsorbed from the bulk phase to the newly created interface up to a surface area of 8–9 m^2/ml emulsion. For the soy protein (0–7) and WPC (0.2–7) stabilized emulsions spreading of already adsorbed molecules becomes more favorable than adsorption of proteins from the bulk at fat surface areas as low as ≈ 3 m^2/ml emulsion. This change in the way of covering the interface occurs for WPC (0–7) stabilized emulsions at surface areas of about 4 m^2/ml emulsion.

INTRODUCTION

FUNCTIONAL PROPERTIES of proteins can be used to define how proteins can be supplemented into existing foods, and how they can replace more expensive proteins in traditional foods. One of these properties, an evaluation of proteins as emulsifiers, is of great importance. In a series of publications (Tornberg and Hermansson, 1977; Tornberg and Lundh, 1978; Tornberg 1978a, b) named functional characterization of protein stabilized emulsions, attempts have been made to understand the emulsifying properties of proteins. In the first paper (Tornberg and Hermansson, 1977) the severe effect of processing parameters, such as different emulsifying equipment, emulsifying time and intensity, on the properties of protein stabilized emulsions was shown. This led to the set up of a standardized emulsifying system (Tornberg and Lundh, 1978), where flow conditions, power and energy input are controlled and measured. Three emulsifying apparatus, such as a turbomixer, a valve homogenizer, and an ultrasonic device, were compared with regard to different creaming stabilities of protein stabilized emulsions (Tornberg, 1978a). But for evaluating the emulsifying characteristics of a protein product on a more general basis, additional methods of characterizing the emulsions formed are needed. Therefore, the drop size distribution and the amount of protein adsorbed on to the fat surface (protein load) of protein stabilized emulsions made in a valve homogenizer have been measured (Tornberg, 1978b). This was done as a function of power input and number of passes. The results from a similar study, using a sonifier instead as emulsifying apparatus, are presented in this paper. The results obtained from emulsification with both the apparatus are compared, and from the results a more general pattern has been deduced for selecting proteins as emulsifiers. Three protein products have been used as emulsion stabilizers: a soy protein isolate, a sodium caseinate, and a whey protein concentrate (WPC).

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MATERIALS & METHODS

Proteins

The proteins soy protein isolate, sodium caseinate, and whey protein concentrate used throughout this study were suspended in distilled water and 0.2M NaCl at pH 7 (denoted as (0–7) and (0.2–7), respectively). The source, protein content and solubility in (0–7) and (0.2–7) are given fully elsewhere (Tornberg, 1978b).

Soybean oil

A commercially available soybean oil (AB Karlshamns Oljefabriker, Karlshamn, Sweden) was used. Analysis: fatty acid composition 18:2 54%, 18:1 23%, 16:0 10%, 18:3 8%; FFA 0.02% (w/w) (average molecular weight = 282). The amount of monoglycerides was so small as to be undetectable with thin-layer chromatography.

Preparation of samples

Protein dispersions (2.5% (w/w), based on the protein content) in distilled water or sodium chloride solution were made with the Sorvall omni-mixer. The pH was adjusted with 0.2M NaOH or 0.2M HCl. Soybean oil was added directly to the protein dispersion to attain 40% oil by weight.

Emulsion formation

A quantity of 50g of the oil and the protein dispersion was emulsified in a recirculating system as earlier described (Tornberg and Lundh, 1978). The emulsifying apparatus used was a Branson Sonifier Cell Disruptor model B-12 (Branson Sonic Power Co.). The description of the apparatus and the measurement of the power and energy input have been given in a previous paper (Tornberg and Lundh, 1978). The distance from the tip of the ultrasonic horn to the outlet of the continuous flow attachment was set to 0.8 mm. The flow rate of the emulsion through the recirculating system was held constant at 250 ± 25 ml min^{-1}, giving an average residence time of an emulsion droplet during one passage, t_p, of 12.5 ± 0.4 sec. "Pre-emulsified" emulsions were recirculated through the system at maximum speed of the motor, i.e. 1050 ml min^{-1} for 30 sec prior to sonification.

Emulsions were held at 25 ± 2°C by chilling during the emulsification process. 30g of the emulsion was stored for 24 hr after formation at 20°C prior to drop size and protein load determination.

Particle size determination

A spectroturbidimetric method for drop size determination was used in this study, as it offers a rapid, easy and reproducible method of analysis. The method is thoroughly described elsewhere (Walstra, 1965, 1968), and for its application on soybean oil-water emulsions

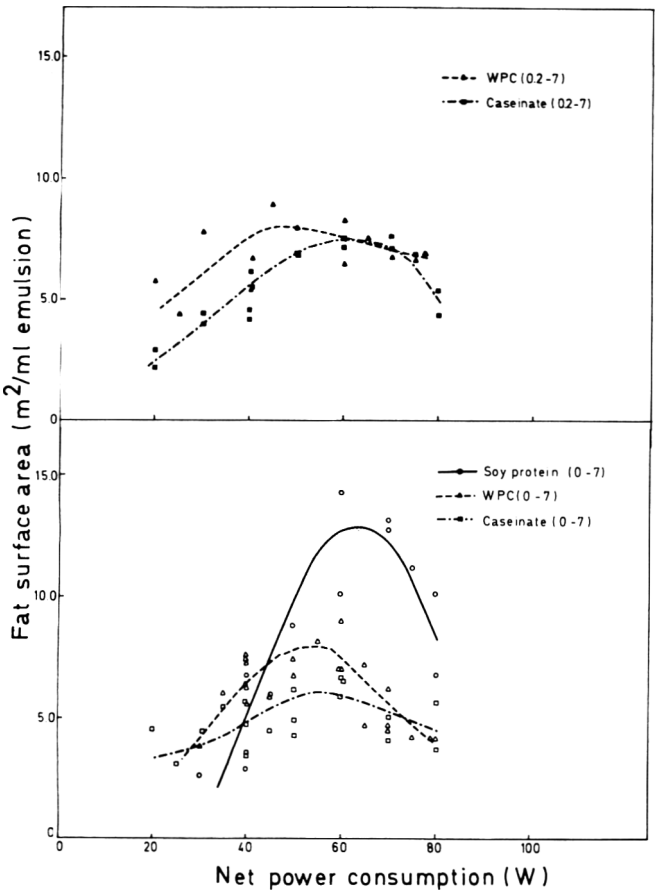

Fig. 1—Fat surface area of protein stabilized emulsions as a function of net power consumption after emulsification with a sonifier, without pre-emulsification, using 10 passes through the recirculating system.

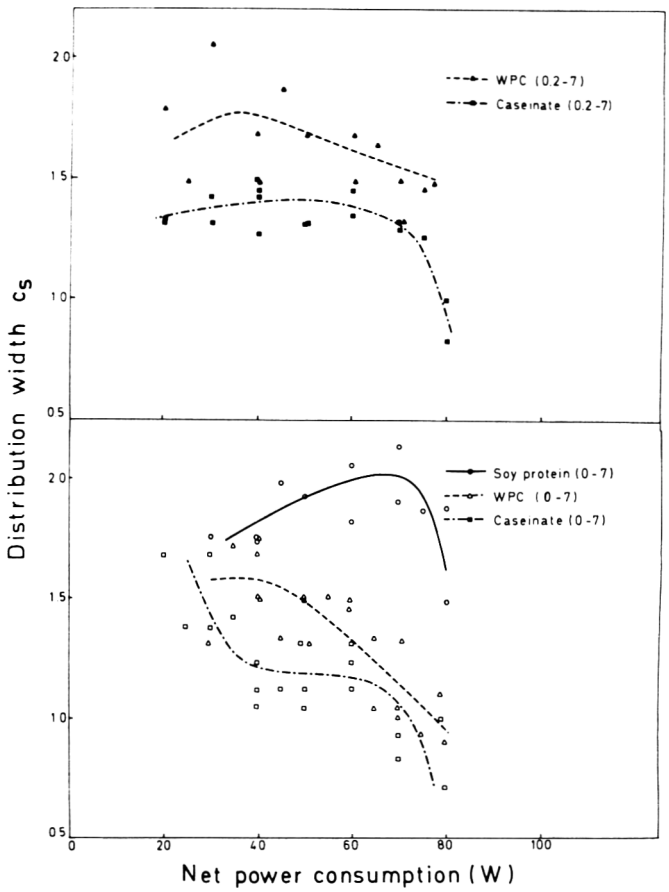

Fig. 2—Distribution width, c_s, of the protein stabilized emulsions as a function of net power consumption, on emulsification with a sonifier, without pre-emulsification, using 10 passes through the recirculating system.

stabilized by proteins the reader is referred to a previous paper (Tornberg, 1978b).

The optical density, E, of the diluted emulsions is measured as a function of the wavelength, λ, between 0.38 and 1.70 μm. With these data and the knowledge of the volume fraction of the disperse phase (ϕ), optical path length (ℓ) and the refractive indices of the continuous (n_2) and the disperse phase (n_1) the reduced turbidity, Z, can be calculated. Z is plotted against log $[2\pi (n_1 - n_2)]/\lambda$ and the experimentally derived curve is compared with theoretical spectra, representing a wide range of size distributions, being log normal and truncated log normal. The theoretical curve of best fit gives a good approximation of the spread given as c_s, which is the relative standard deviation of a surface-weighted distribution. From the shift of abscissa the logarithm of the average globule size is obtained, which is the arithmetic mean of the surface-weighted distribution, denoted as d_{vs}. This mean value is directly related to the specific surface area, A, by the following formula:

$$A = \frac{6\phi}{d_{vs}} \quad m^2 \text{ fat/ml emulsion}$$

where ϕ is the oil volume fraction. The mathematical background of d_{vs} and c_s have been given by Walstra et al. (1969). The degree of fit has been ranked in a scale from 1 to 10, where the degree of fit increases with higher scores. The average value obtained is 7.1 ± 1.8 for all the sonified emulsions, except for the soy protein (0–7) stabilized emulsions, which had a degree of fit of 3.9 ± 1.3. The curves which could not be fitted to any theoretical spectrum, formed, on average, 13% of the total, which is a markedly higher number than 5% for the valve homogenized emulsions. The reproducibility of the measurements is good (≈ 1%), but the major source of error lies in the difficulty of fitting the theoretical spectra to the experimental data, expecially in the case of sonified emulsions, as the size distributions are mostly wide and d_{vs} is very small.

Before measuring the optical density of the diluted emulsions, eventually occurring flocs must be disintegrated into single globules. Because flocculation of the emulsions depends on the emulsifying protein and on the emulsification conditions, the type and amount of disintegrating agent has to be found individually for every protein stabilized emulsion made in different ways in the ultrasonic device. In the case of the caseinate emulsions, i.e. caseinate (0.2–7) and (0–7), which produce almost nonflocculated emulsions, it was sufficient to dilute the emulsions according to the recommended concentrations of solution A as a function of the concentration of the disperse phase, according to Walstra (1965). The WPC (0–7) stabilized emulsions needed a concentration of the dispersing agent twice that of solution A. The more flocculated WPC (0.2–7) stabilized emulsions needed solutions of SDS as a dispersing agent, with concentrations ranging from 0.02–0.03%, depending on the emulsification conditions. The soy protein (0–7) stabilized emulsions were the most flocculated emulsions formed, and were consequently dispersed in 0.12–0.20% solutions of SDS, to which a few drops of a 10% solution of β-mercapto-ethanol was added. The dispersing solution for each emulsion was found by checking in the microscope the lowest level of dispersing agent at which the flocs were just broken. This was done in order to avoid redispersion or coalescence occurring, due to the presence of excess dispersing agent.

Protein load determination

The amount of protein adsorbed per unit area of fat surface (protein load) was obtained by subtracting the residual protein in the bulk phase after emulsification from the original protein content, and dividing that value by the amount of interfacial area created. Discussion of the most suitable separation technique between the two phases is given elsewhere (Tornberg, 1978b).

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The following procedure was used for the sonified emulsions. All the proteistabilized emulsions were centrifuged at 40,000 × G for 30 min, the lower water phase was sucked off with a syringe and compared in absorbance with the original protein solution centrifuged at the same speed and with the same protein content. If the lower water enriched phase was more turbid than the original protein solution, this was taken as an indication of fat droplets occurring in the water phase, in which case the water enriched phase was diluted thirtyfold in three equal stages, and filtered either through a Millipore filter of 0.22 μm (GSWP) or, if still turbid, through a Millipore filter of 0.10 μm (VSWP). Whereas soy protein (0–7) stabilized emulsions did not need any filtration, it was found necessary for the other protein stabilized emulsions. A 0.22 μm filter was needed for the 'pre-emulsified' emulsions, and a 0.10 μm filter was needed, when the emulsions were not 'pre-emulsified.' The determination of the protein content in the aqueous phase was made by analyzing for nitrogen by the general Kjeldahl procedure with an accuracy of ± 0.5%.

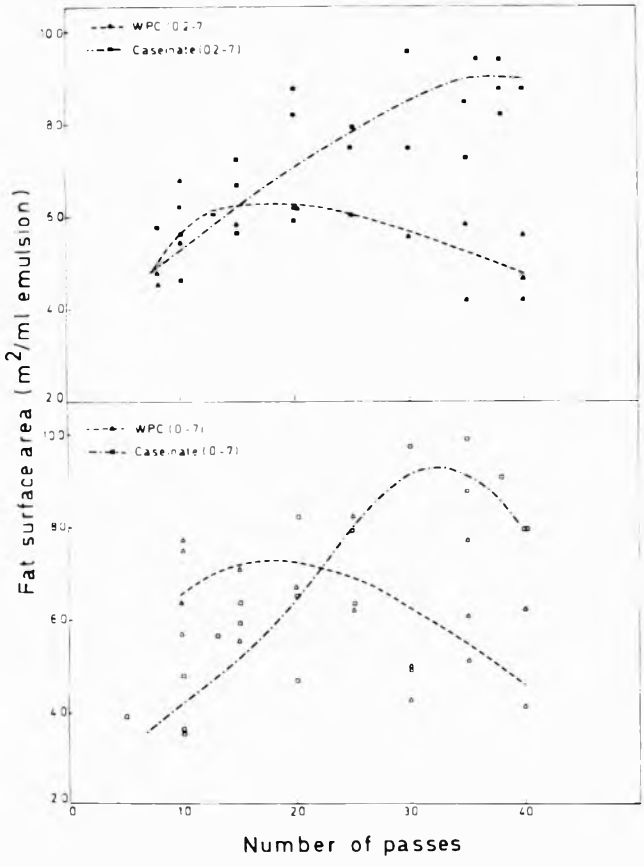

Fig. 3—Fat surface area of protein stabilized emulsions as a function of number of passes after sonication, without pre-emulsification, at a constant power supply of 40W.

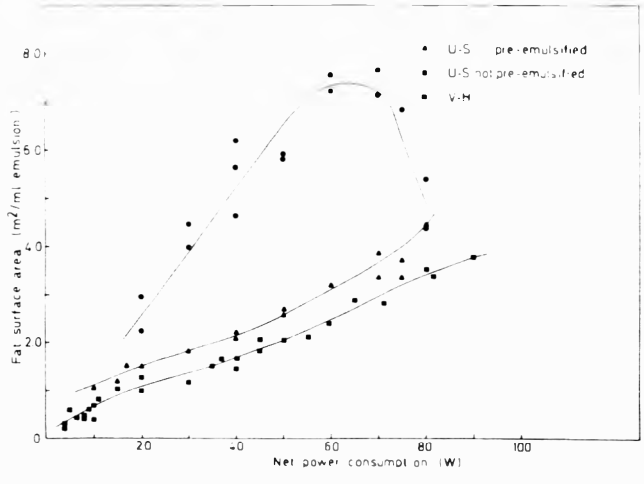

Fig. 4—Fat surface area of caseinate (0.2–7) stabilized emulsions as a function of net power consumption, on emulsification with a valve homogenizer and a sonifier with and without pre-emulsification, using 10 passes through the recirculating system.

Coalescence of fat globules, obvious as free fat, was not observed after centrifugation for any of the protein stabilized emulsions. Errors due to desorption of membrane material and/or sedimentation of protein particles during centrifugation have been estimated and are discussed elsewhere (Tornberg, 1978b).

Filtration of the lower water phase, in the case where fat globules are present, can introduce errors such as protein being retained on the filter. To estimate the maximum error, the protein retention was measured for protein dispersions of 2.5% (w/w) protein content, which were centrifuged, diluted thirtyfold in three equal stages, and filtered through 0.22 μm and 0.10 μm Millipore filters. The retention of protein on the 0.22 μm filter could vary from 1–5% of the protein filtered, whereas this variation lays between 6 and 11% for filtration through a 0.10 μm filter.

RESULTS

Comparison between sonified emulsions stabilized by different proteins

In Figures 1 and 2 the fat surface area, A, and distribution width, c_s, obtained with 10 passes are plotted versus power input during sonication, without 'pre-emulsification.' This is done for all the protein stabilized emulsions, except for the soy protein (0.2–7) stabilized emulsions. They became too viscous to be recirculated in the emulsification system. The reproducibility of A and c_s is relatively poor, but this is also the experience of an earlier investigator (Walstra, 1969). Due to the scarce reproducibility only general trends can be interpreted from Figures 1 to 3. It is seen from Figure 1 that all the curves have a similar pattern with a maximum in fat surface area in the medium power range. The decrease in fat surface area at the highest power inputs is called overprocessing. This is most pronounced for the most flocculated emulsions stabilized by soy protein (0–7). Using this protein as an emulsifier, emulsions of largest fat surface areas and broadest size distributions are produced according to Figures 1 and 2. This behavior is in agreement with the trends obtained using a valve homogenizer (Tornberg, 1978b). In these experiments it was shown that the more flocculated emulsions, such as those stabilized by soy protein (0–7) and WPC (0.2–7), have larger fat surface areas and braoder size distributions than the more or less non-flocculating emulsions stabilized by WPC (0–7), caseinate (0.2–7), and caseinate (0–7). Figure 2 shows that the size distribution spectrum has a tendency to be independent of power consumption at low power inputs and tends to narrow as a function of power consumption at high power inputs.

Figure 3 gives the fat surface area of the WPC and caseinate stabilized emulsions as a function of number of passes when sonified, without 'pre-emulsification,' at a power consumption of 40W. The results obtained for the soy protein (0–7) stabilized emulsions are not given in the figure, due to the wide scatter. Distribution width as a function of number of passes for the emulsions given in Figure 3 are not illustrated, but the pattern for the four types of emulsions is rather similar giving a decrease of c_s from 1.6 to 1.0 for increased number of passes. On the contrary the fat surface area-passes-dependence for the WPC and the caseinate-stabilized emulsions is not similar, as shown in Figure 3. The former proteins produce emulsions with areas, which are rather independent of passes at low numbers and de-

crease slightly at high numbers. However, the caseinates form emulsions, where the surface area increases almost linearly with number of passes with a slight overprocessing at the highest number of passes.

Sonifier and valve homogenizer, a comparison in emulsifying efficiency

To illustrate the difference in droplet size and distribution width of the emulsions formed in the sonifier with those obtained in the valve homogenizer, the caseinate (0.2–7) stabilized emulsions were chosen as an example. In Figures 4 and 5 the fat surface area, A, and the distribution width, c_s, respectively, of caseinate (0.2–7) stabilized emulsions made with both the sonifier and the valve homogenizer are presented as a function of power consumption. The divergence in fat surface area obtained between the pre- and non pre-emulsified sonicated emulsions (see Emulsion formation) is larger than that between those emulsions emulsified in the valve homogenizer and in the sonifier with pre-emulsification. These results really demonstrate the irreversibility of the process, and confirm how important the emulsifying procedure is in governing the properties of the emulsion formed (Tornberg and Hermansson, 1977). This is an observation to underline, because pre-emulsification is often performed industrially, where pre-emulsifying procedure cannot give a higher emulsifying efficiency (using a sonifier) in terms of droplet size.

According to Figures 4 and 5 sonication gives smaller fat globules and wider size distributions than valve homogenization with the same power input. This is in accordance with the interpretations made from the studies of the creaming behavior of differently emulsified protein stabilized emulsions (Tornberg, 1978a).

In Figures 6 and 7 the fat surface area and the droplet size distribution width of caseinate (0.2–7) stabilized emulsions made with different emulsifying apparatus are plotted against number of passes at a power supply of 40W. It can be seen from Figure 6 that by increasing the number of passes the fat surface area of the sonicated emulsions enlarges, especially in the case of non pre-emulsified emulsions. For the valve homogenized emulsions independence of number of passes within the limits of error can be deduced from the figure, which is in accordance with the results obtained by other investigators (Mulder and Walstra, 1974; Walstra and Oortwijn, 1975). Increasing the number of passes causes a remarkable decrease in the spread of globule size for valve homogenized emulsions, which is shown in Figure 7. By assuming that smaller globule size generally decreases creaming rate, the difference in emulsifying behavior of the two apparatus obtained in this study seems to be in accordance with the observations made regarding the creaming stability of the present protein stabilized emulsions (Tornberg, 1978a). Generally, the results of these last mentioned studies seemed to show that it was more benefical to increase the power input rather than number of passes in order to enhance the creaming stability of emulsions, using a valve homogenizer. For the sonifier a higher number of passes was as effective as an increase in power input. The results presented in this study do not contradict these findings.

Protein load and percentage protein adsorbed as a function of fat surface area of emulsions made with different procedures

The amount of protein adsorbed in mg per m^2 fat surface area (protein load) and the percentage protein adsorbed from bulk are plotted against the fat surface area created in Figures 8 and 9, respectively, for all the protein stabilized emulsions. The results obtained by variation of both power supply (at constant number of passes of 10) and number of passes (at constant power supply of 40W)

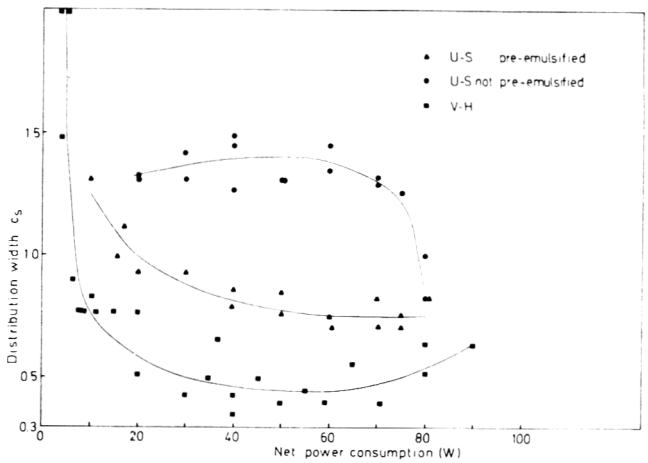

Fig. 5—Distribution width, c_s, of caseinate (0.2–7) stabilized emulsions as a function of net power consumption, on emulsification with a valve homogenizer and a sonifier with and without pre-emulsification using 10 passes through the recirculating system.

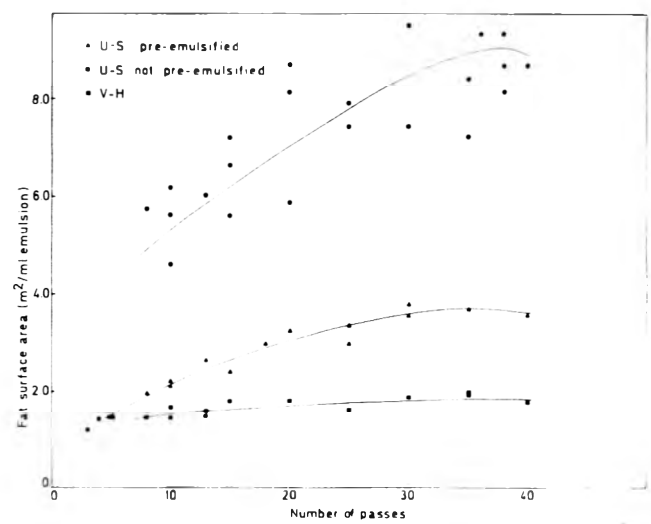

Fig. 6—Fat surface area of caseinate (0.2–7) stabilized emulsions as a function of number of passes, on emulsification with a valve homogenizer and a sonifier with and without pre-emulsification at a constant power supply of 40W.

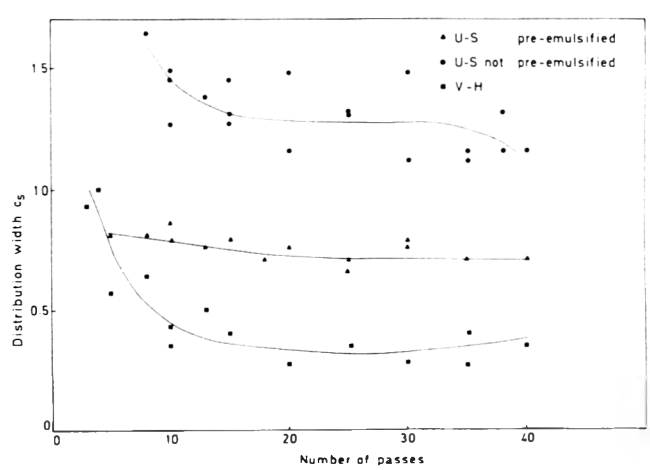

Fig. 7—Distribution width, c_s, of caseinate (0.2–7) stabilized emulsions as a function of number of passes, on emulsification with a valve homogenizer and a sonifier with and without pre-emulsification at a constant power supply of 40W.

for valve homogenization and sonication are included in the figures. The most striking feature to be observed both in Figures 8 and 9 is that for every type of protein stabilized emulsion all the results obtained with few exceptions can be fitted into one curve. The exceptions are mainly represented by the caseinate (0–7) and caseinate (0.2–7) stabilized emulsions, when varying the number of passes in the valve homogenizer, and when overprocessing occurs in the sonifier (marked with arrows in the figures). Otherwise it can be stated that the properties of the protein membrane formed is mainly governed by type of protein used as emulsifier and by the fat surface area created, or more correctly expressed the protein/fat surface area ratio. The overwhelming evidence in Figures 8 and 9 shows that the emulsifying conditions, i.e. the emulsifying equipment, power consumption and number of passes, is of minor importance in controlling the formation of the interfacial film. But these variables are of course very important in controlling the amount of fat surface area created, as suggested by all the former figures.

From Figure 8 it can be seen that at very small surface areas below 1.0 m^2/ml emulsion, the soy protein and WPC stabilized emulsions have the highest protein load. But as the surface area increases the interfacial film thickness diminishes drastically. For larger surface areas than ≈ 3 m^2/ml emulsion the protein load for the soy protein (0–7) and WPC (0.2–7) stabilized emulsions is almost equal and also the least of all the protein stabilized emulsions. For these two latter proteins there is a continuous increase of percentage protein adsorbed from the bulk as surface area expands up to about 35% at 3 m^2/ml emulsion, whereafter it levels off and is independent of surface area created (Fig. 9). For the whey proteins dispersed in distilled water a higher maximal percentage adsorbed (≈ 50%) is obtained according to Figure 9 than in 0.2M NaCl dispersion. The percentage protein adsorbed for WPC (0–7) stabilized emulsions does not start to level off until a surface area of 4 m^2/ml emulsion is obtained. The caseinates show a behavior, where the independence of fat surface area for the percentage protein adsorbed starts at large fat surface areas as 8–9 m^2/ml emulsion. According to Figure 9, this gives the caseinates the highest protein load of all the protein stabilized emulsions.

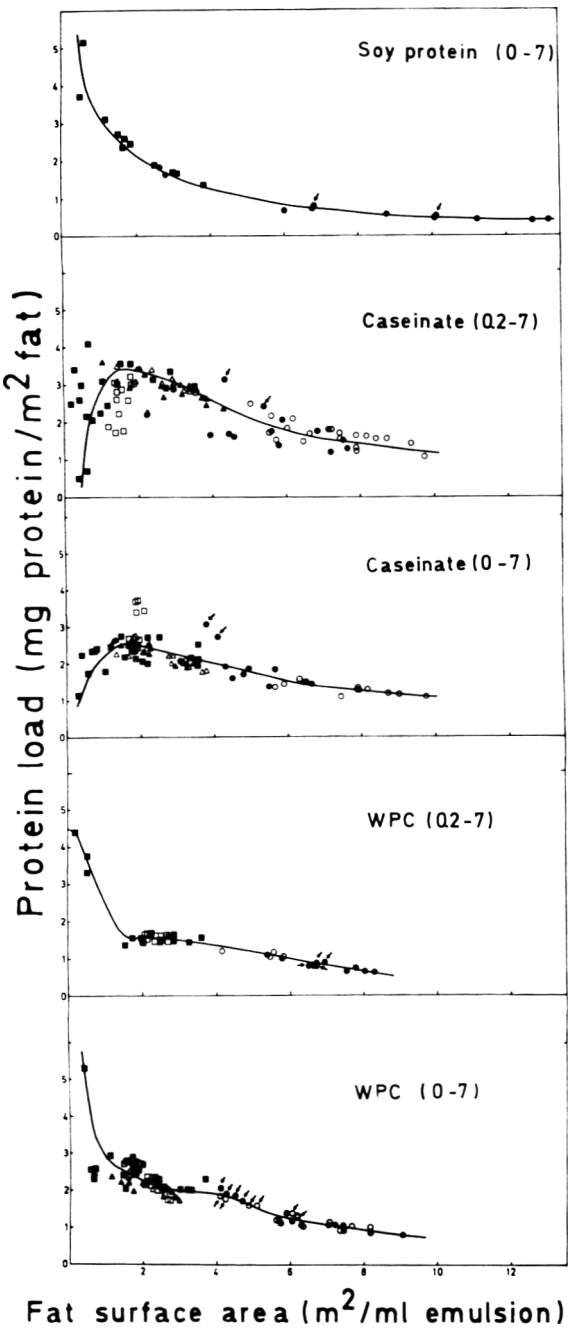

Fig. 8—Protein load of the protein stabilized emulsions as a function of the fat surface area created, on emulsification with a valve homogenizer (■ □) and a sonifier with (▲ Δ) and without pre-emulsification (● ○) and with variation of both power supply (filled symbols) and number of passes (unfilled symbols).

DISCUSSION

EMULSIFICATION is a dynamic process involving disrup-

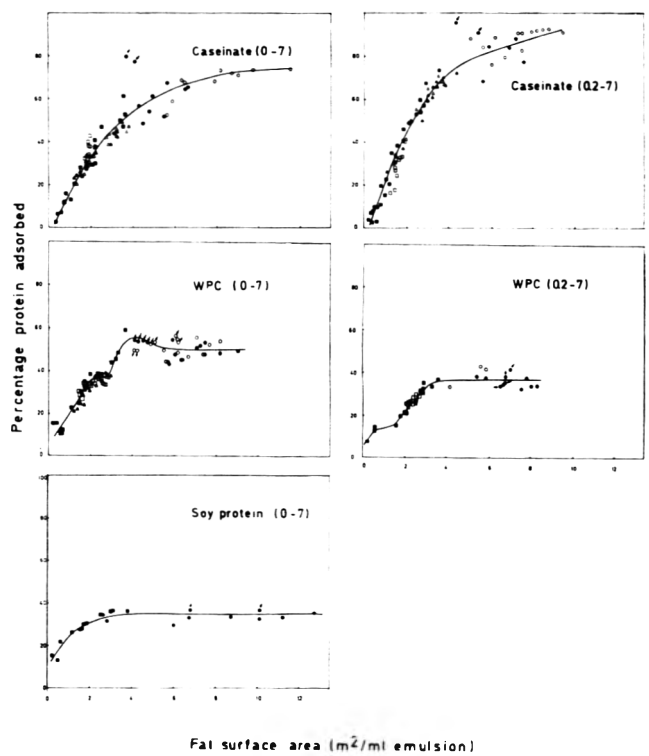

Fig. 9—Percentage protein adsorbed from the bulk phase to the interface of the protein stabilized emulsions as a function of the fat surface area created, on emulsification with a valve homogenizer (■ □) and a sonifier with (▲ Δ) and without pre-emulsification (● ○) and with variation of both power supply (filled symbols) and number of passes (unfilled symbols).

tion and recombination or coalescence of fat globules. Thus, the final droplet size distribution and other properties of the emulsion formed will be governed by the detailed conditions of the balance between disruption and coalescence during emulsification. The probability for the newly created droplets not to coalesce depends on the time available for the interfaces to be covered by proteins in such a way that coalescence is prevented. This time is of course determined by the probability of encounter between droplets, which in turn depends on the fat content of the emulsion and on the emulsifying intensity and time (emulsifying apparatus, power and energy consumption). It is also determined by the rate at which protein molecules cover the interface, either by supply from the bulk or by spreading or unfolding of already adsorbed molecules at the interface. The rate of supply of protein molecules from the bulk depends on the number of collisions of protein molecules with fat particles per unit volume and time (protein/fat surface area ratio and emulsifying conditions), on the adsorption barrier of the film, and on the probability of the protein molecule adsorbing after colliding with the interface (molecular properties such as hydrophobicity, flexibility and charge density). The rate of spreading of an already adsorbed protein molecule will depend on its flexibility on the surface, charge density and on the coverage of protein at the interface. With this reasoning in mind an attempt to elucidate some of the mechanisms inherent in the results given in Figures 1 to 9 has been made.

In Figure 4 there is an almost linear dependence of power consumption for the fat surface area of valve homogenized and sonified, pre-emulsified emulsions, whereas overprocessing occurs at power inputs higher than 65W for sonication without pre-emulsification. This corresponds in Figure 5 for the valve homogenized emulsions to an abrupt decrease in c_s up to around 20W, whereafter it levels off. The emulsions, which are sonicated but pre-emulsified, show a rather similar c_s-power-pattern to the valve homogenized ones, though having broader droplet size distributions and a flatter curve. For sonicated emulsions without pre-emulsification c_s is more or less independent of power supply lower than 65W but decreases abruptly, as overprocessing occurs. These differences in fat surface area and distribution width obtained for emulsions made in differing ways seem to suggest the following mechanism.

The abrupt decrease of c_s with simultaneous increase in fat surface area up to $\approx$ 20W for valve homogenized emulsions, suggest that the final droplet size is mostly determined by the splitting process, as larger particles are more liable to disrupt than smaller ones. The independence of c_s of power supplies higher than 20W indicates that coalescence then starts to be the dominant factor governing the final droplet size of the emulsion. The flatter curve in Figure 5 obtained for sonified and pre-emulsified emulsions suggests that coalescence appears as a determining factor in the emulsification process at lower power consumptions when sonicated, than when valve homogenized. The existence of overprocessing at the highest power inputs for the sonified emulsions, might be explained as a very large fat surface area/protein ratio initially created. This reduces the rate of protein adsorption, leading to coalescence before enough protein has been adsorbed at the interface. Therefore larger droplets and a smaller final fat surface area are formed than at somewhat lower power consumptions. The existence of this type of coalescence mechanism for overprocessing is further substantiated by the results which show that the protein load and the percentage adsorbed protein are generally higher for the same protein/fat surface area ratio. This can be seen in Figures 8 and 9, where results obtained for overprocessed emulsions are marked with arrows. It is evident for both the caseinates, when used as emulsifiers, whereas it is not significant for the WPC and the soy protein stabilized emulsions. Eventually, the hunch visible in Figure 9 for the WPC (0–7) stabilized emulsions might be attributed to increased adsorption, due to coalescence caused by overprocessing. Both Lankveld and Lyklema (1972) and Oortwijn and Walstra (1979) have shown that coalescence favors high protein load.

Figure 9, where percentage protein adsorbed is plotted against fat surface area, is so constructed that one can determine whether supply of molecules from the bulk or spreading of already adsorbed molecules at the interface is the dominant way of covering the interface. It is assumed that when the percentage protein adsorbed increases as a function of fat surface area, covering of the fat surface area is mostly carried out by a supply of proteins from the bulk. When, however, the percentage protein adsorbed becomes more or less independent of fat surface area, spreading of already adsorbed molecules is preferentially performed in order to cover the created interface.

At small surface areas, i.e. when the protein/fat surface area ratio is high, adsorption of proteins mainly from the bulk is obtained for all the protein stabilized emulsions, as revealed by Figure 9. However, as the fat surface area expands, the protein/fat surface area ratio diminishes and the rate of supply of molecules from the bulk decreases until spreading of molecules at the interface becomes more rapid. For WPC (0.2–7) and soy protein (0–7) stabilized emulsions this happens at a surface area as low as ≈ 3 m^2/ml emulsion. Spreading does not become the dominant way of covering the interface until a surface area of ≈ 4 m^2/ml emulsion for WPC (0–7) stabilized emulsions, whereas for the caseinate stabilized emulsions this does not occur until as large fat surface area as 8–9 m^2/ml emulsion. Evidently, these types ofcurves give a characteristic property of the protein itself as an emulsifier.

During the emulsification process the fat surface area enlarges and the bulk phase becomes depleted of proteins, as the process proceeds. This slows down adsorption of molecules from the bulk and favors spreading of already adsorbed proteins. With an initial protein content of 2.5% (w/w) in the bulk phase for the emulsions made in this study and a fat surface area varying up to 12 m^2/ml emulsion, the protein/fat surface area ratio calculated from these figures corresponds to the initial protein bulk concentrations of 10^{-2} wt % down to 10^{-4} wt % in the interfacial studies of the three proteins made in an earlier investigation (Tornberg, 1978c). So, that during the emulsification process protein depletion could not be any greater than two decades in wt %.

According to this previous investigation (Tornberg, 1978c) and in the concentration range concerned the following interfacial behavior was evaluated for the three food proteins. The soy proteins diffuse slowly to the interface and an adsorption barrier is built up at a low protein coverage of the interface compared to the other proteins. Both the whey proteins and the caseinates diffuse relatively quickly to the interface, but an adsorption barrier is obtained at a lower protein coverage for the whey proteins than for the caseinates. The addition of 0.2M NaCl to all the protein solutions studied enhanced the rate of the diffusion controlled adsorption of the proteins at the interface.

Adsorption of proteins from the bulk during the emulsification process can be considered to be convection controlled. In the interfacial tension measurements, however, the migration of proteins to the interface is only governed by molecular diffusion, which makes a direct comparison difficult between the interfacial and the emulsification studies. The rate of the diffusion controlled adsorption in the interfacial tension measurements gives, however, a hint

of the probability of successful collisions of the molecules at the interface, as it is of great importance in speeding up the diffusion controlled process (Graham, 1976; Tornberg, 1978d). The preferentially performed spreading of soy protein (0–7) molecules at a surface area as low as $\approx 3\ m^2/ml$ emulsion, as shown in Figure 9, seem to be in accordance with the interfacial behavior, such as slow diffusion rate and building up of an adsorption barrier at low amounts of protein adsorbed at the interface. The diffusion rate is relatively equal for the whey proteins and the caseinates in the concentration range concerned, but the smaller adsorption barrier obtained for the caseinate film, might explain the high percentage adsorbed from bulk of 75 and 90% for caseinate (0–7) and caseinate (0.2–7), respectively, at large surface areas.

When salt is added the electrical double layer surrounding the protein colloid is suppressed, and the electrostatic repulsion during adsorption and between the adsorbed molecules is reduced. This might be the reason why caseinate (0.2–7) molecules can more densly pack at the interface, resulting in higher percentage protein adsorbed than for caseinate (0–7). But for the whey proteins this reasoning is not applicable as higher percentage adsorbed is obtained for WPC (0–7) than for WPC (0.2–7) stabilized emulsions at the same fat surface area.

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STARCH GELATINIZATION PHENOMENA STUDIED BY DIFFERENTIAL SCANNING CALORIMETRY

C. G. BILIADERIS, T. J. MAURICE, and J. R. VOSE

ABSTRACT

Differential Scanning Calorimetry (DSC) was used to study gelatinization phenomena of corn, potato, acid-modified corn and smooth pea, and various legume starches. Two endothermic transitions were observed for starches heated in the presence of limited amounts of water (starch/water = 46–48% w/w). The effect of starch concentration on the shape of these two endotherms was studied for three legume starches. Considering the starch granule as a spherulite, the experimental data were treated thermodynamically by applying equations describing phase transitions of semi-crystalline polymers. Experimental evidence suggested that a solvation-assisted melting of starch crystallites took place during gelatinization and that the process was facilitated by the presence of the amorphous parts of the granule. The enthalpy change, ΔH, associated with the gelatinization varied among these starches within a range of 2.6–4.4 cal/g. A comparison between DSC transition temperatures and characteristic gelatinization temperatures obtained by other methods was also made.

INTRODUCTION

STARCH gelatinization is an important phenomenon occurring in various food processing operations. Processes such as baking of bread and cakes, extrusion of cereal based products, thickening, and gelling of sauces and pie fillings are all dependent on proper starch gelatinization. It is not surprising, therefore, that considerable attention has been paid to the technological implications of starch gelatinization, especially from the rheological point of view. Various methods, which are based on the properties of granular starches, have been used to study starch gelatinization. These include viscosity measurements, microscopic examinations of granule swelling and loss of birefringence, and light transmittance (Collison, 1968). These methods, however, are limited by certain parameters such as starch/water ratio and temperature range over which the gelatinization process can be studied.

Differential Scanning Calorimetry (DSC) is particularly well suited to investigate the phase transitions of starch/water systems because it allows: (1) study of starch gelatinization over a wide range of starch/water ratio; (2) determination of gelatinization temperatures above 100°C; and (3) estimation of transition enthalpies. Although earlier DSC work on starch gelatinization is reported on native and modified cereal and tuber starches (Stevens and Elton, 1971; Donovan, 1979; Wada et al., 1979; Wootton and Bamunuarachchi, 1979a, b), there are no comparable studies on legume starches. Furthermore, very little research has been carried out (Lelievre, 1973; Marchant and Blanshard, 1978; Donovan, 1979) to study starch phase transitions from a physicochemical approach.

We have attempted, therefore, to study the gelatinization of various legume starches using DSC. Corn and potato starches were also used for comparison. The significance of the amount of water on the mechanism of starch gelatinization was further investigated.

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MATERIALS & METHODS

Starch

Corn, high-amylose corn and potato starches were obtained from a commercial source (A.E. Staley Inc. Decatur, Ill.). Corn starch was also prepared according to Adkins and Greenwood (1966). The following legume starches were prepared from their seeds by a wet-milling process (Biliaderis et al., 1979): smooth pea (*Pisum sativum* L. cv. Trapper), adzuki bean (*Phaseolus chrysanthos*), garbanzo bean (*Cicer arietinum*), mung bean (*Vigna radiata*), green lentil (*Lens culinans*), red kidney bean (*Ph. vulgaris*), navy bean (*Ph. vulgaris*) and faba bean (*Vicia faba equina* L. cv. Diana).

Acid-modified (lintnerized) corn and smooth pea starches were prepared by immersing granules in 2.2N HCl at 35°C for 10, 24, and 48 hr. The acid-treated granules were recovered on a sintered glass filter and then washed repeatedly with deionized water. The extent of lintnerization was then determined by measuring the total carbohydrates in the filtrate (Dubois et al., 1956) and expressing that as percent of the initial starch. Smooth pea amylopectin was isolated according to Banks and Greenwood (1967).

Chemical analysis

Standard AACC (1976) methods were used for proximate analysis. Protein was estimated as %N × 6.25. Starch was assayed according to Banks et al. (1970). Amylose contents were determined by measuring the iodine affinities of the defatted (hot extraction in 85% aqueous methanol for 48 hr) starches according to Schoch (1964) and assuming that pure amylose has an iodine affinity of 20.0%. For phosphorus analysis, the samples were successively digested with conc H_2SO_4 and H_2O_2; phosphorus in digests was measured using Murphy and Riley's method (1962).

Starch gelatinization temperatures

Gelatinization temperatures were determined according to Schoch and Maywald (1956). The recorded temperatures correspond to loss of birefringence by 5, 50, and 95% of the granules. To obtain initial pasting temperatures amylograms were prepared on a Brabender Visco-Amylograph with a 700 cm/g sensitivity cartridge at 75 rpm using 8% w/w slurries. Recorded temperatures were those at which an initial increase of 10 Brabender units (BU) in viscosity was reached (Medcalf and Gilles, 1966).

Differential scanning calorimetry

Starch of known moisture content was mixed with the appropriate amount of distilled water and allowed to stand for 1 hr at room temperature before heating. Samples of 10 ± 2 mg were heated in Du Pont coated aluminium hermetically sealed pans using a Du Pont 990 thermal analyzer and a 910 DSC cell base. A known weight of sand (SiO_2) was used in the reference pan to balance the heat capacity of the sample pan. Samples were heated from 25° to 150°C at a rate of 5°C/min. The instrumental sensitivity was 0.02 mcal/s/in; analyses were performed in a nitrogen atmosphere (54 psi). The calibration coefficient, E, of the cell was determined from thermograms of weighed amounts of materials with known enthalpies of fusion. Transition temperatures were obtained from a temperature plot which was calibrated from the baseline in mvolt/in. The conversion to °C was made by using thermocouple tables. Heats of transition, ΔH, were calculated by measuring the areas by means of a planimeter and using the equation:

–Continued on next page

$$\Delta H = \frac{A \cdot 60 \cdot B \cdot E \cdot \Delta qs}{M \cdot C} \quad (1)$$

where A = area (in.^{2}), ΔH = transition enthalpy (mcal/mg); M = sample mass (mg); C = sample concentration (% w/w); B = time base setting (min/in.); E = cell calibration coefficient (no units); and Δqs = Y-axis range (mcal/sec/in.). The obtained ΔH values had an average standard deviation of ±0.25 cal/g for repeated analyses (n = 5–10) of the same sample. The gelatinization characteristics of starch in a DSC thermogram can be presented by various temperatures: the onset temperature (To); the peak temperature(s) (Tp); and the melting point (Tm) of the most perfect crystallites at the upper temperature limit of the thermogram. These temperatures and the areas taken for calculating ΔH are shown in Figure 1. For calculations of the volume fraction of water, v_1, the density of water was taken as 1.00 and that of starch an average value of 1.5 g/ml (Banks and Greenwood, 1975). The v_1 was then calculated as the ratio of the volume of water divided by the total volume of starch plus water.

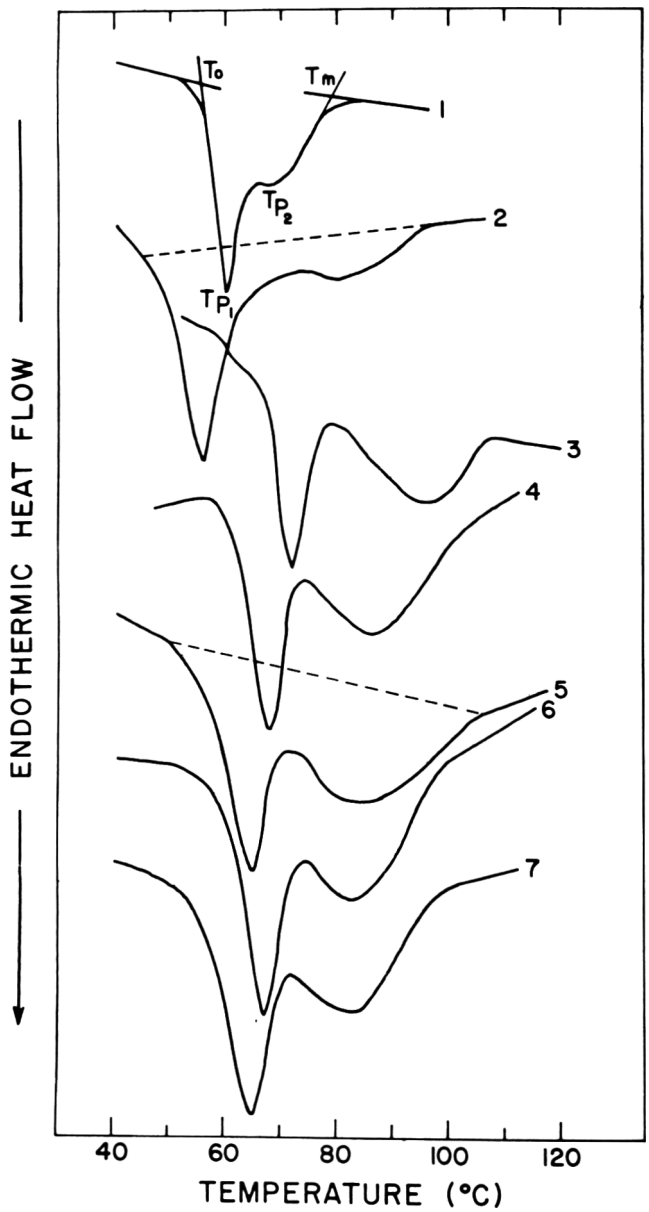

Fig. 1—DSC thermograms of starches: 1 Potato, 2 Lentil, 3 Garbanzo bean, 4 Red kidney bean, 5 Mung bean, 6 Navy bean, 7 Faba bean: To = onset temperature; Tp_1, Tp_2 = peak temperatures; Tm = melting point. Thermograms 2 and 5 indicate areas taken for ΔH calculations. Percent concentrations of starch (w/w) for these experiments, from top to bottom, were: 46.3, 47.5, 45.6, 46.3, 47.5, 46.1, 46.6.

RESULTS & DISCUSSION

PROXIMATE ANALYSIS of the native starches indicated that all samples had protein, lipid, fiber and ash contents less than 0.49%, 0.23%, 0.51% and 0.18%, respectively. Their starch assay showed starch contents greater than 93.5% in all cases.

The DSC thermograms of the starches are illustrated in Figures 1 and 2. Amylose and phosphorus contents, characteristic endothermal temperatures, transition enthalpies, initial pasting temperatures and gelatinization temperatures are given in Table 1. Two endothermic transitions were exhibited by all native starches when heated under similar intermediate water contents (45.6–48.2% w/w). The influence of water content on the appearance of these two endotherms was studied for smooth pea, adzuki bean and lentil starches.

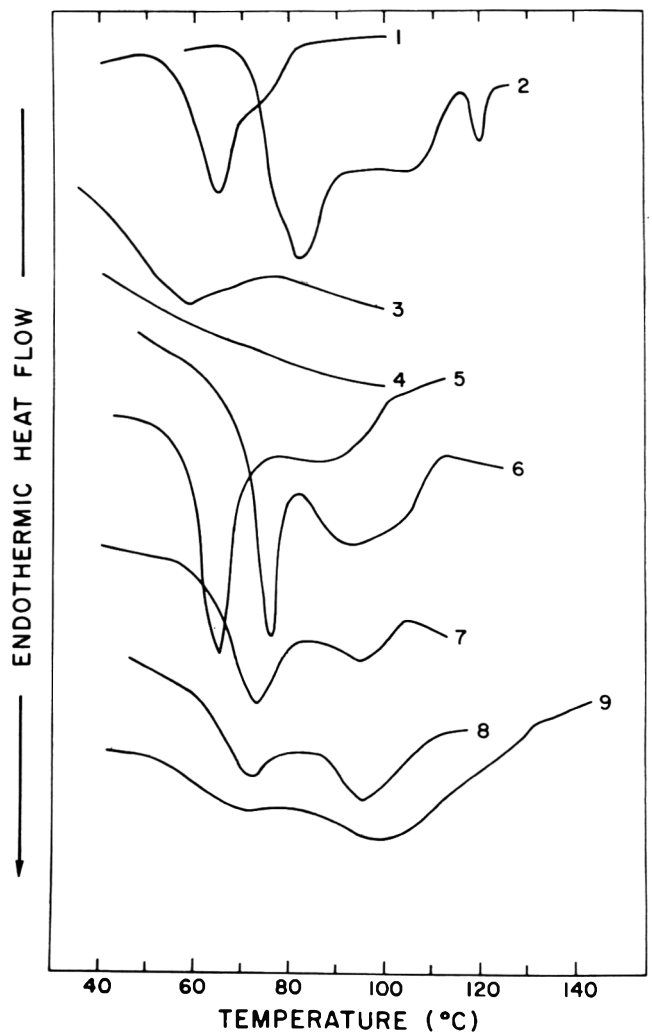

Fig. 2—DSC thermograms of starches: 1 Lab.-isolated corn, 2 High-amylose corn (commercial), 3 Smooth pea amylopectin, 4 Gelatinized smooth pea, 5 Smooth pea, 6 Adzuki bean, 7 Acid-modified smooth pea (5.1% lintnerized), 8 Acid-modified smooth pea (9.6% lintnerized), 9 Acid-modified smooth pea (20.2% lintnerized). Percent concentrations of starch (w/w) for these experiments, from top to bottom, were: 47.3, 48.2, 48.2, 48.0, 47.5, 47.7, 47.8, 47.9, 47.6.

Table 1—DSC characteristics and other physicochemical properties of the studied starches

Starch	Phosphorus content (%)	Amylose content (%)	Initial pasting temp (°C)	Gelatinization temp (°C)	Starch conc for DSC exp. (%, w/w)	To (°C)	Tp_1 (°C)	Tp_2 (°C)	Tm (°C)	ΔH (cal/g)
Adzuki bean	0.013	34.9	78	83–(85)–89	47.7	69	75	89	112	4.4
Smooth pea	0.006	33.1	73	65–(67)–69	47.5	56	64	87	101	3.5
Acid-modified, 5.1[a]	–	26.8	–	–	47.8	60	73	95	103	2.4
Acid-modified, 9.6[a]	–	23.0	–	–	47.9	60	72	96	109	2.2
Garbanzo bean	0.010	34.1	75	65–(68)–71	45.6	68	72	96	108	3.1
Red kidney bean	0.016	35.0	73	64–(66)–68	46.3	61	68	86	100	2.6
Lentil	0.008	45.5	66	58–(59)–61	47.5	48	56	80	95	2.6
Navy bean	0.011	36.0	75	68–(71)–74	46.1	59	67	83	99	3.5
Mung bean	0.016	34.9	73	63–(65)–69	47.5	57	65	83	99	3.9
Faba bean	0.010	32.5	72	61–(63)–66	46.6	56	65	83	97	3.3
Potato, commercial	0.075	20.1	51	64–(65)–67	46.3	55	60	68	85	4.4
Corn, commercial	0.019	22.6	74	63–(65)–68	46.4	60	67	78	89	3.3[b]
Corn, lab prepared	0.012	22.4	73	62–(65)–67	47.3	53	63	75[c]	86	2.7
Acid-modified, 6.5[a]	–	22.6	–	–	47.9	54	73	99[c]	89	2.4
High-amylose corn comm.	0.029	50.3	96	82–(86)–99	48.2	71	82	105	114	4.2[b]
Waxy corn, commercial	0.002	00.0	72	64–(68)–70	47.6	64	71	88	97	4.0[b]

[a] Numbers represent percent lintnerization
[b] For calculations of ΔH values only P_1 and P_2 were used
[c] Shoulder

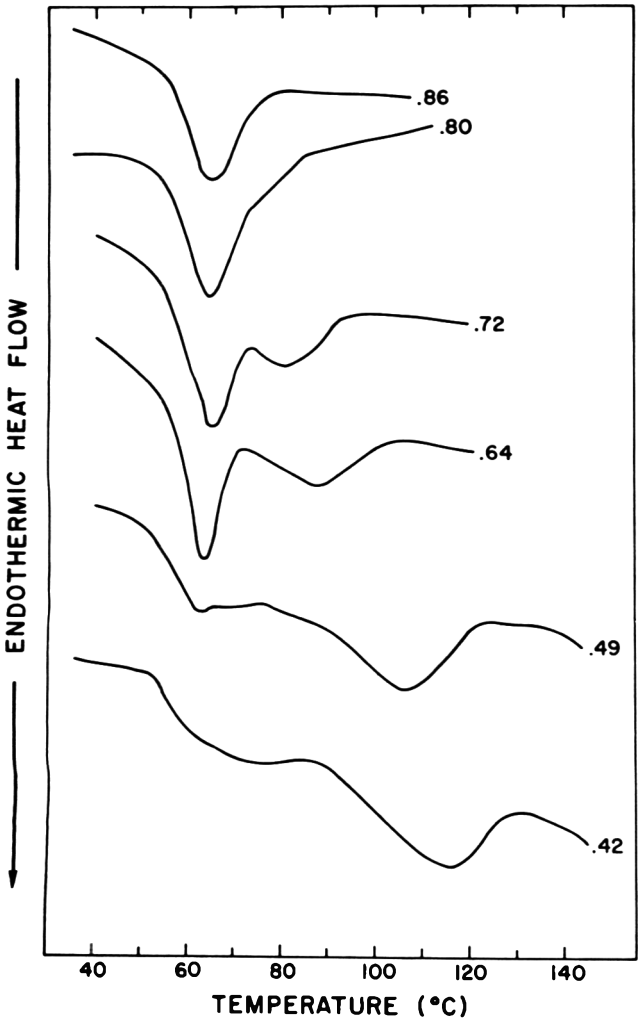

Fig. 3—DSC thermograms of smooth pea starch heated at different water concentrations; numbers represent volume fraction of water. Percent concentrations of starch (w/w) for these experiments from top to bottom, were: 19.0, 26.6, 37.0, 45.6, 60.8 and 67.5.

When smooth pea starch was heated at high water concentrations, a single endothermic transition, P_1, was observed (Fig. 3) at about 64°C (Tp_1). As the ratio of starch/water increased the second endotherm, P_2, started to develop at higher temperatures (Tp_2) and became predominant at low water contents. Although the P_1 endotherm occurred essentially at constant temperature (63–65°C), it was of interest to observe a progressive shift of the P_2 endotherm as ν_1 was decreased. Similar results were also exhibited by both adzuki bean and lentil starches (Table 2). This concentration dependent shift of both Tp_2 and melting point, Tm, suggested a thermodynamic treatment of these results by employing equations that characterize other polymer systems. Similar theoretical treatments of starch gelatinization data have also been used by Lelievre (1973) and Donovan (1979). The former measured the loss of bire-

Table 2—DSC characteristics of three legume starches heated at different water concentrations

Starch	Volume fraction of water, ν_1	To (°C)	Tp_1 (°C)	Tp_2 (°C)	Tm (°C)	Melting temp range (Tm–To)	ΔH (cal/g)
Adzuki bean	0.87	70	76	–	87	17	5.2
	0.80	70	76	–	93	23	3.7
	0.73	70	76	–	98		4.0
	0.62	69	75	89	112		4.4
	0.50	69	76	102	128		4.5
	0.43	66	75	115	138		2.8
Smooth pea	0.86	55	64	–	80	25	3.5
	0.80	55	64	–	85	30	3.8
	0.72	54	65	81	93		3.6
	0.64	56	64	87	101		3.5
	0.49	52	63	107	122		3.1
	0.42	50	65[a]	116	130		2.7
Lentil	0.86	47	57	–	77	30	3.4
	0.79	47	56	71	82	35	2.9
	0.68	46	56	73	89		3.0
	0.62	48	56	80	95		2.6
	0.50	47	55	93	111		3.0
	0.43	45	55	94	116		1.4

[a] Shoulder

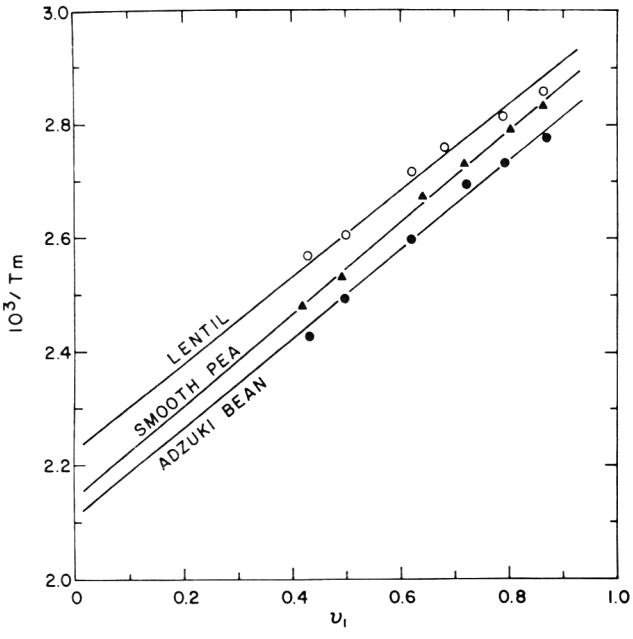

Fig. 4—The reciprocal melting point, $\frac{1}{Tm}$ (°K), plotted against the volume fraction of water, v_1, for three legume starchesEach line is a linear least-square fit of the experimental data.

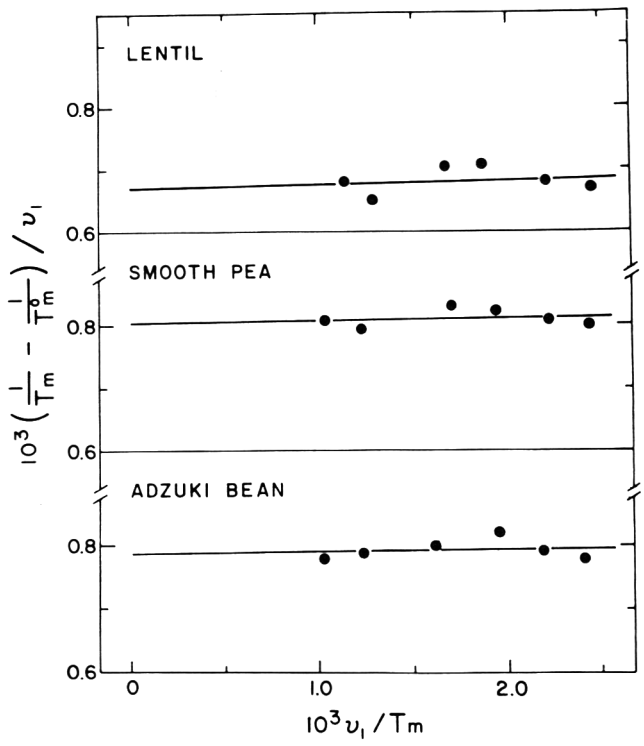

Fig. 5—Plot of experimental data according to Eq. (2). Each line is a linear least-square fit of the experimental data.

fringence in wheat starch granules as a function of temperature and water content, while the later employed DSC to study the gelatinization of potato starch in the presence of different amounts of water. According to the Flory equation (Flory, 1953) the following relation holds between melting point of a polymer and diluent concentration:

$$\frac{1}{Tm} - \frac{1}{Tm^\circ} = \left(\frac{R}{\Delta Hu}\right) \cdot \left(\frac{Vu}{V_1}\right) \cdot (v_1 - X_1 v_1^2) \qquad (1)$$

where ΔHu is the change in enthalpy of fusion per repeating unit (glucose), Vu/V_1 is the ratio of the molar volume of the repeating unit (glucose) in the chain to that of the diluent (water), R is the gas constant, Tm (°K) is the melting point of the diluent-polymer mixture, Tm° (°K) is the true melting point of the undiluted polymer, v_1 is the volume fraction of the diluent, and X_1, is the Flory interaction parameter. The melting point, Tm, of the heated starch/water mixtures was taken as the melting point of the most perfect crystallites at the upper temperature limit of the endotherm, according to Flory (1953). For an ideal solution, $X_1 = 0$; hence Eq (1) gives a linear relation between v_1 and 1/Tm. The intercept at $v_1 = 0$, therefore, gives the reciprocal of the melting point of the most perfect crystallites of the undiluted polymer, $1/Tm^\circ$. A plot of 1/Tm against v_1 for the three starches is given in Figure 4. Linear regression analysis of the experimental data gave significant correlations ($r_{len.} = 0.993$, $P = 0.01$; $r_{s.pea} = 0.998$, $P = 0.01$; $r_{adz} = 0.997$, $P = 0.01$). The obtained Tm° values by extrapolation at $v_1 = 0$ were 439°K (166°C) for lentil, 467°K (194°C) for smooth pea and 476°K (203°C) for adzuki starch. Corresponding values of 210°C for wheat (Lelievre, 1973) and 168°C for potato (Dovonan, 1979) starches have been reported previously. These values are significantly lower than the temperature range of 260–330°C, where extensive thermal decomposition of starch occurs (Greenwood and Muirhead, 1967). Differences in the Tm° values among the three legume starches may be related to various factors, in which the granular organization and its inherent crystallinity are probably the most important. In order to develop this hypothesis, the structural characteristics of the amylopectins (the principle component of the starch crystallites) of these starches were determined by enzymic methods (Biliaderis, 1980). Indeed, some differences in the degree of branching among these amylopectins were detected. The order of increasing degree of branching was adzuki bean< smooth pea<lentil. Considering that branching is detrimental to crystallization and, therefore, broadens the melting temperature range (Tm–To) and lowers the Tm°, as found in the field of synthetic polymers (Cowie, 1973), one would expect that the higher the degree of branching, the wider the melting temperature range and the less resistant the starch is to gelatinization. The obtained values for Tm–To (Table 2), gelatinization temperature, initial pasting temperature (Table 1) and Tm° were indeed in accord with this concept.

An alternative plot of Eq (1) has also been suggested by Flory to estimate the ΔHu values. Considering X_1 to be inversely proportional to temperature ($X_1 = BV_1/RT$, where B is the energy of polymer-diluant interaction), the Eq (1) can be written in the form:

$$\left(\frac{1}{Tm} - \frac{1}{Tm^\circ}\right)/v_1 = \left(\frac{R}{\Delta Hu}\right) \cdot \left(\frac{Vu}{V_1}\right) \cdot \left(1 - \frac{B \cdot V_1}{R \cdot Tm} v_1\right) \qquad (2)$$

A plot of the left-hand side of Eq (2) against v_1/Tm is shown in Figure 5 for the three legume starches. From the intercepts the ΔHu values were calculated to be 18.9 for lentil, 15.8 fro smooth pea, and 16.2 Kcal/mole D-glucose unit for adzuki bean starch. Donovan (1979) reported a value of 13.5 Kcal/mole D-glucose unit in the case of potato starch. From the slopes, the energy of interaction, B,

was computed to be 1.03 for lentil, 0.30 for smooth pea, and 0.60 cal/ml for adzuki bean starch; the positive values of B suggest that the water is a poor solvent for starch (Flory, 1953). The accuracy of the above calculated thermodynamic parameters depends first on the validity of the extrapolations used in Figure 4 and, second, on various experimental limitations. However, one must emphasize the qualitative aspect of the above treatment which demonstrated that the starch granule may indeed be treated as a polymer spherulite; relations analogous to the Flory equation may be also used to predict starch gelatinization behavior in heated food systems of different water activities.

To investigate the reversibility of the heat effects on the starch samples the following experiments were carried out. Samples (corn and smooth pea starches) were heated at both high and intermediate water concentrations, until the complete thermogram was obtained. Cooling to 40°C and immediately reheating showed no transition endotherms. This suggested that complete gelatinization was achieved during the first heat treatment and that no significant recrystallization (annealing) in the gels took place over the time of the experiment. In other experiments, the heating process was stopped after the completion of the first endotherm and the starch samples were cooled quickly. Upon reheating the thermogram obtained was still biphasic with the Tp_1, however, shifted to higher temperatures. The second thermogram represents that portion of the sample that did not gelatinize during the first heating and, therefore, exhibited a higher Tp_1 upon reheating. The shift of Tp_1 must, therefore, be due to the heterogeneity of the starch granules and was observed in all cases regardless of the amount of water present. Gough and Pybus (1971) have also reported that although gelatinization of individual granules occurs over a range 0.5–1.5°C, the overall response of the population, because of its heterogeneity, extends over 10–15°C. In conclusion, then, two suggestions can be made. First, it seems unlikely that the observed biphasic endothermic transition, occurring at intermediate water levels, emmerges from two discrete granule or crystallite populations. A normal distribution of granules and crystallites, in terms of their energy characteristics, would rather be expected. Second, a partial heat treatment (i.e., process stopped between P_1 and P_2) does not induce significant changes in the system, which will eventually terminate the gelatinization process, without any further increase in temperature.

The thermograms of acid-modified smooth pea starch (starch/water:47.6–47.9% w/w) at three different levels of lintnerization, are shown in Figure 2 (Thermograms 7, 8, 9). It has been recognized that lintnerization selectively hydrolyzes the amorphous parts of the granule (Kainuma and French, 1971; Robin et al., 1974). It was of interest to observe that progressive lintnerization resulted in obtaining themograms quite similar to those of the native smooth pea starch heated at low water concentrations. It appears, therefore, that the amorphous parts destabilize the system during heating and cause a large part of the starch crystallites to melt at low temperatures (first endotherm, P_1). We suggest that this effect, exercised by the amorphous regions, is due to their swelling upon hydration, and increased chain mobility upon heating to finally cause a solvation-assisted melting of the starch crystallites.

It is necessary at this stage, to give a brief description of the structure of the starch granule which will alow a subsequent discussion on the mechanism of gelatinization from a theoretical approach. Treatment of the starch granule as a semi-crystalline entity (spherulite) which contains both crystalline and amorphous regions, is widely accepted among starch chemists (Banks and Greenwood, 1975). It has been suggested (French, 1972; Robin et al., 1974) that starch crystallinity is due to the presence of parallel clusters of short chains (~50–60 Å) packed side by side. These chains could be either the external amylopectin chains (the principle crystalline component) or parts of the amylose molecules (less important) which can give analogous parallel chain structures by chain folding. Double helical structures have been poroposed in both cases (French, 1972). It is the presence of such regularly oriented crystallites that makes the starch granules birefringent and have distinct x-ray diffraction patterns. On the other hand, the amorphous regions are those where chain folding or multiple branching occur and prevents the formation of ordered polymer structures. Recent experimental evidence suggests that water absorbed by the granule is associated only with its amorphous parts (Kainuma and French, 1972). When starch granules are heated in the presence of water, gelatinization takes place. This phenomenon is associated with: (a) loss of crystallinity as indicated by the disappearance of both birefringence and x-ray pattern: (b) extensive swelling of the granule, predominantly after the loss of birefringence.

From the above considerations and data, we suggest the following concepts as possible explanations of the overall gelatinization process. If a large amount of water is present, extensive hydration and swelling of the amorphous regions facilitate melting of the starch crystallites upon heating. This occurs over a very narrow temperature range, and therefore results in a single endothermic transition, P_1. The melting of the crystallites may involve a solvation assisted helix-coil transition of their chains, which is an analogous phenomenon to that found in heated solutions of other biopolymers. It is the large gain in entropy, in going from the ordered to the random conformation, that offsets the attractive stabilization forces (hydrogen bonding) occurring in the crystallites. Extensive swelling must also be associated with such crystallite disruption, to account for the observed increase in viscosity following the loss of birefringence in the granule. In more concentrated starch solutions, however, the destabilizing effect of the amorphous regions decreases and, because of the limited amounts of water present, only partial melting of crystallites occurs according to the previous mechanism (first endotherm). Subsequent redistribution of the water, however, around the unmelted crystallites will assist their melting upon further heating at higher temperatures (second endotherm). Jaska (1971) found a rapid drop in NMR line width, after the onset of gelatinization (an analogous state to Tp_1) in 40% (w/w) wheat starch solutions. This was interpreted as a sudden increase in mobilization of water *and* starch molecules. Our calorimetric data and those reported by Donovan (1979) further suggest that water mobilization may take place toward the unmelted crystallites; this is supported by the fact that the Tm values appeared to smoothly fit the plot of 1/Tm vs ν_1 over the entire range of water activities studied. Finally, under extreme conditions, where no moisture is present in the system, and the only denaturant is heat, melting of the starch crystallites would be expected to occur at temperatures close to the estimated Tm° ("true-melting" of crystallites). The above suggested mechanism is schematically summarized in Figure 6. It is obvious therefore, that water plays an important role in the overall process by assisting the melting of the starch crystallites.

The transition enthalpies for the legume starches varied over the range 2.6–4.4 cal/g. The ΔH values for corn and potato starches were in good agreement with those reported by Stevens and Elton (1971), but significantly lower than those obtained by Wootton and Bamunuarachchi (1979a). Differences may reflect variations between starch samples. Moreover, the ΔH values are significantly affected by the heating rate, amount of water and starch damage (Stevens

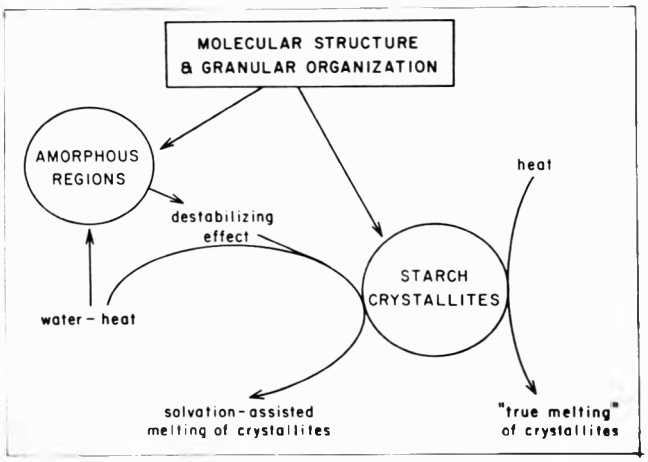

Fig. 6—Schematic representation of the possible interrelationships among the various parameters involved in phase transition phenomena of granular starch.

and Elton, 1971; Wootton and Bamunuarachchi, 1979b; Donovan, 1979). Our data (Table 2), as well those of Wootton and Bamunuarachchi (1979b) and Donovan (1979) have indicated a pronounced decrease in the gelatinization enthalpies in the presence of limited amounts of water. A lower degree of disorder achieved by the starch during gelatinization of concentrated starch/water systems was proposed as the reason for the decreased ΔH values (Stevens and Elton, 1971). Donovan (1979) has further suggested that thermodynamic quantities from different processes are measured by the calorimeter, at different amounts of water present in the system. At low water content, the ΔH value mainly represents the enthalpy of melting of the starch crystallites; at high water levels, however, the ΔH value can account for granule swelling, crystallite melting and extensive hydration of starch molecules. More detailed thermodynamic measurements, however, are required to provide an adequate understanding of the processes contributing to the enthalpy factor of the gelatinization process. Finally, it is of interest to note that similar effects of water content on both the magnitude and temperature shift of the "melting endotherms" of other crystalline biopolymers have been also reported (Morita, 1966).

All commercial corn starches showed additional endothermic peaks (Fig. 2, thermogram 2) at temperatures over 100°C. Since the laboratory isolated corn starch gave the normal biphasic endothermic transition (Fig. 2, thermogram 1), it is suggested that these additional peaks might reflect granular changes introduced during the isolation of these commercial starches.

All ΔH values of the acid-modified corn and smooth pea starches were lower than those of their native starches (Table 1), in agreement with similar results reported for wheat starch by Wootton and Bamunuarachchi (1979a).

Smooth pea amylose and gelatinized smooth pea starch showed no endothermic transitions. Smooth pea amylopectin, however, gave a small endothermic transition, ΔH: 0.9 cal/g (Fig. 2, thermogram 3), suggesting a partial crystalline ordering of the isolated polysaccharide. In a poor solvent, such as water, the requirement for a minimum energy state will favor such compact conformations. This is further supported if one considers that the extensive branching of amylopectin facilitates an ordered conformation, by restricting the rotation about individual glucosidic bonds (Banks and Greenwood, 1975). Retrogradation of the amylopectin during bread staling (Gilles et al., 1961) is further evidence that recrystallization of this polysaccharide may possibly occur.

The observation that the first endotherm (P_1) appeared at a constant temperature, regardless of the amount of water present, suggested that Tp_1 can be used as an index to characterize the resistance to gelatinization of a particular starch sample. The obtained Tp_1 values, although not directly correlated, were close to the average gelatinization temperatures, i.e. 50% loss of birefringence, and distinctly lower than the initial pasting temperatures (Table 1). Apparently, it is difficult to make direct comparisons between these values because of their dependence on various experimental parameters (heating rate, thermal lags in the equipment, etc.)

In conclusion, the thermodynamic treatment of the DSC data indicated that the gelatinization process can be explained in terms of the theory of polymer spherulites. Although quantitation of the thermodynamic parameters is subject to several potential sources of error, one must emphasize the versatility of the DSC technique to measure the extent of gelatinization over a wide range of temperatures. The influence of the amount of water and various food ingredients on starch gelatinization can also be studied under conditions resembling those employed in the preparation of various heat-processed foods.

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INFLUENCE OF VARIOUS PURIFIED AND ISOLATED CELL WALL FIBERS ON THE UTILIZATION OF CERTAIN NUTRIENTS BY SWINE AND HAMSTERS

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ABSTRACT

Four metabolism trials were conducted using swine and hamsters to evaluate the effects of isolated and purified dietary fibers on the utilization of fiber, nitrogen, and minerals. Swine fed a 16% crude protein diet and various types of purified celluloses absorbed less nitrogen than did pigs fed a control diet containing silica flour. No differences in nitrogen metabolism were noted when the crude protein concentration was reduced to 9% although fiber digestion was decreased at the lower protein level. Animal performance, nutrient digestibilities, nitrogen balance, and mineral balance were markedly affected when hamsters were fed silica flour, cellulose, and up to 30% lignin or lignin-hemicellulose.

INTRODUCTION

INCREASED DIETARY FIBER intakes have been credited with a reduction in diverticulosis symptoms, an increase in fecal bile acid excretion, and possible control of diabetic symptoms (Kelsay, 1978). These suggested positive effects have sparked a great deal of enthusiasm for fiber research using various nonruminants as animal models. Various workers (Salter, 1973) feel that fibers may be nutritionally beneficial because of the microbial influence on fibrous residues present in the distal small intestine, the cecum, and the colon. These microbes ferment fibrous residues to volatile fatty acids which can be absorbed and possibly contribute to the energy needs of the animal.

Two negative aspects of fiber ingestion by nonruminants have been noted. Reinhold et al. (1975) hypothesized that consumption of fiber-rich unleavened breads was a factor in the occurrence of zinc deficiency in humans although the phytic acid content of the breads may also play a role in the onset of this deficiency. Also dietary fiber has been shown to increase the metabolic fecal nitrogen excretion of rats (Meyer, 1956) and pigs (Whiting and Bezeau, 1957); therefore, nitrogen retention would be expected to decrease due to a decrease in apparently absorbed nitrogen. However, various workers have conducted experiments which refute this hypothesis. Saito et al. (1959) reported a 28% increase in nitrogen retention of growing chicks when 9.5% cellulose was added to the basal diet. Corley et al. (1978) reported a 24% increase in nitrogen retention of growing pigs when 4% Solka Floc was added to a 19% crude protein, corn-soybean meal basal diet. Kornegay (1978) found that when soybean hulls were substituted in a corn-soybean meal basal diet, nitrogen retention as a percent of nitrogen intake was not affected while nitrogen retention as a percent of absorbed nitrogen was increased at the higher soybean hull levels.

Comparative studies elucidating those metabolic parameters affected by feeding various types and sources of purified and isolated fibers are few (Fahey, 1979). This investigation was designed to (a) determine the effects on fiber digestion and nitrogen balance of feeding various purified sources of cellulose to swine; and (b) to determine the effects on animal performance, nutrient digestibility, nitrogen and mineral balance of feeding a purified cellulose, a purified lignin, and an isolated lignin-hemicellulose complex to hamsters.

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EXPERIMENTAL

Swine trials

Two metabolism trials involving 16 pigs each were conducted. The average initial weights of pigs in trial 1 and trial 2 were 12.5 and 11.0 kg, respectively. Four littermate barrows in each of four blocks were randomly assigned to each of the four dietary treatments. Composition of the basal diets used in the two trials is presented in Table 1. In trial 1, 80% crude protein, crude casein was used to provide 16% crude protein in the basal diet. In trial 2, 90% crude protein, edible-grade casein was used to provide 9% crude protein in the basal diet. The mineral mix and vitamin mix used in both trials was added at sufficient levels to provide the dietary requirement for those minerals and vitamins known to be required by the young pig (NRC, 1979). The diets also contained corn oil to improve the palatability of the diets. Antibiotic, in the form of tylosin, and antioxidant, ethoxyquin, were also included in the basal diets. In both experiments, the control treatment consisted of the basal diet plus 10% silica flour, a relatively inert product made from diatomaceous earth (Wedron Silica Division, Park Ridge, Ill.). The fiber diets contained 10% of either Solka Floc (Brown and Company, Chicago, Ill.), Alphacel or acid hydrolyzed Alphacel (ICN Nutritional Biochemicals Corp., Cleveland, Oh.). The additions of silica flour and the commercial cellulose preparations to the basal diets were made at the expense of cornstarch such that at equal feed intakes, the intakes of dry matter, nitrogen, calories, and other nutrients would be the same for all treatments. The four diets contained, respectively on an as-fed basis, 0.7, 10.5, 10.3, and 10.4% neutral detergent fiber; 0.3, 8.7, 8.8, and 9.9% acid detergent fiber, which consisted almost exclusively of cellulose with only traces of

Table 1—Composition of basal diet fed to all pigs

Ingredient	Trial 1	Trail 2
Casein (%)[a]	20.0	10.0
Cornstarch (%)	70.0	80.0
Corn oil (%)	5.0	5.0
Mineral mix (%)[b]	4.0	4.0
Porcine vitamin mix[c]	1.0	1.0
Tylosin (55 ppm)	+	+
Ethoxyquin (50 ppm)	+	+

[a] Crude casein (80% crude protein) was used in trial 1. Food grade casein (90% crude protein) was used in trial 2.

[b] Composed of the following minerals (% of mineral mix); $CaHPO_4$, 65%; NaCl, 15.4%; K_2CO_3, 14.0%; $MgCO_3$, 3.3%; $FeSO_4 \cdot H_2O$, 1%; $MnSO_4 \cdot H_2O$, 0.05%; $CoCl_2 \cdot 6H_2O$, 0.02%; $CuSO_4$, 1%; NaF, 0.02%; $ZnCO_3$, 0.2%; KI, 0.01%; $NaSeO_3$, 0.0001%.

[c] Each kilogram of mix contained the following: vitamin A palmitate, 500,000 IU; vitamin D_3, 284,000 IU; vitamin E (alpha-tocopherol), 12,000 IU; vitamin K, 20 mg; riboflavin, 660 mg; d-pantothenic acid, 2.64g; nicotinic acid, 4.4g; vitamin B_{12}, 4.4 mg; choline chloride 220g; folic acid, 200 mg; biotin, 20 mg; ascorbic acid, 30g; pyridoxine, 250 mg; thiamin, 260 mg; and powdered cornstarch to 1 kg.

lignin; and 0.4, 1.8, 1.5, and 0.5% hemicellulose.

Prior to initiation of the balance trials, a 5-day adaptation period was conducted to allow the pigs to become accustomed to the metabolism crates and the experimental diets. The pigs were fed twice daily at 0800 and 1600 hr the amount of feed which each pig would consume in the period of 1 hr. Each diet was mixed with an equal quantity of water to increase palatability and additional drinking water was provided after each meal was consumed. The level of feed intake during the metabolism trials for all pigs within a block was determined by the feed consumption of the pig of that block consuming the least amount of feed during the adaptation period. The collection period was conducted for 5 days. On day 1, ferric oxide, used as a marker to determine fecal passage, was fed with the 0800 meal to all pigs. Twenty-four hours later, the urine collection began along with the collection of red feces resulting from the previous morning's meal. Daily urine collections were diluted to a constant volume and a 100 ml aliquot was sampled from each and stored in a refrigerator at 5°C. Total daily fecal collections were stored in plastic bags in a freezer. At 0800 on day 6, the fecal marker was again fed with the morning meal. Fecal collections were discontinued when red feces were passed. The final urine collection and sampling was made at 0800 on day 7. Daily urine samples for each pig were pooled and analyzed for total Kjeldahl nitrogen (AOAC, 1979). Daily fecal samples for each pig were pooled and mixed in a Hobart mixer to obtain a homogeneous sample which was freeze-dried and subsequently analyzed for total Kjeldahl nitrogen (AOAC, 1970), neutral detergent fiber (Goering and Van Soest, 1970), acid detergent fiber (Goering and Van Soest, 1970), and dry ash (AOAC, 1970).

Hamster trials

Two metabolism trials were conducted with growing male Syrian hamsters (Engle Lab Animals, Inc., Farmersburg, Ind.) averaging approximately 85g in weight at the beginning of each trial. The animals were placed in galvanized metabolism cages and fed a cereal-based stock diet (Purina guinea pig chow, Ralston-Purina Co., St. Louis, Mo.) for 2 days prior to the initiation of the experiments. During a 3-day preliminary period, the hamsters were randomly allotted to the experimental treatments and gradually adjusted to the semipurified rations by mixing with the cereal-based stock diet. This was followed by a 7-day period during which time complete consumption of rations by all hamsters was required before initiating a 7-day period for collection of feces and urine. Feed and water were provided ad libitum and eight animals were assigned to each treatment in each experiment.

In trial 1, hamsters were fed rations where the sources of fiber were either silica flour, Alphacel or lignin (indulin ATR-CK 1, a Kraft lignin isolated from wood and obtained from Westvaco Corporation, Charleston, S.C.). Silica flour and Alphacel were added to the rations at the 10% level whereas lignin was added at either the 10%, 20% or 30% levels.

In trial 2, the hamsters were fed semipurified rations where the sources of fiber were silica flour or the lignin-hemicellulose (LH) complexes isolated from either cornstalks (the residue left after harvesting the corn grain, cobs and leaves; usually contains 70–80% neutral detergent fiber), or soybean residue (a collective term used to describe that part of the plant composed of leaves, stems and pods; usually collected in a wagon pulled behind the combine at the time of harvest and averages approximately 60–75% neutral detergent fiber). The LH complexes were isolated using a 10% sodium hydroxide extraction of the crude plant material (1 part plant material to 10 parts sodium hydroxide solution). The mixture was sealed under nitrogen gas for 24 hr, neutralized with acetic acid, and filtered. The filtrate was placed in three volumes of 95% ethanol and the resulting precipitate was recovered by vacuum filtration. By employing this method, all of the LH in the plant material is theoretically recovered. Both LH fractions were then pooled, dialyzed to remove excess salts, and dried at 55°C. All fibers were added to the diets at the 30% level on an as-fed basis. This is the approximate level of LH present in cornstalks and soybean residue. These two low quality roughages were used since previous work in our laboratory had shown that major differences between the two roughages occurred in regard to cell wall polysaccharide utilization by ruminants (Fahey, unpublished data).

Fiber determinations were carried out according to the methods of Goering and Van Soest (1979). Nitrogen was measured using the macro-Kjeldahl analysis as outlined by AOAC (1970). Calcium and magnesium were measured by wet-ashing feed, fecal, and urine samples with concentrated nitric acid followed by oxidation with hydrogen peroxide. The resulting material was then diluted with distilled deionized water and subjected to atomic absorption spectrophotometric analysis (Perkin-Elmer model 306 atomic absorption spectrophotometer).

Statistical analysis

Analysis of variance was performed on all data collected. In the swine experiments, an orthogonal set of single degree of freedom comparisons, as outlined by Steel and Torrie (1960), was used to compare the effects of the silica flour control diet vs the pooled effects of the fiber diets, the effects of the Solka Floc diet vs the pooled effects of the Alphacel and hydrolyzed Alphacel diets, and the effects of the Alphacel diet vs those of the hydrolyzed Alphacel diet. Differences between means in the hamster experiments were determined using Duncan's Multiple Range test as outlined by Steel and Torrie (1960).

Table 2–Apparent fiber digestibilities and nitrogen balance data for pigs in trial 1[a]

Item	Diet				
	Silica flour	Solka Floc	Alphacel	Hydrolyzed Alphacel	Pooled SE
Digestibility (%)					
ADF	–	16.7[b]	19.5[b]	3.7[c]	3.0
Hemicellulose	–	32.8	32.3	–	3.7
Nitrogen balance					
Intake (g/day)	14.76	14.76	14.76	14.76	–
Excretion (g/day)					
Fecal[d,e]	0.44	0.87	1.01	0.76	0.06
Urinary[f,g]	2.20	2.06	2.28	2.83	0.16
Total[h]	2.64	2.94	3.29	3.58	0.20
Absorbed (g/day)[d,e]	14.32	13.89	13.75	14.01	0.06
Retained (g/day)[h]	12.12	11.82	11.46	11.17	0.20

[a] Each value represents a mean of 4 pigs.
[b,c] Significantly different ($P < 0.05$).
[d] Silica flour control differs from pooled effects of fiber diets ($P < 0.005$).
[e] Alphacel and Hydrolyzed Alphacel diets differ ($P < 0.025$)
[f] Alphacel and Hydrolyzed Alphacel diets differ ($P < 0.05$).
[g] Solka Floc diet differs from pooled effects of Alphacel and Hydrolyzed Alphacel diets ($P < 0.05$).
[h] Silica flour control diet differs from pooled effects of fiber diets ($P < 0.05$).

RESULTS & DISCUSSION

Swine trials

Results of trial 1 are shown in Table 2. Fecal nitrogen excretion was increased ($P < 0.005$) by the addition of fiber when the effect of the silica flour control diet and the pooled effect of the fiber diets were compared. This would suggest that the addition of 10% fiber to the 16% crude protein cornstarch-casein diet either increased the excretion of metabolic fecal nitrogen or decreased nitrogen digestibilities. However, studies with pigs (Mason et al., 1976), rats (Mason and Palmer, 1973), and sheep (Mason, 1971) have shown that fecal nitrogen excretion increases due to an increase in the nitrogen of bacterial origin when fermentable carbohydrate is available to the hindgut bacteria. A decrease in true nitrogen digestibility apparently does not occur. Fecal nitrogen excretion was less ($P < 0.25$) for pigs receiving the hydrolyzed Alphacel diet than for those fed the Alphacel diet. No significant difference in fecal nitrogen excretion was noted when evaluating the effect of the Solka Floc diet and the pooled effect of the Alphacel and hydrolyzed Alphacel diets. Urinary nitrogen excretion was greater ($P < 0.05$) for pigs receiving the hydrolyzed Alphacel diet than for those fed the Alphacel diet. When the effects of

the Alphacel and hydrolyzed Alphacel diets were pooled, urinary nitrogen excretion of pigs fed these diets was greater ($P < 0.05$) than for those receiving the Solka Floc diet. The pooled effect of fiber addition on urinary nitrogen excretion was not significantly different from the effect of the silica flour control diet. Total nitrogen excretion was increased ($P < 0.05$) by the addition of fiber when the effect of the silica flour diet was compared to the pooled effect of the fiber diets. No significant differences in total nitrogen excretion were noted when the comparisons outlined in the statistical analysis section were made for pigs fed the three types of cellulose. The apparent absorbed nitrogen values reflect the same effects as did the fecal nitrogen and total nitrogen excretion data, respectively.

The apparent fiber digestibility data for trial 1 are also shown in Table 2. Since the fiber content of the silica flour diet can be considered to be either negligible or artifact, the fiber digestibility values of this diet were not determined. Similarly, the hemicellulose digestibility coefficient for the hydrolyzed Alphacel diets was also not determined since only a trace of hemicellulose occurs in the fibrous material. The apparent acid detergent fiber digestibilities were similar for the Solka Floc and Alphacel diets: however, the value for the hydrolyzed Alphacel diet was significantly ($P < 0.05$) reduced to 3.7% as compared to 16.7 and 19.5%, respectively, for the Solka Floc and Alphacel diets. No significant differences in hemicellulose digestibility were observed although nearly 33% of the hemicellulose of these diets was apparently digested. These data indicate that microbial degradation of the fibrous portion of the Solka Floc and Alphacel diets occurred in the lower gut of the pig.

If fiber has a benefical effect on nitrogen retention, this effect would likely be seen when some amino acid(s) was limiting in the diet. Therefore, the basal diet for trial 2 was formulated to have 9% crude protein and to be limiting in total sulfur amino acids. Nitrogen balance data are presented in Table 3. Using the planned single degree of freedom comparisons, no significant effects of fiber source on fecal, urinary or total nitrogen excretion were noted in this trial. Similarly, values for apparent absorbed nitrogen and retained nitrogen values indicated no significant differences between treatments. These data indicate no beneficial effect of 10% fiber on nitrogen retention when pigs were fed low-protein, low-sulfur amino acid diets. Nomani et al. (1979) found an improved utilization of absorbed nitrogen when rats were fed diets where protein was limited and acid detergent fiber or cellulose was included.

The acid detergent fiber and hemicellulose digestibility coefficients for the diets containing commercial celluloses are shown in Table 3. Pigs fed the Solka Floc, Alphacel and hydrolyzed Alphacel diets digested approximately 0, 5, and 10% of the acid detergent fiber, respectively. These values were not significantly different; however, the values for the Solka Floc and Alphacel diets appeared to be less than for the same diets in trial 1. Hemicellulose digestibilities for the Solka Floc and Alphacel diets were similar in trial 2, 51.6 and 50.0%, respectively; however, these values were greater than those determined in trial 1.

Although no direct comparisons between the two swine trials can be made, pigs in trial 1 which received the Solka Floc and Alphacel diets containing 16% crude protein digested less hemicellulose than did pigs in trial 2 receiving the same diets containing 9% crude protein. Furthermore, those pigs receiving the Solka Floc and Alphacel diets digested approximately twice as much neutral detergent fiber in trial 1 as those in trial 2 although fiber intakes were essentially the same in both trials for each diet. These observations indicate that the level of nitrogen available to the microbes in the cecum and colon may affect the extent of fiber digestion. Decreasing the nitrogen available to the fiber-digesting microbes may influence the numbers and types of microorganisms as well as the substrates utilized for energy by these microbes. Under conditions of limited nitrogen, cellulolytic bacteria may begin to utilize the more easily digested hemicellulose rather than the more difficult and slower digested cellulose. In ruminants, ruminal nitrogen and fiber digestibility have been shown to be positively correlated. Values for daily fecal nitrogen excretion for the pigs fed the fiber diets were 0.88 and 0.56g, respectively, in trials 1 and 2. The higher urinary nitrogen values observed in trial 1 as compared to trial 2 are not only related to a greater intake and absorption of nitrogen but are also conceivably affected by the ureolytic activity of the microbes of the cecum and colon. However since this hypothesis was not evaluated, it is therefore unconfirmed. Since these data are not conclusive, more work should be conducted to investigate the effects of nitrogen on fiber digestion in swine. However, it appeared from these experiments that nitrogen retention was not apparently affected by fiber additions. The fiber sources studied here were the same as those studied by Corley et al. (1978) who reported that the addition of 4% Solka Floc to a 19% crude protein, corn-soybean meal diet resulted in a 24% increase in nitrogen retention by young growing pigs. No such response was noted, however, in our trials where 10% Solka Floc was added to either 9 or 16% crude protein, cornstarch-casein diets for young pigs. Evidently, type of diet (i.e. practical corn-soy vs semi-purified cornstarch-casein) dictates the nature of the response that might be expected in fiber experiments.

Table 3—Apparent fiber digestibilities and nitrogen balance data for pigs in trial 2[a]

Item	Diet				
	Silica flour	Solka Floc	Alphacel	Hydrolyzed Alphacel	Pooled SE
Digestibility					
ADF	–	–0.1	5.1	10.4	3.5
Hemicellulose	–	51.6	50.0	–	5.8
Nitrogen Balance					
Intake (g/day)	8.50	8.50	8.50	8.50	–
Excretion (g/day)					
Fecal	0.48	0.52	0.56	0.60	0.06
Urinary	1.29	1.14	1.18	1.24	0.07
Total	1.78	1.66	1.74	1.84	0.09
Absorbed (g/day)	8.00	7.98	7.93	7.90	0.06
Retained (g/day)	6.72	6.84	6.75	6.66	0.09

[a] Each value represents a mean of 4 pigs.

Hamster trials

The intention of these experiments was to evaluate the more indigestible plant fiber fractions, lignin and lignin-hemicellulose. Since difficulty arises in obtaining sufficient quantities of these fibers to feed to large nonruminants such as pigs, the hamster was chosen as the animal model for the experiments. Also, hamsters possess a forestomach which is histologically and ultrastructurally similar to that of the rumen (Takashi and Tamate, 1976). The forestomach contains microorganisms similar to those identified in the rumen of cattle and sheep and volatile fatty acid production and apparent absorption from the forestomach has been demonstrated (Hoover et al., 1969). It was felt therefore that hamsters could utilize reasonably large quantities of the less digestible fiber fractions.

The composition of the diets fed to all hamsters is presented in Table 4. Casein was used as the source of protein in these studies while cornstarch was the primary energy source. Corn oil was added at the 10% level in both trials to reduce dustiness of the diets and to facilitate binding of

Table 4—Composition of basal diet fed to all hamsters

Ingredient	Trial 1 Treatment					Trial 2 Treatment		
	10% Silica flour	10% Alphacel	10% Lignin	20% Lignin	30% Lignin	10% Silica flour	30% Cornstalk lignin-hemicellulose	30% Soybean residue lignin-hemicellulose
Casein	25	25	25	25	25	25	25	25
Cornstarch	52	52	52	42	32	52	32	32
Corn oil	10	10	10	10	10	10	10	10
Vitamin mix (in glucose)[a]	1	1	1	1	1	1	1	1
Mineral mix[b]	2	2	2	2	2	2	2	2

[a] Composed of the following B-complex and fat-soluble vitamins (g/2 kg glucose): Biotin, 0.05; calcium pantothenate, 4; folic acid, 1; niacin, 20; pyridoxine hydrochloride, 1.5; riboflavin, 1.6; thiamin hydrochloride, 1.6; vitamin B_{12}, 0.005; retinyl acetate, 0.6; alpha-tocopherol acetate, 2; cholecalciferol, 0.004; and vitamin K (menadione), 0.2.

[b] Composed of the following minerals (g/100g diet): $CaCO_3$, 0.90; $CaHPO_4$, 2.04; $MgSO_4$, 0.60; NaCl, 0.26; $Na_2HPO_4 \cdot 7H_2O$, 1.28; KCl, 0.77; Fe citrate, 0.036; $CuSO_4$, 0.002; and KIO_3, 0.0017.

other ration ingredients. Hamsters require from 3–10% fat when fed semipurified diets (Hoekstra, 1972). All of the types and levels of nutrients included in these diets meet NRC specifications (Hoekstra, 1972).

Hamster trial 1 was carried out to examine the effects of dietary additions of Alphacel (10% level) and three levels (10, 20, and 30%) of an isolated Kraft pine lignin on animal performance, nutrient digestibility, nitrogen, and mineral balance. One group of hamsters was fed the bulking agent silica flour. Data from trial 1 are presented in Table 5. Hamsters fed the 10% silica flour, 10% or 20% lignin diets consumed significantly more feed than those fed the remaining diets. Those hamsters fed the highest level of lignin ingested nearly the same amount of feed as those fed Alphacel but lost weight. Depressions in dry matter digestibility were observed when hamsters were fed the 20% or 30% levels of lignin. A significant increase in acid detergent fiber digestibility was noted for those animals fed 10% lignin while no differences were evident between any of the remaining treatments. It appears that at the low level addition of lignin more efficient fermentation of fiber occurs than when silica flour or Alphacel are used as fiber sources. Higher levels of lignin appear, however, to negate this response. A significant linear decrease in acid detergent lignin digestibility occurred with increasing lignin level in the rations. All lignin digestion coefficients were relatively high

Table 5—Weight gains, feed intakes, apparent dry matter, nitrogen and fiber digestibilities, and nitrogen and mineral retentions for hamsters in trials 1 and 2

	Trial 1 Treatment						Trial 2 Treatment			
	10% Silica flour	10% Alphacel	10% Lignin	20% Lignin	30% Lignin	Pooled SE	10% Silica flour	30% Cornstalk Lignin-Hemicellulose	30% Soybean Residue Lignin-Hemicellulose	Pooled SE
Animal performance										
Weight gain/loss (g/day)	+0.4[a]	+0.4[a]	+0.4[a]	+0.3[a]	−0.3[b]	±0.1	+0.5[a]	+0.7[a]	+1.2[b]	±0.2
Feed intake (g/day)	6.9[a]	6.7[b]	6.8[a,b]	6.9[a]	6.6[b]	±0.03	6.8[a]	7.8[b]	8.5[c]	±0.3
Digestibility (%)										
Dry Matter	85.4[a]	84.5[a]	84.7[a]	75.2[b]	70.3[c]	±1.8	84.3[a]	88.7[a]	89.3[a]	±1.3
NDF	–	–	–	–	–	–	–	51.9[a]	53.4[a]	±2.8
ADF	36.2[a]	26.5[a]	58.1[b]	39.4[a]	26.2[a]	±9.7	–	–	–	–
ADL	–	87.4[a]	82.6[b]	74.4[c]	62.9[d]	±0.9	–	–	–	–
Nitrogen	87.2[a]	83.6[a]	83.1[a]	83.6[a]	85.5[a]	±0.5	86.3[a]	84.7[a]	85.2[a]	±0.9
Nitrogen balance										
Intake (g/day)	1.54[a]	1.55[a]	1.55[a]	1.55[a]	1.36[b]	±0.01	1.50[a]	1.82[b]	2.03[c]	±0.05
Excretion (g/day)										
Fecal	0.20[a]	0.26[b]	0.26[b]	0.26[b]	0.19[a]	±0.02	0.21[a]	0.26[b]	0.30[c]	±0.02
Urinary	0.78[a]	0.68[a]	0.73[a]	0.77[a]	0.82[a]	±0.06	0.75[a]	0.84[a]	0.91[a]	±0.06
Total	0.98[a]	0.94[a]	0.99[a]	1.03[a]	1.01[a]	±0.05	0.96[a]	1.10[b]	1.21[c]	±0.06
Absorbed, g/day	1.34[a]	1.29[b]	1.29[b]	1.29[b]	1.17[c]	±0.02	1.29[a]	1.58[b]	1.73[b]	±0.05
Retained, g/day	0.56[a]	0.61[a]	0.56[a]	0.52[a]	0.34[b]	±0.06	0.54[a]	0.73[b]	0.82[b]	±0.07
Mineral balance										
Mineral retention/loss (% of mineral intake)										
Ca	51.2[a]	54.2[a]	89.4[b]	64.3[a]	57.5[a]	±9.5	49.8[a]	72.9[b]	82.4[b]	±2.5
Mg	−15.2[a]	23.0[b]	86.5[c]	80.0[c]	79.4[c]	±12.4	−13.7[a]	54.2[b]	69.4[c]	±2.9

[a,b,c,d] Means in the same row and within the same data set bearing different superscripts differ ($P < 0.05$). SE = ± Standard error of the mean

considering that lignin is oftentimes regarded as an inert fraction of the plant which is supposedly resistant to digestion. The purified lignin used in this experiment was a finely ground, powder-like material with a great deal of surface area. In this form, then, lignin is apparently well fermented by the microbial population in the hamster gastrointestinal tract resulting in a net loss of this moiety with passage through the tract. Normal plant lignin would differ from this purified form in its physical properties (surface area, particle size) rather than its chemical properties but both are important in affecting metabolic responses of animals to fiber additions to diets.

No effect on nitrogen digestibility occurred between treatments although nitrogen balance was affected by treatment. Animals fed the 30% lignin-containing diets ingested significantly less nitrogen than did animals fed any of the remaining diets. Fecal nitrogen was greater ($P < 0.05$) when animals were fed the 10% Alphacel, 10% lignin, and 20% lignin diets while less fecal nitrogen was excreted by hamsters fed 30% lignin. This could be due to the lower nitrogen intake of animals on this diet. Urinary and total nitrogen excretion were unaffected by treatment. Hamsters fed 10% silica flour absorbed more ($P < 0.05$) nitrogen than did those on the other treatments. Significantly less nitrogen was both absorbed and retained by hamsters fed diets containing 30% lignin. It appears that nitrogen retention is unaffected by lignin at levels of 10 and 20% of the diet but is significantly decreased at levels between 20–30%.

The fiber sources fed in this trial markedly affected mineral balance. Animals fed the 10% lignin diet retained significantly more Ca than did those fed the 10% silica flour-containing diet. Silica flour caused a significant depression in Mg balance as compared to hamsters fed 10% Alphacel and the three levels of lignin.

Hamster trial 2 was carried out to evaluate a lignin-carbohydrate complex, namely lignin-hemicellulose (LH) and its effect on the metabolic parameters measured in trial 1. Normally, lignin is tightly bound to hemicellulose in the plant cell wall and drastic chemical treatments, such as those employed in the preparation of the Kraft lignin used in trial 1, are needed to free the carbohydrate from the complex polyphenolic material. These chemical treatments may alter the properties of lignin to such an extent that the characteristics of native plant lignins may be lost. Different metabolic responses may then be elicited when this type of a prepared lignin is fed. Therefore, a natural form of plant lignin (bound to hemicellulose) was isolated and fed to hamsters in this experiment. LH was fed at the 30% level since hamsters have the ability to use relatively large quantities of fiber and since the LH complex comprises approximately 30% of the fiber present in cornstalks and soybean residue (the cornstalks used contained 2.4% acid detergent lignin and 30% hemicellulose; the soybean residues used contained 13.4% acid detergent lignin and 14% hemicellulose). Data from trial 2 are presented in Table 5. Hamsters fed LH isolated from soybean residue gained significantly more weight and ingested significantly more feed than did those fed 10% silica flour or 30% cornstalk LH while animals fed LH isolated from both low quality roughages ingested more ($P < 0.05$) feed than did those fed the 10% silica flour-containing diet. Digestibilities of dry matter, neutral detergent fiber and nitrogen were unaffected by treatment. Nitrogen intake paralleled feed intake in that hamsters fed LH fractions ingested more ($P < 0.05$) nitrogen than did those fed silica flour with soybean residue LH-fed animals consuming more ($P < 0.05$) nitrogen than those fed cornstalk LH. Fecal nitrogen and total nitrogen excretion followed this same pattern while no differences ($P < 0.05$) in urinary excretion of nitrogen were noted between treatments. Equivalent amounts of nitrogen were absorbed and retained by hamsters fed the LH-containing diets and these values were greater than those for animals fed 10% silica flour. This increase in the amount of absorbed and retained nitrogen simply reflects the increased feed intake of hamsters fed the LH fractions. Several studies with humans (Kies and Fox, 1977) indicate a greater negative effect on nitrogen metabolism of hemicellulose than of cellulose or pectin but it appears that this effect is not prevelant in nonruminant herbivores ingesting hemicellulose bound to the lignin moiety. The increased feed intake observed in this trial could be due to a variety of reasons. Hamsters, like other species, eat to meet their energy needs and it is possible that the inclusion of 30% LH into these diets seriously limited the energy available to the animal. However, the hamster possesses the ability to ferment LH to volatile fatty acids and in turn use these to help alleviate their energy deficit. Also, hemicellulose is generally easily fermented by gastrointestinal tract microbes. Another possible reason for the increased feed intake could be due to the inclusion of hemicellulose in the diets. Keim and Kies (1979) observed that mice fed 10% hemicellulose tended to consume more ration and thus tended to gain more weight than did mice fed 5% or 20% hemicellulose. Mice fed 20% hemicellulose gained slightly more weight than did the mice fed 5% hemicellulose.

Mineral balance data for trial 2 are presented in Table 5. Hamsters fed diets containing LH isolated from both cornstalks and soybean residues retained significantly more Ca and Mg than did those fed silica flour. As in trial 1, animals fed silica flour were in negative Mg balance. Animals fed cornstalk LH retained 54.2% of dietary Mg while those fed soybean residue LH retained significantly more, 69.4% Mg.

Spiller and Shipley (1977) point out that an important and controversial issue regarding the advantages or disadvantages of adding fiber to diets centers around its effect on the absorption of certain mineral elements including Ca, Fe, Mg, P, and Zn. In studies with humans, most reports indicate that mineral absorption is decreased by fiber additions to diets (Kelsay, 1978). However, little data exist with regard to the effects of fiber on mineral nutrition of nonruminant animals. From these experiments, it appears that type and level of fiber can have marked effects on mineral metabolism. In trial 1, retentions of Ca by hamsters fed 10% silica flour and 10% Alphacel were approximately equivalent. However, hamsters fed 10% silica flour lost more Mg than they ingested resulting in a body loss of approximately 15% Mg. Retention of Mg by hamsters fed 10% Alphacel was low but the animals were in positive balance (23% retention). Ca retention was highest when animals were fed 10% lignin whereas animals fed diets containing 20% and 30% lignin retained as much Ca as those fed 10% silica flour and 10% Alphacel. Mg retention values were significantly higher at all lignin levels as compared with values for animals fed 10% silica flour or 10% Alphacel. At present, it is unclear whey feeding 10% silica flour-containing diets should cause negative Mg balance. This was observed in both hamster trials and may be related to its high content of SiO_2 which could affect absorption of Mg. The inclusion of LH fractions into diets fed in trial 2 improved ($P < 0.05$) Ca and Mg retention by hamsters. This was surprising in light of the fact that various fibers possess cation exchange activity and workers (Branch et al., 1975; James et al., 1978) have shown that calcium binding is correlated with the unsubstituted uronic acid content of hemicellulose. In this experiment, cornstalk LH contained approximately 30% hemicellulose while soybean residue contained approximately 14% of this polysaccharide. LH's isolated from both residues produced equivalent responses in Ca retention while feeding soybean residue LH elicited a greater ($P < 0.05$) Mg retention as compared to cornstalk LH. Since

both LH's affected mineral retention similarly, it is possible that these complexes isolated from cornstalks and soybean residues do not possess the unsubstituted uronic acid groups that are components of hemicelluloses isolated from grasses and grains. Ability to bind minerals would be reduced considerably in the absence of these groups. Specificity of minerals for binding sites on fiber molecules and selective competition between minerals in the presence of fiber appears to be a complex phenomena and little animal data have been collected on this subject. It would appear, however, that type, source, and level of fiber added to diets might play a major role in mineral balance of nonruminants.

In conclusion, there is little doubt that animal performance, nutrient digestibility, nitrogen balance, and mineral balance are affected by type, source, and level of fiber in the diet. Further work is needed to identify those fibers which maximize each of these parameters.

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MALONALDEHYDE CONCENTRATIONS IN FOOD ARE AFFECTED BY COOKING CONDITIONS

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ABSTRACT

We studied the relationship between cooking methods and the production of malonaldehyde (MA), a putative carcinogen in mice. In hamburgers pan-fried at moderate temperatures, the MA content increased 41–55%; frying at higher temperatures (shorter cooking times) produced smaller increases. Microwave cooking increased MA concentrations by only 15%. In contrast, cooking chicken by microwave, conventional oven, deep-fat-frying, and boiling increased MA concentrations by 60-, 55-, 17–19-, and 22-fold, respectively; the broth contained half of the MA produced in boiled chicken. On an equal portion basis, fried chicken contained less MA than hamburger. Appreciable amounts of MA were also found in smoked trout (2.2–3.8 μg/g), canned chicken broth (2.3 μg/g), bouillon cubes (5.3 μg/g) and cheese (0.44–0.74 μg/g) depending on cooking conditions.

INTRODUCTION

MALONALDEHYDE (MA) is a potential carcinogen. It is mutagenic to *Salmonella typhimurium* (Mukai and Goldstein, 1976; Marnett and Tuttle, 1980) and initiates skin tumors in mice (Shamberger et al., 1974). However, Marnett and Tuttle (1980) reported that much of its apparent mutagenicity may be due to the intermediates in MA preparation, and thus that MA is only a weak mutagen. Yet MA is a highly reactive compound (Mukai and Goldstein, 1976; Hüttel, 1941), and thus in vitro studies of mutagenicity may underestimate its true carcinogenic potential. MA is found in many foodstuffs (Shamberger et al., 1977; Siu and Draper, 1978) and the MA content of meats may have epidemiological significance (Shamberger, 1977), possibly accounting for a part of the strong correlation between per capita meat consumption and cancer incidence in various countries (Cairns, 1975). Therefore, factors influencing the MA content of foods are of concern. Even though this aldehyde is a product of lipid peroxidation, it is not associated only with rancidity; wholesome meats contain significant amounts of the aldehyde. Shamberger et al. (1977) found a range of 1–14 μg/g and 0.3–39 μg/g MA in raw and cooked meats, respectively; Siu and Draper (1978) obtained a range of 0.35–2.9 μg/g in raw meats and 0.7–5.3 μg/g in cooked meats. In some meat products the cooking process itself increases the MA content.

We hypothesized that the formation as well as degradation of this aldehyde would be affected by the temperature, time of cooking, mode of heat transfer to the food, as well as the acidity of the food. Thus, we measured the MA content of selected foods cooked by various means: (a) by conduction i.e., pan-frying and deep-fat frying; (b) by convection (and radiation), i.e., roasting in an oven; (c) by microwave radiation; and (d) by boiling. The production of MA by microwave cooking is of particular interest because of its increasing popularity among consumers.

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EXPERIMENTAL

THE MA CONTENT of food samples was analyzed by a modification of the method prescribed by Shamberger et al. (1977). A 25-g representative sample of each solid food was homogenized with 250 ml of 0.9% NaCl *aq.* in a Waring laboratory Blendor at high speed for 3 min. The homogenate was mixed with 250 ml of a 10% trichloroacetic acid solution (TCA). The acidified suspension was filtered through Whatman #1 filter paper, and the clear filtrate was analyzed directly for MA content. Liquid food samples (250 ml) were homogenized for 3 min, acidified with 250 ml of 10% TCA, filtered, and aliquots of the filtrate were analyzed for MA.

A 5% hot aqueous solution of thiobarbituric acid (Aldrich Chemical Co., St. Louis, Mo.) was purified on a steam jacketed alumina column to eliminate most of the color associated with the crystals. Upon cooling, the crystals were filtered and dried in a vacuum desiccator. A 1% aqueous solution of purified thiobarbituric acid, freshly decolored with activated charcoal, was used for the determination of MA.

One milliliter of the thiobarbituric acid (TBA) solution was mixed with 4 ml of the acidified filtrate of the food sample and then heated in a boiling water bath for 15 min. After cooling in tap water, the absorbance at 532 nm was measured in a Beckman spectrophotometer. The absorbance was compared with that of a series of standards prepared from malonaldehyde *bis*-(dimethylacetal) (Aldrich Chemical Co.). Standard solutions made up in saline were combined with an equal volume of 10% TCA and heated so that from 0.25–1.0 μg of MA per milliliter of final solution would be released. The absorbance was directly proportional to the MA concentration over this range. Final values were corrected to account for the 80% yield of TBA positive material from the standards (Shamberger et al., 1977), and a 62% recovery of TBA positive material from the samples.

A large sample of ground beef was obtained from a local supermarket and formed into 100-g patties 2 cm thick and approximately 9 cm in diameter. These hamburgers underwent the treatments described in Table 1, and all were analyzed simultaneously for their MA content. All hamburgers were cooked to the same degree of doneness, as assessed by a sensory evaluation panel of three individuals.

Fresh chickens were obtained from a single lot at a supermarket; they were cleaned and split before use. The halves were subjected to different treatments, and all were cooked to an internal temperature of 79–80°C. Each chicken sample was boiled in 1050 ml of tap water.

One piece of cheddar cheese was divided into 50-g portions, and each portion was treated as described in Table 1. All cheese portions were cooked in glass casserole dishes.

Also analyzed were: sirloin tip roasts (900–1000g) obtained from a local supermarket; smoked Canadian Arctic char (trout), stored frozen for 6 months; "Campbell's" canned chicken broth; and "Steero" chicken bouillon cubes.

Data are presented as means ± their standard errors; differences between means were analyzed for significance by Student's t test.

RESULTS

TABLE 1 shows the MA content of hamburger, chicken, sirloin tip roast, cheddar cheese, smoked arctic char, canned and freshly prepared chicken broth, and chicken bouillon. The MA content of hamburger cooked in three different ways showed increases over that of raw hamburger. Pan-frying in stainless steel and cast iron resulted in a 41–55% increase of MA ($P < 0.05$). Microwave cooking (650 watts) resulted in an increase of only 15%. However, in sirloin tip roast, cooking by microwave or conventional oven caused a

Table 1—Malonaldehyde content of selected foods cooked under various conditions

Foodstuff	Treatment	No. of samples	Malonaldehyde content (μg/g)
Hamburger	Raw	5	1.67 ± 0.09
	Microwaved for 3 min[a]	3	1.92 ± 0.06
	Fried on stainles steel skillet for 7 min at 163 ± 14°C	5	2.58 ± 0.39
	Fried on cast iron skillet for 7 min at 163°C	3	2.35 ± 0.17
Chicken, whole	Raw	4	0.10 ± 0.03
	Microwaved for 12 min	3	6.13 ± 0.35
	Boi ed for 30 min	3	2.23 ± 0.32
Broth	From whole chicken (above)	3	0.41 ± 0.02
	Deep-fat-fried in cast iron skillet for 45 min at 191°C	2	1.69 ± 0.14
	Deep-fat-fried in stainless steel skillet for 45 min at 191°C	2	1.69 ± 0.14
	Baked in conventional electric oven for 90 min at 177°C	3	5.51 ± 0.45
Chicken, skinned	Raw	2	0.44 ± 0.08
	Microwaved for 12 min	2	2.88 ± 0.14
	Baked for 110 min at 177°C	2	5.23 ± 0.01
	Boiled for 30 min	2	2.43 ± 0.49
Broth	From skinned chicken (above)	1	1.37
Canned chicken broth (Campbell's)		2	2.34 ± 0.14
Chicken bouillon cubes		2	5.29 ± 0.47
Sirloin tip roast	Raw	2	1.62 ± 0.22
	Microwaved for 20 min	1	0.72
	Roasted for 120 min at 163°C	1	0.82
Smoked trout, frozen	Raw	1	3.84
	Microwaved for 4 min	1	2.5
	Baked for 2 hr at 90°C while wrapped in aluminum foil	1	2.24
Cheddar Cheese	Raw	2	0.53 ± 0.11
	Browned in microwave oven for 12 min	2	0.44 ± 0.04
	Melted – 12 min at 177°C (conventional oven)	2	0.47 ± 0.05
	Browned – 34 min at 177°C (conventional oven)	2	0.74 ± 0.08

[a] All microwave cooking exposed food to 650 watts of power

decrease in MA concentrations of approximately 50%.

The MA content of chicken meat appears to be greatly increased by cooking. Deep-fat-frying increases the MA content 17- to 19-fold ($P < 0.001$). But cooking by either microwave or conventional oven produced even greater increases of MA, i.e., 60- and 55-fold, respectively ($P < 0.001$). It is interesting to note that microwave cooking results in as much MA as oven cooking, despite the shorter duration of cooking, i.e., 12 min vs 90 min.

Removal of chicken skin before microwave cooking tends to minimize the formation of MA; only slightly more than half of the aldehyde is formed in skin-free chicken as in whole chicken ($P < 0.005$). This may be due to the fact that the skin of the chicken is subjected to intense heating effects by microwaves. In conventional oven cooking, the MA content in both skinned and whole chicken are essentially the same. It is possible that MA formed in the skin

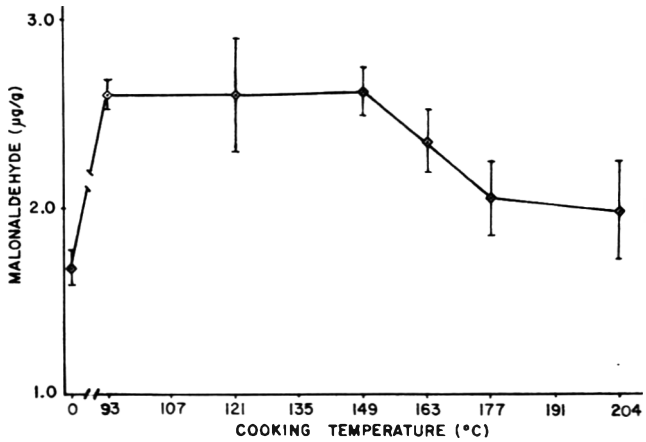

Fig. 1—Malonaldehyde content of hamburger as a function of cooking temperature. (n = 3).

could be more easily lost during prolonged cooking in the oven than that formed within the muscle tissues.

The effect of boiling was tested on chicken, and this cooking procedure (30 min) also increased the MA formation by 22-fold as compared to raw chicken ($P < 0.005$). However, this value represents only part of the total MA formed; a significant amount is found in the broth. About 50% of the measurable MA is found in the broth in the case of boiled skinned chicken; and 25% is found in the broth in the case of boiled whole chicken. When the total amount of MA (i.e., in meat and broth) is considered, boiling skinned chicken produces more MA than cooking it by microwave.

There is also a difference in MA content of broth from chicken boiled with the skin and that boiled without; more MA is formed in the latter. It seems that on a weight for weight basis more MA is formed in the skinned than in the whole chicken. This may be due to greater formation of MA in muscles than in adipose tissues at moderate cooking temperatures. Presumably, the higher iron content in muscle can increase catalysis in the formation of MA from its precursors. Iron is known to catalyze unsaturated fatty acid peroxidation (Bidlack and Tappel, 1972).

Subjecting cheddar cheese to moderate heating does not influence the MA content. This may reflect the low level of unsaturated fatty acid in this cheese. However, browning in the oven tends to increase the level of MA somewhat.

We determined the amount of MA formed in hamburger cooked in the frying pan to the same degree of doneness at 93–204°C; thus the cooking time varied from 10 min at 93°C to 4–4½ min at 177–204°C. This test was done because there is usually such variability in the time and temperature of cooking such products. As shown in Figure 1, the maximum amounts of MA are detected in hamburgers cooked at temperatures between 93 and 149°C; above this temperature, the level of MA decreases and appears to remain constant between 177 and 204°C. The lower level of MA at the higher temperatures may be the result of either decreased formation due to decreased cooking time, increased volatility or destruction, or any combination of these factors. However, it is interesting to note that the MA content in hamburger cooked by microwave for 3 min is comparable to that formed by pan frying for 4 min at 177–204°C.

The high level of MA in smoked Arctic char decreased by approximately 40% when it was heated at 90°C in the oven for 2 hr or cooked in the microwave oven for 4 min. This decrease may be due to volatility. Nevertheless, rela-

tively high levels remain. The finding of high levels of MA in this Arctic trout adds another possible carcinogen to the increasing list found in smoked fish, e.g., the polycyclic aromatic hydrocarbons and the nitrosamines (Grasso and O'Hare, 1976).

When considering the MA in raw meats, it was found that chicken has the lowest concentration, beef significantly more, and smoked frozen Arctic char the most. This is consistent with the increased peroxidation of these foods with increased storage time. Chicken is sold as fresh as possible for optimum palatability. Beef, on the other hand, is aged for up to several weeks for increased tenderness and flavor. Fish stored frozen for prolonged periods is highly susceptible to peroxidation (Kuusi et al., 1975). Thus the MA content of raw food seems quite sensitive to the duration of food handling. This hypothesis is further supported by the following observation: The initial batch of chicken purchased for this experiment had an MA content of 0.07 ± 0.02 μg/g; when additional chickens were purchased from the same lot 5 days later, the MA content had already increased to 0.44 ± 0.08 μg/g, more than a sixfold increase ($P < 0.05$).

DISCUSSION

THE THIOBARBITURIC ACID assay used in this study measures both preformed MA and any that is released when heating acidified food samples containing some putative precursor to MA, presumably hydroperoxides. Thus any change in MA content of cooked foods may reflect a change in the level of MA precursors, free MA or both.

The formation of MA (and possibly its precursors) in foods during cooking seems to be dependent on many factors, including the degree of unsaturation of the fatty acids and the length of time spent in contact with molecular oxygen. However, the amount found in cooked food samples also may depend on the loss of free MA due to its volatility or reactivity.

On the whole, cooking chicken seems to produce higher amounts of MA than cooking beef. This is consistent with the concept that the higher levels of unsaturated fatty acids found in chicken [22% of fat as 18:2, 1.5% as 18:3, and 1.5% as 20:4 (Fristrom and Weihrauch, 1976)] make it more susceptible to attack by molecular oxygen than ground beef [2.5% of fat as 18:2, 1.3% as 18:3 and 0.3% as 20:4 (Anderson et al., 1975)]. Roasting chicken for prolonged periods, which provides for the longest reaction time, seems to produce the highest levels of MA. However, comparing other methods of cooking chicken displays several unexpected results. Microwave cooking provides a much shorter contact time between the chicken and atmospheric oxygen, but when chicken is cooked with its skin, the MA level is comparable to chicken baked in a conventional oven. It appears that the physical and chemical reactions occurring on the surface of foods during microwave cooking require further systematic study.

Another surprising result is the relatively high amounts of MA formed in chicken during boiling. The water had been degassed before the chicken was boiled; however, the fat that floats to the surface of the water does come in contact with air, perhaps accounting for this finding.

The lower levels of MA found in deep-fat-fried chicken may be due to increased losses of MA at higher cooking temperatures.

Hamburgers were cooked to the same degree of doneness at various temperatures, thus cooking times were inversely related to cooking temperatures. A correlation between cooking temperatures and MA concentration in hamburgers is illustrated in Figure 1. The highest temperatures (shortest cooking times) (see Table 1) resulted in the lowest MA concentrations, possibly due to the shorter period of contact with molecular oxygen during cooking, and increased losses of MA, perhaps due to increased volatility or destruction. Lower cooking temperatures (longer cooking times) resulted in higher concentrations of MA, possibly because the rate of formation exceeds the rate of loss. However, it is interesting to note that roasting sirloin beef resulted in lower MA concentrations than were found originally in the raw beef, indicating that in this case the rate of MA loss was greater than the rate of formation, even at this low temperature.

The MA content of smoked frozen fish was quite high before cooking, and also decreased during cooking either by microwave or in a conventional oven at a low temperature. Apparently, the rate of loss of MA (and its precursors) again was greater than its rate of formation under these circumstances.

Because of the unexpected amounts of MA in boiled chicken, we measured the MA in commercial chicken bouillon. In terms of bouillon solids, the MA content was 5.29 ± 0.47 μg/g, or about 22 μg per serving (1 cube). Relatively high levels were also found in canned chicken broth (Campbell's). Thus, in the United States, commercial chicken products may represent a significant source of total MA load in the body. However, fried chicken contained less MA than that prepared by the other methods of cooking that we tested, and indeed one may consume less MA per portion of fried chicken than per portion of fried hamburger. (Both are served in portions of approximately equal weight.)

While it is still too early to definitively assess the oncogenic significance of MA in humans, its chemical and toxicological properties are impressive enough to warrent serious consideration regarding its effects on health. This compound will react with guanine and cytosine in vitro as well as in vivo and thus alter DNA structures (Reiss et al., 1972; Brooks and Klamerth, 1968). Certain compounds similar to MA in structure are strongly oncogenic, e.g., glycylaldehyde (Van Duuren et al., 1965) and β-propiolactone (Palmes et al., 1962). MA may be implicated also in the oncogenic mechanism of such potent carcinogens such as 7,12-dimethylbenz(a)anthracene, 3,4-benzpyrene and 3-methylcholanthrene. MA is one of their metabolic products (Shamberger et al., 1974; Shamberger, 1977), and its great reactivity to DNA suggests that the carcinogenicity of these compounds may be related to their ability to form MA.

This compound was observed to be mutagenic to *S. typhimurium* (Mukai and Goldstein, 1976); however, Marnett and Tuttle (1980) report that much of this mutagenicity was due to intermediates in its preparation and that MA per se is only weakly mutagenic. But MA is a highly reactive compound and may not reach the nuclei of *Salmonella* cells because of interference of various components of the test medium. The less reactive MA precursors perhaps can enter the nuclei more easily and release MA where it will be most damaging. MA that is released only after ingestion (e.g., by means of stomach acid) may be as damaging as the free MA in foods. Our method of analysis does not differentiate between free MA and its precursors.

The ease with which MA (or its precursors) forms in certain beef, chicken, and fish products during storage and especially during the popular forms of cooking may make a significant contribution to the total carcinogenic load of humans in the United States and other countries with high per capita meat consumption. Therefore, consideration should be given not only to the standard end points of food preparation such as nutrient retention, palatability, and microbiological safety, but also to toxicological parameters.

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APPLICATION OF CRYOSCOPY FOR THE MEASUREMENT OF ENZYMATIC HYDROLYSIS OF LACTOSE

H. H. NIJPELS, P. H. EVERS, G. NOVAK, and J. P. RAMET

ABSTRACT

In an earlier report we proposed a new rapid method based on the measurement of the depression of the freezing point of a solution due to the hydrolysis of lactose. In addition, this report describes the influence of acidification on the accuracy and the reproducibility of this method.

INTRODUCTION

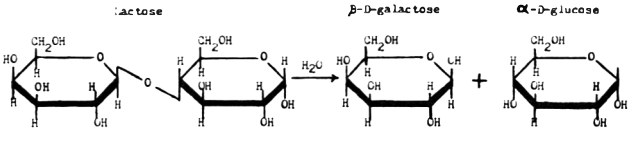
THE utilization of β-galactosidase for the preparation of low lactose milk for use in treatment of lactose intolerance problems in certain populations has been known for several years (Beaufrand et al., 1975; Davis and Bolin, 1967). In addition, fermented dairy products based on lactose hydrolyzed milk (Gyuricsek and Thompson, 1976; Mouillet et al., 1977; Olling, 1972; Ramet, 1978; Tamine, 1977; Thompson and Browers, 1974) and products based on hydrolyzed whey have found applications (Nijpels, 1977; Roger et al., 1976).

Several methods exist for controlling the hydrolysis of lactose. The most well known are classical methods, which are not only quite elaborate like gas chromatography (Nijpels, 1977; Pastuska, 1961) and enzymatic methods (Boehringer-Mannheim, 1977; Cheng and Christian, 1977; Merck-Darmstadt, undated), but which, also, require a relatively long testing time, sophisticated equipment and trained personnel and are, therefore, often less suitable for practical application. In an earlier report (Ramet et al., 1979) we proposed a new and rapid method based on the measurement of the depression of the freezing point of a solution due to the hydrolysis of lactose. In addition, this report describes the influence of acidification on the accuracy and the reproducibility of this method.

MATERIALS & METHODS

Principle of the method

Due to the enzymatic hydrolysis of lactose, the lactose molecule (molecular weight 342) is split into two simple sugars, glucose and galactose (molecular weight 180), according to the following reaction scheme:



This hydrolysis is accompanied by a corresponding decrease in the freezing point of the solution, defined by the relation:

$$\Delta T = -\frac{X}{M}K$$

where X = concentration of the (nonionic) substance in aqueous solution (g/kg); M = molecular weight of the substance; K = cryoscopic constant for water: K = 1.86.

So, for an aqueous solution of 50 g/kg of lactose in water the freezing point will be:

$$\Delta T_1 = -\frac{50}{342} \times 1.86 = -0.272°C$$

If lactose is totally hydrolyzed into glucose and galactose, the freezing point, taking into account a correction for the water bound by the reaction, will be:

$$\Delta T_2 = -\frac{52.6}{180} \times \frac{100}{99.74} \times 1.86 = -0.545°C$$

Complete hydrolysis of 50 g/kg of lactose in solution shows a reduction of the freezing point of −0.273°C. For milk, containing 50 g/kg of lactose, the freezing point, therefore, will decrease from approximately −0.540°C to −0.813°C. The degree of hydrolysis of lactose is, thus, reflected in the freezing point of the milk. If, in addition to the hydrolysis an acidification of the milk or other substrate occurs, this will result in a further decrease of the freezing point and erroneous results will be obtained.

Methods

To verify the hydrolysis we have measured the liberation of glucose and galactose in two different substrates (milk and ultrafiltration permeate) and we have determined the correlation between the proposed cryoscopic method and two other enzymatic methods (Boehringer-Mannheim, 1977; Merck-Darmstadt, undated).

β-Galactosidase preparation. The β-galactosidase preparation utilized for the hydrolysis was derived from the *Kluyveromyces (Saccharomyces) lactis* yeast which is sold by Gist-Brocades N.V. under the tradename Maxilact® (Nijpels, 1977).

Substrate. The milk was made by reconstituting low heat skim milk powder in water. The lactose content in solution was 42–50 g/L depending on the assay. The ultrafiltrate utilized was produced with a P 30 membrane using a Diaflo module yielding a lactose content of 44 g/L. The solutions were preheated to 30 or 35°C and placed in 150 ml flasks in thermostated water.

Conditions of hydrolysis. The β-galactosidase preparation was added to the substrate in concentrations of 500–6000 NLU/L. The chosen temperature was maintained during the hydrolysis.

Measuring the kinetics of the hydrolysis. Two different methods were compared: the enzymatic method and cryoscopic measurement.

Enzymatic method: the hydrolysis was followed by measurement of the galactose formed and measurement of the glucose formed.

Gryoscopic measurement: The kinetics of the hydrolysis were followed by measuring the decrease in freezing point with an Advanced Instruments high precision type Research Osmometer 3R.

The analyses were carried out simultaneously utilizing each method at t_0 (zero time) and then every 15 min during a period of 1.5 or 3.0 hr depending on the assay.

Influence of acidification on the measurement of the degree of hydrolysis of lactose by cryoscope. The acidification of milk causes a decrease in freezing point which can be added to the decrease in freezing point due to the hydrolysis of lactose. In order to separate these two effects, we followed the lowering of the freezing point due solely to the effect of acidification and due to the hydrolysis of lactose alone. To determine the joint effect of acidification and hydrolysis of lactose the lowering of the freezing point was studied

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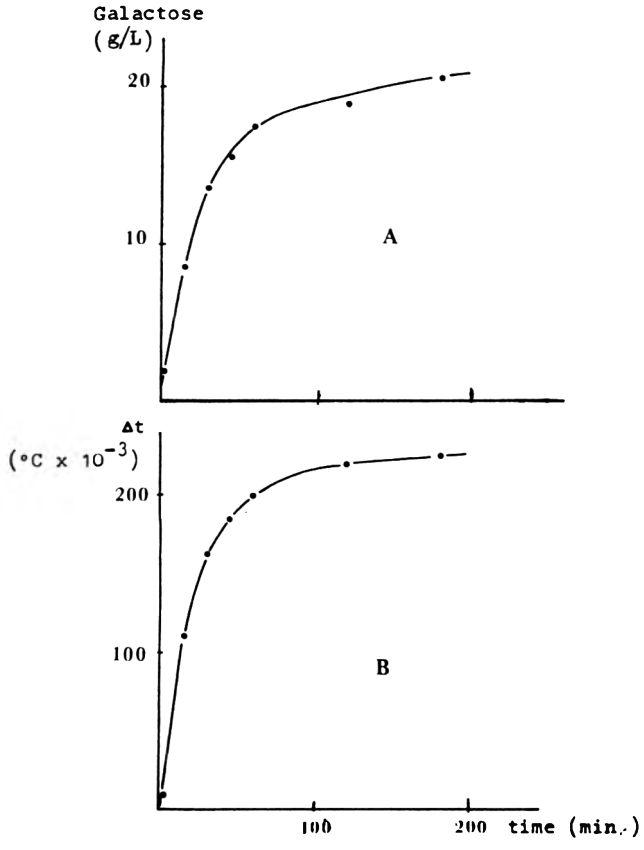

Fig. 1—Kinetics of lactose hydrolysis in milk: (A) Measured by enzymatic method; (B) Measured by cryoscopic method. Conditions of hydrolysis: substrate, reconstituted skim milk; lactose, 44 g/L; temperature, 35° C; lactase enzyme amount, 3000 NLU/L.

Table 1—Comparison of the enzymatic and the cryoscopic method in determining the hydrolysis of lactose. Conditions of hydrolysis: substrate, reconstituted skim milk; lactose, 50 g/L; temperature, 30° C; lactase enzyme amount, 500 NLU/L. CM = Cryoscopic method; EM = Enzymatic method

Incubation time (hr)	Percent hydrolysis									
	Assay 1		Assay 2		Assay 3		Assay 4		Assay 5	
	CM	EM	CM	EM	CM	EM	CM	EM	CM	EM
0	0	0	0	0	0	0	0	0	0	0
1	17	18	17	17	20	18	17	18	18	17
2	29	31	28	30	30	31	28	29	29	29
3	43	40	41	40	42	41	38	39	40	38
4	50	51	49	49	51	49	47	48	49	48

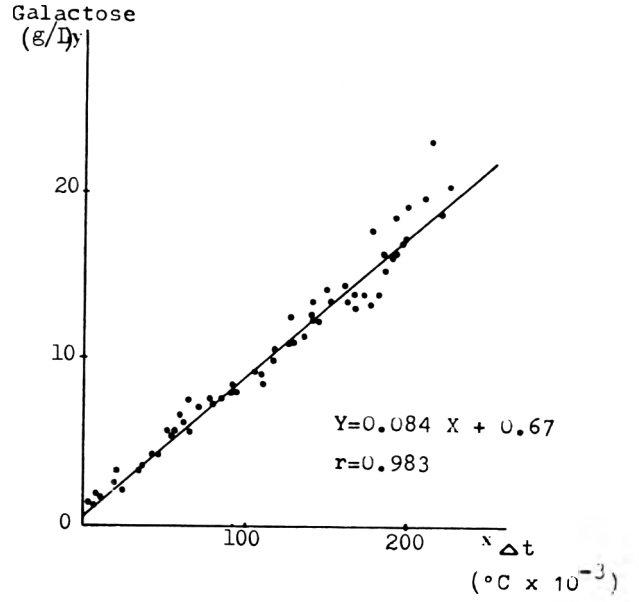

Fig. 2—Measurement of hydrolysis in milk: Correlation between the cryoscopic method and the enzymatic method (galactose dehydrogenase). Equation of regression line: y = 0.084 X + 0.67; Correlation coefficient: r = 0.983; Number of measurements: n = 63, F = 1749.

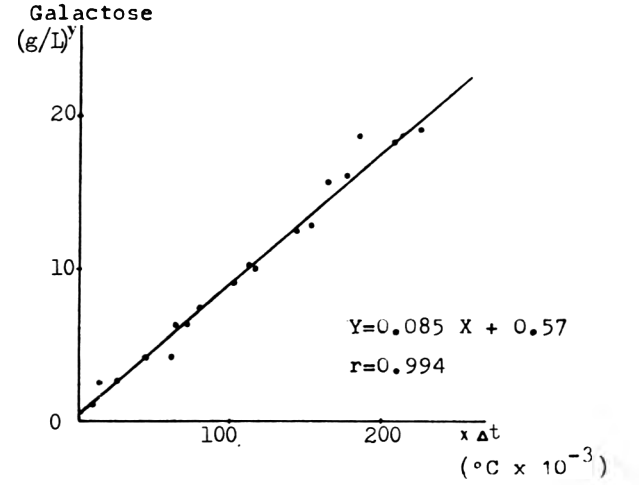

Fig. 3—Measurement of hydrolysis in UF permeate: Correlation between the cryoscopic method and the enzymatic method (galactose dehydrogenase). Equation of regression line: y = 0.085 X + 0.57; Correlation coefficient: r = 0.994; Number of measurements: n = 19, F = 1404.

when both processes were performed simultaneously. The acidification was obtained by using a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* inoculated at 2.5 and 5% and incubated in a waterbath at 45°C. The acidification was measured by a titrimetric method and expressed in ° DORNIC (tenths of a ml N/9 NaOH per 10 ml).

RESULTS & DISCUSSION

Correlation between cryoscopic and enzymatic methods

The cryoscopic method permits one to follow the hydrolysis very accurately (Fig. 1; Table 1).

The regression line between the enzymatic and cryoscopic method shows a very good correlation for the two substrates studied (Fig. 2 and 3); the F values show that the correlation is highly significant.

The similarity of the equations of the regression lines for milk and ultrafiltrate shows that there is no interference of the other constituents and hence that the variation of the freezing point is only a function of the hydrolysis of lactose. After all of the lactose in the milk has been hydrolyzed (lactose 50 g/L; enzyme 5000 NLU/L, 35°C, 4 hr), the freezing point difference is −0.274°C with respect to the control milk. This value is in good agreement with the theoretically calculated value of −0.273°C.

Precision

Taking into account the precision of the cryoscope util-

Table 2—Reproducibility characteristics of the cryoscope method. Hydrolysis conditions: substrate, reconstituted skim milk; lactose, 44 g/L; temperature, 35°C; lactase enzyme amount, 4,500 NLU/L; time, 3 hr

Number of measurements	Mean	Standard deviation	Variation coefficient
m = 10	$\bar{m}$ = −0.7930°C	σ = 0.0034	V = 0.43 percent

ized (5/10,000°C), the maximum relative error for the freezing point measurement is in the order of 1/1,000; this error is, therefore, negligible.

Reproducibility

In a series of 10 measurements of the freezing point of the same sample, measured after total hydrolysis of lactose (Table 2), the variation appeared to be 0.43%. The reproducibility of the proposed method can, therefore, be considered very satisfactory.

Influence of the acidification

The acidification of milk by lactic cultures is an important cause of differences in the freezing point of the milk. In milk subjected to acid production the freezing point varies linearly with the quantity of acid produced according to the equation:

$$Y = 3.25\ X + 4.53\ (°C \times 10^{-3}, °D)$$

The values of the correlation factors r and F show that this relation is highly statistically significant (Fig. 4).

This relationship is not applicable if: (1) an external acidification is performed; and (2) a heterofermentative fermentation takes place in which metabolites other than lactic acid are formed.

On the other hand, variability of the measurement will increase when curd formation and wheying off occur. The cause of this variation is the heterogenic character of two unstable phases (particles of curd dispersed in the whey) due to the acid production.

In the milk which is simultaneously subjected to the enzymatic hydrolysis and acidification by homofermentative bacteria, the lowering of the freezing point corresponds to the lowering of the freezing point due to the hydrolysis *plus* the lowering due to acid production. This cumulative effect has been clearly demonstrated in the experiments in which the hydrolysis proceeded rather quickly while at the same time the acidification proceeded slowly (see Fig. 5).

When the acidification proceeded quickly, the corresponding change in pH modified the activity of the β-galactosidase and the cryoscopic value did not add up to the sum of the values measured separately for the effect of the hydrolysis and the acidification. However, it equalled the lowering of the freezing point due to the acidification plus the lowering of the freezing point due to the hydrolysis modified by the pH effect (Fig. 6). It is possible that in a homofermentative acidification, a correction can be made by estimating the acidification by titrimetic methods.

CONCLUSION

THE PROPOSED cryoscopic method for controlling the enzymatic hydrolysis of lactose is remarkable by its simplicity; the reproducibility and accuracy are very good. For practical purposes the degree of hydrolysis of milk or ultrafiltrate permeate can be directly calculated using the correlation formulas we have derived. It is sufficient then to determine the freezing point of the nonhydrolyzed substrate. Because of the linear relationship between the freezing point depression and the degree of hydrolysis it is also possible to determine the coordinates of the point corresponding to 100% hydrolysis. The graph, then obtained, permits an easy determination of the degree of hydrolysis corresponding with the Δt-value. (Remark: this second method is an approximate method, the line does not fully pass through the origin. However, the error made by this approximation is negligible: 0.10%.) When acidification occurs, a correction for the lowering of the freezing point due to this phenomenon has to be made.

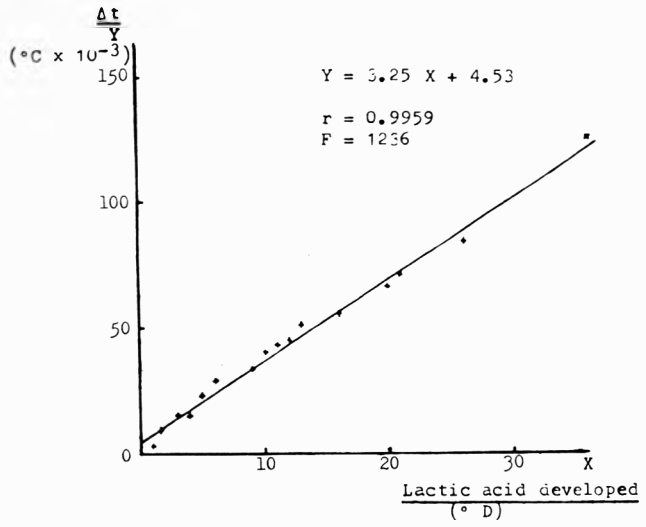

Fig. 4—Measurement of hydrolysis in milk: Correlation between lactic acid produced by homofermentative bacteria and the freezing point.

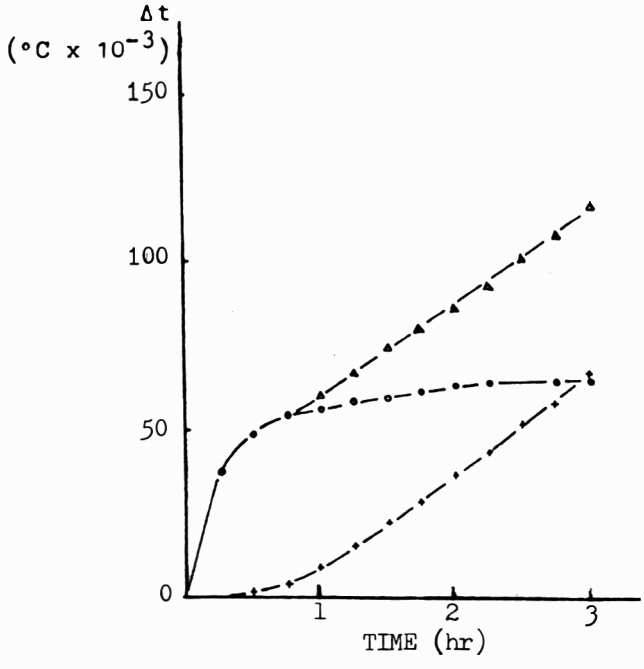

Fig. 5—Measurement of hydrolysis in milk: Simultaneous hydrolysis and acidification (▲), acidification alone (+), and hydrolysis alone (●). Reaction conditions: substrate, reconstituted skim milk; lactose, 44 g/L; temperature, 35°C lactase enzyme amount, 1000 NLU/L; culture, Streptococcus thermophilus and Lactobacillus bulgaricus 2.5%.

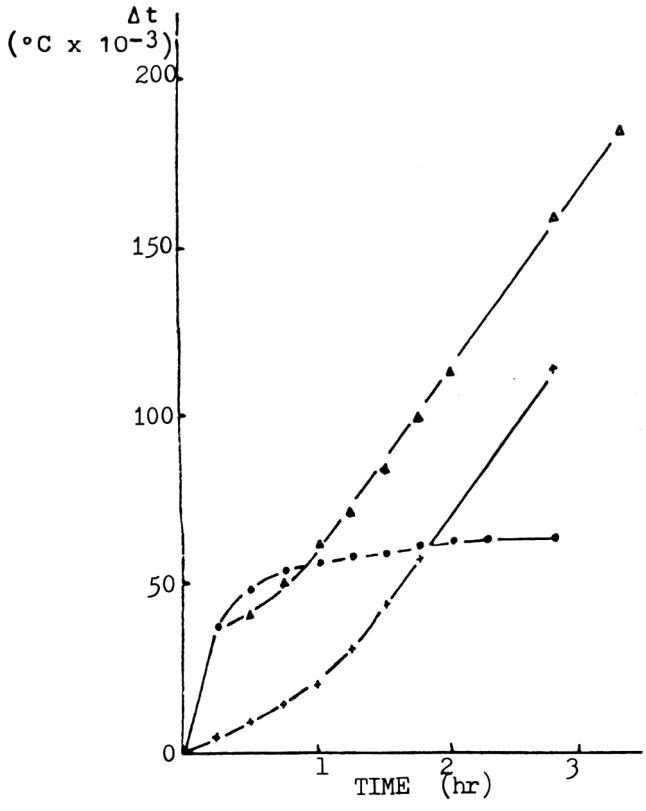

Fig. 6—Measurement of hydrolysis in milk: Simultaneous hydrolysis and acidification (▲), acidification alone (+), and hydrolysis alone (●). Reaction conditions: substrate, reconstituted skim milk; lactose, 44 g/L; temperature, 35°C; lactase enzyme amount, 1000 NLU/L; culture, Streptococcus thermophilus and Lactobacillus bulgaricus 5%.

The essential practical importance of this method is the short time it takes. The measurement of a freezing point can be done in less than 5 min. Moreover, most dairy laboratories are presently equippped with cryoscopes. It is, therefore, not necessary to make additional investments for materials and reagents. The cost of the analysis is consequently very low. For these various reasons this method is very convenient for the dairy industry: (1) to control the hydrolysis of lactose in various substrates; and (2) for testing the activity of β-galactosidase preparations.

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DIETARY FIBER, LIGNOCELLULOSE AND HEMICELLULOSE CONTENTS OF SELECTED FOODS DETERMINED BY MODIFIED AND UNMODIFIED VAN SOEST PROCEDURES

JUDITH A. MARLETT and SUNGSOO C. LEE

ABSTRACT

Neutral detergent residues (NDR) of 6 vegetables, 3 fruits, and 3 grain products were determined by the unmodified Van Soest ND procedure and by modifications which incorporated starch-or protein-hydrolyzing enzymes. Modification of the procedure significantly lowered NDR weights of several foods, with the greatest effect on grain products and high starch vegetables. Incubation with *B. subtilis* amylase and pancreatin (1 hr) yielded NDR which generally were not different from those incubated with hog amylase (18 hr). Modification of the procedure had little effect on lignocellulose values, determined by treating NDR with acid detergent solution, and more effect on hemicellulose values. Results suggest that amylase treatment, but not a long incubation, may be necessary for some foods.

INTRODUCTION

THE VAN SOEST PROCEDURES of fiber analysis, originally developed to determine fiber content of ruminant diets (Robertson, 1978), are being used to estimate the dietary fiber content of human foodstuffs (Robertson, 1978, Shipley, 1978). While the Van Soest methods (Goering and Van Soest, 1970; Robertson, 1978), in contrast to those recommended by Southgate (1969, 1976a), do not provide as much detail about the components of fiber in foods, they are simpler and quicker procedures. The major problem with fiber residues of human foods obtained by the Van Soest neutral detergent procedure is the significant contamination of the fiber with starch and/or protein (Robertson, 1978; Schaller, 1978). Modifications (Kimura, 1977; Robertson, 1978; Saunders and Hautala, 1978; McQueen and NIcholson, 1979) of the method have been proposed in an effort to eliminate these contaminants and at the same time retain the simplicity of the method.

Acid detergent residue (ADR) generated from a food sample will contain most of the cellulose and lignin; a portion of the pectic substances, and variable but small amounts of the hemicelluloses, also may be included in ADR whereas the neutral detergent residue (NDR) of the same food contains cellulose, lignin and water-insoluble hemicelluloses (Van Soest and Wine, 1967; Bailey and Ulyatt, 1970). Acid detergent treatment appears to extract pectin from nonleaf tissue but it does not remove all pectin from leaf tissue, including legumes (Bailey et al., 1978). Because of the differences between the two methods in recovery of hemicelluloses and pectins from different foods, estimation of hemicellulose content by subtracting ADR weight from the NDR weight can yield erroneous values of hemicellulose content. Based on studies of several clovers and grass varieties, Bailey and Ulyatt (1970) have recommended that ADR be quantitated by applying the acid detergent procedure to an NDR.

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The purpose of the experiments reported herein was to compare NDR and ADR contents of selected fruits, vegetables, and grain products obtained by modified and unaltered Van Soest procedures. In an attempt to identify a method which could be completed in 1 day, modifications in which samples were incubated with a mixture of alpha amylase and/or pancreatin at different points in the Van Soest procedure were developed for comparison with the modification which has been suggested by Schaller (Kimura, 1977). Lignocellulose content of several foods was estimated by subjecting residues obtained by modified and unmodified Van Soest neutral detergent procedures to acid detergent solution, as suggested by Bailey and Ulyatt (1970).

EXPERIMENTAL

Sample preparation

Foods were purchased locally and refrigerated less than 1 wk before use. Samples were blended with an equal weight of water, lyophilized, ground to 20 mesh in a Wiley Mill and stored in sealed bottles until analyzed. Cereals were not freeze-dried before analysis. To study the effects of cooking, peaches and apples (1/2 in. cubes) were brought to a boil and boiled for 15 min with a minimum amount of water, while tomatoes were cooked for 15 min without added water. Dry weights of all samples were determined by AOAC procedures (1975).

Sample analysis

Two to six lyophilized samples of each food were analyzed for total fiber by the Van Soest neutral detergent (ND) procedure (Van Soest and Wine, 1967; Goering and Van Soest, 1970), and by one or more of the following modifications; for some modifications two NDR samples were subsequently analyzed for ADR, whereas additional NDR were for nitrogen determinations. The effect of the use of varying amounts of glass wool (0.4–4.0g) as a filtering aid on the amount of NDR obtained by all modifications was also assessed.

Modification A. Lyophilized sample (1g) was preincubated in 100 ml ND solution containing 2 ml of stock bacterial α-amylase solution and 200 mg of pancreatin (Grade II, activity at least equivalent to The National Formulary; Sigma Chemical Co., St. Louis, MO 63178, P-1500) for 1 hr at 40°C, and then subjected to the regular ND procedure. The stock bacterial α-amylase solution was prepared by adding 4g of bacterial amylase, Type III (E.C. No. 3.2.1.1 from *B. subtilis*; Sigma: A-6505) to 90 ml of water and allowing solution to stand for 10 min. The mixutre was then filtered (Whatman #54) and 10 ml of 2 ethoxyethanol was added.

Modification B. Samples (1g) were boiled under reflux in ND solution for 30 min. The sample was removed from the heat and cooled to about 40°C, 2 ml of stock bacterial α-amylase solution and 200 mg pancreatin added, and the sample was incubated for 1 hr at 40°C. The sample was refluxed for another 30 min, washed, filtered and dried according to the unmodified ND procedure.

Modification C. Neutral detergent residue, prepared by the regular ND method, was incubated with α-amylase from hog pancreas (E.C. No. 3.2.1.1; 12 units/mg of α-amylase, 4 units/mg of β-amylase) for 18 hr at 37°C (Kimura, 1977), as suggested by the AACC. The residue was then washed with hot water and acetone and dried. Hog α-amylase solution was prepared by suspending α-amylase (Sigma: A-6880) (2.5% w/v) in 0.1M phosphate buffer, pH 7.0, followed by centrifugation and filtration of the suspension through a coarse sintered filter grit (Kimura, 1977).

Modification D. Bacterial α-amylase (2 ml of 2% solution) was added to a food sample (0.5g) which had been refluxed with ND solution for 30 min and removed from the heat (Robertson, 1978). The sample was returned to the hot plate, refluxed for an additional

30 min and then subjected to the remainder of the regular ND procedure.

When the shortest and simpliest modification, i.e. Modification A, gave values of the fiber content which were comparable to the value obtained with Modification C, the fiber content of the food sample was not always assessed by Modification B.

Lignocellulose contents of all samples were determined by boiling a dried NDR prepared by the Van Soest procedure or one of the above modifications, in 100 ml acid detergent (AD) solution for 1 hr under reflux (Bailey and Ulyatt, 1970; Goering and Van Soest, 1970; Van Soest, 1963). The residue was filtered, washed with hot water and acetone, dried at 100°C for 8 hr and weighed to determine ADR or lignocellulose content.

Starch and nitrogen analyses

Nitrogen content of the NDR was estimated in duplicate by the Kjeldahl method (Scales and Idorrison, 1920). Starch in the NDR generated by the regular ND procedure and by Modifications A and B was detected qualitatively with iodine (AOAC, 1975). A deep blue, a mixed blue, and a weak blue color were interpreted as a high (+++), moderate (++) or low (+) degree of starch contamination, respectively. Starch remaining in NDR which had been obtained using Modification C was quantitated by the AACC method (1978a) since Modification C has been recommended by the AACC (1978b) and was used in the present study as the NDR value against which other modifications were assessed.

Data comparison

Amounts of fiber in the foods are expressed as the mean ± SD percentage of the dry weight of the food. Significant differences (5% level) were determined using Duncan's new multiple range test (Steel and Torrie, 1960). Duncan's test requires use of the formula: standard error of the mean = (error mean square/number of observations in group)$^{1/2}$. When the groups were of unequal size, use of the smallest group size provided a conservative test.

RESULTS

THE USE OF GLASS WOOL as a filtering aid had no consistent or significant effect on NDR values obtained by any of the procedures (Table 1). Varying the amount of glass wool (0.4–4.0g) also had no effect, so the data in Table 1 are means of determinations using different amounts of glass wool. Glass wool was a useful adjunct and the filtration step required less manipulation and time when it was used. However, since variable amounts of glass wool are dissolved by AD solution (Brauer, 1979), NDR which were subjected to AD solution to determine lignocellulose content were done without glass wool.

Neutral detergent fiber analyses

Modification of the Van Soest ND procedure had the greatest effect on the NDR values of vegetables which contain significant amounts of starch or protein (Table 2). The effect of modifying the procedure on the amount of NDR obtained for spinach, carrots, and fresh and cooked tomatoes were small, although some of the differences were statistically significant (Table 2). Nitrogen contents of the Van Soest NDR of these four foods, as well as those of Modification A, were very low (<1%). The Van Soest residues of these foods were positive for starch (+); since those obtained by Modification A were not and since NDR of Modifications A and C were comparable, the NDR of these foods was not assessed by Modification B. Starch in Modification C NDR of these foods was not quantitated.

Because of the higher starch contents of corn and kidney beans, the fiber contents of these vegetables were determined by all three modifications. All three modifications yielded NDR values for corn which were significantly lower than the NDR obtained by the unmodified procedure (Table 2). The moderate amount of starch (++) detected with iodine in the Van Soest NDR of corn could not be detected in the NDR of either Modifications A or B, and starch content of Modification C NDR was low (<1%). The nitrogen content of corn NDR generated by the Van Soest procedure was 2.0 ± 0.09% and in Modification C corn NDR, it was 1.9 ± 0.34%; less than 1% nitrogen was found in Modifications A and B residues.

While all three modifications yielded significantly lower NDR values for kidney beans than did the Van Soest pro-

Table 1—Effect of glass wool filtering aid on neutral detergent fiber content of foods

	PRE-TRT (Mod.A)		MID-TRT (Mod.B)		POST-TRT (Mod.C)	
	%NDF[c]					
Food	–[a]	+[b]	–	+	–	+
Spinach	14.2 ± 0.0	13.9 ± 0.3				
Peach, fresh, peeled	4.3 ± 0.1	4.4 ± 0.1				
Bran Buds					26.0±0.6	26.7±0.3
Whole wheat bread	10.5 ± 0.2	10.1 ± 0.1	7.2 ± 0.2	6.9 ± 0.2	7.2±0.6(3)	7.7±0.4(3)
Apple, fresh, unpeeled			6.7 ± 0.0	6.5 ± 0.3		
Granola			2.9 ± 0.1	2.9 ± 0.6		

[a] No glass wool
[b] 0.4–4g glass wool
[c] Dry weight basis; n=2 except where indicated by number in parentheses.

Table 2—Effects of various modifications of neutral detergent (ND) procedure on ND fiber content of vegetables[a,b]

	Dry weight	Van Soest	PRE-TRT (Mod.A)	MID-TRT (Mod.B)	POST-TRT (Mod.C)
Food	%	%NDF[c]			
Spinach	10.2	15.1 ± 0.7	13.8 ± 0.5(4)[d]		13.4 ± 0.2[d]
Carrots	11.7	8.6 ± 0.1[d]	8.5 ± 0.3(3)[d]		8.2 ± 0.6(3)[d]
Tomato, fresh	5.3	12.8 ± 0.3(3)	11.9 ± 0.2(4)[d]		11.3 ± 0.3[d]
Tomato, cooked	5.2	14.8 ± 0.5(3)	13.5 ± 0.4(4)		
Corn, whole kernel	22.2	9.0 ± 0.0	5.6 ± 0.5[d,e]	5.2 ± 0.2[e]	6.4 ± 0.6[d]
Kidney beans	31.2	62.4 ± 0.0	21.6 ± 1.9	28.3 ± 3.1(4)	13.6 ± 0.4(4)

[a] Mean coefficients of variation: Van Soest, 1.9%; Mod.A, 4.9%; Mod.B, 7.4%; Mod.C, 4.9%.
[b] Values of each food with same superscript (d,e) are not significantly different.
[c] Dry weight basis; n=2 except where indicated by number in parentheses.

cedure, starch contamination of the residue remained high (+++) in both Modifications A and B, and in Modification C NDR, it was 5.0 ± 1.7%. The nitrogen contents of kidney beans NDR generated by Modifications A and B (1.5 ± 0.01 and 1.7 ± 0.13%, respectively) were slightly lower than those NDR obtained by the unmodified method and by Modification C, which were 2.7 ± 0.04 and 2.6 ± 1.0% nitrogen, respectively.

Generally, modifying the Van Soest procedure also significantly changed NDR contents of fruits (Table 3). Modification B yielded NDR values for bananas and apples which were lower than those obtained by the unmodified procedure and which agree with those generated by Modification C (Table 3). The moderate amounts of starch (++) which were detected qualitatively in the apple and banana unmodified NDR were not present in Modifications A or B NDR of banana or Modification B NDR of the apple sample. Modification A was not used to analyze the fresh, peeled, and the cooked, peeled apple samples because results from the fresh, unpeeled apple suggested it was not a suitable method for this food. To determine if there were any differences in the two methods for peaches, two peach samples were analyzed by both methods. Modifications A and B NDR of the three peach samples were comparable to those generated by the unmodified procedure, but not to those obtained by Modification C. None of the Modification B NDR was positive for starch, using iodine, and starch in Modification C NDR of fresh peach and fresh apple were low (<1%). Likewise, nitrogen could not be accurately detected (<1%) in any of the NDR of fruits generated with the unmodified procedure or with Modifications A or B or in fresh apple or peach Modification C NDR.

The three modifications, when applied to Bran Buds or Granola, gave similar NDR values, all of which were lower than those obtained by the unmodified Van Soest method (Table 4). Starch (++) which was detected with iodine in the unmodified method residue, was absent from Modification B residues of Bran Buds and granola and was low (<1%) in Modification C NDR residues. Small amounts of nitrogen (1.0–1.6%) were found in Bran Buds NDR obtained by the unmodified procedure, as well as by Modifications B or C.

Modifications B and C NDR values for whole wheat bread were similar and both were lower than those obtained by Modification A or the unmodified procedure (Table 4). Starch heavily contaminated (+++) the NDR sample obtained by the unmodified procedure and was still present (+) in modification A NDR of bread. No starch was detected with iodine in Modification B, while about 1% was quantitated in the bread Modification C NDR. The amounts of nitrogen in the unmodified and all modification residues were similar (1.7–2.2%).

The fiber content of kidney beans by the unmodified Van Soest procedure was 62.4%, in contrast to 54.9 ± 0.88% obtained by Modification D. While Modification D significantly lowered the NDR weight, it remained much higher than those obtained with Modifications A, B or C (Table 2). Both the unmodified Bran Buds NDR, 32.0% (Table 4) and the Modification D NDR, 30.6 ± 0.97% (n=2) were also significantly higher than those obtained by Modifications A, B or C (Table 2). Therefore, other foods were not analyzed by this method.

Acid detergent fiber analyses

In preliminary experiments, attempts were made to separate cellulose and lignin in several foods by the 72% sulfuric acid method (Goering and Van Soest, 1970). With the exception of spinach, all samples contained less than 1% apparent lignin. Lignin quantitation, therefore, was not continued and data presented are of lignocellulose (Table 5). Differences between ADR values obtained by treating the unmodified and modified NDR with AD solution generally were only about 1%. The greatest differences between methods were among those foods where starch or protein contamination of the NDR, which would be solubilized by the AD solution, might be significant (Table 5). Acid detergent analysis of Modification C NDR was performed on only two food samples, Bran Buds and kidney beans; the

Table 3—Effects of various modifications of neutral detergent (ND) procedure on ND fiber content of fruits[a,b]

Food	Dry weight %	Van Soest	PRE-TRT (Mod.A)	MID-TRT (Mod.B)	POST-TRT (Mod.C)
		%NDF[c]			
Banana, fresh, peeled	25.5	6.7 ± 1.0(4)	4.8 ± 0.8(4)	2.5 ± 0.3(4)	
Apple, fresh, unpeeled	8.6	8.4 ± 0.8(6)[d]	8.3 ± 0.7(4)[d]	6.6 ± 0.2(4)[e]	6.8 ± 0.6(3)[e]
Apple, fresh, peeled	8.1	7.8 ± 0.5(5)		5.6 ± 0.3(6)[e]	5.5 ± 0.0(2)[e]
Apple, cooked, peeled	8.6	9.9 ± 0.5(6)		7.0 ± 0.2(6)	
Peach, fresh, unpeeled	9.1	6.2 ± 0.3(6)[d]		5.9 ± 0.1(6)[d]	7.0 ± 0.3(5)
Peach, fresh, peeled	12.2	5.1 ± 0.4(6)[d]	4.8 ± 0.5(4)[d]	4.8 ± 0.2(6)[d]	2.4 ± 0.3(2)
Peach, cooked, peeled	10.2	7.2 ± 0.5(6)[d]	7.1 ± 0.1(6)[d]	6.5 ± 0.3(5)[d]	8.9 ± 0.9(2)

[a] Mean coefficients of variation: Van Soest, 7.8%; Mod. A, 9.3%; Mod. B, 4.8%; Mod. C, 7.1%.
[b] Values of each food with same superscript (d,e) are not significantly different.
[c] Dry weight basis; n indicated by number in parentheses.

Table 4—Effects of various modifications of neutral detergent (ND) procedure on ND fiber content of grain products[a,b]

Food	Dry weight %	Van Soest	PRE-TRT (Mod.A)	MID-TRT (Mod.B)	POST-TRT (Mod.C)
		%NDF[c]			
Bran Buds	95.4	32.0 ± 1.9	25.7 ± 0.9[d]	24.1 ± 0.7[d]	23.9 ± 0.7[d]
Granola	94.1	8.4 ± 0.4(2)	3.4 ± 0.3[d]	2.8 ± 0.1[e]	3.2 ± 0.0[d,e]
Whole wheat bread	93.2	16.4 ± 0.4(2)	10.5 ± 0.2(2)	7.2 ± 0.2(3)[e]	7.7 ± 0.4(3)[e]

[a] Mean coefficients of variation: Van Soest, 5.3%; Mod. A, 4.7%; Mod. B, 3.1%; Mod. C, 3.1%.
[b] Values of each food with same superscript (d,e) are not significantly different.
[c] Dry weight basis; n=4 except where indicated by number in parentheses.

Table 5—Effects of various modifications of neutral detergent procedure on acid detergent fiber content of foods[a,b,c]

Food	Van Soest	PRE-TRT (Mod.A)	MID-TRT (Mod.B)
	%ADF[d]		
Spinach	10.0 ± 0.3	10.1 ± 0.0	
Carrots	7.7 ± 0.1	7.6 ± 0.1	
Tomato, fresh	11.0 ± 0.1	10.2 ± 0.5	
Tomato, cooked	12.4 ± 0.1	11.1 ± 0.4	
Corn, whole kernel	3.1 ± 0.1	2.3 ± 0.0	2.3 ± 0.1
Kidney Beans	10.7 ± 1.9	8.6 ± 0.3	9.3 ± 0.2
Banana	1.0 ± 0.1	1.0 ± 0.2	
Apple, fresh, unpeeled	4.8 ± 0.1	5.0 ± 0.3	4.8 ± 0.2
Apple, fresh, peeled	4.4 ± 0.0		4.1 ± 0.2
Apple, cooked, peeled	5.0 ± 0.4		5.0 ± 0.1
Peach, fresh, unpeeled	4.3 ± 0.1		4.1 ± 0.0
Peach, fresh, peeled	3.5 ± 0.0	3.1 ± 0.0	3.4 ± 0.4
Peach, cooked, peeled	5.3 ± 0.3	5.4 ± 0.2	4.4 ± 0.4
Bran Buds	8.2 ± 0.3	8.4 ± 0.1	8.3 ± 0.2
Granola	1.2 ± 0.2	0.7 ± 0.4	
Whole Wheat Bread	1.3 ± 0.1	1.9 ± 0.1	1.9 ± 0.1

[a] Obtained by subjecting neutral detergent residue to acid detergent solution

[b] Mean coefficients of variation: Van Soest, 5.1%; Mod. A, 7.3%; Mod. B, 4.4%.

[c] Values for a food are not significantly different except for whole wheat bread where Mods. A and B are significantly greater than Van Soest.

[d] Dry weight basis; n=2 except for kidney beans Van Soest, n=4.

Table 6—Effects of various modifications of neutral detergent procedure on hemicellulose contents of foods[a,b]

Food	Van Soest	PRE-TRT (Mod.A)	MID-TRT (Mod.B)
	%Hemicellulose[c]		
Spinach	4.5 ± 0.5[d]	3.7 ± 0.1[d]	
Carrots	1.6 ± 0.3[d]	1.0 ± 0.4[d]	
Tomato, fresh	3.7 ± 0.5[d]	3.4 ± 0.4[d]	
Tomato, cooked	3.3 ± 0.2[d]	3.9 ± 0.3[d]	
Corn, whole kernel	10.1 ± 0.1	5.0 ± 0.1	4.6 ± 0.2
Kidney Beans	49.9 ± 2.3	12.1 ± 1.2	17.0 ± 1.0
Banana	5.6 ± 1.4[d]	3.7 ± 1.2[d]	
Apple, fresh, unpeeled	3.5 ± 0.9[d]	3.5 ± 0.3[d]	1.7 ± 0.4[d]
Apple, fresh, peeled	3.3 ± 0.8[d]		1.5 ± 0.1[d]
Apple, cooked, peeled	4.8 ± 0.1		1.8 ± 0.0
Peach, fresh, unpeeled	1.8 ± 0.2[d]		1.8 ± 0.1[d]
Peach, fresh, peeled	1.4 ± 0.0[d]	1.3 ± 0.1[d]	1.6 ± 0.2[d]
Peach, cooked, peeled	1.5 ± 0.3[d]	1.9 ± 0.3[d]	2.1 ± 0.1[d]
Bran Buds	24.1 ± 0.2	18.3 ± 0.9	
Whole Wheat Bread	9.5 ± 0.6	4.4 ± 0.1[d]	3.5 ± 0.4[d]
Granola	7.2 ± 0.5	2.8 ± 0.3	

[a] Determined as the weight lost by ND residue when subjected to AD solution.

[b] Values of each food with same superscript (d) are not significantly different.

[c] Dry weight basis; n=2.

Modification C ADR of these 2 foods were comparable to Modification B ADR (Bran Buds: 7.8 ± 0.01%; kidney beans: 8.9 ± 0.5%).

Hemicellulose content of foods

Differences in hemicellulose contents measured by the various procedures were usually statistically significant only for those foods which are higher in starch and nitrogen (Table 6). Hemicellulose contents of Modification C NDR of only two foods were assessed, kidney beans and Bran Buds; the hemicellulose content by Modification C of Bran Buds, 16.7 ± 0.29%, was comparable to that obtained from treatment of Modification B NDR, of 16.4 ± 0.18%, whereas the kidney beans Modification C hemicellulose of 6.0 ± 0.45% was significantly less than that derived from either Modifications A or B (Table 6).

Effects of cooking or peeling on neutral detergent residues

Cooking tomatoes, peeled peaches or apples significantly increased the NDR detected by all unmodified and modified procedures, except for Modification C apple NDR which was not altered significantly by cooking (Tables 2 and 3). Irrespective of the method of analysis, peeled peaches contained significantly less NDR than the unpeeled sample (Table 3). Peeled fresh apple contained significantly less NDR than the unpeeled samples only when analyzed by the Modification B method.

Coefficients of variation

With the exception of analyses of fruits, mean coefficients of variation of all NDR procedures were about 5% or less (Tables 2, 3 and 4). The high mean coefficient of variation of Modification B NDR of vegetables was the result of the kidney beans, despite the fact that four samples were determined. Also, even though more replicates of NDR of fruits were determined than of vegetables or grain products, the coefficients of variation of these foods were higher. Banana NDR determinations were particularly variable and removing their coefficients of variation lowered the means somewhat (Van Soest, 6.8%; Modification A, 7.5%; and Modification B, 3.6%). Granola was primarily responsible for the high mean coefficient of variation observed with the Van Soest and Modification A ADR values. Without granola, mean coefficients of variation of Van Soest ADR was 4.5%, of Modification A, 4.0% (Table 5).

DISCUSSION

COMPARISONS of data generated by gravimetric procedures should be interpreted cautiously. However, taken together, the NDR values determined in this study suggest several points. There are three significant differences between Modifications B and C; in contrast to Modification C, Modification B uses a bacterial, rather than a mammalian source of amylase; pancreatin is used in Modification B; and Modification B is incubated for 1 hr in the middle of the reflux step, instead of for 18 hr after refluxing. Modification A, when compared to either Modification B or C, requires even less manipulation of samples. Little or no differences between Modifications A and C NDR of spinach, carrots, corn, granola or Bran Buds were observed, nor were differences between Modifications B and C NDR values of granola, Bran Buds, whole wheat bread or fresh peeled or unpeeled apple large. Thus, it appears that neither the long incubation period of Modification C nor the pancreatin of Modifications A or B may be necessary for certain classes of foods.

A method similar to Modification D has been recommended as one alternative method of analyzing samples which are high in starch (Robertson, 1978). In our Modification D, the rinsed residue was not treated with a second 2 ml of amylase solution along with 30 ml of 70–80°C water for a few minutes prior to the final washes, as has been suggested (Robertson, 1978). We found, as have others in a study of the NDR of unprocessed grains (McQueen and Nicholson, 1979), that adding the amylase and immediately subjecting samples to boiling does not allow sufficient time for the enzyme to act. While the second treatment of the residue with the enzyme does improve filtration, significant amounts of starch remain in the residues (McQueen and

Nicholson, 1979). A similar modification of the Van Soest procedure also appears to yield inflated NDR values for processed grain products (Jwuang and Zabik, 1979).

The significant difference between the Modifications A and B NDR weights for whole wheat bread suggest preboiling in ND solution may be advantageous in some instances. Agreement between Modifications B and C whole wheat bread NDR, as well as similarity between Modification C values and those reported previously (Baker et al., 1978), suggest prolonged incubation and pancreatin may not be required. Pancreatin did not appear to effect removal of small amounts of nitrogen in either the bread or Bran Buds NDR. This nitrogen probably represents products of the Maillard reaction which occur during certain processing conditions and which are not removed by ND solution or enzymes (Van Soest, 1965; Van Soest and Robertson, 1977), as well as thy apparently indigestible fraction of wheat protein (McCance and Walwham, 1948). Pancreatin appeared to promote removal of nitrogen from corn and kidney beans, although nitrogen was still detected in kidney bean NDR. The use of a protein-degrading enzyme in the fiber analysis of low calorie vegetables and fruits does not appear to be warranted. Pepsin (Hellendoorn, et al., 1975; McQueen and Nicholson, 1979; Schweizer and Wursch, 1979) and pronase (Robertson, 1978; Saunders and Hautala, 1978) have been recommended or used, alone or in conjunction with an amylase, during fiber quantitation. Pronase may be an effective adjunct to remove nitrogen from NDR; fiber values comparable to those yielded by Modifications B and C have been reported for whole wheat bread, which had been delipidated and then incubated with pronase and hog pancreatic amylase (Saunders and Hautala, 1978). However, pepsin is not to be recommended because its activity is optimum at a low pH, which is sufficiently low to hydrolyze some hemicelluloses (Schweizer and Wursch, 1979).

It has been suggested that bacterial sources (*B. subtilis*) of amylase hydrolyze hemicelluloses (Baker et al., 1978). The similarities between NDR values of several different kinds of foods obtained by Modifications A or B, which use bacterial amylase, and those NDR values generated with hog α amylase (Modification C) suggest that hemicellulose hydrolysis, if it does occur, may not be a significant source of error when the incubation period is short and determinations are gravimetric. Alternatively, the source of enzyme we used did not contain significant amounts of impurities proposed by Baker et al. (1978). Others have stated that hemicellulases are not normally produced by *B. subtilis* or other amylolytic bacteria (McQueen and Nicholason, 1979). Incorporation of a starch-degrading enzyme into the Van Soest ND procedure does appear to minimize or eliminate, with the exception of perhaps legumes, i.e. kidney beans, contamination of NDR with starch. However, with the exception of Modification C NDR, starch contamination was assessed qualitatively; differences in the ability of iodine to complex with amylose and amylopectin (Southgate, 1976b) and the possibility that certain hemicelluloses may complex with iodine to produce a blue chromogen (Southgate, 1976b), prevents any conclusions from our data about the ability of the bacterial amylase to quantitatively remove starch. Our results do suggest that failure to detect any starch in NDR with iodine indicates that quantitation of starch in the residue is not necessary.

Kidney beans was the only food in which the Modification C residue weight was significantly lower that either Modifications A or B; thus, long incubations periods may be necessary for some foods. These findings are consistent with the incomplete in vivo digestion of legume starch in some monogastric species. The greater residue weight yielded by Modification B, when compared to Modification A treatment of the beans, hints that preboiling may not be suitable for some samples; it is possible that preboiling caused some of the starch to retrograde, making it less available for enzymatic attack (Collison, 1968).

The effects of peeling on NDR of peaches and apples, except for Modification C peach NDR, was to decrease residue weight about 1% (dry weight basis). The effect of cooking on apparent NDR was greater, probably a reflection of the formation of the products of the Maillard reaction during optimal conditions of heat and moisture (Goering and Van Soest, 1973) and which are not solubilized by ND or AD solutions (Van Soest, 1965). Cooking increased residue weights of tomatoes, peaches and apples from 1.4 to 2.3%, with the exception of Modification C analysis of cooked peaches which was 6.5% higher than the NDR weight of the uncooked sample. The reason for such a large increase in unclear, though Modification C was the only method which involved a long incubation period under conditions suitable for the Maillard reaction.

The reasons for the high variability observed among repeated determinations of fruits are not clear. Fruits are not high in the contaminants of starch and nitrogen. Coefficients of variation comparable to what we observed for grain products are obtained during buffered ADR analyses (Baker, 1977), while those of a straight ADR determination on forages appear to be lower (Van Soest, 1973). Neutral detergent analyses of unprocessed grains appear to have larger coefficients of variation (McQueen and Nicholson, 1979). Hemicellulose values presented in our study are calculated ones and would reflect differences in solubilities of various contaminants and fiber components in the AD solution, as well as differences induced by the treatment modifications (Table 6). The variability in the hemicellulose data as well as that in the fruit values might be a reflection of the fact that the values are low and any gravimetric error would represent a larger proportion of the weight.

For fruits and low calorie vegetables, the effect of modification of the Van Soest ND procedure on NDR values, particularly if expressed on a fresh weight basis, are usually small. Agreement between Modifications A and C NDR values for these foods implies that any differences in residue weights with modification were a result of starch contamination. The reason for the lack of agreement between Modifications B and C NDR values of the peach samples is not apparent, but serve to illustrate the limits of a gravimetric method. Nonetheless, dietary fiber values for such foods obtained by the unmodified Van Soest procedure which are available in the literature are probably a reasonable estimate of fiber content. The significant differences between NDR estimated by the Van Soest procedure and those generated by the modified methods suggest that meaningful fiber estimates by this method must incorporate a starch-degrading enzyme when high calorie vegetables or grain products are analyzed.

Modification of the Van Soest procedure did not alter significantly the ADR content of the foods and the distribution of cellulose and hemicellulose in Bran Buds we observed of approximately 1/3 and 2/3, respectively, agrees with that reported for wheat bran (Rasper, 1979). Values of ADR contents of fruits, apples and bananas, obtained by treatment of a food sample, as opposed to the NDR, with AD solution, are 1.5 to 2 times (Schweizer and Wursch, 1979) the values we observed by applying the two detergent systems sequentially to a food sample. Thus, if an estimate of lignocellulose content is desired, ND and AD treatments need to be applied sequentially to the same food residue. The relatively high variability in hemicellulose data, when considered along with the insignificant changes in ADF obtained by modified ND procedures indirectly suggest that the substances responsible for the variation in

these calculated values are the pectic substances, soluble hemicelluloses or carbohydrates although starch and nitrogen contamination may contribute. Further evaluation of a procedure's ability to retain or eliminate certain kinds of hemicelluloses and pectic substances, as well as starch and nitrogen, requires more detailed analyses.

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LIPASE PRODUCTION, LIPOLYSIS, AND FORMATION OF VOLATILE COMPOUNDS BY Pseudomonas fluorescens IN FAT CONTAINING MEDIA

R. E. ANDERSSON

ABSTRACT

The lipolytic bacterium *Pseudomonas fluorescens* was cultivated in nutrient broth supplemented either with olive oil, sunflower oil or soybean oil. Presence of oil delayed bacterial growth and lipase production, but the finally obtained cell density and amount of lipase was approximately the same as in unsupplemented nutrient broth. The lipase hydrolyzed soybean oil to a greater extent than olive oil and sunflower oil, respectively. Fatty acids were deteriorated into volatile compounds which were detected in the headspace gas over the fat containing media. The volatile fraction was found to contain alcohols, aldehydes, ketones, esters, furans, sulphur compounds, and hydrocarbons.

INTRODUCTION

MOST FOODS contain significant amounts of fat which can be hydrolyzed giving rise to quality changes (Alford et al., 1971). Most microorganisms are able to produce fat hydrolyzing enzymes, lipases, which can contribute to fat degradation and quality changes in fatty foods (Lawrence, 1967).

A number of microorganisms have been reported to produce inducible lipases when fat is present in the growth medium (Akhtar et al., 1974; Iwai et al., 1973; Khan et al., 1967; Tsujisaka et al., 1973). Even constitutive lipases have been found (Jonsson, 1976). However, fat supplemented media can also result in decreased production of lipase (Khan et al., 1967; Mates and Sudakevitz, 1973; Nashif and Nelson, 1953; Smith and Alford, 1966).

Some microbial lipases, e.g. that from *Staphylococcus aureus*, show very low specificity (Alford et al., 1964), while most of them are specific to the position, carbon chain length or degree of unsaturation of the fatty acids. Lipase from *Geotrichum candidum* was shown to be specific for oleic acid independently of its position in triglycerides (Alford et al., 1964), while *Pseudomonas fragi* (Alford et al., 1964), *Aspergillus niger*, and *Rhizopus delemar* (Okumura et al., 1976) produced lipases specific for the 1-position regardless of the fatty acid. Nashif and Nelson (1953) found that a lipase from *P. fragi* hydrolyzed soybean oil and olive oil to the same rate whereas Jonsson (1976) reported that *Saccharomycopsis lipolytica* and *Micrococcus caseolyticus* hydrolyzed soybean oil five times faster than olive oil.

The released fatty acids can be metabolized by the organism and according to Mates (1972) the uptake of free fatty acids by bacteria is independent of lipolytic activity.

Lipases, especially from psychotrophic bacteria, can be remarkably heat-resistant (Cogan, 1977). Andersson et al. (1979) showed that such a lipase from *Pseudomonas fluorescens* can survive pasteurization or even sterilization processes and may therefore cause quality changes in otherwise microbially stable foods when stored at temperatures where such lipases are active.

Most higher fatty acids do not themselves contribute to flavor but can be precursors to the formation of volatile compounds through oxidation. Forss (1972) concluded that hydrolysis of triglycerides and oxidation of lipids produce volatile components including acids, alcohols, esters, aldehydes, ketones, lactones, and furans, and usually cause off flavor. In certain ripening processes, however, such as cheese making (Arnold et al., 1975), and production of dry, fermented sausages (Demeyer et al., 1974), the presence of free fatty acids and their oxidation products is considered positive to flavor. The disposition to oxidation for the fatty acid greatly increases with the number of double bonds in the carbon chain and by splitting the acid from the glyceride. The presence of reaction products like hydroperoxides and carbonyl compounds are often used as over-all indices of rancidity in a fat or an oil (Barthel and Grosch, 1974). Volatile products responsible for flavor or off flavor are, however, more accurately determined by gas chromatography and mass spectrometry.

The present study was undertaken in order to gain background knowledge about the production of lipase during growth of *P. fluorescens* in fat containing media and the fat hydrolysis, caused by the lipase, and production of volatile compounds from the released fatty acids in the media. The importance of the findings in relation to keeping quality of fat containing, heat-treated and microbially stable foods is discussed.

MATERIALS & METHODS

Organisms

Stock cultures of a lipolytic *Pseudomonas fluorescens* SIK W1 and a nonlipolytic *Escherichia coli* were maintained as slant cultures on Tryptone Glucose Extract Agar (Difco, Detroit, Mich.). Before being used as inoculum the organisms were subcultured once in Nutrient Broth (Difco), *P. fluorescens* for 20 hr at 20°C and *E. coli* for 12 hr at 37°C.

Culture media

Supplemented nutrient broth was made by adding either olive oil (purum, AB Kebo, Stockholm, Sweden), refined sunflower oil, or refined soybean oil (AB Karlshamns Oljefabriker, Karlshamn, Sweden) in proportion 4:1 (w:w). These oils represent triglycerides containing oleic acid, linoleic acid, and linolenic acid, respectively. The supplemented nutrient broth was autoclaved at 121°C for 17 min before inoculation.

Culture conditions and controls

The lipolysis was carried out in 3-L flat bottom glass flasks, with cotten plugs, containing 1.5L of the culture media inoculated with 1 ml of a 20-hr subculture of *P. fluorescens*, containing approximately 10^8 cells/ml. The cultures were incubated in a New Brunswick Controlled Environmental Shaker (New Brunswick Co., Inc., New Brunswick, N.J.) at 20°C under reciprocal shaking with 100 strokes per min. Samples were withdrawn regularly for determination of total bacterial count, lipolytic activity, peroxide value, analysis of free fatty acids, and volatile compounds.

Sterile culture media, control samples, were subjected to the same conditions and analyses as the inoculated samples.

Assay of total count

Samples from the flasks were decimal diluted in 0.85% NaCl solution. One ml from appropriate dilutions was mixed with molten

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tryptone glucose extract agar, just above its setting point (45–48°C), in a Petri dish. The colonies were counted after 2 days' incubation at 30°C.

Assay of lipase activity

The lipase activity in the culture media was determined by a previously described pH-stat method (Andersson et al., 1979). Olive oil was used as the substrate and the activity was expressed as μmol of fatty acid released per min and ml of the medium.

Analysis of free fatty acids (FFA)

The lipid material in the culture media was extracted by a method outlined by Jonsson (1976).

The free fatty acid fraction was isolated on a silicic acid column according to McCarthy and Duthie (1962). The fatty acids obtained were converted into their methyl esters as previously described by Metcalfe and Schmitz (1961).

The methyl esters in approximately 1 μl samples wery separated by gas chromatography using Perkin-Elmer 900 gas chromatography equipped with a two column system and flame ionization detectors. Stainless steel columns (2m × 1/8 in.), packed with 10% diethyleneglycosuccinate (DEGS) on HP Chromosorb W, 80–100 mesh (W.G.A. International Ltd., London, England) were used. The temperature was programmed from 130–185°C at 4°C/min. The injector temperature was 220°C and the detector temperature was 200°C. Helium was used as carrier gas at a flow rate of 25 ml/min. The amounts of different fatty acids were calculated from the integrated peak areas, by comparison with the known amount of an internal standard, n-heptadecanoic acid, using a Perkin-Elmer PEP-1 data processor. The degree of hydrolysis for individual fatty acids was expressed as mg free fatty acid, FFA, per g of fat of the culture medium.

Uptake of fatty acids

To study the uptake of released fatty acids in lipid media, a non-lipolytic bacterium had to be used. For this purpose a nonlipolytic *E. coli* was chosen.

Two ml of a sterile filtered (Millipore filter type GS, 0.22μ, Millipore, France) lipase solution from *P. fluorescens*, concentrated 20 times by ultrafiltration as previously described (Andersson et al., 1979) was added to each of three Erlenmeyer flasks containing 100 ml of culture medium, previously autoclaved at 121°C for 17 min. The first flask was inoculated with 1 ml of an overnight culture of *E. coli*. The flasks were incubated at 37°C and after 1 day the second flask was inoculated with *E. coli* while the third flask was kept sterile. The incubation was continued and the release of FFA was repeatedly followed by a titrimetric method (Jonsson and Snygg, 1974a). The concentration of total FFA was expressed as titratable acidity in μmol acid per ml of the culture medium.

Analysis of lipid oxidation

A cell-free solution was obtained by centrifuging a 2-day's culture of *P. fluorescens* at 24.000 × G for 20 min at 4°C. The solution was concentrated 20 times by ultrafiltration (Andersson et al., 1979). The ability of the cell-free solution to oxidize linoleic acid emulsion (2.50g of linoleic acid in 2.5 ml of Tween 20/L of 0.1M phosphate buffer. pH 6.8) was measured using a polarographic method described by Eriksson and Svensson (1970).

The peroxide value, PV, was determined as described by the Association of Official Analytical Chemists (AOAC) (Horwitz, 1975) and expressed as milli-equivalent peroxide per kg of fat.

Analysis of volatile compounds

Volatile compounds were analyzed by a procedure outlined by von Sydow et al. (1970). Liquid samples of the culture media were withdrawn and the head space over this solutions used.

The composition of the volatile fraction was analyzed in a Perkin-Elmer 900 gas chromatograph provided with a flame ionization detector. Separation was carried out on a 181m × 0.76 mm i.d. stainless steel open tubular column coated with 13% SF 96/Igepal CO 880 (95/5). The temperature was programmed 20–140°C at 2°C/min after an initial isothermal period of 6 min. The carrier gas flow was 12 ml He per min. The injection temperature was 120°C and the detector temperature was 140°C. A Perkin-Elmer PEP-1 data processor was used for the quantifications, and the concentration of the compounds was expressed as parts per billion (ppb, v/v) of the headspace volume. Response factors according to Dietz (1967) were used.

For identification of individual compounds a combined gas chromatograph-mass spectrometer, Perkin-Elmer 990 – LKB 9000 was used. Mass spectra were recorded at 70 eV. The separator temperature was 200°C and that of the ion source 270°C. Comparison with reference spectra from the SIK collection and the literature was made for identification of the compounds.

RESULTS

Bacterial growth and lipase production

The growth of *P. fluorescens* was influenced by the presence of oil in the substrate media (Fig. 1). In unsupplemented nutrient broth the viable count reached 10^3 cells/ml in approximately half a day, while the presence of olive oil or sunflower oil delayed the growth to that cell density by 3–5 days. In the soybean oil medium the cell density reached 10^8 cells/ml after 1–2 days.

The lipolytic activity reached maximum values earlier in unsupplemented nutrient broth (2 days) than in supplemented culture media (5–10 days) (Fig. 2). The maximum

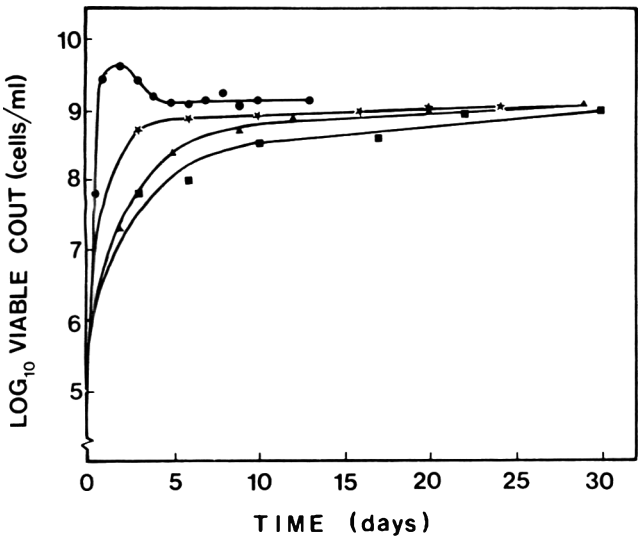

Fig. 1—Growth curves for Pseudomonas fluorescens cultivated at 20°C in pure nutrient broth (•) and in nutrient broth supplemented with olive oil (▲), sunflower oil (■), and soybean oil (★).

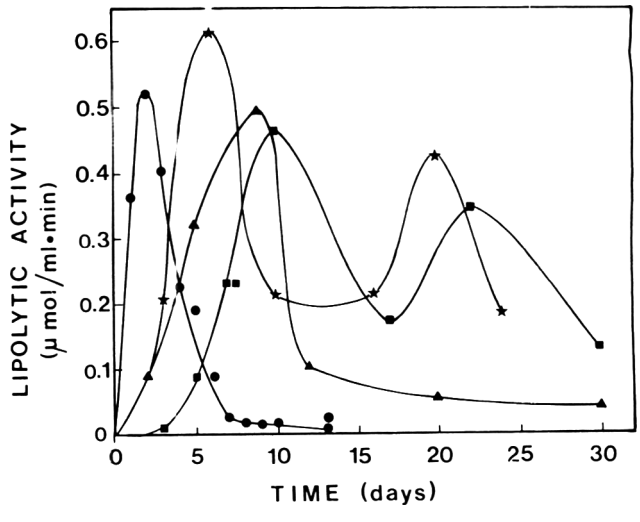

Fig. 2—Lipase production by Pseudomonas fluorescens as a function of time cultivated at 20°C in pure nutrient broth (•) and in culture media containing olive oil (▲), sunflower oil (■), and soybean oil (★).

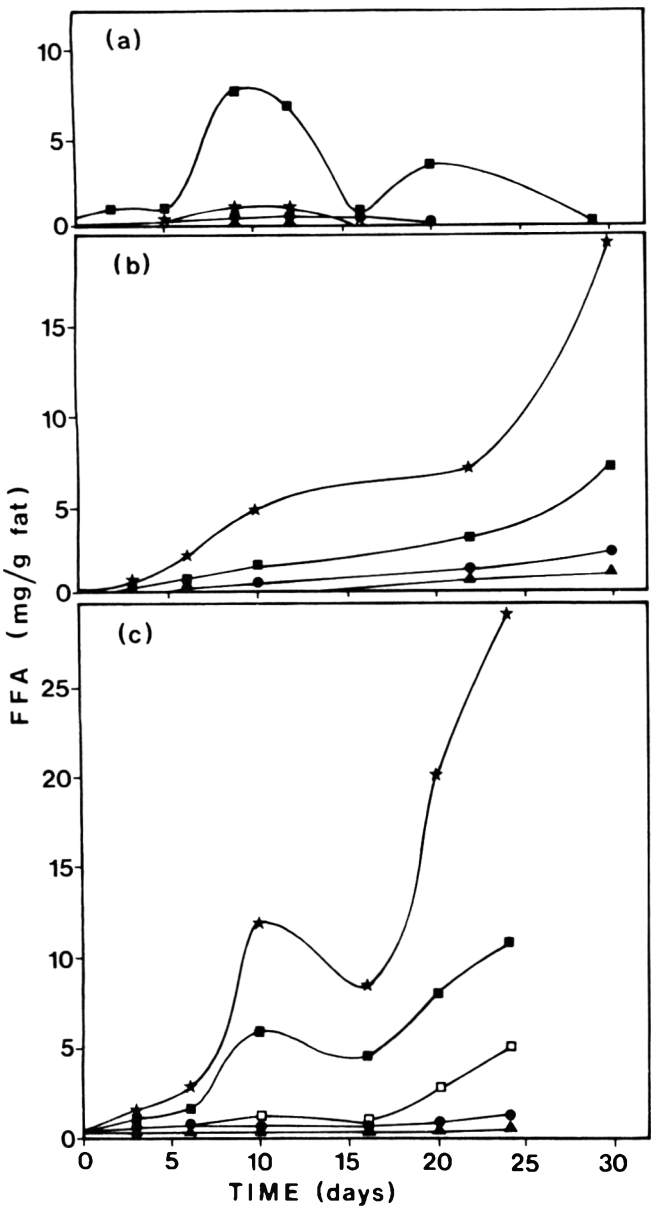

Fig. 3—Composition of the FFA-fraction as a function of time in culture media containing olive oil (a), sunflower oil (b), and soybean oil (c) inoculated with Pseudomonas fluorescens. Palmitic (●), stearic (▲), oleic (■), linoleic (★), and linolenic acid (□).

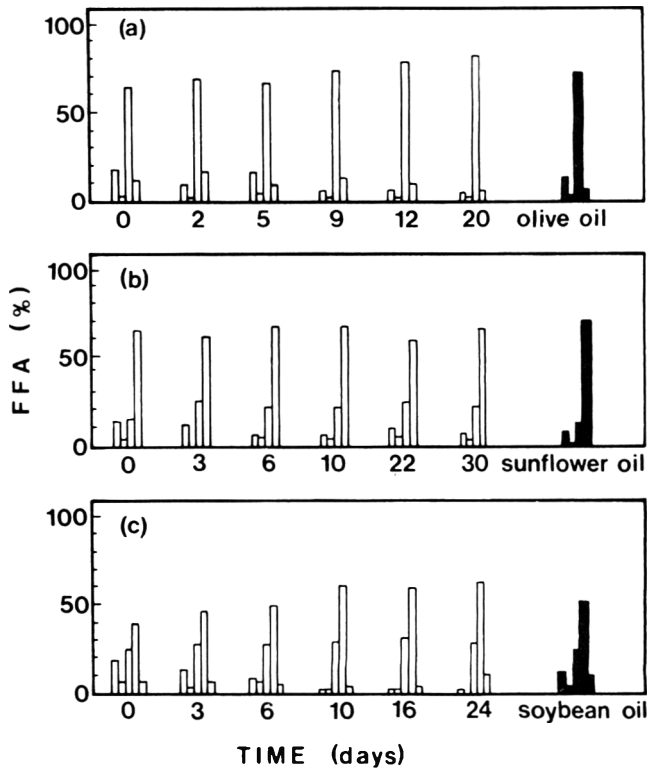

Fig. 4—Percental composition of the FFA-fraction as a function of incubation time in culture media containing olive oil (a), sunflower oil (b), and soybean oil (c). The staples indicate from left to right: palmitic, stearic, oleic, linoleic, and linolenic acid.

Table 1—Autohydrolysis in sterile culture media incubated at 20°C

Incubation time (days)	Total hydrolysis (mg FFA/g fat)		
	Olive oil	Sunflower oil	Soybean oil
0	0.43	0.49	0.30
15	0.32	0.23	0.52
30	0.22	0.19	1.14

Table 2—Peroxide values as a function of time at 20°C

Incubation time (days)	PV (mekv./kg fat)					
	Olive oil		Sunflower oil		Soybean oil	
	Inoculated	Sterile control	Inoculated	Sterile control	Inoculated	Sterile control
0	16	15	17	16	18	14
5	15		21		17	
10	19		23		18	
15	21	25	25	28	18	30
20	21		27		21	
25	20		25		23	
30	21	30	25	42	24	44

lipolytic activity was found to be of the same order both in supplemented culture media and in unsupplemented nutrient broth. In sunflower and soybean oil media a second maximum was observed after 20–22 days.

Hydrolysis

The amount of individual fatty acids liberated during the lipolysis of the fat in the culture media is shown in Figure 3. The lipolytic activity of *P. fluorescens* resulted in significantly higher amounts of FFA in culture media containing soybean oil than sunflower oil and olive oil, respectively. Because of the dynamic hydrolyzing courses it is, however, not meaningful to present ratios in liberated fatty acids in different media in absolute figures. The release of fatty acids in the sterile control samples was found negligible in comparison with that in the corresponding inoculated samples (Table 1).

The percental composition of individual FFA in hydrolyzed oil at some selected incubation times is shown in Figure 4.

Uptake of fatty acids by E. coli

When a nonlipolytic *E. coli* was inoculated into the culture media containing lipase, significantly lower amounts of FFA were found in comparison with those in the sterile controls (Fig. 5). Initial presence of the bacteria inhibited the increment in FFA, and the bacteria could also reduce

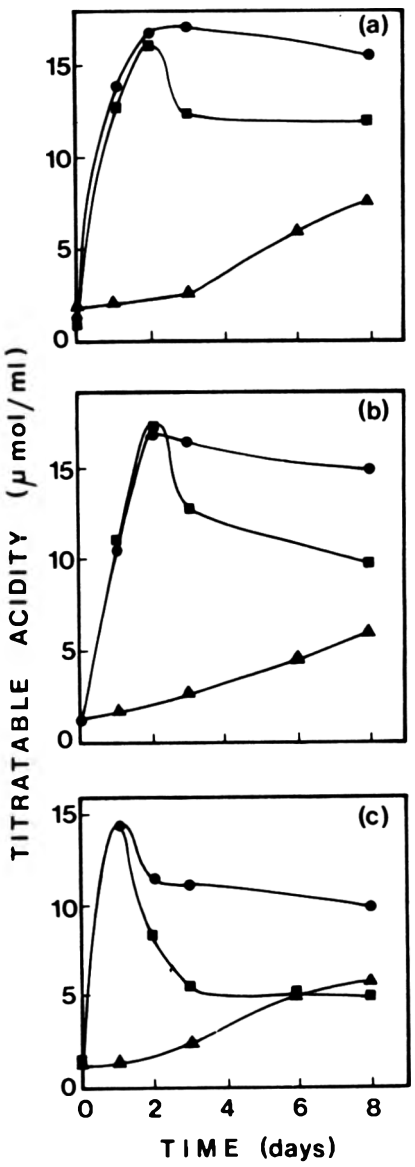

Fig. 5—The amount of FFA in culture media containing olive oil (a), sunflower oil (b), and soybean oil (c) as a function of time at 37° C in sterile control (•) and in samples inoculated with E. coli at the start (▲) and after 1 day of lipolysis (■).

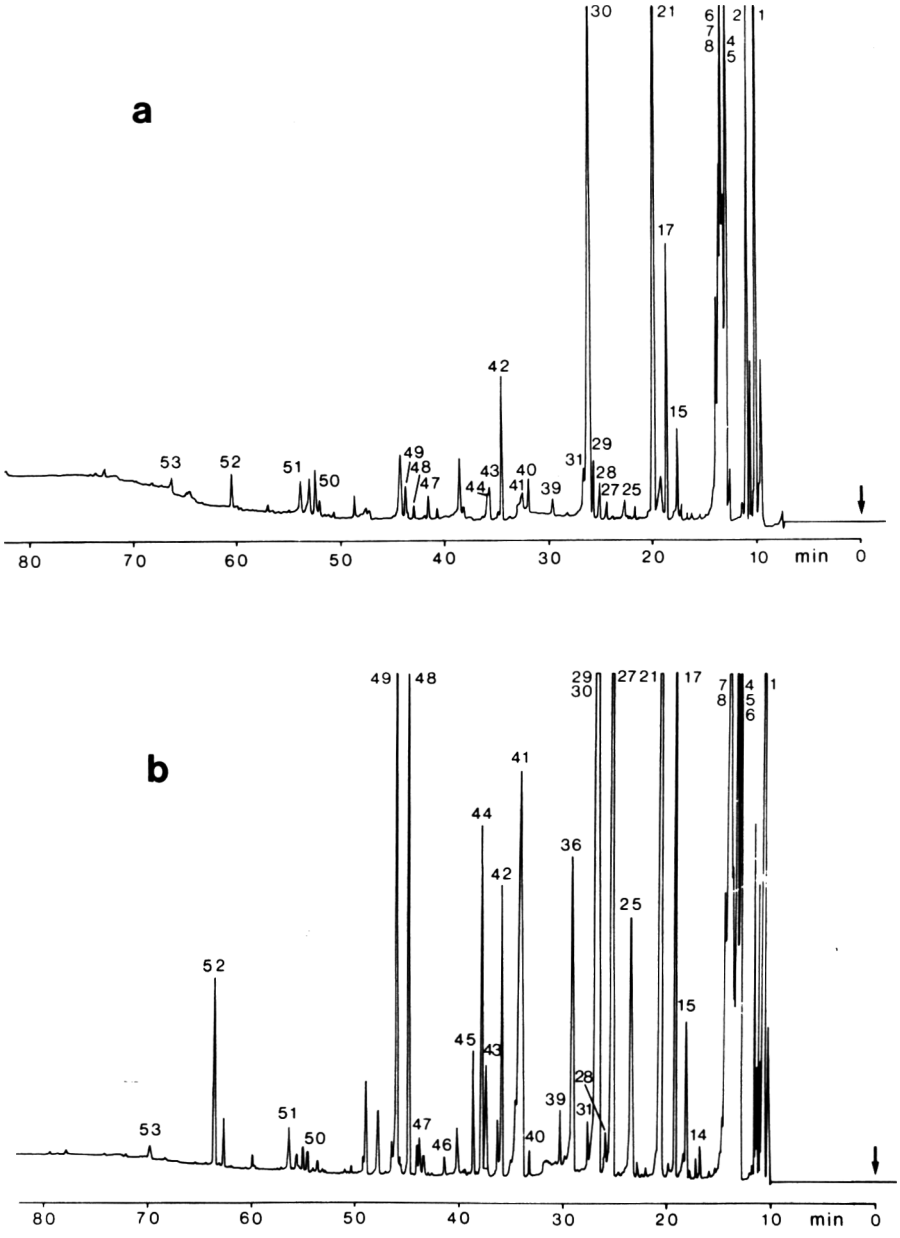

Fig. 6—Representative head-space gas chromatograms of sunflower oil culture media, inoculated with Pseudomonas fluorescens, at start (a) and after 30 days of incubation (b) at 20° C.

the content of FFA when added to culture media containing partly hydrolyzed fat.

Determination of lipid oxidation

Supernatants from *P. fluorescens* were not found to possess any lipid oxidation capacity as measured by an oxygen consumption measuring method.

The PV was at least doubled in sterile culture media after 30 days. In culture media containing *P. fluorescens*, however, the PV remained more constant and always lower than in sterile culture media (Table 2).

Analysis of volatile compounds

More than 50 different volatile compounds were identified and quantified in the head-space gas over the culture media. A representative gas chromatogram is shown in Figure 6. The chromatogram also gives some information about the amount of volatile components in the head-space gas of the culture media after lipolysis compared to that of the starting material. Most compounds were identified by use of the combined gas chromatography and mass spectrometry technique and are listed in Tables 3–5, where also corresponding data from the sterile controls are presented. The quantification of the most volatile compounds at the beginning of the gas chromatograms was difficult to perform because of insufficient separation and, therefore, their amounts were not calculated.

The concentration of some compounds increased as a result of lipolysis in all culture media and those formed in large quantities were e.g. 1-butanol, 1-pentanol, 1-pentanal, 2-methylbutanal, 2-heptanone, and 2-ethylfuran. Hexanone and 1-pentanal were formed in relatively higher concentration in the olive oil culture medium while the sunflower oil medium contained more of 2-methylbutanal and 2-ethylfuran as compared to the other two media. 2-Heptanone reached a higher concentration in the medium containing soybean oil.

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DISCUSSION

THE AMOUNT OF LIPASE present in the medium at a certain time depends on several factors such as choice of bacteria, bacterial growth, lipase productivity and lipase destruction. Thus it is important to separate these effects from each other.

The addition of an oil to nutrient broth reduced the growth rate of *P. fluorescens* but affected neither the final

Table 3—Concentrations (ppb, v/v) of volatile compounds in the headspace gas of samples of olive oil culture medium as a function of time of lipolysis

Compound	Compound no. (see Fig. 6)	Incubation time (days)							Control		
		0	2	5	9	12	20	29	0	11	30
Alcohols											
Methanol	5										
1-Butanol	27	1	3	19	38	103	161	195	1		
1-Pentanol	41	2	6	13	26	28	37	51	1	2	
2-Pentanol	39	1	1	2	2		2	3	1		
1-Heptanol	51	2	1	2	2	1	2	2	2	2	1
3-Methylbutanol	47					1	3	1	0.5		
Aldehydes											
Ethanal	1										
1-Butanal	14	2		3	5	6	2				
1-Pentanal	36	2	1	2	3	9	18	41	3	1	
2-Pentanal	42	10	3	5	4	1	2	2	8	5	3
1-Hexanal	44										
1-Nonanal	53	1	1	2	1	1	1		1	1	1
2-Methylpropanal	13	23					3		1	1	2
2-Methylbutanal	25	15	5	3	7	3	6	13	3	1	1
3-Methylbutanal	24	32	1	5	1	2	5	3	11	4	
Benzaldehyde	50	1	2	2	1	1	1	3	3	1	
Ketones											
Propanone	6										
2-Butanone	15	6	6	9	13	11	8	9	5	2	1
2-Pentanone	28	5	5	3	1	1	1	1	4	2	2
2-Hexanone	43	5	7	8	7	11	13	21	6	4	2
2-Heptanone	48		1	2	4	9	12	14	0.5		
3-Pentanone	19										
3-Methyl-2-butanone	23								1	2	
4-Methyl-2-pentanone	38										
Acetofenone	52	1	1	1	2	3	3	3	1	1	1
Esters											
Methylacetate	12	33	31	20	12						
Ethylacetate	20	20	22	19	11	5	9	10	18	5	
Ethers											
Diethylether	10										
Furans											
Furan	9										
2-Methylfuran	21	3		4	13	7	9	2	5	9	10
2-Ethylfuran	30	47	35	31	91	155	129	122	40	38	32
2-Pentylfuran	37										
Sulfur compounds											
Dimethylsuflide	11										
Dimethyldisulfide	40	2	2	2	3	3	3	3	2	2	2
Alifatic hydrocarbons											
n-Pentane	8										
iso-Pentane	7										
n-Hexane	17	7	8	7	5	3	2	2	6	5	2
n-Heptane	31	2	2	4	2	2	1	2	2	1	
n-Octane	45										
C_4H_8	2										
C_4H_{10}	3										
C_5H_{10}	4										
C_6H_{12}	16			3	4	3	4				
C_6H_{14}	18										
C_7H_{12}	32										
C_7H_{16}	33										
C_8H_{18}	46										
Aromatic hydrocarbons											
Benzene	26	1			1				1		
Toluene	35										
Styrene	49		1	2	4	6	6	7	1	2	2

bacterial number nor the amount of lipase formed during the first two weeks, which suggests that the lipase is a constitutive enzyme. This has also been reported for *S. lipolytica* and *M. caseolyticus* by Jonsson (1976).

The lipolytic activity during the cultivation of *P. fluorescens* reached a maximum at the beginning of the stationary phase. Such a maximum has been observed previously both for *Pseudomonas* (Björklund, 1970; Smith and Alford, 1966), and other microorganisms (Jonsson and Snygg, 1974b; Somkuti and Babel, 1968; Sugiura et al., 1975).

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Table 4—Concentrations (ppb, v/v) of volatile compounds in the headspace gas of samples of sunflower oil culture medium as a function of time of lipolysis

Compound	Compound no. (see Fig. 6)	Incubation time (days)						Control		
		0	6	10	17	22	30	0	17	30
Alcohols										
Methanol	5									
1-Butanol	27	1	13	33	47	122	141	2	2	4
1-Pentanol	41	1	7	13	21	24	42			
2-Pentanol	39	1	1	1	1	1	1	1	1	1
1-Heptanol	51	2	3	3	2	3	3	2	2	2
3-Methylbutanol	47	1	3	2	3		7	1	1	2
Aldehydes										
Ethanal	1									
1-Butanal	14			2	2	1			1	
1-Pentanal	36			1	5	10	20			
2-Pentanal	42	8	5	4	8	3	1	15	30	31
1-Hexanal	44	3	1	1	1	2	3			
1-Nonanal	53	1	2	2	1	2	1	1	1	1
2-Methylpropanal	13							2	1	1
2-Methylbutanal	25	2	3	7	12	11	19	2	5	3
3-Methylbutanal	24				1					
Benzaldehyde	50						0.5	1	1	1
Ketones										
Propanone	6									
2-Butanone	15	4	5	7	7	6	9	1	4	2
2-Pentanone	28	1	1	1	1		2	1		7
2-Hexanone	43	3	1	1	1	2	3	1	2	2
2-Heptanone	48		2	3	6	14	15	1	5	1
3-Pentanone	19									
3-Methyl-2-butanone	23				2	3		1		2
4-Methyl-2-pentanone	38						0.5			
Acetofenone	52	1	2	3	4	5	5	1	4	1
Esters										
Methylacetate	12									
Ethylacetate	20	5	0.5		2		2	2		7
Ethers										
Diethylether	10									
Furans										
Furan	9									
2-Methylfuran	21	22	30	6	17	8	13	23	26	3
2-Ethylfuran	30 + 29	37	272	3132	3617	3787	2897	4	7	6
2-Pentylfuran	37									1
Sulfur compounds										
Dimethylsulfide	11									
Dimethyldisulfide	40	10				2	1	4	3	5
Alifatic hydrocarbons										
n-Pentane	8									
iso-Pentane	7									
n-Hexane	17	7	3	3	3	3	3	6	12	7
n-Heptane	31	0.5	1	1	1	1	2			1
n-Octane	45					1	2			
C_4H_8	2									
C_4H_{10}	3									
C_5H_{10}	4									
C_6H_{12}	16		1	6	5	3	3		2	
C_6H_{14}	18									
C_7H_{12}	32									
C_7H_{16}	33									
C_8H_{18}	46									
Aromatic hydrocarbons										
Benzene	26									
Toluene	35									
Styrene	49	1	3	18	24	29	28		2	1

According to Sugiura et al. (1975) the decrease in lipolytic activity after the maximum may be explained by the fact that the lipase is very labile and/or that the level of lipase is highly regulated. Björklund (1970) and Jonsson and Snygg (1974b) suggested that proteolytic enzymes present in the medium may cause destruction of the lipase. Another possibility might be that products formed in the culture media inhibit the lipase activity or the lipase production. Smith and Alford (1966) showed that fatty acids inhibited microbial lipases and Mates (1974) found that the lipase synthesis

Table 5—Concentrations (ppb, v/v) of volatile compounds in the headspace gas of samples of soybean oil culture medium as a function of time of lipolysis

Compound	Compound no. (see Fig. 6)	Incubation time (days)							Control		
		0	3	6	10	16	20	24	0	15	30
Alcohols											
Methanol	5										
1-Butanol	27	1	2	19	47	45	57	83	1	3	4
1-Pentanol	41	2	5	12	18	29	38	71			1
2-Pentanol	39		1	1	2	1	1	1	2	1	2
1-Heptanol	51	2	2	2	3	2	2	2	2	2	2
3-Methylbutanol	47	1	1	1	1	1	1	1	2	2	1
Aldehydes											
Ethanal	1										
1-Butanal	14						1	3			
1-Pentanal	36	3	2	3	5	8	9	14	1	2	3
2-Pentanal	42	17	4	1	1	7	2	2	7	5	5
1-Hexanal	44	2				1	1	1			
1-Nonanal	53	1	1	2	2	1	1	1	2	2	1
2-Methylpropanal	13								1		1
2-Methylbutanal	25	2	2	3	4	6	6	7	3	1	1
3-Methylbutanal	24	5	1	2	1	1	3	4	10	3	1
Benzaldehyde	50	1	1	1	2	2	2	2	2	5	2
Ketones											
Propanone	6										
2-Butanone	15	6	5	6	7	5	6	7	5	3	2
2-Pentanone	28	3	1	2	2	5	3	2	2	3	2
2-Hexanone	43	5	2	1	1	1	1	2	1		1
2-Heptanone	48		2	2	4	7	11	25	2	2	1
3-Pentanone	19										
3-Methyl-2-butanone	23	13	1	1	1	1	1	2	10	5	
4-Methyl-2-pentanone	38						1	1			
Acetofenone	52	1	1	1	2	2	2	2	2	1	1
Esters											
Methylacetate	12										
Ethylacetate	20		2	2	5	2		3	4		6
Ethers											
Diethylether	10										
Furans											
Furan	9										
2-Methylfuran	21	9	12	7	46	62	22	13	9	20	16
2-Ethylfuran	30	17	25	71	398	327	297	152	29	18	11
2-Pentylfuran	37										
Sulfur compounds											
Dimethylsulfide	11										
Dimethyldisulfide	40	3	2	2	4	3	2		2	2	2
Alifatic hydrocarbons											
n-Pentane	8										
iso-Pentane	7										
n-Hexane	17	2	2	2	2	2		1	2	2	1
n-Heptane	31	1	1	1	1	1	1	1			
n-Octane	45										
C_4H_8	2										
C_4H_{10}	3										
C_5H_{10}	4										
C_6H_{12}	16			1	1	2	1				
C_6H_{14}	18										
C_7H_{12}	32										
C_7H_{16}	33										
C_8H_{18}	46										
Aromatic hydrocarbons											
Benzene	26										
Toluene	35										
Styrene	49	2	1	1	2	2	2	2	2	2	1

by *S. aureus* was reduced by added alcohols. The two maxima in lipolytic activity during growth were also observed by Björklund (1970) and Jonsson and Snygg (1974b). Björklund (1974) suggested that this was due to the lability of the systems which control the synthesis and secretion of the lipase. Another explanation could be that sunflower oil and soybean oil induce the formation of a second lipase thus explaining the difference between these two oils and olive oil.

P. fluorescens lipase hydrolyzed soybean oil to a greater extent than the two other oils. Since the amount of lipase produced was of the same magnitude in all media, the differences in hydrolysis are probably due to specificity of the lipase. Such a specificity has also been reported for other bacterial lipases by Alford et al. (1971) and may depend either on positional specificity or specificity for the particular fatty acids.

The concentration of released fatty acids decreased in some cases during the lipolytic course. This may be caused by autoxidation of the FFA or by the microorganisms themselves. Since *P. fluorescens* seemed not to produce any strong lipid oxidation catalyst, the decrease is probably due to active incorporation of fatty acids by the bacteria, which was shown to occur in *E. coli* in the present investigation.

The presence of *P. fluorescens* in the culture media kept the PV at a lower level compared to a sterile control. According to Smith and Alford (1969, 1970) this is probably due to the formation of antioxidative compounds by the bacteria or by a metabolic decomposition of the peroxides. Grosch et al. (1971) have shown that microorganisms, e.g. *P. fluorescens*, break down hydroperoxides.

The volatile fraction was found to have a complex composition with a number of potential aroma compounds present. According to Forss (1972) 1-pentanol, 1-pentanal, and 2-heptanone are generally regarded as fat degradation products, originating from the oxidation of unsaturated acids and contributing to off flavor. During lipolysis by *P. fluorescens* the concentration of these compounds increased considerably, and corresponded roughly to the increase in FFA caused by hydrolysis by the lipase. This indicates that lipolysis caused by microorganisms can increase the breakdown of fatty acids to carbonyl compounds without a corresponding increase in the PV. The composition of the head-space of the 3 culture media was almost identical, except for some quantitative differences. This is probably a result of that oils that mainly contained unsaturated fatty acids had been selected. Variations in concentrations, especially of some carbonyl compounds, can be a result of bacterial action, and Lilly et al. (1970) and Smith and Alford (1968, 1969, 1970) have shown that some microorganisms can decompose carbonyl compounds in fats.

CONCLUSIONS

1. The Pseudomonas bacterium grows and produces lipase in fat containing media.

2. During bacterial growth the fat is hydrolyzed to free fatty acids.

3. Some of the released fatty acids can either be taken up and metabolized by the bacteria or broken down into volatile compounds.

4. Formation of volatile compounds in fat containing media is favored qualitatively and quantitatively by fat hydrolysis.

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PHENOLIC COMPONENTS OF RAPESEED MEAL

T. W. FENTON, J. LEUNG, and D. R. CLANDININ

ABSTRACT

The phenolic components of several rapeseed meals were studied in base hydrolyzed extracts as free acids and in 70% aqueous acetone extracts as intact compounds. Thin-layer chromatography, gas chromatography, and mass spectroscopy were used for compound purification and identification. The free acids in the hydrolyzed extracts in order of concentration were: sinapic >> protocatechuic > ferulic > p-hydroxybenzoic > vanillic > syringic. Sinapic acid was the only free phenolic acid detected in the 70% aqueous acetone extracts although there was at least seven other compounds that upon hydrolysis yielded sinapic acid. Neither chlorogenic acid or caffeic acid were detected in any of the meals although they had previously been reported.

INTRODUCTION

PHENOLIC COMPOUNDS can be important in terms of palatability and the general suitability of rapeseed meal (RSM) as a protein source (Kozlowska et al., 1975). Recently Durkee and Thivierge (1975) reported the presence of a variety of phenolic compounds in *Brassica* and *Sinapis* oil seeds. Based on paper chromatography and thin-layer chromatography (TLC) of hydrolyzed extracts from such seeds they demonstrated the presence of caffeic, p-coumaric, ferulic, p-hydroxybenzoic, and sinapic acids. They also identified chlorogenic acid based on its behavior on two-dimensional chromatograms and its cleavage products. Chlorogenic acid was also reported in RSM by Lo and Hill (1972).

Kozlowska et al. (1975) tentatively identified the principle phenolic components of dehulled rapeseed flour as p-hydroxybenzoic, trans-cinnamic and sinapic acids and lesser components as p-coumaric, ferulic, caffeic, and chlorogenic acids by gas chromatography (GC) using comparative retention times as the means of identification. Noda and Matsumoto (1971) have reported sinapic acid and methyl sinapate as naturally occurring components in rapeseed lipid extracts. We found that there were many short comings in the procedures used such as interference by lipids, failure to consider cis- and trans-isomerization and the formation of artifacts during the extraction and separation procedures. Therefore, the object of this study was to identify the phenolics present in rapeseed meals taking into account the aforementioned problems.

MATERIALS & METHODS

Materials

Rapeseed samples of the cultivars Midas, Target, Tower, Span, Torch, Echo (the same samples used by Mueller et al., 1978) and Candle were used for this study. Samples of commercial RSM from Bronowski and low erucic acid type rapeseed (LEAR) and of hulls of the cultivar Tower were also investigated. The samples were finely ground in a micromill, defatted by a 16-hr hexane extraction in a soxlet apparatus, reground and extracted a second time. Individual phenolic acids used for standards for TLC and GC were obtained from the Aldrich Chemical Company, Inc. (Milwaukee, Wisc.). Sinapine bisulfate was obtained from RSM by the method of Clandinin (1961). Goitrin was prepared according to the method used by Clandinin et al. (1966).

Chromatography of phenolic acids and esters

The TLC plates used throughout were silica gel G-25 UV-254 (Macherey-Nagel). Four solvent systems were used:

(1) Chloroform-methoxyethanol-formic acid (88%)-acetic acid (CMFA) 60:20:8:12
(2) Benzene-diethylether-acetic acid (BEA) 50:50:0.2
(3) Butanol-acetic acid-water (BAW612)6:1:2
(4) Butanol-acetic acid-water (BAW 415) 4:1:5.

Solvents 1, 3, and 4 were used to separate the polyphenolics in the unhydrolyzed extracts. Solvent 2 was used to separate the free phenolic acids. All the phenolic acids tested had an R_f value less than free fatty acids and trans-cinnamic acid and greater than gallic acid. Therefore the phenolic acids were obtained in relatively pure form for gas chromatography by scraping areas of the plate bounded by cinnamic and gallic acid or scraping off the individual bands. Cinnamic and gallic acids were visualized on the TLC plates as dark spots under short wave (254 nm) UV light while caffeic, ferulic and sinapic acids or esters of these acids show up as blue fluorescent bands under long wave (360 nm) UV light. The sprays employed for visualization included:

(1) 1% aqueous solution of $FeCl_3/K_3Fe(CN)_6$ for phenolics (Ribéreau-Gayon, 1972),
(2) 2,7-dichlorofluorescein in ethanol followed by UV light for fatty acids (Stahl, 1969),
(3) anisaldehyde/sulfuric acid for sugars (Stahl, 1969),
(4) 5% sulfuric acid and charring for organic compounds, and
(5) 2N NaOH for sinapic acid and related compounds.

The gas chromatograms were done on a Bendix 2500 GC equipped with flame ionization detectors. The column was 3m × 6 mm OD glass tube packed with 6% OV-1 on 80–100 mesh Chromosorb W and operated isothermally at 210°C or programmed from 130–210°C at 5 degrees per min with a carrier gas flow rate of 40 ml/min of nitrogen.

A column containing 3% OV-17 on 80–100 mesh chromosorb W was also used to help confirm peak identification by retention time comparisons. GC-MS was done by the Mass Spec lab, Dept. of Chemistry, Univ. of Alberta, using the OV-1 column.

Derivatives for GC analysis were prepared by adding N,O-bis-(trimethylsilyl)-acetamide (BSA) to the dried samples and heating on a steam bath for a few minutes.

The relative amounts of the phenolic constituents in the hydrolysates were calculated on the basis of peak areas of the TMS derivatives while those of sinapic acid obtained from hydrolysis of individual esters were determined by adding a known amount of methyl heptadecanoate as internal standard.

Extraction

Hydrolyzed extracts. Initially hydrolyzed extracts were prepared by letting the defatted RSM samples sit in 2N NaOH at room temperature for 6 hr. The solution was then made slightly acidic and the phenolic components were extracted by stirring with hot 80% methanol for 20 min. The mixture was centrifuged and the supernatant was concentrated in a rotory evaporator to remove the methanol. This aqueous extract was then subjected to a 48 hr liquid-liquid extraction with diethyl ether (Ribéreau-Gayon, 1972). The ether was evaporated and the residue redissolved in methanol for GC analysis (Kozlowska et al., 1975). As will be shown later, it was found that we had to include a TLC purification step to eliminate long chain fatty acids and had to eliminate alcohol from the hydrolysis procedure to prevent the formation of artifacts. Therefore

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the hydrolysis was performed by adding 4 ml of 1N NaOH to 0.75g of defatted sample and heating on a steam bath for 15 min. The mixture was then centrifuged and the supernatant decanted. The residue was washed three times with distilled water, the supernatants combined, adjusted to pH = 2 and extracted with diethyl ether. The ether extract was dried with anhydrous sodium sulfate and reduced to a small volume and applied to a TLC plate which was developed in the BEA solvent system. Two areas of the plate were then scraped off (A = the area including ferulic acid and t-cinnamic acid; B = the area including gallic acid and sinapic acid) and eluted with acetone. Both eluants were dried under vacuum and TMS derivatives were prepared for GC analysis.

Intact polyphenolics. Samples of Midas and Echo were used for estimation of the relative amounts of the various sinapic esters present in RSM. One gram of meal was extracted four times with 10 ml of 70% aqueous acetone by homogenization using a Brinkmann polytron. The pooled extract was evaporated to near dryness and taken up in a small amount of 70% aqueous acetone for TLC using solvent CMFA. The several fluorescent bands were scraped separately from the plate and eluted with 70% aqueous acetone. The eluates were evaporated to dryness under reduced pressure and hydrolyzed on a steam bath for 50 min using 4N NaOH to ensure complete hydrolysis. After acidifying (pH = 2) and evaporating under vacuum to dryness the residue was taken up in acetone and applied to a TLC plate and developed in solvent BEA. The sinapic acid band was scraped off, eluted with acetone, evaporated to dryness, derivatized with BSA and run on the GC.

Transesterification of sinapine

A drop of a saturated solution of sinapine bisulfate was mixed with a drop of a solution of a sugar (sucrose, glucose, arabinose, xylose, mannose, or galactose) and a drop of a saturated solution of Na_2CO_3 was added. This was heated on a steam bath for 15 min, neutralized with HCl and diluted with methanol. To prepare the methyl ester, sinapine was dissolved in methanol, a drop of saturated Na_2CO_3 added and the solution heated for 15 min. It was then neutralized with HCl. These methanolic solutions were used directly for TLC.

RESULTS & DISCUSSION

Hydrolyzed extracts

Our initial attempts to determine the polyphenolic components of a hydrolyzed RSM extract resulted in GC chromatograms similar in appearance to those reported by Kozlowska et al. (1975) but peak retention times did not exactly correspond to those of the standards. Further investigation showed these peaks to be long chain fatty acids, presumably present in the extract due to some hydrolysis of polar lipids that would not have been extracted by hexane. The C-16 and C-18 fatty acids had retention times very close to ferulic and sinapic acids, respectively. Using TLC (BEA) to purify the extract before GC analysis eliminated interference from long chain fatty aicds, but we still had peaks which did not correspond to the standards. It was found that cis-trans isomerism of sinapic acid and its methyl ester (Fenton et al., 1978) were responsible for three unknown peaks. The trans-methyl ester of sinapic acid had a retention time within a few seconds of trans-ferulic acid. By eliminating methanol from the sample preparation we eliminated the peaks due to cis and trans methyl sinapate, indicating that the methyl ester is not a naturally occurring component of RSM. Under slightly acidic conditions we found methyl sinapate could be extracted from an aqueous phase by hexane.

Noda and Matsumato (1971) reported methyl sinapate to be present in the acidic lipid fraction of a lipid extract of rapeseed. We prepared an acid fraction from our hexane extraction and were unable to detect any methyl sinapate which made us suspect that the methyl sinapate found by Noda and Matsumoto (1971) could possibly have been due to transesterification of sinapine with traces of methanol which can be a common impurity in the solvents they used.

TLC in BEA of the alkaline hydrolysates of the RSM samples showed similar patterns for each variety. The phenolics could be identified by their UV fluorescence, color reaction with different spray reagents and R_f values. Sinapic and ferulic acids were easily identified by their characteristic blue fluorescence and R_f values by comparison with standards. Caffeic acid, which had been reported in free (Kozlowska et al., 1975) and ester form as chlorogenic acid (Durkee and Thivierge, 1975; Lo and Hill, 1972) was not detected in any of the RSM samples. Our TLC detection limit for caffeic acid was 0.06 μg.

Durkee and Thivierge (1975) report that they found chlorogenic acid by extracting it into ethyl acetate, leaving sinapine in the aqueous phase. We believe this is in error since the *Merck Index* (9th ed.) reports that chlorogenic acid is only slightly soluble in ethyl acetate but will make about a 4% solution in water. Further, upon repeating their method we were not able to extract a chlorogenic acid standard from an aqueous phase with ethyl acetate. In RSM samples this ethyl acetate extraction almost quantitatively removed from the aqueous phase sinapic acid and an unknown compound (Band 6, Table 2) that upon basic hydrolysis yielded sinapic acid as identified by both TLC and GC. Kowzlowska et al. (1975) reported the existence of cinnamic acid in RSM but we were not able to confirm this. With the GC conditions they used it is unlikely they would have been able to separate cinnamic acid from goitrin, a decomposition product of glucosinolates which we found (and confirmed by GC-MS) in the alkaline hydrolysate of all the RSM samples tested. Esters of p-coumaric acid have also been reported to be present in RSM (Durkee and Thivierge, 1975; Kozlowska et al., 1975) but again this acid was not observed in this study. The R_f values of goitrin and the benzoic and cinnamic acid derivatives identified in the hydrolysates are given in Table 1.

GC proved to be a valuable complement to TLC in identification of the phenolics. Good resolution of their TMS derivatives was achieved as shown in Figures 1 and 2. The retention times and relative concentrations are given in Table 1.

The GC chromatogram (Fig. 1) of the eluate from the TLC section which covered the area which included cinnamic and ferulic acid revealed numerous peaks. On comparison with the TMS derivatives of the standard acids A1, A2, A5, A6, and A10 were found to be p-hydroxy benzoic,

Table 1—Identity, retention time, R_f and relative concentration of the compounds found in hydrolyzed RSM extracts

Peak	Identity	Retention time (min)	R_f of the free acid in BEA	Avg Relative conc in RSM
A1	p-OH-Benzoic acid-diTMS	13.46	0.762	5
A2	Vanillic acid-diTMS	16.17	0.770	3
A3	unknown	16.77	>0.6	
A4	unknown	17.90	>0.6	
A5	syringic acid-diTMS	18.93	0.688	1
A6	cis-ferulic acid-diTMS	19.53	0.723	
A7	unknown	20.17	>0.6	
A8	unknown	21.17	>0.6	
A9	cis-sinapic acid-diTMS	24.00	0.599	
A10	trans-ferulic acid-diTMS	25.47	0.723	13*
A11	unknown	27.07	>0.6	
B1	unknown	8.52	<0.6	
B2	goitrin-TMS	11.13	0.514	11
B3	protocatechuic acid-tri TMS	17.57	0.585	15
B4	cis-sinapic acid-diTMS	24.00	0.599	
B5	trans-sinapic acid-diTMS	33.67	0.599	1000*

* includes cis isomer.

Table 2—R_f, identity, and relative amount of sinapic acid liberated upon hydrolysis from the intact polyphenolics in RSM

Band No.	Rf	Identity	Relative amount of sinapic acid	
			Echo	Midas
Origin	0	unknown	100	90
1	0.12	sinapine	1000	1000
2	0.22	unknown	10	30
3	0.51	1-sinapoyl glucose	50	260
4	0.65	unknown	–	–
5	0.74	unknown	2	–
6A	0.81	unknown	–	150
6	0.87	unknown	230	230
7	0.93	sinapic acid	10	2

vanillic, syringic, cis-ferulic and trans-ferulic acids, respectively. With the exception of syringic acid, these phenolics had been previously found (Durkee and Thivierge, 1975). Two unknowns, A3 and A11, were detected in all alkaline hydrolysates but we were unable to identify them. A3 had a retention time very close to that of cis-p-coumaric acid but the expected trans isomer could not be detected by GC and the molecular weight of A3 did not correspond to that of the TMS derivative of p-coumaric acid, further indicating the absence of this acid in the hydrolysate and was in line with the results provided by TLC.

The identities of the benzoic and cinnamic acid derivatives in RSM hydrolysates were confirmed by GC-MS on the basis of molecular weights and fragmentation patterns of their TMS derivatives through comparison with standards. It was noted that the amount of the various phenolic acids with the exception of ferulic acid remained relatively constant in all samples examined.

It is evident from Table 1 and Figures 1 and 2 that sinapic acid is the most abundant compound released from RSM through alkaline hydrolysis. B4 and B5 (Fig. 2) were identified as cis and trans sinapic acid, respectively. The cis-trans isomerism of cinnamic acid derivatives had been previously established (Fenton et al., 1978; Hartley and Jones, 1975) and shown to occur in solution. Therefore it appears that trans-sinapic acid is the naturally occurring isomer and the detection of the cis isomer is a result of its formation after hydrolysis of the sinapic esters. The other major peaks, B2 and B3, on the same chromatogram were goitrin and protocatechuic acid, respectively. The amount of goitrin varied between varieties, being very low in Candle and high in Midas and Target. This observation was in line with the different levels of glucosinolate reported in RSM (Clandinin and Robblee, 1978). Protocatechuic acid which was found in the hydrolysate of the variety Oro by Durkee and Thivierge (1975) was also found in all seven of the varieties we tested, being highest in concentration next to sinapic acid.

A further observation that may be significant is that no gallic acid was detected in the basic hydrolysates either by TLC or GC. This would indicate there are no gallotannins in RSM although it may be difficult to detect gallic acid as it is subject to oxidation under basic conditions. Attempts to find gallic acid in acid hydrolysates of RSM were also unsuccessful.

Intact polyphenolics

TLC of the 70% aqueous acetone extracts of RSM developed in CMFA showed several bands which had fluores-

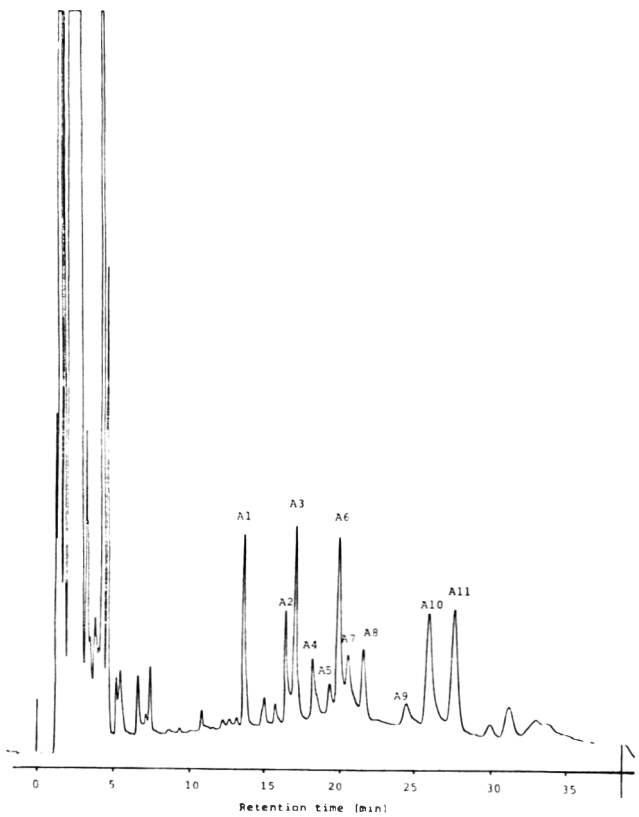

Fig. 1—Gas chromatogram of the TMS derivatives of the compounds from section A of the TLC plate.

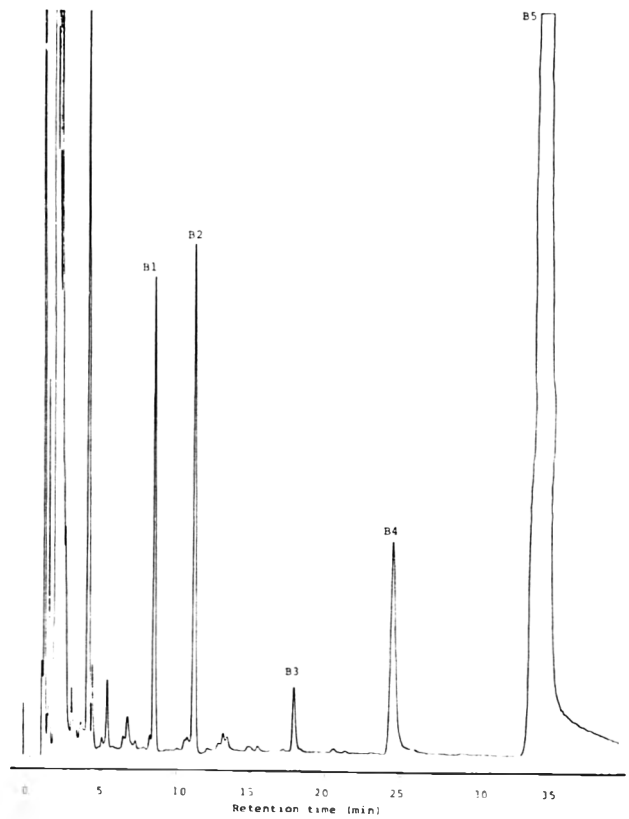

Fig. 2—Gas chromatogram of the TMS derivatives of the compounds from section B of the TLC plate.

cent properties similar to that of sinapic acid. Their R_f values are summarized in Table 2. Based on their intensities of fluorescence bands 1, 3, 6, and 7 existed in more significant amounts and were common constituents in all the meals examined. Band 7 was confirmed to be sinapic acid by GC. Band 1 was sinapine, the most abundant phenolic in RSM, the concentration averaging 1.5% (Mueller et al., 1978). Band 3 appeared to be generally the next highest in concentration. It had a similar R_f value to that of chlorogenic acid in CMFA but a blue rather than a blue-gray fluorescence and on alkaline hydrolysis or treatment with B-glucosidase only sinapic acid and glucose could be detected. This indicated it to be 1-sinapoyl glucose which has been reported by Austin and Wolff (1968). Further, transesterification of sinapine with glucose resulted in three bands, one corresponding to sinapine, one to band 3 and one to sinapic acid. The same reaction with sucrose resulted in five bands which were due to sinapine, the sucrose ester of sinapic acid, the glucose ester of sinapic acid, the fructose ester of sinapic acid and sinapic acid. The sucrose ester had the same R_f as band 2 and the glucose ester again had the same R_f as band 3 while the fructose ester had an R_f between bands 3 and 4. Bands resulting from transesterification with other sugars did not exactly correspond to any of the RSM bands. The similar R_f values for band 2 and the sucrose ester of sinapic acid gives a possible identity to band 2 but certainly does not confirm it. No chlorogenic acid could be detected by TLC in CMFA or BAW(612), this agreeing with the absence of caffeic acid in the alkaline hydrolysates.

Bands 4 and 5 were mainly observed in the Lear and Bronowski meals and appeared to be in low concentration relative to the other bands. As these were processed meals, these compounds may be artifacts formed during the oil extraction process.

Band 6 yielded sinapic acid on alkaline hydrolysis without the detection of sugar. It had acidic properties as it could be extracted by ethyl acetate or diethyl ether from acidic aqueous solution. Work is being done in our laboratory to further establish its identity. Band 6A was exclusively found in Midas meal. It had the typical blue fluorescent color of sinapic acid when viewed under long wave UV, yet it was seen as a dark spot when dried and as a brown spot when viewed under short wave UV light. It also released sinapic acid by alkaline hydrolysis. Its identity was not further pursued since it was limited to one meal only. Contrary to the observation reported by Kozlowska et al. (1975) only sinapic acid was detected when the 70% aqueous acetone extract was examined by TLC using BEA system. GC of the acetone eluant of sections of the TLC plate also supported this finding. Thus it could be concluded that the only free phenolic acid in RSM was sinapic acid.

Besides R_f values, Table 2 gives the relative amount of sinapic acid found in each of the bands after hydrolysis for the Echo and Midas samples. It is apparent that there is also some compound or compounds remaining at the origin that upon alkaline hydrolysis liberate sinapic acid.

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WATER ACTIVITY OF AQUEOUS LACTULOSE SOLUTIONS

JORGE CHIRIFE and CONSTANTINO FERRO FONTÁN

ABSTRACT

This work investigated theoretically and experimentally the water activity lowering behavior of lactulose in the intermediate moisture range (about 0.95–0.80 a_w). Lactulose was found to have a negative deviation from Raoult's law slightly larger than that showed by sucrose. These results indicate that lactulose does not have the exceptionally good ability for depressing water activity recently suggested in the literature.

INTRODUCTION

IT IS WELL KNOWN that the possibilities of adjusting the water activity (a_w) of intermediate moisture foods (IMF) for human consumption with most food additives in use today, are quite limited (Benmergui et al., 1979; Chirife et al., 1980). The main reason is that the high level of solutes required for a_w control imparts undesirable flavors to foods and also may adversely affect their physical properties. By this reason, unconventional additives have been explored mainly looking for compounds which exhibit very large negative deviations from Raoult's law. The results, however, have not been successful, as discussed by Chirife and Ferro Fontán (1980).

Very recently, Ross et al. (1979) examined the water activity lowering behavior of a number of compounds derived from lactose looking for potential humectants for IMF for human use. They reported that lactulose (4-O-β-D-galactopyranosyl-β-D-fructofuranose), which is half as sweet as sucrose (Parrish et al., 1979) and much more soluble than lactose, was the best humectant. Compared to sucrose it gave an a_w = 0.85 at half the concentration, which constitutes a remarkable a_w lowering ability for a nonelectrolyte compound having the same molecular weight as sucrose. These results stimulated our interest for measuring the water activity of lactulose solutions.

EXPERIMENTAL

Materials

Pure lactulose was obtained from E. Merck, Darmstadt (West Germany).

Lactulose is usually available as a concentrated syrup obtained from lactose by isomerization (Parrish et al., 1979). This syrup has laxative properties and is marketed by the pharmaceutical industry. Thus, and in addition to pure lactulose, the behavior of this syrup was also investigated; brands studies were: "Duphalac" (Ferrosan, Malmö, Sweden), "Lactulon" (Lab. Dr. Lazar, Buenos Aires, Argentina), and "Cephulac" (Merrel Lab., U.S.A.). The main characteristics of these syrups are shown in Table 1. They have in common that lactulose comprises about 68–72% of the dry matter with the rest consisting in a mixture of galactose, glucose, lactose, and other sugars in minor proportions.

Analytical methods

Solids content was determined by a vacuum oven method at 65°C over magnesium perchlorate. Density was determined by pycnometry.

The identity and amount of sugars accompanying lactulose in the pharmaceutical syrups was verified (only for "Duphalac") by gas-liquid chromatography of trimethylsilyl derivatives of the sugars. It was performed on a 120 cm × 0.20 cm column of 2% OV-101 on Chromosorb W. AW. DMC S (60–80 mesh) with temperature programming from 100–250°C and a flame ionization detector. Other parameters were injector temperature 250°C and detector temperature 300°C.

Water activity

The water activity of lactulose solutions and syrups was determined using the "a_w-Wert Messer" manufactured by Firma LUFFT, Stuttgart, West Germany. This is a simple cup device where the a_w value is read directly from a dial (Leistner and Rodel, 1976) after the sample has been equilibrated. However, in order to improve the reliability of the measurements, the instrument was operated following the procedure of Chirife and Ferro Fontán (1980). For this purpose the instrument was carefully checked against five different standard saturated salt solutions in the a_w range of interest to this study (about 0.80–0.97), and a calibration curve was obtained at a constant temperature of 25 ± 0.4°C. An excellent linear correlation was obtained between measured and "real" a_w values. For a_w measurements the dial readings were converted to a_w through the calibration curve. Every 24 hr the calibration was checked. In order to insure thermal equilibrium, lactulose solutions were always kept at 25°C for 3–4 hr in a constant temperature oven before placing in the instrument for a_w measurement.

RESULTS & DISCUSSION

NORRISH (1966) proposed the following equation for correlating a_w data in nonelectrolyte aqueous solutions

$$a_w = X_1 \exp(-K X_2^2) \qquad \text{Eq. (1)}$$

where X_1 and X_2 are molar fractions of water and solute, respectively, and K is the correlating constant. For K = 0, Eq (1) reduces to Raoult's law, while positive and negative deviations are indicated by negative and positive values of K, respectively.

Figure 1 shows the a_w data for lactulose solutions and the various lactulose syrups: it can be seen that the experimental data are very well fitted by Eq (1). A computer program was utilized to calculate the best K values for lac-

Table 1—Characteristics of the various pharmaceutical lactulose syrups

Brand	Lactulose content (nominal, g/100 ml)	Solids content (% w/w)	Density (g/ml, 24°C)
"Duphalac" (Sweden)	66.7	68.94	1.327
"Lactulon" (Argentina)	65.0	71.70	1.339
"Cephulac" (U.S.A.)	66.7	70.2	1.338

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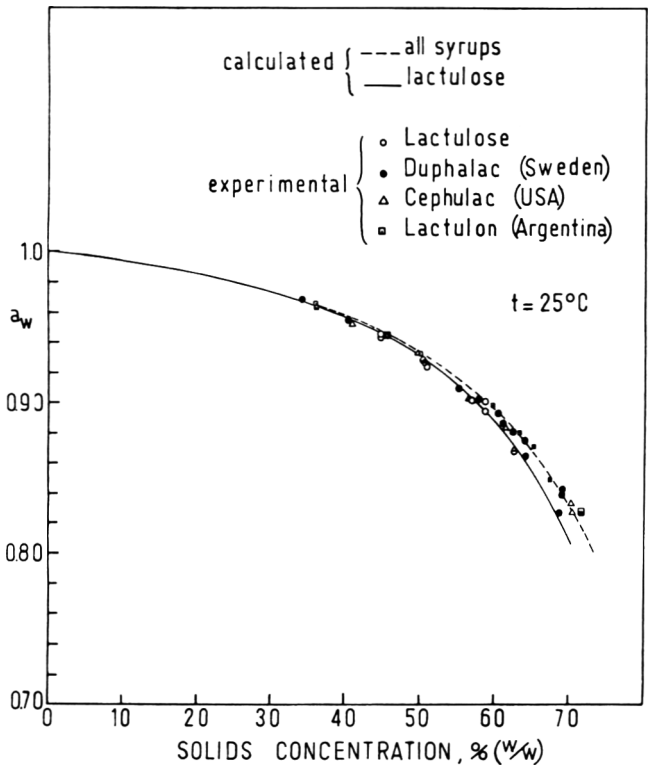

Fig. 1—Comparison of measured and predicted water activity in aqueous lactulose solutions and lactulose syrups at 25° C.

tulose and lactulose syrups. For lactulose, K was found to be 8.0 ± 0.3, a value which is cimparable to that found for other sugars as it is shown in Table 2. The a_w data for the three pharmaceutical lactulose syrups fall approximately on the same correlation with the expected small variations due to the fact that they are multicomponent mixtures which may have small differences in composition. Nevertheless they were analyzed together in order to calculate unique K value for all syrups. This requires the knowledge of the molecular weight of the syrup. As they are multicomponent mixtures a "working" molecular weight was calculated on the basis of the sugar composition as revealed by the gas-liquid chromatographic analysis. The analysis (performed on the "Duphalac" sample) indicated (on dry basis) that lactulose and lactose comprised 81.8%, glucose 3.84%, galactose 5.16%, other disaccharides 5.73% and 3.3% unknowns. The "working" molecular weight was calculated as

$$\bar{M}^{-1} = \Sigma\, w_i M_i^{-1}$$

where w_i is the weight fraction (dry basis) of sugar component i. The calculated value was found to be $\bar{M}$ = 312.7 and a value of K = 4.1 ± 0.2 was thus obtained. This value is also shown in Table 2 as compared with K values for different sugars.

Ross et al. (1979) suggested that lactulose exhibited a remarkable a_w lowering ability; they advanced that, as compared to sucrose, lactulose gave an a_w = 0.85 at half the concentration. On the basis of a_w-concentration literature data for sucrose (Chirife et al., 1979) this means that a lactulose solution having a concentration of about 33.5% (w/w) should give an a_w = 0.85. An inspection of Figure 1 indicates that this is very far from present results.

Table 2—Values of K (Eq 1) for various sugars at 25° C

Sugar	K	
Lactulose	8.0 ± 0.3	This work
Sucrose	6.47 ± 0.06	Chirife et al. (1980)
Maltose	4.54 ± 0.02	Chirife et al. (1980)
Lactulose syrups (average)	4.1 ± 0.2	This work
Glucose	2.25 ± 0.02	Chirife et al. (1980)
Xylose	1.54 ± 0.04	Chirife et al. (1980)

CONCLUSIONS

THE DATA obtained in this work indicate that lactulose has a negative deviation from Raoult's law somewhat higher than that exhibited by sucrose, but very far from the behavior suggested by the data of Ross et al. (1979) which apparently showed that lactulose has a large negative deviation from Raoult's law.

Present data of a_w of lactulose solutions and lactulose syrups are very well fitted by Norrish's (1966) model and K values obtained are those which may be expected for sugar molecules (Chirife et al., 1979).

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EFFECTS OF FERMENTATION ON PHYTATE PHOSPHORUS AND MINERAL CONTENT IN BLACK GRAM, RICE, AND BLACK GRAM AND RICE BLENDS

N. R. REDDY and D. K. SALUNKHE

ABSTRACT

Quantitative changes in phosphorus compounds and mineral content of black gram, rice, and black gram and rice blends were investigated during fermentation. Black gram cotyledons and polished Texas long-grain rice had phytate phosphorus content of about 4.80 and 0.97 mg/g, respectively, on a dry weight basis. Phytate phosphorus accounted for about 51% of total phosphorus in rice. Fermentation of rice alone for 8 hr resulted in complete hydrolysis of phytate phosphorus with simultaneous increase in the quantities of nonphytate phosphorus. At 45 hr fermentation, about 13.33% and 48.80% of phytate phosphorus was hydrolyzed in black gram and black gram and rice blends, respectively, with subsequent increases in nonphytate phosphorus. No apparent changes were observed in the concentrations of calcium, magnesium, zinc, and iron; the inorganic sulfur content was decreased in black gram, rice, and black gram and rice blends as the fermentation progressed to 45 hr. The 20-hr fermented black gram and rice batter was judged to produce an optimum quality of steamed product (Idli) which contained about 1.51 mg/g of phytate phosphorus. Scanning electron microscopy was employed to study the physical changes in the black gram and rice fermented batters and Idli. The Idli had a fine net work (spongy) type of texture and did not contain the starch granules compared to corresponding unsteamed samples.

INTRODUCTION

FERMENTED FOODS are essential components of diets in many parts of the work, especially Southeast Asia, the Near East, and parts of Africa. They are also important in the diets of Western World, because of their high nutritive value and organoleptic characteristics (Stainkraus and van Veen, 1971). Black gram is one of the choice ingredients in the fermented steamed food product (Idli), and plays a major role in the fermentation as a source of microorganisms and fermenting substrate (Radhakrishnamurthy et al., 1961). Rice is the other ingredient of Idli. This product enjoys a great popularity in India due to its textural and other sensory attributes (Steinkraus et al., 1967). Recently, Susheelamma and Rao (1974, 1979) isolated two components namely, surface active protein (globulin) and a polysaccharide (arabinogalactan) from black gram. They indicated that highly surface active (foam forming) protein, globulin was responsible for the soft and porous texture, while the arabinogalactan was essential for raising and stabilization of the texture caused by globulin. They also showed that surface active protein, globulin serves in holding the gas in a network of stable bubbles and upon steaming the bubbles and the network expand and produce the porous (spongy) texture.

Phytate phosphorus makes up approximately 79% and 89% of the total phosphorus in black gram seeds and cotyledons respectively (Reddy et al., 1978; Reddy and Salunkhe, 1980). It is primarily present as a complex salt of minerals or complexes with proteins in seeds (Rackis and Anderson, 1977). Sharma et al. (1978) reported that myo-inositol hexaphosphate (phytate) strongly inhibits the α-amylases of peanuts and chick peas. Phytate plays a significant role in decreasing the bioavailability of multivalent cations such as Ca^{++}, Mg^{++}, Zn^{++}, Fe^{++}, and Fe^{+++} (Harrison and Mellanby, 1939; Sharpe et al., 1950; Roberts and Yudkin, 1960; O'Dell, 1979) by forming insoluble phytate-metal complexes and render them unavailable to man and animals. Some of the processes such as germination, soaking, fermentation, and other autolytic treatments are known to reduce and/or eliminate the phytate in legume seeds (Kon et al., 1973; Becker et al., 1974; Change et al., 1977; Sudarmadji and Markakis, 1977; Kumar et al., 1978). Our earlier studies (Reddy et al., 1978; Reddy and Salunkhe, 1980) investigated the changes in phytate phosphorus and minerals during germination and cooking of black gram and phytates interactions with protein(s) and minerals of black gram.

The present investigation was undertaken to study the effects of fermentation on phyate phosphorus and certain mineral content of black gram, rice, and black gram and rice blends.

MATERIALS & METHODS

BLACK GRAM COTYLEDONS were purchased from Indo-European Inc., Hollywood, Calif. Polished Texas long-grain rice was procured from a local grocery store.

Fermentation

Essentially the following procedure was followed. Equal parts of black gram cotyledons (500g) and polished Texas long-grain rice (500g) were employed in the fermentation. Each of the ingredients was washed with distilled water to remove surface microorganisms, and soaked for 2 hr in distilled water, then ground to a fine paste in a Waring Blendor at high speed for 2 min. Water to beans and/or rice ratio was 1.5:1.0 i.e., 1500 ml distilled water was used per 1000g bean cotyledons and/or rice. They were then mixed and 1% salt added (calculated from the weight of the batter). The black gram and rice mixed batter was distributed into containers, covered, and allowed to ferment in a 30°C incubator. Samples in duplicate were taken at 4-hr intervals. One set was used for pH determinations. The pH determinations were made directly on the batter. Other sets of samples were freeze-dehydrated immediately and analyzed for phytate phosphorus, total phosphorus, inorganic phosphorus, sulfur, calcium, magnesium, zinc, and iron. Individual fermentation experiments with black gram, and rice alone were also conducted under the same above conditions and employed for chemical analyses.

Phytate phosphorus and other phosphorus compounds

Phytate phosphorus was determined by a combination of three methods as described previously (Reddy et al., 1978). Total phosphorus was estimated by the colorimetric method of Fiske and Subbarow (1925). Inorganic phosphorus was determined by the method of Pons and Guthrie (1946).

Minerals

For minerals analyses, the samples were ashed according to the AACC method (1962) and dissolved in dilute HCl. Calcium, magnesium, zinc, and iron were determined by Jarrel Ash 800 Atomic Absorption Spectrophotometer (Fisher Scientific Co., 590 Lincoln St., Waltham, Mass.). The sulfur was determined by turbidimetric method (Chesnin and Yien, 1950).

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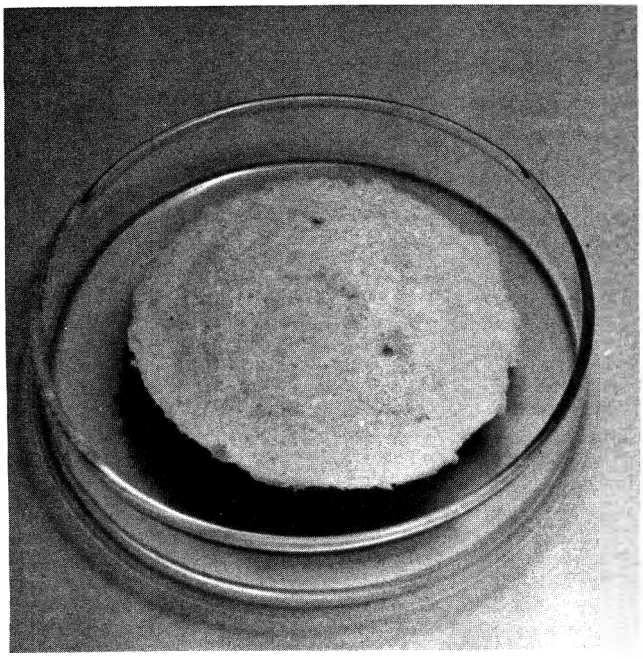

Fig. 1—Photograph of a fermented steamed product (Idli).

Scanning electron microscopy

The fermented samples of black gram and rice blend and Idli were prepared for scanning electron microscopic studies as follows: A small piece of sample (freeze-dehydrated) was placed on an aluminum stub (which had a double-stick tape on it) and was coated with gold-palladium alloy by using Polaron E 5000 Sputter Coater (England, U.K.). The coated samples were viewed and photographed in an AMR 1000 B Scanning Electron Microscope (Cambridge, Mass.) at an accelerating voltage of 20 kv and suitable magnification(s).

RESULTS & DISCUSSION

Fermentation

The two most important physicochemical changes, leavening and acidification, are known to occur during fermentations of black gram, rice, and black gram and rice blends. Similar results for leavening (batter volume), acidification (pH), and microbial counts were obtained as previously reported by Padhye and Salunkhe (1978). The 20-hr black gram and rice (in 1:1 ratio w/w) fermented batter was judged as optimum for preparation of steamed product, Idli, on the basis of volume increase, appearance, and appealing sour flavor. The black gram and rice fermented batter at 20 hr was steamed for 5 min to obtain an Idli, which had soft and spongy (porous crumb) texture and sour flavor (Fig. 1).

Phytate phosphorus and other phosphorus compounds

The changes in the phytate phosphorus (phytate P) and nonphytate phosphorus (nonphytate P) contents in black gram, rice, and black gram and rice fermentations are shown in Table 1. No significant changes were observed in the contents of total phosphorus during 45-hr fermentations of black gram, rice, and black gram and rice blends. Raw black gram cotyledons and polished Texas long-grain rice had phytate P of 4.80 and 0.97 mg/g, respectively, on a dry weight basis. The phytate P in rice accounted for about 51% of total phosphorus. At 45 hr, only 13.3% of phytate P was hydrolyzed in black gram fermentation compared to 100.0% and 49.0% hydrolysis in rice and black gram and rice fermentations, respectively. The decrease in phytate P was accompanied by an increase in nonphytate P content in all the fermentations. In black gram fermentation, the phytate P hydrolysis and/or disappearance was slow. Reinhold (1975) reported the similar results on slow hydrolysis of phytate P by phytases in Tanok (wheat) meal fermentation. He accounted the following reasons for slow hydrolysis of phytate P: (1) Presence of metal ions, and other salts, which form stable salts resistant to enzyme hydrolysis. (2) The existance of phytate P in the form of metals salt and/or in combination with proteins in seeds and these forms may not be available for enzyme attack. (3) Presence of high concentration of phytate P in the medium and which inhibits phytase action. These may explain the slow hydrolysis of phytate P in black gram fermentation observed in the present investigation, because the beans contain 70–80% of phosphorus in the form of phytate P, and which is present in the form of metal salts.

The phytate P completely disappeared in rice within 8 hr presumably its hydrolysis by phytase and which was demonstrated by an increase in nonphytate P content from 0.92 to 1.89 mg/g during that period. The disappearance of phytate P in rice fermentation was faster compared to black gram and rice fermentation. During the first 20-hr black gram and rice fermentation, 40.1% phytate P was hydrolyzed, with simultaneous increase in inorganic phosphorus from 0.39 to 1.13 mg/g (Table 2). The increased hydrolysis of phytate P in black gram and rice blend was possibly due to the additive effect of rice. The 20-hr black gram and rice fermented batter produced Idli, on steaming, which had 1.51 mg/g of phytate P (which accounted for 44% of total phosphorus). The steaming of 20-hr black gram and rice

Table 1—Effects of fermentation on total phosphorus, phytate P, and nonphytate P contents in black gram, rice, and black gram and rice blends

Fermentation time (hr)	Total P mg/g	Phytate P mg/g	Nonphytate P mg/g	% Phytate P hydrolyzed
Black gram				
Raw	5.60 ± 0.05[b]	4.80 ± 0.22	0.80	0.00
0[a]	5.61 ± 0.14	4.80 ± 0.23	0.80	0.00
4	5.59 ± 0.27	4.76 ± 0.16	0.84	0.83
8	5.43 ± 0.14	4.66 ± 0.09	0.94	2.92
12	5.62 ± 0.02	4.66 ± 0.08	0.94	2.92
16	5.57 ± 0.22	4.62 ± 0.16	0.98	3.75
20	5.61 ± 0.17	4.62 ± 0.13	0.98	3.75
24	5.69 ± 0.13	4.22 ± 0.10	1.38	12.08
45	5.77 ± 0.14	4.16 ± 0.32	1.44	13.33
Rice				
Raw	1.89 ± 0.06	0.97 ± 0.06	0.92	0.00
0[a]	1.96 ± 0.13	0.65 ± 0.10	1.31	32.99
4	1.75 ± 0.08	0.07 ± 0.00	1.89	92.78
8	1.79 ± 0.12	0.00 ± 0.00	1.89	100.00
12	1.83 ± 0.00	ND[c]	1.89	–
16	1.79 ± 0.04	ND	1.89	–
20	1.96 ± 0.12	ND	1.89	–
24	1.96 ± 0.12	ND	1.89	–
45	1.96 ± 0.13	ND	1.89	–
Black gram and rice				
0[a]	3.71 ± 0.27	2.52 ± 0.12	1.19	0.00
4	3.84 ± 0.25	2.15 ± 0.09	1.56	14.70
8	3.75 ± 0.15	1.93 ± 0.12	1.78	23.40
12	3.70 ± 0.10	1.61 ± 0.08	2.10	36.11
16	3.71 ± 0.11	1.55 ± 0.03	2.16	38.50
20	3.74 ± 0.08	1.51 ± 0.06	2.20	40.08
24	3.72 ± 0.16	1.38 ± 0.05	2.33	45.24
45	3.89 ± 0.20	1.29 ± 0.04	2.42	48.80

[a] Refers to the samples, which were soaked for 2 hr before blending
[b] Each value is the average of four determinations and expressed on a moisture-free basis; mean plus or minus standard deviation.
[c] Not detectable

Table 2—Total P, phytate P, inorganic P, and nonphytate P contents in unfermented and 20-hr fermented batters and 20-hr fermented, steamed product (Idli)

Sample	Total P mg/g	Phytate P mg/g	Inorganic P mg/g	Nonphytate P mg/g	% Phytate P hydrolyzed
Unfermented (0 hr)	3.71 ± 0.27[a]	2.52 ± 0.12	0.39 ± 0.01	1.19	0.00
20 hr fermented	3.74 ± 0.08	1.51 ± 0.06	1.13 ± 0.02	2.20	40.08
20 hr fermented, steamed product (Idli)	3.80 ± 0.21	1.51 ± 0.07	1.36 ± 0.04	2.20	40.08

[a] Each value is the average of four determinations and expressed on a moisture-free basis; mean plus or minus standard deviation.

fermented batter caused an increase i.e., 20% in inorganic phosphorus in the Idli, than the corresponding unsteamed batter (Table 2). The increase may have been due to degradation of other phosphorus compounds by moist heat treatment (steaming) because phytate P content remained constant i.e. 1.51 mg/g in both the product, Idli, and unsteamed batter. In this study, a decrease of 41% phytate P was obtained in the final product, Idli, and in which black gram and rice were present in 1:1 (w/w) ratio. Rajalakshmi and Vanaja (1967) reported a decrease of 30.0% phytate P in Idli which was prepared by mixing black gram and rice in a ratio of 1:2. The variations observed could be due to use of different conditions and materials.

Minerals

The data on calcium, magnesium, zinc, iron, and inorganic sulfur in fermented samples of black gram, rice, and black gram and rice blends are presented in Tables 3 and 4. No apparent changes in the concentrations of Ca, Mg, Zn, and Fe were observed during the fermentations of black gram, rice, and black gram and rice blends (Table 3). The small differences in the concentrations of minerals during fermentations may be due to experimental errors. The inorganic sulfur content was decreased in its concentration as the fermentation progressed to 45 hr in each case (Table 4). At 45 hr, the black gram, rice, and black gram and rice fermented samples contained about 1.81, 0.02, and 0.26 mg/g of inorganic sulfur, respectively. The decrease in inorganic sulfur content noticed may be due to its metabolism by microorganisms during fermentation, which might have been lost either as gases such as SO_2 or utilized in the synthesis of other sulfur containing organic compounds including thiolesters.

Scanning electron microscopy

The scanning electron photomicrographs of 0-hr and 20-hr black gram and rice (1:1 w/w) fermented batters and 20-hr fermented steamed product, Idli, are shown in Figures 2, 3, 4, and 5. The starch granules can be seen in 0-hr and 20-hr fermented batters (Fig. 2 and 3) compared to the fermented steamed product, (Idli) (Fig. 4 and 5) where the complete disappearance of starch granules may be attributed to steaming. There was a significant difference between the textural characteristics of 20-hr fermented batter and Idli. The air voids and fine network (spongy texture) of the product can be seen in Figures 4 and 5. The soft spongy texture observed in Idli may be due in part to two components namely surface active protein (globulin) and a polysaccharide (arabinogalactan) of black gram. These two constituents were isolated from black gram and studied extensively by Susheelamma and Rao (1974, 1979). They showed that these two components were responsible for soft textural properties of leavened steamed pudding, which was prepared out of black gram. The product showed porous and broken surfaces (Fig. 5-B), perhaps because of stress in excess of that they could withstand during steaming and resulted in rupture at the weak surfaces.

—Text continued on page 1712

Table 3—Mineral content in black gram, rice, and black gram and rice blends during fermentation

Fermentation time (hr)	Calcium mg/g	Magnesium mg/g	Zinc µg/g	Iron µg/g
Black gram				
Raw	0.76 ± 0.03[b]	2.57 ± 0.02	35.20 ± 0.60	66.60 ± 0.80
0[a]	0.75 ± 0.02	2.58 ± 0.03	35.13 ± 1.27	72.67 ± 3.50
4	0.75 ± 0.04	2.59 ± 0.00	33.83 ± 0.00	64.79 ± 0.00
8	0.71 ± 0.03	2.56 ± 0.03	33.54 ± 0.48	65.57 ± 1.76
12	0.69 ± 0.00	2.53 ± 0.00	33.66 ± 0.84	68.02 ± 1.20
16	0.70 ± 0.01	2.54 ± 0.05	33.33 ± 1.37	64.76 ± 0.00
20	0.69 ± 0.02	2.52 ± 0.02	33.49 ± 0.73	64.94 ± 0.85
24	0.78 ± 0.02	2.56 ± 0.04	33.09 ± 0.67	63.29 ± 1.73
45	0.78 ± 0.03	2.60 ± 0.17	33.39 ± 0.65	65.47 ± 1.50
Rice				
Raw	0.15 ± 0.01	0.42 ± 0.03	19.65 ± 0.27	45.67 ± 0.57
0[a]	0.15 ± 0.00	0.41 ± 0.01	20.34 ± 0.94	50.51 ± 1.95
4	0.14 ± 0.01	0.41 ± 0.02	19.65 ± 0.50	46.73 ± 1.18
8	0.14 ± 0.01	0.38 ± 0.00	20.86 ± 0.66	40.61 ± 1.35
12	0.15 ± 0.00	0.39 ± 0.01	20.06 ± 0.68	50.18 ± 3.25
16	0.15 ± 0.01	0.40 ± 0.01	20.77 ± 0.13	45.34 ± 0.00
20	0.15 ± 0.00	0.38 ± 0.03	19.03 ± 0.81	46.60 ± 2.80
24	0.15 ± 0.01	0.42 ± 0.02	19.36 ± 0.00	45.87 ± 4.80
45	0.15 ± 0.01	0.42 ± 0.04	17.90 ± 0.80	44.38 ± 0.00
Black gram and Rice				
0[a]	0.42 ± 0.00	1.36 ± 0.01	23.52 ± 0.85	48.90 ± 0.79
4	0.42 ± 0.01	1.36 ± 0.02	23.50 ± 0.56	45.45 ± 0.80
8	0.42 ± 0.02	1.40 ± 0.02	24.15 ± 1.32	48.80 ± 3.73
12	0.41 ± 0.01	1.37 ± 0.01	22.86 ± 0.00	45.39 ± 0.00
16	0.42 ± 0.00	1.41 ± 0.02	23.61 ± 0.00	46.07 ± 4.15
20	0.41 ± 0.01	1.43 ± 0.00	22.56 ± 0.12	45.30 ± 1.67
24	0.41 ± 0.00	1.38 ± 0.02	23.37 ± 1.00	46.38 ± 2.15
45	0.40 ± 0.01	1.41 ± 0.04	21.83 ± 0.68	48.37 ± 0.80

[a] Refers to the samples which were soaked for 2 hr before blending
[b] Each value is the average of four determinations and expressed on moisture free basis; mean plus or minus standard deviation.

Table 4—Effects of fermentation on inorganic sulfur content in black gram, rice, and black gram and rice blends

Fermentation time (hr)	Inorganic sulfur mg/g		
	Black gram	Rice	Black gram and Rice
Raw	2.56 ± 0.03[b]	0.08 ± 0.01	–
0[a]	2.54 ± 0.00	0.07 ± 0.00	1.26 ± 0.00
4	–	–	0.95 ± 0.00
8	–	–	0.74 ± 0.00
12	2.20 ± 0.06	0.04 ± 0.00	0.42 ± 0.07
16	–	–	0.36 ± 0.00
20	1.95 ± 0.06	0.04 ± 0.00	0.28 ± 0.00
24	–	–	0.28 ± 0.00
45	1.81 ± 0.00	0.02 ± 0.00	0.26 ± 0.00

[a] Refers to the samples which were soaked for 2 hr before blending
[b] Each value is the average of four determinations and expressed on a moisture-free basis; mean plus or minus standard deviation.

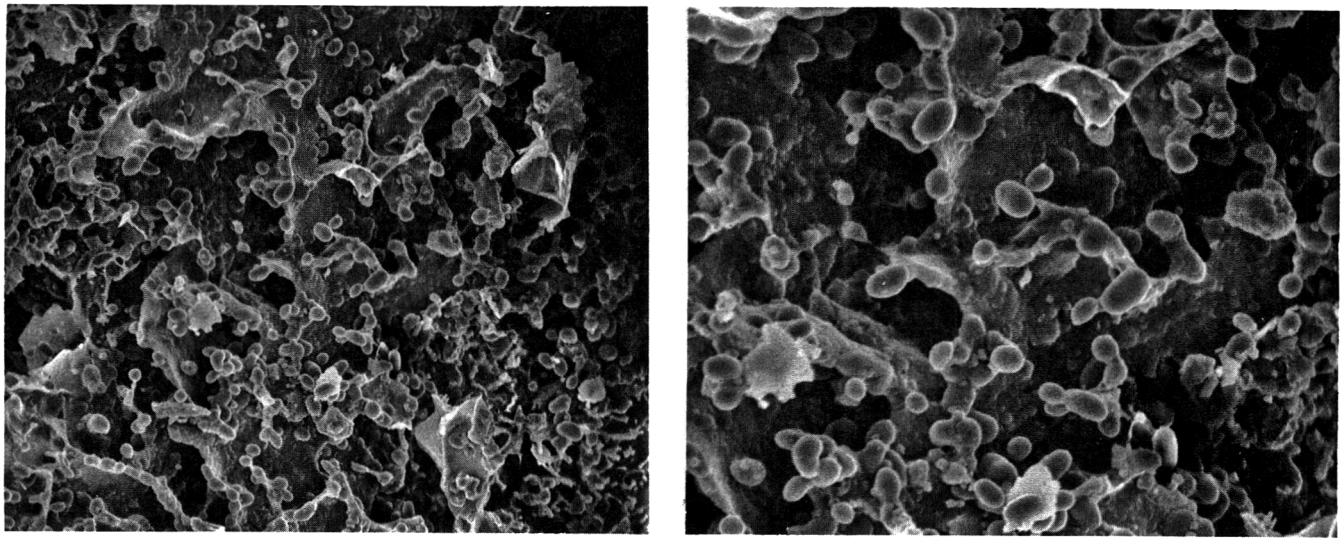

Fig. 2—Scanning electron photomicrograph of (A) zero hr black gram and rice blend at 370X and (B) at 750X, showing starch granules.

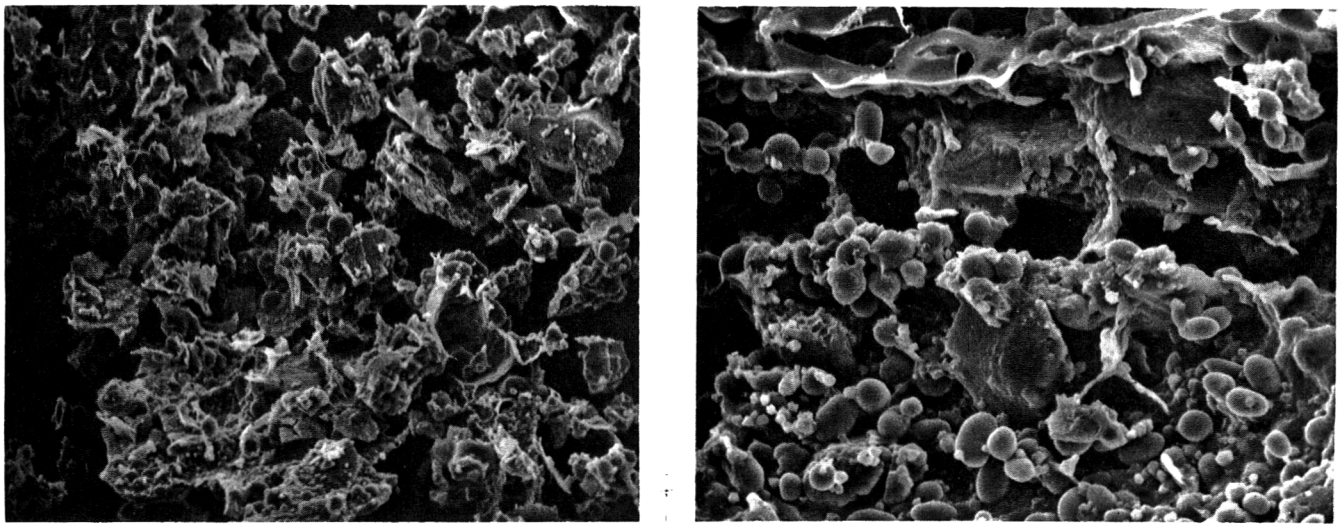

Fig. 3—Scanning electron photomicrograph of (A) 20-hr black gram and rice fermented blend at 370X and (B) at 750X, showing liberated starch granules (taken at two different spots).

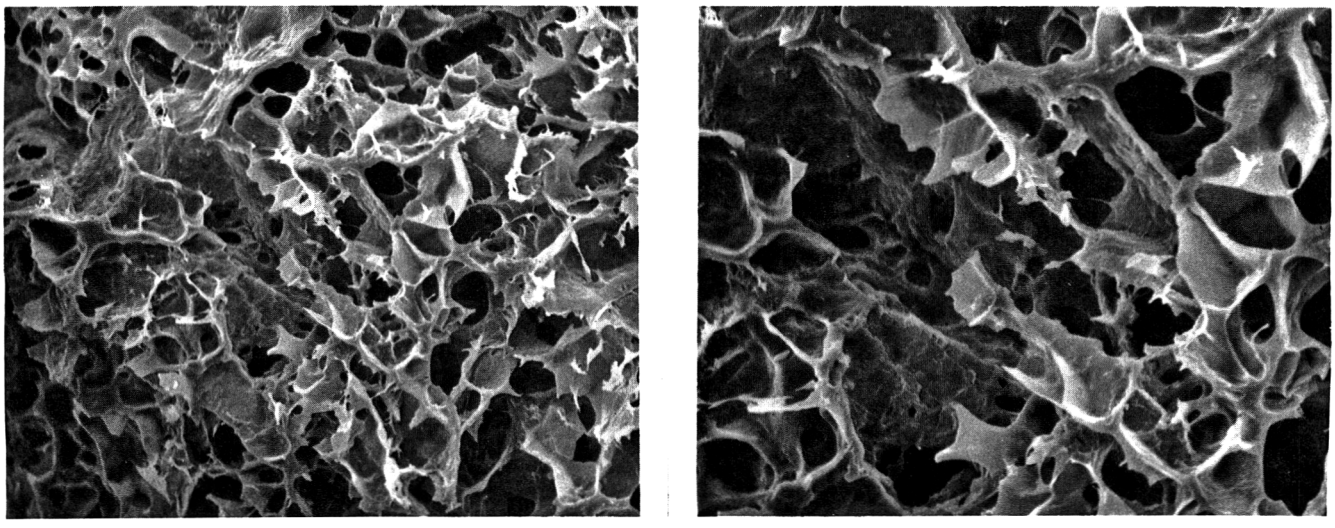

Fig. 4—Scanning electron photomicrograph of (A) 20-hr black gram and rice fermented steamed product (Idli) at 370X and (B) at 750X, showing fine network (spongy type of texture) and complete absence of starch granules.

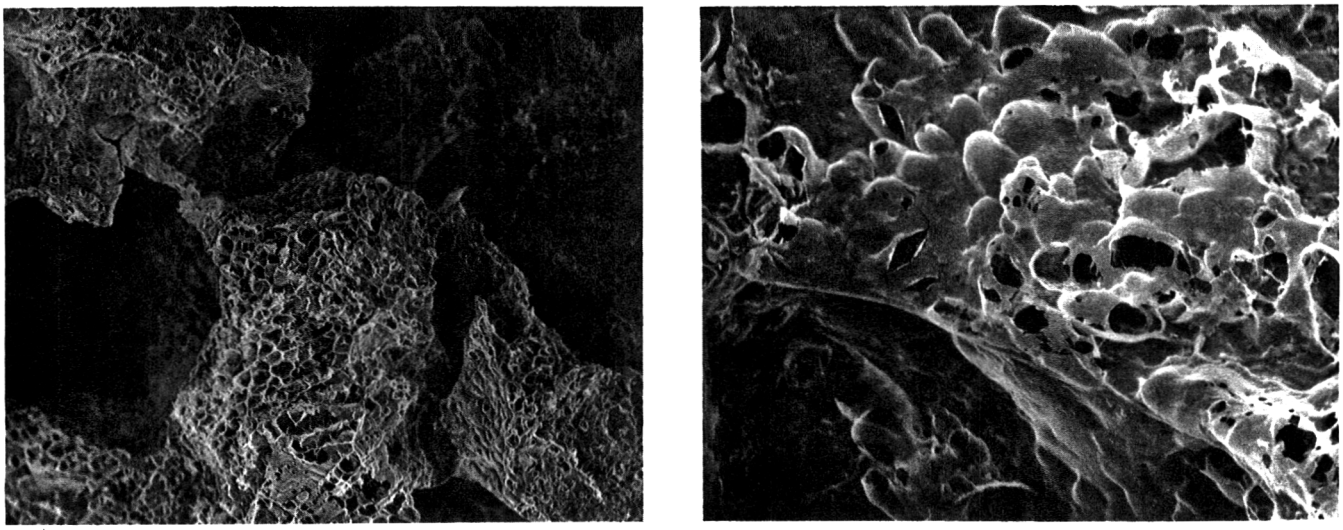

Fig. 5—Scanning electron photomicrograph of (A) 20-hr black gram and rice fermented steamed product (Idli) at 75X showing air void and (B) at 750X, exhibiting ruptures at the weak surfaces.

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EMULSIFICATION IN SYSTEMS CONTAINING SOY PROTEIN ISOLATES, SALT AND STARCH

MARTHA BARNES STONE and ADA MARIE CAMPBELL

ABSTRACT

Emulsification in systems containing commercial soy protein isolates, salt and starch was studied at ambient temperature and 4°C. Three levels each of sodium chloride and pregelatinized waxy maize starch were tested; emulsion stability and apparent viscosity were measured. Nine salt-starch combinations were selected for testing in a salad dressing containing one of the isolates; emulsion properties were evaluated by objective and sensory methods. Soy protein isolate, temperature, salt, and starch, along with numerous interactions, affected emulsion stability and viscosity of model systems. Results with the model systems were reasonably predictive of results with the food system.

INTRODUCTION

SOYBEAN PROTEIN PRODUCTS are available in a variety of forms suitable for use in the fabrication of foods. Soy protein isolates are considered promising as a source of dietary protein because of their high protein content, bland flavor and ease of incorporation into a broad variety of food systems. The functional properties of soy protein isolates as well as of other protein products have important effects on food properties and were reviewed extensively by Kinsella (1976, 1979).

Soy protein isolates are supplied by several manufacturers and vary significantly in physical properties (Wolf, 1976) although they do not differ greatly in composition. Specific processing conditions influence the extent of denaturation during the isolation process and thus influence the response to treatment.

Functional properties of soy protein isolates have been studied extensively in model systems and considerable testing of soy protein isolates in food systems has been conducted. Fewer studies have dealt with functional properties as predictors of performance in food systems. Hermansson and Åkesson (1975), working with a soy protein isolate in addition to two other proteins, did find functional properties to be reliable predictors of effects in model meat systems, as did Hermansson (1975) in a meat product. Hutton and Campbell (1977), studying emulsion stability and viscosity, found that the response of a soy protein isolate to pH variation in model systems was predictive of performance in soy-containing dips; the response of a soy protein concentrate to pH variation in model systems was not predictive of performance in dips under the conditions of the investigation.

This study was part of a project dealing with the effects of varying levels of selected food ingredients on properties of model and food systems containing plant protein products. This phase was a study of emulsification in model systems and in a salad dressing containing soy protein isolates with varying amounts of salt and pregelatinized starch. Information concerning fat absorption of the isolates also was obtained.

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EXPERIMENTAL

FOUR SOY PROTEIN ISOLATES were obtained from their commercial sources. The isolates and their percentage protein contents (N × 6.25, as-is basis) were as follows: Pro-Fam 90/HS (Grain Processing Corp.), 89.4; Promine-D (Central Soya), 92.0; Promine-F (Central Soya), 91.5; and Supro 620 (Ralston Purina Co.), 86.5. The corresponding moisture contents were 3.9, 4.8, 4.5, and 6.0%. Measurements of emulsified oil, emulsion viscosity, and fat absorption of the four isolates were made at ambient temperature (22–25°C) and 4°C. Emulsified oil and emulsion viscosity were studied in model systems containing all combinations of the four isolates and three levels each of salt (sodium chloride) and a pregelatinized waxy maize starch (Amaizo 721A, American Maize-Products Co.). Measurements were made at the as-is pH. (Average emulsion pH values for the model systems were 7.16, 6.95, 7.12, and 6.88 for Pro-Fam 90/HS, Promine-D, Promine-F and Supro 620, respectively.) One isolate and nine combinations of salt-starch levels were selected for the food system investigation. Measurements of emulsion separation and apparent viscosity were made on the nine base products and the corresponding salad dressings at 4°C. The salad dressings were evaluated by a trained six-member sensory panel.

Model systems

Emulsified oil. Throughout the preparation of emulsions a blender was operated through a rheostat at a relatively low rate of speed (ca. 1500 rpm). Each emulsion contained 240g of distilled water and 13.28g of soy protein isolate, which were blended for 15 min. The salt (0, 4.0 or 8.0g) and starch (0, 4.0 or 8.0g) were added and blending was resumed for 1 min. After addition all at once of 66.2g of corn oil, the mixture was blended for 10 sec. Duplicate 35-g samples, in stoppered 50-ml centrifuge tubes, were held for 15 min in a shaker water bath (89 cycles/min) at ambient temperature or 4°C. The amount of oil that did not separate out during centrifugation for 30 min at 1500 × G was determined as described by Hutton and Campbell (1977) and calculated as g oil/g soy isolate, dry weight basis. Values are reported as "emulsified oil" although a distinction was not made between emulsified and bound oil.

Viscosity. The remainder of each emulsion was used for duplicate measurements of apparent viscosity at ambient temperature or 4°C. A Brookfield model LVF viscometer on a Helipath stand was used with spindle T-C at 6 rpm (length of crosspiece on inverted T = 2.7 cm).

Fat absorption. The method was essentially that described by Lin et al. (1974) as modified by Hutton and Campbell (1977) with further modification necessitated by a floating layer of soy protein isolate after centrifugation. A Vortex mixer was used 15 sec for redispersing the floating layer and the sample was recentrifuged (1100 × G, 15 min). The centrifuge tube with sample then was inverted in a Büchner funnel and the supernatant oil was filtered through Whatman No. 1 filter paper with suction. Residue on the filter paper was recovered with a scalpel and added to the centrifuge tube contents for weighing. The absorbed oil, determined as the difference between the weight of isolate with absorbed oil and the weight of isolate used, was expressed as a percentage based on the sample dry weight.

Treatment of data. Data for emulsified oil and for viscosity in the 4 × 2 × 3 × 3 factorial study were collected according to a randomized complete block design with two replications. Analyses of variance were applied to the data and sums of squares for the main effects and interactions were partitioned by the use of or-

thogonal polynomials. All polynomial terms deemed significant from this analysis were included in a reduced model; the data then were fitted to this reduced model and coefficients were estimated. From the resulting equations contour and response surfaces were drawn with a Hewlett-Packard flat-bed plotter. The fat absorption data for four isolates at two temperatures were collected in a randomized complete block design with two replications and analysis of variance was applied to the data.

Food system

The base product was an emulsion that contained Pro-Fam 90/HS and was prepared according to the procedure used for model systems. For selection of nine salt-starch combinations, the model system contour surface for emulsified oil with Pro-Fam 90/HS at 4°C was overlaid on that for viscosity. A point representing an "ideal" combination of stability and viscosity was located and eight additional points around it were selected. This selection process resulted in combinations of 2.0, 4.0, and 6.0g of salt and 3.3, 5.6, and 7.9g of starch for use with 13.28g of soy isolate, 240g of water, and 66.2g of oil.

After each emulsion was prepared, duplicate portions were removed for assessment of emulsion stability and apparent viscosity. A salad dressing was made from the remainder of each base product by the addition of tomato paste (5%), distilled vinegar (8%) and a seasoning mix (0.5% of a 2:1:1:1 mixture of onion powder, garlic powder, paprika, and dry mustard). All base products and dressings were prepared on each of three days.

Evaluation of emulsion stability of the base products and salad dressings was similar to that used for the model systems except that the centrifuged samples showed creaming rather than oil separation and thus the volume of the translucent aqueous layer was read from the centrifuge tubes. The results were expressed as percentage of separated liquid, based on the total volume; relatively high values thus reflect instability. Apparent viscosity was measured according to the procedure specified for model systems.

Sensory evaluation. The salad dressings were transferred into coded 22-ml paper cups for evaluation. Four textural parameters of the nine dressings were evaluated by the sensory panel on each of three days. During the training period the panelists had been familiarized with the terms used in the scoring device; agreement on the definitions of terms was reinforced by the evaluation of some of the base products. The score card was developed according to the guidelines of Civille and Liska (1975) and Civille and Szczesniak (1973) for sensory texture profile techniques. Specific instructions were included for evaluation of each of four characteristics: viscosity (force required to draw liquid over the tongue at a steady rate, degree to which the product does not spread over the tongue, degree to which the product is a thick liquid); slipperiness (degree to which the tongue can slide under the product); graininess (presence of particles in the product); and mouth coating effect (degree of coating in the mouth after manipulation). In each case scoring consisted of marking a continuum that was labeled only at the ends—e.g. "very slippery" and "not slippery." For evaluation purposes the continuum was divided into five units. The panelists were graduate students in food science, nutrition, and food systems administration.

Treatment of data. The data for objective measurements and sensory evaluation for the nine dressings in each of the three replications were collected in a randomized complete block design. Analysis of variance was used to assess the effects of salt and starch levels.

RESULTS & DISCUSSION

Model systems

The dynamic nature of the relationships among the model system constituents with regard to emulsified oil and apparent viscosity is reflected in numerous significant interactions. These interactions, which make simple generalizations as to effects of individual factors impossible, are expressed in the response surfaces presented in Figures 1 and 2. Functional relationships as shown by response surfaces rather than values for specific treatment combinations are emphasized in the following discussion; the polynomial equations that describe the response surfaces are available upon request.

Emulsified oil. Emulsified oil was affected significantly ($P < 0.01$) by kind of soy protein isolate in systems containing varying amounts of salt and starch. Differences among isolates at ambient temperature are seen in the differing shapes of response surfaces a–d in Figure 1; differences among isolates at 4°C are seen in the differing shapes of response surfaces e–f. The response surfaces for Pro-Fam 90/HS (1a and e) and Promine-D (1b and f) are most nearly alike in overall appearance for either temperature, although differences are apparent.

The effect of temperature on emulsified oil is evidenced by comparison of the shapes of the following response surfaces: 1a vs e, 1b vs f, 1c vs g and 1d vs h. The effect of temperature on emulsified oil differed among the isolates: the temperature-isolate interaction was significant ($P < 0.01$).

The effects of salt and starch and of their interaction were significant ($P < 0.01$). With all of the isolates except Promine-F (1c and g) and Supro 620 at ambient temperature (1d) salt can be seen in Figure 1 to have had its greatest effect on emulsified oil in the absence of starch, and the amount of emulsified oil generally increased with increased starch. These two findings could be related in that the starch itself had an emulsifying effect or that it increased emulsification through its effect on viscosity. If the effect of sodium chloride is primarily on protein and if the starch contributes to emulsification directly and/or through an effect on viscosity, the effects of sodium chloride could be masked at the higher levels of starch. The shapes of the response surfaces show that the effects of salt and starch on emulsified oil also were dependent on the soy isolate (e.g. 1c vs d) and on temperature (e.g. 1b vs f). These interactions, as well as all 3- and 4-factor interactions were significant ($P < 0.01$).

Apparent viscosity. The response surfaces for apparent viscosity at 4°C (Fig. 2) also show differences among isolates, although they do not differ as much as do those for emulsified oil. The apparent viscosity response surfaces for ambient temperature, not shown, were similar in shape to those for samples at 4°C but viscosity tended to be somewhat lower at ambient temperature than at 4°C except in the case of Pro-Fam 90/HS. The effects of kind of isolate and temperature were significant ($P < 0.01$), as was their interaction.

Although the effects of both salt and starch on apparent viscosity were significant ($P < 0.01$), so also were the salt-starch interaction and the interaction of each with type of soy and with temperature. With salt, even the direction of the effect depended on the other variables. As could be expected, the effect of starch was an increase in viscosity with increased concentration; the magnitude of the effect depended on the other variables.

Fat absorption. Although fat absorption values (Table 1)

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Table 1—Fat absorption and emulsified oil values for four soy protein isolates at two temperatures

Measurement and temp	Soy protein isolate			
	Pro-Fam 90/HS	Promine-D	Promine-F	Supro 620
Fat absorption[a]				
Ambient	150.6	146.6	141.7	150.4
4°C	154.2	144.0	142.0	150.3
Emulsified oil[b]				
Ambient	3.82	3.74	2.05	0.59
4°C	0.97	1.58	0.66	0.84

[a] Expressed as percentage of isolate dry weight; each value is a mean for two replications.

[b] Expressed as g oil/g isolate dry weight; each value is a mean for two replications.

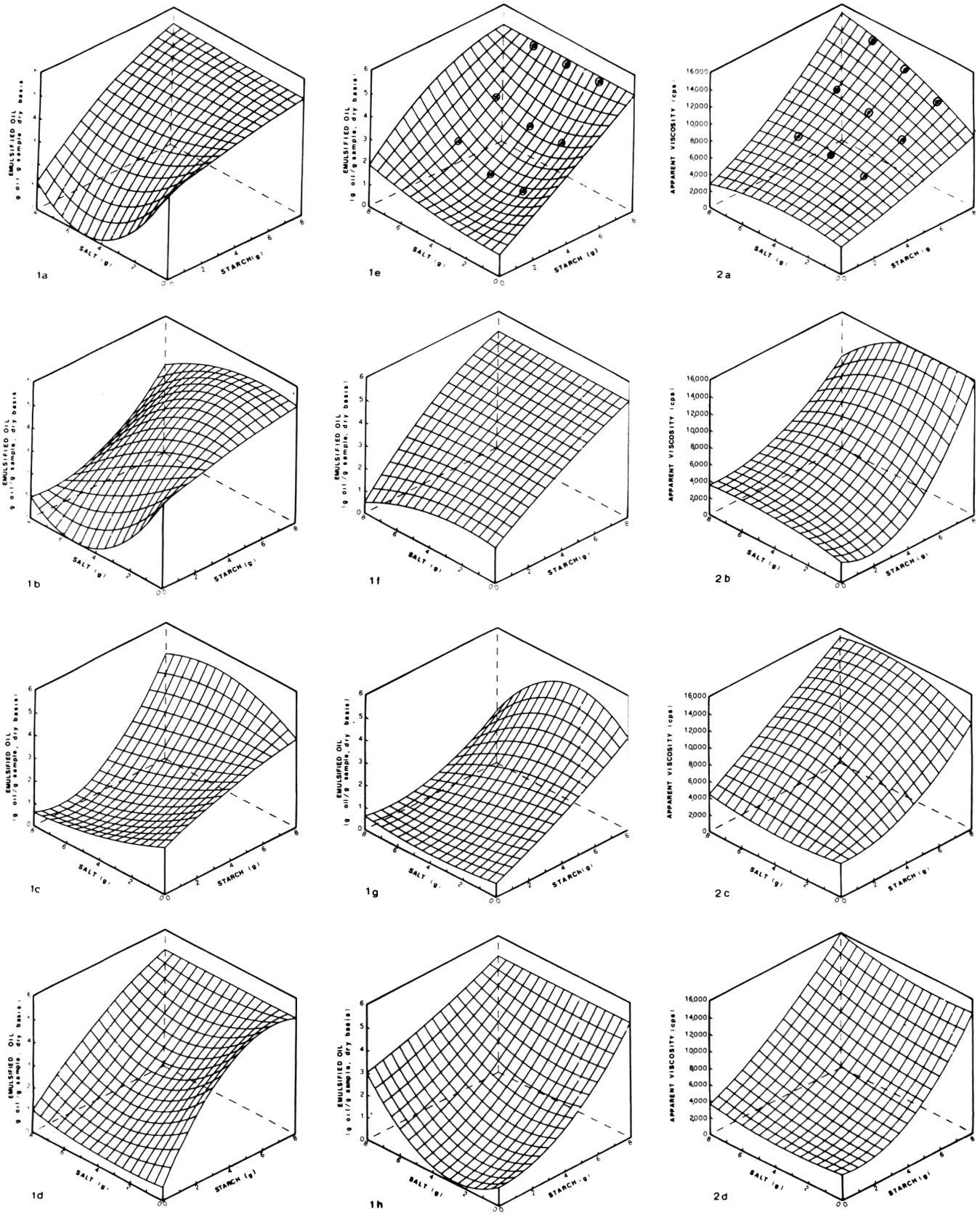

Fig. 1—Emulsified oil response surfaces for Pro-Fam 90/HS (a,e), Promine-D (b,f), Promine-F (c,g), and Supro 620 (d,h) at ambient temperature (a–d) and 4°C (e–h) in model systems with variations in salt and starch. (The 9 points marked in e represent the treatment combinations used in the food system.)

Fig. 2—Viscosity response surfaces for Pro-Fam 90/HS (a), Promine-D (b), Promine-F (c), and Supro 620 (d) at 4°C in model systems with variations in salt and starch. (The 9 points marked in a represent the treatment combinations used in the food system.)

Table 2—Objective measurements on base products containing Pro-Fam 90/HS with varied levels of salt and starch and on salad dressings prepared from base products; panel scores for textural characteristics of the dressings

Objective measurements and textural characteristics	Salt, g								
	2.0			4.0			6.0		
	Starch, g								
	3.3	5.6	7.9	3.3	5.6	7.9	3.3	5.6	7.9
Objective measurements[a]									
Liquid sepn (% of total vol)									
Base	36.0	23.0	10.7	31.8	21.9	9.1	30.1	16.8	6.9
Dressing	39.6	32.7	23.0	37.7	30.0	20.7	36.3	29.4	18.3
Apparent viscosity (cps)									
Base	2,263	4,139	9,108	2,341	4,167	9,639	2,512	4,510	11,935
Dressing	3,326	11,891	>16,600	3,835	13,014	>16,600	4,167	15,604	>16,600
Textural characteristics[b,c]									
Viscosity	1.3	2.9	3.6	1.5	3.0	3.8	2.0	3.0	4.0
Slipperiness	1.9	2.8	3.4	1.7	3.1	3.5	2.1	2.8	3.7
Graininess	3.8	3.2	3.3	3.9	3.8	3.2	3.2	3.3	3.6
Mouth coating effect	2.2	2.4	2.7	2.4	2.5	3.0	2.2	2.8	3.4

[a] Each value is a mean for duplicate measurements in three replications.
[b] Each value is a mean for six trained panelists in three replications.
[c] On a 1–5 scale 5 = thick, *not* slippery, *not* grainy, *no* coating.

differed significantly ($P < 0.01$) among the isolates, the overall range of values was small. Temperature had essentially no effect on fat absorption by the isolates under the conditions of the study. Fat binding probably affected the values reported herein as "emulsified oil." However, fat absorption by the isolates varied much less than did emulsified oil for the isolates in the absence of salt and starch (Table 1).

Food system

For the nine salt-starch combinations used, stability of both the base and the salad dressing emulsions increased (as indicated by decreased liquid separation) with increased salt and with increased starch (Table 2). Both effects were significant ($P < 0.01$), though the effect of starch was greater in magnitude than that of salt. Similarly, apparent viscosity increased with both increased salt and increased starch ($P < 0.01$) and again the magnitude of the effect of starch was greater than that of salt. The effects of starch on emulsion stability and viscosity in the food system were similar to those in the Pro-Fam 90/HS model system (Fig. 1e and 2a). The effects of salt were more consistent in the food system than in the Pro-Fam 90/HS model system; the points representing the nine salt-starch combinations used in the food system, marked on the response surfaces for Pro-Fam 90/HS at 4°C in Figures 1e and 2a, show a trend toward an effect of salt in model systems similar to that observed in the food system.

The lower stability and higher viscosity of the salad dressing than of the base product (Table 2) probably is attributable to greater aggregation of protein with the use of vinegar in the dressings. The salad dressings had a pH of about 4.5, as compared with about 6.9 for the base products.

Sensory evaluation of the salad dressings included, in addition to viscosity, three textural characteristics that were thought to have potential for reflecting differences in emulsion stability. Viscosity of the dressings, as judged by the panelists (Table 2), increased slightly with increased salt ($P < 0.05$) and considerably with increased starch ($P < 0.01$). These results are in agreement with those obtained by objective measurement. Slipperiness, which was expected to decrease with increased emulsion stability, was not affected by salt and was decreased ($P < 0.01$) with increased starch (as indicated by increased average scores for lack of slipperiness). Graininess was not affected by either salt or starch and the products were not considered very grainy. The extent of mouth coating tended to decrease along with increased viscosity (as indicated by increased average scores for lack of a mouth coating effect), although the scores covered a narrower range than did those for viscosity. The effect of salt ($P < 0.05$) was less than that of starch ($P < 0.01$).

The use of starch as an added ingredient probably masked some specific effects of salt on the properties observed in this study. However, the response surfaces for the four isolates do show differing responses to the treatments, indicating the importance of obtaining this type of information for the specific protein isolate and additional ingredients to be used. The model system data were reasonably predictive of results in the food system.

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A STUDY OF THE WATER ACTIVITY LOWERING BEHAVIOR OF POLYETHYLENE GLYCOLS IN THE INTERMEDIATE MOISTURE RANGE

JORGE CHIRIFE and CONSTANTINO FERRO FONTAN

ABSTRACT

This work reports on the experimental determination of water activity (a_w) in aqueous solutions of polyethylene glycol 200, 400, and 600, respectively. The measurements were made in the a_w range of most interest to intermediate moisture foods formulation (about 0.80–0.95). The observed behavior was very well fitted by Norrish's (1966) equation and a simple relationship was found between Norrish's correlating constant and molecular weight of polyethylene glycols. It was found that this relationship may be explained through a theoretical analysis of the thermodynamic behavior of macromolecules in solution.

INTRODUCTION

VARIOUS SOLUTES, also called "humectants," are usually incorporated in intermediate moisture foods (IMF) to lower the water activity (a_w) to the desired range, for instance 0.70–0.90. These solutes include salts, sugars, and polyols, and among compounds most used at present are sodium chloride, glycerol, propylene glycol, sucrose, corn syrup, sorbitol, and dextrose (Kaplow, 1970; Heidelbaugh and Karel, 1975; Flink, 1978).

Sloan and Labuza (1975) noted the various reasons necessitating finding alternative humectants for use in IMF. The selection of a new "humectant" for the formulation of IMF is, however, a difficult task; it involves considerations of texture, cost, safety, a_w lowering ability, pH, compatibility with food components, etc. (Bone, 1973). Within the three general classes of compounds above mentioned, Sloan and Labuza (1975) indicated that the polyols were the most desirable humectants from a water-holding standpoint and in some cases the fact that they are liquids.

The utilization of any solute for IMF formulation requires, among other things, a precise knowledge of its water activity lowering ability; e.g. an equation for predicting the a_w of its aqueous solutions at various concentrations.

This work reports on the experimental determination of a_w in aqueous solutions of polyethylene glycols of varied molecular weight in the a_w range of most interest to IMF. A theoretical analysis of the observed behavior is also presented.

EXPERIMENTAL

Materials

BDH laboratory reagent (BDH Chemicals Ltd., Poole, England) polyethylene glycols (PEG) (average molecular weight 200, 400 and 600) were used without further purification. Solutions of varied concentration were made up using distilled water.

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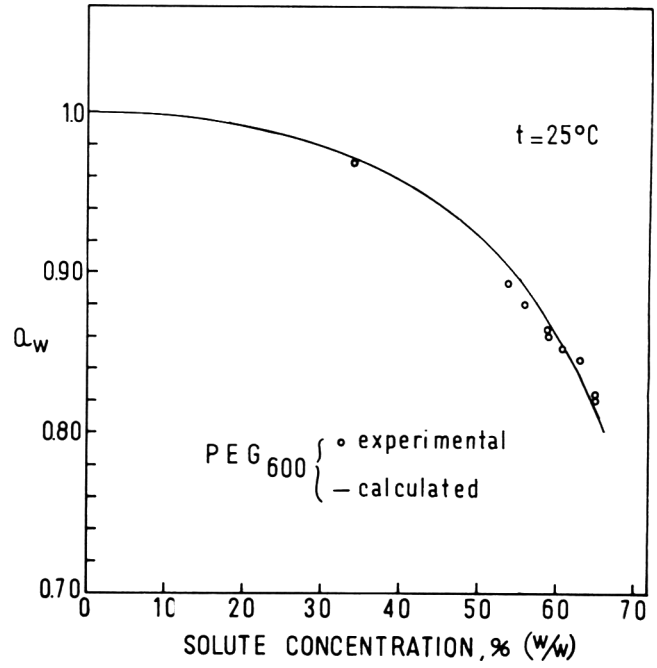

Fig. 1—Experimental and calculated a_w data for solutions of PEG 600 at 25° C.

Determination of water activity

The a_w of polyethylene glycol solutions was determined using the "a_w-Wert Messer" manufactured by Firma LUFFT, Stuttgart, West Germany. The instrument was carefully checked against five different standard saturated salt solutions in the a_w range of interest to this study (about 0.80–0.97). The instrument was operated following the procedure described in detail by Chirife and Ferro Fontán (1980a, b). The a_w measurements were made at 25 ± 0.4°C.

RESULTS & DISCUSSION

Analysis of data

Norrish (1966) proposed a correlating equation for predicting a_w in binary nonelectrolite solutions:

$$a_w = X_1 \exp(-K X_2^2) \quad (1)$$

where X_1 and X_2 are molar fractions of water and solute, respectively, and K is the correlating constant. Chirife et al. (1980) and Chirife and Ferro Fontan (1980a, b) successfully applied Eq (1) to about 20 different nonelectrolyte binary aqueous solutions of compounds relevant to IMF formulation. These compounds included sugars, polyols, amino acids, amides, and organic hydroxy-acids. They concluded that Norrish's (1966) model was an excellent tool for describing the a_w lowering behavior of nonelectrolyte aqueous solutions in the range of most interest to IMF. Figures 1 and 2 show experimental and calculated a_w data for polyethylene glycol 200, 400, and 600, respectively, at 25°C. Calculated data were obtained by applying Norrish's (1966) equation to the experimental data, and it can be

observed that they are well fitted by this simple equation. A computer program was used for calculating the best values of K.

It is to be noted that positive values of K indicate a negative deviation from Raoult's law and "vice-versa." The calculated values of K for PEG 200, 400, and 600 are

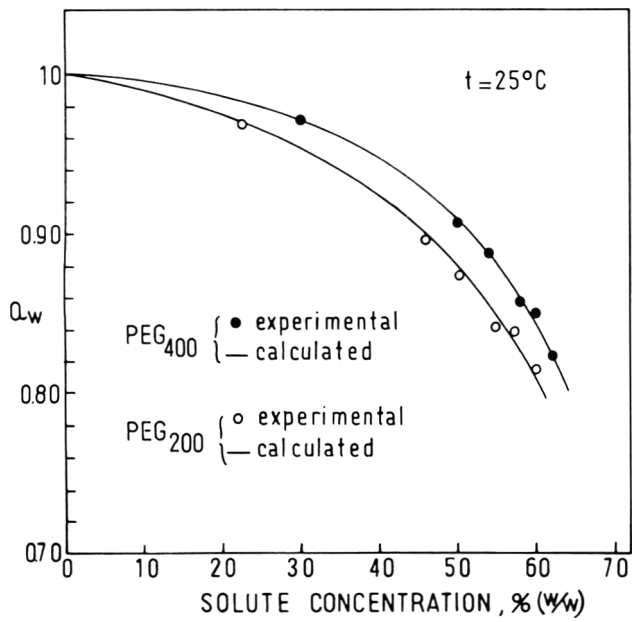

Fig. 2—Experimental and calculated a_w data for solutions of PEG 200 and 400 at 25°C.

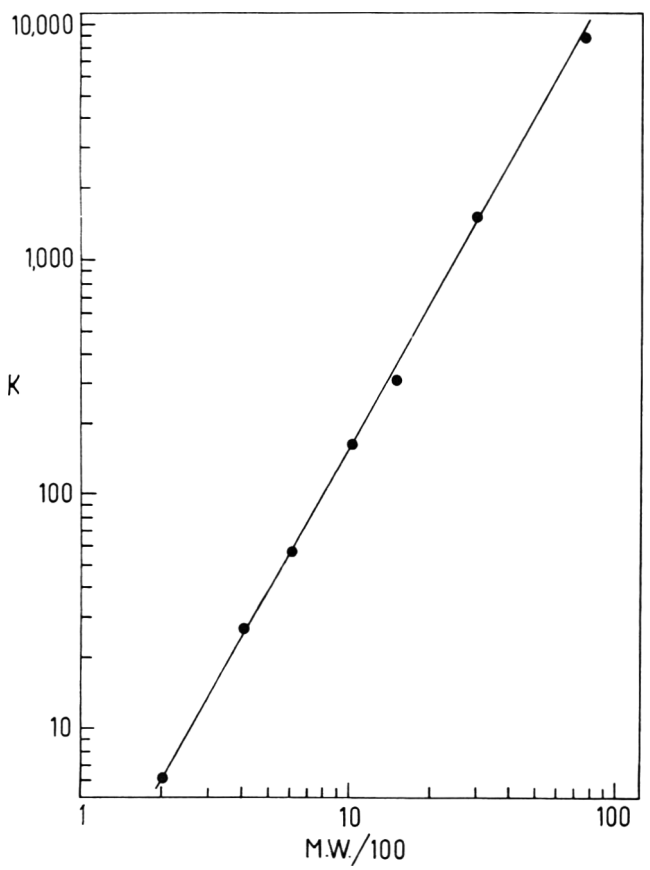

Fig. 3—The dependence of K (Eq 1) on the molecular weight of polyethylene glycols.

Table 1—Comparison of K values (Eq 1) for various polyols at 25°C

Polyol	K	Reference
PEG 600	56 ± 2.0	(this work)
PEG 400	26.6 ± 0.8	(this work)
PEG 200	6.1 ± 0.3	(this work)
Sorbitol	1.65 ± 0.14	(Chirife et al., 1980)
Erythritol	1.34 ± 0.05	(Chirife et al., 1980)
Glycerol	1.16 ± 0.01	(Chirife et al., 1980)
Propylene glycol	1.0	(Chirife et al., 1980)

shown in Table 1 as compared to the values obtained by Chirife et al. (1980) for other polyols of lower molecular weight. It is noteworthy that all three PEG exhibit a very strong negative deviation from Raoult's law as compared to the rest of polyols; further, K values appear to increase substantially with increasing molecular weight of the glycol. This behavior was further investigated as follows. Rogers and Tam (1977) examined the aqueous solution behavior of several polyethylene glycols of higher molecular weight (above 600) than those studied here, employing vapor pressure osmometry. The molecular weight of PEG's used extended up to 7980. Their data were also well fitted by Eq (1) allowing the calculation of K values; the results of the calculation have been plotted in Figure 3 as log K vs log M.W./100. It can be seen that a very good linear correlation exists for the whole range of PEG molecular weight investigated (200–7980); K dramatically increases with the molecular weight of the polymer. A linear regression analysis of the data in Figure 3 yielded the following equation:

$$K = 1.6\ (M.W./100)^2 \qquad (2)$$

Theory

The understanding of the physical behavior of polyethylene glycols in aqueous solutions is certainly an important prerequisite for predicting accurately the behavior of these compounds. It is well known that polymer solutions present large deviations (always negative) from Raoult's law, this being due mainly to the inequalities in the volumes of the molecules of water and polymer (Denbigh, 1964). The thermodynamic analysis of solution behavior of macromolecules was put forward many years ago by Flory and by Huggins independently (Champetier and Monnerie, 1973). Although various refinements have been incorporated into the theory, the basic thermodynamic equations still apply, and are known as the "Flory-Huggins relationships." According to Flory-Huggins,

$$\Delta \mu_1 = RT\ [\ln (1 - \varphi_2) + \varphi_2 (1 - \frac{1}{x}) + X_1 \varphi_2^2] \qquad (3)$$

where, x = ratio of molar volumes $= \frac{M_2}{M_1} \frac{\rho_1}{\rho_2}$; φ_2 = volume fraction of polymer; φ_1 = volume fraction of water; and X_1 = interaction constant.

In terms of water activity, Eq (3) becomes

$$\ln a_w = \ln (1 - \varphi_2) + \varphi_2 (1 - \frac{1}{x}) + X_1 \varphi_2^2 \qquad (4)$$

using a series expansion of the logarithm

$$\ln a_w \approx -[\frac{\varphi_2}{x} + (\frac{1}{2} - X_1)\varphi_2^2] \qquad (5)$$

As it has been shown, Norrish's (1966) equation

$$a_w = X_1 \exp (-K X_2^2)$$

is also able to describe the thermodynamic behavior of aqueous solutions. This means that Norrish's (Eq 1) and Flory-Huggins (Eq 4) equations should be equivalent in some way. This is shown in the following form. Norrish's equation may be written

$$\ln a_w = \ln (1 - X_2) - K X_2^2 \quad (6)$$

expanding the logarithm in series

$$\ln a_w \approx -[X_2 + (\frac{1}{2} + K) X_2^2] \quad (7)$$

It may be shown that

$$X_2 = (\frac{X_1}{\varphi_1})\frac{\varphi_2}{x}$$

Then it follows

$$-\ln a_w \approx (\frac{X_1}{\varphi_1})\frac{\varphi_2}{x} + (\frac{X_1}{\varphi_1})^2 (\frac{K + 1/2}{x^2}) \varphi_2^2 \quad (8)$$

If $\frac{X_1}{\varphi_2} = [X_1 + x(1 - X_1)]$ is not too far from unity (e.g. low concentration) both Eq (8) and (5) are equivalent. The relationship between interaction constants is then

$$K = x^2 (\frac{1}{2} - X_1) - \frac{1}{2} \quad (9)$$

and

$$X_1 = \frac{1}{2} - \frac{K + \frac{1}{2}}{x^2} \quad (10)$$

Eq (9) indicates that K should increase with the second power of x, or

$$K \propto (\frac{M_2 \rho_1}{M_1 \rho_2})^2$$

for $K \gg \frac{1}{2}$, and as $\frac{\rho_1}{\rho_2} \simeq$ constant for the different PEG of varied molecular weight (Dow, 1974), it results that K should be proportional to the second power of PEG molecular weight, as was experimentally found.

Strictly speaking, the above equalities should only apply at low concentrations and mainly for the higher molecular weight polyethylene glycols. This is due to the fact that the classical theory of macromolecule solutions considers that the size of the solute is above 100 times the size of the solvent (water). Nevertheless, within of the above limitations and simplifications we may conclude that the a_w lowering behavior of PEG solutions may be, at least for our purposes, reasonably interpreted and predicted with the very simple equation developed by Norrish (1966).

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A RESPONSE SURFACE METHODOLOGY APPROACH TO THE OPTIMIZATION OF WHIPPING PROPERTIES OF AN ULTRAFILTERED SOY PRODUCT

CAROL L. LAH, MUNIR CHERYAN, and RICHARD E. DeVOR

ABSTRACT

Response Surface Methodology and the Path of Steepest Ascent was of enormous value in rapidly determining optimum conditions for whipping a full-fat soy protein product produced by ultrafiltration, especially since seven variables were considered. In the region of optimum overrun response, overruns in excess of 500% were obtained at 3.5–4.0% w/v protein concentration, 1.67% w/w sugar, 150 rpm whipping speed and 3.5–4.0 min whipping time, at levels of 0.1758M NaCl, preheat temperature of 74°C and pH 6.8. The latter three factors exerted little effect under these conditions, but had large effects in regions away from the optimum response. Preheating to 70–80°C prior to whipping was essential to the formation of good and stable foams. Only the first four variables mentioned above were considered in the second order response surface model for overrun (R^2 = 0.9927), which also indicated slight interaction between protein and time and protein and sugar. The foam stability model (R^2 = 0.9142) indicated protein concentration was the most important factor. Significant interactions occurred between whip speed and time; stable foams (zero fluid leakage) were produced only at high (5.5–7.0% w/w) protein concentrations and high speed/short time or low speed/long time combinations, under which conditions maximum overrun was 250–375%.

INTRODUCTION

WHIPPABILITY, the capacity to form stable foams with air, is an important functional property of proteins for applications in food products such as angel food and sponge cakes, whipped toppings, meringues, souffles, frozen desserts and a variety of candy confections (Kinsella, 1976). The terms "whippability" and "foamability" are used interchangeably in the literature although whippability usually suggests that tests were conducted using a household-type mixer with a wire whip, while foamability refers to the intensive shaking/agitation of a protein solution in a cylinder (Yasumatsu et al., 1972) or the bubbling of air through a protein solution via a fritted disc. Foaming methods require less protein and are measured under milder and more controlled conditions (Wang and Kinsella, 1976), although the mixing method for whipping is simpler and gives more reproducible results, which are more easily translated from laboratory bench to pilot plant (Gunther, 1979; Mansvelt, 1979).

Stability of the whip or foam is its ability to retain its maximum volume and is usually measured by the rate and/or amount of leakage of fluid from the foam. Both whippability and stability are expressed in many different ways in the literature, which makes comparison between products and between investigators difficult. Even more confusing is the fact that whipping properties are affected not only by the mode of creating the foam, but by many other factors that must be considered or controlled in experiments. Protein concentration, source of protein, method of processing and its effect on physico-chemical properties (such as solubility), pH, temperature during whipping, extent of preheat treatment, presence of salts, sugars and lipids and the amount of shearing during whipping are among the many factors affecting the whipping properties of a protein ingredient. Combinations of levels of variables that are optimum for one protein may be quite different for other proteins. Because of the many variables and corresponding interactions, a statistical technique which takes this into account should be used to determine optimum whipping conditions. One technique particularly suited to this application is Response Surface Methodology (RSM), which merges the methods of planned and efficient experimental design with least squares modelling to identify optimum conditions for the process response. When many variables are involved, two-level fractional factorial designs may be usefully employed to determine the Path of Steepest Ascent (PSA) toward the neighborhood of the optimum process response. Then second order experimental designs such as the Central Composite Design (CCD) may be employed to fully elucidate the response surface near the optimum and provide a basis for a second order polynomial approximation to the true response. It is important to point out that the PSA determination phase is essential since it provides for a full exploration of the true response function and leads to a better approximation of the response in the vicinity of the optimum by focusing final attention only in that region.

This paper presents a study of the whipping properties of a full-fat soy protein product produced by ultrafiltration (UF), using the Response Surface Methodology technique. The UF Soy product is produced by a novel process and has excellent and somewhat unusual solubility characteristics (Lah and Cheryan, 1980a) and emulsifying properties (Lah and Cheryan, 1980b), related in part to its composition and method of production, which indicated it might also have good whipping properties. The experimental design and procedures for determining the optimum conditions have been described in some detail because the manner in which the experiments are planned and executed has a bearing on how the results are interpreted; also, few papers in the food science literature using "RSM" provide enough detail on the mechanics of the modelling to enable the reader to judge the usefulness of the data or the technique and almost none discusses the specific application of the Path of Steepest Ascent to optimize the variables.

MATERIALS & METHODS

Production of UF soy product

The UF Soy product was produced from whole soybeans as described earlier (Lah and Cheryan, 1980a). Water extracts of soybeans were prepared by a series of unit operations such as soaking of soybeans, blanching, grinding, and prefiltration of the slurry in a plate-and-frame filter press. The water extract was then ultrafiltered in a Romicon hollow fiber unit equipped with the XM-50 membrane at 50°C and pH 6.7. The initial volume was reduced fivefold, the retentate was diluted back to the original volume with water and reultrafiltered as before to a fivefold volume reduction. The retentate was freeze-dried and stored in covered plastic containers at 4°C

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Table 1—Definition and levels of independent variables in experiment

Independent variable	Symbol	Levels					
		Set 1		Set 2		Set 3	
		−1	+1	−1	+1	−1	+1
Protein (% w/v)	X_1	3	5	3	5	2.5	5.5
Preheat temperature (°C)	X_2	50	80	40	80	70	80
Salt added (molar)	X_3	0.1	0.2	0	0.2	0.15	0.2
Whipping speed (rpm)	X_4	160	195	140	160	140	160
Whipping time (min)	X_5	5	6	4	8	3	5
Sugar added (g/300g slurry)	X_6	0	50	0	50	0	10
pH	X_7	5.5	6.0	6.2	6.7	6.5	7.0

until evaluation. Typical proximate analysis of the product was 60.2% protein (N × 6.25), 33.8% fat, and 3.0% ash (dry basis).

Whip overrun

Whip overrun was measured as the decrease in weight of a given volume of liquid after whipping under the following specified conditions:

1. The UF Soy was dispersed according to the PDI method (Lah and Cheryan, 1980a) to make 300g of slurry, at the desired concentration of protein. The pH was adjusted as necessary at the end of the blend.
2. An aliquot of the UF Soy dispersion was poured into a preweighed 100 ml beaker, all the way to the top, and weighed. The aliquot was poured back into the stock dispersion.
3. All 300g of the stock soy dispersion was transferred to a 600 ml beaker and placed in a hot water bath set at about 80°C. The contents of the beaker were stirred gently until it reached the desired temperature which generally took about 10–15 min. The soy dispersion was then whipped immediately.
4. All 300g of soy dispersion was transferred to a Hobart Kitchen Aid (Model K5-A) mixing bowl and whipped at room temperature using the wire ship attachment. A hand-held tachometer (Metron Instrument Co., Littleton, Colo., Type 26C) was used to measure the whip speed (RPM) of the shaft rotating the whip attachment. A variac was used to control the rpm. If used, sodium chloride was sprinkled over the dispersion at the start of whipping. When needed, sugar was added when half of the whipping time was completed.
5. After the given time, the mixer was stopped and the same 100 ml beaker was filled with a representative portion of the foam, the top leveled and re-weighed. Percent overrun was calculated as follows:

$$\text{Overrun (\%)} = \frac{\text{weight of initial mix} - \text{weight of final whip}}{\text{weight of final whip}} \times 100$$

Whip stability

Whip stability was determined by placing 100 ml of the final whip in a 16 cm tall, 14 cm diameter (at the rim) metal funnel with wire mesh opening at the bottom of the cone. Any liquid from the foam was drained into a 100 ml volumetric cylinder. Stability was measured as the amount of leakage (ml) per 100 ml foam obtained in 30 min. The stability test was conducted at room temperature (25–27°C).

Experimental procedure

The performance of a protein ingredient as a whipping aid is measured primarily by two responses, overrun and stability. At the outset it was unclear exactly how the seven variables selected would affect the responses. Since it was likely that the location of the first set of experiments would be far from the optimum, relatively narrow ranges of the independent variables were chosen so that a plane would adequately approximate the response surface at the initiation point of our studies. A 2^{7-3} fractional factorial was used as a basis to determine the initial Path of Steepest Ascent. In this design, main effects are confounded with third and higher order interactions while two factor interactions are confounded with each other and higher order interactions (Davies, 1956; Box et al., 1978). When second and higher order interactions are neglected for the reasons stated above, the 2^{7-3} fractional factorial design produces "clear" estimates of the main effects and makes it possible to approximate the response surface by the model

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 \quad (1)$$

Table 1 is a listing of the seven independent variables; the levels of the variables for the first set of experiments were based on preliminary experiments and/or literature sources.

Table 2 shows the design and results of the first set of experiments. The order in which each trial was performed was randomized (Lah, 1980). As expected, the location of this set of independent variables is far from the optimum snce overruns (Y_1) were very low, mostly below 100%, and stability (Y_2) very poor. The method of least squares was used to estimate the coefficients of the first order approximation, Eq (1), and these are listed in Table 3. Prediction of responses Y_1 and Y_2 may be calculated by substituting appropriate values of coded independent variables and estimated parameters into the model. For example, the set 1 experiment fitted equations are:

$$Y_1 = 57.4 - 24.4X_1 - 2.01X_2 + 10.7X_3 - 25.1X_4 + 6.7X_5 - 21.6X_6 + 3.1X_7 \quad (2)$$

$$Y_2 = 82.0 - 1.2X_1 + 3.13X_2 + 6.0X_3 + 7.9X_4 + 2.1X_5 + 11.0X_6 - 7.5X_7 \quad (3)$$

The above models are useful in indicating the direction in which to change variables in order to improve Y_1 and Y_2 the fastest. Overrun would be most significantly improved by decreasing protein

Table 2—Design of 2^{7-3} Fractional factorial, experiments 1–3 and results of Exp. Set 1

Trial no.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Y_1	Y_2
1	−1	−1	−1	−1	−1	−1	−1	97.0	60
2	+1	−1	−1	−1	+1	+1	−1	47.0	100
3	−1	+1	−1	−1	+1	+1	+1	76.5	93
4	+1	+1	−1	−1	−1	−1	+1	50.2	0
5	−1	−1	+1	−1	+1	−1	+1	195.0	57
6	+1	−1	+1	−1	−1	+1	+1	60.4	93
7	−1	+1	+1	−1	−1	+1	−1	68.3	90
8	+1	+1	+1	−1	+1	−1	−1	64.5	100
9	−1	−1	−1	+1	−1	+1	+1	−6.1	100
10	+1	−1	−1	+1	+1	−1	+1	16.0	55
11	−1	+1	−1	+1	+1	−1	−1	83.0	100
12	−1	+1	−1	+1	−1	+1	−1	9.3	100
13	−1	−1	+1	+1	+1	+1	−1	40.3	68
14	−1	−1	+1	+1	−1	−1	−1	24.9	98
15	−1	+1	+1	+1	−1	−1	+1	101.0	98
16	+1	+1	+1	+1	+1	+1	+1	−10.4	100

Generators:

5 = 123
6 = 124
7 = 234

Defining relationship:

I = 1235 = 1246 = 2347
= 3456 = 1457 = 1367
= 2567

Table 3–Linear estimators of the Path of Steepest Ascent

Measured variable	Exp. Set	Mean b_0	Prot. b_1	Temp b_2	NaCl b_3	Speed b_4	Time b_5	Sugar b_6	pH b_7
Overrun (Y_1)	1	57.4	−24.4	− 2.01	10.7	−25.1	6.7	−21.6	3.1
	2	197.6	−12.6	34.75	55.4	−38.75	−67.0	−54.4	10.6
	3	350.0	−40.3	− 1.13	2.13	−25.13	−23.75	−12.3	12.9
Stability (Y_2)	1	82.0	− 1.2	3.13	6.0	7.9	2.13	11.0	− 7.5
	2	64.3	6.2	−11.7	−14.2	14.3	11.7	16.0	1.1
	3	12.4	− 9.5	− 3.0	1.63	1.63	2.63	1.63	0

Table 4–Trials made along Path of Steepest Ascent

Test no.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Y_1 Predicted	Y_1 Observed
Set 1									
17	2.9	63.6	0.1742	158	5.65	0.4	5.79	142	169
18	2.34	62.9	0.1863	148	5.73	0	5.81	174	269
19	0.92	61.0	0.2197	125	5.94	0	5.86	259	44
20	1.79	62.3	0.1984	138	5.8	0	5.84	251	30
Set 2									
17	3.7	75	0.22	149	3.0	0	6.51	484	453
18	3.73	73.9	0.21	151	3.25	0.33	6.51	465	459
19	3.75	72.4	0.20	151.5	3.5	0.67	6.50	443	486
20	3.8	69.8	0.18	152.2	4.0	5.74	6.49	393	486
21	3.84	67.2	0.16	153	4.5	10.8	6.47	342	479
22	3.93	62.0	0.12	154	5.5	21.0	6.46	245	312
23	4.0	60.0	0.10	155	6.0	25.0	6.45	198	194
Set 3									
17	3.76	74.8	0.1752	149	3.55	4.76	6.76	384	482
18	3.04	74.2	0.1758	146	3.30	4.04	6.80	486	532
19	2.8	74.0	0.1760	145	3.20	3.8	6.82	520	504

(X_1), whip speed (X_4) and sugar (X_6) while increasing salt (X_3). Stability would be improved (i.e., Y_2 minimized) by decreasing whip speed, sugar and salt and increasing pH (X_7). The Path of Steepest Ascent, based on Eq (2), was determined as outlined by Davies (1956) and Box et al. (1978), and additional tests were done along this path, with the variables set as shown in Table 4 for Set 1. Since there was a significant improvement in overrun to 269% with Test 18, it indicated that additional testing along this path was warranted. Therefore Test 19 was performed still further along the path, but his resulted in a drastic drop in both overrun and stability. Coming back along the PSA (Test 20) did not improve the situation much, indicating a significant departure from the first order approximation of the true response surface at this point, and that further testing along this path would not be fruitful. A new series of trials should be run around Test 18, so that the PSA may be redefined.

From previous work in our laboratory, it was strongly suspected that the pH levels selected were to blame for the generally poor responses, since protein solubility in this region was low (Lah and Gheryan, 1980a). Hence the pH region for the second set of experiments was moved to pH 6.2–6.7 (Table 1). A design similar to that shown in Table 2 was also performed for Set 2 (not shown; Lah, 1980) and Table 3 shows the estimated coefficients for the PSA for Y_1 and Y_2. The average overrun is much larger than the first set and the average leakage is lower. The magnitudes of the slopes are larger and some of them have changed signs. As pH was the only variable whose levels were far removed from the original base of experiments in Set 1, this means that the product behaved differently to the whipping and stability tests at the isoelectric point than at neutral pH. This is not unexpected, as mentioned earlier, due to differences in solubility, indicating that this property has a very important bearing on whippability.

The coefficients of Set 2 listed in Table 3 enabled us to develop a new path of steepest ascent as shown in Table 4, and Tests 17–23, Set 2, were made along this path. Based on these tests and interpreting the results as before, a new 2^{7-3} factorial experiment was conducted in the response region of Test 20 in order to define yet another PSA. Fortunately, the coefficients of Set 2 (Table 3) indicate that to optimize both Y_1 (i.e., to maximize it) and Y_2 (i.e., to minimize it) simultaneously, we needed to: decrease protein (X_1), increase temperature (X_2), increase NaCl (X_3), decrease speed (X_4), decrease time (X_5) and decrease sugar (X_6); pH (X_7) should be increased for overrun and decreased for stability, although the effect of pH on stability appears to be very small. The levels selected for each variable for Set 3 are shown in Table 1 and the corresponding coefficients of the PSA in Table 3.

In Set 3, the average overrun is much larger than any of the previous sets and the stability is also much improved. Except for b_2 in Y_1 and b_1 and b_3 for Y_2, there were no changes in any of the signs of the coefficients and the coefficients were also becoming smaller. This was interpreted as signalling the presence of higher order effects which were overshadowing the main effects and causing extensive curvature in the response surface. The estimated coefficients were determined to be large or small by plotting them on normal probability paper, and examining the relationship between the group of effects which appeared to be normally and independently distributed around 0 mean with homogeneous variance, ($NID(0,\sigma^2)$) and those which have non-zero mean (Box et al., 1978; Daniel, 1959).

Three tests along the PSA were made as shown in Table 4 (Set 3). The predicted and experimental overruns were very high, and the highest overrun was reached at Test 18 Set 3, at 532% overrun. As the highest overrun did not result from levels of variables outside of Set 3, it was concluded that we had encompassed the region of the optimum response, and that further tests along this path would not be fruitful.

To fully describe the nature of the response surface in the neighborhood of the optimum, an experimental design with more than two levels of each factor is required, so that a second order approximation to the response surface can be developed. The last fractional factorial experiment was augmented with eight star points and seven replications of the center point to permit a second order model to be estimated. At this point it became clear that the response surface

Table 5—Definition and levels for central composite design

Independent variable[a]	Symbol	Level				
		−2	−1	0	1	2
Protein (% w/v)	X_1	1.0	2.5	4.0	5.5	7.0
Speed (RPM)	X_4	130	140	150	160	170
Time (min)	X_5	2	3	4	5	6
Sugar (g/300g slurry)	X_6	0	0	5	10	15

[a] Variables kept constant: Salt (X_3) = 0.1758M, Temperature (X_2) = 74.2°C, pH (X_7) = 6.80

in this region is quite "flat" with respect to variables X_2, X_3, and X_7. Hence these factors were held constant and the Central Composite Design was developed using only the variables X_1, X_4, X_5, and X_6.

Table 5 shows definition and levels of independent variables and Table 6 the design and results of the trials for the second order model. The coefficients were obtained with a computer and the final second order response models for Y_1 and Y_2 are shown below:

$$Y_1 = 531 - 28.6X_1 - 20.9X_4 - 24.3X_5 - 12X_6 - 3.3X_1X_4 + 11.9X_1X_5 + 9.4X_1X_6 - 4.8X_4X_5 + 3.3X_4X_6 + 1.13X_5X_6 - 80.0X_1^2 - 31.6X_4^2 - 31.5X_5^2 - 14.9X_6^2 \quad (4)$$

$$Y_2 = 7.3 - 9.6X_1 + 0.66X_4 + 1.25X_5 + 0.92X_6 - 1.75X_1X_4 - 2.75X_1X_5 - 1.5X_1X_6 + 2.87X_4X_5 + 0.13X_4X_6 - 0.38X_5X_6 + 3.18X_1^2 + 1.4X_4^2 + 0.03X_5^2 + 0.28X_6^2 \quad (5)$$

Trial 17. Table 6, defined a set of test conditions (1% protein, 150 rpm, 4 min, and 5g sugar) which produced essentially no foam. Hence, this trial was not included in the modelling.

The model for overrun (Y_1) accounted for 99.27% of the total variation (Table 7). The R^2 corrected, which is calculated as

$$R^2\text{corr} = \frac{\text{Model SS} - \text{Mean SS}}{\text{Total SS} - \text{Mean SS}}$$

and describes the actual variation which the model (terms involving X_1, X_4, X_5, X_6) accounts for when corrected for the mean was 0.85944. The model for Y_2 (stability) accounted for 91.42% of the toal variation and had an R^2 corrected of 0.81332 (Table 8). Both models were significant at the 1% level.

The ANOVA (Tables 7 and 8) indicated that although both models were highly significant, a marginal lack of fit was evident for the overrun model, and a more significant lack of fit was apparent for the stability model. It is probably safe to say that the overrun model does not suffer greatly from lack of fit but that the stability model does to a certain extent. The use of third order terms and/or log transformations did not markedly improve the model fit.

The lower R^2 for the stability model is not surprising considering that certain factors, such as viscosity of the protein solution, surface tension lowering abilities of the protein, salts, temperature treatments, or the pH were not included in this model, and these have all been listed as factors contributing to foam stability (Horiuchi et al., 1978; Richert, 1979). In a sense, stability was not optimized, but rather the experiment was designed to maximize the overrun. Hence the stability model is actually the response surface of stability over the levels of highest overrun. As a result, factors such as salts and temperature were not included in the final model because they did not appear to contribute to an increase in overrun, at the levels in which they were used. However, these same factors may affect the stability of the foams and would probably have improved the fit had they been included.

The second order polynomial approximations above were used to develop contour plots of the response surfaces in the region of the optimums. Each plot shows the overrun or stability response as a function whipping speed and protein concentration (Fig. 1 and 2), at different levels of whipping time and sugar concentration. Through the use of seven such plots (not shown: Lah, 1980), the effects of each of the variables in combination with another could also be revealed.

—Continued on next page

Table 6—Results of trials forming a composite design with those given in Table 1, Set 3.

Trial no.	Order	X_1	X_2	X_3	X_4	Y_1	Y_2
17	14	−2	0	0	0	17.6	100
18	8	2	0	0	0	247	0
19	5	0	−2	0	0	476	15
20	6	0	2	0	0	426	10
21	1	0	0	−2	0	502	10
22	3	0	0	2	0	401	4
23	10	0	0	0	−2	541	9
24	12	0	0	0	2	495	7
25	2	0	0	0	0	515	9
26	15	0	0	0	0	545	6
27	4	0	0	0	0	533	8
28	13	0	0	0	0	545	7
29	9	0	0	0	0	526	7
30	11	0	0	0	0	502	7
31	7	0	0	0	0	552	7

Table 7—Analysis of variance for Y_1 (Overrun) of the central composite design

Source	df	Sums of squares	Mean squares	F
Model	15	5,371,536	358,102.4	135.2214*
Residual	15	39,734	2648.27	
Lack of fit	9	37,765	4196.1	12.8518*
Real error	6	1958.5	326.5	
Total	30	5,411,260.0		

* Significant at the 1% level
R^2 = 0.99266 R^2_{corr} = 0.85944.

Table 8—Analysis of variance for Y_2 (stability) of the central composite design

Source	df	Sums of squares	Mean squares	F
Model	15	5211.01	347.403	10.657*
Residual	15	488.99	32.59	
Lack of fit	9	483.56	53.73	59.39*
Error	6	5.43	0.905	
Total	30	5700.0		

* Significant at the 1% level
R^2 = 0.91421; R^2_{corr} = 0.81332.

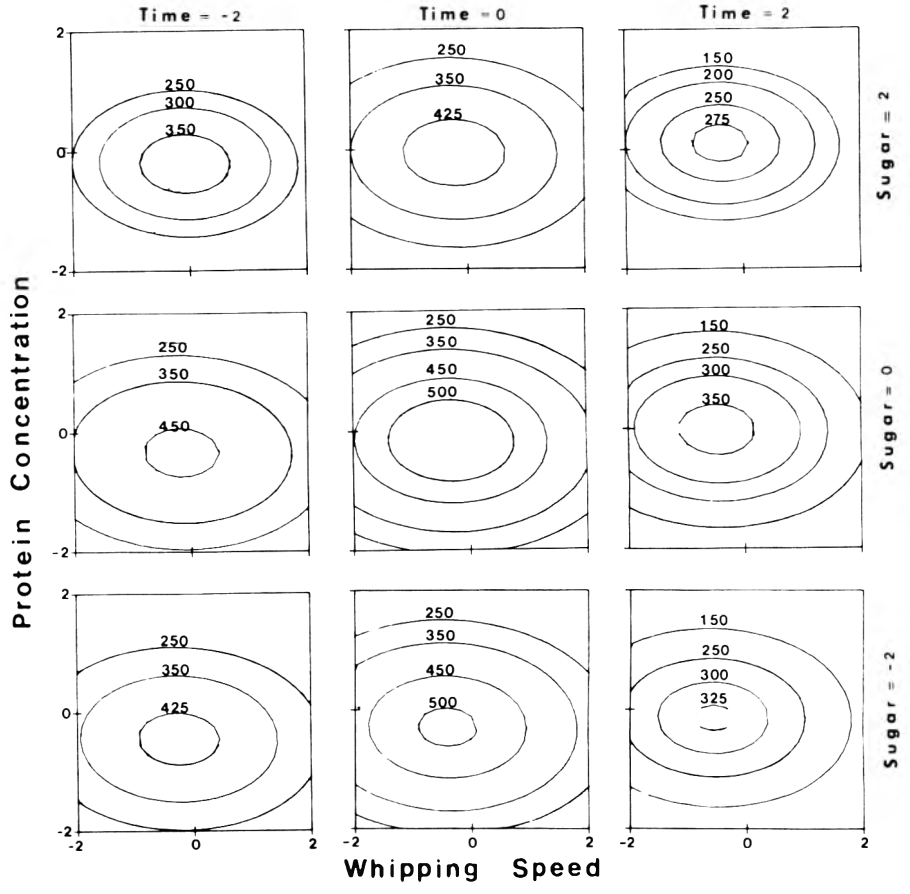

Fig. 1—Response surface contours for overrun (%) in the region of optimum response as a function of protein concentration, whipping speed, sugar concentration, and whipping time. Levels of variables correspond to those in Table 5.

DISCUSSION

Protein concentration (X_1)

This was the most important factor determining overrun and stability. Figure 1 is a typical response surface contour plot for overrun in the region of optimum response and Figure 2 is a typical response surface contour plot for stability in the region of optimum overrun response. The optimum protein concentration for maximum overrun was 3.5–4.0% under all treatment conditions. Others (Eldridge et al., 1963; Hansen and Black, 1972; Huffman et al., 1975; Richert, 1979; Tamsama et al., 1969) have also found that an optimum protein concentration does exist for maximum overrun, and deviating from it lowers foam volume. When the coefficients of the model were plotted on normal probability paper (not shown here; Lah, 1980), strong interaction effects were observed for protein and time (X_1X_5) and protein-sugar (X_1X_6). Interaction effects between two independent variables imply that the effect of one variable depends on the specific levels of the other variable.

To develop a foam, a certain amount of protein is necessary to sufficiently lower the surface tension and to create an adequately thick monolayer to retain the air cell (Graham and Phillips, 1974). If the protein concentration is too high, however, the viscosity may be high enough to decrease air incorporation by reducing the rate of diffusion of the protein to the surface, thus preventing the protein from unfolding sufficiently to form the needed protein monolayer for the formation of the air cell (Bull and Neurath, 1937; Graham and Phillips, 1974). In the case of the UF Soy, it should also be remembered that an increase in protein concentration results in a corresponding increase in lipid concentration. On a dry basis, the UF Soy has about 34% lipid and, at the 3.5–4.0% protein levels in the whipping solutions, this represents 1.5–2% lipid. Lipid has been cited as a foam depressant (Eldridge et al., 1963; Gunther, 1979; Mansvelt, 1979; Wang and Kinsella, 1976; Yasumatsu et al., 1972) and preliminary tests indicated that the UF Soy had extremely poor whipping properties. However, the detrimental effect of lipids could be overcome by the appropriate preheat treatment (see later).

The stability model [Eq (5)] indicated that while protein concentration was the most important factor, the optimum protein concentration for maximum stability (zero leakage) was dependent upon its interaction effects with time, speed and sugar (Fig. 2). In general, the higher the protein concentration, the better the stability, but the lower the overrun above 3.5–4.0% protein.

Shearing effects

A certain degree of surface protein denaturation is required in the formation of the protein monolayer which presumably acts to encapsulate air cells by spreading as a monolayer at the liquid/air interface (Cumper, 1953). If the protein isn't sufficiently denatured, the soluble protein returns to solution and the foam collapses (Bull and Neurath, 1937). If there is too much surface denaturation, the protein monolayer loses its elasticity and cohesive structure and is unable to envelope the air, causing the foam to collapse (Wang and Kinsella, 1976).

Protein denaturation during whipping is primarily a result of shearing, and the amount of shearing is a function of speed (RPM) and time of mixing. Considering the need to obtain just the right amount of protein denaturation for optimum overrun, it is not surprising that both parameters would be significant in the overrun model [Eq (4)]. Figure 1 indicates that a definite optimum of 150 rpm exists for reaching maximum overrun, essentially regardless of the levels of protein, time and sugar. Slightly lower overruns were

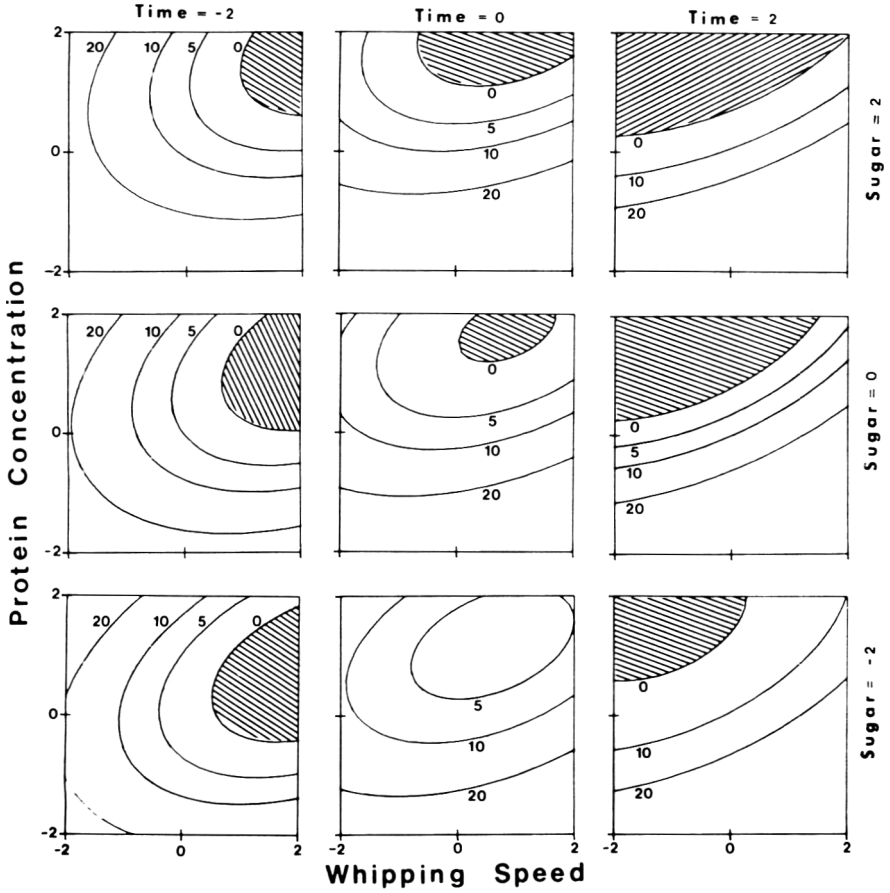

Fig. 2—Response surface contours for stability (ml liquid leakage per 100 ml foam) in the region of optimum overrun response. Shaded areas indicate zones of maximum stability. Levels of variables correspond to those in Table 5.

obtained at 130 rpm and a large drop in overrun was observed when speed was increased to 170 rpm. It is possible that at very high shear rates, the time of contact between air bubble and protein solution during each revolution of the wire whip may not be sufficient for the necessary unfolding and absorption of the protein in the monolayer (Bull, 1947). Unless a reasonably thick film is adsorbed, a foam will not form.

These results point out the necessity of carefully choosing a mixing speed when making comparisons, especially between different products, since each product may have a different optimum speed. Conversely, experimenters conducting whipping tests on the same product at different speeds will arrive at results different from each other. In the case of UF Soy, if it had been whipped at the highest speed on the Kitchen Aid mixer (as is frequently reported in the literature), results would have indicated that it would be a poor whipping agent, since at the highest speed, no foam was produced and a whole body of information may have been overlooked. These results also point out how critical it is to control whipping speed accurately during manufacture, since a small difference of 20 rpm resulted in large differences in overrun. The UF Soy may not be unusual in its sensitivity to small differences in shear rates; rather, most published studies do not take this very important variable into account or neglect this factor completely.

Whipping time

This is obviously another parameter that would govern the rate of surface denaturation. The overrun model, Eq (4) indicates that it is the second most important factor. Figure 1 shows that the foam volume increases to a maximum at 3.5–4.0 min and then decreases rapidly at higher whip times, regardless of the level of protein, sugar, and speed. The model for Y_1 indicates a slight interaction of time with protein as explained earlier. Similar optima in whipping times have been found for soy protein foams (3–4 min) and egg white (5 min) by Eldridge et al. (1963) and for sunflower meal (6 min) by Huffman et al. (1975).

Just as with whipping speed, these results emphasize the fact that there is no one best whipping time, and that it varies significantly with the protein product involved, as well as with the levels of other variables. One should not arbitrarily pick a whipping time to make comparisons of several protein products in order to find best overruns, as the results may be misleading if each protein reaches maximum overrun at different whipping times and also has different beat-down characteristics.

The stability second order model indicates a strong interaction of time and speed, which is not unexpected; this can clearly be seen in Figure 2, as stable foams were only produced by whipping at high speeds for short times or at low speeds for long times. Intermediate times (0 level) produced unstable foams unless protein was high and sugar and speed at intermediate-to-high levels.

Sugar

Sugar was shown to be only a moderately significant parameter in the overrun model, as compared to protein concentration, whipping time and speed (Fig. 1). At 150 rpm, it appears that there is an optimum of 5g (1.67%) of sugar, since no sugar produces slightly less overrun and 15g (5%) sugar produces even less overrun. Hansen and Black (1972) also found that levels of 3% sugar actually improved foam volume, while levels of 10% sugar greatly depressed volume. Possibly the small amounts of sugar retard the rate of coagulation at the air/water interface (Kinsella, 1976), thus permitting more air incorporation before beat-down begins. In earlier trials, with runs away from the optimum, sugar at levels of 50g showed a very large depressing effect

on overrun (Table 3) which also agrees with the literature (Franzen and Kinsella, 1976; Richert, 1979; Kelly and Burgess, 1978).

Sugar has been reported to increase stability, although the effect on UF Soy was quite small in comparison to other factors in the region of optimum overrun response (Fig. 2). Sugar and protein had a mild interaction; high sugar levels were most beneficial at high protein concentration.

Salt

Although NaCl (X_3) was not included in the second order model, it was a fairly important factor. It was observed that in the regions away from the optimum, e.g., Set 2, Table 1 (0.2M vs no NaCl), large values of the linear estimator were obtained (Table 3, set 2, b_3). In set 3 the linear estimator was small probably because the difference in effect between 0.15M and 0.20M NaCl was not large enough to affect overrun or stability very much. A certain amount of NaCl does appear to be beneficial in the formation of foam. Bull (1947) reported that surfactants whose surface activity are negatively charged (such as proteins above the isoelectric point) show an increased rate of surface tension lowering on addition of an electrolyte. Tornberg (1978) noted that soy protein diffusion is faster in 0.2M NaCl than in distilled water and spreading of molecules at the interface is most easily performed in 0.2M NaCl. Since both surface tension lowering and protein diffusion and spreading at the interface are important aspects of foamability, it explains why the addition of NaCl is beneficial to the whipping properties, as is evident from our data.

Temperature

Temperature (X_2) was also not included in the second order model, but it should not be considered as unimportant. As mentioned earlier, when preliminary tests were performed at room temperature without any preheat treatment, the UF Soy did not whip at all, presumably due to its lipids. The data indicate an apparent optimum in preheating temperature of 70–80°C. The benefits of prior heat treatment on whippability have been demonstrated in a number of studies quoted by Richert (1979). Heat treatment was particularly beneficial in lipid-containing systems. Heating to 60°C improved foaming of yolk-contaminated egg albumin, while at least 7% trihexanoate could be added to whey protein concentrate if the sample was preheated; in contrast, less than 0.5% trihexanoate impaired foaming ability if the sample was not preheated (Richert, 1979).

In general, a heat treatment that results in mild denaturation of the protein is optimal (Richert et al., 1974; Eldridge et al., 1963; Kelly and Burgess, 1978; Tamsma et al., 1969), as this may expose the hydrophobic regions of the protein, yet keep it in solution, in such a way that a better stabilizing foaming protein results (Cumper, 1953; Richert, 1979; Horiuchi et al., 1978; Bull, 1947). Heat treatment may also have resulted in the formation of lipid-protein complexes that favor protein adsorption at the air-liquid interface within the foam lamella (Richert et al., 1974). In all likelihood, by leaving temperature out of the second order model, an important factor governing stability may have been overlooked, which is reflected to a certain extent in the lower R^2 for the stability model.

pH

As noted earlier, pH was not included in the models, but did exert its influence on overrun. Using pH of 5.5–6.0 (Table 1, Set 1), overruns did not exceed 200% (Table 2), pointing out the importance of high solubility in whipping properties. Kinsella (1976) cautioned that the true effects of pH are observed when the preponderance of the protein is still soluble, since part of the pH effect may be related to the denaturability of a portion of the soluble proteins involved in membrane formation. Ideally a certain minimum amount of protein should be soluble which should denature slightly during whipping so that the protein will unfold to form the necessary membrane structure around the air cell (Bull and Neurath, 1937; Bull, 1947). If the protein is already insoluble, the necessary unfolding process which must take place during air incorporation does not occur and foam is not formed.

In this study, in the region of optimum overrun response, the pH range selected (6.5–7.0) appeared not to make a large difference, probably because there were no significant differences in protein solubility (Lah and Cheryan, 1980a). Hence, since other factors resulted in much larger effects on overrun, pH was dropped from the second order model. These results confirm Richert's (1979) analysis that pH is an important variable, but needs to be considered simultaneously with other variables in designing experiments.

CONCLUSIONS

IN THIS RESEARCH, the technique of RSM was especially useful because of the complexity of the physics and chemistry of the problem and the number of interrelated variables which governed the process. In particular, the use of two-level fractional factorial designs during the Path of Steepest Ascent provided for the consideration of a large number of potentially important variables and the identification of the specific ranges/levels of these variables which may produce very high overruns while maintaining excellent stability.

Despite tne relatively high fat content, the UF Soy product produced very good foams, provided it was preheated to 70–80°C. Only four of the seven variables considered had significant effects in the region of optimum overrun response. Optimum conditions for overrun were: 3.5–4.0% w/v protein concentration, 150 rpm whipping speed, 3.5–4.0 min whipping time and 5g sugar/300g soy dispersion (1.67%), with levels of 0.1758M NaCl, temperature of 74°C and a pH of 6.8 held constant. An average overrun of 531% was obtained under these conditions. The texture and viscosity of these foams resembled beaten egg white, and formed semi-stiff peaks.

Maximum stability (zero ml leakage) however, was obtained at higher protein concentration (5.5–7.0%), combinations of high speed/short times or low speed/long times and a minimum of 5g sugar per 300g soy dispersion, under which conditions overruns of 250–375% could be obtained. Results clearly showed the importance of careful control of the rate and amount of shearing that the protein experiences during whipping. Since the processing conditions for producing the UF Soy product are mild, good solubility characteristics (Lah and Cheryan, 1980a) were partly responsible for its whipping characteristics. In addition, despite its high fat content, the product was bland with no painty off-flavors and no sign of rancidity even after storage for 18 months at 4°C in the freeze-dried form. This combination of physical, chemical, and sensory properties should make it very useful as a whipping aid in non-dairy toppings and frozen desserts.

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THERMAL DIFFUSIVITY OF MODEL MEAT ANALOG SYSTEMS

S. S. H. RIZVI, J. L. BLAISDELL, and W. J. HARPER

ABSTRACT

Model meat analogs composed of soy spun fiber, egg albumen, and wheat gluten were thermally processed at five different temperatures under transient heat transfer conditions. Thermal diffusivity values were obtained during this period using Dickerson's method. The subject samples were held at the final temperature for 1 hr and thermal diffusivity values were obtained using the method of Pflug et al. (1965); values obtained by the two methods matched favorably within the experimental variations. The thermal diffusivities of the system varied with water content in the temperature range 71.1–93.3°C. Above this range, water alone could not reliably predict the thermal diffusivity values. Increase in processing temperature lowered the thermal diffusivity values.

INTRODUCTION

MEAT ANALOGS or "meatless meat" composed of soy protein, egg albumen, wheat gluten, and other proteins are becoming a commercial feasibility at a rapid rate. USDA estimates for utilization range from 10–20% of the meat product market (Hammonds and Call, 1972; Butz, 1974). Several progressive organizations are manufacturing a wide variety of meat analogs and extenders (used in combination with meat). The combining and processing of the protein blends to achieve meat-like texture has become a technological possibility. To date, however, little is known of the thermal properties as affected by formulation variables and processing parameters. These relationships are needed for selecting the optimal combination to meet the constraints of texturization process and microbial inactivation.

The present investigation (Rizvi, 1976) was undertaken, therefore, to study the changes in the thermal characteristics of model analogs made from soy spun fiber, egg albumen, and wheat gluten. The intent of this paper is to report the factors which significantly influence thermal diffusivity of the analogs made under different formulation and processing conditions.

EXPERIMENTAL

Experimental design

Figure 1 schematically describes the formulation and process variables studied in the production of meat analogs and responses monitored on the end product. Each of the six independent variables going into the process was studied at five different levels.

The choice of the independent variables was limited to the basic parameters of meat analogs: primary protein sources, pH, and temperature. Fat and salt systems were not included. Soy spun fiber was chosen to provide the meat-like fibrous characteristics. Egg albumen and wheat gluten served in the supplementary role in holding the fibers together in the matrix. Temperature and pH are the primary process variables. They were included because of their anticipated influence on protein behavior, and consequently on the thermal and textural properties.

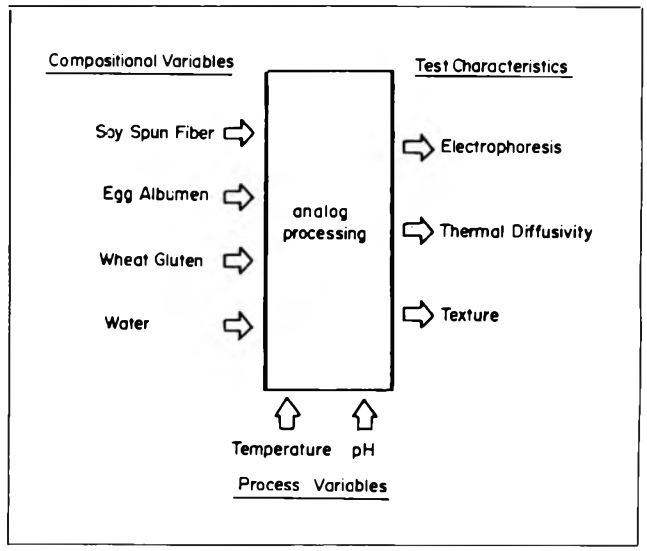

Fig. 1—Analog production process showing input variables and output product characteristics studied.

In order to minimize the number of experiments required and to study the interactions within the independent variables, a Rotable Central Composite Design was used (Chochran and Cox, 1957; Meyers, 1971). For five levels of each of the five independent variables, the number of individual experiments required was calculated by the following equation:

$$N = 2^n + 2n + 1$$

where N = number of experiments, and n = number of independent variables. Thus for five independent variables, the total number of experiments required was 43.

The independent variables and the ranges for the 43 experiments that were carried out are listed in Table 1. Five equally spaced levels were used. The independent variables were not replicated but the dependent properties measurements were made thrice.

Apparatus

The heating bath was a 10-L capacity glass container insulated on the outside with 1-in. thick fiber glass wool. Heating was done using a 115v, 20 amp immersion heater-circulator (B. Braun Melsungen, Bronwill Scientific, Inc., Rochester, N.Y.). The temperature

Table 1—The range of independent variables studied[a]

Variable	Range
1. Egg albumen	0–15% wet basis
2. Wheat gluten	0–15%
3. pH	4–8
4. Temperature	71.1–115.6°C
5. Added water	15–35%

[a] Sixth variable soy spun fiber = 100–(Egg albumen + wheat gluten + water)

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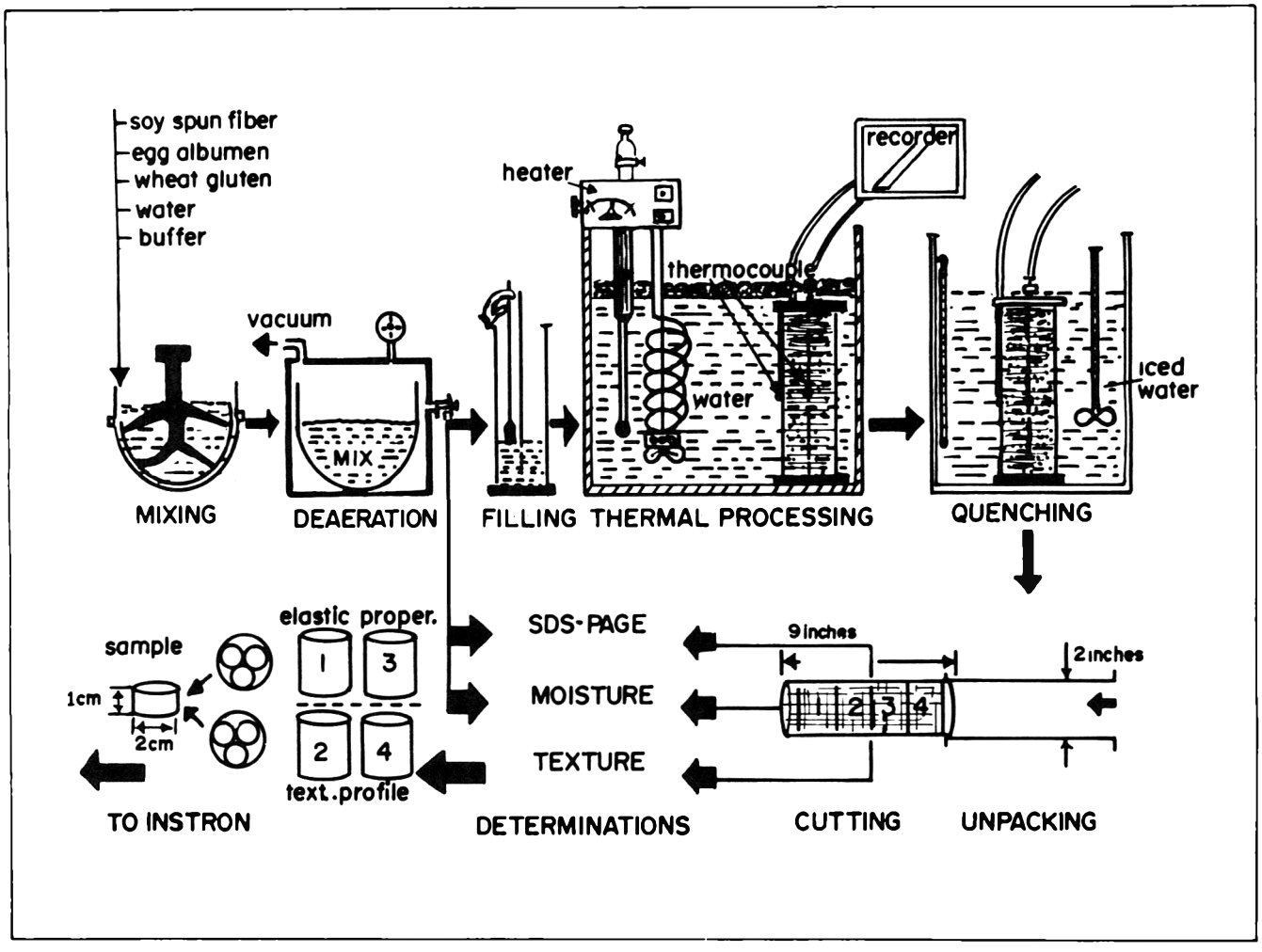

Fig. 2—Schematic flow diagram of the process for meat analog production and response measurement.

range of the heater was 0–200°C, accuracy ± 0.2°C.

A 120°C multipoint potentiometer recorder (least division 1.0°C) with a 24 gage copper-constantan thermocouple was used. The recorder was calibrated to within 1.0° with chilled water at 4°C and boiling water at 100°C.

The 22.86 cm (9 in.) long × 5.08 cm (2 in.) diameter thermal diffusivity tube (volume 425 ml) made of chrome-plated brass and stainless steel described by Dickerson and Reed (1975) was used. The tube had a wall thickness of 0.124 cm (0.049 in.) and inside radius of 2.41 cm.

Procedures

Experimental process. Figure 2 outlines the flow of the process used for analog production.

Raw ingredients. The egg albumen used was from Milton G. Weldbaum Company (P.O. Box 324, Birmingham, MI 48012); the wheat gluten was obtained from Industrial Grain Process (4 Lansing Square, Suite 209, Willowdole, Ontario, Canada), and the soy spun fiber was supplied by Worthington Foods, Inc. (Worthington, OH 43085). The three raw ingredients were obtained in bulk from a single lot to minimize variations.

pH adjustments. 1M phosphate buffer and 1M phosphoric acid solutions were used to adjust the pH. First, a 1-g sample of each of the three raw ingredients was dispersed in 20 ml of water and titrated against the acid and buffer solution to attain the final pH of 4, 5, 6, 7, and 8. Then, the quantity of buffer required for the above-mentioned pH adjustment was subsequently utilized to calculate the amount of buffer needed for a lot of 1 kg analog mix. Consequently, the volume of water going into a 1 kg batch of analog mix was correspondingly decreased.

Mixing. Predetermined quantities of each raw ingredient, calculated amount of buffer solution and all but 100 ml of the water required for a 1 kg mix were first mixed in a Hobart Kitchen-Aid Mixer Model K5-A according to the instructions in the mixer manual for 15 min at the lowest speed. The pH was measured at the end of 15 min and adjusted to the nominal value. The balance of the water was added, then the sample was mixed for an additional 5 min.

Deaeration. To reduce froth formation during mixing the bowl with the mix was transferred to a vacuum chamber for deaeration. After a come-up time of about 10 min, the mix was held at more than 27" Hg vacuum for half an hour and released slowly.

Filling. The deaerated mix was next packed in the vapor proof thermal diffusivity tube. The empty tube was tared and about 430g of analog mix was added—about 100g at a time—and packed in with a plastic plunger to insure uniform apparent density (1 ± 0.005 g/cm^3) after packing. In order to make a passage for the axial thermocouple a metallic rod (1/16-in. diam) was held in the center of the tube during the filling operation then removed. The packed tube was accurately weighed. Then, the apparent density of the packed mix was determined. The top end-plate and thermocouples were secured in position and the assembly was immersed in the bath, as shown in the flow diagram.

Thermal processing. A water bath was used for temperature below 100°C and an identical mineral oil bath was used for higher temperatures. The filled thermal diffusivity tube was equilibrated in the bath to 25 ± 1°C. The bath was heated at a constant rate of about 1 ± 0.2°C per minute. The temperatures at the periphery and center of the tube were recorded continuously. After the bath attained the final temperature, it was maintained at that temperature for 1 hr.

Quenching. At the end of the holding period, the thermal dif-

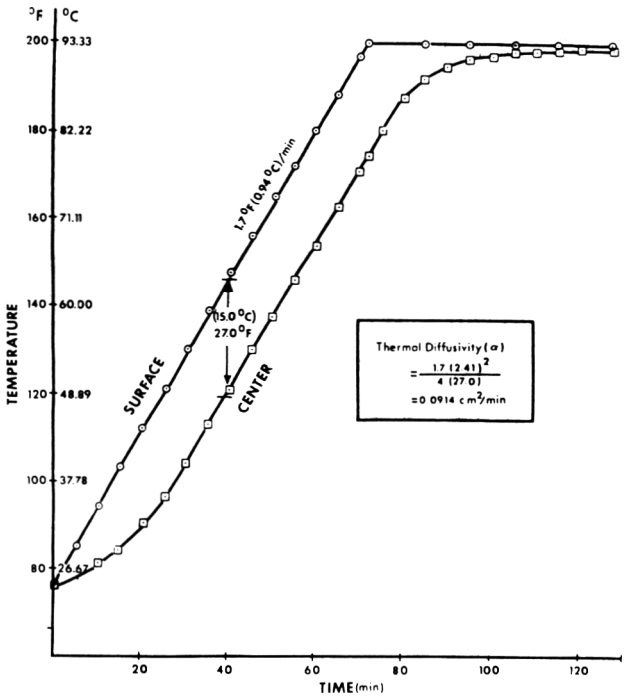

Fig. 3—Plot of temperature and time for thermal diffusivity (α) determination by Dickerson's (1965) method during heating period of model meat analog production.

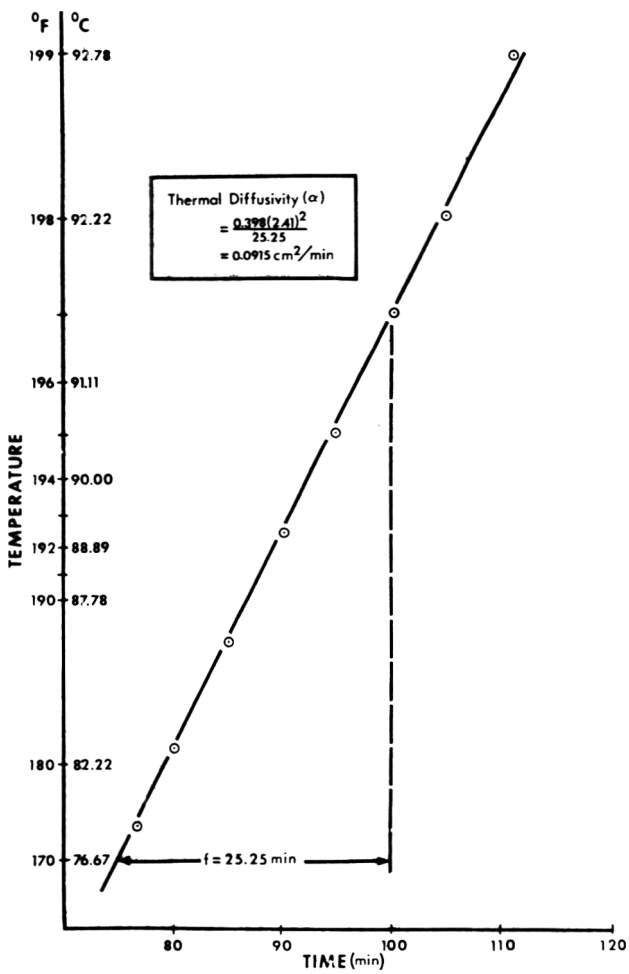

Fig. 4—The plot of temperature and time for determination of thermal diffusivity (α) during holding time of model meat analog production.

fusivity tube along with its contents was transferred to an ice-water bath maintained at about 5 ± 1°C and held for 10 min.

Unpacking and cutting. The cooked meat analog was removed from the tube by loosening it with a long, thin knife blade and pushing it gently with a wooden cylindrical block.

Samples for moisture determination and electrophoresis were taken from the center.

Moisture determinations. The water content of the mix and the final product was determined by drying the sample to constant weight at 95°C as outlined in AOAC.

Thermal diffusivity measurements. The thermal diffusivity during the heating period was calculated using the transient state method of Dickerson (1965). The method of Pflug et al. (1965) was used to determine the thermal diffusivity during the holding period. The accuracy of the apparatus was calibrated with distilled water containing 0.3% (by weight) Ion agar to suppress convection; this was one of the calibration fluids used by Dickerson.

A typical time-temperature history during the heating and holding period of meat analog is shown in Figure 3. This shows that the temperature difference between the surface and the center of the tube was constant during heating period after initial lag time. During holding, the center temperature approached the surface temperature asymptotically. The f value was calculated as the number of minutes required for the straight line portion of the curve to pass through one log cycle (Fig. 4). A value of 0.398 was used for calculation of thermal diffusivity when temperature was expressed in °F.

Statistical analysis. When k factors (or independent variables), $x_1 \ldots x_k$ are being studied in an experiment, the response (or dependent variable) will be a function of the levels at which these factors are combined (x_k). Mathematically, this can be expressed as: $y = f(x_1, x_2, \ldots x_k)$, where y is the response function (Davies, 1954). Based on this, a stepwise multiple linear regression analysis was run for each of the response functions (dependent variables) of Texture Profile, elastic, and thermal parameters. This can be expressed as:

$$y_j = a_{oj} + \sum_{i=1}^{6} a_{ij} x_{ij}$$

where, a_{oj} = constant, and a_{ij} = regression coefficients for egg albumen, wheat gluten, spun fiber, water, pH, and temperature.

RESULTS & DISCUSSION

DETERMINATION of thermal diffusivity on meat analog was made during the heating period using Dickerson's (1965) transient state method. Tests on distilled water with 0.3% (by weight) Ion agar were done to verify the accuracy of the apparatus. Thermal diffusivity was found to be 0.0942 cm^2/min. This is in good agreement with the value of 0.094 cm^2/min reported by Dickerson and Reed (1975).

Figure 3 illustrates a typical time-temperature relationship obtained on a formulation of meat analog and shows the calculated value of thermal diffusivity.

Dickerson's method of thermal diffusivity calculation could not be extended to the entire heat treatment process since it can be used only when the surface and center temperature increase linearly with time. During the heating period, surface temperature became constant and center temperature approached the surface temperature asymptotically (Fig. 3).

In order to calculate the thermal diffusivity value during the holding period, the method of Pflug et al. (1965) was used. Figure 4 illustrates the result obtained. The values of thermal diffusivity obtained during heating and holding periods by two different methods matched very favorably. The thermal diffusivity values for all the formulations calculated by the two methods compared well within the experimental variations (r = 0.97).

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Table 2—Linear regression coefficients and standard error for thermal diffusivity of model meat analogs[a]

	Thermal Diffusivity[b] (cm^2/min)
Constant	0.052
Egg albumen (g dry wt)	0.000,005,7 ± 0.000035 (−0.36)[c]
Wheat gluten (g dry wt)	−0.000,0.14 ± 0.00038 (−0.54)[c]
Soy spun fiber (g dry wt)	−0.000,005,4 ± 0.00005 (0.30)[c]
Water (%)	0.000,70 ± 0.00033 (0.73)[c]
pH (units)	−0.000,98 ± 0.0004 (−0.27)[c]
Temperature (°C)	−0.000,062 ± 0.00002 (−0.28)[c]
Multiple correlation coefficient	0.82

[a] The computor program used for the stepwise linear expression analysis was that of Scientific Process and Research, Inc., made available by Worthington Foods, Inc., Worthington, Ohio.

[b] On dry mass basis of egg albumen, wheat gluten, and soy spun fiber.

[c] Figures in parentheses refer to simple single-factor correlation coefficients.

A stepwise six-way linear regression analysis (Table 2) on the thermal diffusivity value gave a signficant overall correlation coefficient of 0.82. Of the six independent variables, water content was found to be the most significant variable ($r = 0.73$) with the thermal diffusivity value increasing as the water content was increased. Wheat gluten level and temperature of heat treatment had the opposite effect.

The simple single-factor correlation coefficient of egg albumen was found to be negative (−0.36) while the regression coefficient was positive. The opposite trend was exhibited by soy spun fiber. This suggests that in a complex blend of proteins, the factors together especially of temperature and pH and their interactions confounds the simple single-factor correlation.

Thermal diffusivity data obtained in three temperature ranges were plotted against water content (Fig. 5–7). Thermal diffusivity varied linearly with water content but the values were generally higher than those reported by Riedel (1969) for ham at 55°C. Dickerson and Read (1975) have reported a value of 0.082 cm^2/min for thermal diffusivity of ham in the temperature range 40–65°C at a water content of 65% by weight.

A regression equation correlating thermal diffusivity with water content for each of the three temperature ranges has been given (Fig. 5–7). This illustrates that the correlation between the two variables decreases at elevated temperatures and that the thermal diffusivity values also decline with increase in temperatures from 93 to 116°C for the same water content in the analog system. Follow-up of the protein-protein interaction in the system by electrophoresis (to be published) supports the evidence that at higher temperatures water content alone cannot reliably predict thermal diffusivity values. For accurately predicting heat transfer rates at a temperature above 93°C in a highly interactive system, like a meat analog, the thermal diffusivity values should be experimentally obtained.

CONCLUSIONS

(1) Measurement of thermal diffusivity during the holding period of heat treatment using Pflug et al.'s method has provided an extension to Dickerson's method. The thermal diffusivity values obtained by the two methods agreed well within the experimental variations.

(2) Thermal diffusivity values of model meat analogs varied directly with water content in the temperature range

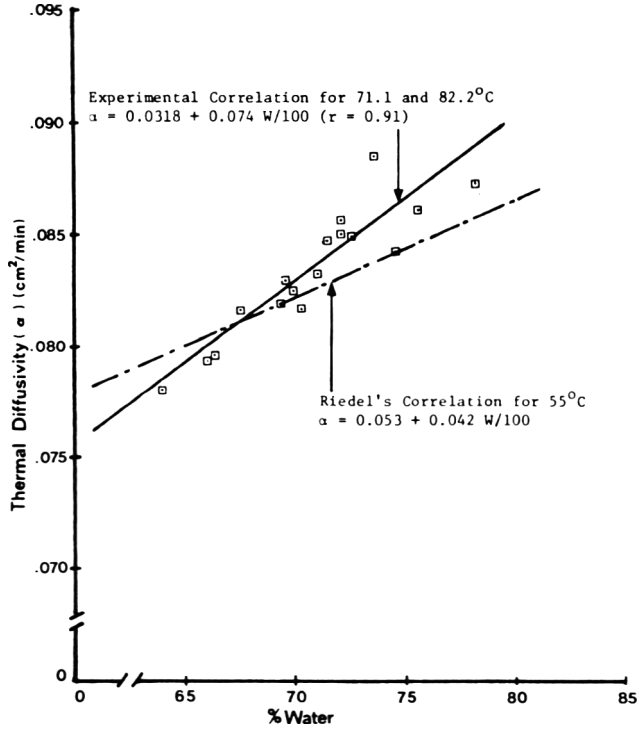

Fig. 5—Thermal diffusivity (α) of model meat analogs at 71.1 and 82.2°C as a function of water content.

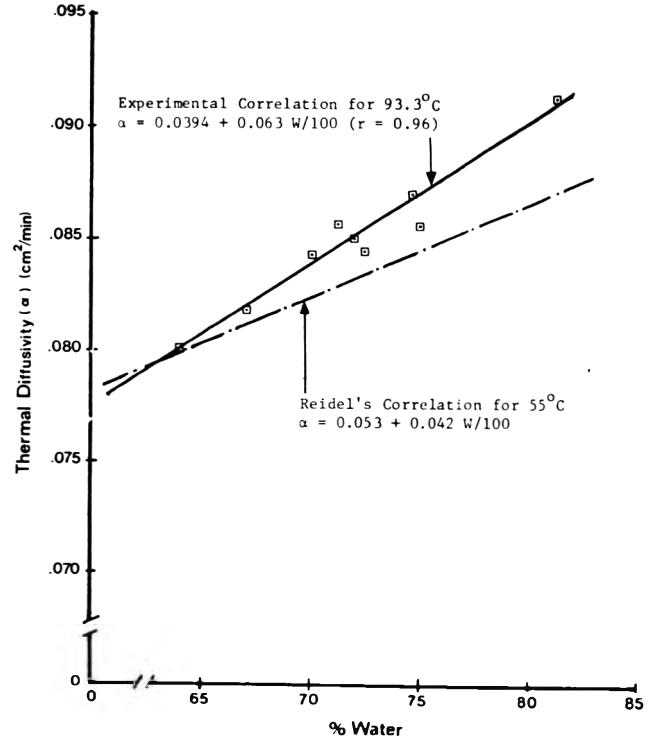

Fig. 6—Thermal diffusivity (α) of model meat analogs at 93.3°C as a function of water content.

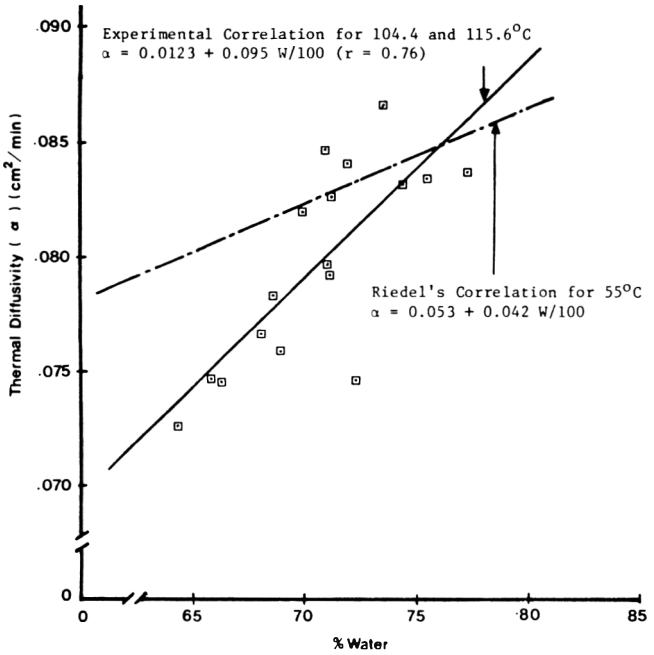

Fig. 7–Thermal diffusivity (α) of model meat analogs of 104.4 and 115.6°C as a function of water content.

71.1–93.3°C and can be described by simple straight line equations as given in Figures 5 and 6.

(3) At 104.4°C and above, water alone could not reliably predict the thermal diffusivities.

(4) Thermal diffusivities of model meat analogs decreased with rise in texturizing temperature above 93°C.

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INFLUENCE OF RAW INGREDIENTS, NITRITE LEVELS, AND COOKING TEMPERATURES ON THE MICROBIOLOGICAL QUALITY OF BRAUNSCHWEIGER

CHU-YING CHYR, HOMER W. WALKER, and JOSEPH G. SEBRANEK

ABSTRACT

Of the main ingredients (pork liver, pork-trim, and salt-spice mixture) used for manufacturing braunschweiger, pork liver had the most influence on the microbial numbers in the raw emulsion. The microflora of the raw sausage emulsion consisted mainly of enterococci, bacilli, lactobacilli, and pediococci. At 5°C, the raw emulsion underwent souring within 4 wk. Freshly cooked liver sausage usually contained 10^3 organism/g; the most frequently recovered bacteria, in this instance, were bacilli, enterococci, and pediococci. Nitrite was effective in inhibiting growth of enterococci capable of producing a perfumy odor in liver sausage. Sausage made from raw emulsion of good bacterial quality and stored at 5°C had a shelf life exceeding 16 wk.

INTRODUCTION

BRAUNSCHWEIGER, when stored at temperatures of 10°C or less, can be expected to have a shelf life of 12 wk or more (Steinke and Foster, 1951a, b; Ertle, 1970). Commercial, freshly-made liver sausage and chopped liver products will usually contain 10^3-10^5 aerobic mesophiles/g and mesophilic anaerobes at levels of 10^2/g (Steinke and Foster, 1951a, b; Surkiewicz et al., 1977). Steinke and Foster reported that the predominant organisms in the fresh product were *Micrococcus* and *Bacillus* spp. that were strictly aerobic and did not grow at 5°C. Upon prolonged storage, these authors observed spoilage as a result of the growth of Gram-positive, nonsporeforming, catalase-negative, microaerophilic rods and some facultative yeasts.

Little information is available on the influence of processing procedures on the microbiological quality of braunschweiger. The purposes of the present study were to determine the numbers and types of organisms in the raw ingredients and the influence thay have on the microbiological quality of the cooked product, to examine the role of nitrite in the control of bacterial growth and to evaluate the influence of cooking temperatures on the microbial quality of braunschweiger.

MATERIALS & METHODS

The sausage was made in the Meat Laboratory at Iowa State University according to the procedures and formulation described by Chyr et al. (1980). For studies on the influence of levels of nitrite, the formulation was varied by adding 0, 50, 100 or 156 ppm of sodium nitrite. Calcium-reduced dried skim milk (CRDSM) was added at a level of 3.5% to some batches. Four types of liver were used in different batches of the product: fresh liver, frozen liver, liver given a scalding treatment and liver soaked in brine (Chyr et al., 1980).

After vacuum-packaging, the raw emulsion was cooked in water held at 74°C until the internal temperature reached 68°C. The internal temperature of the product was measured by inserting a metal thermometer through the end of the sample into the geometric center. The cooking time required to reach an internal temperature of 68°C was about 28–30 min. This treatment is referred to as T2. Other cooking treatments consisted of heating in water at 68°C to an internal temperature of 63°C (T1), heating in water at 79°C to an internal temperature of 74°C (T3) and cooking in water at 85°C to an internal temperature of 74°C (T4). After cooking, the sausages were chilled immediately by immersion in cold water (10°C) for 30 min, after which they were drained and stored at 0° for 2 days. The final product was stored at 5°C unless otherwise stated.

Raw ingredients (liver, pork trim and the salt-spice mixture), the raw emulsion before it was stuffed into the casings, the cooked, freshly-made liver sausage, and the stored sausage were examined microbiologically. Except for liver, 30-g samples of the materials were blended for 2 min with 270 ml of 0.1%-peptone water, and appropriate dilutions made for plating. Livers were sampled by swabbing a 10-cm^2 area with a moistened cotton swab.

Counts were determined for the following organisms: aerobic mesophilic bacteria, anaerobic mesophilic bacteria, enterococci, and aciduric bacteria. Media, plating techniques and incubation conditions are listed in Table 1. Triplicate samples of each treatment were examined. Representative colonies were selected and characterized to determine the proportion of types present.

For the identification of isolated organisms the following tests, when appropriate, were used. Gram stain, catalase test, carbohydrate fermentation tests, motility tests, nitrate reduction, gelatin liquefaction, indole formation, hydrogen sulfide production, casein digestion, starch hydrolysis, MR-VP tests and reaction on litmus milk. The procedures for these tests are described by Durand et al. (1973). Additional tests included the OF test (Evans and Kloos, 1972), arginine hydrolysis (Collins and Lyne, 1970) resistance to 40% bile (Facklam, 1972), pyruvate fermentation (Gross et al., 1975), anaerobic fermentation of glycerol (Gunsalus and Sherman, 1943), 0.04% tellurite tolerance (Cavett, 1963), growth on LBS agar (Cavett, 1963) and growth on KF medium (Kenner et al., 1961). The pH of the sausage was measured on a homogenate of 50g of sample and 50 ml of distilled water blended for 2 min in a Waring Blendor.

RESULTS & DISCUSSION

THE FIRST PART of this study was concerned with determining the types of organisms in the raw ingredients used for making braunschweiger and in the cooked product. The formulation for these studies contained 156 ppm sodium nitrite, and the product was cooked in water at 74° C to an internal temperature of 68°C. Such treatment is commonly encountered in the manufacturing of this product (Rust, 1976).

An evaluation of the number of organisms on the surface of fresh, raw pork livers showed an average count of 2.8×10^4 aerobic mesophiles/cm^2. Numbers of anaerobic mesophiles were essentially the same as the counts for aerobic mesophiles. A survey of these organisms showed that they consisted mainly of enterococci, *Lactobacillus, Pediococcus, Bacillus, Micrococcus* and coryneform bacteria. These findings are compatible with those of Gardner (1971) who reported that the surface of fresh pork live harbored 6.4×10^4 aerobes/cm^2. He found that the microflora consisted of *Micrococcus, Flavobacterium*, coryneform bacteria, *Acinetobacter*, lactic streptococci and *Leuconostoc*. Shelef (1975) recovered approximately 1.0×10^5 aerobes/g of ground beef liver. The isolates in this instance consisted of Gram-positive cocci, lactic acid bacteria, enterococci, and Gram-negative rods.

Comparison of the microbial loads on frozen-thawed

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Table 1—Methods and media used for enumeration of bacteria

Determination	Growth medium	Plating technique	Incubation conditions
Aerobic mesophilic count	Trypticase Soy Agar	Spread p ate	30°C, 48 hr
Anaerobic mesophilic count	TSA + 0.5% Na Thioglycollate (TT) (Difco)	Spread p ate	30°C, 72 hr (anaerobic, nitrogen atmosphere)
Enterococcal count	KF Streptococcus agar (Difco)	Spread p ate	35°C, 48 hr
Aciduric bacterial count	LBS agar (BBL)	Spread p ate w th overlay	30°C, 5 days

Table 2—Comparison of bacterial counts (anaerobic mesophiles/g) of different batches of raw emulsion and cooked liver sausage during storage. The batches included in this table contained fresh liver, 156 ppm sodium nitrite, and were cooked to an internal temperature of 68°C in water held at 74°C

Batch	Raw emulsion numbers/g	Storage temp	Cooked sausage Numbers/g after 1–2 wk	after 8–10 wk	after 12–16 wk
I. (Liver pretreatments)	7.2×10^4	5°C	1.5×10^3 (2)[a]	4.0×10^3 (10)	2.2×10^3 (16)
II. (Nitrite levels)	7.5×10^5	7°C	2.2×10^3 (1)	2.9×10^3 (8)	$10^3 - 10^6$ (12)
III. (Cooking Temperatures)	2.1×10^6	5°C	6.5×10^4 (1)	4.3×10^4 (8)	1.5×10^4 (12)

[a] Numbers in parentheses indicate wk of storage after which samples were analyzed.

liver with those on fresh liver showed that freezing and thawing influenced the count very little (3 to 4 $\times 10^4$ organisms/cm^2). Soaking in brine and washing of the liver reduced the microbial load by two log cycles or more to counts of 2–3 $\times 10^2$/cm^2. The scalding treatment was the most efficient pretreatment for reducing the microbial load on the surface of raw liver (<50 aerobes or anaerobes/cm^2).

The pretreatments of the liver had little influence on the total numbers of organisms found in the raw emulsion. In these and subsequent studies only those organisms that grew under anaerobic conditions were estimated since these types would be the likely cause of spoilage in the final product. The highest total anaerobic count was 7.2 $\times 10^4$ organisms/g for the sample containing fresh liver, and the lowest count was 1.5 $\times 10^4$ organisms/g for the sample containing scalded liver. The small difference between bacterial counts for the raw emulsions containing fresh liver and scalded liver suggests that other ingredients added to the final microbial load. Ingredients contributing the most organisms were probably the pork liver, pork trim, and spice-salt mixture. The pork trim, which constituted 50% of the sausage ingredients, had an anaerobic mesophilic count of 2.4 $\times 10^3$ organisms/g and was judged to be of good microbial quality. The spice-salt mixture, which was added at a level of 3.3%, had an anaerobic mesophilic count of 1.5 $\times 10^5$ organisms/g. All the microorganisms isolated from the spice-salt mixture belong to the genus *Bacillus*.

Another possible source of bacteria in the emulsion could have been the presence of organisms in the deep tissues of the liver. Gardner (1971) reported that the deep tissue of liver contained about 200 organisms/g and that the microflora consisted of *Flavobacterium*, coryneform bacteria, *Micrococcus*, lactic streptococci and *Leuconostoc*. He also found that, during refrigeration at 5°C for 7 days, lactic streptococci and *Leuconostoc* grew well and became the predominant organisms in the deep tissues of liver.

Cooking of the sausage in water at 74°C to an internal temperature of 68°C reduced the microbial load by more than 90%. The number of organisms was reduced from 7.2 $\times 10^4$ organisms/g in the raw emulsion to 1.5 $\times 10^3$ organisms/g in the cooked product (Batch I, Table 2); experience showed that numbers of organisms in the product did not change during the early weeks of storage at 5°C. Identification of isolates from anaerobic plates indicated that facultative bacilli were the main survivors in freshly cooked sausage. Aerobic counts were slightly higher than anaerobic counts; identification of isolates from aerobic plates indicated that this difference was attributable to the inability of certain bacilli and micrococci to grow under anaerobic conditions.

Little change occurred in the numbers of anaerobic mesophiles during storage at 5°C for 10 wk. After 16 wk of storage all samples except those containing scalded liver had a reduced count; a reduction of one log cycle (from 10^3 organisms to 10^2 organisms/g) was observed. Samples containing scalded liver showed growth of enterococci between the 10th and 16th wk of storage in four of six samples; anaerobic mesophilic counts of 10^5 to 10^6 organisms/g were observed in these instances.

The addition of 3.5% CRDSM to the formulation had little influence on the numbers of organisms present in the freshly cooked product even though the raw emulsion for these particular batches contained the greatest numbers of bacteria of any of the batches made. Cooking reduced the counts to $10^3 - 10^4$ organisms/g. Enterococci were predominant in these raw emulsions and occurred at a level of 1.0 $\times 10^5$/g Characterization of the organisms appearing on the anaerobic mesophilic plates showed that enterococci occurred much more frequently than would be indicated by recoveries on KF medium. During storage at 5°C, these enterococci survived but did not grow, indicating that most of the enterococci were sensitive to selective agents in the KF medium; only 6% of the enterococci were capable of initiating growth on KF plates.

After 2 wk of storage at 5°C, the raw emulsions with and without CRDSM showed about 20-fold increases in numbers of bacteria present. The microflora at this time included *Pediococcus* (80%), *Lactobacillus* (10%), and enterococci (10%). Aciduric bacteria as determined on LBS agar were not detected during the first 2 wk of storage; but after 4 wk, lactobacilli and pediococci were predominant in the spoiled samples with or without CRDSM. Development of large numbers of these organisms in the raw emulsion

Table 3—Changes in numbers of anaerobic mesophiles, and enterococci and changes in pH of raw emulsion stored at 5°C and of cooked sausage stored at 7°C containing different levels of nitrite

Nitrite levels (ppm)	Determinations	Raw emulsion (5°C) Weeks 0	Raw emulsion (5°C) Weeks 1	Raw emulsion (5°C) Weeks 2	Cooked sausage (7°C) Weeks 1	Cooked sausage (7°C) Weeks 2
0	Anaerobic mesophiles	8.4×10^5	1.5×10^6	3.9×10^6	3.7×10^3	3.6×10^3
	Enterococci	4.1×10^4	1.2×10^5	5.1×10^5	0 – 100	0 – 200
	pH	5.95	5.94	5.92	6.15	6.32
50	Anaerobic mesophiles	7.9×10^5	4.9×10^6	1.6×10^7	2.5×10^3	4.2×10^3
	Enterococci	7.4×10^4	2.3×10^5	7.4×10^5	0 – 50	0 – 100
	pH	5.98	5.92	5.89	6.19	6.32
100	Anaerobic mesophiles	7.5×10	1.1×10^6	1.1×10^7	2.9×10^3	3.0×10^3
	Enterococci	5.1×10^4	2.2×10^5	9.7×10^5	0 – 150	0 – 100
	pH	5.98	5.94	5.88	6.18	6.31
156	Anaerobic mesophiles	1.5×10^5	6.5×10^5	1.7×10^6	2.2×10^3	3.1×10^3
	Enterococci	6.3×10^4	2.2×10^5	9.9×10^5	50	0 – 200
	pH	5.98	5.95	5.90	6.21	6.35

Table 4—Effect of cooking temperatures on the pH and bacterial counts of Braunschweiger liver sausage

Sample	pH	Anaerobic mesophilic count No./g	Microflora (%) Bacillus	Microflora (%) Enterococci	Microflora (%) Aciduric bacteria	Enterococci/g (KF Medium)
Raw mixture[a]	5.94	2.1×10^6	3	92	5	1.1×10^5
T1[b]	6.17	2.8×10^5	0	73	27	8.0×10^3
T2	6.23	6.5×10^4	5	85	10	2.5×10^2
T3	6.28	1.5×10^3	100	0	0	< 50
T4	6.28	2.7×10^3	83	17	0	< 50

[a] Raw mixture refers to raw emulsion after being stuffed into casings but previous to cooking
[b] T1 cooked in water at 68°C to an internal temperature of 63°C. T2 cooked in water at 74°C to an internal temperature of 68°C. T3 cooked in water at 79°C to an internal temperature of 74°C. T4 cooked in water at 85°C to an internal temperature of 74°C.

and inadequate heating could result in the survival of these organisms in the cooked product. The numbers of aciduric bacteria surviving in cooked products tended to decrease during refrigerated storage.

Pork liver and pork trim used in the second phase of this study, which entailed the effect of nitrite levels on the quality of the sausage, contained 5.3×10^5 organisms/cm^2 and 9.0×10^3 organisms/g, respectively. Determination of the number of enterococci able to grow on KF medium indicated that pork liver contained 3.5×10^3 enterococci/cm^2; pork trim contained about 30 enterococci/g. During storage at 5°C for 2 wk (Table 3), the raw emulsions containing different levels of nitrite showed an increase in numbers of anaerobic mesophiles and enterococci of one or more log cycles. Spoilage was not evident at the end of this time, however.

The microbial content of the cooked sausage containing different levels of nitrite was determined during storage in a display case at 7°C (Table 3). During storage at 7°C for 4 wk, the microbial content changed little. After 8 wk of storage, however, the results showed that no growth (10^3 organisms/g), initiations of growth (10^4 organisms/g) and abudant growth (10^5 organisms/g) had occurred in different samples. When the counts were high, the predominant organisms were mainly enterococci; when the counts were low, bacilli predominated. The enterococcal counts on KF medium were comparable to those for the anaerobic mesophiles on Trypticase Soy agar. Most of the samples with low nitrite levels (0 or 50 ppm) showed some initial growth or abundant growth of enterococci; most of the samples formulated with 100 ppm or 156 ppm nitrite showed no growth or very little growth of the surviving enterococci. Samples without nitrite had the most abundant growth; samples containing 156 ppm had the least growth.

Two samples containing no nitrite contained the greatest number of microorganisms after storage for 12 wk; one sample had an anaerobic mesophilic count of 1.0×10^8/g and a unique perfumy odor. That numbers of enterococci recovered on KF medium equaled the number of anaerobic mesophiles indicated that enterococci predominated in the sample. The other sample, which had the same perfumy odor, had a total anaerobic mesophilic count of 2.2×10^8. In this instance, identification of the isolates indicated that both enterococci and pediococci grew in about equal numbers. Our observation led us to the conclusion that, when other variables such as raw materials, salt content and heat treatment are the same, nitrite produced an inhibitory effect on growth of surviving enterococci that increased as the concentration of nitrite was increase. Stoychev and Djejeva (1971) also observed that increasing levels of sodium nitrite exhibited increasing inhibitory action on *Streptococcus faecalis*.

In a third phase of our work the effect of cooking temperatures on microbial loads in braunschweiger was studied. The heat processing temperatures not only affected the residual microbial content of the cooked sausage but also played a decisive role in the kinds of survivors. Product cooked to an internal temperature of 63°C contained predominantly Gram-positive streptococci as the survivors (Table 4). Whereas enterococci account for 73% of the anaerobic mesophiles, only 8×10^3 organisms/g or 4% could be cultivated on KF medium in contrast to a calculated

number of 2.0 x 10^5/g. The calculated number was obtained by multiplying the number of anaerobes by 73%. From this observation, we assumed that 96% of the enterococci which survived cooking were heat injured (Clark et al., 1968; Duitschaever and Jordan, 1974).

An extended incubation time overcame this injury factor to some extent. One additional day of incubation showed a 2- to 3-fold increase in plate counts; counts continued to increase up to 5 days of incubation and then remained constant.

Cooking to an internal temperature of 68°C resulted in a 97% reduction of numbers of organisms. In samples from this treatment, enterococci constituted 85% of the anaerobic mesophilic population. Only 0.5% of the surviving enterococci (estimated on the basis of the calculated number) formed colonies on KF medium. In other words, 99.5% of the surviving enterococci were heat injured. The products heated to an internal temperature of 74°C showed lesser microbial loads than did products processed to the lower internal temperatures. In products treated at these higher temperatures, *Bacillus* predominated.

It is not unusual to observe the survival of enterococci in mildly heat-processed meats. For example, canned ham, a pasteurized product, is processed to an internal temperature of 66°C. Kafel and Ayres (1969) examined 4241 commercially canned hams and were able to recover enterococci from 45.6% of them. Kafel and Majewska (1961) also reported that enterococci are tolerant of curing salts in pasteurized cured ham and grow during refrigerated storage.

Among the studies for determining the effect of different variables on the microbial content of braunschweiger, one common treatment in each study was used. This common treatment was the formulation consisting of fresh pork liver, 156 ppm of sodium nitrite and cooked in water at 74°C to an internal temperature of 68°C. The initial microbial load of the various raw emulsions differed significantly from each other (Table 2). These differences were attributed mainly to variation in the contamination of the pork livers since microbial contamination in the other ingredients was similar for the different studies. Small variations in the length of time to reach the required internal temperature occurred, but despite these variations, the destruction of microorganisms during cooking exceed 97%. Cooked sausages stored at 7°C had a shorter shelf life than those stored at 5°C. Pasteurized sausage made from raw materials of good microbial quality and stored at 5°C had a shelf life in excess of 16 wk.

In summmary, the treatment of liver (fresh, frozen, scalded or brine-soaked liver) affected the numbers of organisms recovered from the surface of the liver but had little influence on the numbers occurring in the raw emulsion. Cooking of the emulsion caused reduction in numbers of organisms to levels of $10^3 - 10^4$/g. Enterococci predominated in those samples receiving the lower heat treatments and bacilli predominated in those exposed to the higher heat treatments. Nitrite inhibited growth of enterococci which caused a spoilage characterized by a perfumy odor.

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EFFECT OF PRERIGOR PROCESSING ON THE OXIDATIVE RANCIDITY OF GROUND LIGHT AND DARK PORCINE MUSCLES

M. D. JUDGE and E. D. ABERLE

ABSTRACT

The extent of oxidative rancidity developed during storage of ground samples of light and dark porcine semitendinosus muscles has been estimated with the thiobarbituric acid (TBA) test. The prooxidant effect of NaCl was observed in both pre- and postrigor ground and salted samples but the prerigor samples were much less susceptible to lipid oxidation over a 10-day storage period. Unsalted samples ground in the prerigor state were also less susceptible to autoxidation than similarly ground postrigor muscles. When prerigor ground and salted samples were stored in bags purged with nitrogen, the rate of rancidity development was greater than when such samples were stored in atmospheric oxygen. Prerigor oxygenated light muscle samples were more susceptible to oxidation than the dark muscles and vice versa for postrigor oxygenated samples. No differences due to muscle type were observed in samples stored in nitrogen. The partial inhibition of the prooxidant effect of NaCl observed with prerigor grinding and salting is suggested to be the result of an induced high ultimate pH. The improved stability of unsalted ground prerigor muscle as compared to similarly treated postrigor muscle may also reflect a limited pH decline.

INTRODUCTION

INTEREST in prerigor meat processing has become widespread for reasons of energy savings, speed of processing, superior binding properties and other attributes. Yet, little information has been reported about the flavor stability of such meat. Of special concern is the rate of oxidative rancidity development in muscle tissue that is exposed to oxygen prior to its postmortem metabolic and physical transformations.

Several influences on lipid oxidation could be expressed when meat is processed in the prerigor state. Prerigor salting (NaCl) is necessary to preserve the superior water-binding capacity of prerigor meat (Honikel and Hamm, 1978) but the prooxidant effect of salt is well known (Chang and Watts, 1950; Ellis et al., 1968). On the other hand, prerigor salting inhibits glycolysis, particularly at near-freezing temperatures (Honikel and Hamm, 1978), and high pH is associated with inhibition of lipid oxidation in stored meat (Keskinel et al., 1964; Owen and Lawrie, 1975).

The objective of this research was to determine the effect of prerigor salting of ground porcine muscle on the development of oxidative rancidity.

EXPERIMENTAL

SAMPLES of the light and dark portions of both semitendinosus muscles of 30 pigs weighing approximately 90–120 kg were used. Ten pigs were used for each of three processing conditions. Table 1 shows the variables included in each of the three conditions.

The muscles were dissected from the carcasses within 45 min or at approximately 24 hr postmortem for the pre- and postrigor samples, respectively. The distinctly light and dark muscle sections were used and the intermediate portion was discarded. The samples were ground once through a 3 mm plate and those to be salted were immediately mixed with 2% reagent grade NaCl (weight to weight basis). All samples were mixed with approximately 1% of antibiotic powder (Polymyxin B-bacitracin-neomycin).

Oxygenated conditions were attained in aerobically stored samples by pressing the samples into thin sheets (0.5 cm) and wrapping them in oxygen permeable, moisture impermeable polyethylene film (Borden Chemical, RMF-61HY). The samples to be aerobically stored were placed in gas impermeable bags purged with nitrogen. All samples were stored in their respective atmospheres for 10 days at 2°C except for intermittent opening of the packages for sampling. (Nitrogen was replaced after each sampling of anaerobically stored samples).

After 0, 3, 7, and 10 days of storage, the TBA (thiobarbituric acid) test of Tarladgis et al. (1960) was performed. At 24 hr postmortem the pH of all samples was measured with a glass electrode.

The data were analyzed by analyses of variance wherein the samples stored aerobically were analyzed for rigor, salt and muscle effects and those ground prerigor were analyzed for storage environment, salt, and muscle effects. Correlation analyses were conducted for salted and unsalted samples within the three processing conditions.

RESULTS & DISCUSSION

Postrigor ground, aerobic storage

The TBA numbers for the samples ground postrigor and stored in atmospheric oxygen reveal distinctive differences in rate of lipid oxidation due to the presence or absence of salt and the muscle type (Fig. 1; Tables 2 and 3). Even though the variation in the data is great, the prooxidant effect of the salt is apparent. Such observations have been made previously (Chang and Watts, 1950; Ellis et al., 1968) but the mechanism for this catalytic activity of NaCl is unknown.

In the unsalted samples, the TBA numbers agree closely with those reported by Keskinel et al. (1964) for pork. Those investigators also showed that dark turkey meat has higher TBA numbers after storage than light meat, a finding that is confirmed by the present study for pork. If the

Table 1—Experimental design

Identification	Processing conditions: Rigor state during grinding	Storage environment	Salted[a] or unsalted	Muscle type[b]	N
I	postrigor	aerobic	salted	light	10
	postrigor	aerobic	salted	dark	10
	postrigor	aerobic	unsalted	light	10
	postrigor	aerobic	unsalted	dark	10
II	prerigor	aerobic	salted	light	10
	prerigor	aerobic	salted	dark	10
	prerigor	aerobic	unsalted	light	10
	prerigor	aerobic	salted	dark	10
III	prerigor	anaerobic	salted	light	10
	prerigor	anaerobic	salted	dark	10
	prerigor	anaerobic	unsalted	light	10
	prerigor	anaerobic	unsalted	dark	10

[a] NaCl
[b] Semitendinosus

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heme proteins are major catalysts to lipid oxidation in meat as suggested by several workers (Tappel, 1952; Watts, 1954; Younathan and Watts, 1959; Maier and Tappel, 1959), the higher quantity of pigments in dark muscle than light muscle could explain the result. On the other hand, Allen et al. (1967) reported that dark porcine muscles exceed light muscles in content of phospholipids, the major contributors of oxidized flavor in cooked meat (Igene and Pearson, 1979). The brown color observed in both the salted and unsalted postrigor samples of the present study suggest that myoglobin could have been an active catalyst of oxidation.

The pH values for postrigor ground samples appear to be unaffected by the salt treatment but were related to muscle type (Table 4). However pH was negatively correlated with TBA numbers at a low but significant level (Table 5).

The extent of lipid oxidation in the postrigor ground and stored samples suggests that rancid flavor development in raw pork could significantly reduce its palatability as well as severely limit the time available for distribution of such products to consumers. —*Continued on next page*

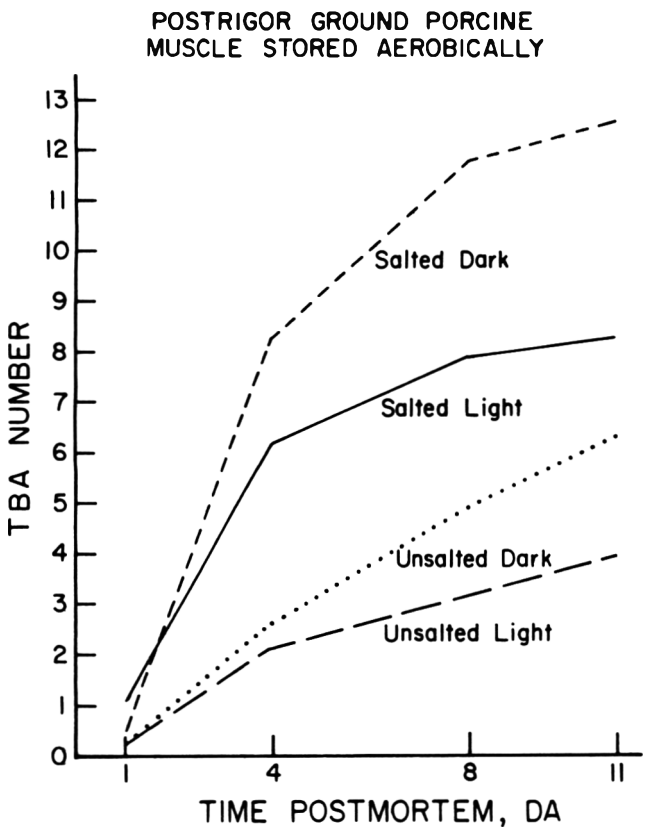

Fig. 1—Effect of NaCl and muscle type on TBA numbers in postrigor ground porcine muscle stored in atmospheric oxygen.

Table 3—Influence of processing conditions, salting and muscle type[a] on TBA numbers[b] in ground porcine muscle

Processing condition	Duration of storage, days			
	0	3	7	10
Postrigor ground-aerobic storage				
Salted light	1.1 ± 0.7	6.2 ± 2.0	7.9 ± 2.3	8.3 ± 2.3
Salted dark	0.5 ± 0.3	8.2 ± 3.9	11.8 ± 5.3	12.6 ± 5.2
Unsalted light	0.2 ± 0.1	2.1 ± 1.6	3.2 ± 2.2	3.9 ± 2.9
Unsalted dark	0.2 ± 0.2	2.6 ± 3.0	4.9 ± 5.5	6.3 ± 6.6
Prerigor ground-aerobic storage				
Salted light	0.7 ± 0.4	4.3 ± 1.8	5.5 ± 1.6	5.7 ± 1.6
Salted dark	0.2 ± 0.1	2.2 ± 1.1	4.7 ± 1.8	5.6 ± 1.5
Unsalted light	0.2 ± 0.1	1.1 ± 0.6	1.6 ± 0.9	1.8 ± 1.0
Unsalted dark	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
Prerigor ground-anaerobic storage				
Salted light	0.5 ± 0.3	3.6 ± 2.0	6.5 ± 2.2	7.7 ± 2.8
Salted dark	0.4 ± 0.4	4.1 ± 2.4	6.9 ± 3.2	9.7 ± 3.6
Unsalted light	0.3 ± 0.2	1.3 ± 1.3	2.2 ± 2.2	2.7 ± 2.5
Unsalted dark	0.2 ± 0.1	0.9 ± 0.8	1.8 ± 1.9	2.6 ± 2.8

[a] Light and dark semitendinosus
[b] Mean ± standard deviation

Table 4—Influence of processing conditions, salting and muscle type[a] on ultimate pH[b] of porcine muscle

Processing conditions	Salted		Unsalted	
	Light	Dark	Light	Dark
Postrigor ground—aerobic storage	5.77 ± 0.22	5.92 ± 0.25	5.73 ± 0.31	5.90 ± 0.21
Prerigor ground aerobic storage	6.14 ± 0.07	6.26 ± 0.07	5.93 ± 0.20	6.06 ± 0.16
Prerigor ground anaerobic storage	6.07 ± 0.12	6.22 ± 0.11	5.91 ± 0.24	5.99 ± 0.20

[a] Light and dark semitendinosus
[b] Mean ± standard deviation

Table 2—Significant effects of rigor conditions,[a] salting,[b] and muscle type[c] on TBA numbers and pH of aerobically stored ground porcine muscles

	TBA numbers after storage, da				pH, 24 hr
	0	3	7	10	
Rigor (R)	*	**	**	**	**
Salt (S)	**	**	**	**	**
Muscle (M)	**				**
RXS	*	*			*
RXM		**	**	**	
SXM	**				
RXSXM					

[a] Pre- vs postrigor
[b] Salted vs unsalted
[c] Light vs dark semitendinosus
** $P < 0.01$
* $P < 0.05$

Table 5—Correlation coefficients between ultimate pH and TBA number in porcine muscle[a]

Processing condition	Duration of storage, da			
	0	3	7	10
Postrigor ground-aerobic storage				
Salted	−0.36	−0.47*	−0.41*	−0.41*
Unsalted	−0.38*	−0.45*	−0.40*	−0.38*
Prerigor ground-aerobic storage				
Salted	−0.61**	−0.72**	−0.52**	−0.27
Unsalted	−0.41*	−0.63**	−0.70**	−0.71**
Prerigor ground-anaerobic storage				
Salted	−0.07	−0.02	−0.01	−0.18
Unsalted	0.15	−0.50*	−0.61**	−0.57**

[a] Light and dark semitendinosus combined
** $P < 0.01$
* $P < 0.05$

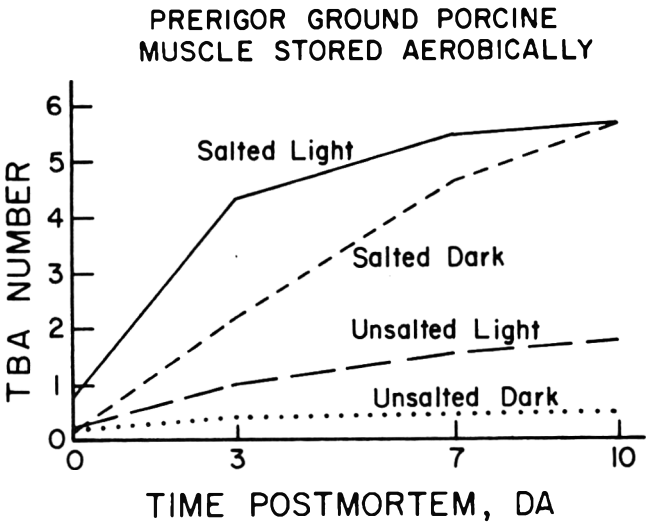

Fig. 2—Effect of NaCl and muscle type on TBA numbers in prerigor ground porcine muscle stored in atmospheric oxygen.

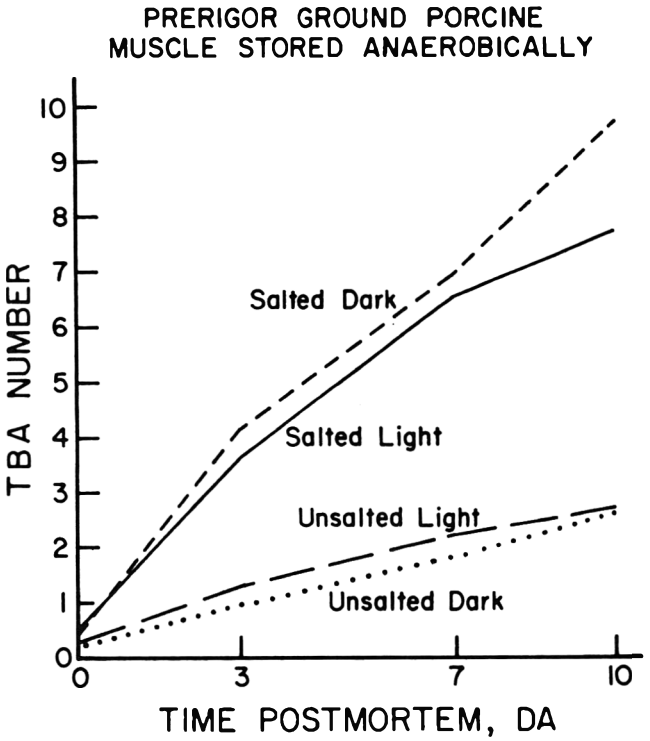

Fig. 3—Effect of NaCl and muscle type on TBA numbers in prerigor ground porcine muscle stored in nitrogen gas.

Prerigor ground, aerobic storage

The salted and unsalted samples that were ground in the prerigor condition developed TBA values that were much lower than the corresponding postrigor samples (Fig. 2; Tables 2 and 3). Although the prooxidant effect of the salt was still evident, it is clear that prerigor salted tissue was much less susceptible to autoxidation than postrigor salted tissue. Such observation is consistent with reports of diminished rancidity development in prerigor salted, massaged, nitrite-free hams (Judge and Cioch, 1979) and prerigor salted, frozen pork sausage (Drerup, 1979).

The light portion of the semitendinosus muscle underwent oxidative change more rapidly than the dark portion when salting and grinding occurred prerigor. This finding seems to minimize the importance of either heme protein or phospholipid level in prerigor salted meat as catalyst or regulator of lipid oxidation. (Visual observation of the samples suggested that the pigments remained in the oxymyoglobin state throughout the storage period).

Further insight may be gained by consideration of the pH values achieved in the samples after glycolysis was complete (Tables 2 and 4). The ultimate pH was influenced both by prerigor salting and by muscle type. As reported by Honikel and Hamm (1978), prerigor salting of ground meat inhibits lactic acid production from glycolysis. Of course the light and dark muscles differed in pH because of their different metabolic capabilities.

Grinding alone as a treatment for prerigor muscle appeared to limit the extent of pH decline as compared to postrigor muscle. Although the rate of pH decline is normally increased by grinding prerigor muscle (Pearson et al., 1973), the extent of pH decline in the present study may have been limited by a prolonged aerobic metabolism supported by the oxygen introduced during grinding as suggested by Lawrie (1979). Newbold and Lee (1965) and Pearson et al. (1973) found no significant difference in ultimate pH of prerigor minced and intact ox and sheep muscles but Drerup (1979) found higher pH and lower lactate concentrations in prerigor than postrigor ground porcine muscles.

Correlation analyses conducted within each processing condition revealed that the ultimate pH was more closely associated with TBA number in prerigor ground aerobically stored muscles than under any other processing condition (Table 5). High pH was associated with low TBA values.

Table 6—Significant effects of storage environment,[a] salting,[b] and muscle type[c] on TBA numbers and pH of prerigor ground porcine muscles

	TBA numbers after storage, days				pH
	0	3	7	10	24 hr
Environment (E)			**	**	
Salt (S)	**	**	**	**	**
Muscle (M)	**	*			**
EXS					
EXM		*			
SXM	*				
EXSXM					

[a] Aerobic vs anaerobic
[b] Salted vs unsalted
[c] Light vs dark semitendinosus
** $P < 0.01$
* $P < 0.05$

Other literature supports the contention that high pH is a deterrent to rancidity development. Keskinel et al. (1964) reported a strong inverse relationship betwee pH and TBA number in pH adjusted ground beef samples stored for 10 days. Owen and Lawrie (1975) reported suppressed peroxide values in frozen pork with induced high pH. Uebersax et al. (1978) and Mast et al. (1979) showed that mechanically deboned poultry meat which had been chilled or frozen with CO_2 snow developed lower pH and higher TBA values than control product.

The mechanism by which pH is associated with lipid oxidation could involve non heme iron as a catalyst. Wills (1966) concluded that both heme and non heme iron were capable of catalyzing the oxidation of fatty acids but non heme iron was more active at acid pH values whereas hemoproteins were less pH sensitive. Non heme iron was also more important than heme proteins in catalyzing the oxidation of endogenous lipids in tissue homogenates.

Adding salt to the muscle samples in the prerigor state as compared to postrigor reduced the variability of the TBA numbers even though the storage conditions (atmospheric oxygen) were identical for both (Table 3). This tendency for less variation in the slowly oxidizing lipids is consistent with the well known mechanism of self-propagation in which the reaction products initiate the formation of several free radicals from one original event.

Prerigor ground, anaerobic storage

The absence of atmospheric oxygen in prerigor ground muscles (or the presence of nitrogen gas) altered the TBA values in both salted and unsalted samples as compared to prerigor oxygenated samples (Fig. 3; Tables 3 and 6). The prooxidant effect of the salt was more pronounced and the effect of muscle type was largely abolished. The basis for these effects is unclear but it is interesting that oxidation seemed to occur at rates that were intermediate between those of pre- and postrigor aerobically stored samples. The exclusion of oxygen from the environment in which the samples of this phase of the study were stored may have partially simulated the conditions to which the postrigor ground samples were exposed immediately postmortem.

Even though the samples were stored in nitrogen gas, it is likely that some oxygen was incorporated into the tissues during grinding resulting in very low oxygen pressure in localized areas. Such conditions would favor the formation of metmyoglobin and, indeed, the color of many of the samples was indicative of this pigment state. In this respect the prerigor anaerobically packaged samples were similar to those processed postrigor and stored aerobically. However the rapid rate of autoxidation in postrigor aerobic tissue may have been due to a combination of a low pH and metmyoglobin whereas the favorable effects of high pH in prerigor nitrogen covered tissues may have been masked by the presence of metmyoglobin. It is notable that the pH influence in anaerobically stored muscles was expressed only in the unsalted samples (Table 5).

SUMMARY & CONCLUSIONS

THE RESEARCH reported herein shows that prerigor ground porcine muscles are more stable with regard to lipid oxidation than postrigor ground muscles, especially when the prerigor muscle is stored under aerobic conditions. In porcine muscle the ultimate pH is higher in dark than light tissues and may be raised in both types by grinding prerigor or addition of salt prerigor. Under aerobic storage conditions of prerigor ground tissue, metmyoglobin does not appear to be abundant, a protective effect of the high pH against lipid autoxidation becomes predominant, and, if salt is present, its prooxidant effect is diminished. Additional research is needed to identify the mechanism by which high pH inhibits such oxidation.

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DIFFUSION OF CHLORIDE, NITRITE, AND NITRATE IN BEEF AND PORK

JAY B. FOX, JR.

ABSTRACT

The diffusion of sodium chloride, nitrite, and nitrate into beef, pork, and pork fat was studied by the porous disc technique. Total salt uptake and boundary concentration analyses were performed on beef. Various salt combinations and concentrations and various muscles were studied. Chloride diffusion was normal by all three methods of analysis, but skew boundaries and decreased uptake of nitrite were observed in the boundary measurements; diffusion rates of nitrite were constant in the porous disc method. Nitrite diffused slower than chloride in meat, but the rate of nitrate diffusion was sometimes greater than that of chloride. Comparison of the results of the steady state diffusion with the total uptake and boundary concentration showed the diffusion of nitrite to be governed by both physical and chemical factors.

INTRODUCTION

THE RATES of diffusion of curing salts in meats determine the length of time required for processing and the uniformity of cure distribution. This is as true for rapid ham curing by stitch pumping and tumbling or massaging as it is for long term brine and dry salt cures. A thorough understanding of the diffusion process is necessary to the interpretation of the effects and interactions of the various salts used in curing and the effects of techniques such as tumbling and massaging. The basic data for this understanding are the diffusion constants of the curing salt anions. Although the two most important anions are chloride and nitrite, determinations of diffusion constants for curing salts have been limited to sodium chloride in pork (Wistreich et al., 1959, 1960; Körmendy and Gantner, 1958; Wood, 1966). The determinations were made with cylinders of meat enclosed in glass tubes so that diffusion proceeded in one plane. While the technique is suitable for the study of single diffusing solutes, if more than one solute is used and interactions occur, it is difficult or impossible to separate individual effects. Chemical reactions in the tissue may take place; nitrite disappears in meat during curing through reaction with tissue components (Mirna and Hofmann, 1969; Fox and Nicholas, 1974). Nitrate has been said to increase the permeability of muscle tissues to water and to affect the penetration of other salts (Jensen, 1942). These effects may be separated operationally by a steady state diffusion system in which a concentration gradient is established across a membrane or porous disc (Crank, 1967; Bull, 1951). Under these conditions, the effect of one solute may be observed by varying its concentration in a series of experiments in which the concentration of the other solutes remain constant. We therefore undertook a study of the diffusion of sodium chloride, nitrite, and nitrate into both beef and pork, using the porous disc technique to determine the effect of salt-salt and salt-tissue component interactions. For purposes of comparison, we ran diffusion experiments in cylinders as previously described (Wood, 1966), analyzing both for total uptake and the shape of the diffusing boundary.

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EXPERIMENTAL

THE VARIOUS CUTS and/or muscles as indicated were purchased from local suppliers. For the preparation of either cylinders or discs, the meat was vacuum packaged and immersed in an alcohol bath at −3 to −4°C until it was firm. For cylinders, the meat was cut into 7-cm lengths and cores were cut out parallel to the fiber with a 1.4-cm cork borer. The cylinders so formed were aspirated into 1.4-cm i.d. glass tubes 10 cm long until one surface of the meat was flush with the end of the glass tube. The exact dimensions were such that a close fit was obtained. Bundles of five to six of the glass tubes were suspended in 500 ml of cure solution with the flush surface of meat immersed half way down in the cure solution. The solutions were stirred occasionally, although the absence of light refraction gradients due to concentration variation indicated stirring was not necessary (cf. Wood, 1966).

For discs, the firmed meat was sliced into 1-cm thick sections perpendicular to the fibers. A stainless steel cutter was used to cut out 5.0-cm discs. The discs were then placed into 5.0 cm i.d. × 1 cm thick Lucite rings, and cellulose film was smoothed over both surfaces. The ring was incorporated as the center member of a 3-component cell (Fig. 1). The lower compartment, enclosed on the bottom, was of the same diameter as the ring, had a filling tube on the side, and held 140 ml of cure solution. A magnetic stir bar was placed in the cell before it was assembled. The upper compartment of the cell was an open cylinder of the same diameter as the ring. The joining surfaces of the upper and lower compartments with the center ring were beveled so they would not slip. The cellulose membranes acted as gaskets to seal the beveled joints. The upper and lower compartments were fitted on the outside with retainer rings so the assembly could be bolted together. After the cell was assembled, the lower compartment was filled with the appropriate cure solution and the filling tube was plugged. The upper cell was filled with water. The cell was placed on a magnetic mixer and a

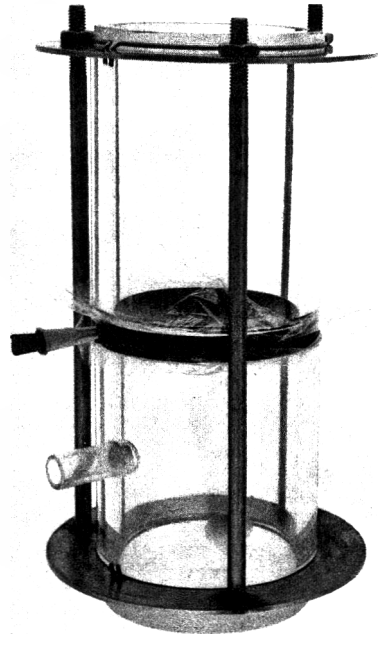

Fig. 1—Perspective view of diffusion cell.

glass stirrer rotated by a 60-rpm motor was placed in the upper compartment. Samples were withdrawn as desired from the upper compartment. All experiments were run at 4–5°C. To determine the rate of diffusion through the cellulose membranes the two sheets of cellulose were set in place to seal the beveled joints, and then the top membrane was cut out.

Chloride concentration was measured with an Orion solid state chloride electrode. Nitrite concentration was determined by the AOAC method of analysis (AOAC, 1976). Nitrate concentration was measured with an Orion nitrate electrode. Standard for chloride, nitrite, and nitrate were run with each set of samples. The curing salt concentrations were those used by a commercial dried beef manufacturer (4.27M Cl^-) and a ham cure brine suggested by Kramlich et al. (1973).

Calculation of diffusion constants

The defining equations for the diffusion constant, D, for the three methods of measurement are:

Total uptake (Wood, 1966)

$$S_t = 2C_o (Dt/\pi)^{1/2} \quad (1)$$

where S_t is the total salt uptake in time, t, per unit area of exposed surface. The slope of a plot of S/C_o as a function of $t^{1/2}$ is $2\sqrt{D/\pi}$.

Diffusing boundary (Crank, 1967)

$$C_{(d,t)} = \tfrac{1}{2}C_o \operatorname{erfc} \frac{d}{\sqrt{Dt}} \quad (2)$$

The error function, erfc x ($x = \frac{d}{\sqrt{Dt}}$), is calculated from the equation for a given value of concentration, C, at d, the distance from the meat surface. The value of x is determined from tabular values of the erfc x (Crank, 1967; WPA, 1941; Milne-Thomson and Comrie, 1944). The diffusion constant was then calculated for given values of d and t.

Porous disc (Crank, 1967)

$$M_t = \frac{D(C_o - C_h)At}{h} \quad (3)$$

where M_t is the total amount of salt diffusing through the disc in time t, h is the thickness of the disc, A is the area of the disc, C_o is the initial concentration of the salt in the lower cell, and $C_o - C_h$ is the concentration difference between the two sides of the disc. Because in the early stages of the diffusion process C_o changes minimally and $C_h \cong 0$, C_o is used in the equation (Bull, 1951).

To convert diffusion constants from one temperature to another, the following equation was used (Bull, 1951).

$$D_{T_1} = D_{T_2} \frac{T_1}{T_2} \times \frac{\eta_{T_2}}{\eta_{T_1}} \quad (4)$$

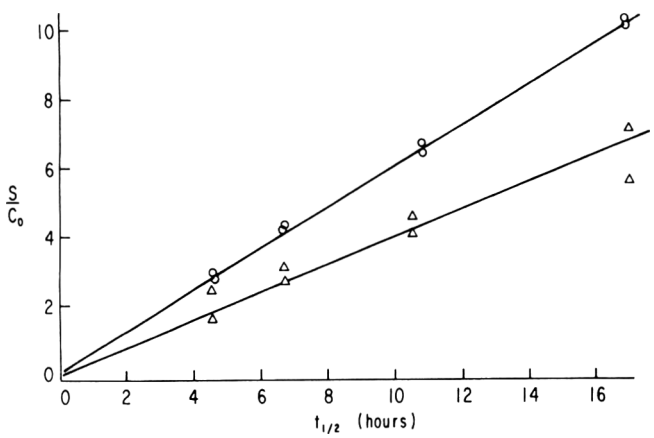

Fig. 2—Total uptake of sodium chloride and sodium nitrite in cylinders of beef semitendinosus at 5° C. [NaCl] = 4.27M, [$NaNO_2$] = 32.6 mM. ○, Cl^-; △, NO_2^-.

where T = °K and η is viscosity of the medium. Since the viscosity term is relative, we assumed that the relative viscosities of water in muscle are the same as those in free water.

Relative flow resistance

Because cellulose membranes were used to hold the meat discs in place, we needed an assessment of their effect on the diffusion rates. According to Crank, the resistance of individual sheets to flow is given by the equation:

$$\frac{Fh_1}{D_1} + \frac{Fh_2}{D_2} + \cdots \frac{Fh_n}{D_n} = \left[\frac{h_1}{D_1} + \frac{h_2}{D_2} + \cdots \frac{h_n}{D_n}\right] F \quad (5)$$

The flow, F, is constant across each sheet in the steady state, and h_i/D_i is the resistance to flow of each sheet. The total resistance to flow is the sum in brackets, so the total resistance, R, may be calculated from the known values of h, the thickness of the sheets, and the diffusion constants, D. For chloride, the values are:

$$R_t = \frac{2 \times 0.0025}{0.024} + \frac{1.00}{0.28} = (0.20 + 3.57) = 3.77 \quad (6)$$

The first term is for the two cellulose membranes, where 0.0025 is the thickness of each in cm and D is the diffusion constant of chloride through the membrane. The second term is the resistance to flow through the 1.0 cm meat sample. For chloride, and nitrite and nitrate as well, this meant a reduction of 4 to 5.5% between the measured value and the true value. All reported values we corrected for the cellulose membrane resistance.

RESULTS

FIGURE 2 shows the results of measuring total uptake of both chloride and nitrite in cylinders of beef semitendinosus. The diffusion constants were calculated from the slopes of the curves according to Eq (1), giving values of (0.26 ± .02) and (0.11 ± 0.035) × 10^{-5} cm^2 sec^{-1} for chloride and nitrite, respectively. To obtain further insight into the process, we analyzed the cores in sections across the diffusing boundary for chloride nitrate and nitrite, with the results shown in Figure 3. The diffusion constants for the sample shown in Figure 3 and a second sample are given in Table 1. The coefficients of variation for the individual chloride diffusion constants for both methods of determination were 8, 9, and 15% compared with a value of 15% reported by Wood (1966) and a range of 0.3 to 21.6% reported by Körmendy and Gantner (1958).

The nitrate and chloride curves of Figure 2 and the data of Table 1 show an anomaly in that the concentrations of both salts are too high near the meat surface, giving abnormally high diffusion constants. Apparently, the concentration of the salts approaches a near maximal value which does not stay at the meat surface X = 0, but moves into the cylinder with time. Except for these high values near the cut surface, the diffusion constants did not vary significantly across the diffusing boundary for chloride and nitrate. The nitrite boundaries, however, diffused faster at the leading edge. The concentration of nitrite was relatively higher than expected from a normal diffusion curve at low nitrite levels and relatively lower at high nitrite concentrations. A chemical reaction, monomolecular in nitrite, may be ruled out because it results in a uniform decrease in the measured diffusion constant (Crank, 1967). On the other hand, a chemical reaction, 2nd order or higher in nitrite or chloride, would result in the relatively lower nitrite values observed at higher nitrite and salt concentrations. The differences at 1.05 and 1.75 cm between the nitrite diffusion constants for the two samples may represent a difference in chemical reactivity of the two pieces of meat. Alternatively, since the diffusion of nitrite takes place in the presence of a

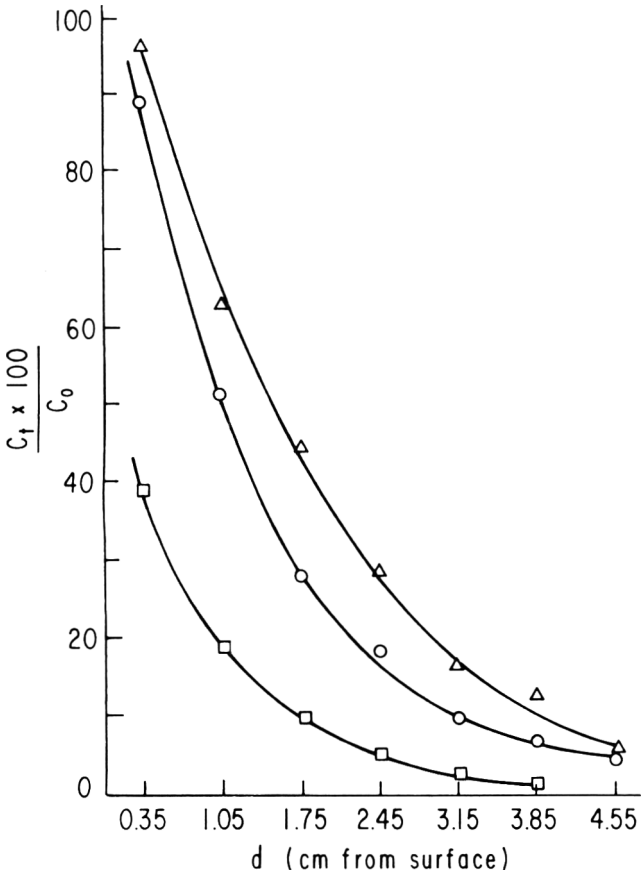

Fig. 3—C_t X 100/C_o for nitrite, chloride, and nitrate as a function of the distance from the surface of beef semitendinosus exposed to a cure composed of 4.27M sodium chloride, 36 mM sodium nitrite, and 103 mM sodium nitrate. △, Cl^-; □, NO_2^-; ○, NO_3^-.

Table 1—Diffusion constants at 5°C calculated from the concentration of anions at distance d from the meat surface measured across the diffusing boundary

	D x 10^5 cm^2 sec^{-1}					
	Sample 1			Sample 2		
d cm	Cl^-	NO_2^-	NO_3^-	Cl^-	NO_2^-	NO_3^-
–	0.35	1.80	0.020	0.46	1.80	–[a]
0.12	1.05	0.36	0.048	0.19	0.26	0.43
0.14	1.75	0.41	0.084	0.19	0.23	0.38
0.13	2.45	0.39	0.12	0.24	0.27	0.34
0.15	3.15	0.37	0.15	0.27	0.30	0.37
0.19	3.85	0.45	0.19	0.34	0.32	0.41
0.25	4.55	0.45	–	0.28	0.35	0.42
–	D[b]	0.405	–	0.252	0.288	0.392
		± 0.039		± .057	± 0.044	± .034

[a] Measured concentration was the same as that of the brine.
[b] Omitting the 0.35 cm values.

sodium chloride gradient, the nitrite may have diffused slower because of some physical changes induced by the chloride. To separate these effects, we turned to the steady state conditions of the porous disc technique. This technique was also expected to improve the precision of replicate analyses. In total uptake and diffusing boundary analyses, each diffusion constant is the result of only one analysis per meat sample since the sample is homogenized for the analysis. With the porous disc technique, a large number of determinations may be made on each disc. Since any set of cylinders or discs are made from one piece of meat, a larger number of determinations may be made for the discs than for the cylinders.

Table 2—Diffusion constants at 5°C for chloride, nitrite, and nitrate by porous disc measurements

		D x 10^5 cm^2 sec^{-1}					
		Cl^-		NO_2^-		NO_3^-	
Exp	Meat	[M]	D	[mM]	D	[mM]	D
1	Beef semi-tendinosus	4.27	0.26	36	0.16	103	0.16
			0.27		0.18		0.18
			0.25		0.16		0.17
2	Beef semi-tendinosus	4.27	0.26	36	0.17	–	–
		3.20	0.26	27	0.18		
		2.14	0.27	18	0.18		
		1.71	0.26	14.4	0.19		
		1.07	0.24	9.0	0.20		
		1.07	0.26	9.0	0.20		
		0.68	0.26	5.8	0.22		
		0.34	0.26	2.9	0.25		
		0.17	0.26	1.4	0.24		
3	Beef semi-tendinosus	4.27	0.26	14.4	0.17		
			0.26	9.0	0.18		
			0.26	9.0	0.18		
			0.26	4.3	0.17		
			0.26	2.9	0.15		
			0.26	1.4	0.17		
4	Beef semi-tendinosus	4.0	0.25	20	0.18	10	0.17
			0.24		0.18	50	0.18
			0.25		0.18	90	0.16
			0.24		0.17	130	0.15
5	Beef semi-tendinosus	3.08	0.24	32.6	0.17	26.4	0.16
			0.23		0.17		0.14
	+ 40.7 mM STPP		0.24		0.18		0.15
			0.25		0.19		0.16
6	Beef semi-tendinosus salt grade						
	Reagent	3.08	0.28	32.6	0.16	26.4	0.20
	Cullinox 999		0.27		0.16		0.20
	Morton Kosher		0.27		0.15		0.18
	Acme Table noniodized		0.26		0.15		0.22
7	Pork semi-membranosus I	3.08	0.19	32.6	0.12	26.4	0.22
	II		0.22		0.13		0.24
	Semitendinosus		0.22		0.13		0.24
	R. femorus		0.22		0.13		0.23
	L. dorsi I		0.20		0.13		0.22
	II		0.22		0.13		0.23
	Back fat		0.028		0.019		–

The results of studying a number of curing variables are shown in Table 2. The first set of data are for a triplicate run to test the precision of the technique. The coefficient of variation of the individual diffusion constants was ±11% (n = 8) and the variation of the replicate analysis was ±4%. This precision was observed for the chloride values determined during the rest of this study. The precision of the nitrite values was somewhat lower, about ±20% for individual diffusion constants and ±7% for the cumulative values. The effect of varying the concentration of cure is shown in Experiment 2, Table 2. The concentration of both chloride and nitrite was intentionally varied simultaneously, as a first approximation to the conditions that take place during diffusion of the salts into meat, only under steady state conditions. The diffusion constant for nitrite increased at

lower nitrite and chloride concentrations. The effect was due to chloride concentration; at constant chloride concentration, the diffusion constant for nitrite remained the same with varying nitrite concentration (Experiment 3). Upon removal of the samples from the cell, swelling was evident depending on salt concentration. As the sodium chloride concentration increased, the samples fit progressively tighter in the retainer ring. At 0.34M Cl^-, the disc, after the diffusion study, had the same degree of tightness of fit that the original sample had; at 0.17M Cl^-, the disc was loose and fell out upon removal of the cellulose membranes.

The next three experiments show that nitrate, tripolyphosphate (STPP), and different grades of salt had no significant effect on the diffusion rates. The observed differences between the corresponding diffusion constants of the three experiments may be attributed to the muscle tissue itself, since the differences were common to one whole set of samples. The last set of data on pork was determined to compare the results of the porous disc technique with values obtained by us and previous workers using the cylinder technique. Muscle type did not appear to make any difference, and the diffusion constants for chloride in pork by the porous disc method are close to those reported by Wood. Converting his value for the chloride diffusion constant from −2 to 5°C, a value of 0.18×10^{-5} cm^2 sec^{-1} is obtained, compared with our values of $0.19–0.22 \times 10^{-5}$ cm^2 sec^{-1}.

Since we do not know the variables involved in converting the diffusion constants in fat for temperature, we cannot make a direct comparison of our chloride value with that reported by Wood (1966), 0.007×10^{-5} cm^{-2} sec^{-1}. In Wood's study the rate of diffusion in fat was about 1/20 of that in muscle tissue; in our study it was about 1/10.

DISCUSSION

OUR DIFFUSION CONSTANTS for chloride in pork agree with the data of both Wood (1966) and Körmendy and Gantner (1958). However, Wood calculated a value of 0.41×10^5 cm^2 sec^{-1} from the data of Wistreich et al. (1959) and hypothesized that fat content may have been a controlling factor since Wistreich et al. used leg muscles which, presumably, were lower in fat content. Both we and Körmendy and Gantner used semitendinosus, a leg muscle, and our disc diffusion data are in agreement with those of Wood. Furthermore, we did not find any difference in rate of diffusion of chloride with different pork muscles. Fat streaks were present in varying quantities in both the discs and cylinders of meat but the observed variation in adipose tissue content had no effect on the calculated diffusion constants. The answer to this point could be obtained only by studying muscle tissues with different intracellular fat contents.

Diffusion constants for chloride and nitrite in pork were lower than the corresponding constants for beef, but the nitrate diffusion constants were higher, especially by comparison with the free diffusion constants. The values of the free diffusion constants for chloride and nitrate at 5°C are 0.84 and 0.79×10^{-5} cm^2 sec^{-1}, respectively (International Critical Tables, 1927, corrected for temperature). As discussed below, chemical reaction did not appear to be a factor in the diffusion of nitrite in the disc experiments, hence the high values for nitrate are not the result of conversion of nitrite to nitrate. Diffusion may be hindered (Crank, 1967) or facilitated (Wittenberg, 1965; Margalit and Schejter, 1976) by interaction with specific groups in the substrate. Of the two ions, chloride and nitrate, the latter would be expected to be the least affected by interactions, since from their positions in the Hofmeister series, chloride reacts more strongly with proteins than does nitrate (Jencks, 1969). Because of the muscle-to-muscle variation, it is not evident which of the possible effects of tissue swelling, protein denaturation, ion binding, and/or chemical reaction cause the variations in the rate of diffusion. If nitrate diffuses with little or no tissue component interaction, then, on an average, its diffusion would be the most uniform and fastest, which may be the basis for Jensen's (1942) statement concerning increased permeability of meat tissues with nitrate. However, our results do not show any effect of nitrate on facilitating the diffusion of chloride or nitrite.

The increase in diffusion rate of nitrite with decreasing chloride concentration in the disc experiments is a physical phenomenon rather than a chemical one. Irreversible chemical reaction of solute with substrate results in a reduction of the measured diffusion rate (Crank, 1967), as observed in the boundary analysis experiments. In the steady state with a sample of finite thickness, chemical reaction during diffusion would result in an upward curving of the diffusion curve with time, due to slowing of the reaction, allowing more nitrite to diffuse. Since the curves were linear with time at all concentrations and the nitrite diffusion constant did not vary with nitrite concentration at constant chloride concentration, we conclude chemical reaction was not the controlling factor for steady state diffusion. The physical change involved is related to tissue swelling. Körmendy and Gantner (1958) observed the swelling due to the salt, determined the diffusion rate in tubes of two different radii and concluded that size of tube did not affect the diffusion rate. This conclusion does not mean that swelling of tissue due to salt did not have an effect on the rate of diffusion. The relative amount of tissue compression induced by swelling would be the same in any area size, since the expansion in any dimension is a proportion of the magnitude of the dimension. This is true even in the relatively large surface area of the discs. Our results show that tissue swelling is a factor in the rate of diffusion. Chloride itself did not show the effect of increased resistance to diffusion, since the diffusion constant did not vary with distance. The result was probably due to two factors operating across the boundary, the first being a slowing of diffusion due to chloride interaction with the substrate. After the chloride had saturated the active sites, the diffusion would then be slowed by the increased resistance to flow due to the swelling, the two processes resulting in the rate of diffusion remaining constant across the boundary. The maximal effect was attained at chloride concentrations between 1.5 and 2.0M, which suggests an interaction with the peptide bonds of the muscle proteins because this is the approximate concentration of the bonds. A partial unfolding of the peptide chain by such an interaction would explain the observed swelling of the tissues.

The nitrite diffusion constant calculated for the total uptake experiments fell to 0.12×10^{-5} cm^2 sec^{-1} or lower as measured across the boundary from lower to higher nitrite concentrations. This value is lower than the constant for the steady state, 0.18×10^{-5} cm^2 sec^{-1}. Since the former represents diffusion into an infinite substrate with an excess of tissue reactants available, the difference in the diffusion constants represent nitrite reacted with tissue components. Based on calculation of the theoretical diffusion curve for the two experiments of Table 2 with $D = 0.18 \times 10^{-5}$ cm^2 sec^1, the loss of nitrite through reaction with tissue components was calculated to be 50% for sample 1 and 30% for sample 2 at 1.05 and 1.75 cm for each sample. The value of $D = 0.11 \times 10^{-5}$ cm^2 sec^{-1} for the total uptake experiment indicates a 30% loss of nitrite in that experiment also. These values agree with losses of 25–35% calculated by various authors from total nitrite analysis (Sebranek et al., 1978; Emi-Miwa et al., 1976).

Effect of phosphate

In addition to Experiment 5 in Table 2, other disc dif-

fusion experiments, as well as diffusing boundary analysis, also showed no difference between the rates of diffusion of either chloride or nitrite with or without phosphate. Conversely, Krause et al. (1978) reported that sodium tripolyphosphate increased the rate of diffusion of nitrite. However, there was a high loss of nitrite in their samples without phosphate which, as shown in the diffusing boundary analyses, can result in an apparent lowering of the rate of diffusion. The samples with phosphate showed little loss of nitrite, which may have been the result of raising the pH of the cure. Körmendy and Gantner (1958) had reported larger diffusion constants at higher pH values, so we adjusted the pH of our cure solutions to that of the meat to eliminate the effect.

The decrease in the rate of diffusion due to tissue swelling may explain the phenomenon observed by Ockerman and Organisciak (1978). They were following the rate of diffusion by the increase in area of cure penetration from a point source of cure injection, and found that after about 10 hr the rate of diffusion began to increase. This observation may be explained on the basis of an initially slow diffusion due to localized internal swelling caused by high salt concentrations at the site of injection. A lowering of the salt concentration due to diffusion would decrease the swelling and result in faster salt diffusion with time.

SUMMARY

THE RESULTS of this study show that the diffusion of nitrite, as measured by Griess analysis, is governed principally by two factors, one physical, due to changes induced in the tissue by chloride, and the second chemical, caused by reaction of nitrite with endogenous tissue components. The first may be an artifact due to confinement of the tissue sample. From the results of this and other studies, diffusion of salts in meats has been found to be a variable process, dependent upon specific interactions between individual meat samples and the diffusing ions.

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A COMPARISON OF Microbacterium thermosphactum AND LACTOBACILLI AS SPOILAGE ORGANISMS OF VACUUM-PACKAGED SLICED LUNCHEON MEATS

A. F. EGAN, A. L. FORD, and B. J. SHAY

ABSTRACT

The spoilage of vacuum-packaged sliced luncheon meats at 5°C by *Microbacterium thermosphactum* and lactobacilli was studied using analytical taste panels. *M. thermosphactum* caused rapid spoilage. "Off" aroma was significant at about the time the bacterial population reached 10^8/g and "off" flavor 2–3 days later. In contrast homofermentative lactobacilli caused spoilage much more slowly. No "off" aroma was detected with products which had carried a population of 10^8/g for 21 days, the time at which "off" flavor first became significant. Heterofermentative lactobacilli were intermediate in their effect. Thus shelf life is dependent upon the types of bacteria present. Under such circumstances a "total count" microbiological standard is of little value.

INTRODUCTION

LACTIC ACID BACTERIA are commonly the dominant organisms in the microbial flora which develops on vacuum-packaged sliced cooked meats (luncheon meats) stored at refrigeration temperatures (Allen and Foster, 1960; Kempton and Bobier, 1970; Mol et al., 1971; Surkiewiez et al., 1977; Paradis and Stiles, 1978). *Microbacterium thermosphactum* also occurs on products of this type but usually not as a major component of the flora (Mol et al., 1971; Paradis and Stiles, 1978).

A number of studies on the significance of lactic acid bacteria in the spoilage of these products have been carried out. Allen and Foster (1960) observed that these bacteria caused atypical flavor and slime formation when the count reached 10^8/g. Mol et al. (1971) examined samples obtained from retail outlets and found that products with a total count greater than about 10^8/g were unacceptable. In further experiments in which they inoculated sliced Berliner sausage with individual strains of lactic acid bacteria, organoleptic deterioration occurred shortly after the count exceeded 10^8/g. In contrast Surkiewicz et al. (1977) found that vacuum-packaged sliced ham remained organoleptically acceptable after 68 days storage at 3°C, even though the count exceeded 10^8/g after about 48 days.

Recently we surveyed the microbiological status of vacuum-packaged, sliced luncheon meats obtained from local supermarkets (Shay et al., 1978). Lactic acid bacteria comprised 50% of the flora but in addition high numbers of *M. thermosphactum* were present. Overall this organism comprised 33% of the flora but it averaged 42% on sliced ham and corned beef. In controlled storage trials a very short shelf life was found for sliced corned beef on which *M. thermosphactum* was a major component of the flora (Shay et al., 1978).

In the present study vacuum-packaged corned beef and ham were inoculated with *M. thermosphactum* and selected strains of lactic acid bacteria and stored at 5°C. At various times, the organoleptic changes produced by the presence of these bacteria were evaluated by a trained analytical taste panel to determine the relative significance of these bacteria as spoilage organisms.

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EXPERIMENTAL

Bacteria

Strains of lactic acid bacteria were selected from a collection of isolates obtained from vacuum-packaged sliced luncheon meats (Shay et al., 1978). All strains chosen were gram-positive, nonmotile, nonspore forming, catalase-negative, oxidase-negative, benzidine-negative rods and hence were members of the genus *Lactobacillus*. All grew well at 5°C and 25°C but failed to grow at 45°C. It was not possible to identify the isolates down to a species level using the methods of Sharpe et al. (1966). The four homofermentative strains chosen (BL128, BL130, BL132, and BL137) were thus atypical streptobacteria and the four heterofermentative strains (BL106, BL135, BL179, BL248) were atypical betabacteria. The type culture of *M. thermosphactum* (ATCC 11509) was used throughout.

Meat samples

Meat was obtained from a local manufacturer after partial processing. The pickle used contained 2.5% polyphosphate, 1.0% glucose, 1.5% sucrose, 5.5% sodium chloride, 0.1% sodium nitrate, and 0.04% sodium nitrite. Whole silversides (biceps femoris and semitendinosus muscles) were pumped with pickle to a weight increase of 40%. Each was then sealed in a plastic bag and cooked to an internal temperature of 68°C. This produced a weight loss of 25–30%. Boneless shoulder ham was similarly prepared by pumping and was not smoked. After rumbling ham was filled into moulds lined with plastic, and cooked to an internal temperature of 72°C. After cooking meat samples were chilled to 0°C (data supplied by manufacturer).

The water activity of the samples was in the range 0.954–0.963 (total dissolved solids equivalent to 6–8% w/v NaCl) and they contained residual concentrations of ca 240 ppm of nitrate and 20 ppm of nitrite.

Preparation of samples

Meat samples were transferred to the laboratory where they were removed from their plastic wrappings and placed in a sterile cabinet. Using aseptic techniques pressed hams were cut into slices about 2 mm thick. The biceps femoris muscle was excised from each whole corned silverside and also cut into slices. About 1/3 of the sliced meat from each muscle or pressed ham was divided into lots of about 250g and immediately packaged. The remaining 2/3 was retained for inoculation with bacteria.

Inocula were grown at 5°C in nutrient broth no. 2(Oxoid) supplemented with 0.25% w/v glucose. Cells in the exponential phase of growth (ca 5.0×10^8 cells per ml) were harvested by centrifugation at 3000 × G for 10 min, suspended in distilled water to give ca 1.0×10^6 cells per ml and placed in a sterile glass chromatogram sprayer. In the case of the mixed cultures of lactobacilli, each strain was grown separately and appropriate aliquots were used so that all strains were present in approximately equal numbers in the suspension to be used for inoculation. One-third of the slices cut from each muscle or pressed ham were then inoculated by spraying both sides of each slice so that an initial viable count of ca 10^4 per g was produced. The meat was then packaged.

The packaging film used consisted of nitrocellulose-coated cellophane laminated to polyethylene. It had an oxygen permeability of ca 1000 $cm^3/m^2/24$ hr/atm at 25°C and 90% RH. Sliced meat was vacuum-packaged in 12 × 20 cm pouches by heat sealing using a chamber-type evacuator (Dixie-Union EK101) operated at a pressure of 18 kPa. Samples were stored at 5°C and 90% RH in the dark.

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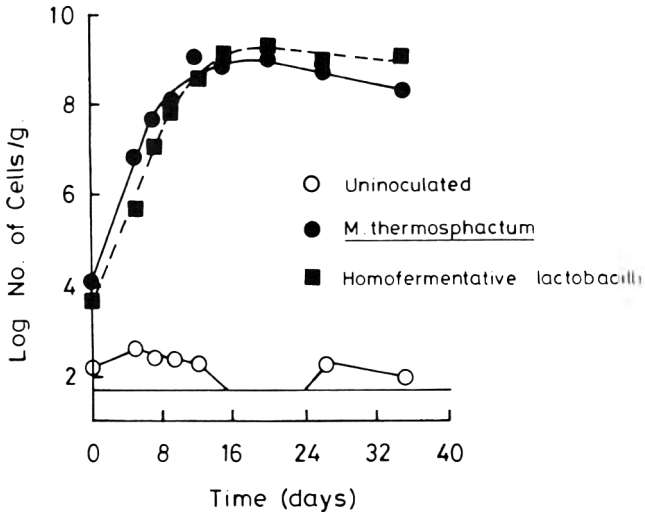

Fig. 1—Growth of bacteria on vacuum-packaged sliced corned beef at 5°C. At zero time meat samples were inoculated with the organisms shown. The horizontal line indicates the lower limit of detection (50/g).

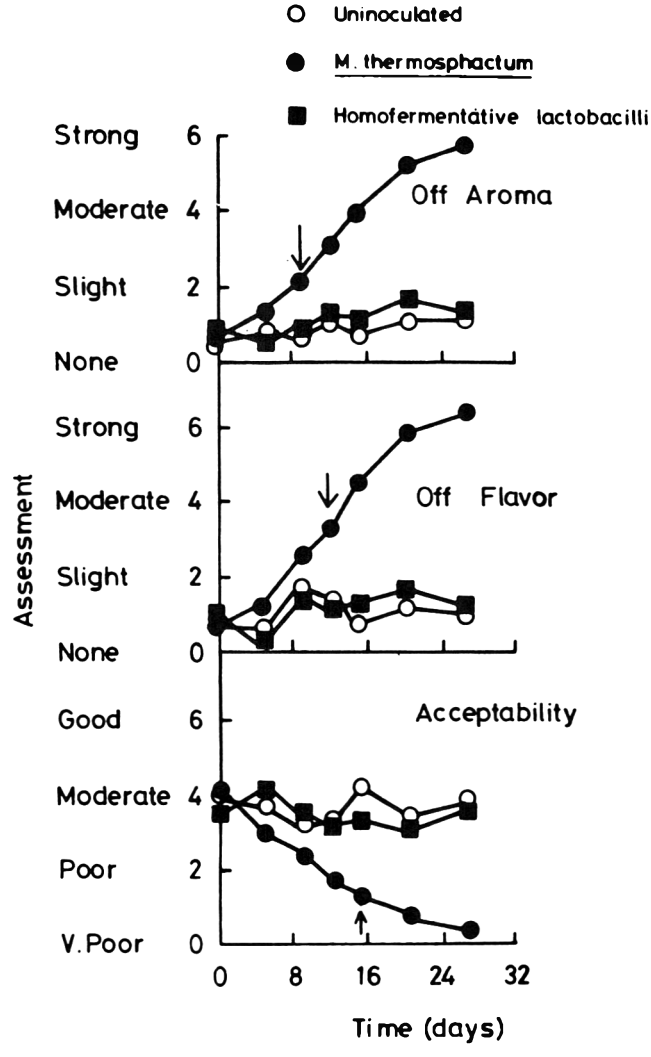

Fig. 2—Taste panel assessments of vacuum-packaged sliced corned beef stored at 5°C. Samples inoculated with either M. thermosphactum (population reached 10^8/g at 9 days) or homofermentative lactobacilli (population reached 10^8/g at 12 days) were compared to uninoculate controls. Arrows indicate the times at which samples first became statistically significantly different from the controls.

Taste panel assessment of stored samples

Each experiment consisted of three treatments: two sets of inoculated samples were compared to an uninoculated "sterile" control. To reduce variability due to differences in test material, three separate silversides or pressed hams were used in each experiment. Each time point thus comprised three separate tasting sessions. At each session packs from the same muscle or ham were compared. Fourteen tasters, experienced in meat flavor assessment, scored a sample from each treatment for "meat" and "off" aromas and flavors and for overall acceptability. Aroma and flavor intensities were rated on 9-point structured scales (0—none, 2—slight, 4—moderate, 6—strong, 8—very strong) and overall acceptability was rated using a 9-point hedonic scale (0—very poor, 2—poor, 4—moderate, 6—good, 8—very good). Sample packs were selected at random for tasting at each time point. The order of sample assessment was randomised amongst panelists to cancel out any sequential effects. Tasting was done under white light so that panelists could include appearance in their acceptability scores.

At each time point, analyses of variance were performed on "off" aroma and flavor and on overall acceptability to compare the scores for each treatment. Results were analyzed with the use of a one-tail test of significance (Snedecor and Cochran, 1976). The muscle by treatment interaction was used as the error term for testing for significant differences.

Microbiological analysis

At each time point, sub-samples from each of the three packs from each treatment being tasted were pooled to produce a 10-g sample. Samples were placed in a blender bowl which contained 90 ml of 0.1% w/v peptone water, blended at 12,000 rpm for 30 sec and appropriate dilutions made in peptone water. All samples were plated on plate count agar (Oxoid). In addition, samples inoculated with *M. thermosphactum* were plated on the medium of Gardner (1966) and those with lactic acid bacteria on the medium of Mann et al. (1960). Duplicate plates were incubated under aerobic conditions for 4 days at 25°C and also for 35 days at 0°C.

RESULTS

Microbiological counts

The results obtained in an experiment using corned beef are shown in Figure 1. At zero time, the count on the inoculated meat samples was in the range 2×10^3 to 2×10^4/g. On these samples the bacteria grew rapidly at 5°C and reached ultimate populations in excess of 2×10^8/g. There was good agreement between corresponding counts on plates incubated at 0°C and those at 25°C and on non-selective and the appropriate selective medium.

M. thermosphactum grew with no detectable lag phase, had a generation time of 12—16 hr and reached 10^8/g after about 9 days storage. The corresponding values for the homofermentative strains were 18—22 hr and 12—20 days, respectively. These latter organisms sometimes had a lag phase of up to 5 days before growth commenced. The heterofermentative lactobacilli had a generation time of 13—16 hr and reached 10^8/g in 9—12 days. No significant differences in growth rates or ultimate population levels were observed during experiments using ham. The results described above and in Figure 1 are typical of those obtained with both types of product studied.

Uninoculated samples used as "sterile" controls were contaminated with low numbers of micro-organisms initially, but this count declined with time. On most samples the count was less than 50/g (the limit of detection) and on no occasion did it exceed 2×10^3/g.

Taste panel evaluation

The results of an experiment in which the storage life of corned beef inoculated with either *M. thermosphactum* or the mixed culture of homofermentative lactobacilli was compared to that of uninoculated control samples are shown in Figure 2. (Fig. 1 presents the corresponding microbiological data). Meat was first downgraded by the taste panel 9 days after it had been inoculated with *M. thermosphactum*. At this time the meat had developed a

slight "off" aroma which was significantly different ($P < 5\%$) to the aroma of samples from the other two treatments. By 12 days significant "off" flavor ($P < 5\%$) was also noted. After 15 days, the product was severely downgraded due to a moderate "off" aroma ($P < 0.1\%$) and "off" flavor ($P < 1\%$) and overall acceptability was greatly reduced ($P < 1\%$). After 20 days acceptability was rated "very poor" ($P < 0.1\%$), and some members of the taste panel, after smelling the meat, refused to taste it.

In contrast, meat inoculated with the homofermentative lactobacilli did not develop any detectable "off" aroma or flavor during 26 days storage and remained as acceptable as sterile control samples. As shown in Figure 1 the population of *M. thermosphactum* first exceeded 10^8/g at the 9-day point and that of the homofermentative lactobacilli at 12 days. Similar results were obtained using ham.

In the second series of experiments the mixed culture of heterofermentative lactobacilli was used in place of the homofermentative organisms. Again, *M. thermosphactum* caused rapid deterioration of the meat (Fig. 3). With samples inoculated with the heterofermentative lactobacilli no "off" aroma was detected during 25 days storage, however, a slight "off" flavor was found after 20 days. After 25 days this became significantly different from the uninoculated control ($P < 5\%$) and caused a reduction in acceptability ($P < 5\%$). On further storage (35 days) samples inoculated with the heterofermentative lactobacilli were significantly different from the controls in all attributes ($P < 1\%$ in each case). At this time "off" aroma was rated slight and "off" flavor slight to moderate. In this experiment the population of both types of organism on the meat reached 10^8/g in 9 days. Again similar results were obtained using corned beef and ham.

These experiments showed that *M. thermosphactum* caused spoilage much more rapidly than the lactic acid bacteria. It appeared possible however that because of the three-way nature of the tests used, meat inoculated with lactobacilli could have been rated more acceptable than it really was because of bias introduced by tasting it in comparison with samples which were much more highly spoiled. To overcome this source of possible error, a third series of experiments was done. In these experiments uninoculated samples of corned beef were compared to samples inocu-

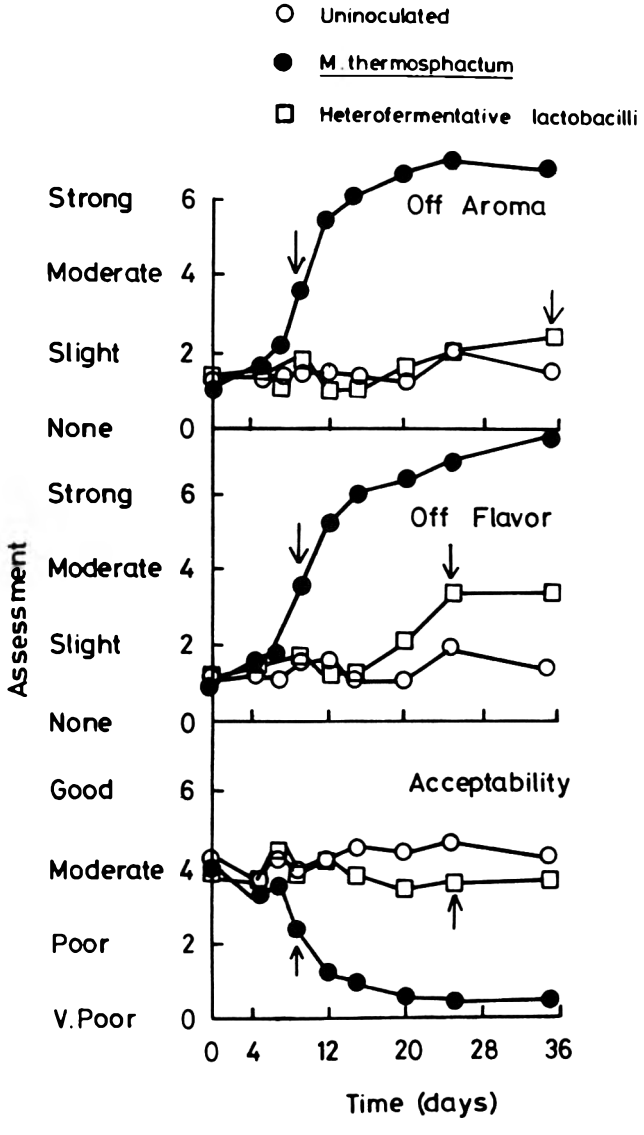

Fig. 3—Taste panel assessments of vacuum-packaged sliced corned beef stored at 5° C. Samples inoculated with either M. thermosphactum or heterofermentative lactobacilli were compared to uninoculated controls. In both inoculated treatments the bacterial population reached 10^8/g at 9 days. Arrows indicate the times at which samples first became statistically significantly different from the controls.

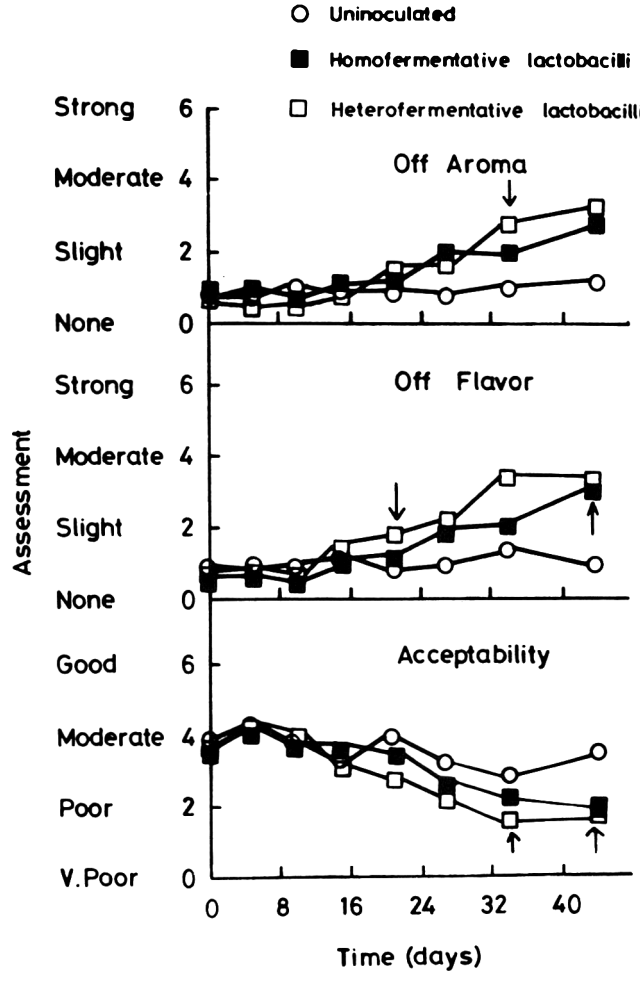

Fig. 4—Taste panel assessments of vacuum-packaged sliced corned beef stored at 5° C. Samples inoculated with either homofermentative lactobacilli (population reached 10^8/g at 20 days) or heterofermentative lactobacilli (population reached 10^8/g at 10 days) were compared to uninoculated controls. Arrows indicate the times at which samples first became statistically significantly different from the controls.

lated with either homofermentative or heterofermentative strains of lactic acid bacteria (Fig. 4).

The first significant effect noted was on "off" flavor ($P < 5\%$) in the corned beef inoculated with heterofermentative strains at 21 days. At 34 days, samples inoculated with these bacteria were significantly different from the controls in all parameters ($P < 5\%$ in each case). Samples inoculated with homofermentative strains were not rated significantly lower than the sterile controls until after 42 days. Then, both "off" flavor and acceptability were downgraded ($P < 5\%$) but no significant "off" aroma was detected. The population of heterofermentative and homofermentative strains reached 10^8/g after 10 and 20 days respectively.

No formation of slime or no significant color changes were observed throughout the experiments. Spoilage was confined to aroma and flavor defects.

DISCUSSION

THESE EXPERIMENTS clearly demonstrate significant differences in the abilities of the organisms tested to spoil luncheon meats at refrigeration temperatures. *M. thermosphactum* is obviously an organism of major importance as it caused very rapid spoilage. In contrast, the lactobacilli appear to be of less importance. This can readily be seen by comparing the time at which significant spoilage was first noted. In the case of *M. thermosphactum* this occurred at about the time the population reached 10^8/g. With the homofermentative lactobacilli, no significant "off" aroma was ever detected during the experiments and "off" flavor first occurred 21 days after the population reached 10^8/g. Heterofermentative strains were intermediate in their effect. Significant "off" flavor first appeared about 11 days after the count reached 10^8/g but "off" aroma not until about 24 days after that time.

There appears to be a good correlation between the nature and degree of spoilage produced by each type of organism and the nature of the end products of its carbohydrate metabolism. Homofermentative lactobacilli predominantly produce lactate, which has a low volatility and heterofermentative strains produce, in addition, some acetate (London, 1976). When these organisms caused spoilage it was first noted as a flavor defect. In contrast, *M. thermosphactum* produces the more pungent compound acetoin as the major end product of the aerobic metabolism of carbohydrate (Brownlie, 1969) and the first spoilage defect noted was an "off" aroma. However, the nature of the end products produced by these organisms from other chemical components of the meat are not known and these will presumably complicate any attempt to interpret the chemical basis of spoilage.

The present results agree with those of Allen and Foster (1960) and Mol et al. (1971) to the extent that they show that sliced luncheon meats may be spoiled when the count reaches 10^8/g. However they also show that a count of this magnitude does not necessarily mean that the product is spoiled.

Our observation that products contaminated with lactobacilli may be acceptable for up to 21 days after the count reached 10^8/g is in agreement with the results of Surkiewicz et al. (1977) but appears to differ from the finding of Mol et al. (1971). These differences are presumably due to a number of factors including (1) the exact nature of the product under test, (2) the strains of bacteria chosen, (3) the conditions of storage and (4) the expectations the tasters hold concerning the product under evaluation. With regard to this last point, for example, one taster stated that the lactobacilli gave the luncheon meats a slightly "pickled" flavor. This agreed with his expectations of the products and increased his appreciation of them.

It is often assumed that the presence of a particular organism as a major component of the microbial flora on food means that it is important in the spoilage of the food. In the case of lactobacilli on vacuum-packaged sliced luncheon meats this is not necessarily true. The fact that the storage life of these products is dependent upon the nature of the bacterial flora makes it difficult to set a meaningful shelf life and makes a "total count" standard of little practical value.

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EFFECT OF BONING, ELECTRICAL STIMULATION AND MEDICATED DIET ON THE MICROBIOLOGICAL QUALITY OF LAMB CUTS

NORMAN J. STERN

ABSTRACT

Seventy-three lambs were divided into four treatment groups and fed for 3 months either (1) control diet, (2) control plus 25 ppm of a tertiary amine (N,N-dimethyldodecanamine), (3) control plus 50 ppm of the amine, or (4) control plus 25 ppm chlortetracycline (CTC). The lambs were slaughtered at the end of the feeding trial. Half of the 146 sides were electrically stimulated (ES). Subsequently, half of the ES and half the nonstimulated sides were hot-boned and the other halves were cold-boned at 6 days postmortem. At 6 days postmortem, all racks were vacuum packaged and held at 3°C until 35 days postmortem. Cold- and hot-boned carcasses were processed into lamb chops and packaged in oxygen-permeable wrap and kept under retail market conditions (5°C and 12 hr of lighting/day). Surface swabs were taken from lamb chops initially and after 3 days of retail storage and from lamb racks at 35 days postmortem. The following statistically significant ($P < 0.05$) results were obtained. Standard plate counts (SPCs) and psychrotrophic plate counts (PPCs) were consistently higher for hot-boned lamb chops initially and after 3 days of storage compared with the cold-boned lamb chops initially and after 3 days of storage. The situation was reversed for the lamb racks after 35 days of storage: hot-boned racks had lower counts than the cold-boned racks. PPCs from lamb chops from lambs fed CTC-supplemented diets were lower than PPCs from lamb chops from lambs fed unsupplemented (control) diets after 3 days of storage. Electrical stimulation exerted no significant effect on surface bacterial numbers.

INTRODUCTION

SEVERAL PRACTICES that influence the cost, quantity and quality of meat products from ruminants have been introduced during the past 25 years. Fabrication of carcasses before chilling (i.e., hot-boning), and electrical stimulation of carcasses offer promising possibilities for increasing the marketing efficiency and the conservation of energy and space (Henrickson, 1975). Patties prepared from hot-processed beef were equal to or superior to patties prepared from conventionally chilled beef in palatability and physical and chemical properties (Cross et al., 1979). Emswiler and Kotula (1979) reported that aerobic plate counts in ground beef from hot-boned beef were less than or equal to counts in ground beef from cold-boned carcasses.

The toughening problems related to hot-boning of carcasses may be offset by postmortem electrical stimulation (Gilbert and Davey, 1976; Gilbert et al., 1977; Chrystall and Hagyard, 1975; Chrystall and Hagyard, 1976). It has also been reported that shelf life of ground beef from electrically stimulated carcasses was prolonged by 3 days as compared to that from control carcasses (Raccach and Henrickson, 1978).

In this investigation, I examined the influences of hot-boning and electrical stimulation upon the microbiological quality of lamb muscle tissues. The carcasses used in this study were of lambs fed control diets and antibiotic-supplemented diets. This use of dietary antibiotics allowed me to also study their influence on retail meat cuts.

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EXPERIMENTAL

SEVENTY-THREE Western crossbred lambs from a feeding trial conducted by the Ruminant Nutrition and the Milk Secretion Laboratories, U.S. Department of Agriculture, Beltsville, Md., averaging from 41–46 kg in weight per lot, were used in this study. The lambs were fed a diet comprising 49% corn meal, 25% straw wheat, 15% soybean oil meal (44% protein), 10% alfalfa meal, 0.5% trace mineral salts, and 0.5% limestone to which was added 1900 IU of vitamin A and 150 IU of vitamin D per kg diet. Control lambs were fed this diet (Diet 1) with no additives. Other lambs were fed the control diet with 25 (Diet 2) or 50 (Diet 3) ppm of N,N-dimethyldodecanamine (a C-12 tertiary amine), or (Diet 4) 25 ppm of chlortetracycline. Diet 1 was estimated to contain 14.4% crude protein, 73.9% total digestible nutrient, 0.4% calcium, and 0.3% phosphorus. Distribution of weight frequencies for lambs was similar for each of the four diets. Lambs were fed one of the regimens ad libitum for 3 months. At the Meat Science Research Laboratory Abattoir, Beltsville, Md., the animals were slaughtered, then exsanguinated, skinned, eviscerated and split into sides. Half the sides were electrically stimulated at about 60 min after exsanguination. Lamb sides were evenly distributed among the four diets for stimulated and unstimulated samples. For electrical stimulation, a probe was placed near the Achilles tendon and a second probe between the scapula and vertebral column of a side. Each stimulated side received an input of 1.5 A of alternating current for 1 min in intervals of 1 sec on and 1 sec off. Half of the electrically stimulated and half of the unstimulated sides were immediately hot-boned, again, evenly distributing lamb sides for both diets and weight frequencies.

The cuts from the hot-boned carcasses were placed into Cryovac B 620, polyvinylicene bags (W.R. Grace and Co., Duncan, S.C.) and evacuated in a Multivac, model #3696-24, type A 6500 (Maulburg, West Germany) vacuum packager. Hot-boned, vacuum-packaged legs and loins along with sides designated for cold-boning were transferred to a 3°C cooler and held for 6 days. The 6-day interval was used to simulate commercial conditions that might be necessary for chilling, transporting, and distributing from a central storage area. After the 6-day interval, they were fabricated into lamb chops and packaged in oxygen-permeable polyvinyl chloride film (Alcoa, Pittsburgh, Pa.) on plastic foam trays after microbiological sampling. After packaging, the lamb chops were placed in a retail display case held at 5°C, with 12 hr of fluorescent lighting at 90 ft-c luminosity followed by 12 hr of darkness, for 3 days.

A representative sample of the bacteria present on 12.3 cm^2 of the surface of each lean lamb chop was obtained with an alginate swab moistened in 0.1% peptone diluent (Lazarus et al., 1977). After sampling, swabs were placed into 10.0 ml of 0.1% peptone diluent and analyzed for microbiological composition (Table 1).

From these same processed lamb sides, lamb racks were produced. Racks from hot-boned carcasses were vacuum-packaged along with the leg and loins. These hot-boned racks were held in a 3°C cooler for 35 days. Racks from cold-boned carcasses were fabricated after 6 days postmortem, vacuum packaged, and were held an additional 29 days at 3°C. After 35 days of storage at 3°C, the exposed surfaces of 46 vacuum-packaged lamb racks were swabbed and analyzed as described in the preceding paragraph, except for the omission of tests for *Clostridium perfringens*. Data for standard plate counts (SPC), psychrotrophic plate counts (PPC), and coliform counts were calculated per square centimeter of lamb tissue and converted to base 10 logarithms with 0.1 added to the counts to avod logarithms of 0 counts. Logarithmic means were analyzed according to the Duncan multiple range test (1955) at the 5% level. The logarithmic means are presented as their antilogarithms in the tables.

RESULTS

THE INFLUENCE of dietary regimen on bacterial num-

Table 1—Bacteriological Procedures[a,b]

Determination	Medium	Plating technique	Incubation	Confirmatory tests
1. Standard plate count (SPC)	Plate count agar[c]	Pour plate	35°C/48 hr	–
2. Psychrotrophic plate count (PPC)	Plate count agar[c]	Pour plate	5°C/10 days	–
3. Coliform count	Violet red bile agar[c]	Pour plate with overlay	35°C/24 hr	Growth and gas evolution in brilliant green bile broth within 48 hr at 35°C
4. Coagulase-positive *Staphylococcus aureus*[a]	Three-tube MPN in trypticase soy broth[d] with 10% NaCl	Positive tubes streaked onto Baird-Parker agar	35°C/48 hr each	Coagulase tube test
5. *Clostridium perfringens*[b]	TSC agar[c]	Spread plate with overlay	35°C/24 hr	Growth and gas evolution in fluid thioglycolate, nonmotility nitrite and gelatinase tests

[a] Speck (1976)
[b] Harmon et al. (1971)
[c] Difco Laboratories, Detroit, Mich.
[d] BBL, Cockeysville, Md.

bers, initially and after three days of retail storage, can be seen in Table 2. After 3 days storage, all bacterial counts were lower in chops from lambs fed chlortetracycline (Diet 4) than in chops from controls (Diet 1). The differences for PPCs and coliform counts were significant ($P < 0.05$). Feeding lambs the C-12 tertiary amine (Diets 2 and 3) did not result in significantly lower bacterial counts; SPC values for the 50 ppm regimen (Diet 3) were significantly higher than the values for Diets 2 and 4 and higher than values for Diet 1. The only statistically significant differences in bacterial counts on vacuum-packaged lamb racks were seen in coliform counts. However, these differences were extremely small. The number of bacteria per square centimeter on lamb racks from lambs fed selected diets and held for 35 days at 3°C in vacuum-packaging were as follows: SPCs, $8.9 \times 10^6 - 2.8 \times 10^7$; PPCs, $1.2 \times 10^7 - 2.8 \times 10^7$; and coliform counts, 0.1–1.1. There was no statistically significant variation in the numbers of bacteria attributed to variation in feeding regimen.

Hot- and cold-boning of lamb carcasses resulted in consistent and significant ($P < 0.05$) differences in bacterial numbers on resulting lamb chops (Table 3). Levels of SPCs, PPCs, and coliform counts revealed large differences in counts initially and after 3 days of simulated retail storage. The initial SPC on lamb chops from hot-boned carcasses were more than three times the SPC on lamb chops from cold-boned carcasses. After 3 days of retail storage, the SPC on hot-boned chops were fivefold the SPC on cold-boned chops. PPCs on hot-boned chops were sixfold and ninefold the values for cold-boned chops, initially and after 3 days storage. Even greater differences attributable to boning were observed in coliform counts. The initial ratio of coliform counts on hot-boned lamb chops to counts on cold-boned chops was 5:1; the ratio after 3 days of retail storage was 20:1. On lamb racks from hot- and cold-boned carcasses held for 35 days at 3°C in vacuum packaging, SPCs and PPCs were significantly ($P < 0.05$) greater on cold-boned racks. A ratio of about 20:1, cold- to hot-boning, was determined for SPCs, and a ratio of about 15:1 for PPCs. Although significant at the 5% level of testing, the differences in coliform counts from hot- and cold-boned lamb racks were small.

Electrical stimulation had no effect on the SPC, PPC, or coliform counts from cuts of lamb held for 0, 3, or 35 days.

Table 2—Number of bacteria per square centimeter on lamb chops, from lambs fed selected diets, stored for 3 days under simulated retail market conditions[a]

Diets	No. of samples	Standard plate counts		Psychrotrophic plate counts		Coliform counts	
		Day 0	Day 3	Day 0	Day 3	Day 0	Day 3
1. Control—1900 IUs vitamin A and 150 IUs vitamin D per kg	(38)	1.5×10^2 a	5.4×10^3 ab	4.1×10^1 a	9.5×10^3 a	0.3 ab	2.8 a
2. Same as control with 25g C-12 tertiary amine per 1000 kg	(38)	1.1×10^2 a	3.7×10^3 b	3.1×10^1 a	4.0×10^3 ab	0.2 b	0.8 ab
3. Same as control with 50g C-12 tertiary amine per 1000 kg	(36)	1.1×10^2 a	9.3×10^3 a	4.7×10^1 a	9.5×10^3 a	0.4 a	0.9 ab
4. Same as control with 25g chlortetracycline per 1000 kg	(34)	1.0×10^2 a	2.2×10^3 b	4.3×10^1 a	2.0×10^3 b	0.1 b	0.3 b

[a] Means in the same column followed by different letters are significantly different ($P < 0.05$).

Table 3—Number of bacteria per square centimeter of surface on chops and racks, from hot-boned and cold-boned lamb carcasses, held at 3°C

	No. of samples	Standard plate counts			Psychrotrophic plate counts			Coliform counts		
		Day 0	Day 3	Day 35	Day 0	Day 3	Day 35	Day 0	Day 3	Day 35
Lamb chops										
Hot-boning	(73)	2.2×10^2 a	1.0×10^4 a	–	9.5×10^1 a	1.6×10^4 a	–	0.5 a	4.2 a	–
Cold-boning	(73)	6.6×10^1 b	2.1×10^3 b	–	1.6×10^1 b	1.7×10^3 b	–	0.1 b	0.2 b	–
Vacuum-packaged lamb racks										
Hot-boning	(23)	–	–	3.1×10^6 b	–	–	4.3×10^6 b	–	–	0.6 a
Cold-boning	(23)	–	–	6.1×10^7 a	–	–	6.5×10^7 a	–	–	0.1 b

Means in the same column followed by different letters are significantly different ($P < 0.05$).

This is in agreement with the findings of Kotula and Emswiler-Rose (1980). The number of samples in which coliforms, *Staphylococcus aureus*, and *Clostridium perfringens* were recovered were relatively few. Coliforms were detected in 24 of 146 samples initially, in 48 of 146 samples after 3 days of retail storage, and in 8 of 46 samples after 35 days of storage at 3°C. Although coliforms were recovered from less than one-third of the samples tested, confirmed coliform counts ranged as high as $5.0 \times 10^4/cm^2$, but most sample values were considerably lower. *S. aureus* was detected in 8 of 146 samples at initial testing, in 7 of 146 samples after 3 days of retail storage, and in only 2 of 46 samples after 35 days at 3°C in vacuum packaging. When recovered, coagulase-positive *S. aureus* was detected at levels of $3.5/cm^2$ or less. The same held true for recovery of *C. perfringens*, i.e., the pathogen was detected on only 2 of all samples tested, at levels of about 1 cell/cm^2.

DISCUSSION

KOTULA and Emswiler-Rose (1980) found PPCs higher on primals from hot-boned beef sides than from cold-boned sides before storage. This difference was not evident after 20 days storage at 2°C. The findings of this present research paper are in accord, for the most part, with those of Kotula and Emswiler-Rose. The present study reports significantly higher bacterial levels on hot-boned cuts than on cold-boned cuts at both initial sampling and after 3 days of retail storage at 3°C. I postulate that a higher initial bacterial load is placed on hot-boned meat than on cold-boned meat because of the inherently greater amount of handling and, that this higher initial load has a greater multiplication rate because the carcass remains warm for several hours, thus accounting for the higher numbers of bacteria found 6 days postmortem on hot-boned cuts. Although both hot- and cold-boned cuts were processed into lamb chops at the same time and under the same conditions, the hot-boned chops carried higher initial counts than the cold-boned chops at day 0 retail storage. This difference was maintained and even increased over the 3-day storage period at 5°C.

Although fewer bacteria were present on cold-boned cuts initially, cold-boned, 35-day old vacuum-packaged lamb racks had significantly higher SPC and PPC values, and significantly lower coliform counts, than did the hot-boned racks. Therefore, I postulate that certain microflora, i.e., psychrotrophic bacteria on cold-boned meat, have growth advantages over microflora present on hot-boned cuts. Those bacteria that can multiply more rapidly at cold temperatures, including microaerophilic to anaerobic lactic acid bacteria, would thus be selected because of the 6-day hanging period in the cooler (for cold-boned meats) and subsequent vacuum packaging of the racks. Once the meat was vacuum-packaged, the psychrotrophic lactic acid bacteria on the cold-boned racks would have favorable growth conditions for an additional 29 days. This was demonstrated by the bacterial numbers increasing more rapidly on the cold-boned lamb racks than on their hot-boned counterparts.

It should be pointed out that although the SPCs and PPCs on hot-boned lamb chops were higher than on cold-boned lamb chops, counts for both boning methods were well within consumer acceptability standards. However, if a meat product is to be vacuum-packaged and held for an extended period (of approx 1 month) in cold storage, then hot-boning would be more desirable than cold-boning from a microbiological standpoint. However, both hot- and cold-boning processing appear to be acceptable for meats held up to at least 3 days of retail storage when only microbiological factors are considered. Quality studies on the effects of boning and electrical stimulation are ongoing and shall be reported in the future.

The decision to evenly distribute lamb carcasses according to weight frequency among postmortem processing was in response to the report by Sauter and co-workers (1979) that thickness of fat cover is inversely related to the number of psychrotrophic bacteria after storage. Thus, even distribution in lamb weight frequencies should neutralize the reported influence of fat on bacterial numbers.

Bacterial numbers per square centimeter reported in this study were similar to those reported by Sauter and co-workers (1979) but substantially different from those reported by Al-Delaimy and Hoshiar (1978), who found mean surface counts of $1.1 \times 10^6/cm^2$ and mean coliform counts of $1.1 \times 10^4/cm^2$ from raw lamb. These higher values could be attributed to the different handling practices in Iraq and the United States.

Antibiotics are fed to animals for three general reasons: (1) the improved rate of animal growth and feed efficiency; (2) disease prophylaxis, and (3) disease therapy (Solomons, 1978). Medical scientists and consumers are concerned about possible human allergic effects and the development of bacteria with increased resistance to the antibiotics that are fed to animals. In contrast, it has been estimated that meat prices would rise between 3 and 28% if a FDA proposal for limiting antibiotic use were put into effect (Marshall, 1979). The influence of antimicrobials in feed on microbial levels in retail meat cuts is unexplored, and should be an important consideration in ultimate risk/benefit evaluation.

Residual levels of chlortetracycline in the tissue might have an antimicrobial affect. However, lamb muscle tissue that had been taken at slaughter and subsequently frozen did not contain detectable levels of CTC. It is possible that the 2 months of storage of the frozen tissue depleted any CTC that might have originally been present in the lamb tissue. No significant differences in PPCs after 35 days of refrigerated storage further suggests deterioration of residual CTC in muscle tissue. Since the only known experimental difference was the addition of CTC to lamb feed, it is difficult to find another explanation for significant differences

in reported PPCs after 3 days of storage. Addition of CTC to lamb feed seemed to contribute to possible extension of lamb chop shelf life. Certainly, further data will be necessary before final conclusions are drawn about the effects of CTC in feed on shelf life extension. Thomas et al. (1977) determined that feeding regimen can effect significant influence on PPCs on surfaces of beef carcasses. Culler and coworkers (1979) reported on the efficiency of alkyl amines as in vitro antimicrobial agents. No antibacterial trends on meat cuts from animals fed either 25 or 50 ppm N,N-dimethyldodecanamine in feed were seen in this study.

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Reference to a brand name does not constitute endorsement by the U.S. Department of Agriculture.

EFFECTS OF MICROWAVE COOKING ON FOOD FATTY ACIDS: NO EVIDENCE OF CHEMICAL ALTERATION OR ISOMERIZATION

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ABSTRACT

Because of the current interest in the potential physiological effects of trans fatty acids and a brief report that microwave cooking causes isomerization of unsaturated fatty acids, we examined the effects of microwave treatments on the fatty acid composition of several food lipids, i.e., chicken fat, beef tallow, bacon fat, rainbow trout, peanut oil, and potato lipids. The data indicate that microwave cooking per se does not change the fatty acid pattern of these lipids nor cause isomerization of the unsaturated fatty acids.

INTRODUCTION

CURRENT TRENDS indicate that about 25% of American households will have a microwave oven by 1990 and this is reflected in the increasing number of microwave compatible products and the expansion of frozen food products suitable for preparation by either conventional or microwave cooking (Anonymous, 1979). Some disadvantages of microwave cooking include the inadequate browning, lack of texture development, "crisping" in certain products, and uneven heating. Advantages are convenience, celerity, and reduced nutrient loss via elution or chemical alteration. Thus, compared to conventional methods, microwave cooking resulted in greater retention of ascorbic acid in fruits and vegetables (Thermador, 1978), and less destruction of lysine in bread (Tsen et al., 1977). Beef, pork, and lamb roasts cooked by microwave retained less sodium chloride, phosphorus, and iron compared to the same types of roast prepared by the conventional method (Baldwin et al., 1976). Maga et al. (1977) reported that microwave cooking resulted in cis-trans isomerization of the unsaturated fatty acids of potatoes.

Because of the current interest in the physiological effects of trans fatty acids (Kummerow, 1974; Hwang and Kinsella, 1979), we examined the effects of microwave treatments on the fatty acid composition of several foods including baked potatoes.

EXPERIMENTAL

CHICKEN, roast beef, bacon, and rainbow trout (*Salmo gairdneri*) were prepared as described earlier (Armbruster and Haefele, 1975). Two varieties of baking potato, Maine Russet U.S. No. 1 baking white potato ("Golden Harvest" Brand) and N.Y. red potato U.S. No. 1 ("Golden Maid" Brand), and culinary peanut oil were purchased locally.

Cooking

The chicken, beef, bacon, and filets of fresh rainbow trout were cooked in 2450 MHz (600 watt) appliances as described previously (Armbruster and Haefele, 1975). The peanut oil (100g each) was exposed to 675 watts for 5, 7, 10, and 15 min, respectively. Whole

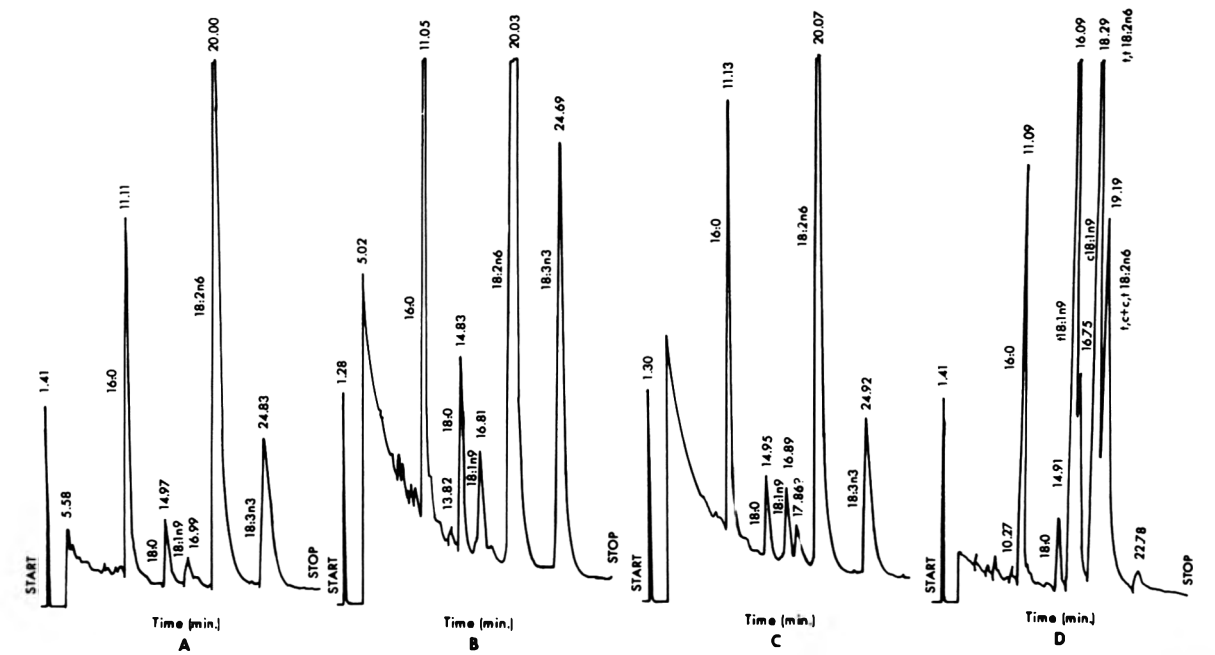

Fig. 1—Gas chromatograms showing separation of fatty acids from raw and microwave cooked potatoes and of trans fatty acids from isomerized safflower oil: (A) Raw red potato; (B) Microwave cooked red potato; (C) Microwave cooked red potato with lipid extracted by refluxing with ether (Maga et al., 1977); (D) Isomerized safflower oil. The numbers appearing on the top of each peak indicate the retention time.

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Table 1—Fatty acid composition of raw and microwave cooked chicken fat, beef tallow, and bacon fat

Fatty acid (wt %)	Chicken fat				Beef tallow	
	Raw		Microwave cooked		Raw	
	SP-2340[a]	Silar 10C[b]	SP-2340	Silar 10C	SP-2340	Silar 10C
14:0	0.65 ± 0.01	0.71 ± 0.04	0.58 ± 0.03	0.62 ± 0.03	2.67 ± 0.03	3.55 ± 0.13
15:0	0.30 ± 0.01	–	0.30 ± 0.00	0.22 ± 0.02	0.87 ± 0.08	0.92 ± 0.03
16:0	25.88 ± 0.20	27.41 ± 0.08	24.41 ± 0.05	25.00 ± 0.10	25.62 ± 0.13	26.43 ± 0.14
16:1n7	8.53 ± 0.09	8.59 ± 0.05	8.95 ± 0.04	8.74 ± 0.01	3.74 ± 0.13	4.37 ± 0.11[c]
17:0	–	–	–	–	1.08 ± 0.03	–
iso 18:0	0.14 ± 0.03	–	0.14 ± 0.03	–	0.35 ± 0.08	–
18:0	5.95 ± 0.07	5.94 ± 0.12	5.41 ± 0.09	5.57 ± 0.13	17.99 ± 0.03	18.52 ± 0.06
cis 18:1n9	36.10 ± 0.12	36.81 ± 0.15	37.09 ± 0.08	37.78 ± 0.04	41.07 ± 0.06	41.48 ± 0.09
trans, trans 18:2n6	–	–	–	–	1.00 ± 0.02	0.85 ± 0.03
cis, trans 18:2n6	–	–	–	–	0.23 ± 0.03	0.29 ± 0.04
cis, cis 18:2n6	22.22 ± 0.88	20.54 ± 0.12	22.30 ± 0.11	21.46 ± 0.24	2.61 ± 0.09	1.73 ± 0.01
20:1n9	0.55 ± 0.07	–	–	–	–	0.22 ± 0.02
20:2n6	0.10 ± 0.04	0.27 ± 0.07	0.09 ± 0.07	0.34 ± 0.00	–	–

[a] 190–245°C at 1.5°C/min; SP-2340 denotes column packing.
[b] 210–235°C at 1.0°C/min
[c] C16:1n7 + C17:0

Table 2—Effect of microwave cooking on the fatty acid composition of lipids of filet of rainbow trout

Fatty acids[a] (Wt. %)	Treatments	
	Raw filet	Microwave-cooked filet
14:0	1.72 ± 0.12	1.52 ± 0.06
16:0	12.40 ± 0.89	13.56 ± 1.46
16:1n7	4.79 ± 0.14	4.02 ± 0.67
18:0	4.39 ± 0.13	4.68 ± 0.89
18:1n9	24.57 ± 1.52	23.67 ± 2.01
18:2n6	18.37 ± 0.98	18.42 ± 1.09
18:3n6	1.35 ± 0.06	1.15 ± 0.03
18:3n3	1.03 ± 0.06	0.89 ± 0.04
18:4n3	0.59 ± 0.04	0.57 ± 0.03
20:0	1.93 ± 0.10	1.76 ± 0.10
20:1n9	5.02 ± 0.38	4.17 ± 0.23
20:2n6	0.84 ± 0.04	0.72 ± 0.04
20:3n6	1.19 ± 0.08	1.27 ± 0.08
20:4n6	1.71 ± 0.10	2.56 ± 0.06
22:1n9	2.54 ± 0.09	2.18 ± 0.09
20:5n3	1.67 ± 0.03	2.72 ± 0.21
22:4n6	0.16 ± 0.01	0.28 ± 0.03
22:5n6	0.58 ± 0.02	0.74 ± 0.13
22:5n3	0.42 ± 0.03	0.10 ± 0.02
22:6n3	15.44 ± 1.12	15.23 ± 0.96

[a] Fatty acid composition determined using 10% silar 10C (12 ft × 1/8 in. stainless steel column) at 200°C and 10% EGSS-X (6 ft × 1/8 in. stainless steel column) started at 160°C for 2 min and then programmed at 4°C/min up to 210°C.

potatoes were baked individually in a consumer model microwave oven (Vendmaster Microwave Oven, Litton) at 650 watts for 6 min. At 3 min, all the potatoes were turned over.

Fatty acid analysis

The adipose trimmed from both raw and microwave cooked chicken, beef, bacon, and the intact trout filets were extracted by the modified procedure of Bligh and Dyer (Kinsella et al., 1977). The lipids of potato were extracted by the procedure of Folch et al. (1957). For comparison, one batch of the microwave cooked red potatoes was extracted by the procedure used by Maga et al. (1977). The peanut oil was processed by saponification and methylation without extraction. The fatty acid methyl esters (FAME) were prepared from the extracted lipids by a procedure described earlier (Kinsella et al., 1977).

A Hewlett-Packard Model 5830A gas chromatograph was used for the fatty acid analyses. Two 12 ft × 1/8 in. stainless steel columns packed with 3% SP-2340 (Supelco Co., Supelco Park, Pa.) and 10% silar 10C (Applied Sci. Lab., State College, Pa.), respectively, and a 20 ft × 1/8 in. stainless steel column packed with 15% OV-275 (Supelco Co.) were used for FAME separation. These are so far the best liquid phases commercially available for the separation of *trans* fatty acids. The carrier nitrogen gas, hydrogen and air were maintained at 10 ml/min, 30 ml/min, and 250 ml/min, respectively. The specific analytical conditions for each analysis are given with the data.

FAME standards were obtained from Nu-Chek Prep. Co. (Elysian, Minn.) for the comparison of retention data. The authentic elaidic (t 18:1n9) and linoelaidic (tt 18:2n6) acid methyl esters were obtained from Supelco Co. (Belafonte, Pa.). The trans-cis and cis-trans isomers of linoleic acids were prepared from commercial safflower oil by the procedure of Harlow et al. (1963).

Triplicate analyses were carried out on each sample and the data are reported as mean ± standard deviation.

RESULTS & DISCUSSION

A COMPARISON of fatty acids from raw and microwave cooked chicken, beef, and bacon fats is given in Table 1. No difference in fatty acid composition between the raw and microwave cooked fats was observed. No trans fatty acid was found in either chicken or bacon fat, which contain high concentrations of oleic (18:1n9) and linoleic (18:2n6) acids. Trans-trans linoleic and cis-trans (trans-cis) linoleic acids were observed in both the raw and the microwave cooked beef tallow. These trans isomers of linoleic acid originate via hydrogenation in the rumen of the bovine (Noble et al., 1974). These trans acids showed an apparent slight increase following cooking.

Only minor changes in fatty acids occurred following microwave cooking of trout filets and no trans isomers were detected (Table 2).

In order to further test the effect of the time of cooking on the possible production of trans fatty acids, peanut oil (rich in both oleic (48%) and linoleic (32%) acids) was chosen as the model fat. The fatty acid components remained unchanged after microwave cooking up to 15 min. No trans fatty acid was detected even after the peanut oil was microwave cooked for 15 min (Table 3).

The fatty acid composition of the raw and the microwave cooked potatoes are presented in Table 4. The fatty acid composition of the raw and cooked samples were very similar. There was no major change in fatty acid composition of potatoes after microwave cooking and no measureable amount of trans fatty acids was detected following microwave baking. There was an apparent increase in oleic acid (18:1n9) in the baked red potatoes from which the lipids were extracted by the procedure of Maga et al. (1977). An unidentified peak, which eluted after oleic acid, was observed i the lipids of baked red potatoes extracted by the same procedure (Fig. 1C). Typical chromatograms of fatty acids from both raw and microwave cooked red pota-

Table 1—Continued

Beef tallow continued		Bacon fat			
Microwave cooked		Raw		Microwave cooked	
SP-2340	Silar 10C	SP-2340	Silar 10C	SP-2340	Silar 10C
2.58 ± 0.08	3.05 ± 0.10	0.95 ± 0.02	1.03 ± 0.10	0.96 ± 0.09	1.04 ± 0.12
0.70 ± 0.28	0.70 ± 0.08	–	–	–	–
25.60 ± 0.64	25.99 ± 0.19	26.68 ± 0.14	24.80 ± 0.67	23.85 ± 0.32	24.74 ± 0.62
3.30 ± 0.21	4.26 ± 0.07*	2.30 ± 0.02	2.17 ± 0.12	2.70 ± 0.06	2.56 ± 0.11
1.10 ± 0.00	–	–	–	–	–
0.39 ± 0.02	–	–	–	–	–
17.99 ± 0.12	18.97 ± 0.12	16.52 ± 0.45	16.60 ± 0.20	14.81 ± 0.27	15.00 ± 0.60
43.84 ± 0.32	43.68 ± 0.08	44.65 ± 0.18	45.45 ± 0.32	45.44 ± 0.09	46.21 ± 0.28
1.14 ± 0.02	1.12 ± 0.02	–	–	–	–
0.51 ± 0.02	0.33 ± 0.05	–	–	–	–
2.68 ± 0.06	1.66 ± 0.11	10.32 ± 0.33	9.21 ± 0.13	10.34 ± 0.08	9.36 ± 0.11
–	0.26 ± 0.04	1.37 ± 0.13	0.75 ± 0.03	1.38 ± 0.10	0.80 ± 0.05
–	–	–	–	0.33 ± 0.02	0.42 ± 0.01

Table 3—Fatty acid composition of commercial peanut oil treated by microwave heating

Fatty acid[a] (wt %)	Heating time (min)				
	0	5	7	10	15
16:0	10.36 ± 0.23	9.47 ± 0.90	9.72 ± 0.91	10.42 ± 0.85	9.79 ± 0.89
16:1n7	0.15 ± 0.02	0.15 ± 0.03	0.14 ± 0.02	0.13 ± 0.02	0.11 ± 0.01
18:0	2.73 ± 0.11	2.78 ± 0.15	2.70 ± 0.13	2.62 ± 0.14	2.69 ± 0.40
cis 18:1n9	48.47 ± 1.12	48.74 ± 1.38	48.81 ± 1.45	49.06 ± 1.29	49.17 ± 1.25
cis, cis 18:2n6	32.27 ± 0.95	32.57 ± 1.12	32.28 ± 1.12	32.36 ± 1.13	32.15 ± 1.13
20:1n9	1.28 ± 0.08	1.28 ± 0.14	1.27 ± 0.08	1.12 ± 0.09	1.20 ± 0.12
22:0	3.23 ± 0.14	3.26 0.19	3.43 ± 0.21	3.24 ± 0.23	3.07 ± 0.23
24:0	1.52 ± 0.10	1.66 0.03	1.78 ± 0.11	1.36 ± 0.09	1.77 ± 0.12

[a] Determined by OV-275 20 ft × 1/8 in. stainless steel column at 220°C isothermally.

Table 4—Fatty acid composition of lipids from raw and microwave cooked potatoes

Fatty acid (wt %)	Red Potato			White Potato	
	Raw	Cooked		Raw	Cooked
		A[b]	B[c]		
C16:0 (RT[a] 11.08)	20.54 ± 0.96	20.99 ± 1.52	18 31 ± 0 03	18.08 ± 0.56	17.53 ± 0.40
C18:0 (RT 14.92)	4.08 ± 0.12	4.10 ± 0.43	4 21 ± 0 08	4.09 ± 0.38	4.22 ± 0.22
C18:1n9 (RT 16.85)	1.10 ± 0.02	0.83 ± 0.12	4 49 ± 0 48	1.57 ± 0.04	1.88 ± 0.10
C18:2n6 (RT 20.05)	58.75 ± 0.69	58.31 ± 2.67	55 55 ± 0 03	60.22 ± 1.06	61.07 ± 1.04
C18:3n3 (RT 24.89)	15.84 ± 1.16	15.78 ± 3.62	14 97 ± 0.85	16.54 ± 0.50	15.30 ± 0.21
unknown (RT 17.86)	trace	trace	2 50 ± 0 12	trace	trace

[a] Retention time in min. See Fig. 1D for comparison.
[b] A = Lipid extracted as outlined by Folch et al. (1957).
[c] B = Lipid extracted directly with ether refluxing by the method of Maga et al. (1977).

toes as well as a chromatogram from trans isomerized safflower oil are given in Figures 1A, 1B, and 1D for comparison. The data indicate that microwave-cooking per se does not change the fatty acid pattern of the potatoes nor cause isomerization of the unsaturated fatty acids.

In conclusion, contrary to the report of Maga et al. (1977), there was no detectable formation of trans isomers of fatty acids following microwave cooking of several foods.

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CHEMICAL AND PHYSICAL PROPERTIES OF APOLIPOPROTEIN OF VERY LOW DENSITY LIPOPROTEIN FROM EGG YOLK GRANULES

J. T. KOCAL, S. NAKAI, and W. D. POWRIE

ABSTRACT

Tetramethylurea (TMU) was used to delipidate very low density lipoprotein from egg yolk granules [VLDL(MF)] and dissociate the apoproteins into subunits prior to gel electrophoresis. Electrophoretic analysis indicated the presence of three apoproteins, one of which was a complex of the other two. SDS disc gel electrophoresis of the proteins extracted from the TMU gels demonstrated that each band was heterogeneous, containing many polypeptide bands. SDS polyacrylamide gel electrophoresis dissociated apoVLDL(MF) into 22 polypeptides (molecular weights ranging from 9,600–136,000) in the presence of urea, dodecyl sulphate and 2-mercaptoethanol. Fourteen of these bands were glycoproteins reacting positively with the Schiff reagent. This heterogeneity of polypeptides is due in part to slight differences in the carbohydrate bound to the apoprotein. Electrophoresis devoid of 2-mercaptoethanol resulted in resolution into only 18 bands, most of which contained higher molecular weight aggregates not present in the reduced samples. The apoVLDL(MF) was fractionated on 6% agarose columns containing 8M urea or 2 mM dodecyl sulphate. Two apoprotein fractions were eluted from the SDS column, and three from the urea column. The protein eluted at the void volume of the urea column was a complex aggregate of the other two proteins; urea was not as good a dissociating agent as dodecyl sulphate. A similarity was found between the fractions eluted from the SDS column and the proteins extracted from the TMU gels; the polypeptides identified by SDS gel electrophoresis had identical electrophoretic patterns. The molecular weights of the two apoproteins from the SDS column were analyzed by sedimentation equilibrium techniques. The molecular weight range for the high molecular weight component was 33,300–165,800 and 5,800–28,100 for the low molecular weight component. It appears that the apoprotein isolated from egg yolk granules has similar protein moiety to that from egg yolk plasma and hen's plasma.

INTRODUCTION

IN A PREVIOUS PAPER (Kocal et al., 1980), the very low density lipoprotein, VLDL(MF), was isolated from the granules of hen's egg yolk, then delipidated to isolate apoprotein.

The objective of this paper was to investigate the chemical and physical properties of this apoprotein in comparison to the apoprotein of very low density lipoprotein from hen's egg yolk plasma.

EXPERIMENTAL

METHODS used for separation of the very low density lipoprotein, VLDL(MF), and total delipidation were reported in the previous paper (Kocal et al., 1980).

Gel filtration on Sepharose 6B containing 8M urea

The VLDL(MF) apoprotein was dissolved in 0.1M Tris-HCl buffer, pH 8.6, containing 0.03M EDTA and 8M urea (Edelstein et al., 1972). The apoprotein solution (25 mg protein) was applied to a 80 × 2 cm water jacketed column of Sepharose 6B containing 8M urea and eluted with the same buffer at a flow rate of 80 ml/hr at 10°C. Protein in the effluents was monitored by absorbance at 280 nm. The eluted protein fraction was rapidly freed of urea by dialysis against water.

To avoid formation of cyanate in the concentrated solutions of urea employed, these solutions were always freshly made and filtered through a mixed ion-exchange resin before use.

Gel filtration on Sepharose 6B containing dodecyl sulphate

VLDL(MF) apoprotein was dissolved in 0.1M Tris-HCl buffer, pH 8.0 containing 0.01% EDTA. After dissolving dodecyl sulphate into this solution to make 0.1M, the solution was incubated for 6 hr at 37°C prior to gel filtration (Brown et al., 1969). The apoprotein-dodecyl sulphate mixture (25 mg protein) was applied to a 100 × 2 cm calibrated column of Sepharose 6B containing 2 mM dodecyl sulphate. The sample was eluted at a flow rate of 30 ml/hr with 2 mM dodecyl sulphate in 0.1M Tris-HCl buffer and absorbance was monitored at 280 nm. The fractions were concentrated by ultracentrifugation at 78,000 × G for 8 hr at 20°C and the sediment was dialyzed against Tris-HCl buffer or against distilled water.

Polyacrylamide disc gel electrophoresis in tetramethylurea

Urea gels were prepared according to the method of Kane (1973) or Kane et al. (1975), both are a modification of the Davis (1964) method.

The VLDL(MF) was dialyzed against 0.4M NaCl, 1 mM EDTA (pH 8.6) for 72 hr at 4°C. Delipidation of VLDL(MF) was carried out either on top of the stacking gel or in a test tube prior to application to the gel.

Dialyzed samples (50–200 μl) were pipetted onto the stacking gels. An equal volume of pure glass distilled tetramethylurea (TMU) and one-tenth volume of freshly prepared standard reducing solution (a solution containing 0.01 ml/ml thiodiglycol, 0.025 ml/ml mercaptoacetic acid, and 0.02 g/ml EDTA), 10 mM dithioerythritol (DTE) or 0.1% β-mercaptoethanol (ME) were added to the sample and the mixture was incubated for 60 min at 20°C. Following this delipidation, one-tenth volume of tracking dye (0.001% Bromphenol blue) and one-tenth volume of 80% sucrose (w/v) were added to the mixture and mixed to increase the density of the TMU-water phase. The upper tank buffer was then layered over the sample mixture.

Alternatively, the delipidation and reduction were accomplished by incubating the VLDL(MF) solution with the TMU and reducing agents (the same proportions as above) in 10 × 1 cm glass centrifuge tubes. The supernatant and the insoluble protein were rapidly separated from each other by centrifugation at 5,000 × G and the supernatant was then applied to the gel.

Some samples of VLDL(MF) were denatured with 8M or 10M urea and reduced with 10 mM DTE, 0.1% ME, or with the standard reducing agents as alternatives to using TMU as the dissociating agent.

Staining of glycoproteins on polyacrylamide gels

To remove free and bound dodecyl sulfate from unstained gels, each gel was immersed overnight at 20°C in about 80 ml of a solution containing 25% (v/v) isopropyl alcohol and 10% (v/v) acetic acid. The gel was transferred into a mixture of 10% (v/v) isopropyl alcohol and 10% (v/v) acetic acid and held for 6–9 hr then left overnight in 10% acetic acid.

The carbohydrates on the gels were then oxidized and stained in the dark at 20°C. Each gel was incubated in at least 80 ml of 0.5% (w/v) periodic acid (H_5IO_6) for 2 hr, followed by immersion in a solution containing 0.5% (w/v) sodium arsenite and 5% (v/v) acetic acid for 60 min. Two more immersions of gels for 20 min each in a fresh mixture of 1% (w/v) sodium arsenite and 5% (v/v) acetic acid completed the periodic acid treatment. The gels were then transferred to tubes containing 20 ml Schiff reagent, left overnight, then

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rinsed in 0.01N HCl containing 0.1% (w/v) sodium metabisulfite to remove excess stain and retard fading.

Chemical analyses: Amino acid analysis

Freeze-dried apoproteins were hydrolyzed with p-toluene sulphonic acid solution (Liu and Chang, 1971) for 20, 24, and 36 hr at 110°C. then analyzed on a Phoenix model M6800 amino acid analyzer (Phoenix Precision Instruments Corp.) utilizing a single column elution technique.

RESULTS & DISCUSSION

Electrophoresis of VLDL(MF) in the presence of tetramethylurea

Kane (1973) demonstrated that TMU delipidates lipoprotein and dissociates into subunits. The whole apoprotein (apoB) of human serum low density lipoprotein as well as that fraction of the apoprotein of human serum VLDL which represents its LDL content were found to be precipitated by this reagent. The remaining apoprotein (apoC) of human VLDL are soluble in TMU.

Yolk granule VLDL(MF) was delipidated by TMU at the top of the stacking gel and electrophoresed on 10% polyacrylamide gel. Three protein bands were detected when the gels were stained with Coomassie blue. Band 1 remained on top of the stacking gel and was the fraction precipitated by TMU. Band 2 was located at the interface of the stacking and the running gels and band 3 appeared to be a small molecular weight protein travelling through the running gel with the tracking dye. The presence of band 3 was confirmed by experiments in which no tracking dye was used. Bands 2 and 3 represented the apoprotein fractions soluble in TMU.

Alternatively, VLDL(MF) was delipidated with TMU in a test tube and the precipitated protein was separated from the soluble protein by centrifugation. Both the TMU soluble and the TMU insoluble fractions were then subjected to electrophoresis. The TMU insoluble fraction produced only band 1, which remained at the top of the stacking gel. The TMU soluble fraction produced 2 bands which had mobilities identical with bands 2 and 3 obtained from whole VLDL(MF) when delipidated in the gel by the TMU method.

Band 2, which remained at the interface of the stacking gel and running gel, was believed to be a complex aggregate of proteins which were not dissociated by TMU or the reducing agents (thiodiglycol and mercaptacetic acid) used in this system. Further dissociation using 8M or 10M urea, in combination with ME or DTE followed by electrophoresis, failed to dissociate this protein. Decreasing the polyacrylamide concentration in the gels to 7.5% and 5% did not affect the mobility of this protein.

A resemblance was found between these protein bands from the TMU electrophoresis procedure and the SDS gel electrophoretic patterns of the fractions of apoprotein eluted from the SDS-Sepharose 6B column which will be shown later. The three protein bands from the TMU electrophoresis were sliced from the urea-polyacrylamide gels, extracted with dodecyl sulphate and electrophoresed in the presence of dodecyl sulphate on 10% polyacrylamide gels. The protein precipitated by TMU (band 1) was soluble in dodecyl sulphate and was found to have the same polypeptide pattern as fraction A eluted by SDS gel filtration (Fig. 1). TMU-soluble band 3 had an identical polypeptide pattern to the low molecular weight fraction B from the SDS column (Fig. 1). The interfacial protein (band 2) appeared to be an aggregate of the two proteins, and was found to contain polypeptides common to both fractions A and B from the SDS column (Fig. 1). The molecular weights of these polypeptides will be discussed in the next section.

Complete dissociation of the apoproteins with TMU was reported in studies on the subunit pattern of the VLDL of human serum (Kane et al., 1975) and rat plasma (Marsh, 1976; Mahley and Holcombe, 1977) using this technique. It appears, however, that dissociation of egg yolk apoVLDL was incomplete since the interfacial protein (band 2) was a complex aggregate of the other two proteins as seen in Figure 1.

The protein component of VLDL, which appears to represent the LDL-like protein, was precipitated. The LDL apoprotein classified as apoB (Scanu et al., 1974) appears to be present in the VLDL fraction isolated from human, rat, and chicken serum. Chan et al. (1976) isolated from hen plasma apoVLDL a fraction resembling the major apoprotein from human VLDL designated apoB (Gotto et al., 1972). This apoB may be similar to the fraction precipitated by TMU in this study, since Hillyard et al. (1972) demonstrated that VLDL isolated from egg yolk was identical with the VLDL isolated from chicken serum. Positive identification of the egg yolk apoB-like protein using immunological techniques would clarify this point.

Molecular weight estimation of apoVLDL polypeptides by SDS polyacrylamide gel electrophoresis

SDS gel electrophoresis of apoVLDL(MF) was conducted in the presence of ME on 7.5% or 10% polyacrylamide gels. The gels were stained for the presence of glycoprotein using the PAS staining procedure and for protein with Coomassie Blue when apoVLDL was reduced with ME (Fig. 2A). The most intensely stained bands had molecular

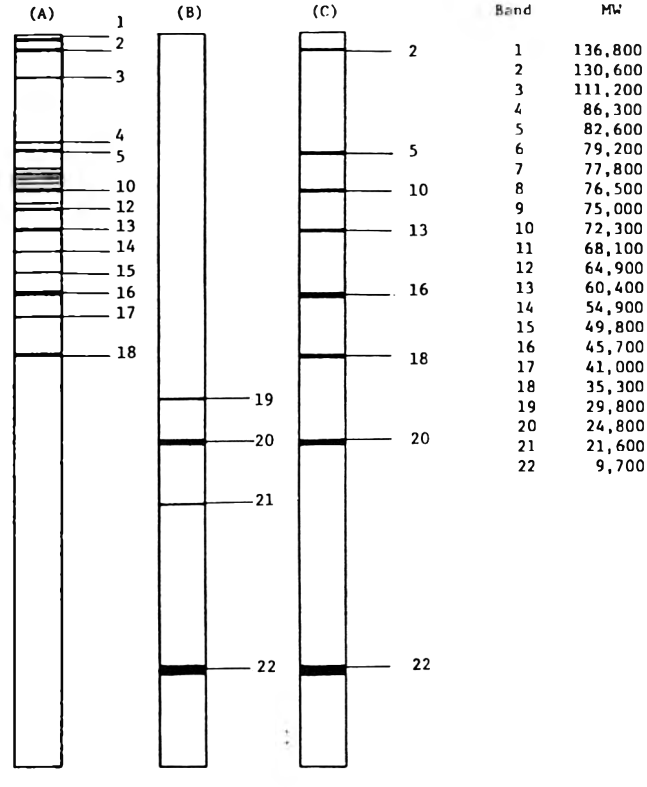
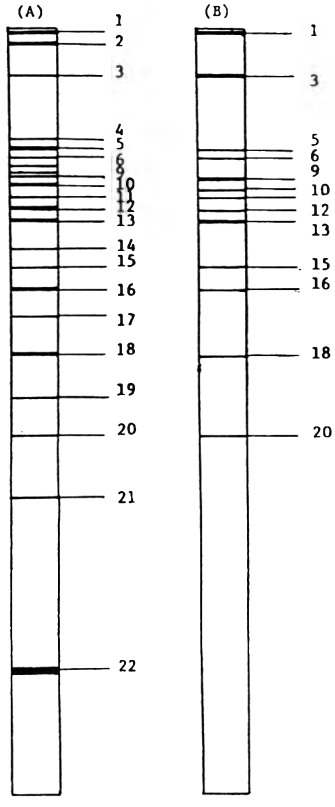
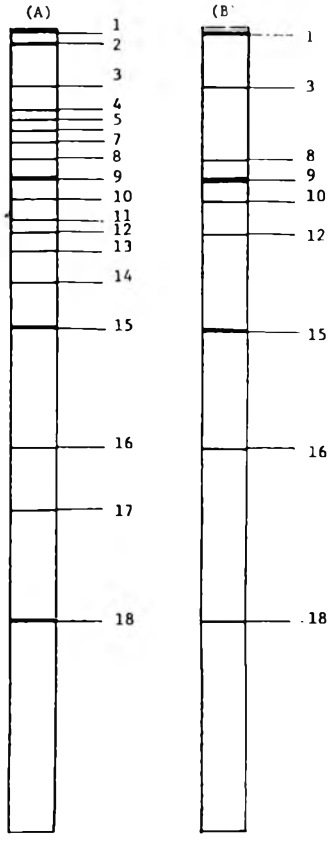


Fig. 1—SDS polyacrylamide gel electrophoresis in the presence of ME for (A) TMU insoluble Band 1/Sepharose 6B, Fraction A; (B) TMU soluble Band 3/Sepharose 6B, Fraction B; (C) TMU soluble Band 2. [The protein in gels from TMU electrophoresis was extracted by the method of Weiner et al. (1972). The protein samples, 0.1 ml, were boiled for 5 min with 0.9 ml of 8M urea containing 15–30 mg SDS and 0.01 ml ME prior to SDS gel electrophoresis (Deutsch, 1976).]



Band	MW
1	136,300
2	131,000
3	111,700
4	86,200
5	82,800
6	79,600
7	78,000
8	76,500
9	75,000
10	72,000
11	67,900
12	65,200
13	60,200
14	54,500
15	50,400
16	45,600
17	41,300
18	35,200
19	30,000
20	24,600
21	21,800
22	9,600

Fig. 2—SDS polyacrylamide gel electrophoresis of apoVLDL in the presence of ME and stained with (A) Coomassie Blue and (B) PAS.



Band	MW
1	136,800
2	130,600
3	112,000
4	99,100
5	95,200
6	91,500
7	87,800
8	81,300
9	73,500
10	66,700
11	62,700
12	59,100
13	54,000
14	48,400
15	36,500
16	24,100
17	21,000
18	12,500

Fig. 3—SDS polyacrylamide gel electrophoresis of apoVLDL conducted in the absence of ME and stained with (A) Coomassie Blue and (B) PAS.

weights of 136,300; 131,000; 82,900; 72,100; 65,200; 60,200; 45,600; 35,200; and 9,600. The remaining bands stained only lightly but were present in all preparations. Fourteen bands stained positively for glycoprotein. The most intensely PAS-reactive bands corresponded to molecular weights of 136,300; 111,700; 75,000; and 60,200 (Fig. 2B). The other bands always stained positively with PAS but were much less intense.

In the absence of ME, 18 protein bands and 9 glycoprotein bands were resolved. The major proteins had molecular weights of 136,800; 130,600; 73,500; 36,500; and 12,500 (Fig. 3A). The intensely stained PAS bands had molecular weights of 136,800; 73,500; and 36,500 (Fig. 3B). All other bands were consistently detected but were stained less intensely.

The most noticeable change in the apoVLDL(MF) pattern due to the omission of ME from the sample was the absence of the low molecular weight protein (9,600) and the formation of several higher molecular weight proteins (12,500; 48,400; 62,700; 91,500; 95,200; and 99,100). These proteins may be aggregates joined together by disulfide bonds which disappear upon addition of ME, resulting in the formation of smaller molecular weight proteins.

The above results indicate that apoVLDL(MF) contains 9 major polypeptides that range in molecular weight from 9,600 to 136,000. Other proteins were detected, some of which could be polymorphic forms of the major or minor bands caused by slight variations in the content of sugar residues. This heterogeneity was demonstrated by the fact that 14 bands were PAS-reactive and sialic acid was found in the apoprotein upon carbohydrate analysis. Brown et al. (1969) have reported that differences in the sialic acid content of otherwise identical polypeptides could account for this type of polymorphism.

Hillyard et al. (1972), using a phenol-urea-acetic acid electrophoresis sytem, detected only 4 protein components in chicken serum VLDL. The major fraction had a molecular weight of 21,800 which dropped to 9,000 in the reduced form. The other protein components were of molecular weight 51,200; 60,800; and 70,700. The major protein found in the present study (molecular weight 9,600) may be similar to the reduced major fraction of chicken serum VLDL (Hillyard et al., 1972). Chan et al. (1976) characterized the hen plasma apoVLDL and found that 43% of the total apoprotein consisted of a protein which migrated in an electrophoretic field as if it had a molecular weight of 12,000–13,000. In the presence of dithiothreitol, the molecular weight was reduced from about 12,000 to approximately 6,000.

Yamauchi et al. (1976) isolated apoVLDL from egg yolk plasma and detected 18 polypeptides (ranging from 10,000–135,000) of which 5 were major components (135,000; 82,000; 71,000; 62,000; and 16,000). The 135,000 and 71,000 molecular weight components were glycoproteins, similar to those found in this study.

Raju and Mahadevan (1976) found 13 protein bands when apoVLDL from egg yolk plasma was subjected to SDS gel electrophoresis in the presence of ME. A similar protein (12,500) was found in this study when ME was omitted. However, it did not stain as intensely with Coomassie Blue as did the 9,600 molecular weight protein found in the reduced apoprotein.

Agarose gel filtration of apoVLDL

The VLDL(MF) apoprotein obtained by delipidation with ethanol-ether or sodium deoxycholate was completely soluble in 0.1M Tris-HCl buffer (pH 8.6) containing 8M urea. Sepharose 6B-8M urea gel chromatography resolved the apoVLDL(MF) into 3 distinct peaks (Fig. 4). The first peak (V_I), eluted in the void volume, followed by a second well separated peak (V_{II}). The third peak (V_{III}) eluted at a

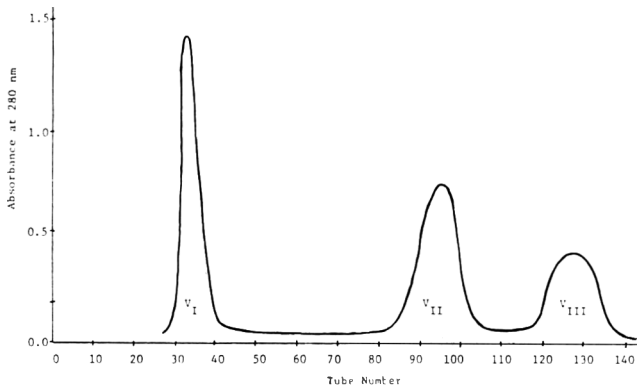


Fig. 4—Gel filtration of apoVLDL on Sepharose 6B containing 8M urea. Fraction, 2 ml.

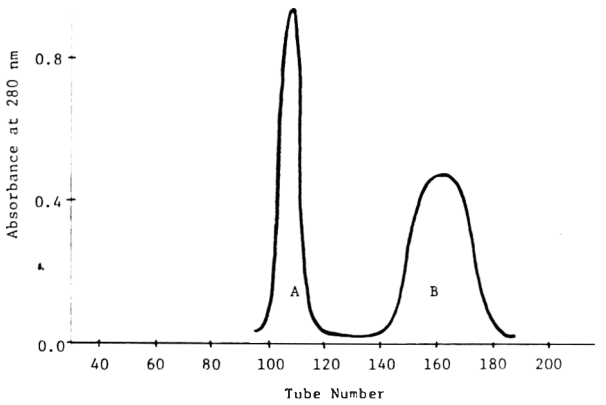


Fig. 5—Gel filtration of apoVLDL or fraction V_I from 8M urea column on SDS Sepharose 6B. Fraction, 2 ml.

volume equal to the total bed volume.

Two protein peaks were observed when fraction V_I from the 8M urea column or whole apoVLDL (Fig. 5) was passed through a calibrated column of SDS-Sepharose 6B. The first fraction (A) was eluted at a volume equivalent to a molecular weight of approximately 110,400. The second fraction (B) was eluted from the column as if it were a small protein at a volume near the total bed volume and had a molecular weight of 26,800. The elution volumes of the 2 peaks obtained from the void volume peak V_I from the 8M urea column were identical to those of whole apoVLDL applied to the SDS column. These results indicated that fraction V_I was an aggregate of two proteins which was not completely dissociated by 8M urea, but was completely dissociated by dodecyl sulphate.

These results are in agreement with those from the TMU electrophoresis. The protein which was found at the interface of the stacking and running gels was a complex of the other two proteins (Fig. 1C). Polypeptides of molecular weights ranging from 9,700–130,600 were evident; some of these were common to both band 1 (fraction A) and band 3 (fraction B). All samples of apoVLDL(MF) were fractionated only on the SDS column due to the incomplete dissociation of apoVLDL(MF) in urea solutions and their possible chemical modification by carbamylation.

SDS gel electrophoretic patterns, in the presence of ME, of fractions A and B from the SDS column corresponded to the proteins extracted from the TMU gels (Fig. 1A and 1B). The calibration curve using known molecular weight markers indicated significant differences in the apparent molecular weights of the fractions. Fraction A (band 1 from the TMU gel) consisted mainly of the high molecular weight proteins. Eighteen bands were detected, six of which stained intensely with Coomassie Blue (136,800; 130,600; 64,900; 60,400; 45,700; and 35,500). The remaining proteins were present in small quantities and therefore stained faintly. Fraction B (band 3 from TMU gels) contained 1 major protein with an apparent molecular weight of 9,700 and 3 faint bands of molecular weights 21,600; 24,800; and 29,800. These figures compared well with those derived from the calibration curve obtained from Sepharose 6B chromatography in the presence of dodecyl sulphate.

Molecular weight determinations using calibrated gel filtration columns do not detect such heterogeneity in molecular weights of the fractions but give molecular weights corresponding to the elution volumes of the fractions. The combination of the greater resolving power of the gel electrophoresis method and the dissociation of the apoproteins in the presence of 8M urea, dodecyl sulphate and ME provide a more accurate determination of the molecular weights of the isolated polypeptides.

The molecular weights of fractions A and B from the SDS column were also determined by sedimentation equilibrium techniques. Although the amount of detergent bound to the apoprotein fractions was not known, all free dodecyl sulphate was removed prior to ultracentrifugal analysis of the apoproteins by dialysis. The molecular weights calculated reflect the state of aggregation of the apoproteins at the time of the experiment. The partial specific volumes ($\bar{\nu}$) of the apoproteins were unknown and assumed to be 0.724 ml/g, with the realization that this is an approximate value which may be affected by carbohydrate bound to the apoproteins.

Plots of $\ell n\ c$ versus r^2 exhibited substantial curvature and were therefore fitted to a least squares quadratic function. Values of $d\ \ell n\ c/dr^2$ were then obtained by taking the first derivative of the polynomial equation at each radial distance This allowed calculation of the weight average molecular weight (M_w) at each point.

Weight average molecular weights of the apoproteins were estimated to range from 33,300–165,800 and from 5,800–28,100 for fractions A and B, respectively. These values were taken from the molecular weights at the meniscus and at the cell bottom. The apparent molecular weights ($M_{w,app}$) of these fractions were 55,800 (A) and 13,200 (B), about one-half that determined by SDS-gel filtration. These values approximately represent the molecular weights of the apoproteins since considerable heterogeneity in molecular weights was observed. The weight average molecular weights calculated at each radial distance in the centrifuge cell more correctly represent the heterogeneity and aggregation state of the sample and are in close agreement with the range of molecular weights of fractions A and B as determined by SDS gel electrophoresis.

Raju and Mahadevan (1976) separated yolk plasma apoVLDL by gel filtration on Sephadex G-200 in 0.5% dodecyl sulphate and obtained 3 protein peaks. The second peak was a shoulder of the first, but the third peak was well separated from the first two. SDS gel electrophoresis of the eluted proteins demonstrated that each peak was heterogeneous. The major proteins in these 3 fractions were arbitrarily designated apoA, apoB and apoC. ApoA consisted of 2 major bands of molecular weights of 180,000 and 100,000, and a few minor bands. ApoB consisted of 4 bands; the 2 major bands contained proteins of molecular weights of 78,000 and 64,000. ApoC contained 2 bands of molecular weight 12,500 and 9,400. It was indicated that the 12,500 molecular weight component was a dimer formed by disulfide linkage of two monomers of 9,400 molecular weight. In this study a similar range of molecular

Table 1—Amino acid composition of apoproteins from yolk granule, yolk plasma, hen serum and human serum VLDL.

Amino acid	Weight % (w/w) Yolk granule apoVLDL	Yolk plasma[a] apoVLDL	Yolk plasma[b] apoVLDL	Hen serum[b] apoVLDL	Human serum[c] apoVLDL
Asp	10.65	11.16	11.40	10.90	10.94
Thr	8.72*	5.69	6.48	6.26	6.55
Ser	8.29*	6.57	7.50	7.09	8.35
Glu	12.15	10.53	11.30	11.30	17.27
Pro	3.00	3.36	3.02	3.62	4.50
Gly	4.65	4.24	4.61	4.44	2.79
Ala	7.15	7.35	7.79	7.65	6.10
Val	5.08	6.67	6.73	6.60	5.06
Met	1.79	2.11	1.48	2.02	N.D.**
Ile	5.79	6.47	6.85	6.54	3.19
Leu	9.29	11.30	10.30	10.40	11.10
Tyr	4.00	4.60	2.12	3.41	2.81
Phe	4.57	4.09	4.19	4.25	5.50
Lys	8.86	9.93	7.70	7.02	9.20
His	1.43	1.36	1.40	1.12	1.56
Trp	0.00	N.D.**	0.61	1.77	N.D.**
Arg	4.57	5.76	5.35	4.58	1.56

* Values for threonine and serine extrapolated to zero hydrolysis time

** Not determined

[a] From Raju and Mahadevan (1976)

[b] From Hillyard et al. (1972)

[c] From Kane et al. (1975)

weights was observed for fractions A and B, although only 2 peaks were resolved on the Sepharose 6B column. This could be due to the large pore size of the agarose column compared to the Sephadex G-200 gel. The higher molecular weights determined by Raju and Mahadevan (1976) for the apoVLDL fractions could be the result of irreversible aggregation of the apoproteins in the chloroform-methanol delipidation of VLDL(MF) resulting in irreversible denaturation of the apoproteins.

Chan et al. (1976) fractionated apoVLDL from hen plasma on a Sephadex G-150 column containing 2 mM dodecyl sulphate. Two peaks were eluted from the column, one of which eluted at the void volume. This large molecular weight fraction represented the LDL-like protein which is present in VLDL. The second fraction had a molecular weight of 12,000–13,000, which upon reduction dropped to 6,000. These two fractions appear to correspond to the two fractions separated in this study.

Amino acid composition of apoVLDL

The results of amino acid analysis of 24 hr hydrolysates of yolk granule apoVLDL are presented in Table 1. The amino acid composition of apoVLDL isolated from yolk plasma, hen serum, and human serum are included for comparison.

The yolk granule apoVLDL has an amino acid composition typical of the VLDL with respect to high levels of aspartic acid, threonine, serine, glutamic acid, alanine, and lysine, and little or no tryptophan. The lack of tryptophan residues in yolk granule apoVLDL would account for its low absorbance at 280 nm after subtracting the contribution of absorbance due to light scattering.

It is concluded that the very low density lipoprotein separated from hen's egg yolk granules has similar apoprotein to that of yolk plasma and hen's plasma.

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PREPARATION OF APOLIPOPROTEIN OF VERY LOW DENSITY LIPOPROTEIN FROM EGG YOLK GRANULES

J. T. KOCAL, S. NAKAI and W. D. POWRIE

ABSTRACT

The very low density lipoproteins VLDL(MF) were isolated from the yolk granules of hen's egg by a combination of preparative ultracentrifugation and agarose gel filtration chromatography. Ultracentrifugal, electrophoretic and chromatographic analyses were performed to characterize and assess the purity of the VLDL(MF) and delipidated VLDL. Flotation velocity analysis of VLDL(MF) indicated a heterogeneous preparation with two major floating boundaries with S_f° rates of 75S and 207S. Alkylation of VLDL(MF) with iodoacetamide prevented aggregation of VLDL(MF) due to oxidation of sulfhydryl groups and decreased S_f° rates to 41S and 108S. Concanavalin A affinity chromatography of VLDL(MF) produced a retained and an unretained fraction. The retained fraction contained twice as much total carbohydrates as the unfractionated VLDL(MF), while the unretained VLDL(MF) contained half as much carbohydrate. All fractions were glycoproteins containing hexose, hexosamine and sialic acid residues. VLDL(MF) was partially delipidated with n-heptane which preferentially extracted the neutral lipids and preserved the solubility of the phospholipid-protein residues. Two fractions of the partially delipidated VLDL(MF) were isolated by gel filtration and characterized by analytical ultracentrifugation and by phospholipid/protein ratios. VLDL(MF) was totally delipidated with sodium deoxycholate (NaDOC) and ethanol-ether procedures. Removal of lipids by both methods produced aggregated apoproteins; however, the NaDOC apoprotein remained soluble, whereas that obtained from ethanol-ether precipitated, but could be solubilized in sodium dodecylsulfate or urea.

INTRODUCTION

FOR THE FIRST TIME, electron microscopically homogeneous myelin figures (MF) were isolated from egg yolk granules by Garland and Powrie (1978) and found to contain 86% lipid and 14% protein. Electron microscopy of the osmium tetroxide-stained MF fraction revealed one or more electron dense concentric lamellae surrounding a weakly osmiophilic core. The similarity of these figures with brain phospholipid myelin figure structure lead to the name 'myelin figure'. Dimensions of 480–1100A in width and 560–1300A in length were reported for the egg yolk myelin figures (Garland and Powrie, 1978).

Hillyard et al. (1972) examined chicken serum and egg yolk lipoproteins isolated by ultracentrifugal techniques. Immunochemical methods, analytical ultracentrifugation, electrophoresis and amino acid end group analysis showed identical results for serum and egg yolk very low density lipoprotein (VLDL). Due to the similarity in physicochemical property with that of Hillyard et al. (1972) and Alexander and Day (1973) and the physical properties of blood serum VLDL, especially MF having high S_f of 75 and 207, we suggest using the term VLDL(MF) as the most suitable operational definition of MF from egg yolk granules.

Although there have been a number of publications on egg yolk plasma lipoprotein, no reports concerning the physical and chemical aspects of the lipoprotein or protein moiety of the VLDL fraction of egg yolk granules have appeared in the literature to date. The objective of this study was to investigate the purity and physicochemical properties of partially and totally delipidated VLDL(MF) fractions of egg yolk.

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EXPERIMENTAL

Reagents

In all experiments, analytical grade chemicals were used unless otherwise specified. Glass distilled, deionized water was used for preparation of buffers and solutions.

Biogel A-50M (2% w/v agarose, 50–100 mesh) was the product of Biorad Laboratories, Richmond, Calif. Sephadex G-75 and G-200 (superfine) and Concanavaline A-Sepharose 4B were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Potato starch, sodium deoxycholate and sudan black B were obtained from Fisher Scientific Co., Toronto and iodoacetamide was from Sigma Chemicals, St. Louis, Mo. Agarose used for gel electrophoresis was the electrophoretic grade product of ICN Pharmaceuticals, Cleveland, Ohio.

Electrophoretic separation of prestained lipoproteins

A GE-4 gel electrophoresis apparatus (Pharmacia Fine Chemicals) was used for disc gel electrophoresis. All experiments were conducted using an agarose gel concentration of 0.5% (w/v). Sudan black B stain was prepared according to the method of Ribeiro and McDonald (1963).

A 0.5% agarose gel was prepared by adding 0.116g of agarose to 22.6 ml of distilled water, and refluxing for 15 min at 100°C while stirring vigorously to prevent burning. The agarose solution was cooled slowly to approximately 55°C then 6.2 ml distilled water and 3.2 ml buffer A (24 ml 1N HCl, 18.1g Tris, water to 100 ml, pH 8.8–9.0) were added. The warm agarose solution (1.5 ml) was transferred into glass electrophoresis tubes, and layered with water to ensure a flat meniscus. The tubes were cooled to 40–45°C very slowly to allow setting of the agarose gel.

VLDL(MF) was dialyzed for 48 hr against 1.6 mM Tris, 7.9 mM glycine buffer, pH 8.6 containing 0.02% EDTA at 4°C. Prestaining was accomplished by slowly adding a 0.5 ml aliquot of the dialyzed VLDL(MF) to 0.1 ml of the dye solution. The vial was flushed with nitrogen, sealed for 1 hr at room temperature, then stored for 24 hr at 4°C. The excess dye was removed by centrifugation at 6000 × G for 5 min. Aliquots of 10–30 μl of this prestained sample were applied to each gel. Disc gel electrophoresis was conducted at pH 9.5 as described by Ornstein and Davis (1964) except that the sample and stacking gels were omitted.

SDS gel electrophoresis

Using a Pharmacia disc gel electrophoresis system the procedures developed by Weber and Osborne (1969) and Weber et al. (1972) were followed. Bovine serum albumin (monomer and dimer), ovalbumin, α-chymotrypsinogen and lysozyme were used as the molecular weight markers.

Sedimentation and flotation velocity ultracentrifugation

A Beckman L2-65B preparative ultracentrifuge, equipped with a Prep UV scanner (Beckman Instruments, Palo Alto, Calif.), was used. The electric signals from the photomultiplier of the scanner were stored on a magnetic tape in a 733 ASR data terminal (Texas Instrument, Houston, Tex.) through a digital voltmeter and a data transfer unit. The stored data were processed in an 1880 programmable calculator (Monroe, Orange, N.J.) after transfer to the memories in the calculator (Van de Voort and Nakai, 1978).

A program was written for the Monroe calculator to calculate the derivative of concentration and the radial distance from the mV values stored on the magnetic tape. *—Continued on next page*

For flotation analysis, VLDL(MF) dissolved in 0.025M sodium phosphate, pH 7.0, containing 0.01% EDTA were centrifuged at 18,000 rpm in NaCl solutions of density 1.0630 g/ml or 1.0385 g/ml at 20°C. Sedimentation analysis was performed in 0.2M Tris-HCl, pH 8.2 and 0.01M NaCl, 0.025M Tris-HCl, pH 7.2.

The observed flotation or sedimentation coefficient was calculated from the slope of regression line, ln x vs t (x, the distance from the center of rotation to the apex of protein peak; t, time). The observed flotation coefficients were converted to the S°_{F} as outlined by DeLalla and Gofman (1954).

Hydrated density was determined by measuring the flotation coefficients of VLDL(MF) at four different solvent densities, obtained by dialyzing the lipoprotein in various NaCl solutions containing 0.1% EDTA and 0.025M sodium phosphate, pH 7.0. The densities and viscosities of the buffer solutions were derived from polynomial equations (Washburn, 1927). Values for the hydrated density (ρ) were obtained by extrapolating the $s\eta$ vs ρ data to zero flotation, where s and η are flotation rate and solvent viscosity, respectively.

Hexose and hexosamine determination

The method of Abraham et al. (1960) was used.

Sialic acid determination

The thiobarbituric acid assay of Warren (1959) was followed.

Isolation of VLDL(MF)

Preparation of yolk granules. Infertile eggs from White Leghorn hens fed a standard laying ration were provided by the University farm. The eggs were stored for less than 24 hr prior to use. Each yolk was carefully separated from the albumen and rolled on absorbent paper towels to remove any adhering egg white. The yolk membrane was then punctured with a spatula to separate the yolk from the vitelline membrane.

Liquid yolk was diluted 1:1 (v/v) with cold (4°C) distilled water containing 0.02% EDTA, pH 7.0, and stirred for 30 min. The granules were separated from the yolk plasma by centrifugation at 20,000 × G for 30 min at 4°C in a Sorvall RC2-B refrigerated centrifuge. The sedimented granules were resuspended in more EDTA solution, stirred for 30 min and recentrifuged. This washing procedure was repeated two to three more times until all the water soluble plasma was removed (Evans et al., 1968).

Separation of FI and FII fractions. Washed granules were suspended in 0.025M sodium phosphate buffer, pH 7.0 containing 0.01% EDTA and 0.02% NaN_3. The solution was adjusted to a density of 1.063 g/ml with NaCl. All solutions were buffered to pH 7 and contained EDTA to inhibit peroxidation or aggregation of lipoproteins (Mauldin and Fisher, 1970).

The dispersed granules were then centrifuged at 78,000 × G for a period of 16–18 hr at 4°C in a Beckman L2-65B or L3-50 ultracentrifuge. The contents of the tubes were distributed into five zones: two floating and three sedimenting fractions. The semisolid layer on the surface (FI) was peeled from the side of the centrifuge tube with the top of a spatula, and the opaque solution beneath (FII) was removed with a Pasteur pipet for further fractionation.

Preparation of VLDL(MF). Lipoprotein FII concentrate was applied in 60-ml aliquots directly from the ultracentrifuge to a 100 × 5 cm column of Biogel A-50M and was eluted at 113 ml/hr with 1.0M NaCl, 0.025M sodium phosphate buffer, pH 7.0 containing 0.01% EDTA and 0.02% NaN_3. The first peak, eluted at the void volume, contained the VLDL(MF) fraction and was concentrated by ultracentrifugation at 78,000 × G for 8 hr at 4°C. Samples of this VLDL(MF) were dialysed against 0.01% EDTA, pH 7, at 4°C and delipidated. Dialysis tubings were cleaned in advance by boiling in distilled water containing 100 mg EDTA and 2.5g $NaHCO_3$ per liter (Mauldin and Fisher, 1970). The VLDL(MF) fraction was also isolated under the reducing conditions using oxygen-free water containing 0.1% 2-mercaptoethanol (ME) after flushing with nitrogen.

Carboxyamidation of VLDL(MF). VLDL(MF) was alkylated with iodoacetamide according to the procedure of Battel et al. (1968). The sulfhydryl and disulfide contents were determined using Ellman's reagent (Beveridge et al., 1974).

Affinity chromatography on Concanavalin A-Sepharose 4B. Affinity chromatography was carried out according to the method of Shore and Shore (1973). The fractions of retained and unretained VLDL(MF) were extensively dialysed against water containing 0.01% EDTA at 4°C and lyophilized. Some samples were not lyophilized but concentrated by ultracentrifugation.

Delipidation of VLDL(MF)

Partial delipidation using n-heptane. Carboxyamidated VLDL(MF) was partially delipidated according to the method of Gustafson (1965) and separated by gel filtration. The potato starch, which was used for protection of the apoprotein from denaturation, was purified prior to use by washing with distilled water (3–5 times), 0.02M Tris-HCl, pH 8.2 (3 times), 1.0M NaCl, pH 7.0 (3 times), distilled water (3 times) and finally extracted with n-heptane. The insoluble starch was then lyophilized and stored in a desiccator.

The delipidated samples were concentrated by ultracentrifugation at 78,000 × G for 8 hr at 4°C and applied to a 77 × 2.5 cm calibrated column of Sepharose 4B and eluted at a flow rate of 68 ml/hr with 0.2M Tris-HCl buffer, pH 8.2. Protein content was determined by the method of Lowry et al. (1951) and phospholipids by the method of Bartlett (1959). Samples of the unfractionated heptane-delipidated VLDL(MF) were extracted with chloroform-methanol (2:1 v/v). The lipid extract was examined for the presence of neutral lipids by thin-layer chromatography on Silica Gel G using a petroleum ether-diethyl ether (95:5 v/v) solvent system (Scanu, 1966).

Total delipidation with ethanol-ether. Prior to total delipidation, VLDL(MF) was dialysed for 48 hr against 0.15M NaCl, 1.0 mM EDTA, pH 7.0. The standard delipidation conditions outlined by Scanu and Edelstein (1971) were followed.

Total delipidation with sodium deoxycholate. The procedure of Helenius and Simons (1971) was followed. The approximate VLDL(MF) concentration was calculated by multiplying the protein concentration by a factor of 7.14, obtained from the (protein + lipid)/protein ratio. Total cholesterol of the effluents from the Sephadex G-200 superfine column was assayed by the method of Pearson et al. (1953). Sodium deoxycholate in the effluents from a Sephadex G-75 column was determined by the method of Mosbach et al. (1954). The presence of lipids in the totally delipidated samples was examined by the method of Folch et al. (1957).

RESULT & DISCUSSION

Isolation of VLDL(MF)

Preparation of yolk granules. Yolk granules were separated from the plasma by low speed centrifugation of whole yolk diluted with pH 7 water containing EDTA. The granules were found to sediment rapidly and this proved to be a more efficient means of their isolation than ultracentrifugation of whole undiluted yolk as perfomed by Garland and Powrie (1978).

Agarose gel chromatography. FI and FII fractions from yolk granules were subjected to gel filtration with Biogel A-50M (Fig. 1). The 280 nm absorbance pattern of VLDL(MF) is a function not only of the concentration of protein but also of the light scattering effects of these turbid dispersions. The granule low density lipoprotein (LDL_g) from the FI fraction eluted earlier than that of the FII fraction, indicating that the LDL_g in FI had a higher molecular weight. Chang et al. (1977) observed LDL_g in both fractions by electron microscopy and found that FI contained globules which were much larger than LDL_g micelles in FII.

When the entire floating fraction containing both FI and FII fractions was used for gel filtration chromatography (Fig. 1, granules), the early elution of the LDL_g peak (tube number 130–190) limited the volume of VLDL(MF) at void volume that could be separated without contamination.

When whole yolk granules were applied to the column, the VLDL(MF) peak was eluted in the void volume, followed by the LDL_g, high density lipoprotein and phosvitin. The VLDL(MF) used in this study was isolated only from the FII fraction.

Carboxyamidation. During preliminary study it was observed that as VLDL(MF) samples aged, aggregates were formed. Raju and Mahadevan (1976) reported that the apoproteins from yolk plasma VLDL were insoluble in aqueous buffer systems, however, soluble in a buffer containing

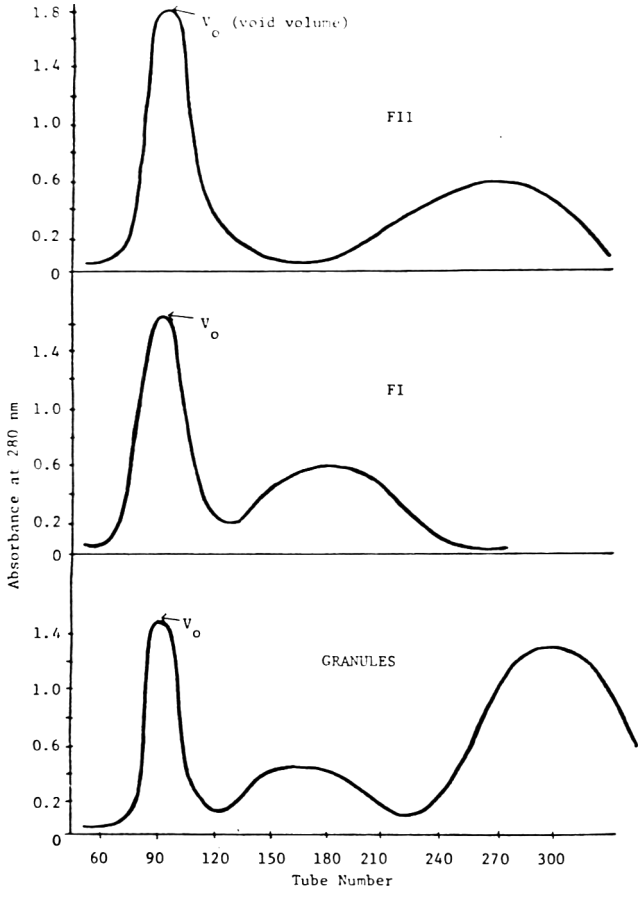

Fig. 1—Elution profiles of yolk granules, FI and FII filtered on Biogel A-50M columns. (Buffer = 1.0M NaCl, 0.025M sodium phosphate, 0.01% EDTA, pH 7.0).

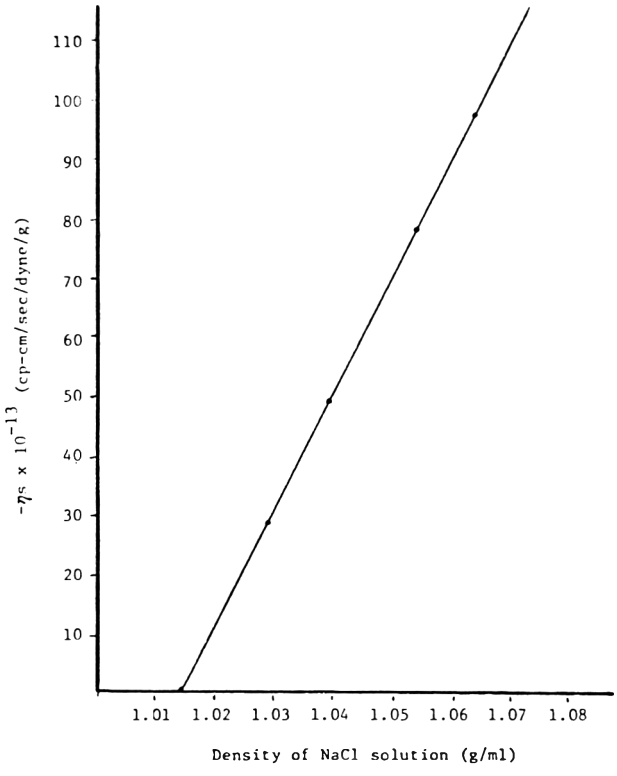

Fig. 2—Hydrated density of VLDL (MF) estimated from ηs and solvent density at 20° C.

0.01M ME. It was suggested that the insolubility of the proteins is partly due to the existence of inter- and intramolecular disulfide bridges. The aggregation of VLDL(MF) could have been due to oxidation of free sulfhydryl groups. The VLDL(MF) was therefore prepared from yolk diluted with deoxygenated, nitrogen flushed water containing 0.1% ME and all subsequent handling of granules and lipoproteins also performed in degassed buffers containing 0.1% ME. After isolation by gel chromatography, the VLDL(MF) was alkylated with iodoacetamide.

Iodoacetamide was chosen as the sulfhydryl-blocking agent since the reactivity of sulfhydryl groups with iodoacetamide far exceeds that of other reactive groups on proteins under almost all conditions (Means and Feeney, 1971). Modification of native lipoproteins with iodoacetamide has not been reported before. Experimental conditions were chosen such that the VLDL(MF) remained in its unaggregated state.

The SH and SS contents of the alkylated VLDL(MF) and unmodified control are shown in Table 1. Sulfhydryl content was determined after the addition of dodecyl sulphate which alters the native protein conformation exposing buried SH groups. As expected, the control VLDL(MF) had a high content of disulfide groups compared to the alkylated sample. Even after alkylation to block SH groups, the VLDL(MF) had a much greater SH content than that of the control. This would indicate that most of the SH groups were inaccessible to the iodoacetamide when the reaction was carried out in the absence of dissociating agents. These data suggest that most of the SH groups in the protein of the unmodified VLDL(MF) may have been oxidized to SS group during separation and storage of VLDL(MF).

Flotation velocity ultracentrifugation. Native VLDL(MF) revealed two major peaks with S_f^o rates of 75 and 207S, which were decreased to 41 and 108S upon carboxyamidation. When 0.1% ME was added to the native VLDL(MF) the flotation rates decreased to 45 and 116S. These results suggest that the native VLDL(MF) particles were disrupted by carboxyamidation of the exposed sulfhydryl groups or reduction of the disulfide bonds with ME.

The hydrated density of the slow floating component of VLDL(MF) was obtained by measuring the flotation rates of the lipoprotein at different solvent densities (Fig. 2). Extrapolation of the plot of the product of solvent viscosity and flotation rate as a function of solvent density gave values of 1.014 g/ml and 0.986 ml/g for hydrated density and partial specific volume, respectively. Accurate measurement of the faster floating component was difficult to obtain due to its high flotation rate.

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Table 1—Estimation of SH and SS contents of VLDL using Ellman's reagent

Sample	SH content* (μM/g protein)	SS content* (μM/g protein)
Unmodified VLDL (MF)	71.05 ± 2.50	308.85 ± 15.62
Alkylated VLDL (MF)	434.53 ± 20.25	83.47 ± 4.40

* Data reported as a mean of 6 determinations ± standard error of the mean

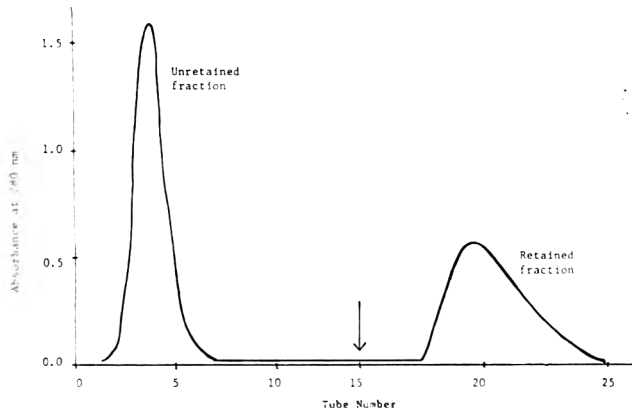

Fig. 3—Elution profile of alkylated VLDL (MF) from Con-A Sepharose 4B column (↓ indicates application of 0.2M methyl α-D mannopyranoside).

Turner and Cook (1958) studied the effect of lipid content on the partial specific volume of apoproteins and found that a 1% increase in the lipid portion of the lipoprotein caused an increase in the partial specific volume by 0.003 ml/g. Using this factor, and assuming a partial specific volume of 0.724 ml/g for the protein moiety of VLDL(MF), a theoretical partial specific volume of 0.982 ml/g corresponding to a hydrated density of 1.018 g/ml was calculated for VLDL(MF). These theoretical values are close to the experimentally derived values. The carbohydrate bound to the protein moiety of VLDL may have caused a decrease in the partial specific volume of the protein moiety (Gibbons, 1966).

The VLDL fraction of human serum is considered to possess a continuous distribution of molecules with diameters varying from 300–900 A (Scanu et al., 1974). Pig plasma VLDL (Fidge. 1973), rat plasma VLDL (Koga et al., 1969), rabbit VLDL (Shore et al., 1974), egg yolk plasma VLDL (Yamauchi et al., 1976), and chicken serum VLDL (Azuma et al., 1976) were also heterogeneous, showing a broad distribution in flotation rates with two or three major peaks when analyzed using the schlieren optics.

Gel electrophoresis. Samples of native VLDL(MF) and carboxyamidated VLDL(MF) were applied for electrophoresis using 1% agarose film and discs of the mixed gel of 3% acrylamide and 0.5% agarose. However, only one broad band at the origin that did not penetrate into gel was observed. Experiments were carried out using 0.5% agarose gel only and the native VLDL(MF) was prestained with sudan black B to trace the migration of the lipoproteins. Unstained samples run in parallel with the prestained samples were later stained for protein with Coomassie Blue. Two bands were detected for both VLDL(MF) samples. The first band migrated about three-quarters of the way through the gel, while the second band remained at the origin and did not enter gel. Prestaining had no effect on electrophoretic mobility. This technique facilitates immediate observation of the result and eliminates the staining and destaining steps thus requiring only less than 2 hr for completion of the entire process.

Affinity chromatography on Concanavalin A-Sepharose 4B. Carboxyamidated VLDL(MF) was applied to the Concanavalin A-Sepharose column and eluted with 0.2M methyl α-D-mannopyranoside (Fig. 3). Similar patterns have been reported with human serum VLDL (McConathy et al., 1974) and pig serum VLDL (Azuma et al., 1976). When samples of native VLDL(MF) were applied, no adsorption occurred. The sugar residues which would normally bind to the column may be unavailable for binding due to the aggregation of the lipoprotein.

Carbohydrate analysis of the totally delipidated fractions revealed that the apoVLDL contained a small amount of carbohydrate (2.51%), the retained VLDL almost twice as much (3.83%) and the unretained VLDL had only 1.03% (Table 2). The major carbohydrates detected in all three fractions were hexoses, with lesser quantities of hexosamine and sialic acid. The ratios of these sugars obtained from the three fractions are very similar. Raju and Mahadevan (1976) determined the carbohydrate composition of yolk plasma VLDL. They found the sugars present in a hexose:hexosamine:sialic acid ratio of 1.1:0.27:0.20, very similar to the results of the present study of egg yolk granule VLDL(MF).

Abraham et al. (1960) studied the S_f 20S and higher lipoprotein class of whole egg yolk and chicken serum. The carbohydrates bound to the lipoprotein obtained from both sources were present in the same ratios (1.1:0.5:0.3). They concluded that the serum lipoprotein was the source of the egg yolk lipoproteins.

The whole VLDL(MF) and retained and unretained VLDL(MF) fractions were examined by sedimentation velocity ultracentrifugation (Table 3). Two components were detected in all three samples with observed sedimentation coefficients of 13.87S and 56.48S for whole VLDL(MF), 13.29S and 53.16S for unretained VLDL, and 15.45S and 61.45S for retained VLDL indicating similar particle size for all samples.

The polypeptide compositions of the unretained and retained VLDL(MF) were analyzed by SDS gel electrophoresis in the presence of ME. These results are compared with those obtained on PAS stained gels (Fig. 4). The polypep-

Table 2—Carbohydrate composition of apoproteins from whole and fractionated lipoproteins

Sample	Hexose*	Hexosamine* (% of protein)	Sialic acid*	Total (%)	Hexose/hexosamine/ sialic acid ratio
VLDL (MF)	1.68 ± 0.08	0.58 ± 0.03	0.25 ± 0.03	2.51	1.0/0.35/0.15
VLDL (MF)-U	0.67 ± 0.03	0.22 ± 0.02	0.14 ± 0.01	1.03	1.0/0.33/0.21
VLDL (MF)-R	3.16 ± 0.15	1.08 ± 0.06	0.59 ± 0.03	4.83	1.0/0.34/0.19
VLDL[a] (yolk plasma)	1.91	0.52	0.39	2.82	1.0/0.27/0.20
VLDL[b] (chicken serum)	1.00	0.53	0.33	1.86	1.0/0.50/0.30
VLDL[b] (whole egg yolk)	1.30	0.67	0.38	2.35	1.0/0.50/0.30
VLDL[c] (pig serum)	2.23	1.67	0.83	4.73	1.0/0.75/0.37
VLDL-U[c] (pig serum)	0.93	0.47	0.14	1.54	1.0/0.50/0.15
VLDL-R[c] (pig serum)	4.20	3.21	2.05	9.46	1.0/0.76/0.49

* Mean of 3 determinations ± standard error of the mean
[a] From Raju and Mahadevan (1976)
[b] From Abraham et al. (1960)
[c] From Azuma et al. (1976)

Table 3—Sedimentation coefficients (S_{obs}) of VLDL (MF) and VLDL (MF) fractions from Concanavalin A affinity chromatography

Sample	Sedimentation coefficients (S_{obs})	
	Slow component*	Fast component*
VLDL (MF)	13.87 ± 0.68	56.48 ± 2.52
VLDL(MF)-U	13.29 ± 0.65	53.16 ± 2.34
VLDL(MF)-R	15.45 ± 0.76	61.45 ± 3.15

* Mean of 3 determinations ± standard error of the mean

tide bands of the two fractions of VLDL(MF) differed from each other fraction although few polypeptide bands were common. It can be seen that bands 1, 2, 4, 7, and 8 were predominant in the unretained VLDL(MF), while bands 1, 2, 5, and 10 were predominant in the retained VLDL(MF). Only band 1 in unretained VLDL(MF) reacted strongly with the PAS stain for glycoprotein. Bands 1, 5, 7, and 8 in the retained fraction were strong PAS positive bands, but other weakly positive PAS bands were observed in both fractions.

Delipidation of VLDL(MF)

Partial delipidation using n-heptane. The native VLDL(MF) particle could not be separated into subfractions by gel filtration techniques. However, there have been several reports concerning the fractionation of partially delipidated lipoproteins (Brown et al., 1969; Kalab and Martin, 1968; Augustyniak et al., 1964). Total delipidation of lipoprotein usually results in the formation of insoluble apoproteins. Partial delipidation with diethyl ether or n-heptane, however, results in the preferential removal of the neutral lipids and does not insolubilize the phospholipid-protein residues.

Carboxyamidated VLDL(MF) was delipidated with n-heptane according to the method of Gustafson (1965). Thin-layer chromatography of chloroform-methanol extracts of the partially delipidated lipoproteins indicated the absence of all neutral lipids and the presence of only one spot, corresponding to phospholipids. The partially delipidated VLDL(MF) was separated into two fractions by gel filtration on a calibrated Sepharose 4B column (Fig. 5). Peak I eluted at a volume equivalent to a molecular weight of 285,700 while peak II emerged at a volume corresponding to a molecular weight of 34,000. Partial delipidation produced soluble residues characterized by phospholipid: protein ratios of 0.59 and 0.83 for peak I and II, respectively. Neither of the fractions were homogeneous ultracentrifugally and contained two sedimenting fractions. Peak I contained 15.6S and 10.5S components and peak II 7.2S and 5.0S components in 0.2M Tris-HCl buffer, pH 8.2, at 45,000 rpm. A third peak was eluted at the void volume from unalkylated heptane-delipidated samples, which was likely to be an aggregate since it did not appear in the alkylated samples.

Gustafson et al. (1966) extracted human serum VLDL with heptane in the presence of insoluble starch. Three soluble phospholipid-protein residues were isolated by gel filtration. These fractions had sedimentation rates of 4S, 7S and 14S and their phospholipid:protein ratios were 0.9, 2.4, and 0.7, respectively.

The extraction of VLDL(MF) by the relatively nonpolar solvent heptane allowed the preparation of two partially delipidated VLDL(MF) fractions without insolubilization of the residual phospholipid-protein complex. In the case of LDL, it has been reported that the aggregation coincides with the removal of water from the medium, and compounds such as glucose, glycerol and sucrose in water have been found to protect serum and membrane lipoproteins from aggregation (Gustafson, 1965). The water-insoluble potato starch used in this study has many hydroxyl groups which offer the same protection to VLDL(MF) as water-soluble carbohydrates. Starch has the advantage that the phospholipid-protein residues can be easily separated from the insoluble starch by centrifugation.

Partial delipidation of aqueous suspensions of lipoproteins with diethyl ether usually results in incomplete removal of neutral lipid (Hayashi et al., 1959). Complete removal of neutral lipids from VLDL(MF) was achieved by extraction of the freeze-dried starch-VLDL(MF) mixture with n-heptane. The extraction of the lipoproteins in the

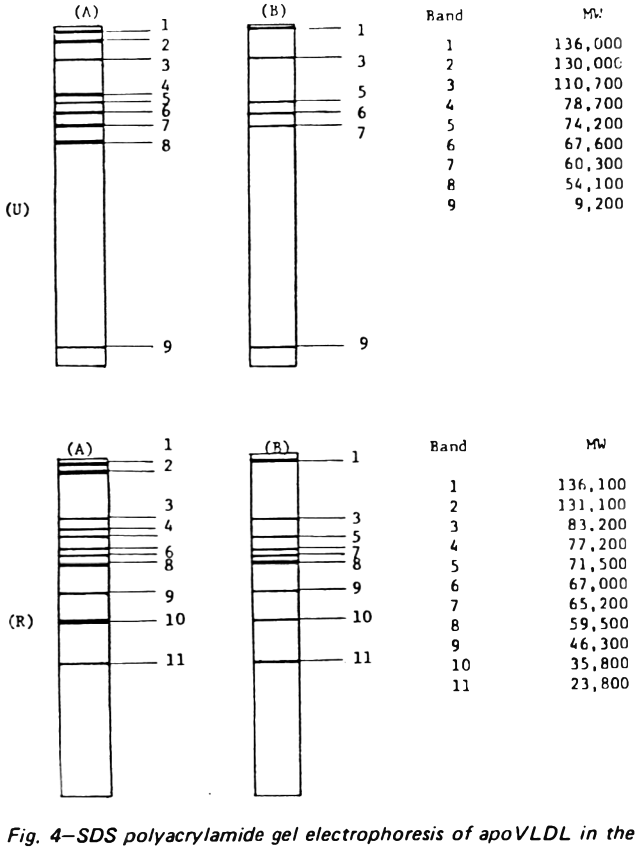

Fig. 4—SDS polyacrylamide gel electrophoresis of apoVLDL in the presence of ME of (U) Unretained fraction and (R) Retained fraction from Con A Sepharose 4B column stained with (A) Coomassie Blue and (B) PAS. Acrylamide concentration, 7.5%.

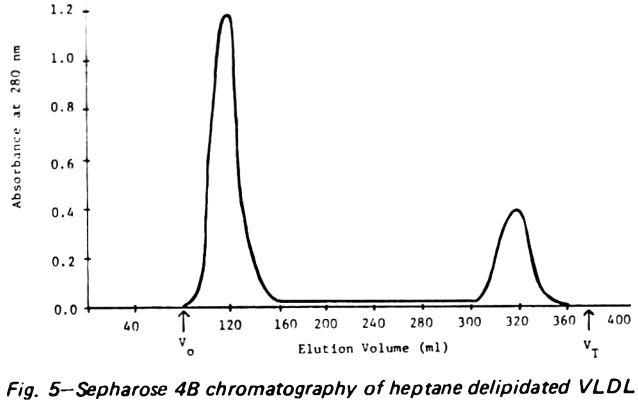

Fig. 5—Sepharose 4B chromatography of heptane delipidated VLDL (MF)

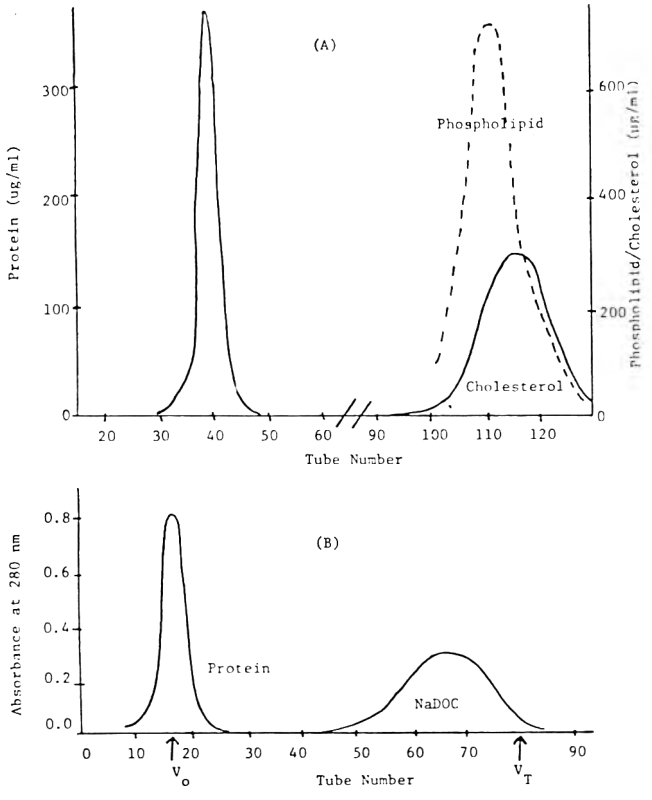

Fig. 6—(A) Separation of apoVLDL and lipids by gel filtration in the presence of NaDOC. (B) Removal of NaDOC from apoVLDL by gel filtration.

dried state is a more efficient means of extraction because of the increased surface area and closer contact between the heptane and VLDL(MF). Zilversmit (1964) demonstrated this by heptane extraction of cholesterol from lipoprotein monolayers spread between phosphate buffer and heptane.

Total delipidation with ethanol-ether. The method of Scanu and Edelstein (1971) using 3:1 (v/v) ethanol-ether has become one of the standard methods for delipidation of VLDL. Since small molecular weight peptides from VLDL are soluble in the ethanol-ether, complete recovery of N-containing compounds cannot be expected. These peptides may be recovered by changing the solvent ratio to 3:5 (Scanu and Edelstein, 1971). A long extraction time, the need to work at a low temperature and the utilization of large volumes of solvents are several disadvantages of this delipidation procedure.

The ethanol-ether treatment rendered even alkylated VLDL insoluble upon removal of lipid. This insolubility in aqueous solution is effected by protein-protein interactions which are of a reversible nature. Solubilization is presumably effected by interaction of urea or dodecyl sulphate with the more hydrophobic areas of the protein which become exposed when the lipid is removed (Kane et al., 1970). The apoVLDL(MF) produced by ethanol-ether delipidation was perfectly soluble in 8M urea or 1.0 mM dodecyl sulphate in Tris-HCl buffers. Only trace amounts of phospholipids were detected by thin layer chromatography in chloroform-methanol extracts of the apoprotein.

Total delipidation with sodium deoxycholate. ApoVLDL(MF) was prepared in a soluble but aggregated form using the NaDOC delipidation procedure and the viscous, clear amber solution produced was applied to a column of Sephadex G-200. The protein eluted at the void volume as a clear solution while the phospholipids and cholesterol eluted later in a separate peak (Fig. 6A). The protein contained no detectable neutral lipids or cholesterol when examined by chemical assay or by thin-layer chromatography. Less than 0.5% phospholipid remained bound to the apoprotein.

The detergent was removed from the apoprotein by gel filtration on Sephadex G-75 in a detergent-free medium. The protein eluted at the void volume and the detergent eluted very close to the total volume of the column (Fig. 6B). No NaDOC was detected when the apoprotein was assayed for the presence of the bile salt. The detergent-free apoprotein was highly soluble in pH 9.0 Tris-HCl buffer.

Sedimentation velocity analysis of the apoprotein, however, showed the presence of a rapidly sedimenting boundary, which indicated that the protein was in highly aggregated state. This aggregation may have been caused by the removal of the lipid from the lipoprotein or by the pressure and constant stirring as the sample was concentrated by ultrafiltration.

The detergent delipidated apoprotein was subjected to polyacrylamide gel electrophoresis in the presence of dodecyl sulphate; a sample of VLDL(MF) delipidated with ethanol-ether 3:1 was coelectrophoresed for comparison. The electrophoretic patterns of the two samples were identical.

Chen and Aladjem (1974) and Helenius and Simons (1972) used deoxycholate delipidation in conjunction with gel filtration to obtain a lipid free protein from human serum low density lipoprotein. Lipid removal by detergent treatment was postulated to be caused by an exchange in which deoxycholate is bound to hydrophobic regions in the protein previously occupied by lipid. The presence of micellar concentrations of detergent caused the formation of apoprotein-detergent complex micelles as well as detergent-lipid complex micelles. The protein-detergent complex can be separated from the mixed micelles by gel filtration in the presence of the detergent; the deoxycholate-lipid micelles are of smaller size and therefore elute as a separate peak. This delipidation procedure was examined because deoxycholate is thought to be a nondenaturing detergent and therefore a much milder treatment than organic solvent delipidation. Helenius and Simons (1971) demonstrated that deoxycholate delipidated LDL from human serum did not undergo any conformational changes; SDS-delipidation of LDL, however, caused denaturation of the protein moiety.

Delipidation with NaDOC was the mildest and most efficient method in the case of VLDL(MF) derived from egg yolk granules.

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STUDIES ON RELEASE OF COMPONENTS FROM FROZEN-THAWED LOW-DENSITY LIPOPROTEIN (LDL) OF EGG YOLK

T. WAKAMATU and Y. SATO

ABSTRACT

This experiment was carried out to confirm that the components of low-density lipoprotein (LDL) are not released from LDL during freezing and thawing. With the aid of ultrafiltration and gel filtration with Sephadex G 25, it was found that low molecular weight material was not liberated from frozen-thawed LDL. However, in centrifugation, a small amount of precipitate was found in unfrozen LDL and frozen-thawed LDL, and this amount was greater in the frozen-thawed LDL than in the unfrozen LDL. Specific gravities ranged from 1.02–1.04, and the phospholipid and protein contents of frozen-thawed and unfrozen LDL were higher than in the parent LDL. However, the SDS polyacrylamide gel electrophoretic patterns of the precipitates were the same as that of apo-LDL.

INTRODUCTION

WHEN EGG YOLK is frozen below −6°C and thawed, it loses its fluidity. This phenomenon is known as gelation. It has been shown that, as with the case of egg yolk, the viscosity of low-density lipoprotein (LDL) is increased and its solubility is extremely reduced by freezing and thawing. Thus, it has been generally accepted that LDL is mainly responsible for gelation of egg yolk (Powrie et al., 1963; Saari et al., 1964b; Sato and Aoki, 1975; Sato and Takagaki, 1976). Several investigators have suggested that the increase in the apparent viscosity of LDL solutions and the decrease in LDL solubility after freezing and thawing were caused by LDL aggregation (Kumar and Mahadevan, 1970; Kamat et al., 1976). However, the mode or mechanism of the aggregation is obscure.

Holdsworth and Finean (1972) suggested that the liberation of some components in the surface layer of LDL caused this gelation, based on their finding of a fraction rich in protein and phospholipid liberated from repeatedly frozen and thawed LDL. On the other hand, Kamat et al. (1976) suggested that gelation was caused by nonspecific aggregation due to cross-linking of peptides or phospholipids, but they did not take into account the possibility that some LDL components might be liberated by freezing and thawing.

The present investigation was undertaken to determine if any LDL components were released during freezing and thawing.

EXPERIMENTAL

Materials

LDL was prepared from White Leghorn hen's egg yolk by two methods. In method I, LDL was prepared according to Sato and Aoki (1975) except for the use of 0.15M KCl containing 1 mM EDTA and 0.02% sodium azide (NaN_3) instead of 0.15M NaCl. The floating LDL was solubilized in 10% KCl solution (d = 1.063 at 20°C) and centrifuged at 75,000 × G for 16 hr at 4°C. The semisolid floating LDL was then dialysed against 0.15M KCl solution containing 1 mM EDTA and 0.02% NaN_3.

In method II, LDL was prepared according to Martin et al. (1964), that is, the yolk was diluted with an equal volume of distilled water and centrifuged at 75,000 × G for 1 hr at 4°C. The supernatant NaCl concentration adjusted to 1M was centrifuged at 75,000 × G for 16 hr at 4°C. The floating LDL was diluted with 1M NaCl and centrifuged by the same procedure described above. The final LDL was dialysed against 1 mM EDTA-0.02% NaN_3 solution for 72 hr at 5°C.

Livetin and the granules were prepared according to Sato and Takagaki (1976).

Freezing treatment

In freezing treatment A, 10 ml of 5% LDL solution prepared by method I was frozen at −20°C for 48 hr in a stainless steel vessel (3 × 3 × 3 cm) and thawed at 25°C in a water bath. In freezing treatment B, 30% LDL solution prepared by method II was frozen in the same vessel as used in treatment A at −40°C for 6 hr and 72 hr and thawed at 25°C.

Viscosity measurement

The viscosity of LDL and frozen-thawed LDL (FLDL) was measured at 25°C by a cone-plate rotational viscometer (Models ELD and EHD, Tokyo Keiki Co., Tokyo).

Turbidity measurement

LDL solution and FLDL suspension were diluted to 0.3% of the protein with 1M NaCl. After being left for 30 min at room temperature, their absorbance at 660 nm was measured by electrophotometer (Model 200-20, Hitachi Seisakusho, Tokyo).

Ultrafiltration

Frozen-thawed LDL by treatment A (FLDL I) was diluted with a volume of distilled water and filtrated using Amicon DIAFLO equipment with XM 100 A membrane at 5°C. Compounds with a molecular weight of less than about 100,000 are considered to pass through this membrane.

Chemical analysis

Phosphorus was determined by the method of Bartlett (1959). Ninhydrin-positive materials (NPM) were determined according to the method of Yemm and Cocking (1955), after hydrolyzing the sample with 2.5N NaOH. Sugars were determined by the method of Dubois et al. (1956) and expressed as the mole equivalent of glucose. Nitrogen was measured by the Kjeldahl or Nessler method, and protein content was obtained by multiplying the nitrogen value by 7. (This value was calculated through analytical experiment). Total lipid was extracted by the method of Bligh and Dyer (1959). The phospholipid content was given by multiplying the lipid phosphorus value by 25. Triglyceride was determined by the method of Van Handel (1961) and expressed as a triolein equivalent. Cholesterol was determined by the method of Zak (1957).

Gel filtration of the filtrate

Sephadex G 25 was equilibrated with 0.15M KCl and packed in a glass column (1 × 80 cm). A sample was applied on the column and eluted with 0.15M KCl solution and fractionated by 5 ml portions. The void volume of the column was 75 ml as determined by using Blue Dextran 2000.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was carried out with a current of 2 mA per gel at pH 8.9 in 5% gel. After electrophoresis, protein and lipid were detected by staining with Amido Black and Oil Red 0 solution, respectively. Carbohydrate was stained with periodic acid Schiff (PAS) reagent according to the method of Zacharius et al.

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Table 1—Composition of the filtrate and the methanol-water phase of LDL and FLDL I

	Ultrafiltrate		Methanol-water phase	
	LDL	FLDL I	LDL	FLDL I
Total P (μg)	16	14	13	9
NPM (μmole)[a]	0.48	0.32	0.25	0.16
Sugar (μg)[b]	N.D.[c]	N.D.[c]	468	593

[a] NPM Ninhydrin-positive materials, leucine equivalent
[b] Glucose equivalent
[c] N.D. Not detected

Table 2—Fractionation of aqueous phase obtained by centrifugation of LDL and FLDL I[a]

	O.D. (280 nm)	
Portion	LDL	FLDL I
1	0.160	0.178
2	0.078	0.072
3	0.060	0.060
4	0.045	0.120
5	1.50	0.660

[a] Thirty ml of 5% LDL and FLDL I in 10% KCl solution or suspension were centrifuged for 16 hr at 75,000 × G and at 4°C. After centrifugation, the aqueous phase was eluted through the pinhole at the bottom of the tube and fractionated by 5 ml portions. Fraction 1 contained the lowest portion and Fraction 5 contained the uppermost portion.

(1969). Stained gels were traced by densitometer (Model CS 910, Shimadzu Co., Kyoto).

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was carried out essentially according to the method of Weber and Osborn (1969), in the presence of 2-mercaptoethanol, with a current of 6 mA per gel at pH 8.0 (0.2M Tris-HCl buffer containing 0.5% SDS) in 5% gel. A sample was applied on the gel after removal of lipid. Protein was detected by staining with Coomassie brilliant blue R 250. As molecular weight standards, cytochrome C (12,400), chymotrypsinogen (25,000) and Molecular Weight Marker (53,000 ~ 265,000) purchased from Daiichi Pure Chemicals Co., were used.

RESULTS

Chemical analysis and gel filtration of ultrafiltrates

The number of viable microorganisms in fractions LDL and FLDL I were extremely low, and thus it was assumed that microbial decomposition of the fractions was insignificant. The ultrafiltrates obtained from 50 ml of 5% FLDL I suspension and 50 ml of 5% LDL solution were analysed for phosphorus, sugars and NPM (Table 1). Sugars were not detected in either filtrate. Trace amounts of phosphorus and NPM were detected, but the differences between them were negligible. After their concentration by vacuum evaporator, the filtrates were fractionated with a Sephadex G 25 column by 5 ml portions (Fig. 1). In their gel filtration patterns, trace amounts of phosphorus and materials, absorbed at 280 nm, were found in some fractions. The phosphorus and NPM in both the filtrates are probably attributable to a few impurities in the prepared sample and/or the reagents used.

To reconfirm whether liberated materials were trapped in the FLDL mass, the residue of LDL and FLDL I obtained by ultrafiltration was delipidated by chloroform-methanol-water (1:2:0.8) according to the method of Bligh and Dyer (1959), and the methanol-water phase was evaporated to dryness under vacuum at about 60°C. The dry matter was dissolved in distilled water, diluted to 10 ml and

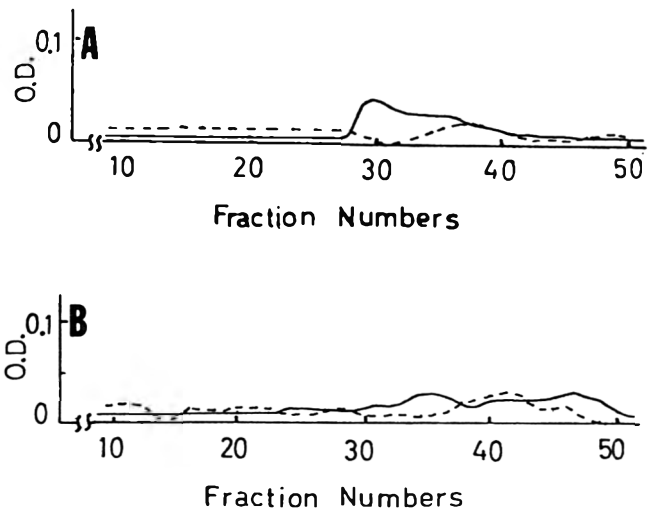

Fig. 1—Fractionation of the filtrates of (A) LDL and (B) FLDL I by Sephadex G 25 column: ——— O.D. (280 nm), ---- O.D. (660 nm) in phosphorus assay.

analysed for phosphorus, NPM and sugars (Table 1). Trace amounts of phosphorus and NPM were detected, but their LDL contents were higher than in FLDL I. The sugar content in the methanol-water phase of FLDL I was slightly higher than that of LDL. Thin-layer chromatography (TLC) of this concentrated methanol-water phase was carried out according to the method of Hansen (1975), but sugars were, for reasons unknown, not detected by spraying with aniline-diphenylamine-acetone-H_3PO_4. No difference in the TLC-pattern (omitted) of chloroform layers (lipid fraction) was found between the LDL and FLDL I.

Subfractionation of LDL and FLDL by centrifugation

In the centrifugation of 30 ml of the solution containing 10% KCl and either 5% LDL or FLDL I per tube for 16 hr at 75,000 × G and at 4°C, semisolid floating mass, floating yellow aqueous phase and transparent subnatant were found. However, no precipitate was encountered. The semisolid floating mass from the 5% LDL solution was designated as SF-LDL. The floating aqueous phases and the subnatants of both samples were fractionated by 5 ml portion. One ml of each portion was diluted to 10 ml with 1M NaCl, and the optical density of the diluted solution was measured at 280 nm. Based on the findings shown in Table 2, they were classified into two fractions; one was Fraction Aϱ of LDL or Fraction Aƒ of FLDL, which is the mixture of portion 1 ~ 3 containing the subnatant from each sample, and the other was Fraction Bϱ of LDL or Fraction Bƒ of FLDL, which is the mixture of portion 4 and 5 containing mainly the floating yellow aqueous phase from each sample.

The compositions of Fractions Aϱ, Aƒ, Bϱ, Bƒ and SF-LDL were shown in Table 3. The solid content in Fraction Aϱ was 0.2% of the total solid and contained no lipid. Based on the result of the polyacrylamide gel electrophoretic (PAGE) pattern of Fraction Aϱ and Aƒ (described later), this may mean that LDL prepared by method I contains about 0.2% contaminants. Total solid content in Fraction Aƒ was higher than in Fraction Aϱ by 4 mg per g of LDL. The difference in total solid between Fraction Aϱ and Aƒ might be caused by the fact that a part of Fraction Bƒ mingled in with Fraction Aƒ, because of the uncertain boundary between both phases. Assuming that the material in Fraction Aƒ may consist of 2 mg of the contaminant appearing in Fraction Aϱ and in 4 mg of the material which

Table 3—Composition of SF-LDL, Fraction Aℓ, Fraction Bℓ, Fraction Af and Fraction Bf.

	Fraction Aℓ	Fraction Bℓ	Fraction Af	Fraction Bf	SF-LDL
Total lipid (%)	0	86.9 (53.0)	55.0 (3.3)	82.0 (16.4)	88.6 (830)
Phospholipid (%)[a]	0	26.8 (14.2)	24.2 (0.8)	31.7 (5.2)	22.7 (188)
Protein (%)	100 (2.0)[d]	13.1 (8.0)	45.0 (2.7)	18.0 (3.6)	11.4 (107)
Protein P (%)[b]	0.7 (0.014)	0.2 (0.016)	0.2 (0.016)	0.4 (0.014)	0.2 (214)
Total solid (mg)[c]	2.0	61	6	20	937

[a] Percent of lipid
[b] Percent of protein
[c] per g of LDL or FLDL
[d] The values in parentheses indicate the amount per g of LDL or FLDL.

has the same composition as in Fraction Bf, the lipid and protein contents in Fraction Af could be calculated as 3.28 mg and 2.72 mg per g of LDL, respectively, and these values are almost the same as the measured values of Fraction Af.

Total solid, total lipid, phospholipid, and protein contents in the totals of Fraction Af and Bf excluding the contaminant (Fraction Aℓ) were calculated as 24.0 mg, 19.7 mg, 6.0 mg and 4.3 mg per g of LDL. However, these values were smaller than those of Fraction Bℓ by 37.0 mg, 33.3 mg, 8.2 mg, and 3.7 mg per g of FLDL, respectively. This result may indicate that a part of the material that appears in Fraction Bℓ is floated after freezing and thawing, and that the semisolid floating FLDL mass during centrifugation is composed thereof. If this is the case, the total lipid, phospholipid, and protein in the material would be calculated as 90%, 26.5% (percent of lipid) and 10.0%, respectively. The calculated composition of the material was similar to that of the semisolid mass of LDL.

The PAGE patterns of Fraction Aℓ, Bℓ, Af and Bf, livetin and granules were shown in Figure 2. Based on the PAGE patterns shown by Mc Bee and Cotterill (1979), the bands in the livetin PAGE pattern were identified as α, β, and γ-livetin as shown in Figure 2 (C). In Fraction Aℓ [Fig. 2(A) solid line], eight bands were found and designated as 1 ~ 8 in the order of increasing relative mobility. If some components were liberated from LDL by freezing and thawing, they should be fractionated in Fraction Af. However, the PAGE pattern of Fraction Af was almost the same as that of Fraction Aℓ, except that one broad band was shown instead of bands 1, 2 and 3 in Fraction Af [Fig. 2(A,B) solid line]. These bands were slightly stained with both Oil Red O and PAS reagent, and their relative mobilities were the same as those of granules and γ-livetins [Fig. 2(C,D)]. Bands 4, 5, and 6 of Fraction Aℓ and Af were stained with PAS reagents and their relative mobilities were the same as those of β-livetins. Bands 7 and 8 of Fraction Aℓ and Af may be phosvitin or α-livetin. In PAGE patterns of Fraction Bℓ and Bf [Fig. 2(A,B) broken line], most of the material could not pass through the stacking and running gel, so is was considered that the material in Fraction Bℓ and Bf was mainly composed of LDL. Several bands in the PAGE pattern of Fraction Bℓ and Bf, which had the same relative mobilities as those of bands 4, 5, and 6 of Fraction Aℓ and Af, were probably contaminants from Fraction Aℓ and Af. Based on these results, it is reasonable to assume that Fraction Aℓ is the contaminant of the LDL sample and any LDL component whose specific gravity, and molecular weight was less than 1.063 and about 100,000 daltons, was not liberated by freezing and thawing.

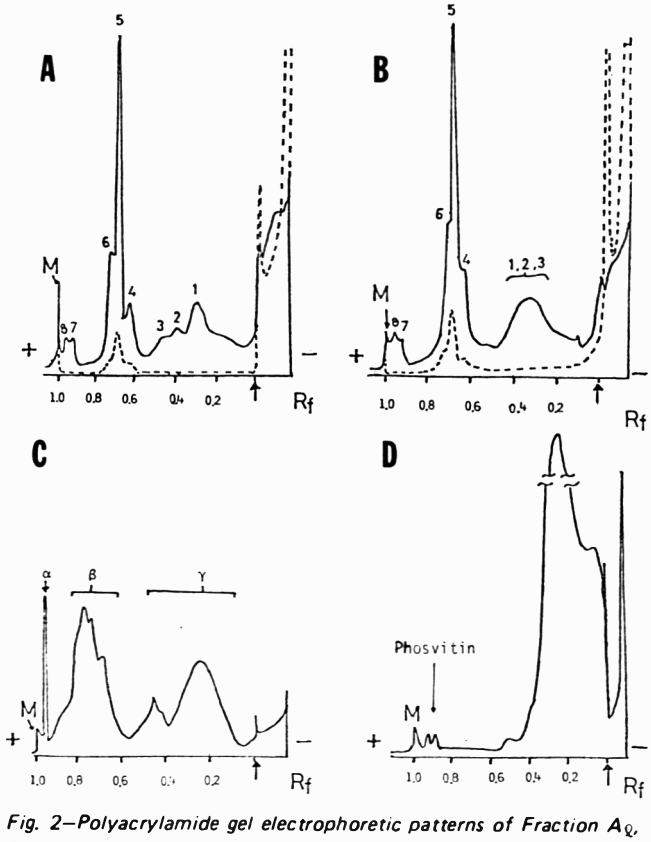

Fig. 2—Polyacrylamide gel electrophoretic patterns of Fraction Aℓ, Bℓ, Af and Bf, livetin and granule. Arrows indicate the boundary of the stacking gel and separating gel. M is the band of Bromphenol Blue used as marker. Gel was stained with Amido Black solution: (A) LDL —— Fraction Aℓ, ---- Fraction Bℓ; (B) FLDL I —— Fraction Af, ---- Fraction Bf; (C) livetin (D) granule.

Characterization of high density fraction from LDL and FLDL

According to the experiment of Holdsworth and Finean (1972), 10g of 30% LDL solution prepared by method II was frozen at −40°C for 3 hr and thawed at 25°C. This freezing and thawing cycle was repeated another 9 times. Frozen-thawed LDL obtained by this procedure was designated as FLDL R. LDL frozen at −40°C for 6 hr and 72 hr and thawed at 25°C was designated as FLDL 6 and FLDL 72, respectively. These FLDLs and LDL were diluted with three volumes of distilled water, then stirred for 16 hr at 5°C. The FLDL suspension and LDL solution were centrifuged for 100 min at 75,000 x G and at 4°C. Under FLDL R centrifugation, floating mass, turbid supernatant and precipitate appeared. In FLDL 6 and FLDL 72, turbid supernatant and a small amount of precipitate were found. In LDL, a trace amount of precipitate was detected, but the supernatant was transparent. The parts other than precipitates from these samples were again stirred for 16 hr at 5°C and then centrifuged in the same way as described above.

The precipitates were washed once by suspending with distilled water and centrifuging at 75,000 X G for 100 min at 4°C. The precipitates obtained in the first and second centrifugation were combined and their compositions were determined (Table 4). The amounts of the precipitates from LDL, FLDL 6, FLDL 72, and FLDL R were 0.52 mg, 1.47 mg, 1.32 mg, and 8.28 mg per g of LDL, respectively. Their compositions were extremely different from that of the parent LDL. Thus, the protein, phospholipid and cholesterol contents of the precipitates were higher than those of the parent LDL. Although there was almost no difference in composition or amount of precipitates of FLDL 6 and FLDL 72, the composition of the precipitates of FLDL R differed from that of FLDL 6 and FLDL 72. The viscosity and turbidity of FLDL R were higher than those of FLDL 6 and FLDL 72 (Table 5). These results indicate that the repeated freezing and thawing has a greater effect on gelation of LDL than continuous freezing.

The SDS polyacrylamide gel electrophoretic patterns of apo-LDL and the precipitates of FLDLs were shown in Figure 3. The electrophoretic pattern of apo-LDL was similar to that shown by Yamauchi et al. (1976) and Raju and Mahadevan (1976), except for the small difference in their estimated molecular weight. The electrophoretic patterns of the delipidated precipitates of FLDLs were almost the same as that of apo-LDL.

In order to investigate the proportionate effect of the dispersed precipitates on viscosity and turbidity, the portion of FLDL other than the precipitate was concentrated to the same solid content as undiluted FLDL, and its viscosity and turbidity were measured (Table 5). Contrary to expectations, the loss of precipitate from the FLDL fraction did not reduce the apparent viscosity significantly. These data indicate that the precipitate does not play an important role in the change of viscosity and turbidity of LDL.

To estimate their approximate specific gravity, these precipitates were suspended in 1 ~ 8% NaCl solutions and centrifuged at 75,000 X G for 60 min at 4°C. They were apparently insoluble in 1 ~ 8% NaCl solutions. The insoluble materials from LDL, FLDL 6 and FLDL 72 were floated in more than 7% NaCl solutions (Table 6). In FLDL R, a part of the insoluble material was found floating in 4% NaCl solution (d = 1.025), and floating materials increased with the increased concentration of NaCl; but in the end, no precipitate was found in 8% NaCl solution (d = 1.054). Based on these findings, the approximate specific gravity of

Table 4—Composition of LDL and Precipitates for LDL and FLDLs[a]

	protein (%)	TG (%)[b]	phospholipid (%)[b]	CH (%)[b]	Total (mg)[c]
LDL (ppt)	48.7	60.0	31.7	8.3	0.52
FLDL 6	39.1	57.2	34.8	8.0	1.47
	(33.8)	(56.0)	(36.2)	(7.8)	(0.95)
FLDL 72	39.3	59.7	32.6	7.7	1.32
	(33.1)	(59.5)	(33.1)	(7.4)	(0.80)
FLDL R	21.5	5.38	38.8	7.4	8.28
	(19.7)	(53.5)	(39.1)	(7.4)	(7.76)
LDL	12.0	68.8	25.7	5.6	–

[a] Figures indicate the average value from duplicate analysis. The values in parentheses were calculated by subtracting the precipitate in LDL from the values of those of FLDL 6, FLDL 72 and FLDL R, respectively. TG = Triglyceride; CH = Cholesterol and cholesterol ester
[b] Percent of total lipid
[c] Per g of LDL solid

Table 5—Viscosity and turbidity of LDL and FLDLs before and after centrifugation

		LDL	FLDL 6	FLDL 72	FLDL R
Apparent viscosity	control	7.6	512	540	737
@ 25°C (c.p.)	ppt removed[b]	5.4	480	512	680
Turbidity[a]	control	0.073	0.930	1.27	2.18
O.D. (660 nm)	ppt removed	0.113	1.05	1.42	2.11

[a] Solid concentration was 0.3% (w/v) in 1M NaCl.
[b] After removing precipitates by centrifugation, samples were concentrated to the same solid content as control.

Table 6—Estimation of specific gravity of the percipitates obtained from LDL and FLDLs

NaCl (%)	s.g.*	LDL	FLDL 6	FLDL 72	FLDL R
1	1.0041	+	+	+	+
2	1.0111	+	+	+	+
3	1.0185	+	+	+	+
4	1.0253	+	+	+	±
5	1.0325	+	+	+	±
6	1.0396	+	±	±	±
7	1.0470	–	–	–	±
8	1.0541	–	–	–	–

* s.g. specific gravity of NaCl solution
+ precipitate detected
± a part of insoluble material was floated
– all insoluble material was floated

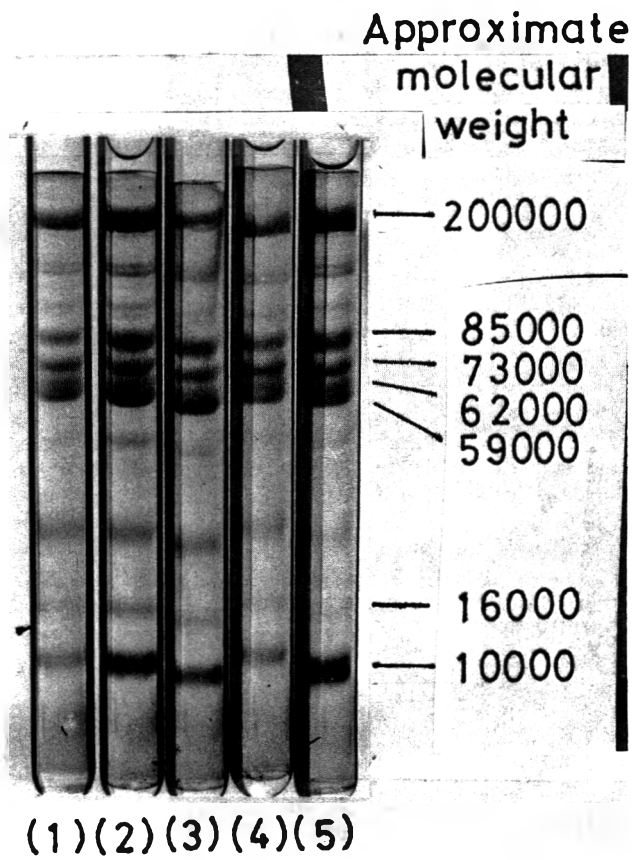

Fig. 3—SDS polyacrylamide gel electrophoretic patterns of (1) apo-LDL and delipidated precipitates of (2) FLDL 6, (3) FLDL 72, (4) FLDL R, and (5) LDL ppt.

the insoluble materials of LDL. FLDL 6 and FLDL 72 was estimated as 1.04, while that of FLDL R ranged from 1.02–1.04. The precipitates may not be contaminants derived from livetin or granules. This is because the specific gravity of high density lipoprotein, as shown by Hara et al. (1973), ranges from 1.25–1.34, and the delipidated precipitates show the same pattern as apo-LDL in SDS polyacrylamide gel electrophoresis. They may be either components liberated from LDL or a LDL subfraction which shows the same apo-protein pattern but less triglyceride than the parent LDL.

DISCUSSION

MAHADEVAN et al. (1969) suggested that the change in the surface charge of LDL might mainly involve its gelation, based on the finding that phospholipase C induced gelation without freezing. However, as shown in Table 1 and Figure 1, liberated material could not be found in the ultrafiltrate and methamol-water phase of frozen-thawed LDL. It is tempting to speculate that the cause of gelation may not be related to the removal of the fragment produced from LDL by the breakdown of the covalent bond during freezing. The mechanism of the gelation induced by phospholipase C is probably different from that caused by freezing and thawing.

Holdsworth and Finean (1972) showed that the protein and phospholipid contents of the precipitates obtained from repeatedly frozen and thawed LDL were higher than those of the parent LDL. As shown in Figure 3, the polypeptide composition of the precipitates from FLDLs was qualitatively the same as that of apo-LDL. These results suggest at least two contrary assumptions, namely: (1) the precipitate may be a by-product produced by liberation of a part of triglyceride which is a component of the LDL particle; and (2) the material precipitated by centrifugation may exist originally in LDL, and the alteration in its solubility and hydrated density may cause the precipitate. In this respect, it should be noted that LDL used in the above-cited experiment, as well as in the present one, is polydisperse, as shown by Martin et al. (1964) and Saari et al. (1964 a). In addition, a LDL subfraction with a composition similar to that of the FLDL precipitate has been found by Evans et al. (1973) and Gornall and Kuksis (1971). Moreover, in the present experiment, a trace amount of the precipitate was found even in native LDL during the 6th centrifugation. Recently, Chang et al. (1977) reported that the majority of globules in micrographs for frozen-thawed plasma had diameters somewhat greater than those for globules in native plasma, and that lipid masses were not found in the electron micrograph.

On the basis of these results, it is reasonable to assume that the precipitate that Holdsworth and Finean (1972) presumed to be a constituent of the surface layer might be a LDL subfraction, and the gelation may not have been caused by liberation or removal of such LDL components. We support the view that LDL gelation is induced only by a conformational change accompanied by a decrease in hydrated water.

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A Research Note

DETECTION OF GRAPE JUICE IN APPLE JUICE

DORA C. SMOLENSKY and CARL E. VANDERCOOK

ABSTRACT

A microbiological assay used by Vandercook and Smolensky (1976) to measure the content of orange juice in orange drinks was modified for the detection of the adulteration of apple juice by grape juice or water. An assay of 24 samples of retail apple juice, diluted 1:10 revealed little or no growth of *Lactobacillus plantarum* after 48 hr incubation, whereas solutions containing 8% of the same apple juice and 2% of a retail grape juice had a growth value in absorbance of 0.176 ± 0.039. When samples were supplemented with vitamin-free casein amino acids (AHC), bacterial growth increased dramatically in 21 of the juices. Assays run on juice prepared in the laboratory from fresh apples gave the same results, eliminating the possibility that the assay was dependent on the extraction procedure. Solutions of two retail samples of apple juice showed no growth with addition of amino acids which might indicate that the products were processed differently, were less than single strength, or were "synthetic" juices. One drink mix labelled "artificially flavored" apple cider did not support any growth either. Thus, the adulteration of apple juice with grape juice, overdilution, or use of synthetic products may be detected with the aid of a microbiological assay.

INTRODUCTION

ADULTERATION of apple juice is an important economic problem in the competitive apple and beverage industries around the world (Wallrauch, 1975). Quality products are sometimes diluted with cheaper additives, to obtain high profits from misrepresented commodities. Grape juice concentrates or deacidified grape juices are often used as a base for these adulterations. Detection of the presence of grape juice in apple concentrates is also important because of tariff regulations for importation of apple and grape juices into the United States. Apple juice may be imported free of duty, whereas mixtures of apple juice and grape juice are assessed at the higher rate applicable to grape juice (Adams, 1978). Some adulterations tend not to adversely affect the relative nutritional value of a beverage. For example, adding grape or pear juice products to apple juice still results in a nutritive beverage, however overpriced. Adulteration with water, on the other hand, may cause a significant drop in the nutritional value. All types of adulteration are, nevertheless, considered illegal and subject to control. Many constituents have been proposed as indices of authenticity, such as proline, arginine (Tanner and Sandoz, 1973), tartaric, and malic acid (Blumenthal and Helbling, 1977). However, many of these individual juice constituents vary significantly in fruit from different geographical regions even though the proportions may be relatively constant in any given area.

We have proposed a microbiological method similar to the one used by Vandercook and Smolensky (1976) to measure adulteration in apple juice. The organism used in the assay is *Lactobacillus plantarum* a species that is very fastidious and requires many nutrients for growth (Ledesma et al., 1977). The assay, therefore, also determines indirectly the presence of some compounds that might be important in human nutrition.

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MATERIALS & METHODS

Apple and grape juice samples

The commercial apple juices used in the assays were purchased from local supermarkets. Unfiltered samples were centrifuged at 34800 × G for 15 min before use. Fresh apple juice was prepared by blending 380g of peeled apples with 600 ml of water in a Waring Blendor. The suspension was centrifuged at 27000 × G for 15 min and filtered by vacuum. White grape juice, also bought at the local supermarket, (Welch's E-3592-12NB-246) was clear and did not have to be centrifuged.

Inoculum

A 5% aqueous solution of acid hydrolized vitamin-free casein (Difco 0288-02) was used as a nutrient supplement. Samples of *L. plantarum* NRRLB-1921 were obtained from the Northern Regional Research Laboratory, USDA, Peoria, IL 61604. The inoculum was prepared by subculturing from a stock culture of *L. plantarum* into a tube containing 10 ml of Bactolactobacilli MRS broth (Difco 0881-02). After 24 hr of incubation at 37°C, the cells were centrifuged under sterile conditions, decanted, and washed twice with 0.85% sodium chloride. Then, 10 ml of the cell suspension was diluted 1:10 with sterile saline. One drop of inoculum was used for each assay tube.

Sample preparation

Ten milliliters of single strength filtered apple juice or a mixture of 8 ml apple juice and 2 ml grape juice was adjusted to pH 6.5 with NaOH, and the volume completed to 100 ml with water.

One set of seven culture tubes (25 × 150 mm) for each type of juice sample was used. The tubes were prepared with 10 ml of a 1:10 dilution of the juices as described above. Three of the tubes had an extra addition of 100 microliters of a 5% solution of vitamin-free casein amino acids. All tubes were then autoclaved for 15 min at 15 lb pressure at 121°C and cooled to room temperature. One drop of the inoculum was added to each of the tubes, except for one control tube. The suspensions were mixed by shaking and incubated at 37°C for 48 hr. Bacterial growth (as turbidity) was deter-

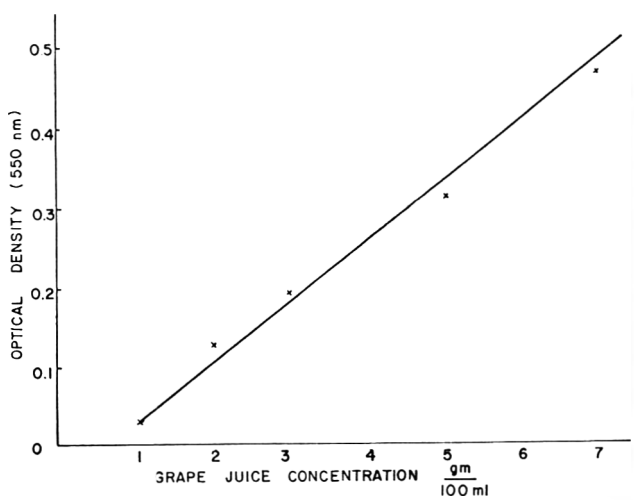

Fig. 1—Relationship between bacterial growth and grape juice concentration.

RESEARCH NOTES

Table 1—Bacterial growth (turbidity) in media containing 24 commercial apple juices after 48 hr of incubation at 37° C

Sample no.	10% Apple juice[a]		8% Apple juice +2% grape juice[b]		Growth due to grape juice	
	10 ml juice	10 ml juice +5 mg AHC	10 ml mixture	10 ml mixture +5 mg AHC	Without AHC[c]	With AHC[c]
1	0.06	0.43	0.20	0.68	0.14	0.25
2	0.05	0.21	0.19	0.66	0.14	0.45
3	0.01	0.12	0.11	0.50	0.10	0.38
4	0.03	0.36	0.15	0.91	0.12	0.55
5	0.02	0.37	0.13	0.62	0.11	0.25
6	0.03	0.33	0.14	0.71	0.11	0.38
7	0.03	0.61	0.21	0.98	0.18	0.37
8	0.05	0.55	0.23	0.87	0.18	0.32
9	0.05	0.39	0.18	0.93	0.13	0.54
10	0.03	0.62	0.22	0.90	0.19	0.28
11	0.03	0.39	0.13	0.63	0.10	0.24
12	0.03	0.41	0.13	0.59	0.10	0.18
13	0.10	0.54	0.22	0.80	0.12	0.26
14	0.06	0.40	0.15	0.52	0.09	0.12
15	0.05	0.26	0.23	0.52	0.18	0.26
16	0.05	0.28	0.17	0.81	0.12	0.53
17	0.04	0.36	0.12	0.53	0.08	0.17
18	0.02	0.39	0.21	0.86	0.19	0.47
19	0.04	0.59	0.18	0.82	0.14	0.23
20	0.04	0.51	0.21	0.69	0.17	0.18
21	0.08	0.47	0.19	0.65	0.11	0.22
Avg	0.043 ± 0.02	0.409 ± 0.131	0.176 ± 0.039	0.722 ± 0.151	0.133 ± 0.035	0.316 ± 0.130
22	0.02	0.07	0.16	0.64		
23	0.01	0.02	0.07	0.52		
24	0	0	0.07	0.49		
Avg	0.01 ± 0.01	0.03 ± 0.03	0.1 ± 0.05	0.55 ± 0.07		

[a] Relates to 100% apple juice
[b] Relates to 80% apple juice + 20% grape juice
[c] AHC = Acid-hydrolyzed casein. Values are the averages of three determinations

Table 2—Bacterial growth (turbidity) in laboratory-prepared apple juice

Concentration (%)	Turbity[a] (OD_{550})
6.7	0.04
13.5	0.02
27	0.03
48	0.02

[a] OD_{550} = optical density at 550 nm.

mined by measuring absorbance at 550 mμ in 1 cm cells of the inoculated assay mixture against the uninoculated control.

RESULTS & DISCUSSION

COMMERCIAL APPLE JUICES and mixtures of apple juices with grape juice were examined by a microbiological assay (Table 1). Addition of essential amino acids (Dunn et al., 1947) did not increase the growth of bacteria (data not shown). However, addition of acid-hydrolysed vitamin-free casein amino acids (AHC) did significantly (P = 0.05) increase the growth of the bacteria (Table 1), suggesting that although pure apple juice lacks some amino acids, it provides other essential and/or stimulatory nutrients required by *L. plantarum* for growth.

The average increase in growth due to 2% grape juice was 0.133 ± 0.035. On an independently prepared standard curve of bacterial growth plotted against grape juice concentration, this value was equivalent to 2.2% grape juice (Fig. 1). Thus, the presence of grape juice in apple juice was detectable with a microbiological assay.

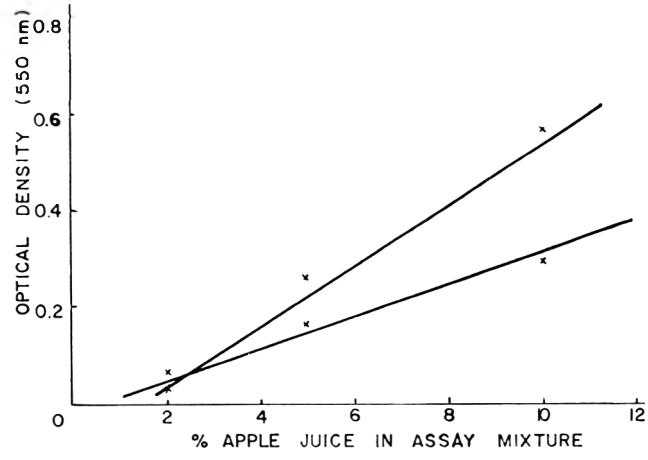

Fig. 2—Standard growth curves of bacteria grown on media containing different concentrations of samples 7 and 9 with AHC.

L. plantarum grown on a medium containing laboratory-prepared apple juice showed essentially no differences in growth at different concentrations of juice (Table 2). Samples 22 and 23 showed no growth when AHC was added which would suggest that they have been diluted with water. Sample 24, a commercial product labelled "artificially flavored apple cider" did not favor any growth either. Because bacterial growth increased significantly when we cultured these samples with added grape juice and AHC, we eliminated the possibility of a growth inhibitor.

—*Continued on page 1780*

A Research Note

RELATIONSHIP BETWEEN ACCEPTABILITY OF PACKAGED FRANKFURTERS AND EATING QUALITY

H. W. OCKERMAN and V. R. CAHILL

ABSTRACT

Four brands of frankfurters were evaluated for packaging and appearance of product in the package. In both catagories, consumers found significant differences ($P < 0.01$) between brands. When these same four brands of frankfurters were coded, cooked and evaluated, only minor differences could be detected and acceptance differences were nonsignificant ($P > 0.05$). This suggested that package appearance, product appearance in the package, and/or prior reputation of product or manufacturer may have a major influence on sale of frankfurters which are very similar in organoleptic or cooked color properties. Sex of consumer and frequency of consumption of frankfurters also influenced evaluation of the cooked frankfurters.

INTRODUCTION

FRANKFURTERS are a very popular meat item and were found to be consumed by 95% of the households sampled (Union Carbide, 1974). This same survey showed that adults eat frankfurters more frequently than children, young children eat more than older children, and that women eat more than men.

Initially food packaging was for product protection, but today packaging is also an intergral part of merchandising and every packaging decision has an important effect on sales (Sacharow and Griffin, 1970). Forrest et al. (1975) stated that the purpose of a package is to attractively display the product to the consumer. Manufacturer identification is also accomplished by package labeling. Sacharow and Griffin (1970) also stated that packaging is now considered a top management marketing tool.

The purpose of this research was to evaluate the influence of visual appearance on consumer acceptance of sausage items and to investigate if preferences noted were substantiated by sensory evaluation of the cooked product. This project was designed to compare consumer acceptance of four commercially packaged brands of frankfurters at the point of sale and also compare by means of a laboratory panel, these same four brands in a coded fashion at the point of consumption.

EXPERIMENTAL

FOUR BRANDS (A, B, C, D) of skinless frankfurters were selected for testing. In each case the label indicated that the product contained beef and pork and did not contain variety meats. All four products also contained the same label ingredients.

Consumer evaluation of brand identifiable packaged frankfurters

Two self-service retail grocery stores (one in a high income area and one in a medium income area) owned by the same organization were selected which offered for sale all four of the brand-name frankfurters tested. Consumer evaluations were conducted in each store on five different days and a total of 861 evaluations were made by 861 different customers. Four 16-oz (453.6g) packages, one of each brand, were displayed on a green tray for consumer evaluation. Relative positions of the brands on the tray were rotated periodically. Shoppers were invited to participate in the evaluation as they approached the meat department of the store. Each consumer was asked to rate each brand on two factors: (1) "appearance of package," including color combinations and package design; and, (2) "appearance of frankfurters" as viewed through the packaging material. The consumers were asked to rate both factors on a 1 (dislike) to 10 (like) hedonic scale. Four consumer forms were utilized to provide rotation of brands on the form.

Panel evaluation of coded cooked frankfurters

Two liters of tap water were heated to boiling in a 2.8 liter aluminum saucepan. Four frankfurters were placed in the water and the pan was removed from the heating element. The frankfurters were allowed to heat for five minutes and then cut into four sections, coded, and placed on a paper plate and served to the panel while still warm. This procedure was repeated for all brands and presented to a laboratory sensory panel for evaluation. Lawrie (1974) stated that the evaluation of a meat product for odor and taste is best accomplished by a taste panel. The panel evaluation procedure was repeated until 425 evaluations were obtained. Fifty-two different panel members were used. Panel members were asked to list age, sex, frequency of eating frankfurters per month, (0–3, 4–5, 6–7, 8 or more) and if frankfurters were one of their favorite foods (yes, no). Panel members were then asked to rate the cooked frankfurters on the following factors: saltiness (10–very salty, 1–not salty); spice flavors (10–very spicy, 1–no spice); smoke flavor (10–strong smoke flavor, 1–no smoke flavor); frankfurter flavor (10–characteristic frankfurter flavor, 1–off flavor); texture or bite (10–firm, 1–mushy); color (10–dark, 1–light); and, total acceptability (10–very acceptable, 1–unacceptable).

Analysis

All data were evaluated by least squares analysis (Harvey, 1968) and Duncan's multiple range (Harter, 1960).

RESULTS & DISCUSSION

Evaluation of package appearance

The least squares analysis of variance indicated no significant difference ($P > 0.05$) for "package appearance" between stores and a nonsignificant difference ($P > 0.05$) for the "product brand" × "store" interaction suggesting that the store in which the consumer shopped or income level (high, medium) had no major influence on the consumer's acceptance of "package appearance" for these four brands. There was a highly significant difference in "package appearance" for "product brands" ($P < 0.01$) as shown in Table 1. Acceptance of "package appearance" of Brand A, was significantly lower than for Brands B, C, or D. This would suggest that Brand A had a less consumer acceptable package than the other brands or that advertising, prior experience with the product, or product manufacturer's reputation was causing a bias in the consumer panel. The panelists were instructed to ignore prior commitments to a particular brand and to evaluate only the package, but this is extremely difficult to insure.

Evaluation of product appearance in package

"Product appearance" in the package also showed no significant ($P > 0.05$) difference between "stores" and a nonsignificant ($P > 0.05$) "product brand" × "store" interaction. The differences between "brands" were less pro-

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Table 1—Retail consumers evaluation (least square means) and coded and cooked evaluation (least square means ± standard errors) for frankfurters by brands[a]

Attributes evaluated	Brands			
Retail consumer evaluation	A	B	C	D
Package appearance**	5.26a	7.52b	6.82b	7.33b
Product appearance in package**	6.56c	7.50e	6.96d	7.83e
Coded & cooked evaluation[b]				
Saltiness[NS]	5.77 ± 0.23	5.99 ± 0.24	5.66 ± 0.23	5.59 ± 0.22
Spiciness[NS]	6.09 ± 0.24	5.17 ± 0.25	5.53 ± 0.24	5.90 ± 0.23
Smoke flavor[AS]	6.27 ± 0.23	6.44 ± 0.24	5.70 ± 0.24	6.38 ± 0.23
Frankfurter flavor[NS]	6.06 ± 0.24	6.10 ± 0.25	5.67 ± 0.24	5.73 ± 0.23
Texture*	6.28 ± 0.24b	6.21 ± 0.25b	5.55 ± 0.24a	5.69 ± 0.23a
Color (cooked)[NS]	5.95 ± 0.23	6.17 ± 0.24	5.78 ± 0.23	5.99 ± 0.22
Acceptance[NS]	6.05 ± 0.23	6.21 ± 0.24	5.64 ± 0.23	5.67 ± 0.22

[a] Mean values in one row which have unlike letters differ significantly ($P < 0.05$).
[b] NS = nonsignificant ($P > 0.05$); AS = Approaching significance ($P < 0.10$).
* Significant ($P < 0.05$)
** Highly significant ($P < 0.01$)

Table 2—Evaluation (least square means) of coded, cooked frankfurters. Separated according to evaluator's age, sex, frequency of consumption and preference for frankfurters[a]

Attribute evaluated	Age[b]	Sex[c]		Frequency of consumption per month*				Favorite food	
		Male	Female	0-3	4-5	6-7	8-more	Yes	No
Saltiness	NS	6.33**	5.48	5.81b	6.18bc	6.85c	4.76a	5.78	6.02
Spiciness	NS	6.06[NS]	5.63	5.77b	6.07bc	6.77c	4.79a	5.77	5.93
Smoke flavor	NS	6.32[NS]	6.09	6.17b	6.30bc	7.03c	5.33a	6.15	6.26
Frankfurter flavor	NS	6.13[AS]	5.60	5.73b	6.01b	6.89c	4.82a	5.79	5.95
Texture	NS	6.38**	5.59	6.36b	6.15b	6.81b	4.61a	6.07	5.90
Color (cooked)	NS	6.79**	5.73	6.49b	6.74b	7.10b	4.71a	6.35	6.17
Acceptance	NS	6.15*	5.59	6.00b	6.12b	6.95c	4.42a	5.97	5.77

[a] Mean values in the same row under "Frequency of Consumption" which have unlike letters differ significantly ($P < 0.05$).
[b] Analysis of variance linear regression of age. NS = Not significant ($P > 0.05$).
[c] NS = not significant ($P > 0.05$); AS = Approach significance ($P < 0.10$).
* Significant ($P < 0.05$)
** Significant ($P < 0.01$)

nounced for "product appearance in the package" than they were for "package appearance", but there was still a highly significant ($P < 0.01$) difference in "product appearance" when different brands were compared. Color is very important in product acceptability and Forrest et al. (1975) stated that its importance is equal to flavor and texture. Price and Schweigert (1960) reported that color is the prime factor in influencing consumer selection of packaged meat. Brand A again was evaluated significantly ($P < 0.01$) lower than Brands B, C or D and Brand C was evaluated significantly ($P < 0.01$) lower than Brands B and D. This would suggest that product appearance was less desirable for Brands A and C. However, prior knowledge of the product may have influenced the consumer. Another factor that might have been important is that "product appearance" was evaluated after "package appearance" and this could have also influenced the evaluation. There was a highly significant ($P < 0.01$) correlation of r=0.44 between "package appearance" and "product appearance" in the package.

Regardless of the causes, the consumer ranked Brand A as the least desirable followed by Brand C in both "package appearance" and "product appearance" in the package.

Evaluation of coded cooked frankfurters

"Consumer age" (Table 2) proved to be nonsignificant ($P > 0.05$) for all attributes evaluated on the coded cooked product. Males evaluated all attributes of the cooked frankfurters higher than females and this difference was significant for saltiness, texture, color, acceptance, and approached significance for frankfurter flavor. Analysis of variance for "frequency of consumption" of frankfurter was highly significant ($P < 0.01$) for all attributes evaluated. Evaluators who consumed frankfurters eight or more times per month consistently ($P < 0.05$) scored all attributes lower than panelists who consumed fewer frankfurters. The next higher scores were recorded by people who consumed 0–3 and then 4–5 frankfurters per month. Differences between these last two groups were not significant ($P > 0.05$). The highest scores were recorded by panelists who consumed frankfurters 6–7 times per month and they were usually (Table 2) significantly higher than the panelists who consumed fewer products. Whether or not panelists considered "frankfurters one of their favorite foods" had no significant influence ($P > 0.05$) on the rating received by any attributes.

A comparison of Brands (A, B, C, D) (Table 1) for acceptance, cooked color, saltiness, spiciness, smoked flavor, and frankfurter flavor resulted in a nonsignificant ($P > 0.05$) difference between brands. The lack of difference in color between brands agrees with Puolanne (1975) who found little color differences in sausages that contained the same ingredients even though quantity relations were different. Texture was significantly different ($P < 0.05$) with Brands A and B being significantly ($P < 0.05$) firmer in texture than C and D. Smoke flavor differences approached significance ($P < 0.10$) with Brand C having less smoke

—continued on page 1778

A Research Note

HARD CHEESE SUBSTITUTE FROM SOY MILK

W. M. ABOU EL-ELLA

ABSTRACT

A substitute for "Ras," a hard cheese, was made successfully from soy milk. The product gained a cheesy flavor during 3-months of ripening. Body and texture and color of cheese made with soy milk were compared to cheese made with a mixture of soy milk and cow's milk. Results showed that body and texture, as well as color, were relatively lower than cheese made from a mixture of soy milk and cow's milk. The latter possessed more acceptable properties.

INTRODUCTION

MILK SUBSTITUTES have received world-wide interest in order to overcome the relatively limited milk supply especially in developing countries.

It has been indicated that soybeans contain a high percentage of protein with balanced amino acid (Smith and Circle, 1972).

During the past few years soy milk has been prepared from soy beans and was used for making some dairy products (Kim and Shen, 1971; Hofi et al., 1976).

The present study was planned to investigate the use of soy milk in making a hard cheese substitute.

EXPERIMENTAL

SOY MILK was prepared from "Wayne variety" soy beans as suggested by Hwa (1967).

The following steps were adopted for making the cheese substitute.

Three replicates were carried out using 25 kg of soy milk which was heated to boil, then cooled to about 37°C. With gentle mixing, 250g of starter *Streptococcus lactis* were added followed by a 500-g emulsion of 30% good quality ripened "Ras" cheese solids.

Coagulation was carried out after 1 hr using 75g calcium lactate in 400 ml water, which was added very slowly to the soy milk.

When the coagulum had settled, it was gently transferred with a ladle to a wooden box lined with a double layer of cheese cloth; corners of the cloth were folded over the coagulum, and the coagulum was then pressed with a light weight until the whey had almost stopped draining (approximately 2 hr). Finally it was pressed, salted in brine, followed by dry salt through the ripening period.

In four comparative treatments of three replicates each, "Ras" cheese was also made using cow's milk and mixtures of soy milk and cow's milk in ratios of 1:3, 1:2 and 1:1. In general, the manufacturing procedure of Hofi et al. (1970) was used.

Methods of analysis

Samples were analyzed for moisture and fat contents according to British Standard Method (1952). Total nitrogen and acidity were determined according to Ling (1963).

Soluble tyrosine and tryptophan contents were estimated by methods given by Vakaleris and Price (1959). Also, cheese samples were scored for flavor as well as body and texture, color, and appearance during ripening according to Nelson and Trout (1951). (The score points were, flavor 50, body and texture 25, color 10, and appearance 5.)

RESULTS & DISCUSSION

TABLE 1 represents the changes in chemical properties of the cheese substitute and cheese made from soy milk-cow's

Table 1—Some properties of cheese substitute from soy milk and soy milk-cow's milk mix comparable to cow's milk cheese

Cheese milk	Ripening period	Moisture % (MNFM)[a]		Fat % (FDM)[b]		Acidity %	Total N %	Soluble tyrosine %	Soluble tryptophan %
Soy milk	Fresh	46.89	51.8	9.50	17.9	0.93	3.58	38.30	24.28
	1 month	44.24	49.2	10.10	18.1	0.98	4.10	40.70	25.80
	2 month	41.54	46.8	11.30	19.3	1.20	4.50	62.00	28.20
	3 month	37.41	42.4	11.80	18.9	1.25	4.90	65.15	33.50
Soy milk:	Fresh	45.65	51.2	10.80	19.9	0.90	4.26	39.04	25.02
cow's milk	1 month	43.07	48.7	11.50	20.2	0.95	4.54	41.53	25.66
mix 1:1	2 month	40.30	46.2	12.75	21.4	1.18	4.91	63.00	29.32
	3 month	37.00	43.4	14.80	23.5	1.21	5.20	65.88	35.11
Soy milk:	Fresh	45.10	51.0	11.60	21.1	0.87	4.46	40.76	24.90
cow's milk	1 month	42.96	48.9	12.20	21.4	0.91	4.70	42.87	26.22
mix 1:2	3 month	35.55	41.9	15.20	23.6	1.15	5.58	67.20	35.61
Soy milk:	Fresh	44.20	50.9	13.20	23.7	0.85	4.57	42.85	25.17
cow's milk	1 month	42.10	48.9	13.90	24.0	0.89	4.82	45.64	26.50
mix 1:3	2 month	38.50	45.3	15.10	24.6	1.10	5.31	65.01	30.62
	3 month	35.00	44.9	16.40	25.2	1.02	5.95	67.90	36.09
Cow's milk	Fresh	42.40	52.4	19.10	33.2	0.84	4.76	46.33	26.38
(Control)	1 month	41.30	51.6	20.00	34.1	0.86	4.91	48.80	27.20
	2 month	35.50	46.6	23.80	36.9	0.88	6.65	65.30	31.40
	3 month	33.40	44.5	25.00	37.5	0.92	7.88	70.00	37.66

[a] MNFM = Moisture in the nonfat matter.
[b] FDM = Fat in the dry matter.

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Table 2–Sensory properties of cheese substitute from soy milk and soy milk-cow's mixtures comparable to cow's milk cheese

Cheese milk	Riping period (mo)	Flavor 50	Body & texture 35	Color 10	Appearance 5	Total
Soy milk	1	25	25	3	2	55
	2	30	27	3	2	62
	3	32	28	3	2	65
Soy milk:	1	30	27	4	2	63
cow's milk	2	32	22	4	3	65
mix 1:1	3	40	30	3	3	76
Soy milk:	1	35	28	5	3	71
cow's milk	2	38	30	4	3	75
mix 1:2	3	40	30	4	2	76
Soy milk:	1	48	28	7	3	86
cow's milk	2	40	27	7	3	77
mix 1:3	3	42	30	6	3	81
Cow's milk	1	40	30	8	4	82
	2	43	32	7	4	86
	3	46	32	7	4	89

milk mixture as well as cheese made from cow's milk.

Using only soy milk helped the product produced to retain more moisture. Increasing the percentage of soy milk to cow's milk, resulted in an increase of moisture content, indicating a relationship between the addition of soy milk and moisture content of cheese. When moisture and fat were estimated in the nonfat matter in the former and fat in the dry matter in the latter, it could be observed that cheese with a lower fat content contained more moisture.

Fat and total nitrogen contents were lower in soy milk and soy milk-cow's milk cheese due to the relatively lower levels in soy milk. These results are in accordance with results reported by Hofi et al. (1976). During ripening the higher fat and total nitrogen contents noticed could be attributed to changes in the moisture contents of the cheese as well as diffusion occurring into the brine and throughout the ripening period. However, the protein breakdown of cheese, as illustrated from soluble tyrosine and tryptophan, indicated that soy milk caused slightly lower contents of these components. The changes of these parameters gradually increased with progressive ripening.

The sensory properties of cheese with corresponding score points are presented in Table 2. Results show that cheese from soy milk was characterized by a flat flavor during 1 month of ripening which turned to a slightly acid and cheesy flavor during 2–3 months, respectively. However, the color was inferior while the body and texture were acceptable. From a technological point of view one could conclude that soy milk could be used successfully in the manufacture of a cheese substitute.

However, flavor and body, as well as texture and color, were improved by increasing the percentage of cow's milk.

Results showed that cheese made from cow's milk possessed a ripened and full-ripened flavor during 2 and 3 months of ripening, respectively.

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ACCEPTABILITY OF PACKAGED FRANKFURTERS... .From page 1776

flavor than the other brands. Both texture and smoke flavor differences were probably due to processing procedure used in manufacturing the frankfurters. Correlation values between acceptance on a coded cooked frankfurter and the other attributes (saltiness, 0.71; spiciness, 0.78; smoke flavor, 0.74; frankfurter flavor, 0.86; texture, 0.66; color 0.60; all values are highly significant, $P < 0.01$) would suggest that factors that contributed to flavor are slightly more important than color and texture in the acceptance of frankfurters. This agrees with the report of Gould (1977) that flavor of processed foods is probably the most important single quality factor of concern to the food technologist.

SUMMARY

THIS RESEARCH would suggest that frankfurters that are very similar (except for minor processing differences) when evaluated by a laboratory sensory panel at the point of consumption may receive a significantly different rating when packaged and offered to the consumer at the point of sale. This emphasizes the importance of package appearance, product appearance and/or prior reputation of the product or manufacturer on the sale of meat items. Sex of the consumer and frequency of consumption of frankfurters also influenced the evaluation of cooked frankfurters.

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A Research Note

AN INDEX OF PEELABILITY FOR SHRIMP

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ABSTRACT

A quantitative measure of force to pull shell from meat was developed as an index of peelability for Pacific shrimp (*Pandalus jordani*). An average, normalized tensile strength measured with a spring force gage, correlated positively (r = 0.98) with weight of unremoved shell found with a prototype mechanical peeler. In an example application, the force index shows shell removal to be influenced by holding shrimp in different refrigerating media for varying lengths of time.

INTRODUCTION

PROCESSORS REPORT that peelability of Pacific shrimp (*Pandalus jordani*) depends in part on the method used for refrigerating the product prior to processing. A simple field technique would be desirable for measuring the variation of this quantity with refrigerating media, times, and pretreatments, both as a research tool and for production.

One measure of peelability is the ease with which heads and shell are removed in the peeling machine. In this paper, we examine the possibility of quantifying an empirical peelability test used by many shrimp processors, which is to pull the shrimp apart by hand.

EXPERIMENTAL

Correlating tensile strength and machine performance

Shrimp held in ice (in a refrigerated room) and in refrigerated seawater spray at 0°C were sampled and processed three times over a 7-day period. Samples were measured for both peelability (shell removal) and for tensile strength, defined as the normalized force required to separate two ends of the shrimp.

Tensile strength generally refers to a force per unit cross sectional area. But because cross sectional areas of shrimp are very difficult to measure, dimensional similarity is assumed and the breaking force is divided by the square of the carapace length (i.e. the main shell covering the body). For each sample, 20 shrimp were broken by first fixing the rostrum (a forward-projecting spine) in a clip attached to a table-mounted spring force gage (Hunter, Div. of AMETEK; Hatfield, Pa.) having a 250g range and specified accuracy of ± 0.5% of full scale. Each shrimp was steadily pulled to the point of fracture. The pull time was on the order of 3–5 sec. At fracture, the gage maintained its maximum reading until reset to zero.

Peelability or shell removal was measured on a roller machine similar in principle to those used commercially (Laitram, 1979). The experimental prototype was built by the Marine Construction Co. (MARCO) of Seattle. In its operation, shrimp pass through a steam pre-cooker, then drop onto a narrow belt which carries them parallel to two moving rollers. The combined rotating motions of the rollers and the oscillating motion of the belt pinches and removes the head and shell of the cooked shrimp. The following conditions applied to the tests reported here:

Roller diameter: 7.5 cm
Roller and belt lengths: 122 cm
In-feed clearance: 0.013 cm
Out-feed clearance: 0.025 cm
Roller speed: 116 rpm
Oscillation rate: 100 strokes/min
Feeding rate: 0.454 kg/min
Cooking time in steam: 110 sec

Drained samples weighing 5 kg were fed into the machine. The peelability was indicated by measuring the weight of heads and shell still clinging to the meat leaving the machine, expressed as percentage of the original 5 kg sample weight.

Example application of tensile strength index

Shrimp initially chilled in refrigerated seawater spray were taken

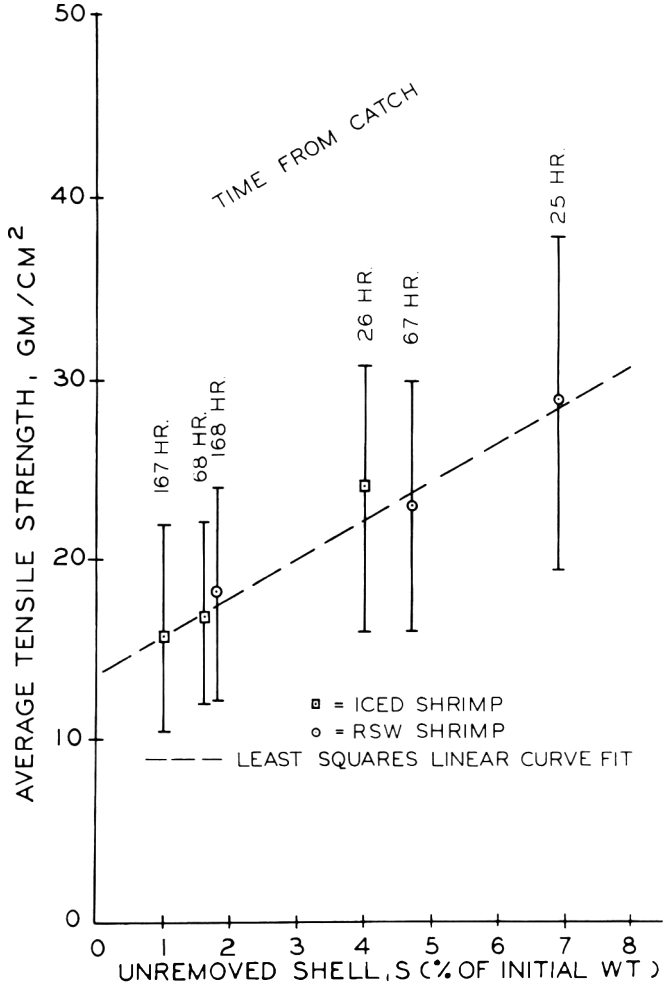

Fig. 1—Average tensile strength of shrimp vs percent weight of unpeeled shell from MARCO prototype peeler. Bands give standard deviation for 20 determinations.

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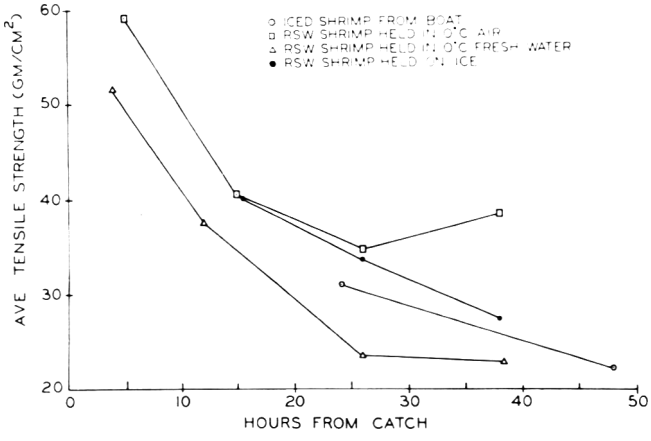

Fig. 2—Average tensile strength of shrimp vs hours from catch, for four holding media.

from a boat approximately 3 hr after catching. They were subsequently held in three media:

Air at 0°C
Melting ice
Fresh water at 0°C

Over a period of 50 hr tensile strengths were measured on samples from the three treatments as well as on samples of conventionally ice-chilled shrimp taken from a different boat.

RESULTS & DISCUSSION

THE DATA of Figure 1 show a linear relationship between average value of tensile strength and weight of unremoved shell. The positive correlation has a correlation coefficient of 0.98. The expected standard deviation of the means will be less than the standard deviation of the data shown by a factor of $1/\sqrt{N}$ where N is the number of determinations for each sample (Meiners, et al., 1959). Thus for 20 determinations, standard deviation of means will be less than data dispersion by a factor of 0.22. In one case (S = 1.6%), a second operator performed an additional series of tensile tests on 20 shrimp. The average value of 15.5 g/cm^2 was within 8% of the original.

Dispersion of data is the result of several factors affecting the quality and condition of the shrimp. The 20 shrimp in each sample, being randomly selected, were of different ages and sizes. Dividing breaking force values by a quantity representing the size of the animal is an attempt to normalize the index for various year classes of shrimp. The variation of shell removal with size was not investigated. Yield (percent weight of meat) found for all MARCO peeler tests varied between 24 and 26% except for 25 hr sample which was 30%.

The example application of Figure 2 shows the tensile test index to generally decrease with time, as expected. Standard deviation of data for 20 determinations ranged from 6.7–12.9 g/cm^2.

No attempt is made to predict performance of the full-scale commercial peeling machines. However, relative values of the peelability index shown in Figures 1 and 2 imply two results:

(a) Shrimp held in refrigerated seawater (RSW) are more difficult to peel than those held on ice;

(b) If shrimp are initially held in RSW, soaking them in 0°C fresh water would rapidly improve shell removal during processing. The low tensile strength value after 1 day (shown in Fig. 2) is comparable to that of 2-day-old iced shrimp, often considered by processors to peel well in full-scale machines.

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DETECTION OF GRAPE JUICE IN APPLE JUICE. . .From page 1774

Standard growth curves of bacteria grown on a medium containing different concentrations of samples 7 and 9 with AHC (a high and an average value, respectively) showed that growth was proportional to the amount of apple juice present in each sample (Fig. 2). Samples 22, 23, and 24 showed no such proportionality with AHC.

Work should be continued in establishing the average growth value for apple juice plus acid hydrolyzed casein amino acids and in finding more of the stimulatory nutrients in apple juice and its application to human nutrition.

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A Research Note

CAULIFLOWER LYSOZYME

K. I. EREIFEJ and P. MARKAKIS

ABSTRACT

An enzyme capable of lysing the cell walls of *Micrococcus lysodeicticus* was isolated from cauliflower. In SDS polyacrylamide gel electrophoresis at pH 7.2, this enzyme behaved like a basic polypeptide with 21,700 MW. The pH optimum for activity was 6.0, the K_m was 0.33 mg dry cell walls per ml, and the V_{max} was 0.2 A_{540} units per min.

INTRODUCTION

THE ANIMAL LYSOZYME (EC 3.2.1.17) has been studied extensively. This work was aptly discussed in a conference held on the occasion of the 50th anniversary of the discovery of lysozyme by Fleming (Osserman et al., 1974). Few studies have been devoted to the plant lysozyme and these deal chiefly with the lysozyme present in the latex of papaya (Smith et al., 1955; Howard and Glazer, 1967; Bradley, 1976) and fig (Meyer et al., 1946; Glazer et al., 1969). The purpose of this communication is to present a few data on a lysozyme isolated from cauliflower.

METHODS & MATERIALS

CAULIFLOWER (*Brassica oleracea*) heads were trimmed and the flower clusters were kept frozen in plastic bags until they were analyzed. A mixed sample was subjected to proximate analysis according to AOAC (1975) procedures.

For the isolation of the lysozyme, 400g of frozen cauliflower was thawed and blended with 500 ml distilled water for 2 min in a Waring Blendor. The slurry was filtered through three layers of cheesecloth, and the filtrate was centrifuged at 14,500 × G for 1 hr. The sediment was dispersed in 100 ml water and centrifuged a second time. The supernatants from the first and second centrifugations were combined and mixed with 20g of Amberlite IRC-50 resin (previously equilibrated with 0.2M phosphate buffer, pH 6.5) for 4 hr at 4°C under continuous stirring. The resin, after sorbing the enzyme, was washed with distilled water and eluted with 0.8M phosphate buffer, pH 6.5. The fractions were tested for lysozyme activity. The active fractions were combined and the enzyme was salted out by adding 25g of ammonium sulfate per 100 ml of eluate. Precipitation was allowed to proceed overnight at 4°C and the precipitated enzyme was separated by centrifugation at 14,500 × G.

This enzyme preparation was dissolved in 0.1M NaCl-0.1M NaAc buffer, pH 6.0, and further purified by passing it through a 1.5 × 50 cm Sephadex G-50 column. The buffer in which the enzyme was dissolved was also used as an eluant and the fractions emerging from the Sephadex column were both monitored at 280 nm for protein content and tested for lysozyme activity.

The purity of the isolated enzyme was tested by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following the Weber and Osborn (1969) procedure. The same procedure was used in determining the MW of the cauliflower lysozyme by comparison with standard proteins of known MW (Pharmacia Co). In order to retest the single band observed in SDS-PAGE for lysozyme activity, the part of the column corresponding to the band was excised from several gels and the lysozyme was eluted with the eluant buffer and tested.

Lysozyme activity was determined by a turbidimetric assay based on the lysis of irradiated cells of *Micrococcus lysodeicticus* obtained from Difco Labs (Smolelis and Hartsell, 1949). A quantitative estimate of the lysozyme content of cauliflower was obtained by the procedure of Parry et al. (1965), using egg-white lysozyme (Difco Labs) as a standard. Proteolytic activity was tested with two substances: N-α-benzoyl-D-arginine-p-nitroalanine hydrochloride and N-benzoyl-L-tyrosine-p-nitroanilide, according to Kakade et al. (1969).

RESULTS & DISCUSSION

THE RESULTS of the proximate analysis of the trimmed

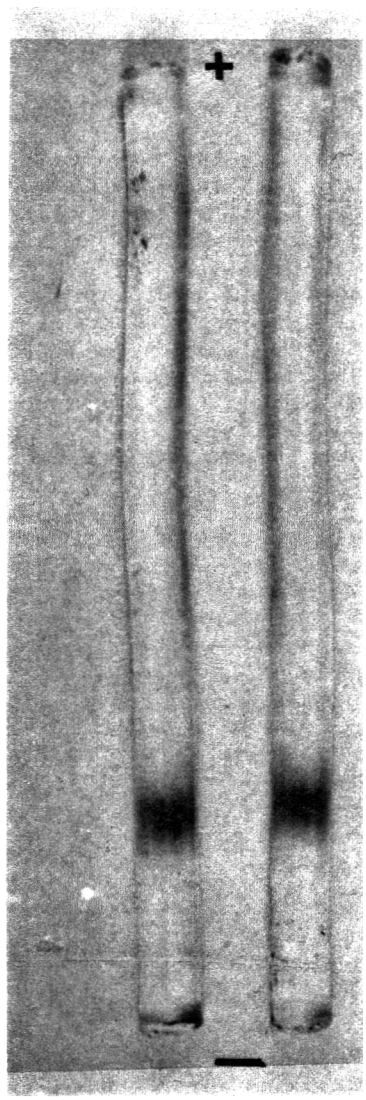

Fig. 1—SDS polyacrylamide gel electrophoresis of cauliflower lysozyme at pH 7.2 and 7.5% gel concentration.

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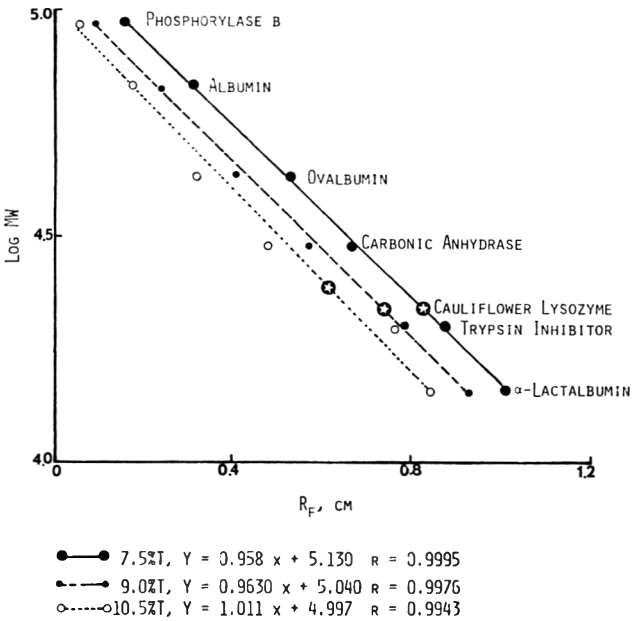

Fig. 2—Molecular weight vs electrophoretic mobility of six standard proteins and cauliflower lysozyme, at three acrylamide gel concentrations, %T. The regression equations and the correlation coefficients are also shown.

cauliflower were: moisture 92.3%, protein 2.5%, lipid 0.2%, ash 1%, fiber 0.5%, and carbohydrate (by difference) 3.5%. The eluate from the Sephadex G-50 column indicated one protein peak and one peak with lysozyme activity. The two peaks practically coincided. When the compound corresponding to these peaks was electrophoresed, only one protein band was observed on the gel as shown in Figure 1. Upon retesting, this band was found to have lysozyme activity, but no proteolytic activity. In an effort to assess the yield in lysozyme obtained by the purification procedure, the protein fraction from the Sephadex filtration was dialyzed against water and freeze-dried; 0.9 mg of active protein per 100g of cauliflower was obtained.

The activity of cauliflower lysozyme reached a maximum at pH 6.0, with pH 3.0 and 8.0 being the limits of the range in which activity was measurable. From a Lineweaver-Burk plot, a K_m equal to 0.33 mg *M. lysodeicitus* dry cell walls per ml and a V_{max} equal to 0.2 A_{540}/min were deduced. From the comparative mobility in SDS-PAGE of the cauliflower lysozyme and the five standard proteins (phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and α-lactalbumin), at three concentrations of polyacrylamide (% T equal to 7.5, 9.0, and 10.5), a MW close to 21,700 was estimated for the cauliflower lysozyme (Fig. 2). It should be mentioned that when the log of the relative electrophoretic mobilities of the six proteins was plotted against % T, six straight lines were observed, all converging to the same point of the mobility axis, indicating a free mobility, log F_f, equal to 0.23 (Rodbard and Chrambach, 1971).

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A Research Note

GELATINIZATION AND IN VITRO DIGESTIBILITY OF STARCH IN BAKED PRODUCTS

MICHAEL WOOTTON and MUHAMMAD ANWAR CHAUDHRY

ABSTRACT

The extent of gelatinization and in vitro digestibility of starch in shortbread, hard sweet cookies, soda crackers, a crispbread type product based on wheat, sugar wafer base, fruit cake and bread were determined. The first three products differed little in either starch gelatinization or digestibility from raw wheat starch. Among the other products, these two properties increased in the order of crispbread, wafer base, fruit cake, and bread. These variations were explained in terms of prebaking water content, baking time at high moisture level which favored higher values and the presence of other ingredients such as sugar and fat which decreased both parameters.

INTRODUCTION

THE EXTENT of starch gelatinization in baked products is of importance to their physical properties and storage behavior. Previous work has examined the influence of moisture, heating rate, salt and sugar concentration on the gelatinization of wheat starch-water systems using differential scanning calorimetry (Wootton and Bamunuarchchi, 1979, 1980). D'Appolonia (1972) used the Brabender Visco-amylograph to study the influence of a number of bread ingredients in the same context. Although water content is a dominant factor, other factors such as the concentration of salt and sugar, and heating rate also have important roles in play.

Recently Lineback and Wongsrikasem (1980) published findings concerning the extent of gelatinization in a number of commercial U.S. baked products. These workers used loss of birefringence and enzymatic digestion of the starch fraction of these products to measure extent of gelatinization. This work demonstrated wide variation in the extent of gelatinization between product types and available water was implicated as a major factor causing this variation. The present study on the extent of gelatinization in some Australian commercial baked goods was undertaken with a view to expanding the range of products for which such data are available and also to examine further factors which affect this parameter.

EXPERIMENTAL

Materials

White bread, fruit cake, shortbread, hard-sweet cookies, crackers, a crispbread-type product based on wheat, and sugar wafers were obtained from retail outlets. Bread was treated on the day of manufacture to avoid the effects of staling on its starch component as far as possible. Raw and pregelatinized wheat starch samples were commercial products. Porcine pancreatic α-amylase (PPA) specific activity 1000 IU/mg was obtained from Boehringer-Mannheim GmbH.

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Pretreatment of samples

The cookies, crackers, shortbread, and crispbread were crushed in a mortar. Sugar wafer base was carefully separated from the filling before crushing. Fruit cake was crumbled and fruit pieces removed from the crumbs by hand. The outer 1 cm of the bread was cut off and discarded, and the remainder crumbled. Each sample was then extracted for 24 hr in a soxhlet extractor using light petroleum (b.p. 40–60°C) prior to further treatment.

Extent of gelatinization

The extent of gelatinization of starch in each product was determined as follows (Wootton et al., 1971): Defatted product (2g) was macerated with water (100 ml) in a Waring Blendor for 1 min. The suspension was then centrifuged at 2000 × G for 10 min at room temperature. Duplicate aliquots (1 ml) were diluted to 10 ml with water and iodine solution (4% KI, 1% I_2; 0.1 ml) added. Absorbance (A_1) at 600 nm in 10 mm cells was then measured against a reagent blank. A further suspension of defatted product (2g) in water (95 ml) was prepared as described above. To this suspension was added KOH solution (10M, 5 ml) and the mixture allowed to stand for 5 min with gentle agitation. The alkaline suspension was then centrifuged. Duplicate aliquots (1 ml) were then mixed with HCl (0.5M, 1 ml) and diluted to 10 ml with water. Iodine solution (0.1 ml) was then added and absorbance (A_2) at 600 nm determined as described above. Extent of gelatinization was calculated as $(A_1/A_2 \times 100)$ %.

Extraction of starch

Starch was extracted from the defatted crumb of all products by the method of Adkins and Greenwood (1966). The starches isolated had moisture content (AACC method 44-40, 1962) of 8–10%.

In vitro digestibility of starch

The isolated starch was subjected to digestion conditions employed by Leegwater and Luten (1971) which were described by Wootton and Chaudhry (1979) with a digestion time of 60 min. Maltose in the digests was determined as described by the latter workers and in vitro digestibility calculated as mg of maltose produced per mg of dry starch expressed on a percentage basis.

RESULTS & DISCUSSION

THE CONDITION of starch in the baked products was assessed by two methods. The ratio of absorbance at 600 nm of the starch-iodine complex in aqueous and alkaline extracts of each product was used as a measure of extent of gelatinization. This method and its advantages were discussed by Wootton et al. (1971). The in vitro digestibility of starch isolated from each product was also assessed by a method based on that of Leegwater and Luten (1971). The products examined in this study were chosen to cover a range of formulations and baking conditions to allow comparisons between the condition of the starch and variations in these factors. Commercial samples of raw and pregelatinized wheat starch were similarly examined to provide comparative data for both extremes of gelatinization.

These data are presented in Table 1 together with approximate water/flour ratios in the dough or batter of each product prior to baking. These ratios were calculated from standard formulations assuming a flour moisture content of 13%. Some variation in these ratios will occur depending on exact manufacturing procedures but they are indicative of relative moisture levels among the product types. It is apparent from the data in Table 1 that increases in in vitro digestibility follow the extent of gelatinization. The relationship between these two is not linear and merits further investigation.

Table 1—Prebaking water/flour ratio, extent of gelatinization and in vitro digestibility of some wheat flour products

Product	Prebaking water/flour ratio	Extent of gelatinization (%)	In vitro starch digestibility (%)
Raw wheat starch	–	2	25
Pregelatinized wheat starch	–	70	90
Shortbread	0.2	1	18
Hard-sweet cookie	0.2	2	25
Soda crackers	0.5	3	33
Crispbread type	0.7	33	43
Wafer base	1.6	40	56
Fruit cake	0.8	50	66
Bread	0.7	60	69

In spite of its high in vitro digestibility, pregelatinized starch was only 70% gelatinized according to the present method. This is attributed to the greater solubilization of starch by alkali than by heat and moisture. As a consequence, values for extent of gelatinization shown in Table 1 may be underestimates. However, it is the comparative data which are important to the discussion.

Those products of a water/flour ratio of 0.5 or below differ little from raw wheat starch in extent of gelatinization, and in vitro starch digestibility, although crackers are slightly higher in both regards. A minimum ratio of 0.45 between water and wheat starch was found by Wootton and Bamunuarchchi (1979) to be necessary for gelatinization to occur. Hence, because of limited water, it is expected that shortbread, hard sweet cookies, and crackers would undergo little gelatinization during baking and that the values observed for gelatinization and digestibility may reflect starch damage in the parent flour rather than changes due to baking. Because of the minimal changes to starch observed in these products, it is not possible to relate other ingredients or processing conditions to extent of gelatinization.

The remaining products have water/flour ratios of 0.7 and above. Their starch components display gelatinization and in vitro digestibility considerably greater than raw starch, although lower than pregelatinized starch. While the importance of water/flour ratio is clear, comparisons between data in Table 1 for these products reveals that other factors are also involved. The crispbread type product and bread are made from doughs similar in water/flour ratio and levels of salt, sugar, and fat. The lower extent of starch gelatinization and in vitro digestibility in crispbread are probably due to different baking conditions. The crispbread enters the oven as a thin dough sheet and loses moisture rapidly to a final level of about 4%. Products of this type may undergo a parboiling process prior to baking which would contribute to starch gelatinization during a baking time of approximately 5 min. Bread, on the other hand, is exposed th high temperatures and achieves a final moisture content of about 40% during a longer baking period (approx 40 min). Hence, its starch is gelatinized more extensively. Wafer base undergoes a rapid moisture loss during baking since its production involves the spraying of a film of batter onto hot wafer oven plates. The lower extent of gelatinization observed in wafer base as compared to bread, in spite of higher prebaking moisture, is attributed to this factor. The higher moisture content in wafer batter compared to crispbread dough may be the reason for greater starch gelatinization in wafer base. Both products lose moisture at rapid, although probably not identical, rates during baking.

In spite of a somewhat higher prebaking water/flour ratio, fruit cake showed less starch gelatinization than bread. Since both products have similar final moisture contents and comparable baking times, this is attributed to the presence of other ingredients such as sugar and fat in the fruit cake. The restrictive effect of sugar on starch gelatinization is well known (e.g. D'Appolonia, 1972; Bean and Yamazaki, 1978) although the role of fat is less clear.

Several differences between the above results and those of Lineback and Wongsrikasem (1980) are apparent. Loss of birefringence leads to a higher estimate of extent of gelatinization than does the iodine blue procedure, most probably due to the alkali treatment used in the latter method as discussed above. The enzymatic procedure of Lineback and Wongsrikasem (1980), whether applied to isolated starch or to a compounded product, indicated more starch gelatinization in bread and cake, but less in cookies, than does the in vitro digestibility in this study. These disparities may reflect the use of different products and/or methodology. However, a similarity in variation of gelatinization between baked products is shown by both studies.

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Call for Nominations for IFT's 1981 Achievement Awards

□ THE IFT ACHIEVEMENT AWARDS honor outstanding individuals and developments in food science and technology and since the presentation of the first Nicholas Appert Award in 1942, the annual awards presentation has been one of the highlights of nearly every one of IFT's Annual Meetings. (See the June issue of *Food Technology* for details of this year's winners.)

It's time to make the nominations for the 1981 awards, which will be presented at the 1981 Annual Meeting in Atlanta on June 7, 1981. Details are on these two pages. Here are the groundrules:

1. Candidates for any of the awards cannot be considered by the jury unless someone nominates them.
2. Each nomination must be submitted on a prescribed form. Nomination forms for each of the awards are available from: IFT 1981 Achievement Awards, Institute of Food Technologists, 221 N. LaSalle St., Chicago, IL 60601 USA. *When you request an award form, be sure to state the award for which you intend to nominate a candidate.*
3. Except for the International Award, nominees need not necessarily be members of IFT; however only IFT members may make nominations.
4. Members may not nominate themselves for an award. Exception: For the Industrial Achievement Award, a member may nominate his or her own company or organization.
5. Deadlines for submitting nomination forms for each of the awards are given in the following descriptions.

1981 Nicholas Appert Award

Purpose: To honor a person for pre-eminence in and contributions to the field of food technology.

Qualifications: Candidate should fit the purpose of the Award. (Factors of personality or achievement outside the field of food technology cannot be considered.)

Awards and Sponsors: The Awardee receives a special medal and a $1,000 honorarium from the Institute of Food Technologists. Co-sponsored by the Chicago Section, IFT.

Deadline for receipt at IFT: **December 15, 1980.**

1981 IFT Food Technology Industrial Achievement Award

Purpose: To recognize and honor the developers of an outstanding food process and/or product which represents a significant advance in the application of food technology to food production.

Qualifications: The process or product constituting the significant advance must have been in commercial use for no less than six months nor more than four years prior to September 1, 1980. Jurors are charged to require high criteria of merit. Therefore, in any year where none of the nominated processes and/or products meet these criteria, an award may not be made.

Award and Sponsor: Bronze plaque(s) will be presented to the winning organization(s). As with the other achievement awards, public recognition will be given during the 41st Annual Meeting in Atlanta. An award of the Institute of Food Technologists.

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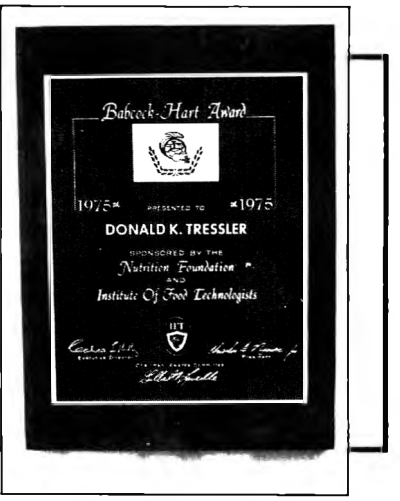
1981 Babcock-Hart Award



Purpose: To honor a person for distinguished contributions to food technology which have resulted in improved public health through some aspect of nutrition or more nutritious food.

Qualifications: Candidate's contributions should fit the purpose of the Award, and should have led to practical application leading to improved nutrition. (Contributions in fundamental studies in nutrition do not qualify for the Award.)

Award and Sponsors: The Nutrition Foundation, Inc., sponsors the Award and provides a $1,000 honorarium. The Awardee also receives an engraved plaque from IFT.

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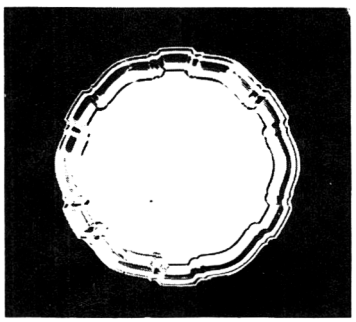
1981 International Award



Purpose: To honor a member of the Institute of Food Technologists who has made outstanding efforts to promote the international exchange of ideas on food technology, or whose work has led to such exchange of ideas or to better international understanding in this field.

Qualifications: Candidate should fit the purpose of the Award.

Award and Sponsors: A suitably inscribed silver salver is furnished by the Australian Institute of Food Science and Technology. A $1,000 honorarium is provided by IFT.

Deadline for receipt at IFT: **December 1, 1980.**

1981 Wm. V. Cruess Award

Purpose: To recognize excellence in university teaching in the field of food science and technology.

Qualifications: Candidate should have taught for at least five years in a department offering a bachelor's or higher degree in food science/technology or the equivalent thereof.

Award and Sponsors: The Institute of Food Technologists provides a special medal and a $1,000 honorarium. Co-sponsored by the Northern California Section, IFT.

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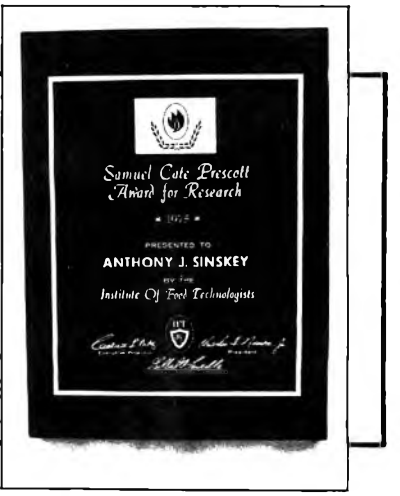

Purpose: To recognize a young research scientist (35 years of age or younger at the time of presentation) who has demonstrated outstanding ability in research in some area of food science and technology.

Qualifications: Candidate should fit the purpose of the Award. Special attention will be given to contributions in methodology, competence shown, and effects of the research on food science advances.

Award and Sponsor: The Institute of Food Technologists provides a $1,000 honorarium and an engraved plaque.

Deadline for receipt at IFT: **January 1, 1981.**

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STYLE GUIDE FOR RESEARCH PAPERS

GENERAL

THE INSTITUTE of Food Technologists publishes the *Journal of Food Science* bimonthly. Research articles must report original work and have a clear relationship with foods. The journal contains three major sections: "Basic Science," "Applied Science and Engineering," and "Research Notes." Abstracts of all papers are also published in *Food Technology*.

AUTHORSHIP

Membership in the Institute of Food Technologists is not a prerequisite for consideration of research articles for publication.

MAILING INSTRUCTIONS

Papers offered for publication should be sent—in triplicate—to:

Dr. Aaron E. Wasserman
IFT Scientific Editor
P. O. Box 6197
Philadelphia, PA 19115

MANUSCRIPTS

Identification. Upon receiving a manuscript, the Scientific Editor determines its general suitability for publication in the *Journal of Food Science*, assigns an identifying number and places it in the review process. The author(s) is so advised, and future correspondence is greatly facilitated if the identifying manuscript number is used.

Acceptance. Each paper is accepted with the understanding that it is the sole document under active consideration for publication covering the work reported. In other words, work reported in a paper submitted

1. Has NOT been previously published;
2. Has NOT been accepted for publication elsewhere; or
3. Currently is NOT submitted for publication elsewhere.

Copyright. Papers accepted for publication become the sole property of the publisher, the IFT, who holds the copyright. United States and convention copyright laws prohibit reproduction (by anyone, including authors) without permission. Requests for permission to reproduce material should be made to:

The Director of Publications
INSTITUTE OF FOOD TECHNOLOGISTS
Suite 2120, 221 N. LaSalle St.
Chicago, IL 60601 U.S.A.

PUBLICATION

Page charges. A page charge of $50 per printed page is effective for research manuscripts and we sincerely hope that authors' sources of financial support share the prevailing view that publication costs are, in fact, part of the cost of doing research.

Where solely personal funds are available for payment of page charges and such payment would be a hardship for the authors, application for "Waiver Under Hardship Clause" should be made—prior to publication—on a separate signed statement addressed to the Director of Publications at the address previously listed.

Reprints. Reprints of published articles may be secured from the Publications Office of the Institute if an order is placed before or at the time of publication. A rate schedule is attached when the galley proof is mailed for checking. (NOTE: After type has been destroyed, reprints can be supplied only at a considerably higher cost.)

IFT meeting papers. The Institute has first claim to the publication of volunteered papers presented at IFT Annual Meetings. Unless released by the Scientific Editor, they cannot be offered for publication elsewhere.

Permission to publish. If the paper has been presented at a meeting of an organization other than the Institute, the author must certify that he has freedom to offer it to IFT for publication.

TYPES OF PAPERS

ARTICLES REPORTING original research, considered for publication in the *Journal of Food Science*, will be classified as either: 1. Basic Science; 2. Applied Science and Engineering; or 3. Research Notes.

Papers offered for publication in an Institute journal are to conform to the style for research articles as set forth in this Style Guide and when applicable, to the Author Guidelines for IFT Papers Reporting Sensory Evaluation Data (available from IFT's Director of Publications).

RESEARCH ARTICLES

A research article should be confined to a coherent well-defined piece of work. Closely related work should be put in one paper and should not be broken down into several papers, as sequential numbering of papers in a series is no longer permitted. Although no limits on length are imposed, authors are expected to report their findings as CONCISELY as possible. The following organization is appropriate.

Title. Informative of the content of the article. Start with a word of value in indexing and general information retrieval.

Author(s). Given name and initials are adequate for proper identification, whether male or female. Omit professional and official titles. Give the complete address for place where work was done and each author's organizational association (company, university, laboratory, etc.).

Page 3 and following are for the text of the article–Introduction, Experimental, Results & Discussion, References, Acknowledgments, Tables (one per page), Figure captions and illustrations–and should follow in unbroken sequence.

Do not use footnotes in the text for descriptive or explanatory information, source of supply, etc. Include the information at the appropriate place in the text.

Acknowledgments for credits should be short and typed after the References.

REFERENCES

General. The references cited should be to the original source which should be examined in connection with the citation. If this cannot be done, a secondary source (e.g., abstract, book, etc.) may be used, but it should be cited along with the original reference.

Only references actually cited in the paper are to be included in this section.

Use lower case letters a, b, c, etc., following the year to distinguish among different publications of the same author(s) in any one year.

Private communications cited in the text are included in the list of references, the same as unpublished data or publications.

When no author (or editor) is indicated, "Anonymous" is cited in the text as well as in the list of References.

In the text. Cite literature in one of the following ways:

1. With last name of the author as part of the sentence, immediately followed by the year of publication in parentheses:
   Example: Smith et al. (1963) reported growth on vinasse. This was demonstrated by Jones (1966).
2. With last name of author and year of publication in parentheses, usually at the end of a sentence:
   Example: The starch granules are normally elongated in the milk stage (Brown, 1956).
3. To indicate more than two authors, use et al. after the name of the first author:
   Example: This was first observed by Smith et al. (1966) and later confirmed by Smith and Jones (1972).

Complete titles of works should not be cited in the text.

In the list of References. This section is headed References. The entire list MUST be typed double spaced. Each individual citation in the list should begin flush left (with no paragraph indentation). If the citation requires more than one line, indent runover lines about six characters (½ in.).

Arrangement shall be strictly alphabetical, by author(s) LAST name, and where necessary, by order of citation in the text in the case of multiple references to the same author(s) in any one year.

REFERENCE EXAMPLES

EXAMPLES of use in a Reference list are given below. The bold-faced parenthetical type of citation above the example is indicated ONLY for information and is NOT to be included in the reference list.

(Book–example)

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.

(Bulletin, circular–example)

Adams, J.Z. and Brakestone, I.J. 1959. Growing apple tissue in a hydrotropic environment. Bull. 1478, Geneva Agric. Exp. Stn., Geneva, NY.

(Chapter of book–example)

Dickerson, R.W. Jr. 1968. Thermal properties of foods. In "The Freezing Preservation of Foods," Vol 2, p. 26. Avi Publishing Co., Westport, CT.

(Journal–example)

Katz, M.H. 1972. Functional properties of carbohydrates. Food Technol. 26(3): 20.

Tsai, R., Cassens, R.G. and Briskey, E.J. 1972. The emulsifying properties of purified muscle proteins. J. Food Sci. 37: 286.

(Non-English reference–example)

Wolfsohn, J.D. 1923. "Apfelgeruch und die Chemische Bestandteile des Apfels." Springer Verlag, Stuttgart, Germany.

(Paper accepted–example)

Delft, J.R. 1972. Apple essence recovery. Food Technol. In press.

(Paper presented–example)

Parrish, F.C. Jr., Young, R.B. and Miner, B.E. 1972. Effect of postmortem conditions on certain properties of bovine muscle. Presented at the 32nd Annual Meeting of the Institute of Food Technologists, Minneapolis, MN, May 22–24.

(Patent–example)

Roth, T.L. 1972. Coating of frozen foods. U.S. Patent 3,607,313.

(Secondary source–example)

Wehrmann, K.H. 1961. Apple flavor. Ph.D. thesis, Michigan State University, East Lansing, MI. Quoted in Wehrmann, K.H. 1966. "Newer Knowledge of Apple Constitution," p. 141. Academic Press, New York.

–or–

Ogandzhanyan, A.M. 1966. Carbohydrate and nitrogenous fractions of corn ears (in Russian). Tr. Arm. Nauch.-Issled. Inst. Zhivotnovod. Vet. 7-8: 283. [In Chem. Abstr. 1968, 68(2): 967.]

(Thesis–example)

Taumer, U. 1965. Identification of volatile constituents of aged Delicious apple juice. Ph.D. thesis, Cornell University, Ithaca, NY.

(Unpublished data/letter–example)

Douglas, B. 1963. Unpublished data. Natl. Bur. Stand., Washington, DC.

Douglas, B. 1963. Private communication. Natl. Bur. Stand., Washington, DC.

Add present address of each author, if different, on the title page as a footnote(s).

Abstract. The abstract should state clearly the objective of the study, a concise description of the experiment(s), major observations, results and conclusions. No references should be cited in the abstract. The abstract SHOULD NOT EXCEED 110 WORDS. The abstract will be published in both the *Journal of Food Science* and *Food Technology*.

Introduction. This should be a review of only pertinent previous work and should cite appropriate references. It should also include a statement of the purpose of the investigation.

Experimental. This section is generally headed Experimental but, depending upon its content, may also be headed Materials & Methods, with appropriate subheads indicated for apparatus, reagents, procedures, etc.

Provide sufficient detail of the method and equipment to allow the work to be repeated. Accepted methods should be cited by adequate references. In reporting procedures, the active voice is preferred.

Results & Discussion. Results should be presented CONCISELY in the form of tables and figures and should give enough data to justify conclusions. Do not, however, present the same information in both forms.

The Discussion should point out the significance of the findings and, if possible, relate the new information to previous knowledge.

References. Complete information to enable the reader to locate a citation should be given. The accuracy of the references is the responsibility of the author. Only references cited in the paper are included in the reference section.

Acknowledgments. Acknowledgments of assistance in the work are appropriate provided they are not related to analyses, or other services, performed for a fee. Acknowledgments indicating permission to publish by the author's employer will not be printed. May include any or all of the following: (1) If presented at a scientific meeting; (2) Article number or thesis fulfillment; (3) financial support; (4) thanks for assistance; and (5) disclaimers.

Appendix. Research papers rarely need an appendix; however, details of calculations, nomenclature, etc., may be placed in an appendix if deemed necessary.

Tables. Numbered with Arabic numerals and each typed on a separate sheet of paper.

Legends for figures. Supply separately from illustrations. Several legends may be included on a single sheet.

Illustrations. Line drawings, photographs, etc., to be reproduced as figures.

RESEARCH NOTES

The principal characteristic of a research note is brevity. It should not exceed four (double-spaced) typewritten pages and two figures (or, two tables).

Research Notes are evaluated for publication by the same high standards as are Research Papers and should be used to report either (a) important preliminary findings in a fast moving research field, or (b) findings of a nature which do not require a full research article. It need not have the more formal organization of a research article, but otherwise should follow the same basic concepts of presentation. An abstract of 110 words or less is required.

Research Notes are given top priority in publication scheduling.

PREPARATION OF THE MANUSCRIPT

LANGUAGE

Only papers written in English will be accepted for consideration. Language usage, including spelling, should be in accord with good American practice. Authors whose primary language is not English should have the paper checked for usage prior to submitting it for publication.

Abbreviations, coined expressions, or trade names shall not appear in titles of papers. Use only abbreviations and nomenclature in the text that are accepted by recognized professional organizations, or that are well known through long and common use. At first appearance of coined expressions or trade names in the text, explain the meaning in parentheses.

Characters from foreign languages, engineering or mathematical symbols and other items liable to misinterpretation or misprinting should be clearly explained in the margin. Carefully draw in any characters not available on a standard typewriter; do not use a typewritten a for Greek α (alpha); u for Greek μ (mu); p for Greek ρ (rho); w for Greek ω (omega), etc.

IFT does not use a dual system of notation; therefore units of measurement (e.g. temperature) should be consistent. Metric units are preferred whenever practicle.

Type formulas and equations clearly, accurately placing subscripts and superscripts. Distinguish between like figures on the typewriter: e.g., 1 (one) and l (el), zero and cap O.

COPIES

Three complete copies are required. One is an original typed copy on good quality white bond of a size 8½ x 11 in., or close to these dimensions. The second and third copies may be carbon copies, on lighter-weight paper, or duplicator copies provided they are clear and easily readable.

TYPING

Use typewriter having elite or pica type. All typing is to be double spaced, including the References. Leave generous margins, a minimum of 1¼ in. at top and at left. At the top of each page type a brief version of the title

PAGINATION

Number all pages consecutively. Do not insert pages not in consecutive numbering; renumber pages, if necessary, to make all consecutive.

Page 1 should contain the following information:

Title
Name(s) of author(s)
Affiliation(s) of author(s), including zip code
Running Head—A shortened form of the title, not to exceed 40 letters and spaces
Footnote Present address of author(s) if different from institution or laboratory where research was conducted.

Page 2 should contain the abstract, complete with the title and author(s). Space (one line) should be left for bibliographic citation. For example:

CONSISTENCY OF TOMATO PRODUCTS. Effects of Acidification on Cell Walls and Cell Breakage. R. BECKER, J.C. MIERS, M-D. NUTTING, W.C. DIETRICH & J.R. WAGNER, complete mailing address of senior author only. *J. Food Sci.*

(Leave one line space here)

The consistency of tomato products can be . . .

- Single author precedes same author with co-authors.
- Use only initials of first and middle names and always place them after the last name.
- Do not use et al. in the reference list. List all authors by name as described above.
- Government agencies or similar organizations may be cited as authors: e.g., ACS, AAAS, IFT, USDA.

Order of listing shall be as follows: author(s); year; title (plus title of larger work, where applicable); bibliographic reference. Cite only the volume number if pages in the source are numbered consecutively; if not, give the issue in parentheses. Use a colon after the volume number (or issue). Use only the page number of the first page of the referenced item in all listings: e.g., J. Food Sci. 37: 621; Food Technol. 26(3): 27.

- In citing titles of articles or chapters, capitalize only the first word and any proper nouns. Place book titles inside quotation marks and capitalize the first letter of all significant words.
- Never underline any part of the reference listing.
- Refer to examples cited in this Style Guide.

Unpublished papers. Reference to papers presented at scientific meetings but not published should indicate the year of presentation. Similarly, references to papers accepted for publication but not yet published should show the journal name and, if known, the probable year of publication, and state "In press." If possible, avoid reference to papers that have been submitted for publication but not (yet) accepted. If reference is made to such papers they should be listed as "Submitted for publication." If the papers so cited are the author's, the author should advise the Scientific Editor of any change of status of his referenced paper during the time his new paper is being processed.

Foreign language publications. References to publications in languages other than English should be given in the original language, where this is possible, using English alphabet characters; transliterations or translations are acceptable in the case of references originally in Cyrillic alphabet. This applies to journal articles, book titles, etc.

Journal abbreviations shall conform to the *International List of Periodical Title Word Abbreviations* prepared by Standards Committee Z39, American National Standards Institute. Do not abbreviate journal titles consisting of a single word. Do not abbreviate personal names when they begin a journal title. Retain the original title order when abbreviating, but omitting articles, conjunctions and prepositions.

TABLES

Type each table on a separate sheet. Use Arabic numerals to number tables. Provide a descriptive caption, with descriptive but short column and/or side heads, giving recognized units. Keep the number of columns to a MINIMUM; do not include columns derived from others by simple arithmetic manipulation. Keep footnotes to a MINIMUM; identify them by a superior lower case letter in the body of the table and preceding the footnote below the table.

Avoid duplication. Do not present the same material in both tabular and graphic form.

ILLUSTRATIONS

General. Each illustration is to be supplied as a separate piece. If it is not approximately 8 x 10 in. in size, it must be mounted on a sheet of paper (one method is to insert its four corners into slits made in the sheet; never use adhesive). Be sure to enclose or otherwise protect illustrative material against bending, breakage or other damage.

Number each illustration with Arabic numerals and identify it by name of first author (use pencil on the reverse side or type on portions not to be reproduced or used as mounting).

Illustrations for the other copies of the manuscript may be photo reproductions provided they are reasonably clear. Authors are responsible for obtaining permission to reproduce previously copyrighted illustrations. Proof or certification of permission to reproduce is required.

Line drawings. Prepare with India ink on good quality white drawing paper or on tracing linen. Good clear glossy photographs of such drawings are also acceptable. Lettering done with India ink (NEVER use typewriter) MUST be of a size proportional to overall size in order that it is still legible after appropriate reduction for publication (never smaller than 1/40th the vertical field; e.g., 1/4 in. on a 10-in. field).

In graphs, draw curves heavier than axes. Label axes clearly, but concisely, and keep such captions as close to each axis as possible. Use distinct geometric symbols (open ○ or closed ● circles, triangles △, etc.) and explain them within the body of the graph. DO NOT put the legend for the figure on the drawing; type it on a separate identifiable sheet of paper.

Photographs. Ordinarily, approximately 8 x 10 in. high-contrast glossy prints of photographs are satisfactory. Original artwork, however, may be requested. Magnification of photomicrographs MUST be indicated.

Illustrations are only printed in black and white.

Unacceptable illustrations. These include slides, transparencies, color prints, original recorder trace chart strips, poorly executed line drawings, mimeograph copies of illustrations and the like.

USE OF TRADE NAMES.

Rules for the use of trade names and for comparing the merits of two (2) or more products of commerce in papers published in *Journal of Food Science* include:

a. Trade names are to be avoided in defining products dealt with, except where, in the judgment of the scientific editor, the maximum value of the paper cannot otherwise be retained.

b. If trade names are used for any of the commodities compared, all of the commodities having trade names should be so designated, if practicable.

c. If trade names are used, the commodities in question must be described in sufficient detail to make the nature of such products sufficiently clear to professionally trained readers.

BIBLIOGRAPHY

American National Standards Institute, Standards Committee Z39. 1970. International list of periodical title word abbreviations. (Available in N. and S. America from Chemical Abstracts Service, Marketing Dept., University Post Office, Columbus, OH 43210; elsewhere, ICSU-AB Secretariat, 17 rue Mirabeau, 75-Paris 16e, France.)

Committee on Form and Style of the Conference of Biological Editors. 1979. "Style Manual for Biological Journals," 4th ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW, Washington, DC 20016.

Editorial Staff, University of Chicago Press. 1969. "A Manual of Style," 12th ed., rev. The University of Chicago Press, Chicago and London.

Spear, M.E. 1952. "Charting Statistics." McGraw-Hill Book Co., Inc., New York.

กำหนดส่ง

Inst ts

The Institute of Food Technologists is a
entists, engineers, educators, and executives
nology. Food Technologists are professiona
engineering to the research, production, pr
bution, preparation, evaluation, and utiliza
who are qualified by education, special tra
tended an invitation to join in professional
group of the food industry's scientific and te
are Institute members. Membership is wor
cost, reflecting positive benefits, stimulation
individual in his/her business or profession.

OBJECTIVES

The Institute, as a non-profit, profession
several major aims: to stimulate investigatio
problems; to present, discuss, and publish th
tions; to raise the educational standards of
promote recognition of the scientific appro
role of the Food Technologist in industry.
the ultimate objective to provide the best p

ORGANIZATION AND PROGRESS

Organized July 1, 1939, at Cambridge, M
less than 100, the Institute has grown to mo
wide in scope with approximately 1500 of it

QUALIFICATIONS FOR MEMBERSHIP

Professional Members. Any person who
mum requirements: (1) Bachelor's degree
university with a major in one or more of
engineering associated with food technolog
sional experience in food technology, for which a master's degree may
be presented as the equivalent of one year's experience; a doctor's
degree, the equivalent to three year's experience.

Members. Any person active in any aspect of the food industry and who evidences interest in supporting the objectives of IFT. Recent graduates who are completing their experience requirement for Professional Member status.

Student Members. Any person who is registered as a full-time student in an educational institution and who is pursuing candidacy for an Associate Degree or higher in one or more of the sciences or branches of engineering associated with food technology shall be eligible for membership as a Student Member. After graduation, a student may convert automatically to Member without obligation for the higher Member dues until the following calendar year.

DUES

Professional Members and Members – $35 a year; includes subscription to *Food Technology* and *IFT World/Directory & Guide*; option to subscribe to the *Journal of Food Science* at members' special rate of $15. Student Members – $7.50 a year; includes subscription to one IFT journal, *IFT World/Directory & Guide*, and option to subscribe to the other journal at $7.50. Emeritus Members – no dues; includes *IFT World/Directory & Guide*, option to subscribe to either or both journals at $7.50 each.

PUBLICATIONS

The Institute publishes two journals. *Food Technology*, issued monthly, is the official journal of the Institute. The *Journal of Food Science*, issued bimonthly, is devoted to basic and applied research papers on fundamental food components and processes. In addition, an *IFT World/Directory & Guide* is published annually.

REGIONAL SECTIONS

Where 25 or more members live within commuting distance of a given geographic area, a regional section may be established. Presently, there are 49 regional sections.

DIVISIONS

Where 50 or more members of the Institute have a common interest

iscipline of food technology, they may
sently 12 divisions serving the areas of
ing, Food Packaging, Food Service, Mi-
trition, Quality Assurance, Refrigerated
valuation, Student, and Toxicology and

ertificate may be granted to a group of
science and technology curriculum in a
anized to form a student club. There are

IS

e granted to food technology organiza-
ere are currently 11 chartered affiliate

Institute provides a specially-organized
ogram, and industrial exhibit of equip-
ingredients. The program is designed to
echnological developments. Special guest

llowing awards, usually annually:
RD. Purpose of this award (medal fur-
and $1,000 by IFT) is to honor a person
utions to Food Technology.
). Purpose of this award ($1,000 fur-
lation and a plaque by IFT) is to honor a
person for contributions to Food Technology that have improved public health through some aspects of nutrition or more nutritious food.

IFT INTERNATIONAL AWARD. Purpose of this award (engraved silver salver furnished by Australian Institute of Food Science and Technology and $1,000 by IFT) is to recognize an IFT Member for promoting international exchange of ideas in Food Technology.

IFT FOOD TECHNOLOGY INDUSTRIAL ACHIEVEMENT AWARD. Purpose of this award (plaque to company) is to recognize and honor the developers of an outstanding new food process and/or product representing a significant advance in the application of Food Technology to food production, successfully applied in actual commercial operation.

WM. V. CRUESS AWARD FOR EXCELLENCE IN TEACHING. Purpose of this award (medal furnished by the Northern California Section and $1,000 by IFT) is to recognize the teacher's role in Food Technology advances.

SAMUEL CATE PRESCOTT AWARD FOR RESEARCH. Purpose of this award ($1,000 and plaque furnished by IFT) is to recognize a research scientist 35 years of age or younger who has demonstrated outstanding ability in Food Science or Technology research.

FELLOWS AWARDS. Any Professional Member who has been active for at least ten years and who has been nominated by the IFT Committee on Fellows for outstanding contributions to the field of food science/technology is eligible to be elected a Fellow of the Institute by the IFT Council.

SCHOLARSHIP/FELLOWSHIP PROGRAM

To attract and encourage students in the field of food science and food technology, the Scholarship/Fellowship program is offered to worthy and deserving students, primarily on the basis of scholastic ability.

IFT sponsors 20 $500 Freshman/Sophomore awards.

One freshman, 34 Junior/Senior Scholarships, and 23 Graduate Fellowships, ranging in amount from $6,000 to $1,000 annually, are sponsored by various food companies and IFT and administered by IFT. Details are available in the booklet, *IFT Administered Fellowship, Scholarship Program–1981–1982*, available from IFT's Scholarship Department.