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Letters

THE LAST WORD – POSITIVELY!

This will be the final communication in a series of exchanges between Dr. George Giddings and the authors of "A reevaluation of the products of gamma irradiation of beef ferrimyoglobin," [J. Food Sci. 46:1814 (1981)].

A.E.W.

In his response [JFS 47(5): vi, (1982)] to our recent letter regarding the gamma-irradiation of deaerated ferriMb solutions [JFS 47(3): iv, (1982)], Dr. Giddings neither refutes our rejection of his published mechanism nor offers any alternative mechanism to explain his earlier results. At issue is whether MbO_2 (red) or deoxy (purple) is the major product when e_{aq}^- and •OH react simultaneously with ferriMb. Dr. Giddings contends that MbO_2 is formed in the oxygen-free system [JFS 37:361 (1972)], whereas we show that deoxyMb is produced in oxygen-free systems in which each of these free radicals reacts separately with ferriMb [JFS 46:1814 (1981)]. In order to settle this issue, we now report that deoxyMb forms from the simultaneous reaction of e_{aq}^- and •OH with ferriMb in the absence of oxygen. A brief account of this study follows.

Two preparations of purified ferriMb were used in this study: one prepared from bovine muscle tissue, as previously reported, and one from a commercially available horse heart sample (Nutritional Biochemical Corp.). Solutions made 15 μM in ferriMb at pH 7.2 were deaerated in an all-glass cuvette assembly by purging with O_2-scrubbed Ar for 60 min at 2°C. Visible absorption spectra were taken of the solutions before irradiation and immediately after gamma-irradiating to a dose of 33 krad at both 3°C and 22°C. A substantial growth of a broad absorbance centered at ~ 550 nm was apparent in the post-irradiated spectra for both ferriMb preparations at both temperatures. The irradiated solutions were then flushed with air, and absorption spectra over the same region were immediately retaken. The absorbance around 550 nm was replaced by a dual-peaked absorbance having maxima at ~540 and 580 nm, clearly characteristic of MbO_2. Spectral-based, quantitative analysis of the irradiated solutions before and after the flushing with air showed that the primary deoxyMb product is converted stoichiometrically to the MbO_2 derivative upon exposure to oxygen. In addition, substantial formation of ferriMb peroxide (ferrylMb), unaffected by the aeration, was observed. Complete details of this study will be published elsewhere.

These results are fully consistent with our original reevaluation of the radiation chemistry of deaerated ferriMb solutions, and show that *deoxyMb* and ferriMb peroxide are the products of the simultaneous reaction of e_{aq}^- and •OH with ferriMb in oxygen-free systems. Moreover, they demonstrate the value of using model systems in which the reactivity of each radical can be separately evaluated in order to understand the more complex system.

These results are also consistent with Dr. Giddings' own observations on the chemically complex meat system in which the brown surface (dominant in ferriMb) of vacuum-packaged beefsteaks irradiated to pasteurizing doses develops a purple color that becomes bright red upon oxygenation [JFS 37: 361 (1972)]. Although Dr. Giddings mentioned these observations in his initial letter to support his contention that oxygen is generated radiation-chemically in the ferriMb system, we have shown previously that this explanation for MbO_2 appearance is kinetically invalid.

– *Kevin D. Whitburn,* Ph.D., Dept. of Chemistry, Boston University, Boston, MA (for)
– *Morton Z. Hoffman,* Ph.D., Dept. of Chemistry, Boston University, Boston, MA, and
– *Irwin A. Taub,* Ph.D., U.S. Army Natick Research & Development Laboratories, Natick, MA.

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Evaluation of the Water Binding Properties of Food Hydrocolloids by Physical/Chemical Methods and in a Low Fat Meat Emulsion

L. WALLINGFORD and T. P. LABUZA

ABSTRACT

The Baumann capillary suction apparatus, the cryoscopic osmometer, and moisture sorption isotherms were used to measure the water-binding capacity (WBC) of several macromolecular food grade hydrocolloids. Although each method was able to distinguish between ingredients of different WBC's, the values obtained by the three methods were very different from each other. A significant correlation was only found between the osmometer data and the moisture sorption data at a_w = 0.98, while neither method correlated with the WBC as determined by the Baumann method. The Baumann method was found to give the best prediction of a hydrocolloid's relative WBC in a low fat meat emulsion food system when compared to the typical WBC methods used for meats. Xanthan gum was found to be the best gum in retention of added water.

INTRODUCTION

MACROMOLECULAR HYDROCOLLOIDS, or gums as some are more commonly known, are used by the food industry as texture modifying agents in many different types of products. The term "gum" refers to a wide variety of compounds, including polysaccharides of plant and microbal origin, animal proteins such as gelatin and some chemical derivatives of cellulose (Andres, 1975). They have a wide range of functional properties and are used for several different functions, including as stabilizers, thickeners and structure forming (gelling) agents. These functional properties are related in part to the ability to imbibe and retain large amounts of water and interact with that water in solution.

The terms "water-holding capacity" (WHC) and "water-binding capacity" (WBC) are often used interchangeably in the literature to refer to the ability of the hydrocolloid to hold water under certain conditions (Labuza and Busk, 1979). Generally, WHC is a measure of a gum's ability to pick up water and retain it and is equal to the moisture content of the gum after equilibrium under a given condition such as humidification, whereas WBC generally refers to the ability of the gum to retain added water when given a physical stress Rey and Labuza, 1981). However, the amount of water held at a given water activity is also sometimes used as a WBC value and both these terms are used interchangeably in the literature.

The WBC of food hydrocolloids is of utmost importance in the classification and usage of these macromolecules in the food industry, and a number of methods have been employed to examine the WBC of these and other food materials. In this capacity many researchers have attempted to distinguish between the amounts of "free" and truly "bound" water in a system. Normally, the term "bound" water is used to describe water held in a gel that has properties differing from those of free or bulk water. These physical properties include lowered vapor pressure (Chou and Morr, 1979) and nonfreezability at low temperatures as determined by differential scanning calorimetry (Ross, 1978), i.e., biophysical properties. Many gums have the ability to form gels at low concentrations, physically binding water into a three-dimensional structure. However, the water held by these gels exhibits physical properties similar to those of free or bulk water and is not easily removed from the structure when physically stressed. Thus, with solidified gels of high moisture contents formed by food gums, the distinction between free and bound water becomes clouded (Labuza and Busk, 1979).

Examples of methods that have been used to measure the WBC of various food materials include centrifugation with small and large amounts of water (Quinn and Paton, 1979; Regenstein et al., 1979; Rasper and DeMan, 1980; Wierbicki and Detherage, 1958), compression by a physical force (Kim et al., 1978), freezing point depression (Rey and Labuza, 1981), suction potential measurements (Lewicki et al., 1978), and to a very limited extent, the Baumann capillary suction apparatus (Rasper and DeMan, 1980; Hermansson, 1972) which measures both the rate of uptake of water and an equilibrium value. The results of the Baumann apparatus have been shown to have a correlation with certain functional properties of food materials such as viscosity (Hermansson, 1979).

The purpose of this research was to: (1) Determine the maximum water absorption or WBC under swelling conditions on the Baumann apparatus; (2) Compare the results of the WBC determinations on the Baumann apparatus with those of biophysical WBC methods such as freezing point depression (cyroscopic osmometer) and moisture sorption isotherm determination; and (3) Use these results to pick four materials with different WBC's and compare how they function in a low fat meat emulsion.

MATERIALS & METHODS

Materials

Low methoxy pectin, karaya gum, high methoxy pectin (lot numbers 483320, 518181 and 8020-103 respectively, F. & Fd. Gums), xanthan gum, locust bean gum (lot numbers A5182A and A5342A respectively, General Mills Chemicals, Inc.), agar-agar (lot number 32171, Tragacanth Importing Co.), gelatin (250 bloom, lot number 09984, General Gelatin Corp.), guar gum (lot number 8534-103, G-3004, Hercules), and carrageenan (lot number RE-7998, Marine Colloids) were selected for analysis as representative examples of various sources of food gums. Dispersions were first prepared by a method similar to that of Persidsky and Luyet (1959). The dry powder was suspended in deionized-distilled water and subsequently heated in a 90°C water bath until the material was completely solvated. These were then transferred to sample cuvettes before evaluation on the cryoscopic oxmometer. A portion of the agar-agar and gelatin slurries were freeze dried before measuring the WBC on the Baumann apparatus, while all other gums were used as packaged by the manufacturer (dry form) for this purpose. Moisture content of the dry material was determined by drying for 18 hr at 65°C and 29 in. vacuum. This was used to correct the sample to dry weight.

Lean ham trimmings (65.5% water, 13.7% fat, 20.7% protein) from 6-month old animals (Dept. of Animal Science, Univ. of Minnesota) were ground finely prior to storage at −21°C. When needed, these samples were thawed at 4°C for 24 hr prior to using in the low fat meat emulsion system. *—Continued on next page*

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Table 1—Equilibrium moisture content of dry gum powders at various water activities (T = 22°C)

	Moisture contents (g water/100g solids)								
a_w	LM pectin	Agar-agar	Xanthan	Locust bean	Karaya	Guar	HM pectin	Carrageenan[a]	Gelatin[a]
0	1.6	0.9	1.3	1.1	1.6	0.3	0.8	C	0
0.11	5.9	10.2	6.8	6.7	5.9	5.7	4.9	8	7
0.22	7.8	14.8	9.7	9.9	7.3	8.7	6.5	14	13
0.33	9.2	18.1	11.0	11.8	9.2	10.3	7.5	18	15
0.40	10.1	19.2	11.9	12.6	10.5	10.9	8.8	19	16
0.44	10.5	20.0	12.6	12.8	11.1	11.1	9.5	22	19
0.52	12.4	21.9	15.4	14.2	12.8	12.6	11.6	23	20
0.65	18.5	24.9	19.8	17.1	15.3	16.2	14.1	26	22
0.75	29.4	28.3	26.3	20.9	18.4	20.2	16.7	33	28
0.85	46.3	32.9	36.7	26.9	30.4	27.5	28.7	48	41
0.94	76.4	40.0	51.3	37.4	46.3	39.7	44.9	78	82
0.98	127.0	50.9	90.3	71.1	97.5	65.1	80.5	101	113

[a] from Busk (1978)

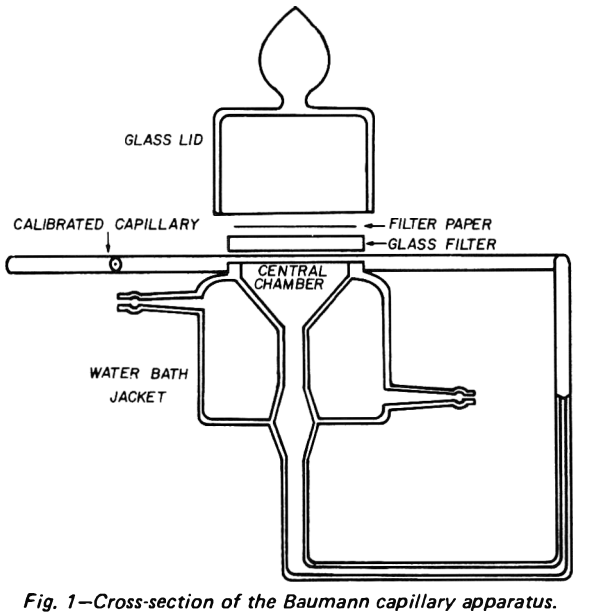

Fig. 1—Cross-section of the Baumann capillary apparatus.

Table 2—Low fat meat emulsion formula

Amount (g)	Component
500	ground ham trimmings
395	ice
20	NaCl
7.5	sucrose
1.6	$NaNO_2$
5.0	liquid smoke
0.3	ascorbic acid
0.7	ground nutmeg
1.4	ground white pepper
2.0	gum (xanthan, locust bean, carrageenan or low methoxy pectin)

Water binding by the Baumann apparatus

Spontaneous water uptake was measured with the Baumann capillary apparatus as shown in Fig. 1 (Baumann, 1967). A small amount of dry, powdered sample, in the range 1–30 mg, was sprinkled in a thin layer onto a piece of filter paper which was then placed on the glass filter of the apparatus. Water uptake, as measured by the calibrated capillary, was recorded as a function of time until equilbirium was reached. A blank value is subtracted from all measurements to account for evaporative losses. This was done by measuring the water uptake by filter paper alone in quadruplicate on each of the two apparatuses. In each case, a value of 0.007 ml/hr uptake was found. Duplicate samples were run on each of the two devices and the average of the four runs reported. Samples were run for most gums at 10, 20 and 30 mg dry weight.

Moisture sorption isotherm

Moisture sorption isotherms were determined at room temperatures on all of the gums by placing triplicate samples over saturated salt slurries of water activities in the range 0–0.98. Final moisture contents were determined after 12 wk. The data are shown in Table 1. The WBC, as related to high moisture systems, was taken as the value at a_w = 0.98.

Cryoscopic measurement of WBC

The osmolality of several low concentration slurries (0.1–3%) was measured using the Advanced Digimatic Osmometer 3DII (Advanced Instruments, Inc.) and the resulting readings of MOsm plotted versus grams of solids. The regression line was extrapolated to 280 mOsm, a point equivalent to a water activity of 0.995 based on Raoult's Law, and the moisture content at that point determined.

Low fat meat emulsion functionality test

A low fat meat emulsion system was used to test the functionality of xanthan gum, carrageenan, locust bean gum and low methoxy pectin as water binders. The meat formulation shown in Table 2, a modification of a formula by Kramlich et al. (1973), was used as the model system. The protein content was set at 11% and the fat volume made up with a gum and ice mixture. Duplicate emulsions were prepared by finely chopping all ingredients in a food processor to a final temperature of 13°C. The mixtures were stuffed into test tubes, then heated in a water bath at 54.4°C for 15 min, 60.0°C for 13 min, and 71.1°C for 15 min. The samples were then removed from the water bath and the emulsion stability and expressible moisture determined.

Emulsion stability determination

The emulsion stability of the low fat meat emulsion system was determined by a modification of the method of Townsend et al. (1968). Immediately after cooking, all free fluid was decanted from the tubes and the tubes reweighed. The emulsion stability was calculated as the grams of fluid released during cooking per 100 grams of emulsion, expressed as a percent of the uncooked emulsion.

Expressible moisture

The expressible moisture of the cooked emulsion was determined by the method of Jauregui et al. (1981) whereby a small amount of cooked sample was centrifuged with two pieces of filter paper. The fluid released was considered to be the expressible moisture under an external force.

RESULTS & DISCUSSION

THE RESULTS of the WBC determination for the nine food hydrocolloids by the three methods are shown in Table 3. The Baumann values, and moisture sorption isotherm values except for carrageenan and gelatin, represent the average and standard deviation of the replicates for each

system. Carrageenan and gelatin isotherm values represent the average value taken from Busk (1978). No variation is shown for the osmometer data since the osmometer was shown by Rey and Labuza (1981) to read less than ±1 mOsm.

Few results of research efforts using the Baumann apparatus for measuring the spontaneous uptake of water by powdered materials have been published to date. Hermansson (1972, 1979), Urbanski et al. (1982), and Rasper and DeMan (1980) have all used the Baumann device successfully to measure the uptake of water by various food materials, including proteins, flours and starches. However, its usefulness has yet to be determined in its application of the WBC value in relationship to functionality in food systems.

This study has found the results of the Baumann WBC determinations to be somewhat repeatable as indicated by the moderate standard deviations shown in Table 3. Although xanthan is shown to have a rather high standard deviation, it should be noted that as a result of this gum's high WBC, it was necessary to run the apparatus with only a 1 mg sample, whereas the lowest sample size used for the other gums was 10 mg. This factor made it more difficult to weigh out the xanthan sample and spread it evenly on the filter paper with the result of increasing the error.

In looking at the WBC data in Table 3, it is clear that there is a large difference between the gums and between the methods. The properties of the different gums in their relationship to the method itself are responsible for these differences, including the effect of molecular structure, water structuring ability, viscosity and solubility. The Baumann results will be discussed first.

Xanthan gum, by far the best water binder by this method, is an extracellular heteropolysaccharide produced by *X. campestris*. The linear portion of this colloid is composed of repeating units of D-glucose and is chemically identical to cellulose. The side chains, which account for xanthan's water solubility, are made up of D-mannose and D-glucuronic acid subunits and also contain approximately 3% by weight pyruvate (McNeely and Kang, 1973). Because of its chemical structure, it is able to form highly viscous, stable solutions at low concentrations at room temperature, a result of the rigid cellulosic backbone that is stabilized by the side chains (Morris et al., 1977). This characteristic results in the high WBC on the Baumann apparatus. Hermansson (1979) and Urbanski et al. (1982) found the Baumann WBC correlated well with rheological properties such as viscosity, pseudoplastic flow behavior and consistency coefficients.

The gum with the second highest WBC using the Baumann apparatus is carrageenan. This gum is not a single homogeneous compound but rather a heterogeneous mixture of several different polysaccharides. Galactose is the most commonly repeated monomer. The solubility of carrageenan depends on the hydrophilic sulfate half-ester groups present and the galactopyranosyl unit and therefore, a range of solubility is found for the various types of carrageenans (Towle, 1973). The carrageenan used in this study was a mixture of iota, kappa and lambda fractions of carrageenan and is therefore difficult to characterize accurately. It is reported to have a molecular weight of about 300,000 Daltons (Marine Colloids, Inc.) which is much lower than that of xanthan. Carrageenans are capable of forming viscous solutions at low concentrations in cold water with the viscosity dependent on temperature. pH, concentration, and type of carrageenan molecules and solutes present.

Guar gum has the next highest WBC in Table 3 with a WBC approximately two-thirds that of carrageenan. This seed gum is composed of linear chains of D-mannose with numerous short side units composed of D-galactose. It has a molecular weight of approximately 220,000 Daltons and froms colloidal dispersions in cold water (Goldstein and Alter, 1973). It is simple to see why guar gum ranks under carrageenan in WBC after noting the branching of guar gum molecules. Linear molecules, such as carrageenan, are more likely to interact in solution and generally give higher viscosities than branched molecules of similar molecular weight. However, guar gum is not highly branched with side chains consisting of single galactose subunits. Therefore, guar dispersions with fairly high viscosities in comparison to more highly branched molecules of equal molecular weights should absorb less water but still give a relatively high WBC.

Karaya gum shows a much lower WBC, approximately half that of guar gum. Although the molecular structure is not completely known, it is believed to be a branched heteropolysaccharide consisting of D-galactose, L-rhamnose and D-galacturonic acid (Goldstein and Alter, 1973; Sanderson, 1981). Karaya gum is capable of forming highly viscous dispersions at low concentration, seemingly in contradiction to the relationship of gum viscosity and Baumann WBC. However, it should be noted that karaya gum hydrates extremely rapidly; a 1% dispersion may reach 50% of its final viscosity within the first five minutes (Goldstein and Alter, 1973) and after that it may pass through the filter.

The next highest water-binding agent, based on the Baumann data in Table 3, is gelatin which is a protein. This was prepared by making a gel first and freeze drying it since the initial dry gelatin gave almost no absorption and will not gel at room temperature as the other gums did.

The WBC for gelatin is about the same as the starting moisture content of the gelatin slurry before freeze drying, indicating that the gelatin merely rehydrated on the Baumann apparatus.

Locust bean gum, which is next on the list in Table 3, has a fairly low WBC at 515g water per 100g dry gum solids. This is not surprising since it is not very soluble in cold water. Locust bean gum consists of a linear chain of D-mannose as does guar gum; however, it differs in the level

Table 3—WBC data for nine food gums as determined by Baumann, osmometer and moisture sorption isotherm methods

	g H_2O/100g solids			
	Baumann	Cryoscopic osmometer (a_w = 0.995)	Moisture sorption isotherm (a_w = 0.98)	(Daltons) Molecular weight
Xanthan	15850 ± 7,460	524	90.3 ± 2.38	2-15 x 10^6
Carrageenan	3290 ± 400	883	101[a]	~ 300,000
Guar	2480 ± 470	108	65.07 ± 1.85	~ 200,000
Karaya	1254 ± 200	681	97.49 ± 3.89	~ 200,000
Gelatin	1100 ± 120	212	113[a]	~ 500,000
Locust bean	520 ± 160	127	71.06 ± 2.54	~ 310,000
Agar-agar	480 ± 120	92	50.96 ± 0.39	
HM pectin	400 ± 90	538	80.51 ± 0.58	
LM pectin	370 ± 70	1584	127.05 ± 2.34	

[a] from Busk (1978)

of substitution of D-galactose on the side chains with one substitution every fourth or fifth molecule of mannose compared to every second for guar gum (Rol, 1973). It has a molecular weight on the same order of guar gum, approximately 310,000. The dispersability of locust bean gum at room temperature appears to be the key to this gum's low WBC. Crystalline regions within the gum's structure fail to solubilize at room temperature, only breaking up as the solution is heated. Cold water dispersions of locust bean gum have a significantly lower viscosity (about ten times) than hot water dispersions (Rol, 1973, Andres, 1975).

Agar-agar is another gelling agent that appears to have a fairly low WBC, binding only approximately five times its own dry weight in water. The exact molecular structure of agar-agar is not completely known, and its determination is complicated by the fact tht it is not a clearly defined substance. It is believed that agar-agar is composed of repeating units of D-galactose. Like gelatin, agar-agar is relatively insoluble in cold water, so a ground freeze-dried preparation was used on the Baumann apparatus to get the WBC. However, if the agar-agar merely rehydrated as the gelatin appeared to do, its WBC would have been approximately 5,000g water per 100g dry solids or ten times the value shown in Table 3. Something seemed to have happened to the agar-agar, preventing it from rehydrating to its original moisture content.

At the bottom of Table 3 are the pectins, both low and high methoxy types. Pectins are polymers of galacturonic acid and are referred to as low or high methoxy pectins according to the degree of methoxylation on the subunits. Generally, HM pectin is very soluble in cold water and is capable of forming a gel when sugar and acid are added whereas LM pectin can gel with calcium ions added (Andres, 1976; Blanshard, 1970). Unfortunately, the Baumann apparatus only allows pectins to solubilize, and not gel, due to the absence of these additives. Therefore, the WBC values listed in Table 3 for low and high methoxy pectins are not indicative of the gums' WBC under conditions of use in jellies. Instead, it is only a peak in the solvation curve of pectin – the moisture content or water uptake increases up to a critical viscosity at which point the pectin has solvated enough to pass through the filter of the apparatus. This point appears as a maximum on the uptake curve, as the sample will seem to lose moisture over time after reaching this critical viscosity. Due to the extreme solubility of pectin, this maximum occurs soon after the sample is placed on the apparatus, and a low WBC value is found.

The WBC results of the Baumann experiments shown in Table 3 have good correlations with general viscosity and molecular structural characteristics of most of the gums tested. However, the limitations of the apparatus are apparent in that it does not measure the WBC of gums under all conditions of usage in food systems. Compounds requiring heat, high or low pH, or polyhydroxyl compounds such as sugars for complete hydration cannot be judged fairly by this method. Only gums capable of structuring water after hydration in cold water can be ranked with any degree of accuracy.

The second column in Table 3 shows the WBC's of the gums as determined by the cyroscopic osmometer. The WBC's, by this method, are based on the ability of a gum to depress the freezing point of a solution. If a hydrocolloid followed Raoult's Law exactly, then it would be expected that hydrocolloids with lower molecular weights would have higher WBC's. However, it should also be noted that the osmometer recognizes "kinetic units" in solution, these units consisting of whole or portions of the hydrocolloid molecule, hydrocolloid-water complexes and any salts that might be present in the sample, giving rise to deviations that change the effective molal concentration preceived by the osmometer. Any deviation from the predicted WBC could be looked at as an indicator of the hydrocolloid's ability to restructure surrounding water. Cooperative hydrogen bonding or hydrophobic interactions, similar to those described by Labuza and Busk (1979), contributing to a gum's WBC could be detected in this manner. However, in this study, the exact molecular weights of the types of gums used were not known. Literature values for the molecular weights of food macromolecules vary considerably, depending on the exact source and manufacture of the gum, method of processing or fraction used. Only crude approximations can be made in this respect. For example, in Table 3, low methoxy pectin appears to have a significantly higher WBC than high methoxy pectin although their structures and molecular weights are similar. This phenomena cannot be explained by the effect of heat used in preparation of the gum slurries on low methoxy pectin. At higher temperatures low methoxy pectin may undergo a depolymerization by way of a β-elimination reaction although this is unlikely in the present conditions (Pederson, 1980). This cleavage would increase the WBC but other factors must be controlling.

Xanthan, reported to have a molecular weight on the order of two million or greater (Kelco, 1978), is only a medium water binder according to this method. As the highest molecular weight compound, it should be ranked as the poorest water binder by this method based on the previous discussion. Again, it must be emphasized that little is known about the chemical properties of the specific gum materials used in this study, thus any correlations have a weak basis.

The moisture sorption isotherm WBC data at $a_w = 0.98$ are shown in the third column of Table 3. It was noted by Kuntz and Kauzmann (1974) that the relative hdyration of proteins in sorption experiments could be significantly different, depending on the relative humidity chosen as a reference. Hermansson (1977) found a positive correlation between samples in the water activities range 0.2–0.6 with uptake data from the Baumann apparatus. It was felt that data from these intermediate water activity ranges were more representative of the swelling behavior of proteins; at higher relative humidities, sorption isotherm data reflect both the swelling and solubility properties. However, that does not seem reasonable when one is investigating hydrocolloids in solution. In our study, 0.98 a_w was the highest we could use, because above that microbial growth destroyed the sample. Since this is an equilibrium measure, a sorption isotherm should reflect the degree of hydrophillic groups on the molecule and the amount of swelling, however it does not represent a solution such as with the cyroscope or Baumann at the end. In any case, the isotherm data seems to correlate well with the cyroscopic results but not at all with the Baumann results.

A significant Spearman rank correlation ($r_s = 0.87$) was found between the cyroscope data and the isotherm data at $a_w = 0.98$, as would be expected, since at high a_w Raoult's Law should apply. However, no correlation was found between either biophysical method and the Baumann apparatus WBC values.

In order to determine which method was the best in predicting the water binding functionality of a gum in a food system, a low fat meat emulsion was used as a model test system using four of the gums as water binders.

The average values (± standard deviation) of emulsion stability and expressible moisture determinations for two runs for each gum are shown in Table 4. They followed the relative pattern predicted by the Baumann WBC results with xanthan binding the highest amount of added water, carrageenan and locsut bean gum a moderate amount and low methoxy pectin the least. Neither the cyroscopic osmometer or the moisture sorption isotherm data would have predicted the actual order shown.

Table 4—Results of low fat meat emulsion trials using food gums as water binders

Gum	Baumann WBC g water/100g dry gum solids	Emulsion stability g water lost/100g uncooked emulsion	Expressible moisture % moisture expressed
Xanthan (X)	15,850	2.91 ± 0.85	49.8 ± 1.28
Carrageenan (C)	3,285	3.99 ± 1.08	51.0 ± 1.34
Locust Bean (LB)	515	8.38 ± 3.26	53.3 ± 2.06
LM Pectin (LMP)	367	20.05 ± 1.85	55.8 ± 2.38
Ratio X/C	5	1.37	1.02
Ratio X/LB	31	3	1.07
Ratio X/LMP	43	7	1.08

It can be seen, however, that the Baumann apparatus was only able to predict the order of the gums' WBC in relationship to the functional test. The relative amounts of water bound by the different gums on the Baumann apparatus compared to the emulsion stability and expressible moisture results were not the same, as seen by the ratios of xanthan to carrageenan, xanthan to locust bean and xanthan to low methoxy pectin presented at the bottom of Table 4. Although xanthan binds 43 times as much as low methoxy pectin, according to the Baumann apparatus, it appears to be only seven times better according to the emulsion stability data and only 8% better according to the expressible moisture data. Similar relationships exist for carrageenan, LM pectin and locust bean gum also. It is clear that factors other than spontaneous water uptake should be considered in order to get accurate predictions.

Xanthan gum has an unusual property in that temperature has little effect on viscosity (Kelco, 1978). This is in contrast to locust bean gum that shows an increase in viscosity with an increase in temperature (Andres, 1975). As previously discussed, low methoxy pectin breaks down when heated, resulting in a decrease in WBC (Pederson, 1980). Carrageenan generally shows an increase in viscosity at higher temperatures due to gelation (Towle, 1973). All of these factors have an effect on the behavior of the gum in the meat emulsion system and yet are not considered when using the Baumann WBC measurements as predictive measurements. In addition, other components of the meat system, such as salt or fat, may increase or decrease the apparent functionality of a gum in comparison to its Baumann WBC.

The data collected show that it is possible to determine the WBC of commonly used food hydrocolloids using the three methods presented here – the Baumann capillary apparatus, the cryoscopic osmometer and the moisture sorption isotherm methods. However, the Baumann apparatus appears to be the method of choice for determining the WBC of food gums as related to their relative functional properties in a meat emulsion system. It appears that the relationship between the WBC, as measured by the apparatus, and the functionality of the gum make the measurements more useful than by those methods involving the principles of water activity. However, it should be noted that the value obtained by the Baumann apparatus is a relative one as evidenced in this study and cannot be taken as absolute.

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An Evaluation of Selective Differential Plating Media for the Isolation of *Yersinia enterocolitica* from Experimentally Inoculated Fresh Ground Pork Homogenate

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ABSTRACT

Seven selective differential plating media were evaluated for their effectiveness in recovering eight serotypes (0:3; 0:8; 0:9; 0:11,24; 0:12,25; 0:16; 0:17; and 0:28) of *Y. enterocolitica* from pure cultures and from artificially inoculated fresh ground pork homogenate. Cefsulodin-irgasan-novobiocin (CIN) agar was the most effective medium for the recovery of *Y. enterocolitica*. However, *Y. enterocolitica* 0:12,25 was slightly inhibited on CIN agar. Bismuth sulfite agar, MacConkey, and MacConkey-Tween 80 agars were acceptable; cellobiose-arginine-lysine, desoxycholate citrate, and *Salmonella-Shigella* agars were the least effective media.

INTRODUCTION

Yersinia enterocolitica has recently emerged as an organism of considerable public health significance. Evidence indicates that this species of bacteria may be transmitted to humans through food (Lee, 1977b), although the first and only definitive food-associated yersinia enteritis outbreak in humans took place in 1976 as a result of consumption of contaminated chocolate milk (Black et al., 1978). Nonyersinia enteritis related isolations of *Y. entercolitica* have been reported from milk and milk products, egg products, raw meats (beef, pork, lamb, poultry), vegetables, and various prepared food products (Mollaret et al., 1977).

The incrimination of *Y. enterocolita* as an infectious agent transmitted through foods has stimulated research on the development of a plating medium upon which colonies of *Y. enterocolitica* can be differentiated easily from other foodborne Gram-negative bacteria. Strains of *Y. enterocolitica* grow well on a variety of nonselective nutritionally complete media such as trypticase soy agar, but they show remarkable variability in the extent of growth on selective media commonly used for the isolation of enteric pathogens (Swaminathan et al., 1982). Examples of selective differential plating media used for the isolation of *Y. enterocolitica* are *Salmonella-Shigella* agar, MacConkey agar (Asakawa et al., 1973; Caprioli et al., 1978; Kaneko et al., 1978; Morris et al., 1977; Toma, 1973; Toma and Deiderick, 1975; Tsubokura et al., 1973; Zen Yoji et al., 1973; 1974), Drigalski agar (Lassen, 1972), lactose-sucrose-urea agar (Ahvonen, 1972; Eiss, 1975; Kapperud, 1977), desoxycholate citrate agar (Szita and Svidro, 1976), and bismuth sulfite agar (Hanna et al., 1977).

Modification of available media and the development of new media capable of increased selection and differentiation for *Y. enterocolitica* have been reported by various investigators. Lee (1977a) modified MacConkey agar by incorporating Tween-80 and calcium chloride to differentiate lipolytic *Y. enterocolitica* from interfering organisms. A cellobiose-arginine-lysine medium was developed by Dudley and Shotts (1979) who reported it useful in the isolation of *Y. enterocolitica* from clinical specimens. Schiemann (1979) reported the development of cefsulodin-irgasan-novobiocin agar for selective isolation of *Y. enterocolitica*. None of these media have been directly compared for selectivity to *Y. enterocolitica*.

The objectives of this study were (1) to determine the abilities of the following seven selective differential plating agars to recover pure cultures of *Y. enterocolitica* compared to brain heart infusion agar (BHI): bismuth sulfite (BS), cellobiose-arginine-lysine (CAL), cefsuldoin-irgasan-novobiocin (CIN), desoxycholate citrate (DC), MacConkey (MAC), MacConkey agar with Tween-80 (MAC-TW80) and *Salmonella-Shigella* (SS); and (2) to compare the seven selective differential plating media for the isolation of *Y. enterocolitica* from artificially inoculated fresh ground pork homogenate.

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MATERIALS & METHODS

THE EIGHT STRAINS of *Yersinia enterocolitica* used in this study were of the 0:3 (IP134); 0:8 (731707); 0:9 (IP383); 0:11,24 (IP841); 0:12,25 (IP490); 0:16 (IP1475); 0:17 (IP855); and 0:28 (IP1474) serctypes. All were from Dr. G. Wauters' collection (Universite Catholique de Louvain, Bruxelles, Belgium) except 0:8 (731707) which was obtained from Dr. T.J. Quan (Center for Disease Control, Ft. Collins, CO). Stock cultures were maintained on tryptic soy agar slants at 4 C.

Plating media

BS, DC, MAC, and SS agars were purchased as dehydrated media from Difco Laboratories, Detroit, MI. CAL, CIN and MAC-TW80 media were prepared after Dudley and Shotts (1979), Schiemann (1979), and Lee (1977a) respectively.

Preparation of *Y. enterocolitica* cultures

Two trials were conducted in which cultures of *Y. enterocolitica* were grown in 5 ml of trypticase soy broth (BBL Microbiology Systems, Cockeyville, MD) for 24 hr at 25°C. Appropriate dilutions (10^{-5}, 10^{-6}, 10^{-7}) were made in buffered peptone water (Oxoid) and 0.1 ml quantities were spread, in quadruplicate, on each of the selective differential plating media (BS, CIN, CAL, DC, MAC, MAC-TW80, and SS), and on brain heart infusion agar. The plates were incubated for 48 hr at 25°C before colonies were counted. In some cases DC and SS plates were read after 4 or 5 days.

Evaluation with artificially inoculated ground pork homogenate

One trial was conducted in which fifty grams of fresh ground pork (obtained from retail stores in Lafayette, IN) were homogenized in 450 ml of Butterfield's phosphate buffered saline at 37°C in a Waring Blendor for 2 min and filtered through two layers of cheesecloth. The filtrate was pipetted in 9 ml aliquots into sterile test tubes. One milliliter of an appropriate dilution of a pure culture of *Y. enterocolitica* in buffered peptone broth was added to each 9 ml of filtered pork homogenate. After mixing, 0.01 ml (calibrated platinum loop) of the artificially inoculated filtrate was streaked (in triplicate) on each of the seven selective differential plating media. The plates were incubated at 25°C for 48 hr.

After incubation, the plates were observed for *Y. enterocolitica*-like colonies. A stereomicroscope with oblique illumination, as suggested by Wauters (1973), was used for the examination of the plates; particularly SS, DC, and MAC agars. Suspect colonies of *Y. enterocolitica* were picked from the plates and inoculated into

triple sugar iron agar, mannitol broth, and lysine decarboxylase broth as suggested by Mehlman and Aulisio (1978). Further confirmation of *Y. enterocolitica* was conducted according to recommended procedures of Mehlman and Aulisio (1978).

All data were analyzed by means of the SPSS package at the Purdue University Computing Center, West Lafayette, IN 47907. One-way analysis of variance was performed and means significantly different from one another ($P < 0.05$) were identified by Student-Neuman-Keuls-Procedure.

RESULTS & DISCUSSION

DATA on the effectiveness of the seven selective media relative to brain-heart-infusion agar in the quantitative recovery of eight strains of *Y. enterocolitica* are shown in Table 1. All media except SS agar were reasonably effective in the quantitative recovery of the strains belonging to serotypes 0:3, 0:8, and 0:9. The various media were significantly different ($P < 0.05$) in their effectiveness in the recovery of serotypes 0:3 and 0:8. No significant differences were observed in the quantitative recovery of serotype 0:9 by the eight media. Significant differences ($P < 0.05$) were noted in the effectiveness of the various selective media when atypical serotypes (0:11,24; 0:12, 25; 0:16; 0:17 and 0:28) were examined (Table 1). The greatest differences were observed for serotypes 0:12,25, and 0:16, and 0:28. The strain of serotype 0:12,25 grew poorly on DC, SS, and CIN agars. Strains of serotypes 0:16 and 0:28 were not effectively recovered on SS and BS agars.

SS, MAC, MAC-TW80, and DC have been utilized most frequently in the isolation of *Y. enterocolitica* (Swaminathan, et al., 1982). Bottone (1977) stated that serotypes 0:3, 0:8, and 0:9 will grow on most of the media listed above while serotypes 0:16 and 0:17 fail to develop well on SS. The strain of 0:16 used in this investigation grew poorly on SS agar while the strain of *Y. enterocolitica* 0:17 was recovered as effectively on SS agar as on CIN, MAC, MAC-TW80, or DC agars.

Two parameters are important in the determination of selectivity of a plating medium: (1) the minimum ratio of *Y. enterocolitica* to other organisms which is required to obtain consistent isolation of *Y. enterocolitica* from the selective medium; and (2) the minimum number of cells required for isolation. With regard to the first parameter, the lower the ratio, the greater would be the effectiveness of the medium. BS and CIN agars yielded very low ratios (Table 2). MacConkey agar modified with Tween-80 was an efficient as CIN in the recovery of *Y. enterocolitica* serotype 0:3. SS, CAL, and DC agars were much less efficient than the other four media.

CIN met the second parameter most closely (Table 3). BS, MAC, and MAC-TW80 were less efficient than CIN; and SS, CAL, and DC were the least efficient. However, CIN medium was slightly less efficient than MacConkey agar in the quantitative recovery of *Y. enterocolitica* (see Table 1). Schiemann (1979) reported that CIN was useful in the recovery of 40 strains of *Y. enterocolitica* representing several serotypes. The advantages of CIN are the distinct appearance of colonies of *Y. enterocolitica* and the inhibition of many competing organisms. A disadvantage of CIN is its slight inhibition of some of the atypical serotypes. The counts for serotype 0:12,25 on CIN agar were significantly lower when compared to counts of BHI agar (Table 1).

Bismuth sulfite agar is a useful medium which yields distinct black *Y. enterocolitica* colonies after incubation for a sufficient length of time. *Y. enterocolitica* develops a dark black sheen on BS on 48 hr; however, 72 hr or longer is not uncommon for the development of this characteristic for atypical strains.

Cellobiose-arginine-lysine agar is useful for isolating *Y. enterocolitica* only in those situations where there are few interfering organisms. Colonies of *Y. enterocolitica* on CAL medium are bright burgandy red or occasionally clear with red centers. They are distinguishable at 48–72 hr of incubation (Dudley and Shotts, 1979). However, a large number of rapidly growing competing organisms can inhibit color development resulting in white or clear *Y. enterocolitica* colonies.

Desoxycholate citrate agar supported the growth of all the strains tested. However, the pinpoint colonies resulting from *Y. enterocolitica* growth may be easily overlooked. For instance, colonies of 0:12,25 required 4 to 5 days of growth on DC before colonies could be observed and counted accurately.

MacConkey agar has the advantage of virtually no inhibition of any strain tested. Various researchers have used MAC alone or in combination with other media, such as, SS or DC agar for the isolation of *Y. enterocolitica.* Colonies of *Y. enterocolitica* on MAC after 48 hr of growth are 1.5–2.5 mm in diameter. The colony morphology is generally flat with slightly rough edges which tend to become smooth with longer incubation time (Bottone, 1977). *Y. enterocolitica* colonies growing on MAC may be easily confused with other lactose negative enteric Gram-negative organisms, especially if the competing organisms are present in large numbers. However, with practice, *Y. enterocolitica* colonies can be recognized quickly.

MacConkey-Tween 80 agar was suggested by Lee (1977a) for the isolation of *Y. enterocolitica.* After approximately

Table 1—Efficiencies of various selective differential media in the quantitative recovery of various serotypes of Yersinia enterocolitica

Serotype of *Y. enterocolitica*	Plating media[a]							
	BHI	CIN	BS	CAL	DC	MAC	MAC TW 80	SS
	Colon forming units per ml (10>)[b]							
0:11,24	660a	640a	740bd	550c	560c	650a	690ab	780a
0:12,25	990ab	580c	1300a	880b	310d	880b	860b	520c
0:16	410a	370ab	180b	280ab	240ab	400a	370ab	<1c
0:17	920a	770b	1000c	840ab	650d	800ab	810ab	750b
0:28	74a	54a	13b	53a	48a	61a	60a	<1b
0:3	100ab	110b	100ab	97ab	94ab	100ab	90ab	78a
0:8	800ab	750ab	850ab	660ab	810ab	810ab	910b	670a
0:9	1010a	1000a	1200a	1100a	1000a	1200a	1200a	1000a

[a] BHI = brain heart infusion agar
BS = bismuth sulfite agar
CIN = cefsulodin-irgasan-novobiocin agar
MAC = MacConkey agar
MAC-TW-80 = MacConkey with Tween 80 agar
SS = *Salmonella Shigella* agar
CAL = cellobiose-arginine-lysine agar
DC = desoxycholate agar

[b] Mean of four values. Numbers in a row not having common letters are significantly different from each other (Student - Newman - Keul's Procedure; $P<0.05$).

48 hr of incubation, strains with lipolytic ability typically develop 2 mm, flat, wrinkled colonies surrounded by a zone of lipolysis and are, therefore, quickly recognizable. Although various researchers have used MAC-TW80 (Seelye and Yearbury, 1979; Lee et al., 1980), its distinct usefulness is limited to the selection of lipolytic strains of *Y. enterocolitica*.

Salmonella-Shigella agar usually has been used in conjunction with MAC. The main advantage of SS agar is that it restricts the growth of many competitive organisms. The lack of efficiency of SS agar is a result of the poor growth of some strains such as 0:16 and 0:28 and the indistinguishable morphology of the pinpoint colonies of *Y. enterocolitica* (Bottone, 1977).

Several investigators have demonstrated the occurrence of *Y. enterocolitica* in pork (Christensen, 1980; Mollaret, 1971). Serotype 0:3, Wauters biotype 4 (a common clinical strain isolated from humans in Canada, Japan, and Europe) has been the predominating pathogenic serotype isolated from pigs in Europe (Lee, 1977b). The most frequently used selective plating media have been MAC, MAC-TW80, and SS. The findings in this investigation indicate that the above media may have a selectivity for serotypes 0:3 and 0:8.

Organisms, which frequently appeared with similar colony morphology to serotypes of *Y. enterocolitica* on the seven selective plating media are listed in Table 4. Occasional organisms, such as *Pseudomonas spp.* grew with colony morphology similar to *Y. enterocolitica* on more than one of the selective medium. This behavior could result in confusion and emphasizes the need to carefully observe the sizes, color, and shape of 48 hr colonies.

Devenish and Schiemann (1981) reported *Citrobacter freundii, Enterobacter agglomerans* and species of *Aeromonas* and *Klebsiella*, displayed similar growth characteristics as those found for *Y. enterocolitica* on CIN. Our results are in agreement with the report by Devenish and Schiemann (1981) (Table 4). *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* are unable to grow on CIN (Schiemann, 1979). This is an important advantage of CIN since each of these organisms is capable of growing on one or more of the remaining six selective plating media (Table 4).

Hanna et al. (1977), in an evaluation of BS as a plating medium for *Y. enterocolitica*, concluded that species other than *Y. enterocolitica* which gave dark colonies (but not as shiny enamel-black) on BS plates included *Erwinia herbicola, Enterobacter liquefaciens (Serratia liquefaciens)*, and some strains of *Escherichia coli* and *Pseudomonas putrefaciens*. Some of these organisms offered confusion in our investigation as well (Table 4). These dark gray smoky colonies without the sheen can be confused with the slowly growing *Y. enterocolitica* colonies (Table 4).

The Tween-positive nature of *Serratia liquefaciens, Pseudomonas sp.* and *Hafnia alvei* (also known as *Enterobacter hafniae*) contributed to occasional mistakes in selection. This was the case although, as suggested by Lee (1977a) differences between well isolated colonies of these organisms and colonies of *Y. enterocolitica* are usually apparent.

It is appropriate to emphasize here that optimal isolation of low numbers of *Yersinia enterocolitica* from foods can be achieved only by the proper combination of appropriate methods of enrichment coupled with selective differential plating techniques. This investigation has been confined to the evaluation of selective differential plating media. Also, *Yersinia enterocolitica* and related bacteria have recently been classified into four species namely,

Table 2—Required ratio of Yersinia enterocolitica to other organisms for consistent[a] isolation of Yersinia enterocolitica from various selective differential media

Plating[b] medium	Serotypes 0:3	0:8	0:9	0:11,24	0:17
BS	0.15	0.001	0.002	0.05	0.06
CIN	0.0015	0.001	0.002	0.005	0.006
MAC	0.015	1.00	0.002	>0.5	0.06
MAC TW 80	0.0015	1.00	>0.2	>0.5	>0.06
SS	0.015	>1.00	>0.2	0.5	>0.06
CAL	>0.15	1.00	>0.2	>0.5	0.06
DC	0.15	1.00	>0.2	>0.5	0.06

[a] The term "consistent" denotes that *Y. enterocolitica* was isolated at that ratio and at all higher ratios of *Y. enterocolitica* to competing organisms in two independent trials.
[b] Legend: Refer to Table 1

Table 3—Minimum number of cells of Yersinia enterocolitica required per gram of fresh ground pork homogenate for isolation when $10^5 - 10^6$ cells/gram of competing organisms are present

Plating[b] medium	Serotypes 0:3	0:8	0:9	0:11,24	0:17
BS	90000	450	4444	10000	78000
CIN	900	450	4444	100	780
MAC	9000	45000	4444	>100000	78000
MAC TW 80	900	45000	>44444	>100000	>78000
SS	9000	>45000	>44444	100000	>78000
CAL	>90000	45000	>44444	>100000	>78000
DC	>90000	45000	>44444	>100000	7800

[a] The numbers are based on the approximate numbers of Y. enterocolitica organisms inoculated into the pork meat homogenate as determined by spread plate counts.
[b] Refer to Table 1

Table 4—Organisms that frequently cause confusion during the isolation of Yersinia enterocolitica

Plating media BS	CAL	CIN	DC	MAC	MAC-80	SS
Acinetobacter calcoaceticus	*Citrobacter freundii*	*Citrobacter freundii*	*Pseudomonas putida*	*Pseudomonas aeruginosa*	*Serratia liquefaciens*	*Klebsiella ozaenae*
Pseudomonas sp.	*Shigella sp.*	*Klebsiella sp.*	*Citrobacter sp.*	*Salmonella sp.*	*Pseudomonas sp.*	*Pseudomonas fluroescens*
Hafnia alvei	*Acinetobacter calcoaceticus*	*Enterobacter agglomerans*	*Chromobacterium violaceum*	*Escherichia coli*	*Hafnia alvei*	
Escherichia coli	*Klebsiella ozaenae*	*Aeromonas hydrophila*	*Klebsiella ozaenae*		*Escherichia coli*	
Shigella sp.	*Proteus sp.*					
Salmonella sp.	*Pseudomonas aeruginosa*					

Y. enterocolitica (sensu-stricto), Y. kristensenii, Y. intermedia and *Y. frederiksenii* (Bercovier et al., 1980). Only the organisms which fall in the reclassified *Y. enterocolitica* speices are thought to be pathogenic. Atypical serotypes, frequently isolated from foods and water have not been shown to possess virulence characteristics (as determined by tests for autoagglutination, calcium dependence, and Hela cell invasiveness (Schiemann and Devenish, 1982). The pathogenicity of such strains remains to be demonstrated.

CONCLUSIONS

IN CONCLUSION, cefsulodin-irgasan-novobiocin agar (CIN) was the most useful selective differential plating medium for the isolation of *Y. enterocolitica*. Since some strains of *Y. enterocolitica* were inhibited on CIN, it may be advisable to use at least one more selective differential plating medium in conjunction with CIN agar. The results of this investigation indicate that bismuth sulfite agar (BS) would complement CIN agar well in the isolation of *Y. enterocolitica*.

ADDENDUM

SINCE THIS RESEARCH was completed, Dr. D.A. Schiemann has modified the composition of CIN medium by changing the novobiocin concentration from 15 μg/ml to 2.5 μg/ml and by substituting sodium desoxycholate for bile salts. The modified formulation of CIN medium has been reported to be less inhibitory to the atypical strains of *Y. enterocolitica* (Schiemann, personal communication).

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Evaluation of Certain Electrical Parameters For Stimulating Lamb Carcasses

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ABSTRACT

The effectiveness of electrical stimulation (ES) at two voltages (50 and 350V) and three frequencies (10, 100 and 250 Hz) was evaluated on lamb sides. Twenty-one lambs were used. Electrical stimulation at 350V with 10 Hz significantly exhibited faster postmortem glycolysis in the Longissimus dorsi (LD) and Semimembranosus muscles and less Ca^{++}-induced shortening in the Semitendinosus muscle than achieved by any other combinations of voltages and frequencies. This treatment also provided the greatest energy per pulse and showed fast and vigorous twitching of most muscles during stimulation. These results suggested that the ES parameters of high voltage with low frequency were more effective in accelerating postmortem glycolysis than low voltage with high frequency. However, the solubility of different protein fractions in LD muscle was not affected by any of the experimental treatments.

INTRODUCTION

THERE IS NOW AMPLE experimental evidence that postmortem electrical stimulation of the carcass provides many benefits over the unstimulated carcass. This aspect has been dicussed at length in recent reviews (Cross, 1979; Bendall, 1980; Asghar and Henrickson, 1982). However, there has been a lot of variation in the electrical parameters (type of current, voltage, frequency, pulse duration, pulse shape, etc.) that have been used by research workers. For instance, voltages as high as 3600 (Chrystall and Hagyard, 1976; Gilbert and Davey, 1976) and as low as 5 (Enamorado et al., 1981) have been used for stimulation of beef and lamb carcasses. In an earlier study, Harshman and Deatherage (1951) used 2000–2500V. Gilbert (1978) suggested 1100V, whereas Bendall et al. (1976) advocated 700V. Savell et al. (1978) employed 440V while McCollum and Henrickson (1977), Tang and Henrickson (1980), and Will et al. (1980) used 300V. Golovkin et al. (1981) reported 15V to be the optimum voltage for stimulation of muscle. Since the use of high voltages required major safety considerations in a commercial abattoir, some researchers were led to examine the effectiveness of low current ranging from 5–110V for accelerating postmortem glycolysis while maintaining meat quality (Westervelt and Stouffer, 1978; Bouton et al., 1978; Taylor and Marshall, 1980).

With regard to the frequency (Hz) of electrical current, Belousov et al. (1981) used as high as 2400 Hz and Bouton et al. (1978) employed as low as 5 Hz for stimulation of the carcasses. Harshman and Deatherage (1951) preferred to keep the frequency close to 60 pulses per second for stimulating beef carcasses while Golovkin et al. (1981) suggested 25 Hz. Henrickson and associates have used 400 Hz (McCollum and Henrickson, 1977; Tang and Henrickson, 1980; and Will et al., 1980). Chrystall and Devine (1978) compare the effect of different frequencies on excised muscles and recommended 5–16.5 Hz to achieve the greatest pH fall. It is likely that there is a relationship between the electrical parameters (i.e. voltage and frequency) for accelerating postmortem muscle glycolysis. However, such a relationship has not previously been studied on the intact carcasses. Hence, this study was conducted to investigate the effect of electrical stimulation of lamb carcasses at different voltage and frequency levels on some biochemical and quality characteristics of lamb muscle.

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MATERIALS & METHODS

Experimental design

This study involved two voltage levels (50 and 350V) with three frequency levels (10, 100 and 250 Hz) within each voltage. Altogether there were seven treatments including the control. Twenty-one Suffolk lambs were randomly assigned to 21 blocks and two treatments were randomly assigned to each block. The two treatments were randomly assigned to the two carcass sides within a block. The animals were randomly removed from the lot and slaughtered in the Meat Science Abattoir at Oklahoma State University, skinned, eviscerated and divided into sides for experimental treatment (carcass weight ranged from 19–25 kg). Thereafter, each side was transferred into a temperature-controlled room at 14 ± 2°C where electrical stimulation was applied. After stimulation, the sides were kept at the same temperature until the pH of the longissimus dorsi (LD) and semimembranosus (SM) muscles reached 6.0, then the sides were subjected to chilling temperature at 2°C until 24 hr postmortem.

Electrical stimulation

The sides received the electrical stimulation treatment as appropriate within 15 min postmortem. The electrical current was applied by two wires each terminating with a spring-loaded jaw type clamp. One clamp was attached to the neck near the 5th and 6th cervical vertebrae as the negative charge, whereas the other clamp was attached to the Achilles tendon as the positive charge (near its muscular attachment). The sides were hung from the Achilles tendon using a track roller so that the rail served as further grounding to complete the circuit. A direct current with a square wave pulse at the desired voltage and frequency was applied to the carcass side for 4 min. The duty cycle was kept constant at 20% in every case. Both voltage and duty cycle were adjusted by using a Dual-Beam Oscilloscope, Type 502, while the frequency was recorded by a BK-Precision Type 1801, Frequency Counter. The resistance of the carcass side was measured using a Simpson Digital Multimeter Model 464. The physical response of the carcass sides (initial responses of the side, twitching of the muscles and duration of twitching) was noted by visual observations during electrical stimulation.

Muscle sampling

Samples from three muscles, namely, LD, SM and semitendinosus (ST), were taken post-stimulation to measure physicochemical changes as described below.

Muscle pH. Sample cores (1.27 cm in diameter) were taken from the intack LD muscle (at level of 9th and 13th thoracic vertebrae) and from the SM muscle at 0, 2, 4 and 24 hr post-stimulation intervals. A 1.5g sample (taken from the center of the core) was homogenized with 15.0 ml of 0.005M sodium iodoacetate (to arrest glycolysis) for 30 seconds in a Brinkmann Polytron (Nichols and Cross, 1980). The pH of the slurry was measured with a Digital Corning-130 pH Meter.

Muscle temperature. The changes in the internal muscle temperature of the intact LD and SM were measured with a temperature probe, Koch Model 1364, at 0, 2, 4, and 24 hr post-stimulation intervals.

Ca^{++}-induced shortening. The ST muscle was removed from the carcass side immediately after electrical stimulation and divided longitudinally into 4 strips of approximately equal weight. The initial length of each strip was marked by inserting straight pins in either end. Two strips selected at random were injected with 0.1M $CaCl_2$ (1 ml/100g muscle) while the other two strips were injected with equivalent amounts of deionized water (controls). The strips were placed in a deep tray, covered with Handi-W food wrap film (Dow Chemical Company, Midland, MI) to guard against evaporation and placed in a cold room at 2°C. After 24 hours, the final length of each strip was measured and the percent shortening was calculated. The shortening caused by $CaCl_2$ injection was referred to as Ca^{++}-induced shortening (Pearson et al., 1973, Asghar et al., 1981).

Protein solubility. The LD muscle adjacent to the 3rd and 8th thoracic vertebrae was removed from the sides at 24 hours postmortem to study the solubility of the different protein fractions. Each sample was prepared following the procedure of Asghar and Yeates (1974). Triplicate, 2g, samples from the homogenous minced meat were extracted sequentially with different buffer systems using protein extraction apparatus as described by Asghar and Yeates (1974). The sarcoplasmic proteins were extracted with 2% glycerol solution (Scopes, 1968). The residue was extracted with 0.3M KCl in 0.1M phosphate buffer to isolate myofibrillar proteins and then with 0.6M KI in 0.1M tris-buffer to isolate the remaining myofibrillar proteins (Asghar et al., 1981). All extractions and centrifugation were performed at 2°C. The resulting residue was washed thoroughly with deionized water, extracted with chloroform-methanol (3:1, v/v) to remove the lipids, and dried at 105°C. The dried residue has been referred to as connective tissue (Asghar and Yeates, 1974), although it does contain desmin and some actin (Lazarides and Hubbard, 1976). The protein content in different extracts was measured by the biuret reaction and the A 540 nm was determined using a Gilford Spectrophotometer (Gornall et al., 1949).

Statistical analysis

The data were subjected to analysis of variance using a balanced incomplete block design, block size 2. The F-test was used to determine if significant variations occurred among treatments. Means were compared using the Duncan Multiple Range Test at the 5% level of significance (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Physical response of carcass sides

The physical response of lamb carcass sides during electrical stimulation at different voltages and frequencies are shown in Table 1. It was noted that cervical and thoracic regions of the sides bend more vigorously in a lateral direction when stimulated at a high voltage (350V) than at a low voltage (50V). It was also observed that the frequency had a pronounced effect on the extent of muscle twitching. Most of the muscles on the carcass surface exhibited fast twitching during stimulation at the low frequency (10 Hz) regardless of whether the voltage was low or high; however, twitching continued for a longer period at the low voltage than at the high voltage. The twitching of muscles was apparent at 100 Hz in a few muscles such as in the leg and neck region. At 250 Hz, the whole side went into a tetanic condition without showing any twitching of individual muscles. This condition occurred independently of voltage.

Table 1—Physical response[a] of lamb carcass sides during electrical stimulation at different voltages and frequencies within 15 min after bleeding for 4 min

Electrical parameters				
Voltage (V)	Frequency (Hz)	Initial responses of the carcass side[b]	Twitching of muscles	Duration of Twitching (sec.)
50	10	Moderate	Fast twitching of most of the muscles	180–240
50	100	Moderate	Very slight twitching in few muscles	60–120
50	250	Moderate	No obvious twitching of muscles	–
350	10	Vigorous	Fast and vigorous twitching of most muscles	120–180
350	100	Vigorous	Very slight twitching in few muscles	20– 80
350	250	Vigorous	No obvious twitching of muscles	–

[a] Based on visual observation
[b] The cervical and thoracic regions bend laterally outward

Time required to reach pH 6.0

How fast the postmortem muscle pH drops to 6.0 has been used as a criterion to determine the effectiveness of electrical stimulation (Chrystall and Devine, 1978; Chrystall et al., 1980). Table 2 shows that both voltage and frequency had a tremendous effect on the time required for the muscle to reach 6.0 when stimulating carcass sides. The data indicated significant differences in time for the LD ($P < 0.005$) and SM ($P < 0.05$) muscles to reach pH 6.0 when carcass sides were stimulated at 350V and 50V, while the frequency was varied from 10 to 250 Hz. Stimulation at 350V with 10 Hz required the shortest period of time for the muscles to reach pH 6.0. For instance, the LD and SM muscles attained pH 6.0, respectively, in 3.91 hr and 3.95 hr, whereas the control sample of the same muscles took 12.22 hr and 11.22 hr, respectively. Other stimulation treatments required more time for the muscles to reach pH 6.0 than stimulation at 350V with 10 Hz, but they were still significantly different from the control for both the LD ($P < 0.005$) and SM ($P < 0.005$) muscles. Carse (1973) found that stimulating lamb with 250V caused the pH to decline to 6.0 in approximately 3 hr while Chrystall and Hagyard (1976) reported that 3600V reduced the pH to 6.0 in less than 1 hr. In this study, high voltage (350V) had a greater effect in reducing the time to reach pH 6.0 than a low voltage (50V) for both the LD ($P < 0.005$) and SM ($P < 0.005$) muscles regardless of the frequency. This agrees with earlier findings of several workers (Carse, 1973; Bendall et al., 1976; Bendall, 1980; Bouton et al., 1980), who have shown that stimulation at high voltage causes a faster drop in muscle pH. High voltage also uniformly accelerated the biochemical reactions throughout the carcass (Chrystall and Devine, 1978; Chrystall et al., 1980; Bouton et al., 1980). Apart from this, the present study showed that the frequency was also an important factor in reducing the time for the muscle to reach pH 6.0. The data in Table 2 show that a low frequency was more effective in accelerating the glycolysis in both LD and SM muscles ($P < 0.005$) than a high frequency. Thus the present study provides further

Table 2—Time required to reach pH 6 for LD and SM muscles as affected by electrical stimulation at different voltages (V) and frequencies (Hz)

E. S. Treatment		Time required to reach pH 6.0 (hr.)[a]	
Voltage (V)	Frequency (Hz)	LD muscle	SM muscle
50	10	8.91bc	8.47c
50	100	8.72c	8.70bc
50	250	10.07b	9.77b
350	10	3.92e	3.91d
350	100	7.12	7.81c
350	250	7.97cd	8.59c
Unstimulated (control)		12.33a	11.22ac
S.D. of Adj. Mean		0.39	0.37

[a] Each value of time required to reach pH 6.0 is averaged from 6 samples in both LD and SM muscles. Means within a column followed by different letters are significantly different ($P < 0.05$).

evidence that frequency along with voltage increases the rate of glycolysis in muscle due to stimulation.

Energy output during stimulation

The data on energy output in lamb carcass sides during electrical stimulation at different voltages and frequencies are shown in Table 3. It can be seen that stimulation of the side with 350V resulted in a much higher total energy output than that at 50V. A change in frequency from 10 to 250 Hz had little effect on the total energy output. The output energy per pulse decreased as the frequency was increased. The decrease in joules per pulse was also affected by voltage. For instance, electrical stimulation at 350V with 10 Hz provided the greatest energy output per pulse (9.2 J/P); thus, the energy per pulse was markedly lower at 50V and further decreased with an increase in frequency. There seems to be a relationship between the output energy per pulse and the extent of postmortem glycolysis.

The electrical stimulation at 350V and 10 Hz resulted in both the least time for the muscle to reach pH 6.0 and the highest output energy per pulse (see Table 2 and Table 3). Also, based on visual observations, the treatment of 350V with 10 Hz showed fast and vigorous twitching of most muscles in the carcass during electrical stimulation which possibly converts more chemical energy at the expense of adenosine tripohsphate (ATP) in the muscle into mechanical energy of movements as compared to other stimulation treatments. Moreover, a high correlation has also been reported between ATP and lactate content, which can be reinterpreted in terms of pH value precisely (Bendall, 1976). Hence, it is quite possible that the output energy per pulse at certain electrical stimulation parameters acts as a governing factor in determining the rate of muscle postmortem glycolysis. More work is needed to substantiate such a proposition.

pH and temperature decline

The effect of electrical stimulation has largely been evaluated by measuring the pH changes in postmortem muscle since altering the pH indicates the extent of glycolysis (Bendall, 1973). Fig. 1 and 2 show the effect of different electrical stimulation parameters (i.e. voltage and frequency) on pH fall in the LD and SM muscles, respectively, at 0, 2, 4 and 24 hr post-stimulation. Current at 350V with 10 Hz caused the greatest pH fall ($P < 0.05$) in the LD and SM muscles at 2 and 4 hours post-stimulation period. However, at the 2 hr interval, the pH of the LD muscle from lamb sides stimulated at 50V with 10 Hz and 50V with 100 Hz was not significantly different from that of the control treatment ($P > 0.05$) while the pH of the SM muscle at the treatment of 50V with 100Hz, 50V with 250 Hz, 350V with 250 Hz and the treatment of control was also not significantly different ($P > 0.05$). This study indicated that the effectiveness of a particular electrical stimulation system, when evaluated on one muscle, may not be adequate since different muscles do not respond alike. At 4 hr intervals, the treatment of 50V and 250 Hz was not significantly different from the control ($P > 0.05$) for both the LD and

Table 3—Total electrical energy output and joules per pulse of various electrical parameters for stimulation of lamb sides within 15 min after bleeding for 4 min

E.S. Parameters			Total electrical energy output	Joules/ pulse
Voltage (V)	Frequency (Hz)	Duty cycle (DC)%	(KJ)	(J/P)
50	10	20	0.41	0.17
50	100	20	0.41	0.02
50	250	20	0.41	0.01
350	10	20	21.84	9.10
350	100	20	21.84	0.91
350	250	20	21.84	0.63

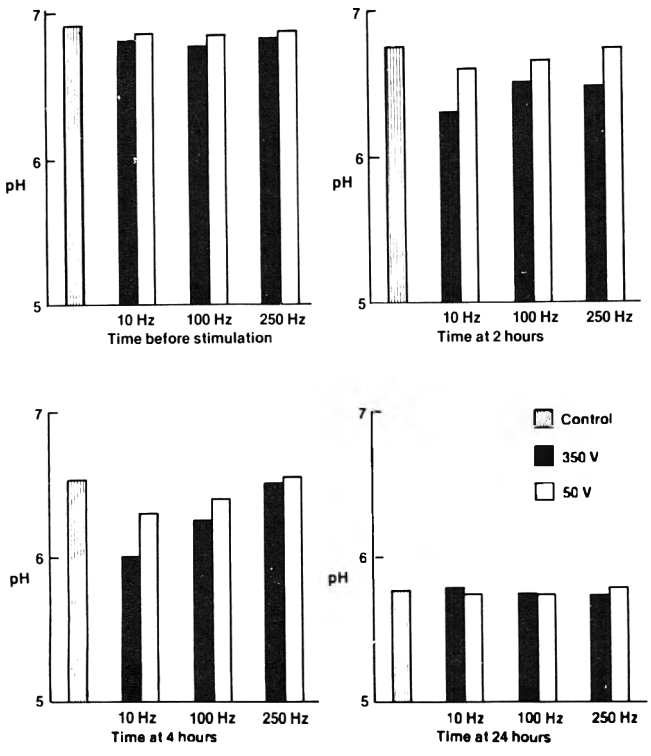

Fig. 1—Postmortem pH fall for intact LD muscle before stimulation and at 2, 4, and 24 hr as affected by electrical stimulation at different voltages (V) and frequencies (Hz).

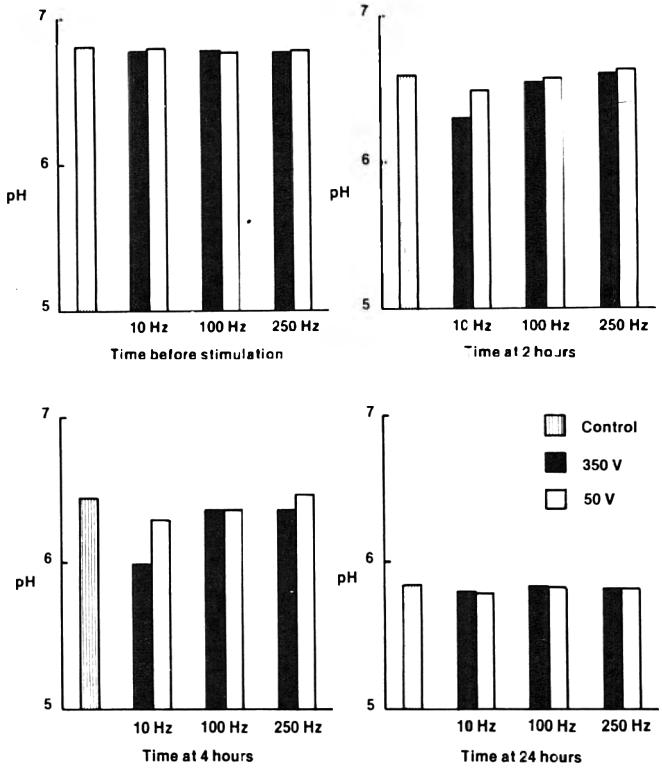

Fig. 2—Postmortem pH fall for intact SM muscle before stimulation and at 2, 4, and 24 hr as affected by electrical stimulation at different voltages (V) and frequencies (Hz).

Table 4—Pattern of post-mortem temperature (°C) changes for intact LD and SM muscles at 0, 2, 4, and 24 hr. when the pH measurements were done

E.S. Treatment		Temperature °C							
		LD muscle				SM muscle			
Voltage (V)	Frequency (Hz)	0 hr[a]	2 hr	4 hr	24 hr	0 hr	2 hr	4 hr	24 hr
50	10	37.4a	16.7b	12.6c	2.8e	39.8f	22.7g	16.0h	3.1i
50	100	37.3a	17.1b	12.9c	2.7e	39.6f	23.5g	16.7h	3.0i
50	250	37.6a	16.8b	12.9c	27.e	39.9f	22.1g	16.5h	3.1i
350	10	37.5a	16.9b	12.7c	2.6e	39.6f	22.9g	16.0h	3.1i
350	100	37.6a	16.6b	13.3c	2.7e	39.6f	22.9g	16.5h	3.2i
350	250	34.4a	16.2b	12.8c	2.7e	39.8f	22.1g	16.0h	3.1i
Unstimulated (control)		37.6a	16.2b	12.4c	2.7e	39.6f	23.1g	16.8h	3.1i
SD of Adj. Mean		0.20	0.64	0.33	0.06	0.12	1.13	0.43	0.06

[a] 0 hr = zero hour, where muscle temperatures were measured immediately before stimulation procedure. Means within each column followed by the same letter are not significantly different ($P > 0.05$).

Table 5—Extent of Ca^{++}-induced shortening of ST muscle strips as affected by electrical stimulation at different voltages and frequencies

E. S. Treatment		Shortening (%) in ST muscle[a]	
Voltage (V)	Frequency (Hz)	Injected with $CaCl_2$	Injected with H_2O
50	10	26.58b	13.78bc
50	100	26.63b	14.22b
50	350	28.35b	13.83bc
350	10	19.53d	8.44d
350	100	24.02c	12.46c
350	350	26.88b	14.23b
Unstimulated (control)		32.68a	16.56a
S.D. of Adj. Means		0.70	0.48

[a] Each muscle shortening value is averaged from 12 samples. Means within a column followed by different letters are significantly different ($P < 0.05$).

SM muscles. This suggested that a low voltage and higher frequency were less effective and less consistent in influencing the postmortem glycolysis than that of high voltage and low frequency. At 24 hr postmortem, the ultimate pH of the muscles was not significantly different between the treatments ($P > 0.05$). The pattern of postmortem temperature changes of intact LD and SM muscles was shown in Table 4. There were no significant differences at 0, 2, 4, and 24 hr post-stimulation (the same time interval as the pH measurement) among the treatments ($P > 0.05$).

Ca^{++}-induced shortening

The effect of electrical stimulation of lamb sides at different voltages and frequencies on the extent of shortening of excised ST muscle strips is summarized in Table 5. Electrical stimulation had a significant influence on the potential of Ca^{++}-induced shortening of muscle. High voltage (350V) had the greatest effect ($P < 0.05$) in reducing the Ca^{++}-induced shortening than stimulation at a low voltage (50V) as the frequency increased from 10 to 100 Hz and from 10 to 250 Hz. At 50V, the frequency had no effect ($P > 0.05$) on the shortening of the ST muscle. The excised ST muscle from carcass sides which were stimulated at 350V and 10 Hz experienced the minimum shortening (19.5%) whereas the same muscle as the control exhibited the greatest percent of shortening (32.7%). The shortening of the ST muscle strips injected with deionized water shows the same trend as was observed with Ca^{++}-ions injection. The review by Asghar and Pearson (1981) indicated that Ca^{++}-ions and ATP content are the major factors which govern cold shortening of muscle. The postmortem release of Ca^{++} -ions from the sarcoplasmic reticulum (Tume, 1979, 1980) and/or from the mitochondria (Cornforth et al., 1980) at the time when the ATP level in muscle is still high results in a significant level of cold shortening. However, if the Ca^{++}-ions are released after some depletion of ATP from muscle has taken place, only a minor amount of shortening will occur. It is known that electrical stimulation causes rapid depletion of ATP (Bendall, 1976; Bendall et al., 1976; Will et al., 1979) which is the primary source of energy for the cold shortening process. The present study suggested that stimulation of carcasses at 350V with 10 Hz will result in a more rapid depletion of energy source from muscle than the other treatments.

Solubility of different protein fractions

The extent of protein solubility of different fractions for the LD muscle from electrically stimulated lamb sides is shown in Table 6. The data show that none of the treatments had any significant effect ($P > 0.05$) on the solubility of sarcoplasmic protein fractions. No change was also noted for the protein fraction, extracted with 0.3M KCl in 0.1M phosphate buffer and with 0.6M Kl in 0.1M tris-buffer sequentially. The total percentages of myofibrillar proteins, intracellular proteins, stroma residue and total protein of muscle were not significantly different ($P > 0.05$).

Many researchers have reported that cold shortening and accompnaying toughness (due to ultimate actomyosin configuration) can be avoided when muscle pH has reached a value of 6.0 before subjecting the muscles to cold temperature (Bendall, 1975; Asghar and Pearson, 1981). In view of this theory, such consideration was undertaken in designing the present study to eliminate the influence of chilling temperature on the muscle protein structure, particularly, myofibrillar proteins, while preserving the effect of ES on the muscle proteins. If such an assumption is true, the effect of ES at different voltages and frequencies showed no influence on the gross solubility of the major protein fractions. If such an assumption is not true, the present study suggested that neither the ES nor the combined effect of ES and chilling temperature exhibited no effect on the solubility of the different protein fractions. This is partly substantiated by earlier observations of McKeith et al. (1980) and Whiting et al. (1981) who found no measurable differences in the solubility of the myofibrillar protein of muscle from electrically stimulated and unstimulated steer and lamb carcasses, respectively.

CONCLUSION

THE CARCASS SIDES which were electrically stimulated with 350V and 10 Hz significantly exhibited fastest postmortem glycolysis (LD and SM muscle) and a less Ca^{++}-induced shortening (ST muscle) than achieved by any other combination of voltage and frequency. Also, the electrical treatment of 350V with 10 Hz provided the greatest energy

Table 6—Effect of electrical stimulation at different voltages (V) and frequencies (Hz) on the solubility of different protein fractions in LD muscle

E.S. Treatment / Protein Fraction %	50 V			350 V			Control	S.D. of Adj. Mean
	10 Hz	100 Hz	250 Hz	10 Hz	100 Hz	250 Hz		
Sarcoplasmic	4.65a	4.57a	4.50a	4.34a	4.25a	4.27a	4.35a	0.12
Myofibrillar I[a]	4.59b	4.99b	5.42b	5.16b	4.75b	4.74b	5.02b	0.28
Myofibrillar II[b]	8.52c	7.87c	7.44c	7.65c	8.11c	8.13c	7.77c	0.42
Total Myofibrillar	13.11d	12.86d	12.86d	12.81d	12.86d	12.87d	12.79d	0.03
Intracellular	17.76e	17.43e	17.36e	17.15e	17.11e	17.14e	17.14e	0.05
Extracellular	2.26f	2.48f	2.51f	2.85f	2.76f	2.79f	2.83f	0.17
Total protein	20.02g	19.91g	19.87g	20.00g	19.87g	19.93g	19.97g	0.08

[a] Extracted with 0.3 M KCl in 0.1M phosphate buffer.
[b] Extracted with 0.6 M KI in 0.1M tris-buffer.
Means within each row followed by the same letter are not significantly different ($P > 0.05$).

per pulse and showed fast and vigorous twitching of most muscles in the sides during electrical stimulation. Hence, at certain electrical parameters, it is quite possible that the output of energy per pulse acts as a governing factor in determining the rate of muscle postmortem glycolysis and possibly converts more chemical energy at the expense of ATP into mechanical energy and motions. More work is needed to substantiate such a proposition. The present study also provided further evidence that the interaction between voltage and frequency has a significant influence in the glycolytic rate in electrically stimulated carcasses. However, there seems to be a linear increase in the rate of glycolysis with a decrease in pulse frequency from 250 to 10 Hz. Therefore the optimum frequency may be less than 10 Hz to achieve the maximum rate of glycolysis in muscles by electrical stimulation.

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Lipolysis in Turkey Muscle: Association of Lipid Hydrolase Activities with Zinc and Copper Metalloproteins in a High-Molecular Weight Lipid-Protein Aggregate

D. SKLAN, ORNA HALEVY, and P. BUDOWSKI

ABSTRACT

Lipids of stored turkey meat undergo lipolytic and oxidative degradation; partial characterization of the lipolytic activity was achieved. Gel filtration of turkey thigh muscle cytosol revealed a lipid-protein aggregate with the major lipolytic activity. This aggregate exhibited a molecular weight of approximately 2×10^6 daltons, and contained approximately 45% lipids, and was associated with zinc and copper. Incubation of this aggregate, or cholate treatment, resulted in the appearance of lower molecular weight lipolytically active fractions. Two of these fractions, one containing copper and zinc, the second, zinc, were partially purified by ion-exchange chromatography and gel filtration, and had molecular weights and amino acid compositions similar to those reported for rat liver copper-chelatins. The inhibition characteristics of the lipolytic activity was similar to other lipases.

INTRODUCTION

ONE of the main problems occurring on frozen storage of turkey meat is the development of off flavors. This has been attributed to oxidation, but lipolysis has been demonstrated in the meat of chickens (Davidkova and Kahn, 1967), turkeys (Acosta et al., 1966, Sklan et al., 1982), and fish (Bosund and Ganrot, 1969; Olley et al., 1962) on frozen storage. Phospholipases have been shown to play a role in the hydrolytic changes observed on storage of fish muscle, and a phospholipase A has been isolated (Audley et al., 1978). Herring muscle phospholipids were reported to contribute 75 and 45% of the free fatty acids (FFA) in light and dark meat, respectively, the FFA fraction being mainly responsible for the rancidity developing in stored herring (Bosund and Ganrot, 1969). In turkey meat, little information is available as the the characteristics and specificity of the agents responsible for the lipolytic activity. The object of this study was to characterise part of the cytosolic lipolytic activity.

MATERIALS & METHODS

PORTIONS OF TURKEY MEAT (fresh or stored at −20°C) from the leg muscle (gastrocnemius) were homogenised at setting 5 for 30 seconds in a high speed homogeniser (Janke-Kunkel, Stauffen i. Br.) with 0.12M KCl (pH 8.0) using 4 ml solution per 3g meat. The homogenate was centrifuged at 45000 *g* for 120 min and the clear supernatant (termed cytosol) beneath any floating fat was taken for further examination. All operations were carried out at 4°C.

Hydrolytic activity towards triglycerides (TG) was determined by incubation with tri[1-^{14}C-oleoyl] glycerol at 37°C for 60 min, separation of the FFA and by phasic solvent partition as described by Prystowsky et al. (1981). The incubation medium includes 0.75% cholate in 50 mM tris buffer at pH 8.0. Activity was defined as μg free fatty acids released/hr.

Hydrolytic activity towards phospholipids was determined by incubation with di(1-^{14}C-palmitoyl)phosphatidylcholine and the FFA were separated by thin-layer chromatography (Sklan et al., 1982).

Gel filtration was carried out on columns of Sepharose 4B, 6B and on Sephadex G-50 (Pharmacia Fine Chemicals, N.J.) eluting with 10 mM tris-HCl buffer containing 25 mM mercaptoethanol at pH 8.0. The columns used were: Sepharose 4B (42 x 1.8 cm), Sepharose 6B (46 x 1.8 cm) and Sephadex G-50 (53 x 2.0 cm). Ion exchange chromatography was done with activated DEAE cellulose (Whatman Co. Ltd, Maidstone, 20 x 2.5 cms) with increasing concentration of tris HCl buffer (Sklan & Donoghue, 1982).

Amino acid composition was determined after hydrolysis of the proteins with 6M HCl at 110°C for 20 hr, with and without the addition of dimethylsulphoxide (Spencer and Wold, 1969).

Vertical slab polyacrylamide electrophoresis was carried out with 20% acrylamide in the presence of 1% sodium dodecyl sulphate. Samples were mixed with dansyl chloride (0.3%) in the presence of 3% sodium dodecyl sulphate in 0.2M tris buffer and boiled with 1% mercaptoethanol before electrophoresis; visualization was with ultraviolet light (Kato and Sasaki, 1975).

Protein was determined by the method of Bradford (1976) and fatty acids by gas chromatography of the methyl esters using heptadecanoic acid as an internal standard (Sklan et al., 1971).

Hydrated density was measured by centrifugation in sucrose solutions of hydrated density of 1.21 for 40 hr at 105000 *g* in the Ti 50 rotor of a Beckman L3-50 centrifuge.

Zinc and copper were determined by atomic absorption using a Perkin-Elmer instrument, model 303.

RESULTS

INCUBATION of a homogenate of muscle or cytosol with either ^{14}C-triolein or ^{14}C-phosphatidyl-choline resulted in the release of labelled FFA. Both fresh and stored meat exhibited lipolytic activity with differences of less than 10% in release of free fatty acids per gram between fresh and frozen samples. Lipolytic activity in cytosol comprised 70–80% of homogenate activity in both fresh and frozen stored muscle. In order to elucidate the nature of the lipolytic agent(s), the cytosol was subjected to gel filtration and lipolytic activity toward labelled triolein was determined in the eluted fractions. Chromatography of cytosol on a Sepharose 6B column yielded three major active fractions (Fig. 1): fraction I eluting close to the void volume, fraction II with molecular weight (MW) of 200,000–400,000 daltons and fraction III, close to the bed volume of the column. Lipolytic activity in these fractions was at least five-fold higher towards triolein than towards phosphatidy-choline. In addition, Fig. 1 shows that zinc and copper co-eluted with the lipolytic activity peaks. Gel filtration of cytosol on Sepharose 4B (not shown) resulted in Fraction I eluting well within the included volume with an elution volume very slightly greater than dextran blue (MW 2×10^6 daltons). Copper and zinc again co-eluted with the lipolytic materials.

Ultracentrifugation of the cytosol (Table 1) showed that about 76% of the lipolytic activity of the cytosol was associated with a fraction of hydrated density less than 1.21, and a similar density distribution was obtained with fraction I isolated on Sepharose 6B (see Fig. 1). Distribution of zinc showed that some 40% of the cytosolic zinc was associated with materials of density less than 1.21 and close to 80% of the zinc in fraction I was in material of density less than 1.21.

The effects of various additives on the lipolytic activity are presented in Table 2. The cytosolic lipolytic activity was stimulated by cholate with maximum activity observed

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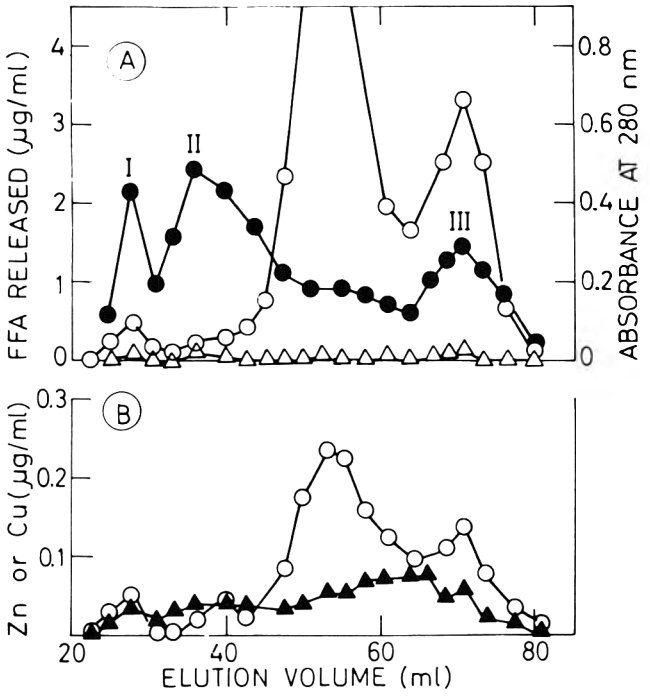

Fig. 1—Gel filtration of cytosol on a column of Sepharose 6B. Release of FFA from triglyceride substrate (µg/ml eluent per hr ●—●), release of FFA from phospholipid substrate (△—△) and absorption at 280 nm (○—○) are shown in the top panel and concentrations of zinc (○—○) and copper (▲—▲) (µg/ml) are shown in the bottom panel.

Table 1—Hydrated density distribution of triolein hydrolysing activity and zinc from both cytosol and the lipid-protein aggregate following ultra-centrifugation in sucrose solutions of density 1.21

	Triolein hydrolyzing activity % in 1.21 top fraction
Cytosol	75.9 41.0
Lipid-protein complex (Fraction I)	85.0 77.8

[a] Results are means of duplicate determinations. Samples were spun at 105,000 g for 40 hr at 4°C. Total lipolytic activity and Zn content were set at 100%.

Table 2—Effect of various additives on the triolein hydrolysing activity of the lipid-protein aggregate (Sepharose 6B Fraction I) Isolated from turkey muscle[a]

Additive	% Activity
No additive	100
0.75% cholate	161.2± 5.4
0.1% Tritor X-100, cholate omitted	5 ± 2
0.75% bovine serum albumin, cholate omitted	124 ±13
0.75% deoxycholate, cholate omitted	81 ± 6
0.75% cholate + 28 units alkaline phosphatase	11 ± 2
0.75% cholate + 1 x 10^{-4} M phenylmethyl sulphonyl fluoride	5 ± 2
0.75% cholate + 1 x 10^{-3} M dithiobis-(2-nitrobenzoic acid)	61 ± 3
0.75% cholate + 1 x 10^{-3} M EDTA	26 ± 6
0.75% cholate + 0.5 M NaCl	219 ± 1.6

[a] Activity µg free fatty acids released/hr. Results are means ± SD of at least three determinations.

0.75% cholate. Higher cholate concentrations did not enhance activity. Bovine serum albumin also stimulated activity but to a lesser extent than cholate (Table 2). Presence of detergents, such as Triton X-100 abolished the activity (Table 2). The optimal pH for lipolysis in the presence of cholate was pH 8.0 for both cytosol and fraction I, (Fig. 2). Hydrolytic activity towards tripalmitin had characteristics which were similar to those observed with triolein.

Incubations were also carried out in the presence of various additives, deoxycholate did not support lipolysis and preincubation with alkaline phosphatase also abolished activity. Phenylmethylsulphonyl fluoride and dithiobis (2-nitrobenzoic acid) also inhibited activity as did ethylenediaminetetraacetic acid. NaCl at concentrations of 0.5M enhanced the lipolytic activity.

The high MW fraction (approximately 2 x 10^6 daltons) isolated from cytosol by gel filtration on Sepharose 6B contained 45% lipids, the bulk of which, usually about 90%, was made up of TG + FFA, the remainder being phospholipids. The fatty acid composition of phospholipids, TG and FFA are shown in Table 3.

Fraction I isolated from Sepharose 6B was incubated at 37°C or treated with cholate (0.75%), this resulted in partial disintegration of the fraction and the appearance of several new lower MW fractions with lipolytic activity. In order to identify some of these fractions, further gel filtration on Sepharose 6B (not shown) and Sephadex G-50 was carried out. A fraction with MW200000 – 400000 daltons as well as low MW (close to bed volume) lipolytic fractions were observed on Sepharose 6B. Lipolytic activity and zinc concentration in the eluate from G-50 are shown in Fig. 3. Lipolytic activity was found at MWs of about 13000, 9500 and 6500 daltons, and these peaks co-eluted with either zinc, copper or both. In order to further purify these low MW fractions, ion exchange chromatography on DEAE cellulose was carried out with stepwise elution with tris-

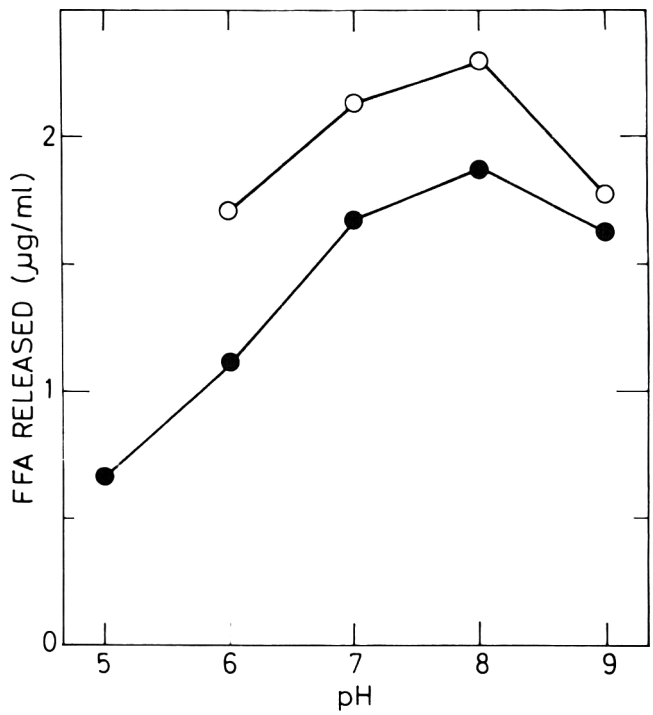

Fig. 2—Influence of pH on the lipolytic activity of cytosol (○—○) and lipid-protein aggregate (Fraction I) (●—●) prepared on Sepharose 6B. The results are means of duplicate determinations.

hydrochloride buffers. Purification of the "9,500 MW" G-50 fraction yielded material that was rich in both copper and zinc, whereas the "6,500 MW" G-50 fraction yielded a product that contained zinc with only traces of copper. Electrophoresis on polyacrylamide gels of these "9500"

Table 3—Fatty acid composition of the lipid-protein complex (Fraction I) from Sepharose 6B[a]

Lipid fraction[b]	Fatty acid (%)					
	16:0	16:1	18:0	18:1	18:2	20:4
Triglycerides	41.8	8.9	8.2	25.8	15.1	0
Phospholipids	37.0	1.0	25.0	16.2	18.6	2.0
Free fatty acids	39.5	6.6	13.2	19.7	20.1	0.6

[a] Results are means of three determinations.
[b] Different preparations yielded contents of triglycerides + FFA that change from 60 to 90% of total fatty acids.

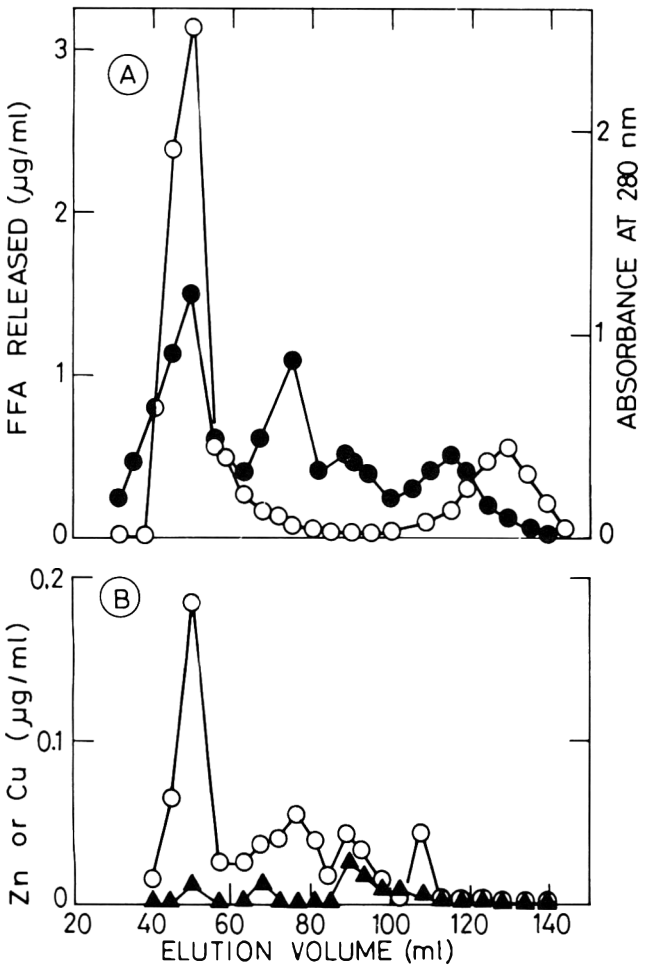

Fig. 3—Gel filtration on Sephadex G-50 of the lipid protein aggregate Fraction I) obtained by chromatographing a Sepharose 6B column. The lipid-protein aggregate (Fraction I) was incubated for 2 hr at 37°C and chromatographed on a column of Sephadex G-50. The top panel shows release of FFA from triolein (●—●) and absorption at 280 nm (○—○). Zinc (○—○) and copper (▲—▲) concentrations (µg/ml) are shown in the bottom panel.

and "6500" fractions in the presence of sodium dodecyl sulphate gave a single band with a MW of 7,500 daltons for both fractions. The UV spectrum of these materials showed no absorption peak at 280 nm and major absorption at 210–220 nm. Amino acid analysis of both fractions (Table 4) revealed that both had similar compositions with low concentrations of phenylalanine, arginine and histidine. Both these G-50, DEAE fractions exhibited lipolytic activity toward triolein and the increasing specific activity at the various stages of purification is shown in Table 5: a purification of 100–500 fold was achieved. Zinc was present in the "6500" fraction at approximately 5 atoms per molecule

Table 4—Amino acid composition of two fractions; one co copper-rich, 9500 MW, and the other, rich in zinc, 6500 MW; isolated from the lipid-protein aggregate[a]

Amino acid	Copper-rich	Zinc-rich
	mol %	
Lysine	4.9	2.9
Histidine	1.7	1.5
Arginine	3.0	2.7
Aspartic acid	9.8	13.7
Threonine	5.1	4.9
Serine	9.5	8.6
Glutamic acid	12.2	15.0
Proline	4.7	4.2
Glycine	14.5	15.5
Alanine	13.1	8.6
Cysteine[b]	5.1	5.1
Valine	5.2	4.2
Methionine	0.5	1.5
Isoleucine	2.6	2.5
Leucine	4.5	5.2
Tyrosine	1.1	2.0
Phenylalanine	1.9	1.4

[a] Results are means of two duplicate determinations. For preparation of fractions, see text.
[b] Cysteine was calculated as cysteic acid from analysis with and without the addition of dimethylsulphoxide.

Table 5—Specific activity of the triolein hydrolase activity and zinc and copper to protein ratios at various stages of purification[a]

Separation stage	Zn µg/mg protein	Cu µg/mg protein	Triolein hydrolase activity µg/hr/mg protein
Cytosol	0.30	0.03	0.106
Fraction I after Sepharose 6B	18.0	0.92	0.91
Copper-rich 9500 MW fraction after DEAE and G-50	24.4	13.0	15.7
Zinc-rich 6500 MW fraction after DEAE and G-50	46.8	0.4	56.2

[a] Results are means of duplicate determinations at each stage of purification.

protein and the "9500" fraction contained 3 atoms of zinc and 2 copper per molecule of protein.

DISCUSSION

THE PRESENT STUDY has demonstrated that the lipolytic activity in turkey muscle cytosol is distributed over at least three protein fractions when examined by gel filtration. The highest MW fraction (MW approximately 2×10^6) contained some 45% lipids which were mainly triglycerides and free fatty acids and had a hydrated density less than 1.21. Lipolytic activity was higher towrad triolein than toward dipalmitoylphosphatidylcholine. On incubation or cholate treatment of this fraction, active materials of lower MWs were detected on gel filtration. These fractions comprised a higher MW fraction (200000–400000 daltons) and fractions with MWs between 6500–13000 daltons, and possibly parallel the whole cytosol pattern. A lipid-protein aggregate similar to that reported here with lipolytic activity and MW in the region of 2×10^6 and hydrated density of 1.06–1.12 has been observed in the tissues of the rat (Sklan et al., 1982), horse (Sklan and Donoghue, 1982a) and chicken (Sklan and Donogue, 1982b). In addition, in chicken liver, zinc and copper metalloproteins with lipolytic activity have been isolated (Sklan and Donoghue, 1982b).

Lipolytic activity from turkey muscle cytosol co-eluted with zinc and copper, and this was also observed in the subfractions obtained following incubation of the lipid-protein aggregate. Moreover, a similar pattern of association of zinc and lipolytic activity was obtained by ultracentrifugation.

—Continued on next page

Low MW metalloproteins with lipolytic activity were purified by ion-exchange chromatography and gel filtrations; these contained copper and zinc or zinc alone. These metalloproteins both had MWs of approximately 7200 and amino acid composition with relatively low concentrations of aromatic amino acids and histidine. This is a characteristic of metallothioneins although the latter also exhibit high concentrations of cysteine which were not observed here (Winge et al., 1975; Anonymous, 1980). The metalloproteins isolated here also appear to be nonglobular on the basis of the discrepancies between MW estimation by gel filtration and SDS electrophoresis. The low MW fractions isolated here are similar in amino acid composition and in MW to the copper-chelatins isolated from the livers of several species (Evans et al., 1975; Riordan and Gower, 1975; Winge et al., 1975), but their identity remains to be conclusively determined.

Most lipases are stimulated by albumin in vitro and all are activated by bile acids (Brockerhoff and Jensen, 1974), although in some lipases, secondary bile salts such as deoxycholate inhibit activity (Hernell, 1975; Levy et al., 1981; Patton et al., 1977; Tuhakova et al., 1980). In addition, most lipases exhibit a pH optimum between 8 and 9 as was observed for the turkey muscle activity (Brockerhoff and Jensen, 1974). Furthermore, many lipases are high MW lipid-protein aggregates which readily decompose into monomers (Ramachandran et al., 1970; Kimura et al., 1972; Hamosh et al., 1979). Lipolytic activity appears to depend on the presence of serine at active sites in lipases (Brockerhoff and Jensen, 1974) and this was shown for turkey muscle activity both by preincubation with alkaline phosphatase and by the use of the inhibitor phenylmethylsulphonylfluoride. Sulphydryl reagents and EDTA also inhibited the lipolytic activity, in common with other lipases (Brockerhoff and Jensen, 1974). Increasing concentrations of NaCl did not inhibit turkey muscle lipolytic activity in contrast to lipoprotein lipase (Brockerhoff and Jensen, 1974). However, in contrast to previous reports, turkey muscle activity in the present study has demonstrated the presence of zinc and copper in low-MW proteins with lipolytic activity. Zinc may be present at the active site of lipolysis and has been demonstrated to catalyse the polarization and scission of C=O bonds by formation of the Lewis acids in studies on carboxypeptidase (Buckingham, 1976) and carbonic anhydrase (Pocker and Serkaven, 1978). In addition, phospholipase C from *bacillus cereus* has also been demonstrated to contain zinc (Ottolenghi, 1965).

Hydrolysis of the lipids of stored turkey meat occurs in both phospholipids and triglycerides (Acosta et al., 1966; Sklan et al., 1982) but the activity demonstrated in the present system in vitro at 37°C showed relatively low activity towards phospholipids. It is possible that the relative rates of activity towards the substrate may depend on the temperature, and also that the intracellular location of the triglycerides may provide protection against hydrolysis in intact muscle, resulting in a more rapid attack on phospholipids during storage.

The significance of the cytosolic lipolytic metalloproteins isolated in this study in the degradation of turkey meat remains to be precisely defined but this study represents the first demonstration of lipolytic zinc and copper metalloproteins in muscle.

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Effect of Electrical Stimulation on Postmortem Property Change of Myofibrillar Proteins

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ABSTRACT

Changes in biochemical properties of myofibrillar proteins of rabbit muscle, which had been subjected to electrical stimulation soon after slaughter, during postmortem storage at 0°C were investigated. Myofibrillar ATPase activity and the ATPase activity of acto-heavy meromyosin (HMM) complex, reconstituted from actin and HMM which had been prepared from at-death and postmortem muscles, decreased at first and then increased slightly during 7 days storage. In addition, the change of the dissociation constant of acto-HMM complex of electrically stimulated muscle during postmortem storage was quite small, i.e., 1.59 x 10^{-4}M for at-death muscle, 1.70 x 10^{-4}M for muscle stored for 1 day and 1.49 x 10^{-4}M for muscle stored for 7 days. This indicates that electrical stimulation treatment minimized the postmortem change of actin-myosin interaction.

INTRODUCTION

THE PHYSICAL and chemical changes of muscle after slaughter are largely dependent upon slaughtering conditions, ante- and postmortem treatments and storage conditions of carcasses as well as genetic traits and physiological and nutritional conditions of animals (Cassens et al., 1975; Forrest et al., 1975), so that physical conditioning procedures, such as high temperature conditioning, have been developed for improving the quality of meat (West, 1979). When muscle is stored at low temperature (0°C), actin-myosin interaction is strengthened with the development of rigor mortis and it continues to increase as measured by an increase of myofibrillar ATPase activity (Ito et al., 1978; Ikeuchi et al., 1978; Goodno et al., 1978). On the other hand, when muscle is stored at high temperature (37°C) the strength of actin-myosin interaction does not change appreciably during first 2 hr storage and then it decreases with increasing storage time (Ikeuchi et al., 1980). Therefore, it is evident that high temperature conditioning weakens the actin-myosin interaction.

Recently, various types of electrical stimulation methods have been developed for accelerating postmortem change of muscle in order to save the time and cost of meat marketing, for improving the quality of meat such as color and texture, and for preventing cold shortening of beef and lamb (Carse, 1973; Locker et al., 1975; Davey et al., 1976; Crystall and Hagyard, 1976; Savell et al., 1977; Cross, 1979; Smith et al., 1980; George et al., 1980; Taylor et al., 1980). It has been elucidated that the major causes for the improvement of meat quality by electrical stimulation are the increased rate of ATP depletion and pH fall (Bendall, 1976; Bendall et al., 1976; Shaw and Walker, 1977; Bouton et al., 1978; Bowling et al., 1978; Will et al., 1979). The rate of postmortem glycolysis and pH fall influence the biochemical properties of myofibrillar proteins, and hence they affect the textural properties of muscle (Asghar and Pearson, 1980). Recently, we have shown that pH and ATP concentration influence the rigor tension development and dissociation of rigor complex (Izumi et al., 1981). Therefore, electrical stimulation may affect the biochemical properties of myofibrillar proteins, especially the interaction of actin and myosin.

In the present study, we have investigated postmortem changes of myofibrillar proteins of electrically stimulated rabbit muscle, with special reference to the postmortem change of the actin-myosin interaction, and discussed the effectiveness of electrical stimulation in modifying the postmortem changes of myofibrillar proteins.

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MATERIALS & METHODS

Materials

Rabbits (Japanese White, male, 14–16 wk old; purchased from Kyudo Animal Breeding Co.) were anesthetized with sodium pentobarbital prior to exsanguination. Electrical stimulation was conducted on carcasses by connecting the cathode to the neck and the anode to the achilles tendon for 15 min under the conditions of 8 msec intervals, 4 msec delay, 3 msec duration and 50 mA current, using an electrical stimulator (NIHON KOHDEN SEN-3201). Carcasses were dipped for a few seconds in cold 10 mM sodium azide solution in order to prevent the growth of bacteria, wrapped in polyethylene bags and stored for 1, 7 and 21 days at 0°C. Longissimus thoracis, semimembranosus and biceps femoris muscles were used in the present study.

Measurement of pH value

Whole longissimus muscle was excised and minced and the minced muscle (2g) was homogenized in 10 ml, 0.15M KCl solution containing 5 mM sodium iodoacetate, pH 7.0, with a blade type homogenizer at a speed of 10,000 rpm for 3 min. The pH of the resulting homogenate was measured with a pH meter (Hitachi-Horiba F-7II).

Myofibrillar proteins

Myofibrils were prepared according to the procedure described by Briskey and Fukazawa (1971). At the first step in the procedure myofibrils were re-homogenized in about 5 vol of KCl-borate buffer (0.1M KCl, 39 mM borate, 5 mM ethylenediamine-tetraacetic acid (EDTA), and 1 mM β-mercaptoethanol, pH 7.1) containing 0.02% triton X-100 to eliminate the ATPase of the sarcoplasmic reticulum and other organelles. Myosin was extracted with 3 vol of a modified Guba-Straub solution (0.3M KCl, 0.15 M phosphate, 2 mM ethyleneglycol-*bis*(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 10 mM $MgCl_2$ and 5 mM ATP, pH 6.5). Extracted myosin was purified according to the procedure of Tonomura et al. (1961). Myosin was stored at −20°C in 50% glycerol solution containing 0.25M KCl, 2 mM β-mercaptoethanol and 2 mM EDTA until used. HMM was obtained by the method of Lowey and Cohen (1962) as described by Ito et al. (1978). Actin was prepared from acetone dried powder as described by Spudich and Watt (1971), except that the polymerization of G-actin was made by adding 50 mM KCl (final concentration) and by following dialysis against 100 vol of 50 mM KCl and 0.5 mM β-mercaptoethanol, pH 7.0.

ATPase activity

Myofibrillar Mg^{++}-ATPase assay was conducted in a water bath at 25°C for 1–5 min with 1 min intervals. Actin-activated HMM ATPase reaction was also made at 25°C. Conditions for the ATPase assays are given in the legends of the individual experiments. The inorganic phosphate liberated was determined by the method of Fiske and Subbarow (1925). *—Continued on next page*

Viscometry

Polymerization rate of G-actin was examined by determining the relative viscosity of actin after the addition of KCl (final concentration; 50 mM) (Mannherz et al., 1975).

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was carried out according to the procedure of Weber and Osborn (1969).

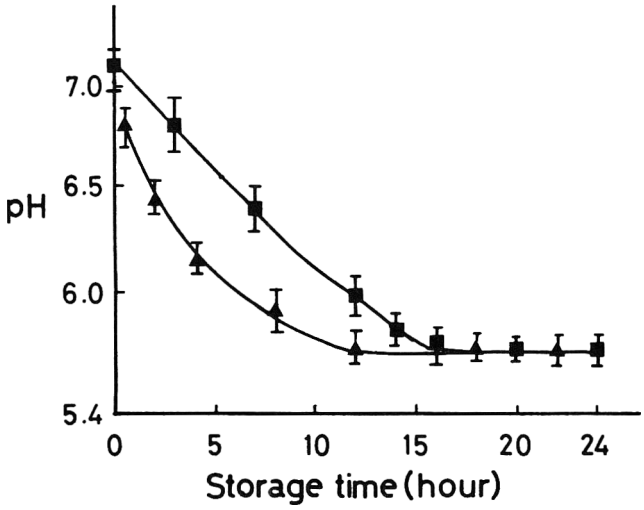

Fig. 1—Time course of pH fall in longissimus muscle during postmortem storage at 0°C. ■, nonstimulated; ▲, stimulated. Vertical lines represent means ± SEM (n=3).

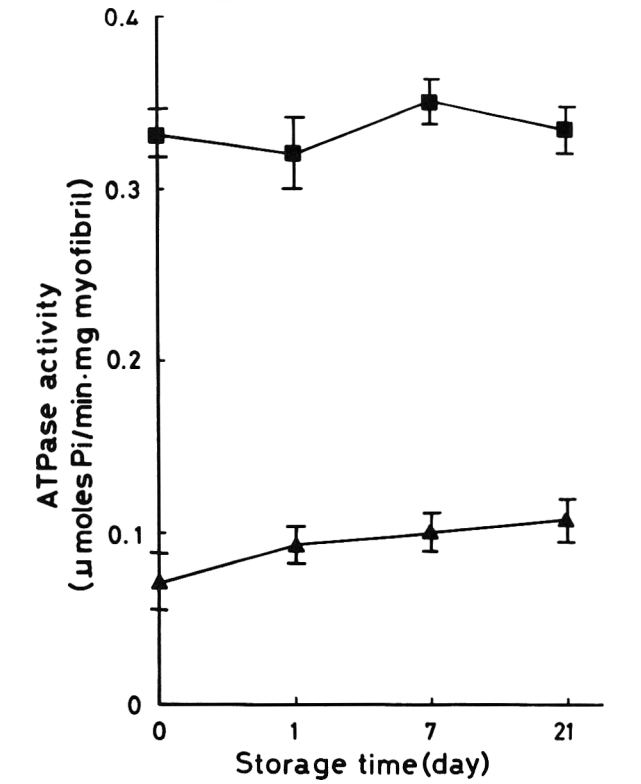

Fig. 2—Postmortem change of Mg^{++}-ATPase activity of electrically stimulated muscle myofibrils. Myofibrils were prepared from at-death and electrically stimulated muscle stored for varying periods at 0°C. Mg^{++}-ATPase activity was determined in a reaction mixture of 50 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 20 mM Tris-maleate (pH 6.8), 0.35 mg/ml protein (final concentration), and either 0.1 mM $CaCl_2$ or 1 mM EGTA. ■, in the presence of Ca^{++}; ▲, in the absence of Ca^{++}. Vertical lines represent means ± SEM (n=3).

Protein concentration

Protein concentration was determined by the biuret reaction (Gornall et al., 1949).

RESULTS

FIG. 1 shows the change of muscle pH during postmortem storage at 0°C. The rate of pH fall of electrically stimulated muscle was much faster than that of control: electrically stimulated muscle attained its ultimate pH value (pH 5.70) after 12 hr storage, while nonstimulated (control) muscle needed another 4–6 hr to reach to its ultimate pH value (pH 5.68) However, there was no appreciable difference in ultimate pH values between control and electrically stimulated muscle, even between different sides of the same carcass. This result is consistent with the findings reported by many workers (Bendall, 1976; Shaw and Walker, 1977; Whiting et al., 1981).

Fig. 2 shows the change of myofibrillar Mg^{++} -ATPase activity of electrically stimulated muscle during postmortem storage. As shown in this figure, myofibrillar Mg^{++}-ATPase activity in the presence of Ca^{++} decreased only slightly during 1 day storage and then increased a little during 7 days storage, although the change of myofibrillar ATPase activity during postmortem storage was quite small. We (Ikeuchi et al., 1978) and Goodno et al. (1978) have shown that myofibrillar ATPase activity increased during postmortem storage of muscle for 1 wk at low temperatures (0 or 4°C). So, it seems that the postmortem changing pattern of myofibrillar ATPase of electrically stimulated muscle (Fig. 2) differs from nonstimulated muscle (Goodno et al., 1978; Ikeuchi et al., 1978). On the other hand, the ATPase activity in the absence of Ca^{++} increased gradually throughout the postmortem storage period. [This was substantiated by statistical analysis; the residual ATPase activity of muscle stored for one and three weeks is significantly higher than the ATPase activity of at-death muscle ($P < 0.05$).]

Fig. 3 shows double-reciprocal plots of actin-activated HMM ATPase activity of electrically stimulated muscle. The change in acto-HMM ATPase activity during storage was small. For instance, the ATPase activity at the same weight ratio of actin to myosin as in situ decreased only slightly after 1 day storage and increased a little during 7 days storage. The maximum velocities (V_{max}) of the ATPase activity of at-death, 1 and 7 days stored muscle were 5.64, 6.25 and 5.56 μmoles Pi/min/mg HMM, respectively, and the apparent dissociation constants (K_{app}), assuming a binding ratio of 4.2×10^4g of actin/mole of HMM (Rizzino et al., 1970; Elzinga et al., 1973), were 1.59×10^{-4}, 1.70×10^{-4} and 1.49×10^{-4}M, respectively.

As shown in Fig. 4, no appreciable change was found in EDTA (K^+)- and Ca^{++}-activated myosin ATPase activity during postmortem storage of electrically stimulated muscle, although it has been found that in the case of nonstimulated muscle the activation energy of Ca^{++}-ATPase of myosin decreases with postmortem storage time (Ito et al., 1978). On the other hand, polymerization rate of G-actin decreased at first and then increased slightly during postmortem storage of electrically stimulated muscle (Fig. 5). This changing pattern is also somewhat different from that of nonstimulated muscle (Ito et al., 1978).

Fig. 6 shows SDS polyacrylamide gel electrophoretic patterns of myofibrils prepared from nonstimulated and electrically stimulated muscle during postmortem storage. A noticeable change was the appearance of 30,000- and 27,000-dalton components during 7 and 21 days storage of muscle, which might be the degradative products of troponin-T (Dabrowska et al., 1973; Olson et al., 1977). In addition, the intensity of an unidentified band (indicated by an arrow) between myosin heavy chain and actin, which might

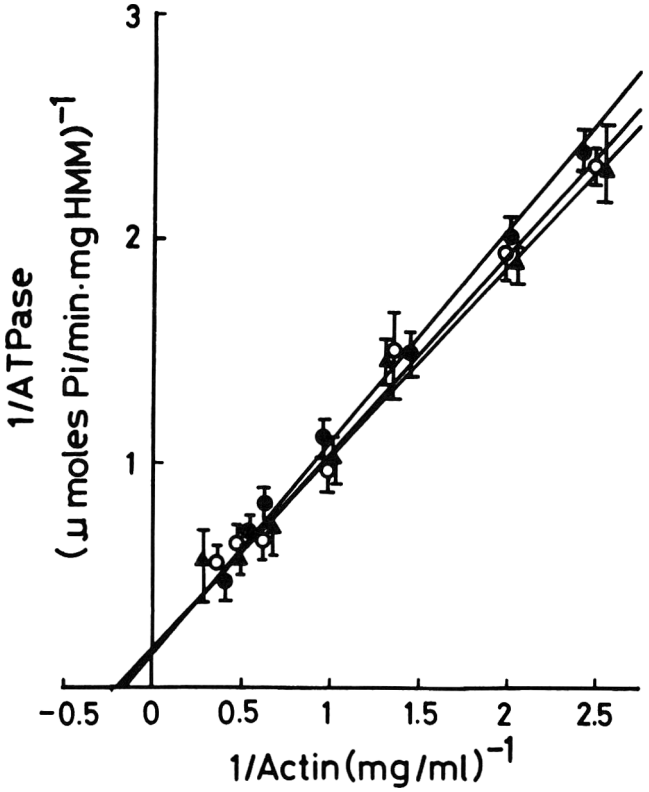

Fig. 3—Double-reciprocal plots of actin-activated HMM ATPase vs actin concentration. Actin and HMM were prepared from at-death and electrically stimulated muscles stored for designated periods at 0°C. ATPase reaction was made under the condition of 40 mM KCl, 1 mM $MgCl_2$, 2mM ATP, 10 mM Tris-maleate (pH 7.0), 0.2 mg/ml HMM and varying concentrations (0.4–2.5 mg/ml) of actin. The linear regression lines were calculated from the data presented in this figure. ○, acto-HMM prepared from at-death muscle, y=0.813x + 0.177; ●, acto-HMM from 1 day stored muscle, y=0.851x + 0.16; ▲, acto-HMM from 7 days stored muscle, y=0.764x + 0.18. Vertical lines represent means ± SEM (n=3).

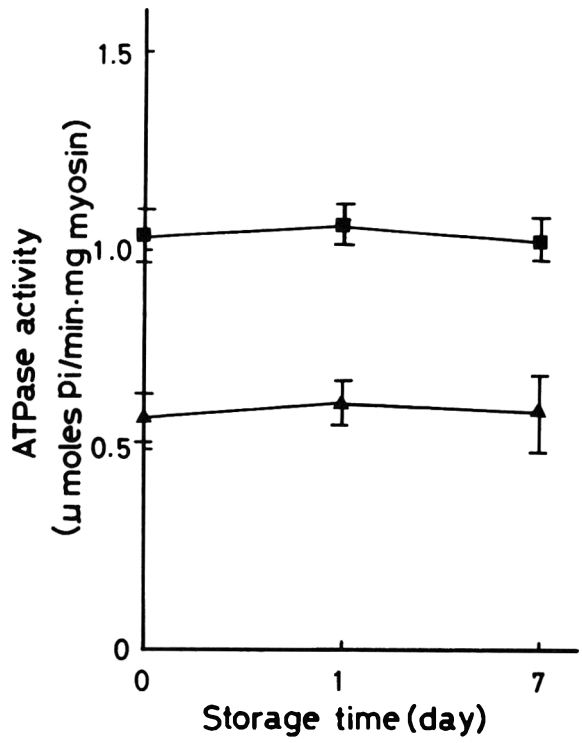

Fig. 4—Postmortem changes of Ca^{++}- and EDTA (K^+)-ATPase activities of myosin prepared from electrically stimulated muscle. The muscles were stored at 0°C for 1 and 7 days. The value shown at 0 day was from at-death muscle (nonstimulated). EDTA (K^+)-ATPase activity was determined in 0.5 M KCl, 1 mM EDTA, 1 mM ATP, 20 mM Tris-HCl (pH 7.5) and 0.5 mg/ml myosin. Ca^{++}-ATPase assay was made in 0.25M KCl, 1 mM ATP, 20 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$ and 0.5 mg/ml myosin. ■, EDTA (K^+)-ATPase; ▲, Ca^{++}-APTase. Vertical lines represent means ± SEM (n=3).

be derived from myosin heavy chain, increased during postmortem storage of muscle. On the other hand, electrophoretograms of nonstimulated muscle myofibrils indicate that the change of the structure of myofibrillar proteins during storage was small, e.g., 30,000- and 27,000- dalton components appeared only slightly after 7 days storage of muscle and after 21 days storage they appeared clearly, while the intensity of the unidentified band between myosin heavy chain and actin did not increase.

DISCUSSION

IT HAS BEEN FOUND that myofibrillar ATPase activity increases during 2 wk storage of muscle at 0°C (Goodno et al., 1978). Ikeuchi et al. (1980) have demonstrated a changing pattern of myofibrillar ATPase activity postmortem similar to that of acto-HMM ATPase activity (as the same weight ratio of actin to myosin as in situ), indicating that myofibrillar ATPase activity is dependent upon the activity of acto-myosin complex in muscle. Ito et al. (1978) have clearly shown from the double-reciprocal plots of acto-HMM ATPase activity that the apparent dissociation constant of acto-HMM complex decreases with increasing postmortem storage time of muscle at 0°C. This finding indicates that the affinity of actin for myosin increases with the development of rigor mortis and it continues to increase until 1 wk storage. In the present study, however, both acto-HMM ATPase (at the same weight ratio of actin to myosin as in situ) and myofibrillar Mg^{++}-ATPase (in the presence of Ca^{++}) activities of electrically stimulated muscle decreased

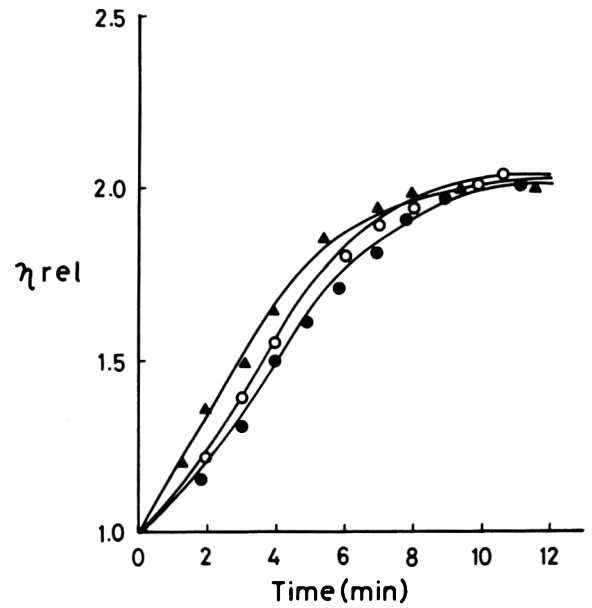

Fig. 5—Polymerization rate of G-actin prepared from electrically stimulated muscle. The reaction mixture contained 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, 10 mM Tris-HCl (pH 8.0) and 2 mg/ml actin. Polymerization reaction was started by the addition of 1/20 vol of 1.0M KCl at 25°C. ○, actin from at-death muscle; ●, actin prepared from electrically stimulated-1 day stored muscle; ▲, actin from electrically stimulated-7 days stored muscle. Each plot shown in this figure is the mean of three determinations.

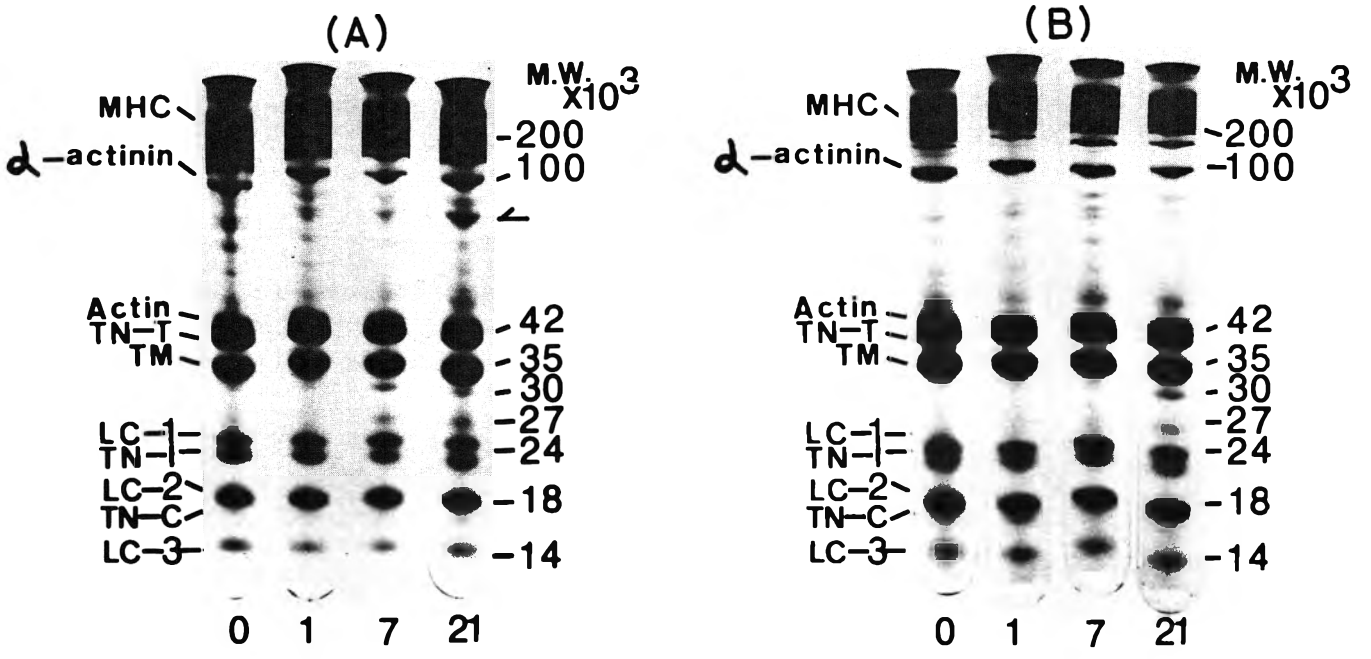

Fig. 6—SDS polyacrylamide gel electrophoretograms of nonstimulated and electrically stimulated muscle myofibrils. (A), Electrically stimulated muscle; (B) Nonstimulated muscle. Myofibrils were prepared from at-death and postmortem muscles. 100 μg myofibril was loaded on each gel. Electrophoresis was made on 7.5% gel at 8 mA current per tube for 5 hr. The number under each gel indicates postmortem storage time (days). MHC, myosin heavy chain; TN-T, troponin-T; TN-I, troponin-I; TN-C, troponin-C; TM, tropomyosin; LC 1-3, myosin light chains 1-3.

during 1 day storage and then increased slightly during 7 days storage. This result reconfirmed that postmortem change of myofibrillar ATPase activity is dependent upon the change of acto-HMM ATPase activity. In addition, no appreciable change was found in the dissociation constant of acto-HMM complex during postmortem storage of muscle, indicating that postmortem change of the actin-myosin interaction in electrically stimulated muscle was quite small. In other words, the electrical stimulation procedure inhibits the increase in the actin-myosin interaction during postmortem storage of muscle.

Mannherz et al. (1975) and Ito et al. (1978) have indicated that acto-subfragment I ATPase and acto-HMM ATPase activities are closely related to the polymerization rate of G-actin. As shown in Fig. 5, the polymerization rate of actin decreased at first and then increased slightly during 7 days storage. Therefore, the present results (Fig. 2, 3 and 5) also indicate that acto-HMM ATPase (at the same weight ratio of actin to myosin as in situ) as well as myofibrillar ATPase activity is closely related to the polymerization rate of G-actin.

Dutson et al. (1980) have suggested that the increased tenderness of electrically stimulated muscles may result from the enhanced activity of autolytic enzymes released from lysosomes. Bendall (1976) and Bendall et al. (1976) reported that electrical stimulation hastens the development of rigor mortis and allows the natural enzymatic process to occur much sooner. As shown in Fig. 6, the 30,000- and 27,000-dalton components appeared more distinctively in stimulated muscle than in nonstimulated muscle during postmortem storage. The distinctive appearance of the degradative products of troponin-T and myosin heavy chain in the stimulated muscles during postmortem storage (Fig. 6) may indicate the enhancement of autolysis in electrically stimulated muscle.

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—Continued on page 32

Postmortem Muscle Metabolism and Meat Tenderness

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ABSTRACT

Left sides of 75 steers were electrically stimulated (ES) and right sides were nonstimulated controls (NES). NES sides had the highest pH, lowest temperature, were slower-metabolizing (lower R values), and produced steaks that had the least desirable sensory ratings compared to ES sides. Carcass weight, fat thickness, temperature and pH accounted for 31, 32, 34 and 16% of the variation, respectively, in overall tenderness ratings for steaks from NES sides and accounted for 0, 0, 0 and 7% of the variation, respectively, for steaks from ES sides. R values accounted for 28 and 32% of the variation in overall tenderness ratings of steaks derived from NES and ES sides, respectively. Thus, metabolic rate (R value) is a good indicator of postmortem tenderness.

INTRODUCTION

IN A REVIEW PAPER addressing the topic of nutritionally controlled tenderness, Tatum (1981) concluded that the primary reason for increased tenderness of beef, as a result of feeding, was increased fatness and carcass weight, both of which reduce the rate of postmortem chilling. Fat thickness has been equated to USDA quality grade in its ability to stratify carcasses according to expected palatability (Dolezal et al., 1982). It is possible that these carcass traits (weight and fatness) exert an influence on tenderness by maintaining higher carcass temperatures while the pH is being reduced. Lochner et al. (1980) found that the temperature decline of beef muscle very early postmortem was related to tenderness, even when no differences were found in sarcomere length.

In a study which evaluated steaks from young bulls selected either for leanness or for tenderness, Field et al. (1970) found that, apart from differences in tenderness, the only major differences between the two groups of bulls were muscle temperature and pH, both obtained at 1 hr postmortem. Similarly, Luckett et al. (1975) identified muscle temperature (1 hr postmortem) and muscle pH (6 days postmortem) as important factors influencing ultimate beef tenderness. Thus, it appears that postmortem metabolic rate (measured by pH), as altered by temperature, has a significant effect on meat tenderness.

It is generally accepted that electrical stimulation (ES) varies in the magnitude of tenderization that it produces (Savell et al., 1981). If these differences are due to varying rates of postmortem metabolism, then tenderness differences between ES carcasses or nonstimulated (NES) carcasses should be explained by measurement of metabolic rate. Therefore, the objective of this study was to evaluate the relationship of direct measures (muscle pH and R value) and indirect measures (carcass weight and subcutaneous fat thickness) of metabolic rate with the tenderness of NES and ES beef.

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MATERIALS & METHODS

BRAHMAN, Angus and Brahman x Angus steers (n=25 in each group) fed a high energy ration for differing lengths of time (0–224 days) were slaughtered at the meat laboratory of Texas A&M Univ. Left sides of each carcass were ES (550 v, approximately 2 a, 15–17 impulses at 2 sec on and 2 sec off per impulse) within 45 min postmortem, and right sides were used as the NES controls. Both sides of each carcass were placed, unshrouded, in a 1°C cooler 20 min after stimulation of the left side. A 10-g muscle sample was removed from the posterior end of the longissimus dorsi (LD) of each side, in the region of the third lumbar vertebra, 2 hr after stimulation of the left side. The muscle sample (wrapped in cheesecloth) was frozen and stored (approximately 8 mo) in liquid N_2 until the R value was determined.

Carcass muscle temperature was recorded 6 hr post-stimulation usng copper-constantan thermocouples (probes) and an Omega digital thermometer. Probes were placed parallel to and 3.2 cm from the split dorsal spinous process of the thoracic vertebrae, between the 12th and 13th ribs, at a depth of 3.8 cm. Muscle pH was also measured at 6 hr post-stimulation using a portable pH meter with either an Ingold or a Cole-Parmer probe-type electrode. The pH measurement was taken near the posterior end of the LD immediately anterior to the origin of the gluteus medius, at a depth of 1 cm.

Both sides were ribbed at 24 hr postmortem, allowed 15 min to bloom, and evaluated for USDA quality (mean marbling score = 218 ± 64; slight minus) and yield grade factors (mean hot carcass weight = 212 ± 58 kg; mean subcutaneous fat thickness = 6.2 ± 4.5 mm). Left (ES) sides were dissected into muscle, bone and fat at 24 hr postmortem for another research project. The dissected LD muscle was wrapped in polyvinyl chloride film and aged on racks in a 1°C cooler. Right (NES) sides were aged as quarters. Seven to 8 days postmortem, two steaks (3.2 cm thick) were removed from the anterior end of the shortloin (or from a comparable location in the dissected muscle) for sensory evaluation and shear force determination. The steaks were wrapped in polyethylene-coated freezer paper, blast-frozen (−34°C) and held approximately 8 mo at −18°C until evaluated.

Steaks were thawed for 24 hr at 2°C and cooked to an internal temperature of 70°C on Faberware open-hearth broilers, prior to sensory evaluation by an experienced (8-member) panel and determination of Warner-Bratzler (WB) shear force values (1.27 cm cores). The sensory panel evaluated juiciness, muscle fiber tenderness, connective tissue amount, overall tenderness, flavor desirability and overall palatability on 8-point rating scales (1 = least juicy, least tender, most connective tissue, least desirable flavor and least palatable overall; and 8 = most juicy, most tender, no connective tissue, most desirable flavor, and most palatable overall).

The R value procedure involved homogenization of 10g of frozen muscle in 50 ml of 0.9M perchloric acid (at room temperature) for 1 min. The resultant slurry was centrifuged for 10 min at 3,000 x *g* and the supernatant was adjusted to pH 6.0–6.5 using 2.0M potassium hydroxide. After chilling on ice for 45 min, the solution was filtered through Whatman No. 1 filter paper. Phosphate buffer (pH 6.5, 0.1M potassium phosphate) was combined with the filtered extract (2.0 ml + 0.1 ml, respectively) and the absorbance of the mixture was recorded at 248, 250, 258 and 260 nm. R value was determined by creating a ratio of two absorbance values: R248 was the absorbance at 248 nm over the absorbance at 260 nm (Davidek and Velisek, 1973); R250 was the absorbance at 250 nm over the absorbance at 260 nm (Honikel and Fischer, 1977); and R258 was the absorbance at 258 nm over the absorbance at 250 nm (Kahn and Frey, 1971).

Means and standard deviations were calculated. Statistical analyses included paired t-test, correlation coefficients and both linear

and curvilinear regression. For all analyses, the Statistical Analysis System of Barr et al. (1979) was used.

RESULTS & DISCUSSION

MEANS AND STANDARD DEVAITIONS for muscle temperature, pH, sensory panel ratings, shear force value and R values of NES and ES beef longissimus are presented in Table 1. NES sides had significantly higher muscle pH and produced steaks that had higher shear force values and lower (less desirable) ratings for muscle fiber tenderness, connective tissue amount, overall tenderness, flavor desirability and overall palatability than those samples from ES sides. These data are in agreement with many reports in the literature regarding the effect of ES on meat palatability (Bouton et al., 1978; Cross, 1979; George et al., 1980; Savell et al., 1977, 1978a, b, 1979; Smith et al., 1977, 1979) and muscle pH (Bendall et al., 1976; Devine et al., 1979).

R values (Table 1) were significantly different between ES and NES samples. Honikel and Fischer (1977) explained the principle behind the use of absorbance ratios to indicate the stage of rigor mortis. The adenine nucleotides (ATP, ADP, AMP) exhibit absorbance maxima near 260 nm while the absorbance of inosine monophosphate (IMP) and inosine peaks near 248 nm and hypoxanthine absorbance peaks near 250 nm. Since the adenine nucleotides are converted to IMP, inosine and hypoxanthine in postmortem muscle, the R value (ratio of absorbance at two wavelengths) of an extract containing these compounds is an indication of the relative amounts of the adenine nucelotides versus the inosine compounds (Honikel and Fischer, 1977). The normal progression of rigor mortis would reduce the concentration of adenine nucleotides (measured by the absorbance at, or near, 260 nm) while maintaining or elevating the concentration of inosine compounds (measured by absorbance at or near 250 nm).

Table 1—Means and standard deviations for muscle temperature, pH, sensory panel ratings, shear force value and R values of nonstimulated and electrically stimulated beef longissimus

Measurement	Stimulation treatment[a]	
	NES	ES
Temperature (°C)[b]	10.76 (3.59)[e]	12.52 (3.80)
pH[b]	6.38 (0.28)	5.77 (0.22)
Muscle fiber tenderness rating[c]	4.10 (1.64)	5.85 (1.11)
Connective tissue rating[c]	7.31 (0.61)	7.53 (0.34)
Overall tenderness rating[c]	4.11 (1.67)	5.88 (1.09)
Flavor desirability rating[c]	5.43 (0.56)	5.61 (0.45)
Overall palatability rating[c]	4.12 (1.51)	5.58 (0.84)
Shear force value (kg)	5.45 (1.80)	3.94 (1.16)
R248[d]	0.82 (0.17)	1.08 (0.14)
R250[d]	0.88 (0.15)	1.12 (0.14)
R258[d]	1.14 (0.10)	0.96 (0.09)

[a] NES = nonstimulated, ES = electrically stimulated.
[b] Measurements taken 6 hr post-stimulation.
[c] Based on 8-point rating scales (1 = extremely tough muscle fibers, abundant amount of connective tissue, extremely tough overall, extremely undesirable flavor and extremely undesirable overall palatability; 8 = extremely tender muscle fibers, no connective tissue, extremely tender overall, extremely desirable flavor and extremely desirable overall palatability, respectively).
[d] R values calculated as the ratios of the absorbance at 248 nm over the absorbance at 260 nm (R248); or 250 nm over 260 nm (R250); or 258 nm over 250 nm (R258). R values were measured on samples excised at 2 hr postmortem.
[e] Parenthetical values are standard deviations. All means are significantly different ($P<0.05$).

Absorbance ratios (although not always referred to as R values) have been used to measure postmortem metabolic rate by several researchers: Kahn and Frey (1971) used the ratio of absorbance at 258 nm over absorbance at 250 nm (corresponding to the R258 values reported in this study) as a simple measure of rigor mortis development; Davidek and Velisek (1973) used a 248 nm reading over a 260 nm reading (R248 in this study) as a method of examining rigor mortis; and Honikel and Fischer (1977) used absorbance at 250 nm over absorbance at 260 nm (R250 in this study) as a method of detecting PSE (pale, soft, exudative) and DFD (dark, firm, dry) pork. Based on the R values obtained in this study, ES sides had a more rapid metabolic rate and, thus, exhibited a more advanced stage of rigor mortis development at 2 hr post-stimulation than did the NES sides (Table 1). This is in agreement with results reported by Calkins et al. (1982) regarding the concentration of adenine nucleotides and inosine compounds, as well as R value changes, during the progression of rigor mortis in NES and ES beef muscle.

The relationships between R value (R248, R250 and R258) and meat tenderness of NES and/or ES beef muscle are presented in Table 2. In all cases, R258 had lower correlation coefficients with tenderness than did R248 or R250, indicating that absorbance at 260 nm was a more definitive measure for incorporation into the R ratio for use as a predictor of tenderness. The R value explained more of the variation in sensory panel ratings and shear force values in the ES group than in the NES group, and larger correlation coefficients were observed for the overall sample population (ES plus NES) than for either the ES or NES groups separately. These data indicate that postmortem metabolic rate, especially as measured by R248 or R250, is significantly related to tenderness of beef from NES and/or ES carcasses.

Results of regression analyses using carcass weight, subcutaneous fat thickness, muscle pH, muscle temperature or R250 to predict tenderness of NES and ES beef muscle are shown in Table 3. All of these measures were significantly related to tenderness of NES beef, although muscle pH was observed to account for the least amount of variability in

Table 2—Correlation coefficients relating R values to sensory ratings and shear force value of ES and NES muscle

Measurement	Sample population	Sensory panel rating			Shear force value (kg)
		Muscle fiber tenderness	Overall tenderness	Overall palatability	
R248[a]	NES[b]	0.51*	0.52	0.48	−0.31
R250[a]	NES	0.52	0.53	0.51	−0.33
R258[a]	NES	−0.36	−0.35	−0.31	0.18
R248	ES[c]	0.56	0.57	0.55	−0.45
R250	ES	0.56	0.57	0.56	−0.46
R258	ES	−0.53	−0.54	−0.53	0.43
R248	NES + ES	0.67	0.68	0.65	−0.52
R250	NES + ES	0.68	0.68	0.66	−0.53
R258	NES + ES	−0.62	−0.62	−0.58	0.47

[a] R value calculated as the ratio of the absorbance at 248 nm over the absorbance at 260 nm (R248); or 250 nm over 260 nm (R250); or 258 nm over 250 nm (R258).
[b] NES = nonstimulated.
[c] ES = electrically stimulated.
* All correlation coefficients are significant ($P<0.01$).

Table 3—Coefficients of determination for selected factors related to tenderness

Sample population	Trait	Carcass weight	Subcutaneous fat thickness	Muscle[a] pH	Muscle temp[a]	R250[b]
NES[c]	Muscle fiber tenderness	31.79[e]	32.47	15.47	34.26	27.04
NES	Overall tenderness	31.23	31.56	16.04	34.31	28.09
NES	Shear force value	21.11	32.13	9.51	36.22	10.89
ES[d]	Muscle fiber tenderness	NS[f]	NS	NS	NS	31.36
ES	Overall tenderness	NS	NS	7.22	NS	32.49
ES	Shear force value	NS	12.89	11.25	6.28	21.16

[a] Muscle pH and temperature were measured 6 hr post-stimulation.
[b] R250 is the R value obtained by calculating the ratio of the absorbance at 250 nm over the absorbance at 260 nm. The value was obtained from muscle samples excised and frozen at 2 hr post-stimulation.
[c] NES = nonstimulated.
[d] ES = electrically stimulated.
[e] Except for NES equations using subcutaneous fat thickness (in a quadratic form), all coefficients of determination are from linear regression equations.
[f] Regression was not signifcantly different from zero.

tenderness. Within the ES sample, however, only R250 was consistently related to sensory panel ratings and shear force values. Since the amount of tenderness variation explained by carcass measurements (fatness, weight, pH or temperature) was low (C.D. = 12.9%) or not significant, it would seem that ES negates the influence of these characteristics. Perhaps this is due to the increase in metabolic activity caused by ES. Increased metabolism of ES muscle has been demonstrated through the measurement of various metabolic indicators: rate and extent of pH decline (George et al., 1980; Hallund and Bendall, 1965; Shaw and Walker, 1977); concentration of lactic acid at specific times postmortem (Bendall, 1976; Bendall et al., 1976; Forrest and Briskey, 1967); and the concentration of the adenine nucleotides (Bendall, 1976; Bowling et al., 1978; Calkins et al., 1982). This increased metabolic rate in ES beef muscle appears to overcome detrimental effects of little subcutaneous fat, light carcass weight, and/or rapid chilling (e.g., relatively cool muscle temperatures at 2 hr post-stimulation).

Since muscle pH has been considered a measure of postmortem muscle metabolism, a similar relationship between R250 and postmortem muscle metabolism might be expected, however, they are not related to tenderness in the same manner. The relatively low coefficients of determination (C.D.) between pH and tenderness (as compared to those of R values and tenderness, Table 3) of NES (C.D. = 14–16%) and ES (C.D. = nonsignificant to 11.3%) beef muscle may be explained in several ways. First, the pH measurement was taken on the outer area of the muscle (approximately 1 cm in depth). Different cooling rates for the internal versus external portions of the muscle probably resulted in different internal versus external pH values. Thus, the mean pH of the muscle may not be highly related to the pH measurement obtained on the exterior muscle by use of a probe electrode. Secondly, there may not be a strong, linear relationship between muscle pH and ultimate meat tenderness. Muscle pH decline occurs via an accumulation of lactic acid. Since muscle is a naturally buffered system, the relationship between pH and lactic acid concentration is not a linear one. As a result, muscle pH may not be a direct measure of the postmortem metabolism of the muscle. If metabolic rate, rather than muscle pH, per se, is the factor that ultimately alters meat tenderness, then a direct relationship between metabolic rate and tenderness would be expected, regardless of the type of treatment employed to alter metabolic activity.

In support of this latter point, R250 exhibits a significant association to tenderness of both NES and ES muscle (Table 3). Perhaps, as suggested by Locker and Daines (1976) in an evaluation of the effects on tenderness of elevated temperature, alterations may occur in actin-myosin bonding to cause increases in tenderness. Elucidation of the means by which rates of postmortem metabolism are related to tenderness requires further study.

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—Continued on page 35

Relationships of Hydrophobicity to Emulsifying Properties of Heat Denatured Proteins

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ABSTRACT

Effects of heating on the emulsifying properties of selected food proteins and the protein surface hydrophobicity (S_o) as a predictor of these properties were investigated. The emulsifying properties of the proteins studied were differently affected by heating. Heat-denaturation was not always accompanied by loss of emulsifying properties, but, on the contrary, in some cases resulted in great improvement. The emulsifying properties could well be predicted solely on the basis of S_o level but not on the basis of solubility level, which indicated that S_o is a very important property determining protein functionality. However, the emulsifying activity, emulsion stability and fat binding capacity of the proteins studied could be explained and more accurately predicted using S_o and solubility together.

INTRODUCTION

TO BE USEFUL and successful in food applications, proteins in addition to providing essential amino acids, should ideally possess several desirable characteristics referred to as functional properties (Wang and Kinsella, 1976). Moreover, according to Johnson (1970) the functional and physical properties, rather than the nutritional value, of protein in protein-containing products will largely determine their acceptability as ingredients in prepared foods.

The ability of protein to aid the formation and stabilization of emulsions is critical for many applications in chopped, comminuted meats, cake batters, coffee whiteners, milk, mayonnaise, salad dressings, and frozen desserts. In these products varying emulsifying and stabilizing capacities are required because of the differing composition and stresses to which these products are subjected (Kinsella, 1979). Moreover, the ability of proteins to bind fats is a very important functional property for such applications as meat replacers and extenders, principally because it enhances flavor retention and reputedly improves mouth-feel (Kinsella, 1976). Soy proteins have been added to ground meats to promote fat absoption or fat binding, and thus decrease cooking losses and maintain dimensional stability in the cooked product (Wolf and Cowan, 1975).

To evaluate the emulsifying properties of a protein its solubility profile is usually determined, because of its usefulness as an excellent index of protein functionality (Kinsella, 1976). Good solubility can markedly expand potential applications of a protein (Kinsella, 1976). Denaturation, on the other hand, implicates losses of protein functional properties and is usually measured as a loss of solubility (Nakai and Powrie, 1981). Generally, surfactant properties are related to the aqueous solubility of proteins (Kinsella, 1976). A positive correlation between solubility and the ability of a protein to emulsify and stabilize an emulsion has been reported in many studies (Crenwelge et al., 1974; Pearson et al., 1965; Swift and Sulzbacher, 1963; Volkert and Klein, 1979; Yasumatsu et al., 1972). Many authors point to evidence, however, that emulsifying properties and solubility are not well correlated (Aoki et al., 1980; McWatters and Cherry, 1975; McWatters and Holmes, 1979a, 1979b; Smith et al., 1973; Wang and Kinsella, 1976).

The protein hydrophobicity has been lately receiving much attention since the hydrophobic interactions are considered to play important roles in the functional properties of food proteins (Kato et al., 1981; Kinsella, 1979). Keshavarz and Nakai (1979) reported a significant correlation between surface hydrophobicity (determined by hydrophobic chromatography and hydrophobic partition techniques) and interfacial tension of the proteins studied. Kato and Nakai (1980) subsequently reported that the surface kydrophobicity (determined fluorometrically) showed significant correlations with interfacial tension and emulsifying activity of the proteins studied. Their results suggest that the emulsification of oil with protein can be explained using the concept of protein hydrophobicity. Nakai et al. (1980b) also reported that the effective (surface) hydrophobicity showed good correlations with interfacial tension and emulsifying activity of the plant proteins studied. It is noteworthy that these authors observed a closer correlation of emulsification capacity with hydrophobicity than with solubility.

While many factors influence the performance of proteins in food systems, heat treatment is one of the most important and is very often used during the processing of protein products. This study was instigated by the observation that the emulsifying capacity of soy protein was not adversely affected even by texturization which caused a loss in solubility. The objectives of this paper, therefore, were to determine the effect of heating on the emulsifying properties of selected food proteins, and to assess the value of surface hydrophobicity as a predictor of the emulsifying properties of these proteins.

MATERIALS & METHODS

Materials

Bovine serum albumin (#A-4503), β-lactoglobulin (#L-6879 from milk) and ovalbumin (#A-5503) were all purchased from Sigma Chemicals (St. Louis, MO). Soy protein isolate was obtained from General Mills, Inc., (Minneapolis, MN). Promine-D was purchased from Central Soya Co. (Chicago, IL). Pea protein isolate (a) (M412-046), Century cultivar field pea, was obtained from POS Pilot Plant Corp., Univ. of Saskatchewan (Saskatoon, Sask.). Pea protein isolate (b), Pro-Pulse W100, was obtained from Griffith Laboratories Ltd. (Scarborough, Ont.). Vital wheat gluten, Whetpro 75%, was supplied by Industrial Grain Products Ltd. (Thunder Bay, Ont.). Canola Protein isolate was prepared by the method of Nakai et al. (1980a). The protein content of isolate batches (a) and (b) was 84% and 88%, respectively. Sunflower protein isolate was prepared by the method of Nakai et al. (1980a). Whey protein concentrate (75%) was obtained from Sodispro Technol. (St. Hyacinthe, Que.). Gelatin, Bloom 300, was purchased from United States Biochemical Corp. (Cleveland, OH).

Whole casein was prepared from skim milk by acid precipitation. Myosin was extracted from the pectoralis superficialis and profundus muscles of a freshly slaughtered male chicken (broiler 11 – 12 wk old) with cold KCl-potassium phosphate buffer, pH 6.5 according to the method of Perry (1955).

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Preparation of protein samples

For hydrophobicity, solubility, emulsifying activity index, emulsion stability index and fat binding capacity determinations, the following protein samples were prepared. To increase the accuracy of regression analysis, as many different extents of denaturation as possible were attempted thus resulting in samples of different solubility. For protein samples which needed constant pH to reproduce the same degree of denaturation, the weakest phosphate buffer (0.01M) was used. For proteins in which the solubility was difficult to change by heating, $CaCl_2$ was used to disperse the proteins.

Bovine serum albumin (BSA). A 1% solution in distilled water. Heat denatured: the pH was adjusted to 4.0 and then the solution was heated at 100°C for 5 min and homogenized in an Omni-Mixer (Ivan Sorvall, Norwalk, CT) for 1 min at speed setting 1 (lowest speed).

β-Lactoglobulin. A 1% solution in distilled water. Heat denatured: the pH of the solution was adjusted to 1.0, heated at 100°C for 15 min and then homogenized for 1 min at speed setting 1.

Soy protein isolate. A 1% aqueous dispersion, stirred on magnetic stirrer for 5 min, and then, homogenized for 1 min at speed setting 5. Samples 1–4: pH 5.5, heated at 100°C for 0.25, 0.5, 1.0, and 2.0 min, respectively. Sample 5: pH 5.5, autoclaved at 121°C for 15 min. Sample 6: pH 7.2, autoclaved at 121°C for 15 min.

Promine-D. A 1% dispersion in 0.01M phosphate buffer, pH 7.4, stirred magnetically for 5 min and homogenized for 1 min at speed setting 5.

Ovalbumin. A 1% aqueous solution. Samples 1–4: pH 5.6, heated at 80°C for 1.5, 2.0, 2.5, and 3.0 min, respectively. Sample 5: pH 5.6, heated at 100°C for 5 min. Sample 6: pH 1.0, heated at 100°C for 15 min.

Pea protein isolate (a). A 1% aqueous dispersion, stirred magnetically for 5 min, and then, homogenized for 1 min at setting 5. Samples 1–4: pH 5.8, heated at 80°C for 1, 2, 4 and 7 min, respectively, and then, homogenized for 10 sec.

Pea protein isolate (b). A 1% dispersion in 0.01M phosphate buffer, pH 7.4, was magnetically stirred 5 min, and then, homogenized for 1 min at speed setting 5.

Vital gluten. Samples 1–5: 1% dispersions in 0.5, 1.0, 1.5, 1.75 and 2.0 N acetic acid, respectively. Then, the samples were magnetically stirred for 5 min, homogenized 15 sec at speed setting 1, and heated at 100°C for 30 min.

Canola protein isolate (a). A 1% aqueous dispersion, was stirred for 5 min, and then, homogenized for 1 min at speed setting 5. Samples 1–4: pH 5.5, heated at 100°C for 0.5, 1.0, 1.5 and 2.0 min, respectively, and then homogenized for 5 sec at speed setting 1. Sample 5: pH 7.2, autoclaved at 121°C for 10 min.

Canola protein isolate (b). A 1% dispersion in 0.01M phosphate buffer, pH 7.4, was stirred for 5 min, and then, homogenized for 1 min at speed setting 5.

Sunflower protein isolate. A 1% dispersion in 0.01M phosphate buffer, pH 7.4, was stirred for 5 min, and then, homogenized for 1 min at speed setting 5.

Whey protein. A 1% solution in 0.03M $CaCl_2$. Samples 1–3: pH 6.0, heated at 80°C for 4, 5 and 15 min, respectively. Sample 4: pH 5.8, heated at 80°C for 15 min.

Whole casein. A 1% solution in 0.01M $CaCl_2$, pH 7.4. Samples 1 and 2: 1% solution in 0.01M $CaCl_2$, pH 7.4, autoclaved at 121°C for 5 and 20 min, respectively. Sample 3: 1% solution in 0.02M $CaCl_2$, pH 7.4, autoclaved at 121°C for 20 min.

Gelatin. A 1% dispersion in 0.01M phosphate buffer, pH 7.4, stirred 5 min, and then, homogenized for 1 min at speed setting 5. Heat denatured: 0.5% dispersion in 0.01M phosphate buffer, pH 7.4, heated at 75°C for 2.5 min with stirring.

Myosin. To form a stock solution for determination of hydrophobicity, solubility, and other functional properties, the original gel was diluted twice with 0.3M NaCl in 0.01M phosphate buffer, pH 7.4. The control sample was unheated and samples 1 and 2 were heated at 75°C for 1 and 5 min, respectively.

Prior to analysis, all heated and unheated protein samples–except Promine-D, pea protein (b), canola protein (b), sunflower protein, gelatin, and myosin–were dialysed against 0.01M phosphate buffer, pH 7.4, containing 0.02% sodium azide. After hydrophobicity, solubility and emulsifying properties were determined, the protein samples were freeze-dried and subsequently used for fat binding capacity (FBC) determinations.

Protein (surface) hydrophobicity

Protein surface hydrophobicity was fluorometrically determined according to the method of Kato and Nakai (1980) after slight modification. Each protein sample (2 ml) was serially diluted with 0.01 M phosphate buffer, pH 7.4, to obtain protein concentrations ranging from 0.00156% to 0.05%. Two sets of protein samples were prepared. Ten liters of cis-parinaric acid solution were added to the first set of tubes. The parinaric acid-protein conjugate was then excited at 325 nm and the relative fluorescence intensity was measured at 420 nm in an Aminco-Bowman spectrofluorometer, using a slit width of 0.5 mm. The fluorescence intensity reading was standardized by adjusting the fluorometer reading to an arbitrary value of 7.4 when 10 liters of cis-parinaric acid solution was added to 2 ml of decane. Then, the fluorescence readings of the protein samples were taken. The net fluorescence intensity at each protein concentration was determined by subtracting the fluorescence intensity of each sample without cis-parinaric acid from the fluorescence intensity of the corresponding sample containing cis-parinaric acid. The initial slope (S_o) of the fluorescence intensity vs protein concentration plot was used as an index of the protein surface hydrophobicity. The initial slope was determined by linear regression analysis using a Monroe (Orange, NJ) 1880 programmable calculator.

Solubility index

Protein samples (1%, w/v, in 0.01M phosphate buffer pH 7.4) were dispersed by stirring with a magnetic stirrer for 5 min and then blended in a Sorval Omnimixer at speed setting 5 for 1 min. The pH of each dispersion was adjusted to 7.4 by adding 1N NaOH. A portion of each protein suspension was then centrifuged at 27,000 × *g* for 30 min. Aliquots of the suspension and the supernatant after centrifugation were diluted and the protein contents were determined by the Phenol-Biuret method (Brewer et al., 1974). The percent solubility index (s) was taken as the ratio of the protein content of the supernatant to that of the suspension.

Fat binding capacity (FBC)

The FBC was determined according to the method of Voutsinas and Nakai (1981).

Emulsifying activity index (EAI)

EAI was determined by the tubidimetric technique of Pearse and Kinsella (1978). To prepare emulsion, each protein sample was diluted to a concentration of 0.5% with 0.01M phosphate buffer, pH 7.4. Two ml of corn oil and 6 ml of the diluted protein dispersion were homogenized together in an Omni-mixer with a microattachment (Ivan Sorvall, Inc., Norwalk, CT) at speed setting 1 for 1 min at 20°C.

Emulsion stability index (ESI)

ESI was determined by a modification of the method of Pearse and Kinsella (1978) as follows: the emulsion, prepared as described above (EAI), was held at room temperature and aliquots (0.1 ml) were taken directly from the bottom of the container, containing the emulsion, at different time intervals and diluted to 50 ml (500 x dilution) with 0.01M phosphate buffer, pH 7.4, containing 0.1% sodium dodecyl sulfate (SDS). The absorbance of diluted emulsions at 500 nm was then recorded with a Beckman DB spectrophotometer. The half-life (min) of the absorbance decay with time, determined graphically, was used as ESI.

Viscosity

Viscosity measurements of 0.5% protein dispersion at 20°C were made using a Brookfield Synchro-Lectric viscometer, Model LVT fitted with a UL adapter, at 60 rpm.

Statistical analysis

Simple and multiple regression analyses were used to determine the relationships between hydrophobicity, solubility and emulsifying properties of the protein samples. These analyses were carried out by using a Monroe 1880 programmable calculator. In addition, backwards stepwise multiple regression analyses and surface visualization plotting were carried out using an Amdahl 470 V/8 computer at the University of British Columbia. Five independent variables

were used in the initial equation in the backwards stepwise procedure including surface hydrophobicity (S_o), solubility index (s), interaction of S_o and s, and quadratic powers of S_o and s. Dependent variables included EAI, ESI, and FBC. The models for the prediction of the emulsifying properties were selected on the basis of the statistical significance of F-probabilities of the partial regression coefficients.

RESULTS & DISCUSSION

Effect of heat treatment on emulsifying properties

Table 1 shows the relationships of hydrophobicity and solubility index of selected proteins with their emulsifying properties. It is evident that, for most proteins under a given set of conditions, protein solubility decreased as heating time increased due to the progressive denaturation of the protein. Gelatin, as expected, was completely solubilized on heating due to the rupture of hydrogen bonds which are responsible for its insolubility (Blanshard, 1970). As protein denaturation progressed, as seen by the decrease in protein solubility, the hydrophobicity usually increased. This is due to the gradual exposure of hydrophobic amino acid residues of native proteins which are usually buried in the interior of the molecules (Tanford, 1973) as a result of the protein unfolding. In the case of whey protein, it can be seen, that excessive heating resulted in a decrease of

Table 1—Relationships between protein hydrophobicity, solubility index, emulsifying activity, emulsion stability and fat binding capacity of various proteins[a]

Protein	Hydrophobicity (S_o)	Solubility index (%) (s)	EAI (m^2/g)	ESI[b] (min) I	ESI[b] (min) II	FBC (%)
Bovine serum albumin – control	325	100.0	148	108.50	91.50	25.0
Bovine serum albumin – heated	304	26.8	140	90.00	109.00	54.4
β-Lactoglobulin – control	426	100.0	96	27.20	37.00	4.2
β-Lactoglobulin – heated	192	6.4	51	25.30	27.50	16.5
Soy isolate – control	95	26.4	42	6.65	25.20	90.0
– sample 1 (pH 5.5, 100°C, 0.25 min)	97	26.4	51	7.30	26.00	86.3
– sample 2 (pH 5.5, 100°C, 0.5 min)	131	24.0	56	5.55	17.00	81.7
– sample 3 (pH 5.5, 100°C, 1.0 min)	150	14.2	48	1.00	5.13	76.0
– sample 4 (pH 5.5, 100°C, 2.0 min)	144	4.2	58	0.98	14.03	73.5
– sample 5 (pH 5.5, 121°C, 15 min)	160	8.2	50	0.76	11.20	68.4
– sample 6 (pH 7.2, 121°C, 15 min)	128	77.2	76	112.50	100.00	54.8
Promine-D	39	29.1	65	1.10	5.80	85.3
Ovalbumin – control	6	100.0	24	0.56	0.70	42.7
– sample 1 (pH 5.6, 80°C, 1.5 min)	38	90.6	34	0.63	1.10	45.9
– sample 2 (pH 5.6, 80°C, 2.0 min)	87	75.8	35	1.10	1.80	57.5
– sample 3 (pH 5.6, 80°C, 2.5 min)	95	70.6	45	1.08	1.70	66.9
– sample 4 (pH 5.6, 80°C, 3.0 min)	142	48.9	49	5.85	8.40	82.5
– sample 5 (pH 5.6, 100°C, 5 min)	269	10.6	78	24.30	138.00	102.6
– sample 6 (pH 1.0, 100°C, 15 min)	296	46.0	136	12.60	108.00	101.3
Pea isolate (a) – control	66	42.6	61	3.67	15.50	66.4
– sample 1 (80°C, 1 min)	77	34.7	66	0.60	0.42	53.9
– sample 2 (80°C, 2 min)	104	29.5	35	0.56	0.31	51.6
– sample 3 (80°C, 4 min)	100	25.2	37	0.85	0.16	50.4
– sample 4 (80°C, 7 min)	121	20.2	25	1.45	0.16	57.5
Pea isolate (b)	59	58.0	54	1.60	17.60	78.9
Gluten – sample 1 (0.5 N acetic, 100°C, 30 min)	65	71.6	70	0.36	17.60	38.0
– sample 2 (1.0 N acetic, 100°C, 30 min)	59	79.0	69	0.55	25.00	41.8
– sample 3 (1.5 N acetic, 100°C, 30 min)	65	85.6	82	0.28	23.80	34.8
– sample 4 (1.75 N acetic, 100°C, 30 min)	60	89.6	74	0.80	26.60	39.9
– sample 5 (2.0 N acetic, 100°C, 30 min)	57	93.3	69	0.78	23.30	32.2
Canola isolate (a) – control	65	28.3	60	0.54	3.76	33.9
– sample 1 (pH 5.5, 100°C, 0.5 min)	75	15.9	63	0.49	2.80	29.1
– sample 2 (pH 5.5, 100°C, 1.0 min)	77	10.5	49	0.17	1.27	29.2
– sample 3 (pH 5.5, 100°C, 1.5 min)	86	3.6	41	0.12	0.45	35.1
– sample 4 (pH 5.5, 100°C, 2.0 min)	78	2.9	40	0.14	0.47	42.5
– sample 5 (pH 7.2, 121°C, 10 min)	101	21.2	50	2.20	8.40	35.7
Canola isolate (b)	55	44.0	66	0.25	3.35	56.0
Sunflower isolate	47	31.0	75	1.40	5.30	105.8
Whey protein – control	182	88.7	87	50.30	87.50	74.5
– sample 1 (pH 6.0, 80°C, 4 min)	211	75.2	98	61.30	102.00	75.2
– sample 2 (pH 6.0, 80°C, 5 min)	164	65.5	89	63.00	104.00	72.5
– sample 3 (pH 6.0, 80°C, 15 min)	128	50.4	82	48.30	104.50	100.8
– sample 4 (pH 5.8, 80°C, 15 min)	132	61.6	85	46.30	108.00	99.4
Casein – control	28	100.0	58	1.70	39.30	11.3
– sample 1 (0.01M $CaCl_2$, 121°C, 5 min)	30	76.2	56	9.50	35.00	18.1
– sample 2 (0.01M $CaCl_2$, 121°C, 20 min)	21	71.5	49	14.75	24.30	16.7
– sample 3 (0.02M $CaCl_2$, 121°C, 20 min)	23	70.2	50	12.50	16.80	14.0
Gelatin – control	5	15.3	46	4.65	3.10	19.1
– heated (75°C, 2.5 min)	6	100.0	59	10.20	9.40	----
Myosin – control	14[c]	100.0[c]	43[c]	36.00[c]	----	----
– sample 1 (75°C, 1 min)	44[c]	50.9[c]	50[c]	22.40[c]	----	----
– sample 2 (75°C, 5 min)	54[c]	16.5[c]	48[c]	10.00[c]	----	----

[a] average of duplicate determination
[b] I: 0.1 M NaCl added; II: NaCl not added
[c] 0.3 M NaCl added

hydrophobicity, due probably to participation of some of the exposed hydrophobic groups in hydrophobic interactions. For casein, heating did not result in any substantial change of its hydrophobicity and this was expected, since casein exists in a random coil conformation (Morr, 1979). The results in Table 1 also indicate that for samples having the same solubility, the more hydrophobic the protein, the greater are its emulsifying properties.

Looking, specifically, at the results of soy protein in Table 1, it can be seen that, the EAI of all heated samples were slightly greater than that of the control. The ESI, however, was initially slightly increased by heating, but subsequently started to decrease as the solubility progressively decreased; that is, solubility became an increasingly important factor controlling this property. The higher EAI values of heated soy protein samples were probably due to their increased hydrophobicity values. On the other hand, the observed decrease in ESI upon heating may be due to a decrease in solubility as a result of aggregation.

The proteins studied here can be divided into four groups according to the effect of heating on their emulsifying properties. The first group includes BSA, gluten, and whey protein whose EAI was not substantially affected by heating, whereas ESI was improved by heating. The second group includes soy protein and myosin whose EAI was slightly improved by heating, whereas the ESI was decreased. The third group includes β-lactoglobulin, pea, canola, and casein, whose emulsifying properties were adversely affected by heating. Finally the fourth group includes ovalbumin and gelatin whose emulsifying properties were markedly improved upon heating. It is evident, therefore, that heating does not have uniform effects on the emulsifying properties of different proteins.

The improvement of emulsifying properties of gelatin upon heating might have been due to the increase in solubility since its hydrophobicity was not changed by heating. Kato and Nakai (1980), and Kato et al. (1981) reported that the emulsifying properties (EAI and ESI) of ovalbumin and lysozyme were markedly improved upon heating. They also found that this improvement was correlated with the higher hydrophobicity of heat denatured protein samples as compared to those of the native proteins. Furthermore, their results indicated that the more hydrophobic the proteins, the greater the decrease in interfacial tensions and the improvement in emulsifying properties. During the handling of the ovalbumin samples we noticed that the apparent viscosities of heat-denatured samples increased. This is evident in Table 2, which shows the apparent viscosity changes of some proteins upon heating. It is suggested, therefore, that higher viscosity appears to be another factor contributing to the great improvement of emulsifying properties of heat denatured ovalbumin samples.

As shown in Table 1, the FBC of different proteins is differently affected by heating. While the FBC of Canola protein is relatively unaffected by heating, the FBC's of BSA, β-lactoglobulin, ovalbumin, whey protein, and casein are positively affected, and the FBC's of soy and pea proteins are adversely affected by heating.

Protein denaturation usually decreases solubility then adversely affects protein functionality. However, as shown in this study, emulsifying and fat binding properties of some proteins (ovalbumin, whey protein) can be improved by denaturation. According to Morr (1979) denaturation of the whey protein molecule, if produced at the proper stage of the protein concentrate isolation/utilization process, can improve the functionality. The improvement in functionality is probably due to an unfolding of the molecule to expose hydrophogic amino acid residues, thus making the protein more amphiphilic and capable of orienting at the oil-water interface (Morr, 1979). A great improvement of emulsifying properties (EAI and ESI) of ovalbumin and lysozyme by heat denaturation was also reported by Kato and Nakai (1980) and Kato et al. (1981).

Another example of protein denaturation resulting in improvement of functionality was reported by Aoki et al. (1981), who determined the effect of alcohol modification of soy protein on its emulsion stabilizing properties. The soy protein was denatured with 50% alcohol by treatment at 35°C for 2 hr. The emulsion stabilizing properties of soy protein modified with ethanol or n-propanol decreased with increasing solubility, whereas the emulsion stabilizing properties of the unmodified soy protein increased. Aoki et al. (1981) attributed the improved emulsion stability brought about by the alcohol modification of soy protein to the perturbation and unfolding of the hydrophobic interior structure of the native soy protein by alcohol, and to the resulting increase in the exposed hydrophobic amino acid residues. It should be noted, that the increased emulsion stabilizing properties of soy protein between pH 2 and 7, achieved through alcohol modification by Aoki et al. (1981), is very important because soy protein can be expected to play a significant role in the stabilization of a wide range of food emulsions, all meat emulsions falling well within these pH limits.

Statistical analysis

Regression equations for predicting the emulsifying and fat binding properties from hydrophobicity and solubility data of heat denatured proteins in Table 1 are presented in Table 3. Simple linear regression analyses showed that the coefficients of determination (r^2) between S_o and EAI, and between S_o and ESI (detetmined without NaCl) were highly significant ($P < 0.001$). No significant correlation was found, however, between solubility and EAI or ESI. Moreover, no correlation was found between S_o or solubility and FBC. Although significant correlations were found between S_o and ESI as well as solubility and ESI determined in the presence of 0.1M NaCl, such correlations are not considered meaningful since the effect of 0.1M NaCl on solubility and S_o were not determined. It is reasonable that 0.1 M NaCl may positively or negatively affect the solubility of a protein. Sodium chloride was used in the ESI test, to obtain a general idea on the salt sensitivity of the emulsions formed by different proteins. Relatively high salt concentrations are used in some foods, e.g. meat products. As seen in Table 1, 0.1M NaCl usually has a detrimental effect upon the emulsion stability. NaCl exerts its negative effect on emulsion stability probably by

Table 2 – Effect of heating on the apparent viscosity of some proteins[a] measured at 20°C

Protein[a]		Apparent viscosity (Pa·s x 10^{-3})
Whey	– control	1.14
	– sample 1 (pH 6.0, 80°C, 4 min)	1.14
	– sample 2 (pH 6.0, 80°C, 5 min)	1.14
	– sample 3 (pH 6.0, 80°C, 15 min)	1.16
	– sample 4 (pH 5.8, 80°C, 15 min)	1.20
BAS	– control	1.13
	– heated (pH 4.0, 100°C, 5 min)	1.24
Ovalbumin	– control	1.10
	– sample 1 (pH 5.6, 80°C, 1.5 min)	1.13
	– sample 2 (pH 5.6, 80°C, 2.0 min)	1.13
	– sample 3 (pH 5.6, 80°C, 2.5 min)	1.16
	– sample 4 (pH 5.6, 80°C, 3.0 min)	1.17
	– sample 5 (pH 5.6, 100°C, 5 min)	1.18
	– sample 6 (pH 1.0, 100°C, 15 min)	1.90

[a]0.5% protein in 0.01 M phosphate buffer, pH 7.4

reducing the surface charge and by withdrawing surface water of oil droplets.

As seen in Table 3, multiple regression analyses between S_o, solubility and EAI or ESI, show highly significant correlations (R^2 values are 0.542 and 0.434, $P < 0.001$, respectively).

Since the Student's t-values of partial regression coefficients are highly significant, both hydrophobicity and solubility significantly affect the emulsifying properties of the proteins. As shown in Table 3, the backwards stepwise multiple regression analysis gives a highly significant correlation between S_o, solubility and FBC of the 48 protein samples in Table 1. Comparing the regression models in Table 3, it is apparent that the models obtained by stepwise regression analysis have higher R^2 values and lower S.E. (standard error of the estimate) values than those of the linear regression models. Our discussion, therefore, will be confined within the models calculated by stepwise regression analysis.

The EAI of the proteins studied was significantly affected by S_o, solubility and the square of solubility (Table 3). The R^2 value for EAI was 0.583, indicating that 58.3% of the variability in EAI of the protein studied could be accounted for by the three independent variables S_o, s and s^2. In this model, the statistical significance of the square of solubility index indicates that as the value of solubility index increases, the effect of that variable declines, i.e., the EAI response to increasing levels of solubility may be depicted as curvilinear rather than linear. Response surface plots (contours) were generated by the computer to visualize the effects of S_o and solubility on the functional properties studied. As shown in Fig. 1, regardless of the solubility, as hydrophobicity increases the EAI is initially increased and then slightly declined. Although at low and medium hydrophobicity values, increasing solubility levels increase the EAI, at very high S_o values, solubility does not appear to play an important role in the emulsifying capacity of proteins.

The ESI of the proteins is significantly affected by S_o, s, the interaction of S_o and s, and the s^2 (Table 3). The R^2 value of the model is 0.584, indicating that 58.4% of the variability in ESI could be accounted for by these 4 variables. As shown in Fig. 2, regardless of the solublity, as S_o increases the ESI is initially increased and then decreased. At low and medium S_o values, increasing solubility index levels increases ESI. However, at very high S_o values, solubility index does not appear to be important for the emulsion stability.

The FBC of the proteins studied was significantly affected by S_o, s, and the squares of these two variables (Table 3). The R^2 of the model was 0.473 ($P < 0.001$), indicating that 47.3% of the variability in FBC could be accounted for by the four variables. The statistical significance of quadratic powers of S_o and solubility in the FBC model in Table 3 indicates that as the value of S_o or s increases, the FBC value increases first then declines (i.e. the response of FBC to increasing levels of S_o or solubility can be depicted as a curvilinear graph).

The finding of this study that with increasing S_o the emulsifying properties initially increase and then decrease can be explained by taking into account the fact that the emulsifying properties of proteins ultimately depend on the

Table 3 — Regression models for prediction of emulsifying and fat binding properties of heat denatured proteins.

Dependent variable	Regression analysis	Variable description	Regression coefficient	F-ratio	F-probability	t-value[a]	β-value
EAI (n=52; r^2=0.464, P<0.001; S.E.[b]=18.95)	Simple linear	Constnat	41.162				
		S_o	0.202				
EAI (n=52; R^2=0.542, P<0.001; S.E.[b]=17.78)	Multiple linear	Constant	29.283				
		S_o	0.207	–	–	7.202***	0.698
		s	0.226	–	–	2.837**	0.280
EAI (n=52; R^2=0.583, P<0.001; S.E.[b]=17.40)	Backwards multiple nonlinear	Constant	16.877	4.531	0.039		
		S_o	0.214	58.77	0.000	–	0.720
		s	0.932	7.83	0.008	–	1.151
		s^2	–0.007	4.73	0.035	–	–0.894
ESI (n=49; r^2=0.377, P<0.001; S.E.[b]=30.98)	Simple linear	Constant	0.880				
		S_o	0.274				
ESI (n=49; R^2=0.434, P<0.001; S.E.[b]=29.53)	Multiple linear	Constant	–14.308				
		S_o	0.278	–	–	5.613***	0.623
		s	0.295	–	–	2.153	0.239
ESI (n=49; R^2=0.584, P<0.001; S.E.[b]=26.70)	Backwards multiple nonlinear	Constant	–69.463	18.02	0.000	–	
		S_o	0.565	34.31	0.000	–	1.268
		s	2.034	13.62	0.001	–	1.651
		S_o x s	–0.004	10.08	0.003	–	–0.779
		s^2	–0.012	5.92	0.019	–	–1.044
FBC (n=48; R^2=0.473, P<0.001; S.E.[b]=20.97)	Backwards multiple nonlinear	Constant	4.895	0.16	0.691	–	
		S_o	0.451	13.84	0.001	–	1.445
		s	1.398	13.84	0.001	–	1.602
		S_o^2	–0.001	10.15	0.003	–	–1.270
		s^2	–0.014	11.79	0.001	–	–1.745

[a] *: P<0.05; **: P<0.01; ***: P<0.001

[b] Standard error of estimate

suitable balance between the hydrophile and lipophile, and do not necessarily increase as the proteins become more lipophilic (Aoki et al., 1981). Excessive denaturation of the soy protein by n-propanol resulted in a lower emulsion stability as compared to the moderate denaturation by ethanol (Aoki et al. 1981). There may be many factors, e.g., molecular size, molecular flexibility and charge, besides the balance of hydrophile and lipophile which participate in determining the emulsifying properties of proteins.

Wolf and Cowan (1975) reported that in ground meat products fat absorption or binding appeared to be involved in formation and stabilization of an emulsion. Thus, they suggested that fat adsorption may simply be another aspect of emulsification. This helps to explain the observation in this study that hydrophobicity has a curvilinear effect also on FBC, as the concept of hydrophile-lipophile balance can be utilized again. Voutsinas and Nakai (1981) already reported that with increasing S_o the FBC was initially increased and then decreased.

Pearson et al. (1965) reported that only that fraction of protein which is soluble can function as an effective emulsifying agent. Moreover, Franzen and Kinsella (1976) suggested that, as a protein becomes more soluble, it forms layers around the fat droplet to facilitate association with the aqueous phase. Granular, insoluble proteins, however, separate from the oil phase or just float on the oil surface where they remain inert and contribute little toward emulsification. Similarly, soluble proteins enclose the fat globules and render the emulsion more stable to heat treatment. Also, according to Bull (1972), the surface activity of a protein is a function of the ease with which the protein can migrate to, adsorb at, unfold, and rearrange at an interface. Therefore, solubility in the aqueous phase, is closely related to surface activity of proteins (Kinsella, 1979). Many authors, on the other hand, have reported that high levels of solubility were not necessarily associated with maximum emulsifying properties (Aoki et al., 1980; McWatters and Cherry, 1975; McWatters and Holmes, 1979a, 1979b; Smith et al., 1973; Wang and Kinsella, 1976). Flint and Johnson (1981) investigated a film formation by soy protein (isolate) at an oil-water interface for the pH range 1–10. Definite film formation was observed at all pH values below the isoelectric point (4.6) of the protein and up to pH 6.5. At pH 5.4, despite the low solubility strong film formation occurred. However, at pH's above 5.4 the strength of films formed gradually decreased until at an upper limit pH of 7.5, the presence of an interfacial layer could barely be seen. The marked pH dependence of film formation on the alkaline side of the isoelectric point was attributed to the fact that with increasing pH the protein becomes more soluble in the aqueous phase and consequently less likely to be brought out of solution (coagulated) at the phase boundary. The ability of soy protein to form a film even at very low pH's, where the solubility of protein is high, suggested that this phenomenon may not be linked solely to solubility but also to the availability of lipophilic groups for binding at the oil-water interface. Changes in the protein conformation may occur at acid pH's which enhance the combination of the protein and oil leading to the formation of interfacial film described (Flint and Johnson, 1981).

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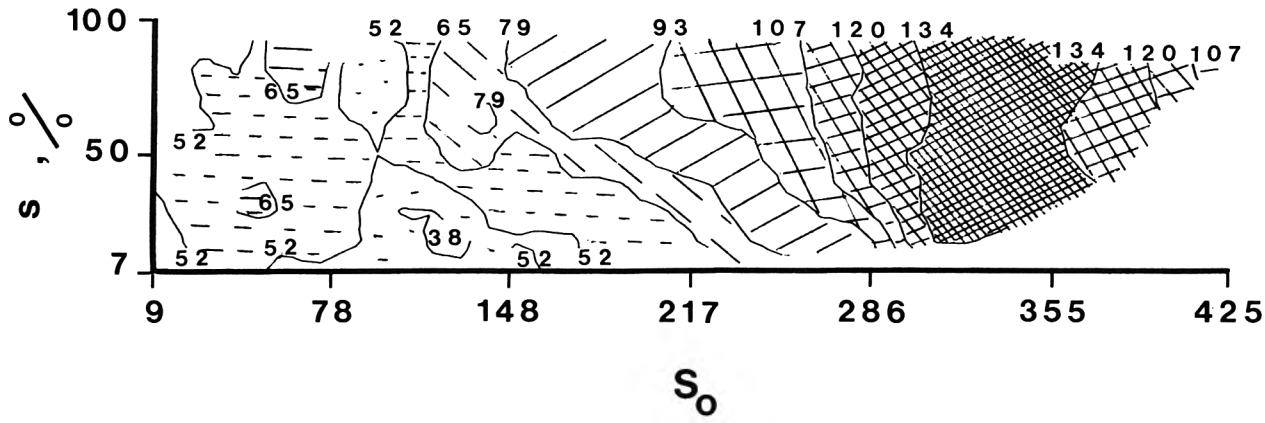

Fig. 1–Emulsifying activity index response surface contour as a function of hydrophobicity (S_o) and solubility index (s).

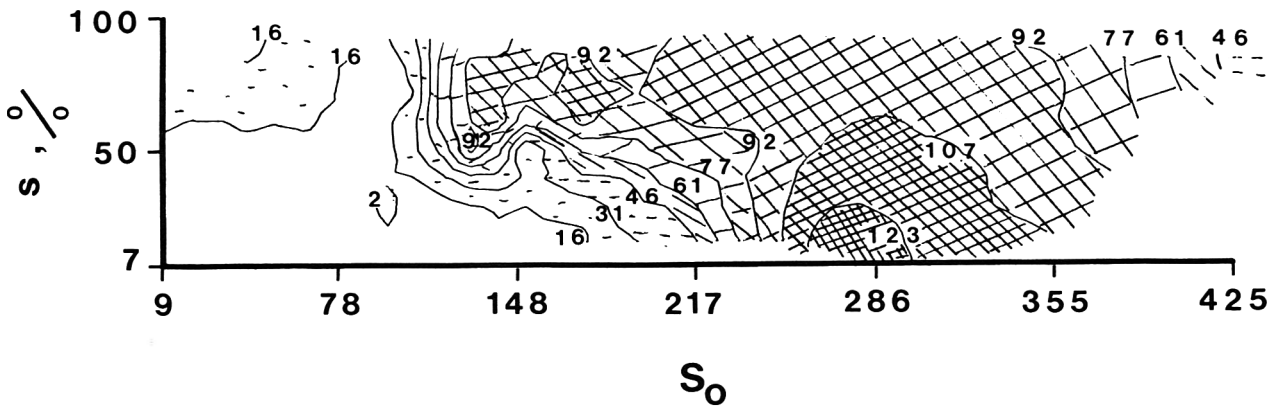

Fig. 2–Emulsion stability index response surface contour as a function of hydrophobicity (S_o) and solubility index (s).

Considering the above contradictory situation, the results of this study showed that high solubility had a negative effect on FBC in agreement with the results of Voutsinas and Nakai (1982). They attributed the low FBC of soluble proteins to their conformation (mainly helical) which may not permit their binding sites to be sterically available for interaction with oil or to the limited access of oil (hydrocarbon chains) to the protein binding sites due to a possible barrier around them formed by the excessive number of protein polar groups.

In conclusion, the emulsifying properties of proteins are influenced by both hydrophobicity and solubility. Although important, solubility alone cannot fully explain the emulsifying properties especially when the proteins are heat denatured.

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ELECTRICAL STIMULATION OF RABBIT MUSCLE. . . From page 22

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Characterization of Shrimp Lipids

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ABSTRACT

Edible shrimp (*Penaeus aztecus*) tissue contains approximately 1.2% extractable lipids, the majority of which are phospholipids. Data from the gravimetric quantitation of lipid classes isolated by column chromatography indicated that phosphatidyl choline was the predominant phospholipid and cholesterol the predominant neutral lipid in edible shrimp tissue. Fatty acid distribution data indicated that sphingomyelins contained the greatest percent by weight of unsaturated fatty acids while cholesterol esters contained the greatest proportion of saturated fatty acids. Enzymatic hydrolysis followed by gas liquid chromatography of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine indicated that fatty acids located at the β position were more highly unsaturated than those at the α position.

INTRODUCTION

PUBLISHED STUDIES concerning shrimp lipids have dealt mainly with cholesterol content (Neal et al., 1976; Sidwell et al., 1974; Thompson, 1964) and total fatty acid profiles (Bottino et al., 1980). These data indicate that species differences have very little effect on fatty acid profiles and only a slight effect on cholesterol content. The effect of seasonal variation on the level of these two classes of lipids is well documented and has been attributed to water temperature, nutrition, stage of development, and photoperiod as well as other factors (Guary et al., 1976; Martin and Ceccaldi, 1977; Bottino et al., 1980). The purpose of this paper is to quantify and characterize the lipids of brown shrimp (*Penaeus aztecus*).

MATERIALS & METHODS

FRESH SHRIMP (*Penaeus aztecus*) were purchased in Crystal River, Florida, in early March, and packed in ice for transport to our laboratory. The shrimp were washed with deionized water and stored at −10°C until analyzed. Maximum storage time was 60 days.

Prior to analysis, the shrimp were thawed at room temperature and the head, shell, tail, and legs removed. The remaining edible portion was chopped into approximately 1 cm sections, weighed into 100g aliquots and subjected to chloroform:methanol extraction (Bligh and Dyer, 1959) using a Lourdes blender. The volume of the total lipid extract (TLE) was reduced to 1.0 ml under reduced pressure rotary evaporation at 30°C.

Twenty grams of Silica Gel 60 (70–230 mesh) were slurried with chloroform and poured into a 1.5 X 20 cm glass column. A 1 cm layer of anhydrous sodium sulfate was placed on top of the packed silica gel. One gram TLE dissolved in 1 ml chloroform was placed on the column. The lipids were eluted with 400 ml each of chloroform, acetone, and methanol.

Each solvent fraction was recovered and reduced to 10 ml under reduced pressure rotary evaporation at 30°C and quantitatively transferred to 25 ml tarred flasks. The remaining solvent was evaporated under a stream of N_2 at 35°C, and the lipid fractions dried to constant weight in an evacuated desiccator over $CaSO_4$. The lipid fractions were dissolved in 2 ml chloroform and 0.5 mg lipid was spotted on 1 X 20 cm lanes on 500 μm Redi-Coat TLC plates (Supelco Inc.). Neutral lipids were developed in petroleum ether: ethyl ether:acetic acid (78:20:2) and visualized by spraying with H_2SO_4:methanol (1:1). The phospholipid and glycolipid fractions were developed in chloroform:methanol:H_2O (65:25:4). Phospholipids were visualized by spraying with molybdenum blue reagent (Applied Science) and glycolipids with diphenylamine (Applied Science) (Moerck and Ball, 1973).

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Separation of neutral lipid components

Approximately 300 mg of neutral lipids in 1 ml hexane were placed on a 1.5 X 30 cm column packed with 25g of 100–200 mesh Florisil which had been activated at 260°C for 8 hr and then hydrated to 7% H_2O w/w prior to use. The neutral lipids were fractionated by eluting with solvent mixtures of increasing polarity (Table 1) and 7 ml fractions were collected. The lipid content of each fraction was determined gravimetrically following evaporation of solvent under N_2 at 35°C and drying to a constant weight in an evacuated desiccator containing $CaSO_4$. The purity and identity of each fraction was assayed by redissolving the lipid fraction in 2 ml chloroform and streaking approximately 200 μg on 1 X 20 cm lanes etched on 500 μm Redi-Coat plates. The plates were developed in petroleum ether:ethyl ether:acetic acid (78:20:2) and visualized by spraying with H_2SO_4:methanol (1:1). R_f values of the detected lipid components were compared to neutral lipid standards which had been applied, developed, and visualized under identical conditions.

Separation of phospholipids

A 2 X 40 cm glass column was packed with 70–230 mesh Silica Gel 60 (hydrated to 7% H_2O) slurried in chloroform. The column was washed first with 200 ml of methanol and then 200 ml chloroform. A 1 cm layer of anhydrous sodium sulfate was placed on top of the packed column and approximately 600 mg of shrimp phospholipids in 1 ml chloroform was applied to the column. The phospholipids were eluted with 400 ml 15% methanol in chloroform (v/v), and 15 ml fractions were collected in tarred test tubes. The column was subsequently eluted with 400 ml each of 35% methanol in chloroform and 100% methanol. The 400 ml fractions were reduced to approximately 10 ml under reduced pressure rotary evaporation and quantitatively transferred to tarred 25 ml flasks. The solvent was evaporated from all fractions under N_2 at 35°C and the lipid content of each fraction was quantitated gravimetrically. The identity of each fraction was determined by a TLC method analogous to that used for neutral lipids except that the developing solvent was chloroform:methanol:H_2O (65:25:4).

Following characterization, the remaining lipids in the subfractions from three independent replicate analyses were pooled. Fatty acid methyl esters of the triglycerides and cholesterol esters were prepared by transesterification of approximately 5 mg aliquots of the appropriate fractions with Meth Prep II (Applied Science). Five milligram aliquots of the free fatty acid fractions were esterified with Meth Prep I by co-injection into the gas chromatograph.

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Table 1—Elution scheme for separation of shrimp neutral lipids by Florisil column chromatography

Fraction	Volume (ml)	Eluting solvent
hydrocarbons	60	hexane
cholesterol esters	80	5% ether in hexane
triglycerides	120	15% ether in hexane
cholesterol	100	25% ether in hexane
diglycerides	100	50% ether in hexane
monoglycerides	100	2% methanol in ether
free fatty acids	150	4% acetic acid in ether

Sphingomyelins were treated with 2N methanolic HCl in order to transesterify the N-acyl fatty acid esters. The fatty acid methyl esters were then extracted with ethyl ether.

The phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidyl choline fractions from duplicate runs were dissolved in 1 ml chloroform, to which 0.3 ml triethylamine was added and the solvents were evaporated to dryness. The resulting triethylammonium salts were dissolved in 0.3 ml ethyl ether, mixed with 3.0 ml pH 7.4 0.005M Tris buffer and 0.2 ml 0.002M $CaCl_2$, and vortexed until a white suspension was achieved. Following the addition of 125 units of phospholipase A_2, each test tube was capped and incubated at room temperature with continuous agitation for 2 hr. The suspensions were then acidified to pH 1–2 with 0.5N HCl and fatty acids were extracted with 5 mL ether. The extraction was repeated twice, the extracts pooled, and the volume reduced to approximately 1 ml under N_2. The aqueous phase was lypholized, dissolved in 0.5 ml toluene, and transesterified with Meth Prep II. The fatty acid methyl esters were extracted with ethyl ether and reduced to a volume of approximately 1 ml.

The fatty acid methyl esters were separated and quantified on a Hewelett-Packrd 5840A gas chromatograph equipped with a 5.5 mm i.d. X 1.8m column packed with 10% Silar 10°C on 100/200 mesh Gas Chrom Q (Applied Science) and a flame ionization detector. The column was operated isothermally at 160°C for 3 min, programmed to 180°C at 2°C/min, held at 180°C for 5 min, programmed to 220°C at 3°C/min, and held at this temperature for 40 min. H_2 and N_2 flow rates were 30 ml/min. The injection port temperature was 250°C and the detector temperature was 300°C. Methyl esters were identified and quantitated by comparing the retention time and peak area of the unknowns with those of the fatty acid methyl esters standards. The identity of peaks with retention times not corresponding to any standards was determined from a semi-log plot of retention time vs carbon number for either saturated or monoenoic fatty acids.

Table 2—Lipid classes isolated from 100g shrimp tissue

Class	Tissue	Percent
	mg/100g	
neutral lipids	430.7 ± 20.1	36.0 ± 1.7
glycolipids	22.8 ± 2.9	1.9 ± 0.3
phospholipids	742.0 ± 40.7	62.1 ± 3.4
TOTAL	1195.5 ± 63.3	

mean ± standard deviation

Table 3—Lipid sub-classes isolated from 100g shrimp tissue

Lipid fraction	mg/100g Tissue[a]	Percent of total lipid extract
Hydrocarbons	0.8 ± 0.3	trace
Cholesterol esters	49.0 ± 2.0	4.1 ± 0.2
Triglycerides	100.4 ± 4.6	8.4 ± 0.4
Cholesterol	200.8 ± 16.6	16.8 ± 1.4
Diglyceride	2.4 ± 0.2	0.2 ± 0.1
Monoglyceride	4.7 ± 0.3	0.4 ± 0.1
Free fatty acid	77.3 ± 0.5	6.5 ± 0.4
Sphingomyelin	286.9 ± 26.2	22.8 ± 2.4
Phosphatidyl serine	69.3 ± 1.9	5.8 ± 0.3
Phosphatidyl choline	310.0 ± 2.9	27.2 ± 1.9
Phosphatidyl ethanolamine	99.3 ± 1.6	7.8 ± 0.9

[a] mean ± standard deviation

RESULTS & DISCUSSION

THE FOLLOWING DATA represent mean values from three independent analyses of 100g portions of edible shrimp tissue. All samples came from shrimp harvested on the same day to avoid possible seasonal variations in lipid profile.

The chloroform:methanol extractable lipid content of edible shrimp tissue was approximately 1.2%. This agrees with previously published values of 1.1 ± 0.2% (Sidwell et al., 1974). The relative weight fractions of the three major lipid classes isolated by column chromatography are reported in Table 2. Neutral lipids, eluted with chloroform, composed 36%, glycolipids eluted with acetone and were 1.9%, and phospholipids, eluted with methanol, constituted 62.1%.

Table 4—Fatty acid distribution (percent by weight) of lipid sub-fractions[a]

Fatty acids	Trigly-cerides	Chol. esters	Free fatty acids	SP	PS		PC		PE	
					α	β	α	β	α	β
9:0	8.32	38.10	nd	nd	8.32	nd	2.0	nd	7.5	nd
12:0	nd	0.89	nd	nd	3.55	nd	nd	2.0	3.27	1.27
14:0	8.76	0.07	4.55	0.56	0.52	0.95	0.46	2.85	0.71	2.49
14:1	nd	nd	nd	nd	nd	nd	0.04	nd	nd	0.19
15:0	nd	42.87	1.53	0.74	1.26	2.74	1.78	1.25	1.70	1.92
15:1	18.42	1.10	nd	0.84	nd	5.23	0.30	5.13	nd	0.34
16:0	7.37	1.41	19.98	10.90	19.61	9.04	17.34	8.01	6.59	16.56
16:1	nd	nd	7.98	3.51	2.88	4.49	2.34	7.84	nd	4.85
17:0	nd	1.32	4.56	3.25	3.54	4.29	5.26	2.01	7.94	3.09
17:1	nd	nd	nd	5.40	nd	2.29	nd	1.92	nd	1.13
18:0	33.04	1.28	13.98	16.36	29.52	12.05	39.88	9.52	26.26	10.59
18:1	5.33	3.98	21.06	13.46	8.14	17.37	6.93	25.39	9.53	20.40
18:2	nd	nd	6.19	4.37	4.49	5.93	1.01	4.57	nd	1.16
18:3	nd	nd	nd	nd	5.16	10.61	nd	nd	8.03	nd
20:0	nd	1.44	nd	nd	nd	nd	13.03	nd	9.97	3.19
19:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.07
20:1	4.61	2.07	5.65	3.51	nd	nd	3.05	8.44	nd	nd
20:2	nd	1.67	nd	1.59	2.38	9.51	1.32	7.05	3.8	7.51
20:3	nd	nd	nd	nd	nd	nd	0.46	0.23	nd	nd
20:4	nd	0.31	4.56	9.55	3.82	10.20	1.23	5.55	3.06	5.60
20:5	nd	nd	3.17	13.00	5.15	5.50	1.06	3.44	3.05	4.69
22:1	14.15	1.39	nd	0.87	nd	nd	nd	2.51	nd	nd
22:2	nd	0.52	2.76	nd	nd	nd	0.52	nd	4.57	8.02
22:4	nd	nd	nd	0.82	nd	nd	0.58	nd	nd	nd
22:6	nd	0.44	nd	10.64	nd	1.59	0.78	1.65	3.62	4.23
Unk rea nk.	0	3	2	3	2	3	1	2	1	2
	0.00	1.14	4.03	0.63	1.72	0.50	0.5	0.54	0.43	0.62

[a] SP = sphingomyelin; PS = phosphatidyl serin; PC = phosphatidyl chlorine; PE = phosphatidyl ethanolamine

Table 5—Double bond distribution of fatty acids (percent by weight in lipid sub-classes

	Trigly-cerides	Chol. esters	Free fatty acids	SP	PS		PC		PE	
					α	β	α	β	α	β
Saturated (total)	57.49	87.38	44.60	31.81	66.23	29.07	79.84	25.64	63.94	41.18
Monoenoic (total)	42.51	8.53	34.69	27.59	11.02	27.09	12.66	51.60	9.53	26.91
Polyenoic (total)	0.00	2.50	16.68	39.97	21.00	43.31	6.96	22.49	26.13	31.29

Separation of the lipid sub-classes was achieved by column chromatography as indicated by the migration of a single band on the TLC plates from each of the fractions collected during column chromatography. Table 3 shows the weight per 100g of edible shrimp and the percentage distribution of the phospholipid and neutral lipid fractions after column chromatography. Florisil was used as the solid support to separate the neutral lipids, as resolution of the di- and mono-glycerides was superior to that obtained using silica gel. It was necessary to separate phospholipids on silica gel because the polarity of Florisil did not permit separation of phospholipids (Carrol, 1961). The weight fraction of cholesterol, 16.8 ± 1.4% is similar to previously published values of 15.95 ± 1.38 (Sidwell et al., 1974), 15.7% (Thompson, 1964) and 18.3% (Neal et al., 1976).

The fatty acid composition and relative distribution with respect to the various lipid sub-fractions is shown in Tables 4 and 5. All but two peaks had retention times comparable to standards. These two peaks were identified as corresponding to 15:1 and 17:1 from a semi-log graph of retention time vs carbon number for monoenoic fatty acids With respect to phosphatidyl choline, phosphatidyl serine and phosphatidyl ethanolamine, the fatty acids at the β position were more unsaturated than α fatty acids.

Stearic (18:0) was the most common fatty acid in sphingomyelin, phosphatidyl serine, phosphatidyl chlorine and triglyceride fractions while oleic acid (18:1) predominated in the free fatty acid fraction. Pentadecanoic acid (15:0) is the most abundant fatty acid associated with cholesterol esters. The most abundant fatty acid in phosphatidyl ethanolamine was 22:6.

In summary, this study expands on published work dealing with fatty acids of shrimp which looked only at total fatty acid profiles of fatty acids with 14 or more carbons. By utlizing column chromatography, enzymatic hydrolysis, and an improved gas chromatography procedure, we were able to ascertain the distribution of fatty acids containing 9 or more carbons with respect to the various lipid sub-classes in addition to gravimetrically quantitating these sub-classes.

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POSTMORTEM METABOLISM AND TENDERNESS. . . From page 25

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Dye Binding Properties of Chitin and Chitosan

DIETRICH KNORR

ABSTRACT

Chitin (β (1→4)-N-acetyl-D-glucosamine) and chitosan (deacetylated chitin) are currently available in large quantities as waste products and by-products of the shellfish industry. Their potential as carriers for food additives was studied. Significant correlations were found between dye concentration ranging from 0.2–1.6 mg dye (FD&C Red No. 40) per g chitin or chitosan and dye-binding capacity of chitin or chitosan. Within a pH range of 2.0–7.0, dye-binding capacity of chitin was stable. Chitosan gelled below a pH of 5.5 and could not be evaluated but its dye-binding capacity was constant between pH 7.0 and 5.5. Above pH 7.0 dye-binding capacity decreased for chitin as well as for chitosan but between pH 2.0 and 6.0 no dye was released from dyed chitin containing 0.77 mg dye/g chitin.

INTRODUCTION

CHITIN, a cellulose-like bipolymer is widely distributed in nature, especially in marine invertebrates, insects, fungi and yeasts (Austin et al., 1981). There is agreement that chitin is essentially a continuous polymer of β(1→4)-N-acetyl-D-glucosamine residues (Dweltz, 1960; Rudall and Kenchington, 1973). Chitosan (deacetylated chitin), which has been recognized in various fungi (Rudall, 1969), is processed industrially from chitin.

The marked expansion and centralization of the U.S. shellfish industry during the last 25 yr has led to a growing disposal problem of shellfish waste which contains chitin as a major constituent (Johnson and Peniston, 1978; Revah-Moiseev and Carroad, 1981). This development has made larger quantities of chitin available with little seasonal fluctuation in supply because different shellfish species have overlapping production seasons (Johnson and Peniston, 1978). Both chitin and chitosan are currently processed industrially in million-kilogram quantities in Japan and in the U.S. (Austin et al., 1981; Bentley, 1982).

Chitosan has been used as an effective agent for coagulation of suspended solids from various food processing wastes (Bough, 1975, 1976; Bough and Landes, 1978) and the potential food application of chitin and chitosan and some of their functional properties have been presented recently (Knorr, 1982). The unusual combination of properties of chitin and chitosan such as water binding capacity, fat binding capacity, bioactivity, biodegradability and toughness makes them "attractive" specialty material. The apparently low digestibility and toxicity of chitosan and chitin (Arai et al., 1968; Watkins and Knorr, 1982), as well as the reported hypolipidemic and hypocholesterolemic activity of chitosan (Nagyuary et al., 1979, Sugano et al., 1980), further attribute to the potential food use of these cellulose like biopolymers.

A wide range of cellulose ingredients is already being used by the food processing industry to improve food functionality and quality, one of them being to serve as carrier for highly concentrated ingredients. Since chitin has been proposed as support for immobilized enzymes (Bough, 1977; Pigott, 1972) and due to its unique binding properties (Knorr, 1982), work on the potential of chitin and chitosan as carrier for food additives has been initiated. The study presented aimed to investigate the dye binding properties of chitin and chitosan.

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MATERIALS & METHODS

Materials

Commercially available chitin and chitosan (pharmaceutical grade, Madera Products Inc., Albany, OR) was ground to pass through a 0.5 mm or a 1.0 mm sieve (Wiley Lab Mill, Model #4, A.H. Thomas Co., Philadelphia, PA) and stored at room temperature. FD&C Red No. 40 (disodium salt of 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfophenyl)azo-2-naphthalenesulfonic acid) was used for the dye-binding experiments.

Dye-binding and water uptake

For the determination of the standard curve, the absorbance of the aqueous dye solutions containing 1.25–20 mg dye/L was measured at 505 nm (Spectronic 20, Bausch and Lomb, Rochester, NY, 13 mm cuvettes) using deionized water as the blank. The regression equation obtained (n = 36) was $y = -0.31 + (19.06)x$.

Dye binding properties were examined by weighing 0.5 or 2.0g of chitin or chitosan in centrifuge tubes, adding 20g of aqueous dye solution (5 mg dye/L to 40 mg dye/L) and then shaking the closed centrifuge tubes for 30 min at 200 rpm at a horizontal position (Gyrotory Shaker, Model G2, New Brunswick Scientific Co. Inc., New Brunswick, NJ). The samples were then centrifuged for 35 min at 4,500 x g, the supernatant decanted and the water uptake of chitin or chitosan was determined after Sosulski (1962). The absorbance of the supernatant was measured at 505 nm (Spectronic 20, Bausch and Lomb, Rochester, NY, 13 mm cuvettes) using deionized water as the blank. The weight of the supernatant was used as a basis for the calculation of the total amount of dye bound or released. The pH adjustment was carried out by using either 10 ml of a commercial buffer solution or by adding 0.1N HCl to a slurry of 0.5g chitin/chitosan and 10 ml of dye solution. After stirring for 15 min the pH was readjusted and deionized water added to reach 20.5g of total weight. Chitosan formed gels at pH values below 5.5 and no dye binding measurements could be obtained.

Dyeing of chitin

The dyeing of chitin was carried out by stirring 50 g chitin and 750 ml of an aqueous dye solution (40 mg FD&C Red #40 per litre) for 15 min, adjusting the pH to 5.5 with 1N HCl, stirring for additional 15 min, readjusting the pH to 5.5 and adding a dye solution to obtain 1,050g of mixture containing an adjusted final concentration of 0.8 mg dye/g chitin. After shaking for 30 min the sample was centrifuged for 35 min at 5,500 x g and the dye concentration of the supernatant as well as water uptake of the residue was determined. The residue was then dried for 48 hr at 65°C.

All experiments were carried out in four replications.

RESULTS & DISCUSSION

Dye binding and water uptake capacity

The effects of dye concentration and chitin/chitosan: dye solution ratios on dye binding capacity and water uptake of chitin and chitosan are given in Table 1. A marked difference between water uptake of chitin and chitosan was found with chitosan having higher water uptake than chitin. This difference can be due to differences in the crystallinity

Table 1—Effect of dye concentration and chitin/chitosan concentration on dye binding capacity and water uptake of chitin or chitosan

Dye conc added (mg/g chitin or chitosan)	Dye-binding capacity (mg dye/mg chitin or chitosan) (Water Uptake %)			
	Chitin conc		Chitosan conc	
	0.5 g + 20 ml dye solution	2.0 g + 20 ml dye solution	0.5 g + 20 ml dye solution	2.0 + 20 ml dye solution
1.6	0.53 ± 0.17* (450 ± 71)**	0.27 ± 0.01 (400 ± 1)	0.74 ± 0.03 (950 ± 71)	0.33 ± 0.02 (538 ± 88)
0.8	0.27 ± 0.01 (550 ± 71)	0.12 ± 0.01 (413 ± 18)	0.34 ± 0.01 (850 ± 70)	0.16 ± 0.01 (513 ± 159)
0.4	0.10 ± 0.00 (550 ± 70)	0.07 ± 0.04 (413 ± 18)	0.15 ± 0.01 (750 ± 70)	0.08 ± 0.00 (525 ± 106
0.2	0.02 ± 0.06 (450 ± 71)	0.01 ± 0.00 (438 ± 18)	0.08 ± 0.01 (850 ± 70)	0.04 ± 0.00 (525 ± 71)

*Dye-binding capacity
**Water uptake

Table 2—Results of regression and correlation analyses of dye concentration vs dye binding capacity of chitin and chitosan

Chitin/Chitosan conc	Regression equation	Standard error	Correlation coefficient (N = 14)
Chitin (0.5g + 20 ml)	y* = −0.047 − 0.731x**	0.017	0.997**
Chitin (2.0g + 20 ml)	y = −0.019 + 0.362x	0.021	0.981***
Chitosan (0.5g + 20 ml)	y = −0.030 + 0.995x	0.022	0.997***
Chitosan (2.0g + 20 ml)	y = −0.005 + 0.417x	0.101	0.996***

* = dye binding capacity
** = dye concentration
***= ($P < 0.01$)

Table 3—Effect of pH on dye binding capacity of chitin or chitosan at constant added dye concentration (0.4 mg/g chitin or chitosan) and constant chitin/chitosan:dye solution ratio (0.5g chitin/chitosan + 20 ml dye solution)

pH	Dye binding capacity (mg/g chitin or chitosan)			
	Chitin		Chitosan	
	pH with buffer	pH with HCl	pH with buffer	pH with HCl
	x ± s (N = 4)*		x ± s (N = 4)*	
9**	0.36±0.00	0.24±0.02	0.38±0.01	0.37±0.03
7.0	0.75±0.01	0.71±0.04	0.77±0.01	0.78±0.00
5.5	—	—	—	0.78±0.01
4.0	0.79±0.07	0.80±0.01	0.78±0.01	***
2.0	0.78±0.00	0.75±0.00	0.78±0.01	***

*Water uptake also determined and considered in calculations.
**Initial pH.
***Gel formation.

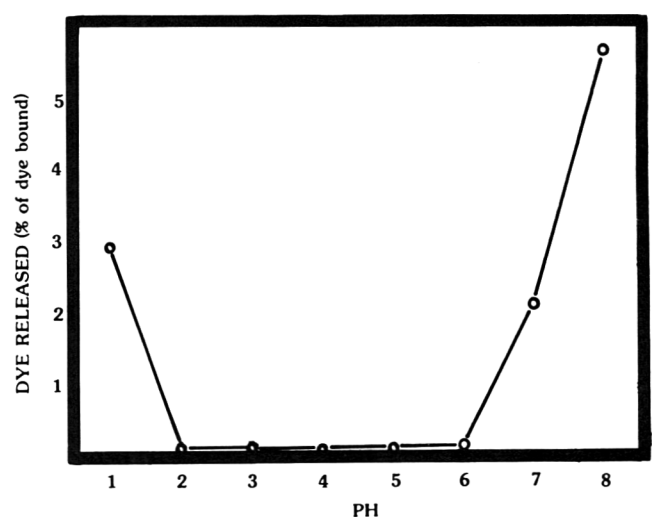

Fig. 1—Effect of pH on the dye released from dyed chitin.

of the products or due to differences in the amount of salt forming groups (Knorr, 1982). Differences in the amount of covalently bound protein residues (Austin et al., 1981) might also affect water uptake. An effect of chitin/chitosan: dye solution ratio on water uptake was also observed being higher at a 0.5g:20ml ratio than at a 2.0g:20ml ratio. Similar trends were found with dye-binding capacity. This difference could be caused by differences in rate of water uptake (wettability) at different chitin to aqueous dye solution ratios.

Dye concentration had no marked effect on water uptake but significantly affected dye-binding capacity of chitin and chitosan. The results of regression and correlation analyses examining dye-binding capacity of chitin and chitosan as a function of dye concentration are given in Table 2. The results indicate that dye-binding capacity of both chitin and chitosan correlated significantly with dye concentration.

The effect of pH on dye-binding capacity of chitin and chitosan is shown in Table 3. These data indicate a decline in the dye-binding capacity of chitin and chitosan above pH 7.0. Within a pH range of 2.0–7.0 dye-binding capacity of chitin was shown to be stable while chitosan formed gels below pH 5.5 and could not be evaluated. With one exception (chitin at pH 9) dye-binding capacity was not affected by adjusting the pH either with hydrochloric acid or with a buffer solution.

The effect of pH on the dye release from dyed chitin previously prepared at pH 5.5 and with a dye-binding capacity of 0.77mg dye/g chitin is shown in Fig. 1. There was no effect of pH on dye release between pH 2–6. The release of dye from dyed chitin was affected below and above this range. Beyond this range 5.7% of bound dye was released at pH 8.0 and 2.9% at pH 1.0 (Fig. 1). This could limit the application of dyed chitin because many foods have a pH higher than 6.0. However, in many cases of

—Continued on page 41

Characterization of Lupine Proteins

B. D. OOMAH and W. BUSHUK

ABSTRACT

Proteins of eight cultivars of two lupine species (*Lupinus albus* and *L. angustifolius*) were examined by several procedures. Differences in protein solubility between the species and among varieties within a species were observed. Defatting of the lupine flour altered protein solubility. Amino acid compositions of total protein and protein fractions showed only minor differences between the species and among varieties within a species (except for cv. Kali). Sodium dodecylsulfate polyacrylamide gel electrophoresis patterns of protein fractions revealed both qualitative and quantitative differences between species and among varieties.

INTRODUCTION

INTEREST in legume crops in the context of world protein needs has prompted many researchers around the world to investigate the feasibility of growing lupines. Data on the composition of the lupine seed are either incomplete or vary widely because of differences in methods of separation and analysis. Gladstones (1970) and, more recently, Aguilera and Trier (1978) have reviewed the literature on the proximate composition of lupines. Hill (1977) discussed variations within and among species in his extensive review on composition and nutritive value of "sweet" lupines.

Lupine proteins consist of a number of different components; globulins comprise the major fraction (Cerletti et al., 1978). Blagrove and Gillespie (1975) resolved the globulins of *Lupinus angustifolius* into conglutins α, β and γ by electrophoresis on cellulose-acetate strips.

The purpose of the present investigation was to determine the solubility, amino acid composition, and electrophoretic mobility of proteins of cultivars from two lupine species *L. albus* and *L. angustifolius* grown in Canada. Such information is fundamental to assessing the potential use of lupine proteins in foods and feeds.

MATERIALS & METHODS

SEEDS of eight cultivars of two lupine species, *L. albus* and *L. angustifolius*, grown in Winnipeg were used. The seeds were ground on a UDY Cyclo-Tec grinder fitted with a 1 mm screen. The meal was stored at −20°C prior to analyses.

Proximate analyses

Moisture content was determined by the AACC (1969) vacuum oven method. Total nitrogen content was determined by the micro-Kjeldahl procedure and converted to protein content using the factor 6.25. Crude fiber was determined by the AOAC (1970) method using acid hydrolysis followed by alkaline hydrolysis. Crude fat content was determined by the AOAC method, using petroleum ether (Skellysolve F, bp 40–60°C) as the solvent. Ash content was determined by the AACC method 46-11. All analytical data are reported on a dry matter basis.

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Protein fractionation

The protein in the meal was fractionated according to the modified Osborne procedure (Chen and Bushuk, 1970). The insoluble residue that results from this procedure was further extracted with 0.1M sodium hydroxide solution. One sample of undefatted flour (cv. Reuscher) was included in the fractionation for comparison. Nitrogen content of fractions was determined by the micro-Kjeldahl procedure; reported values are means of duplicate determinations. Nitrogen recoveries of this procedure were between 98.4 and 100.1%. Fractions were dialyzed against distilled water and freeze-dried.

Proteins of the defatted samples were also fractionated by sequential extraction with distilled water, 0.5M sodium chloride solution, and 0.1M sodium hydroxide solution. Nitrogen recoveries by this procedure ranged from 95.5 to 102.3%.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out at pH 8.4 according to the procedure of Khan and Bushuk (1977). Molecular weights were estimated from a calibration curve obtained from data for cytochrome c (equine heart, MW 11,700), chymotrypsinogen A (MW 25,000), pepsin (MW 35,000), ovalbumin (MW 43,000), and bovine serum albumin (monomer, MW 69,000).

Amino acid analysis

Amino acid compositions of total proteins and protein fractions were determined on a Beckman, model 121, automatic amino acid analyzer using the standard (6N HCl, vacuum, 24 hr, 11°C) hydrolysis procedure. Amounts of individual amino acids were computed in the usual manner using the Beckman standard. The data reported are from single analyses. Nitrogen recoveries ranged from 83.1–91.8%. To facilitate comparison, the amino acid content values were normalized to a nitrogen recovery of 90%.

RESULTS & DISCUSSION

Proximate composition of lupines

There is considerable variation in proximate composition between and within species (Table 1). Neuland had the highest (40%) and Kali the lowest (33.8%) protein content. Crude fibre content of the meal ranged from 9.8% for Neuland to 14.6% for Borre. Crude fiber contents of the angustifolius varieties were higher and crude fat contents were lower than those of the albus species. The results reported here are in general agreement with those published by others.

Protein solubility fractionation

Differences among the solubility distributions by Osborne fractionation were observed within and between the two species investigated (Fig. 1). Values for albumin content ranged from 2–10%. Varieties which were low in albumin had a high globulin content. The amount of protein extracted with salt solution varied from 53–85%. The proportions of prolamins and acetic acid soluble glutelins were neglibible for all varieties. The proportion of the alkali soluble fraction ranged from 10% (Neuland and Kali) to 35% (Reuscher). The amount of the alkali insoluble fraction (insoluble residue) was small (2.3–3.3%) and essentially constant for all varieties examined.

Table 1—Proximate composition of seed of eight lupine varieties

Species/cultivar	Moisture %	Crude protein (N x 6.25) %	Crude fiber %	Crude fat %	Ash %
L. Angustifolius					
Borre	6.9	36.4	14.6	4.8	3.5
Unicrop	6.9	37.4	12.1	4.7	3.9
Mean	6.9	36.9	13.4	4.8	3.7
Standard deviation	–	0.6	1.8	0.1	0.3
L. Albus					
Kali	6.1	33.8	12.1	9.3	4.0
Neuland	5.9	40.2	9.8	8.1	3.8
Reuscher	5.9	36.0	10.8	7.5	3.2
Ultra	6.2	34.8	11.1	8.4	3.6
ACC #2P91	5.9	39.6	11.5	6.6	4.2
Gela	5.7	34.2	11.1	9.1	4.0
Mean	6.0	36.4	11.1	8.2	3.8
Standard deviation	0.2	2.8	0.8	1.0	0.4

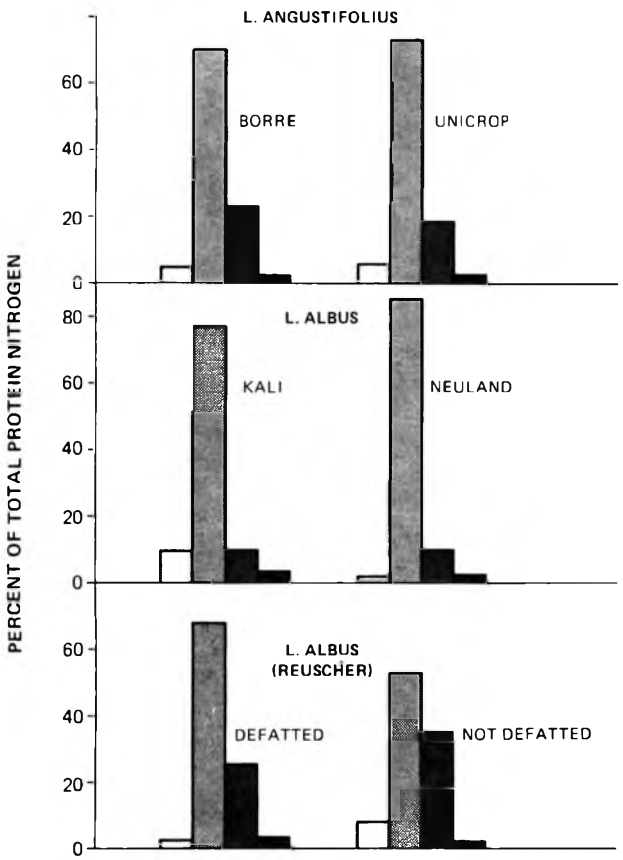

Fig. 1—Osborne solubility fractionation of the proteins of defatted meals of five lupine cultivars and nondefatted meal of one cultivar: ( ) Albumins, ( ) Globulins, ( ) Glutelins and ( ) Residue proteins. [Within each attribute, columns with the same letter are not significantly different (α = 0.01, Duncan's multiple range test).]

The cultivars Neuland and Kali of the albus species had similar protein solubility distributions. Unicrop and Borre, of the angustifolius species gave the same solubility distributions. On the other hand, Reuscher, of the albus species, differed from the other varieties of the same species and resembled those of the angustifolius species in protein solubility distribution.

Defatting lupine seed meal affected protein solubility by decreasing the amount of albumins and prolamins and increasing the amount of globulins.

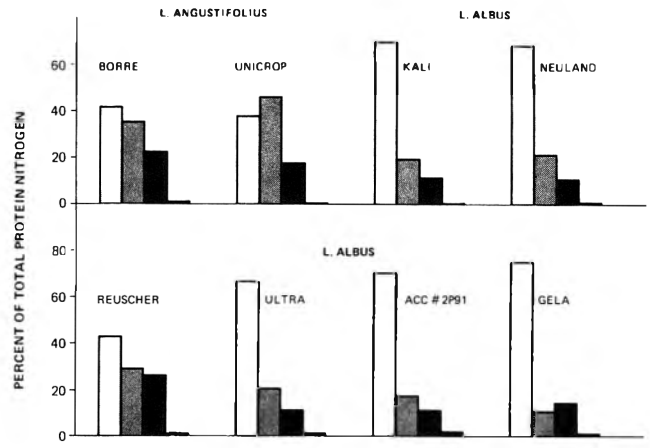

Fig. 2—Solubility fractionation of the proteins of defatted meals of eight lupine cultivars: ( ) Water soluble, ( ) salt soluble and ( ) Alkali soluble proteins. For details, see legend to Fig. 1.

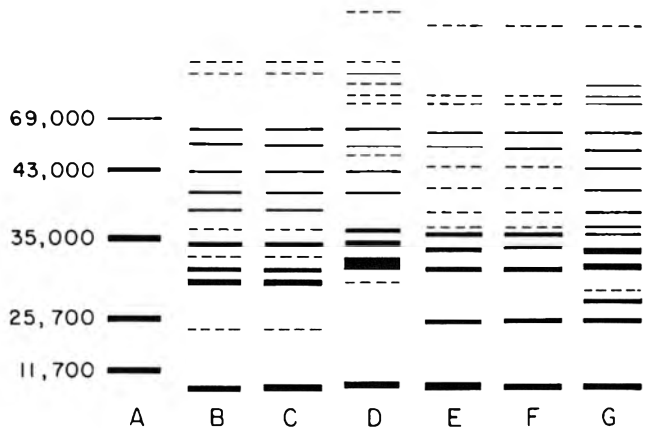

Fig. 3—SDS-PAGE patterns (schematic) of reduced albumins of five defatted and one nondefatted lupine meals: A, standard proteins; B, Borre; C, Unicrop; D, Kali; E, Neuland; F, Reuscher; and G, Reuscher (nondefatted).

With the second fractionation procedure (Fig. 2), varieties of the albus species, except Resucher, contained more water-soluble protein and less salt and alkali soluble protein than those of the angustifolius species. Results for Reuscher were similar to those for the angustifolius varieties. As expected, the sum of the water and salt-soluble fractions obtained by this procedure was approximately equal to the amount of the salt-soluble fraction by the modified Osborne procedure. The amount of the alkali fraction obtained in this case was slightly lower than that obtained by the modified Osborne method.

In relation to preparation of lupine protein isolates from lupine meals, it should be noted that water alone can solubilize up to 75% of the meal protein.

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE results (Fig. 3) showed that the albumin fraction of the varieties of the albus species contained a high molecular weight component which was absent in the two varieties of the angustifolius species. The patterns of Neuland and Reuscher are the same, but are quite different from that of the Kali variety (all of the albus species).

Defatting of the Reuscher flour altered the solubility of two protein components (26,000 and 27,000 daltons) whereby they became insoluble in water (compare patterns F and G).

SDS-PAGE patterns of the globulins (Fig. 4) showed

both quantitative and qualitative differences among the varieties and between and two species. The angustifolius varieties had two bands (60,000 and 68,000 daltons) which were absent in patterns of varieties of the albus species. Defatting caused a loss of three globulin bands in the Reuscher patterns (compare patterns M and N).

The glutelin patterns (Fig. 5) had a high molecular weight band that was common to all the varieties. Patterns were similar for varieties of the same species but different for the two species. The 71,000 dalton band was present only in the patterns of the angustifolius species. Defatting (Reuscher only) had no effect on the patterns of this fraction.

Quantitative differences (judged by band intensity) between varieties were evident in the patterns of all three fractions examined. Molecular weights ranged from 17,000–126,000 daltons for the albumins, 18,000–68,000 for the globulins, and 21,000–130,000 for the glutelins.

Amino acid composition

Amino acid compositions of the eight varieties were generally similar. As found by others, lupine protein has a low methionine content compared with other plant proteins.

The amounts of isoleucine, phenylalanine and tyrosine for both species, were higher, and that of valine was lower, than the FAO levels (FAO, 1970) for a nutritionally adequate protein. Leucine values for the angustifolius species were slightly lower than the FAO level. The Kali variety of the albus species contained more lysine and threonine than the FAO levels.

The Modified Essential Amino Acid index values obtained here agree with those of Cerletti and Duranti (1979). The protein for the albus species showed slightly better nutritional quality than that of the angustifolius species.

Defatting altered the amino acid composition of the albumin fraction, but had no effect on the composition of the other two fractions analyzed (data not shown). The major effect was a decrease in the proportions of the charged polar amino acids, arginine and glutamic acid, and an increase in the hydrophobic amino acids, proline, alanine, valine and phenylalanine. The shift in amino acid composition on defatting is consistent with the observed shift in solubility distribution.

Amino acid compositions of the protein fractions were similar for all varieties except Kali (Table 2). The albumin fraction of Kali had the lowest amounts of lysine, histidine, threonine, proline, glycine, alanine, valine, and methionine and the highest amounts of glutamic acid and tyrosine. Consistent with the literature, the albumin fraction is rich in lysine, histidine, threonine, glycine, alanine, valine, and methionine. Of the three protein fractions analyzed, it has the lowest proportions of arginine, leucine and phenylalanine.

In the context of nutritional quality, the albumin fractions has better amino acid composition than the globulin fraction. Unfortunately the albumin fraction forms only a small proportion of the total seed protein and there-

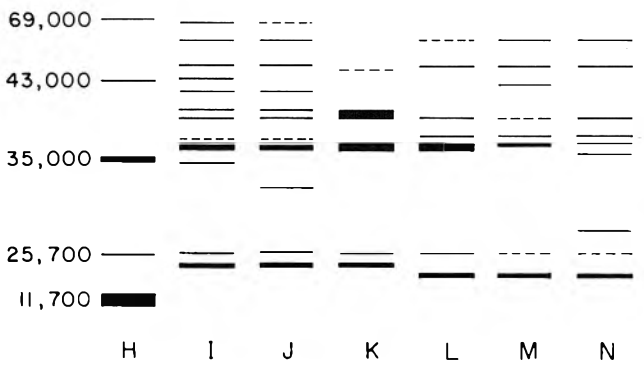

Fig. 4–SDS-PAGE patterns (schematic) of reduced globulins of five defatted and one nondefatted lupine meals: H, standard proteins; I, Borre; J, Unicrop; K, Kali; L, Neuland; M, Reuscher; and N, Reuscher (nondefatted).

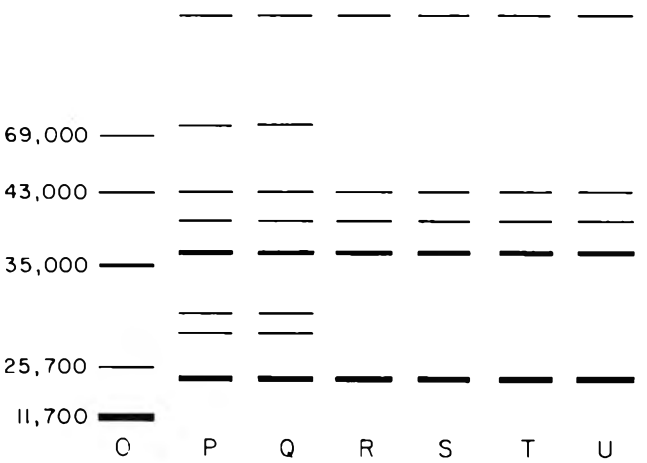

Fig. 5–SDS-PAGE patterns (schematic) of reduced glutelins of five defatted and one nondefatted lupine meals: O, standard proteins; P, Borre; Q, Unicrop; R, Kali; S, Neuland; T, Reuscher; and U, Reuscher (nondefatted).

Table 2–Amino acid compositions of lupine flours and protein fractions (g amino acid/100g protein)

	cv. Borre				cv. Kali			
	Flour	Albumin	Globulin	Glutelin	Flour	Albumin	Globulin	Glutelin
Lysine	5.1	8.0	4.0	3.9	5.0	5.3	4.3	6.0
Histidine	2.7	3.1	2.7	2.5	2.6	1.8	2.2	3.1
Arginine	10.7	8.3	11.6	11.2	9.6	10.1	9.6	7.9
Aspartic acid	10.5	10.1	10.7	10.8	12.5	11.8	11.9	11.6
Threonine	3.5	6.0	3.0	3.9	4.3	3.8	3.7	5.4
Serine	4.4	5.4	5.4	4.9	5.1	5.8	5.9	6.1
Glutamic acid	23.2	20.6	25.4	23.9	27.8	25.2	23.7	17.5
Proline	4.2	5.1	4.2	5.1	4.4	4.0	4.6	4.9
Glycine	4.2	6.5	3.9	4.6	4.5	4.2	3.9	5.0
Alanine	3.6	6.3	2.9	3.7	4.0	3.5	3.2	4.8
Valine	4.2	5.2	3.6	4.3	5.0	3.7	4.2	5.8
Methionine	0.7	1.2	0.4	0.2	0.7	0.3	0.5	1.3
Isoleucine	4.3	3.7	4.4	4.4	5.2	4.4	5.2	5.2
Leucine	6.7	6.1	7.8	7.8	8.6	7.5	9.3	9.6
Tyrosine	2.8	3.4	3.8	3.6	3.7	5.3	5.3	4.9
Phenylalanine	4.0	2.9	4.1	4.2	4.4	3.6	4.4	5.2

fore offers little possibility for nutritional improvement by breeding. The main storage protein (globulin) was rich in glutamic acid, and arginine. Of the three fractions analyzed, it had the lowest amounts of lysine, threonine, proline, glycine, alanine, valine, and methionine. The glutelin fraction is particularly rich in the hydrophobic amino acids, phenylalanine, proline, and leucine.

CONCLUSIONS

SOLUBILITY FRACTIONATION RESULTS suggest that lupine protein isolates can be easily prepared in fairly high yields by precipitating the protein in water extracts. However, the high solubility of the protein in water might restrict its use in food applications. Defatting of lupine meal affects the solubility of some protein fractions more than of others. The electrophoretic patterns of each protein fraction of the albus and angustifolius species were similar. The patterns of the globulins are variety specific and therefore can be used to identify the variety for plant breeding and/or commercial grading purposes.

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CHITIN FUNCTIONALITY. . . From page 37

potential application a dye-release of 2.5% at pH 7.0 might be tolerable.

The results of this study indicate the potential of chitin and chitosan as carrier for food additives which could further contribute to the potential food application of chitin and chitosan as "inert" but highly functional food ingredients. The unstable nature of the -N=N- link of azo dyes to gut microflora was first shown by Sisley and Porcher (1911) and has also been reported by several authors (Daniel, 1962; Furia, 1977). Information on the effect of the gut microflora on the cleavage of the azo bond of dyed chitin (Furia, 1977; Radomski and Mellinger, 1962) could provide further insight into the use of chitin and chitosan. According to Knecht and Hibbert (1926), dyeing properties of chitin are very different from cellulose. This might be due to the high degree of crystallinity of chitin which can affect accessibility of its acetamide (and amino) groups. This mechanical difficulty of penetration of the structure could also affect the release of ions of high affinity (Giles et al., 1958).

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Effects of Ca^{++}, Mg^{++} and Na^{+} on Heat Aggregation of Whey Protein Concentrates

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ABSTRACT

Effect of Ca^{++} on the heat aggregation of whey protein concentrates (WPC) was compared with that of Na^{+} and Mg^{++}. On the alkaline side of the isoelectric zone, aggregation of WPC was increased by the addition of $CaCl_2$, $MgCl_2$ or NaCl, among which $CaCl_2$ showed the greatest effect. The denaturation temperature of WPC determined by differential scanning calorimetry significantly decreased in the presence of $CaCl_2$ or $MgCl_2$, but increased slightly in the presence of NaCl. In the electrophoretic patterns of heated WPC, the most sensitive protein to Ca^{++} was β-lactoglobulin.

INTRODUCTION

SEVERAL STUDIES have been reported dealing with the effect of calcium ions on the heat aggregation of whey proteins. Kenkare et al. (1964) found that acid-prepared serum contained a higher concentration of calcium phosphate than ultracentrifugal serum, and the serum proteins in acid-prepared serum were much less stable under heat than those in ultracentrifugal serum. Hidalgo and Gamper (1977) observed that rennet whey protein concentrates aggregated greatly over the whole pH range (2–12) under heat treatment in the presence of Ca^{++}. Towend and Gyuricsek (1974) showed that Ca^{++} highly promoted the heat aggregation of whey proteins at pH above 6.0. Morr and Josephson (1968) suggested that heat aggregation of whey protein is a multi-reaction, the third reaction of which involves the formation of gross-sized aggregates and is dependent on Ca^{++} concentration. De Wit (1981) showed that the amount of Ca^{++} to induce the aggregates of β-lactoglobulin, a main protein of whey, was equivalent to its net negative charge. This observation can be related to the suggestion made by Zittle et al. (1957) that Ca^{++} binding neutralized the net negative charges of whey proteins and caused isoelectric precipitation.

However, more detailed studies should be made on the role of Ca^{++} in the heat aggregation of whey proteins. The effects of other salt ions on the heat aggregation of whey proteins must also be clarified. Furthermore, the methods for whey preparation in the previous studies were different from one another. Since whey protein concentrates (WPC) prepared by different methods have been shown to possess different composition and properties (Morr et al., 1973), studies should be conducted on WPC with a high level of purity.

In the present study, WPC was prepared by the method of Palmer (1977) with some modification, and the effect of Ca^{++} on the heat aggregation of whey proteins was compared with that of Na^{+} and Mg^{++}.

MATERIALS & METHODS

WPC preparation

Whey was pooled from raw skim milk which was adjusted to pH 4.6 with 1N HCl and centrifuged at 9,000 rpm for 30 min to remove all caseins. WPC was prepared by the method of Palmer (1977) with a slight modification. Carboxymethylcellulose (CMC) was used as an ion exchanger. To suppress denaturation of proteins, acetate buffer (pH 4.0, ionic strength 0.025) was employed instead of HCl for applying the whey to CMC column, and phosphate buffer (pH 8.0, ionic strength 0.025) was used instead of NaOH for eluting the whey proteins from CMC column. Protein fractions were collected and exhaustively dialyzed 10 times against 100 volumes of distilled water for 5 days to assure the exclusion of salts. Dialyzed protein solution was lyophilized.

Heat treatment

Two ml of 1% WPC solution, adjusted to the required pH with 1N NaOH or 1N HCl, was placed in a test tube (1 x 7 cm) and immersed in a controlled temperature water bath (Shimadzu type TB-95), precision ± 0.5°C). The test tube was shaken gently for 30 sec, kept at controlled temperature for 5 min and cooled in ice water.

Aggregation and denaturation measurement

The extent of aggregation was measured by determining the decrease in the solubility of WPC at the heating pH. Aggregated proteins of heated WPC solution were removed by centrifugation at 1,500 x *g* for 15 min. Protein content of the supernatant was measured by the method of Lowry et al. (1961) using bovine serum albumin as a standard.

The extent of denaturation was measured by determining the decrease in WPC solubility in the isoelectric zone (Nielson et al., 1973); pH of heated WPC solution was adjusted to 4.6 by adding 2 volumes of acetate buffer (pH 4.6, 0.025M), and after 1 hr it was centrifuged at 1,500 x *g* for 15 min. Measurements of protein solubility were carried out as described above.

Differential scanning calorimetry (DSC) measurement

DSC curves were recorded on a Daini Seiko Sha model ssc/560 thermal analyzer with a heating rate of 1.5°C per min in the temperature range 25–125°C. Samples of 50 μl of 12% protein solutions, adjusted to pH 6.5 with 1N NaOH, were pressure-sealed in silver pans. An identical sealed pan filled with 53 μl of distilled water, adjusted to pH 6.5 with 0.1N NaOH, was used as a reference. The denaturation temperature was defined as the temperature of the DSC peak maximum. The enthalpy of denaturation was calculated from the DSC peak area with stearic and palmitic acids as calibrants (McClain and Wiley, 1972).

Polyacrylamide gel electrophoresis

A vertical slab polyacrylamide gel electrophoretic method (Reid and Bieleski, 1968) was used. Gel sheets (0.1 x 13 x 13 cm) of 7.5% of polyacrylamide and electrophoresis buffer of Tris-glycine were prepared as described by Davids (1964). Electrophoresis was performed at room temperature with a constant current of 20 mA. After 2 hr of electrophoresis, the gel was stained with 0.25% Coomassie Brilliant Blue R-250 in water/methanol/acetic acid (5:5:1, v/v/v) for 20 min and destained with 7% acetic acid in which a small amount of methanol was added. The relative intensity of the stained bands on polyacrylamide gel was determined by scanning the gel sheets with a Shimadzu dual wavelength chromatoscanner, Model CS-910).

RESULTS & DISCUSSION

THE AMOUNT of undenatured proteins in WPC prepared in this study was 94.0%, which is comparable with that in WPC prepared by Sephadex gel filtration (88.6%) or electrodialysis (94.4%), according to Morr et al. (1973).

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Fig. 1 shows the effect of pH on the aggregation and denaturation of WPC heated at 80°C for 5 min. When WPC was heated without addition of any salts, aggregation occurred in the isoelectric zone of WPC, around pH 5.5, but no aggregation could be found on both sides of this pH zone. This result was similar to those of Townend and Gyuricsek (1974) and Hidalgo and Gamper (1977).

The extent of denaturation, however, was different on both sides of the isoelectric zone; WPC was denatured extensively by the heating of the alkaline side of isoelectric zone, but was very stable under heat on the acidic side. Some WPC solutions heated at alkaline pH remained turbid even after centrifugation, so the actual extent of denaturation would obviously be much greater than the level shown in Fig. 1. A similar result was obtained by Modler and Emmons (1977) and Harwalkar (1979). Since heat denaturation of whey proteins is suggested to be characteristically accompanied by thioldisulfide group reactions (Morr and Josephoson, 1968) and thiol activity is shown to be increased at pH above 6.5 (Tanford, 1968), the thiol-disulfide group reaction could be one of the factors that promote the denaturation of WPC on the alkaline side of the isoelectric zone.

When NaCl was added to WPC solution, both the aggregation and the denaturation of WPC increased slightly in the pH region from 4.5–9.5. When $CaCl_2$ or $MgCl_2$ was added to the WPC solution, both aggregation and denaturation of heated WPC increased much more on the alkaline side of the isoelectric zone. It is of interest that WPC was very stable on the acidic side of isoelectric zone and not affected by any of the salts tested.

As shown in Fig. 2, effects of Ca^{++}, Mg^{++} and Na^{+} on the aggregation and denaturation of WPC could be seen even at a salt concentration as low as 0.01 of the ionic strength. When the heating temperature was lowered, the extent of both aggregation and denaturation of WPC was decreased. Under the heating condition of 60°C or 70°C, the effects of Ca^{++} and Mg^{++} were similar but entirely different from that of Na^{+}; when the ionic strength was higher than 0.03, both Ca^{++} and Mg^{++} suppressed the WPC aggregation and denaturation somewhat, whereas Na^{+} did not. However, this stabilizing effect of Ca^{++} or Mg^{++} decreased at a higher heating temperature. When pH of WPC was adjusted to 8.0, the difference between the effect of Na^{+} and Ca^{++} or Mg^{++} became greater (Fig. 3). It is also seen in this figure that the effect of Ca^{++} is clearly greater than that of Mg^{++}.

When $CaCl_2$ or $MgCl_2$ was added to WPC solution after heat treatment, the difference in the effect of these salts between pH 6.5 and 8.0 became clearer (Fig. 4); although the effect of Ca^{++} or Mg^{++} on the aggregate formation of WPC was quite small at pH 6.5, it became large at pH 8.0. Both results shown in Fig. 3 and 4 tend to indicate that Ca^{++} or Mg^{++} specifically binds to the heat-denaturated whey proteins and forms aggregates at alkaline pH.

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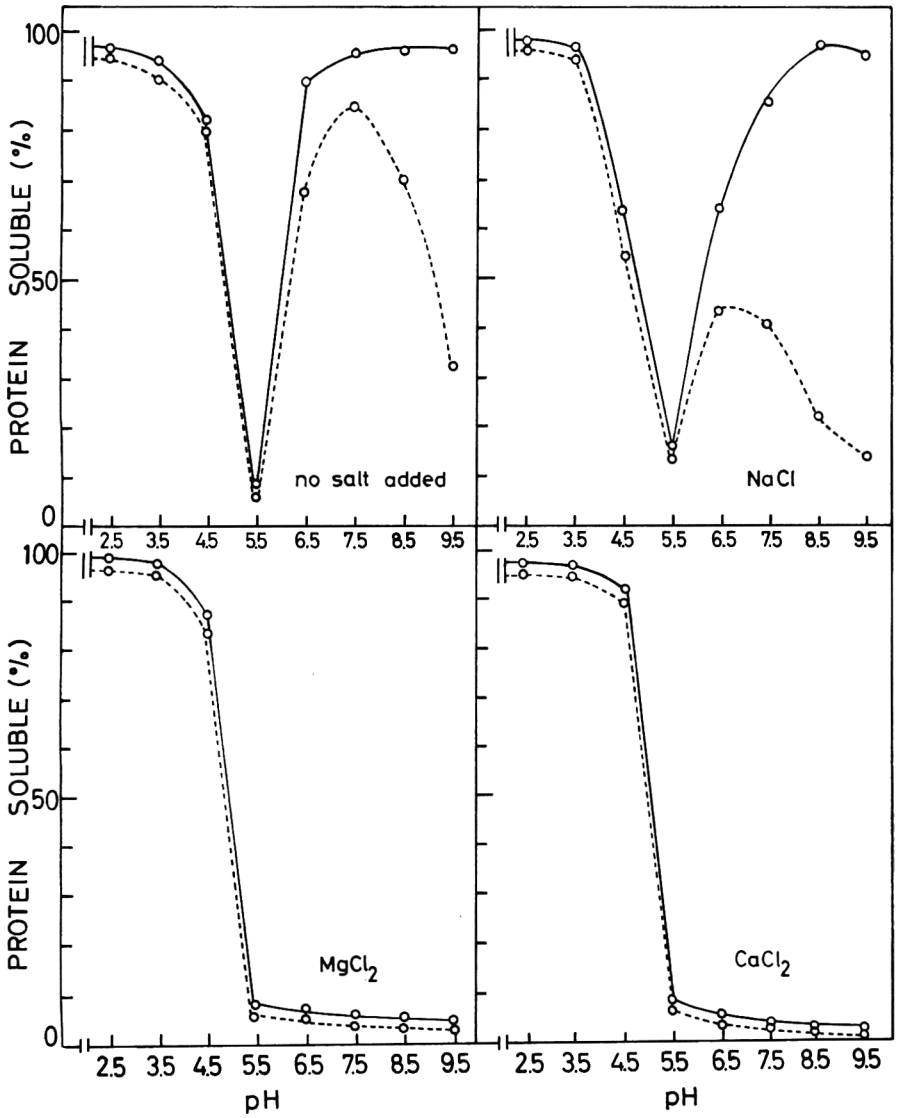


Fig. 1—Effect of pH on the aggregation and denaturation of WPC heated at 80° C for 5 min. ——: soluble protein content of WPC after removing aggregated proteins at the heating pH. - - - -: soluble protein content of WPC after removing denatured proteins at pH 4.6. Ionic strength of salt: 0.05; Protein concentration: 1%.

Fig. 5 shows DSC patterns of WPC heated with or without salts. In this figure, only one peak was noted in every WPC sample. Judging from the protein composition of whey, this peak may be derived from β-lactoglobulin. The denaturation temperature obtained from WPC without addition of any salt was 75°C (Table 1). This value is comparable with that of β-lactoglobulin (De Wit and Swinkels, 1980), near 80°C. In the presence of $CaCl_2$ or $MgCl_2$, the denaturation temperature of WPC was about 2–4°C lower than that of WPC without any salt. In the presence of NaCl, the denaturation temperature of WPC slightly increased. This result coincides well with the general properties of these salts against the denaturation of proteins (von Hippel and Wong, 1965). The presence of Ca^{++} or Mg^{++} clearly promotes heat denaturation of WPC. However, the enthalpy of WPC with $CaCl_2$ added was much less than with $MgCl_2$ or NaCl, so the role of Ca^{++} in the heat denaturation of whey proteins may well differ at least somewhat from that of other salts.

To investigate the effect of salts on each constituent protein of WPC, electrophoresis of heated WPC was performed. The results are shown in Fig. 6 and 7. Among the constituent proteins of WPC, immunoglobulin was the most susceptible to heat. In the electrophoretic patterns of WPC heated with or without NaCl, two remarkable changes were noted. One was the increase of the protein band which did not enter the lower part of gel and the other was the appearance of a thin band in the post-region of α-lactalbumin. These newly formed protein bands could be due to small-sized aggregates of denatured proteins that could not be removed by centrifugation. When WPC was heated with $MgCl_2$ or $CaCl_2$, all aggregated proteins were completely removed by centrifugation and no such newly formed protein bands were noted in the electrophoretic patterns of heated WPC.

Fig. 8 shows the densitometric pattern of Fig. 7. This figure indicates that Na^+ had a destabilizing effect only on bovine serum albumin, whereas both Ca^{++} and Mg^{++} destabilized all the constituent proteins of WPC. Among the latter, β-lactoglobulin was particularly affected by Ca^{++}. Compared with two genetic varients of β-lactoglobulin, β-lactoglobulin A was more sensitive to heat than β-lactoglobulin B under the heating in the presence of $CaCl_2$. Many studies on the heat stability of β-lactoglobulins showed that β-lactoglobulin A is more stable than β-lactoglobulin B (Alexander and Pace, 1971; Sawyer, 1968; Dupont, 1965; Gough and Jenness, 1962), but Hillier and Lyster (1979) reported that the difference in the thermostability of β-lactoglobulin A and B present in cheese whey is very small and varies with heating temperature. Some protein components of WPC might affect the heat denaturation of β-lactoglobulins.

Since β-lactoglobulin is the main constituent of WPC, its remarkable sensitivity to Ca^{++} is interesting. Further

–Text continued on page 70

Table 1–Denaturation temperatures and enthalpies of denaturation of WPC in tne presence of various salts

Addition	T_D (°C)	ΔH(Cal/g)
none	75.2	2.24
NaCl	76.5	2.84
$MgCl_2$	73.5	2.53
$CaCl_2$	71.5	1.56

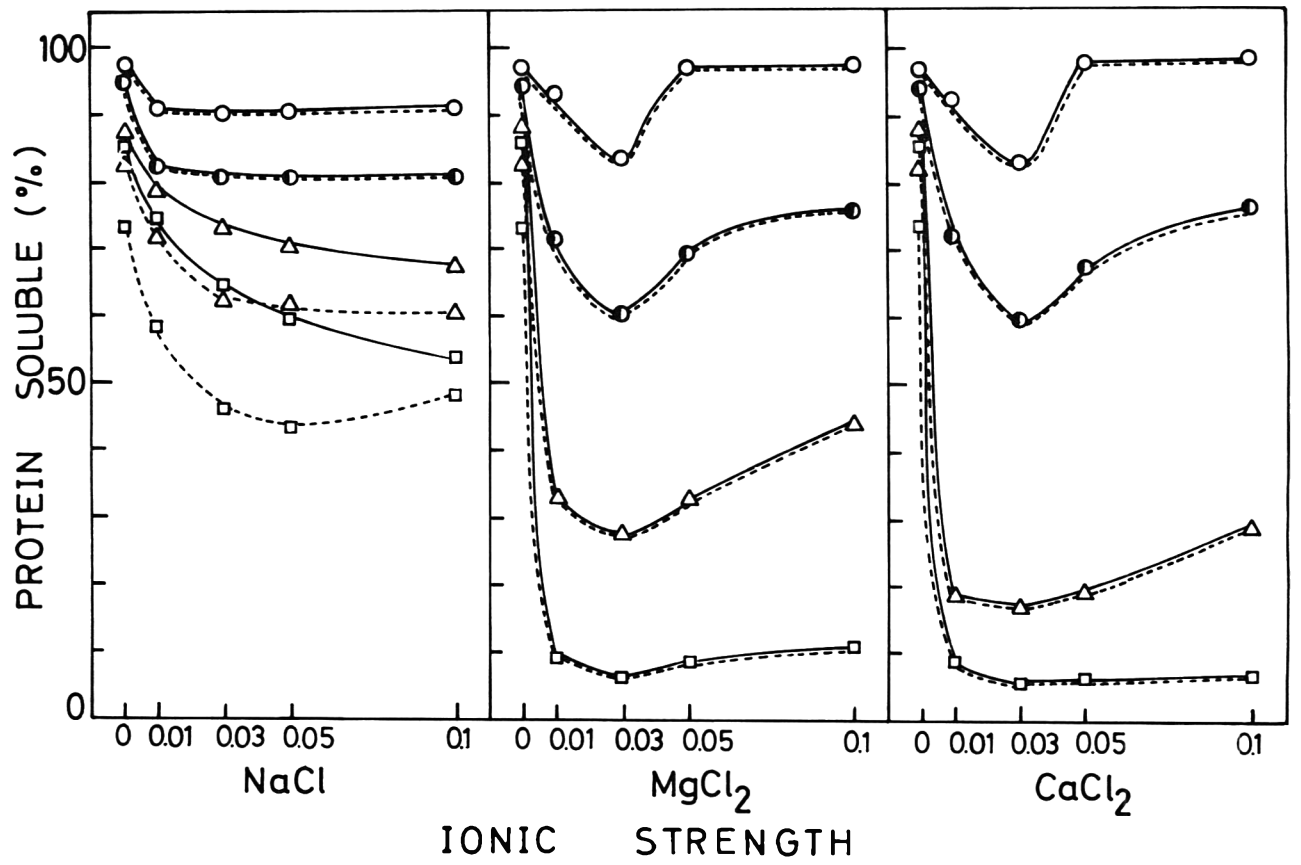


Fig. 2–Effect of salt concentration on the aggregation and denaturation of WPC heated at pH 6.5, at 60°C (○), 70°C (●), 75°C (△) and 80°C (□) for 5 min. ——: Soluble protein content of WPC after removing aggregated proteins at the heating pH. - - - -: soluble protein content of WPC after removing denatured proteins at pH 4.6. Protein concentration: 1%.

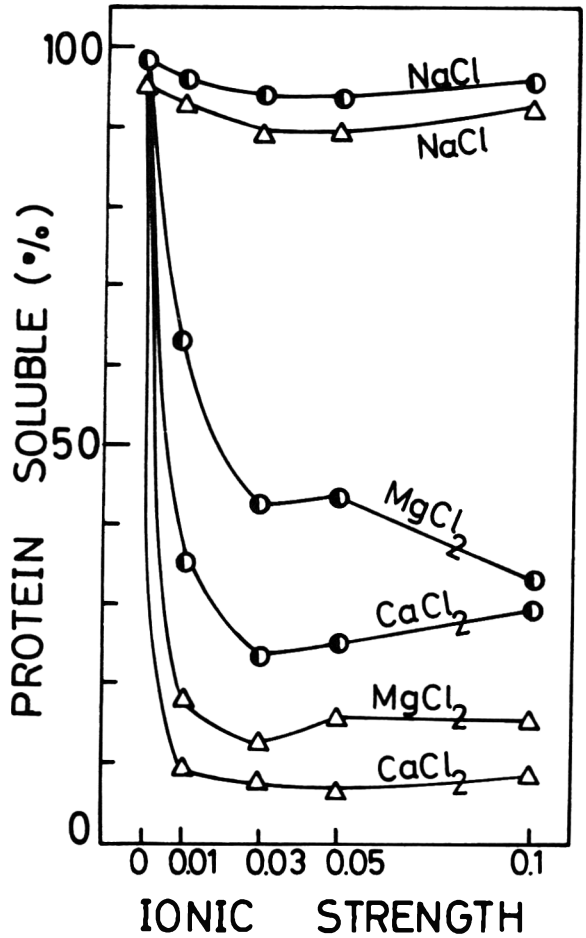


Fig. 3—Effect of salt concentration on the aggregation and denaturation of WPC heated at pH 8.0 at 70°C (○) and 75°C (△) for 5 min. ——: soluble protein content of WPC after removing aggregated proteins at the heating pH. Protein concentration: 1%.

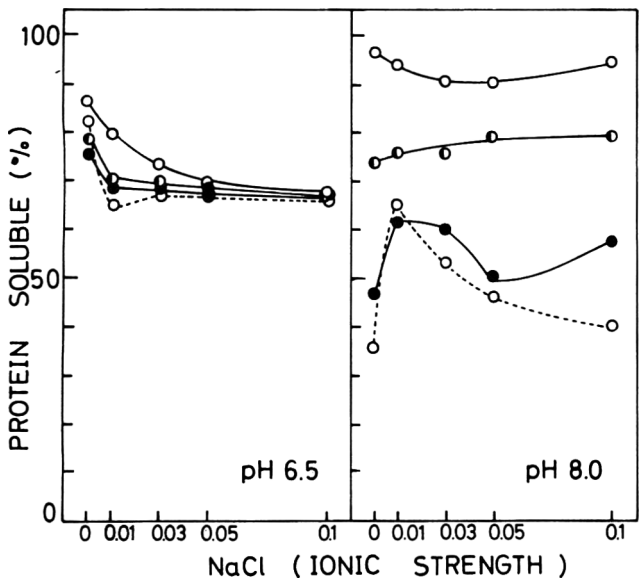


Fig. 4—Effect of Ca^{++} (●) and Mg^{++} (○) on the heat aggregation of WPC dissolved in various concentrations of NaCl solution (0–0.1M), when added after heat treatment of 75°C for 5 min at pH 6.5 or 8.0. (○): heated in NaCl solution with no Ca^{++} and Mg^{++} added. Protein concentration: 1%, concentration of $CaCl_2$ or $MgCl_2$ added: 0.05 of ionic strength. ——: soluble protein content of WPC after removing aggregated proteins at the heating pH. - - - -: soluble protein content of WPC after removing denatured proteins at pH 4.6.

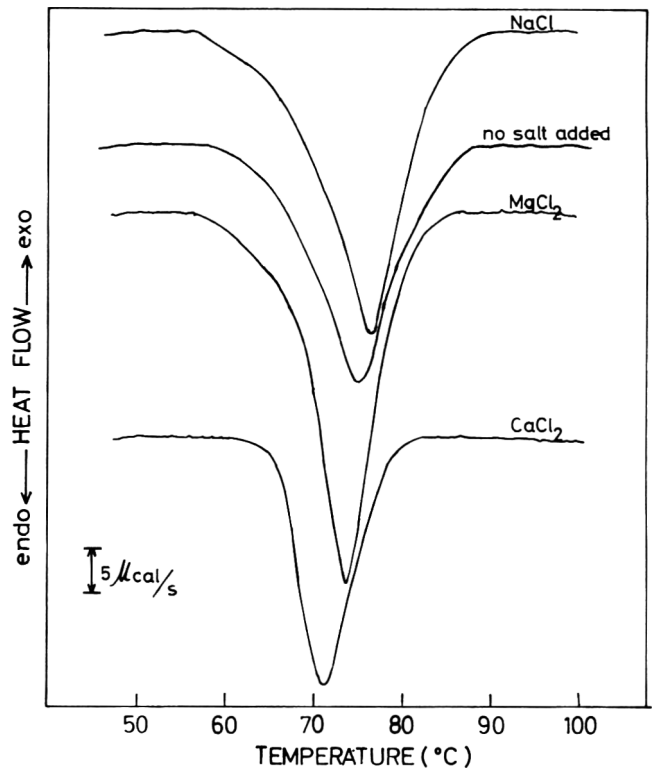


Fig. 5—DSC patterns of WPC in the presence of various salts. Ionic strength of salt: 0.1; protein concentration: 12%; heating rate: 1.5°C/min.

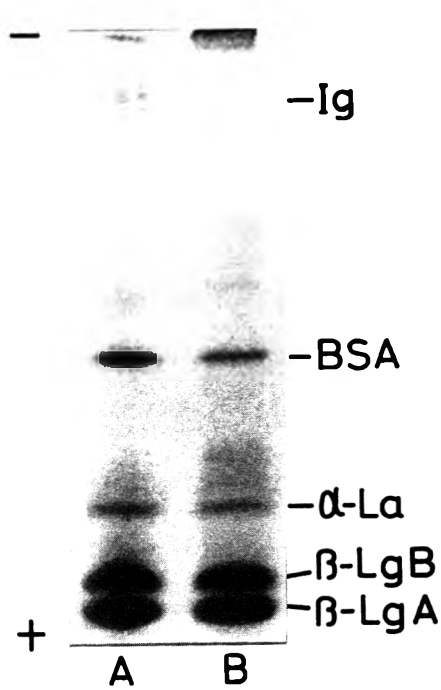


Fig. 6—Electrophoretic patterns of native WPC (pH 6.5) (A) and WPC (pH 6.5) heated at 75°C for 5 min (B). WPC solution was centrifuged at 1,500g for 15 min after heating and the supernatant solution obtained was used for electrophoresis. Abbreviations: Ig = immunoglobulin; BSA = bovine serum albumin; α-La = α-lactalbumin; β-LgA = β-lactoglobulin A; β-LgB = β-lactoglobulin B.

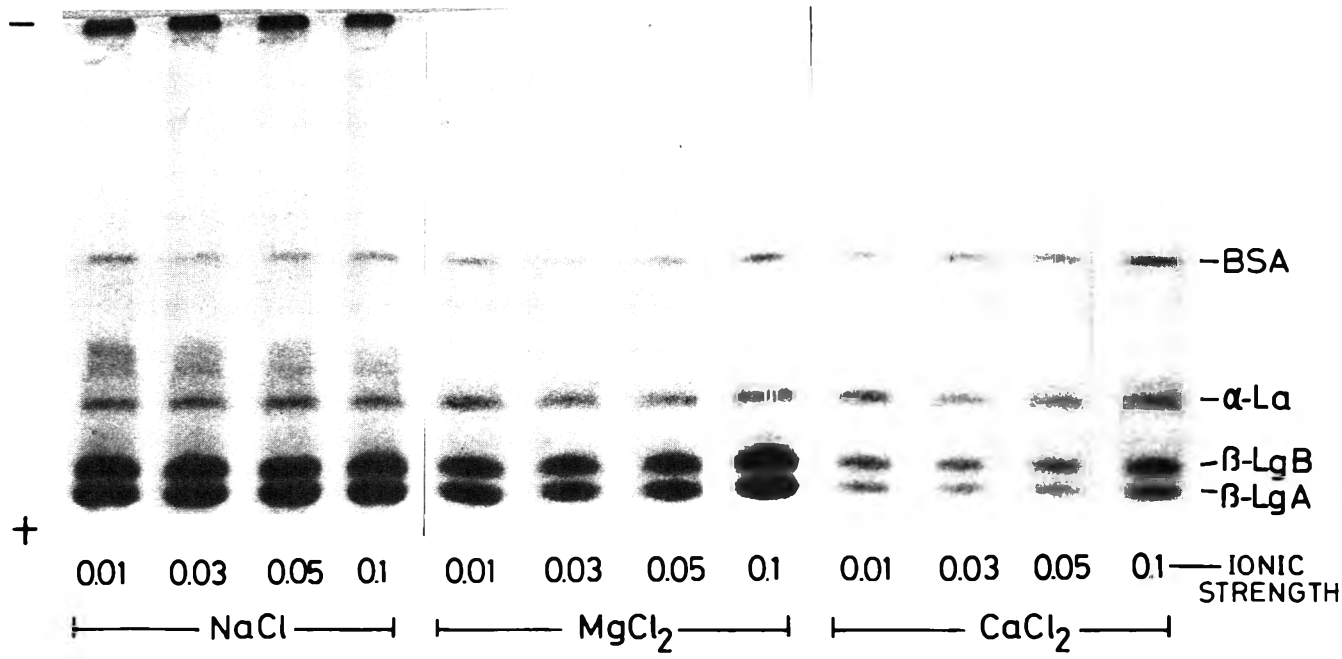
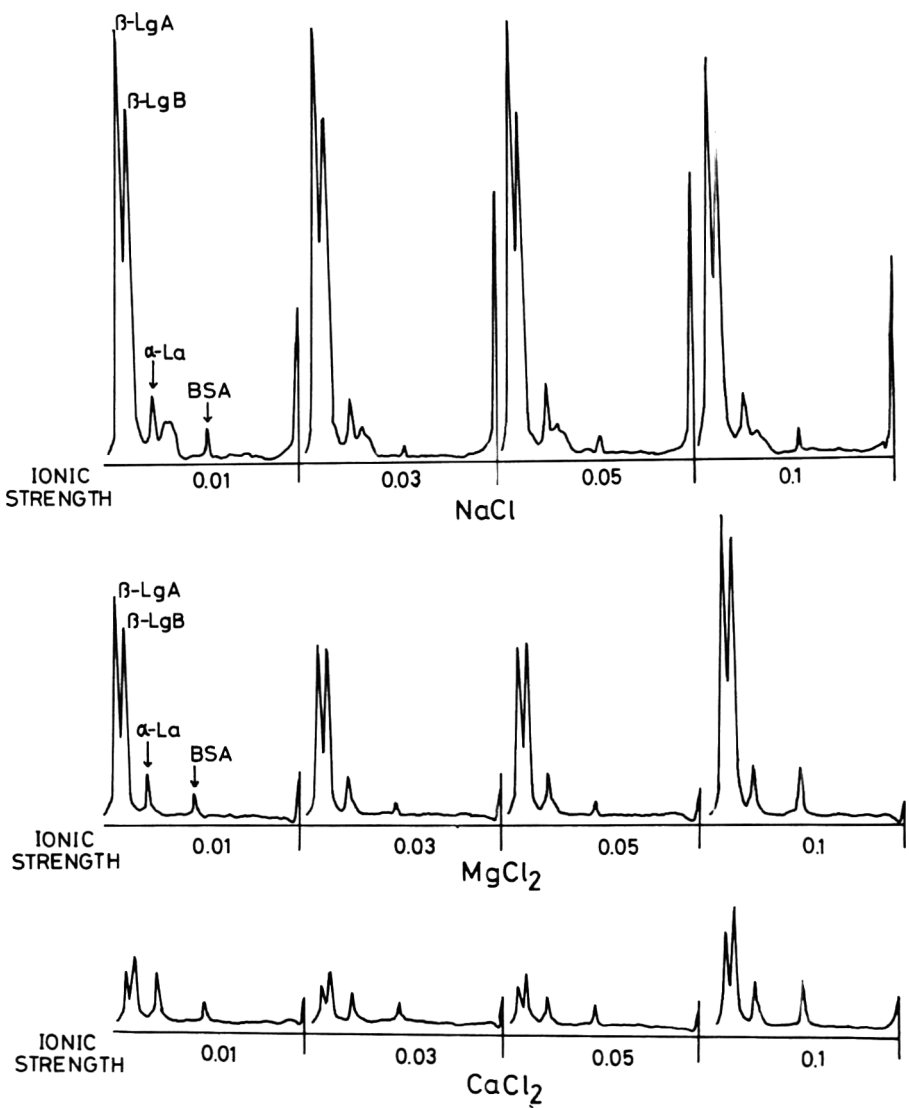


Fig. 7—Electrophoretic patterns of WPC (pH 6.5) heated at 75°C for 5 min in the presence of various salts under various ionic strengths. All WPC solutions were centrifuged at 1,500 x g for 15 min after heating and the supernatant solutions obtained were used for electophoresis. See Fig. 2 for definitions of protein band abbreviations.

Fig. 8—Densiometric patterns of Fig. 7.



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Heat-Induced Changes in the Proteins of Whey Protein Concentrate

EUNICE LI-CHAN

ABSTRACT

Three-level fractional factorial experiments were used to study effects of heating conditions (pH, time, temperature, solids content, calcium addition) on whey protein concentrate. Increasing pH and temperature led to lower solubility at pH 4.6 and 7.0, lower sulfhydryl content, higher hydroxymethylfurfural, generally darker color, lower DNBS-available lysine and altered pepsin pancreatin digestion profiles. Mercaptoethanol and SDS demonstrated relative importance of disulfide and hydrophobic bonds on solubility loss. Polyacrylamide gel electrophoresis indicated heat stability of proteose peptones; susceptibility was greatest at pH 8.0, 95°C for β-lactoglobulin and α-lactalbumin, and pH 4.6, 95°C for bovine serum albumin. HPLC gel filtration showed that heating rendered a high molecular weight fraction undissociable by mercaptoethanol.

INTRODUCTION

INCREASING PRESSURE to utilize whey, a by-product from cheese manufacture, has recently led to development of processes such as ultrafiltration for preparation of undenatured whey protein concentrate (WPC). Although generally recognized to be of high nutritional quality and of potential value for use in bakery products, infant formulae and animal feeds, and as supplements to poor quality proteins (Robinson and Tamine, 1978), the use and nutritional value of WPC may be impaired by its susceptibility to heat (Hidalgo and Gamper, 1977; McDonough et al., 1974). Yet, controlled heating is required for some functional properties such as gelation (Morr, 1979), and milk solids used for breadmaking must be sufficiently heated to denature 80–85% of the whey proteins to promote high absorption and prevent dough slackening and volume depression (Guy, 1978). Detailed studies are urgently needed to investigate and control effects of processing such as heating on WPC (Morr, 1979).

The objective of this work is to study the significance of heating conditions on various properties of WPC, including solubility at pH 4.6 and 7.0, sulfhydryl versus disulfide content, individual protein components (by electrophoresis and HPLC gel filtration), amino acid composition, in vitro enzyme digestibility, color (by diffuse reflectance spectra), hydroxymethylfurfural and "free" lactose contents. Knowledge of the heat-induced changes in WPC will facilitate the control of heat processing for achieving specific functional and nutritional properties.

MATERIALS & METHODS

Materials

Whey protein concentrate (WPC) prepared by ultrafiltration to contain 35% protein was a gift from Sodispro Technologie Ltee., St. Hyacinthe, PQ. Standard proteins were obtained from the following sources: bovine immunoglobulin G, crystallized β-lactoglobulin (A and B, from milk), α-lactalbumin (Grade II from bovine milk), bovine albumin (fraction V, 96–99% albumin), and ovalbumin (chicken egg albumin, Grade V, 99%) were all from Sigma Chemical Co.; myoglobin (from horse skeletal muscle, 1x crystallized) was from Calbiochem; and cytochrome C, ferritin and catalase were from a "Combithek, Calibration Proteins II" kit from Boehringer, Mannheim GmbH (Mannheim, W. Germany).

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Heat treatment

A three-level fractional factorial experimental design [$L_{27}(3^{13})$ design of Taguchi, 1957] was used to study the importance of five variables and some of the possible interactions between them during heat treatment of WPC. The five variables and the three levels assigned to each of them were as follows: pH (4.6, 6.0 and 8.0); time (15, 30 and 45 min); temperature (60, 73 and 95°C); solids content (6, 20 and 40% total solids) and calcium addition (0, 0.015 and 0.030M $CaCl_2$).

Protein solubility at pH 4.6 and pH 7.0

Protein solubility at pH 4.6 has been used as an index of the extent of whey protein denaturation by several workers (e.g. McDonough et al., 1974; Guy et al., 1967). The following modified procedure was used in this study. The WPC sample was brought to 6% total solids content (generally 0.3g in 5 ml), pH was adjusted to 4.6 using 10% acetic acid, then the suspension was centrifuged at 27,000 x *g* for 1 hr. The supernatant was analyzed for protein content using the biuret-phenol method (Brewer et al., 1974) or for nitrogen content on a Technicon Auto Analyzer II system after prior digestion of samples by the micro-Kjeldahl digestion method of Concon and Soltess (1973). Protein content was calculated from nitrogen content using a conversion factor of 6.38. In this work, the pH 4.6 solubility has generally been expressed as a percentage of the total protein content of the sample in dispersion before centrifugation.

To determine the role of hydrophobic forces and sulfhydryl-disulfide reactions on the pH 4.6 solubility, samples were also incubated with 1% SDS (sodium dodecylsulfate) and/or 1% ME (mercaptoethanol) in pH 8.3 10mM Tris glycine buffer for 30 min, prior to pH adjustment to 4.6 for the solubility determination.

Protein solubility at pH 7.0 was also measured to give an indication of solubility of heated WPC for neutral pH food applications. The procedure was essentially similar to that described above for pH 4.6 solubility, except that samples were in pH 7.0 0.05M sodium phosphate buffer.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed in a slab-type vertical gel system using an ATTO SJ 1060 SDH Electrophoresis Unit (Atto Co., Tokyo, Japan), comprised of SJ 1060 SG unit for preparation of 2 mm thick gels, SJ 1060 SD buffer reservoir and running tank, and SJ 1060 SH drying apparatus.

Running gel, concentration gel, marker dye, staining and destaining solutions were prepared essentially according to Suzuki (1977), with some modifications. 9% polyacrylamide gel was used for the running or separation gel (9 cm in length), while 3.75% gel was used for the concentration or spacer gel, containing 8 or 12 sample slots formed by using the Teflon slot-forming combs provided with the SJ 1060 SG unit. Marker dye (0.1% Bromophenol blue in 20% glycerine-0.05M Tris HCl pH 6.8 buffer) was applied to each sample slot, followed by careful layering first of protein samples [0.4% in 15% (v/v) glycerine, pH 7], then of buffer. The buffer consisted of Tris (0.05M)-glycine (0.38M) at pH 8.3. Gels were placed in the SJ 1060 SD running unit attached to an EICO 1030 Regulated Power Supply and electrophoresis was carried out at 125 volts for the first 45 min, then at 95 volts for the remainder of the run (generally 3.5 hr). After removal from the supporting frame, the running gel was stained for 50 min in Amido Black 10B solution (0.025% (w/v) in water:methanol:acetic acid, 4:5:1 by volume), then destained by several changes of water:methanol:acetic acid

solution (9.7:3.6:1 by volume). The protein bands of the unheated WPC control and of a whey control on the electrophoretograms were identified by comparing their mobility to those of protein standards (β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulin G) run on the same gel. An unheated WPC control was always included on each gel for comparison with the heated samples. Densitometric scanning of the gels was carried out on a Kontes Fibre Optic Scanner (Kontes, Vineland, NJ) attached to an Integraph Automatic Integrator Model 49 (Photovolt Corp., NY) and Varicord Model 42B Variable Response Recorder. After scanning, in order to obtain dried gels for easier storage, gels were soaked in 3% (w/v) glycerine for 3 min, then dried under vacuum for 50 to 60 min in a 50°C water bath, using the SJ 1060 SH drying apparatus.

High performance liquid chromatography (HPLC)

HPLC gel filtration was carried out on two Bio-Sil TSK 250 columns (300 x 7.5mm, from Bio Rad Laboratories), connected in series, using a Spectra physics SP 8700 solvent delivery system, SP 8400 uv/vis detector and SP 4100 computing integrator. Samples were 0.5% protein (w/v) in 0.05M sodium phosphate (pH 6) buffer containing 0.025% SDS and 0.02% azide, and were filtered through 0.45 μm millipore filters before injection. In some cases, 0.5% mercaptoethanol was included in the buffer system. Generally, 15–30μl of protein solution was injected. The eluent contained the same buffer used for sample preparation, and was run at a flow rate of 0.8 ml/min. The detector was set at either 225 nm or 420 nm. A calibration plot of log molecular weight versus retention time was prepared using the following standard proteins: immunoglobulin G (mol. wt. 160,000), bovine serum albumin (mol. wt. 69,000), ovalbumin (mol. wt. 45,000), β-lactoglobulin (mol. wt. of dimer 36,000), α-lactalbumin (mol. wt. of 42,450 and 14,150 for trimer and monomer, respectively), myoglobin (mol. wt. 17,000) and cytochrome C (mol. wt. 12,380).

Diffuse reflectance spectra

Diffuse reflectance spectra of powdered samples were recorded on a Unicam SP 800 spectrophotometer equipped with a Unicam SP 890 Diffuse Reflectance Accessory. Freshly prepared magnesium oxide powder was used as the reference surface (100% reflectivity). The tristimulus values, chromaticity coordinates and percent Unreal Primary Y (i.e. % lightness were calculated from the absorbance data by conversion to reflectance, then calculated by the weighted ordinate method using an Amdahl 470 V/8 computer.

Amino acid analysis

After first blocking cysteine residues by reaction with 4-vinylpyridine, as described by Cavins et al. (1972), samples were hydrolyzed for amino acid analysis with *p*-toluenesulfonic acid in the presence of 3-(2-aminoethyl) indole for 24 hr at 110°C according to Liu and Chang (1971). Amino acids were analyzed on a single column system attached to a Phoenix model M6800 amino acid analyzer.

Amino acid analysis was also carried out after an in vitro enzymatic digestion with pepsin and pancreatin according to the method of Stahmann and Woldegiorgis (1975), with modifications as described by Holguin and Nakai (1980).

Chemical analyses

Lactose content. The content of lactose in WPC samples was determined spectrophotometrically by reaction with methylamine after prior removal of protein and fat by precipitation with zinc acetate-phosphotungstic acid solution (Nickerson et al., 1976).

Sulfhydryl and disulfide content. Sulfhydryl (SH) and disulfide (SS) groups were determined using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid), according to Beveridge et al. (1974), with some modifications.

For SH group determination, 0.010g WPC samples were solubilized in 5 ml 0.086M Tris-0.09M glycine-0.004M EDTA (pH 8) buffer containing 8M urea. To a 3 ml aliquot of the sample solution in a cuvette was added 0.03 ml of Ellman's reagent solution (4 mg/ml). The absorbance at 412 nm (A_{412}) was read at 0, 1, 2, 3, 4, 5 and 6 min after addition and rapid inversion mixing of reagent with sample. Color development was complete by 3 min and remained stable for at least 1 hr. Sample and reagent blanks were included for each determination.

For SS group determination, 0.010g WPC samples were solubilized in 5 ml Tris-glycine-EDTA (pH 8) buffer containing 10M urea and 2% 2-mercaptoethanol. After incubation for 1 hr at room temperature, 5 ml 24% trichloroacetic acid (TCA) solution was added. After an additional hour incubation, the samples were centrifuged at 5000 x *g* for 10 min. The pellets were twice resuspended in 5 ml of 12% TCA solution and centrifuged at 5000 x *g* for 10 min to remove 2-mercaptoethanol. The pellets were then dissolved in 10 ml of Tris-glycine-EDTA buffer with 8M urea. To a 3 ml aliquot of this solution in a cuvette was added 0.05 ml Ellman's reagent. After rapid inversion mixing, A_{412} was read. This procedure gives the total content of SH and SS groups (after reduction of SS to SH groups with mercaptoethanol).

The SH and SH + SS content may be calculated using the following equation: $\mu M\ SH/g = (73.53\ A_{412}\ D)/C$ where A_{412} is the net absorbance at 412 nm after correcting for reagent and sample blanks; C is the sample concentration (mg solids/ml); D is the dilution factor, if any; and 73.53 is $10^6/1.36 \times 10^4$ (1.36×10^4 being the molar absorptivity of Ellman's reagent and 10^6 the conversion from mole/mg to μM/g).

Hydroxymethylfurfural content

For detecting early symptoms of the browning reaction in milk products, a modified version of the Keeney Bassette method B (Keeney and Bassette, 1959) was developed to measure hydroxymethylfurfural (HMF) content. Precautions during the digestion step suggested by Della Monica et al. (1967) and Craig et al. (1961) were taken into account to ensure complete condensation of water vapor. WPC samples (0.5g/5 ml H_2O) were digested with freshly prepared oxalic acid in test tubes covered with marbles, which were set in a boiling water bath. The lower parts of the tubes and the pot were shielded by a piece of foil while the upper parts were cooled using a fan. After TCA precipitation of the digest and reaction with thiobarbituric acid, A_{443} was read against a reagent blank. The procedure yields "total" HMF" content (free HMF and potential HMF from browning intermediates) using the following equation:

$$\text{Total HMF } (\mu\text{mole HMF/100g WPC powder}) = (A_{443} - 0.055)\ 87.5$$

Available lysine content

Free ε-amino groups of WPC samples were measured by the dinitrobenzenesulfonate (DNBS) spectrophotometric method of Concon (1975), as modified by Holguin and Nakai (1980). These values were reported as "DNBS-available" lysine content (g/100g protein). In order to remove lactose which may interfere with the DNBS reaction, WPC samples were first dialyzed exhaustively against water. Aliquots of dialyzed solution were taken for DNBS reaction and nitrogen determination.

Statistical analysis

Analysis of variance of the data on the 27 heated samples was carried out on a Monroe 1880 programmable calculator. For factors or interactions found to be significant at the 1 or 5% levels, the average values of the parameters at each factor level were calculated. 95% confidence limits were calculated for these as:

$$\pm \frac{t_{0.05} \text{ at } df_e}{\sqrt{\text{no. data averaged}}} \quad \frac{\sqrt{S_e}}{df_e},$$

where df_e is the degrees of freedom for error and S_e is the sum of squares for error, and are reported with the average values.

Solubility at pH 4.6

Since whey proteins are generally recognized as being the nitrogenous fraction remaining soluble in the supernatant at pH 4.6 after precipitation of casein, the loss of solubility at this pH is commonly used to assess their extent of denaturation. This criterion has also been used in this work. However, although denaturation generally precedes aggregation and loss of solubility, it is important to realize that these are distinct and separate processes. Approximately 90% of the protein remained soluble in the pH 4.6 supernatant of the unheated WPC control used in this work.

Analysis of variance of the solubility data showed that pH and temperature of heating, and the interaction between these two factors were significant ($P < 0.01$) in affecting pH 4.6 solubility of heated WPC samples.

Heating at higher temperatures resulted in progressively lower pH 4.6 soluble protein content. Heating at pH 8.0 resulted in greater loss of pH 4.6 solubility, compared to

heating at either pH 4.6 or 6.0 (Table 1). Generally, as temperature increases, hydrogen bonds and electrostatic interactions are weakened. Hydrophobic interactions, on the other hand, are strengthened with increases in temperature up to 60–70°C, then are gradually weakened with further temperature increase. Sulfhydryl and sulfhydryl-disulfide interchange reactions as well as molecular collision frequency due to increased kinetic energy are enhanced at higher temperatures. Thus, in general, the extents of both denaturation and aggregation of protein molecules with probable insolubilization would be expected to increase with higher temperature. The pH of the samples during heating would also affect ease of unfolding as well as possibility of intermolecular interactions. Sulfhydryl reactivity is dependent on pH. The pK of the functional thiol group being at approximately pH 8, sulfhydryl reactions and sulfhydryl-disulfide interactions would be promoted at pH 8 rather than at pH 6.0 or 4.6. The breaking of intramolecular disulfide bonds would encourage denaturation while the formation of new intermolecular disulfide bonds would favor aggregation and insolubilization.

The effects of pH and temperature were interdependent (Table 1). At a temperature of 73°C, heating samples at pH 8.0 resulted in much greater loss of pH 4.6 solubility than heating at either pH 4.6 or 6.0. Yet at 95°C, the pH 4.6 solubility was not significantly different ($P > 0.05$) between samples heated at the different pH levels. Samples heated at 60°C, pH 6.0 had higher pH 4.6 solubility compared to those heated at pH 4.6, 60°C, as well as to the unheated control. It is possible that by heating at 60°C, pH 6.0, partial unfolding through the breakdown of some intramolecular noncovalent bonds enabled the molecules to assume, upon cooling, a conformation which is more soluble at pH 4.6. Solubilization of protein aggregates, which may have resulted from the ultrafiltration procedure for WPC preparation, may also have been encouraged by these heating conditions, by weakening of intermolecular bonds. On the other hand, by heating at 73°C, pH 8.0, SH and SH-SS reactions would begin to predominate and the covalent bonds formed may have prevented the unfolded molecules from reverting to a soluble state even after cooling; formation of intermolecular disulfide bonds would increase aggregate size and promote insolubilization. Heating at 73°C, at pH 4.6 or 6.0, did not result in such insolubilization probably since SH, SH-SS reactions are less likely at pH below 8, except at very high temperatures, and the changes in noncovalent interactions by heating at 73°C would have been more readily reversible. At 95°C, the extent of changes in noncovalent as well as covalent linkages may have been so severe as to result in nonspecific, irreversible denaturation and aggregation, independently of pH effects.

Table 1—Effects of pH and temperature and the interactions between them on the pH 4.6 and pH 7.0 solubility of heated WPC

Heating conditions	Average pH 4.6 Sol. protein, %[a]	Average pH 7.0 Sol. protein, %[a]
Unheated control	90	100
Temp. 60°C	96 ± 4	91 ± 6
73°C	74 ± 4	71 ± 6
95°C	34 ± 4	34 ± 6
pH 4.6	69 ± 4	69 ± 6
6.0	76 ± 4	71 ± 6
8.0	60 ± 4	56 ± 6
Temp.-pH 60°C pH 4.6	90 ± 6	91 ± 10
60°C pH 6.0	104 ± 6	95 ± 10
60°C pH 8.0	96 ± 6	88 ± 10
73°C pH 4.6	78 ± 6	74 ± 10
73°C pH 6.0	91 ± 6	88 ± 10
73°C pH 8.0	53 ± 6	49 ± 10
95°C pH 4.6	39 ± 6	41 ± 10
95°C pH 6.0	34 ± 6	30 ± 10
95°C pH 8.0	30 ± 6	30 ± 10

[a] "±" values refer to 95% confidence limits

According to Lyster (1979), at temperatures below about 70°C, conformational changes induced in α-lactalbumin and β-lactoglobulin molecules are largely reversible on cooling, whereas at higher temperatures, the denaturation is less easily reversible. The kinetics of irreversible denaturation of β-lactoglobulin in milk shows a rather sharp change in activation energy at about 90°C.

Protein solubility at pH 4.6 was also determined in the presence of 1% SDS and/or 1% ME, to determine the role of hydrophobic forces and disulfide bonds on the loss of pH 4.6 solubility in heated samples. Solubility in the presence of urea or guanidine hydrochloride to determine the role of hydrogen bonding and hydrophobic forces was not attempted since complex, irreversible unfolding and aggregation reactions involving the SH group of β-lactoglobulin have been reported in the presence of these reagents (McKenzie, 1971b).

The percentage of protein soluble at pH 4.6 was greatly affected by the presence of SDS and ME even for the unheated WPC control. Only 30, 50 and 82% of the whey proteins remained in the pH 4.6 supernatant when determined in the presence of 1% SDS, 1%SDS + 1%ME, and 1%ME, respectively, compared to 90% solubility for the pH 4.6 supernatant in the absence of these reagents (see Tables 1 and 2).

The major protein fractions in whey, β-lactoglobulin, α-lactalbumin, and the immunoglobulins, have isoelectric points above pH 5 (Brunner, 1976). At pH 4.6, these proteins would usually bear net positive charge, which may serve to discourage extensive protein-protein interactions. Upon binding of SDS molecules to the proteins, an increase in negative charge would result, which could counter the positive charge on the proteins. The resulting reduction in electrostatic repulsion between protein molecules may have

Table 2—Effects of heating conditions on the pH 4.6 solubility of heated WPC determined in the presence of SDS and/or ME

Heating conditions	Average % pH 4.6 sol. in presence of[a] ME	SDS/ME	SDS
Unheated control	82	50	30
Temp. 60°C	87 ± 4	57 ± 5	34 ± 1
73°C	72 ± 4	50 ± 5	32 ± 1
95°C	38 ± 4	45 ± 5	31 ± 1
pH 4.6	72 ± 4	40 ± 5	30 ± 1
6.0	70 ± 4	54 ± 5	33 ± 1
8.0	55 ± 4	58 ± 5	33 ± 1
Temp.-pH 60°C pH 4.6	96 ± 7	NS	30 ± 1
60°C pH 6.0	88 ± 7		35 ± 1
60°C pH 8.0	78 ± 7		37 ± 1
73°C pH 4.6	78 ± 7		30 ± 1
73°C pH 6.0	83 ± 7		33 ± 1
73°C pH 8.0	55 ± 7		33 ± 1
95°C pH 4.6	43 ± 7		31 ± 1
95°C pH 6.0	37 ± 7		32 ± 1
95°C pH 8.0	33 ± 7		29 ± 1
% total solids 6	NS	41 ± 5	28 ± 1
20		47 ± 5	32 ± 1
40		64 ± 5	37 ± 1
$CaCl_2$ added 0	NS	65 ± 5	35 ± 1
0.015M		50 ± 5	33 ± 1
0.030M		37 ± 5	28 ± 1

[a] "±" values refer to 95% confidence limits; "NS" indicates nonsignificant ($P>0.05$) factor or interaction. ME/SDS concentrations used were 1%.

encouraged interactions leading to aggregation and decrease in solubility. The disruption of intramolecular hydrophobic interactions by SDS resulting in a more unfolded molecular configuration, as well as possible formation of aggregates strengthened by intermolecular hydrophobic interactions linked by SDS may have contributed to the loss of solubility. The tendency for SDS to precipitate out in the acid form at low pH may also have resulted in low protein solubility due to coprecipitation of the acid and protein.

Precipitation of proteins by anionic detergents such as SDS has long been documented, and depends on the detergent/protein weight ratio (Putnam, 1948). Three regions can be classified according to the weight ratio-protein excess, equivalence zone, and detergent excess, corresponding to incomplete precipitation, complete precipitation and dissolution of precipitates, respectively. According to Elkes et al. (1945), protein precipitation occurs only when the protein/detergent ratio lies between 2.5/1 and 5/1, and not above or below this range. In the present study, the level of 1% SDS was chosen for a protein/detergent weight ratio of 2/1, which should allow dissolution of precipitates due to detergent excess. However, the results indicate that some precipitation still occurred.

The addition of the reducing agent ME serves to break disulfide bonds. In the case where the disulfide bond is intermolecular, the decrease in molecular size upon reduction of the bond would generally promote an increase in solubility. However, in the case where the disulfide bond is an intramolecular one stabilizing the protein's globular configuration, the reduction of the bond may result in unfolding and encourage protein-protein interactions (e.g. by hydrophobic interactions of newly exposed areas), leading to a decrease in solubility. The latter process appears to be the major one in this case since pH 4.6 solubility of the WPC was slightly lower in the presence of ME than in its absence.

Heat-treated WPC showed a wide range of pH 4.6 solubility determined in the presence of SDS and/or ME. In some cases, heated samples had greater % solubility than their corresponding unheated controls. The % protein solubility of the 27 heated samples ranged from 27–110% in the absence of SDS and ME, from 22–45% in the presence of SDS, from 32–97% in the presence of ME, and from 24–91% in the presence of SDS and ME.

Table 2 shows the effects of the various heating condition parameters on pH 4.6 protein solubility when determined in the presence of SDS and /or ME. Analysis of variance of the data showed that pH 4.6 solubility of heated samples determined in the presence of 1% SDS was significantly affected by the levels of % total solids ($P < 0.01$), addition of $CaCl_2$ ($P < 0.01$), temperature ($P < 0.05$), pH ($P < 0.05$) and the interaction between temperature and pH ($P < 0.05$) during heating. In the presence of 1% SDS and 1%ME, pH ($P < 0.01$), % total solids ($P < 0.01$), addition of $CaCl_2$ ($P < 0.01$) and temperature ($P < 0.05$) were significant factors. In both cases, samples which had been heated at 60°C, at pH 6.0 or 8.0, or as dispersions containing high total solids, or in the absence of added $CaCl_2$, showed significantly higher pH 4.6 solubility compared to their unheated controls. The presence of ME alone during pH 4.6 solubility determinations did not appreciably change the solubility patterns from those determined in the absence of ME.

Interpretation of these results is confounded by the low pH 4.6 solubilities of the unheated controls in the presence of SDS or SDS/ME. However, the data do suggest that in the samples heated under the conditions mentioned above (60°C, pH 6.0 or 8.0, high total solids, no added calcium chloride) hydrophobic interactions are an important contributing factor in aggregation and loss of solubility. In these samples, much of the insolubilization is reversible by addition of SDS, especially in combination with ME, so that the resulting pH 4.6 solubility of these samples not only surpasses that of the unheated control in the presence of SDS/ME, but also approaches the average solubility (68%) of heated samples determined in the absence of these reagents. In 8 of the 27 heated samples, pH 4.6 solubilities determined with SDS/ME were higher than the corresponding solubilities in absence of SDS/ME. For example, WPC heated as a 40% dispersion at pH 8 for 30 min at 73°C had pH 4.6 solubility of 59% in the absence and 91% in the presence of SDS/ME; WPC heated as a 20% dispersion at pH 8 for 15 min at 95°C had pH 4.6 solubility of 27% in the absence and 68% in the presence of SDS/ME.

Solubility at pH 7.0

Although solubility at pH 4.6 is the common criterion of whey protein denaturation, knowledge of the solubility at pH 7.0 is important for extending the use of WPC in neutral pH food applications. Analysis of variance of the pH 7.0 solubilities of the 27 heated samples indicated that temperature ($P < 0.01$), pH ($P < 0.05$) and the interaction between these two factors ($P < 0.05$) were significant variables during heating. Table 1 shows that, in general, the trends for pH 7.0 solubility of heated samples were similar to those for pH 4.6 solubility.

Protein solubility at pH 7.0 was also determined in the presence of 1% SDS, 1% ME and 1% SDS + 1% ME. In contrast to the lower solubilities exhibited by the unheated WPC control in the presence of these reagents at pH 4.6, solubilities of the control remained in the 95–100% range in the presence of these reagents at pH 7.0. Solubilities of the heated samples were generally improved in the presence of SDS or of ME, but the most dramatic improvements were seen in the presence of both of these reagents. Whereas the average pH 7.0 solubility of heated samples was 65% in the absence of the reagents, this value improved to 70% in the presence of ME, to 74% in the presence of SDS, and to 84% in the presence of both SDS and ME.

Analysis of variance of the data on the heated samples indicated that temperature ($P < 0.01$) and % total solids ($P < 0.05$) were significant factors in influencing pH 7.0 solubility determined in the presence of both SDS and ME. In the presence of either SDS or ME alone, temperature ($P < 0.01$), % total solids ($P < 0.05$), the interaction between temperature and pH ($P < 0.05$) and pH ($P < 0.05$ for SDS alone, and $P < 0.01$ for ME alone) were significant factors influencing pH 7.0 solubility. Fig. 1 to 4 illustrate the effects of these factors on pH 7.0 solubility and show how the solubilities of the heated samples are influenced by SDS and/or ME.

These results indicate that the changes leading to loss of solubility were largely reversible by addition of SDS and ME, provided that the temperature of heating was 60 or 73°C. Samples heated at 95°C, however, still exhibited low solubility (Fig. 1). SDS generally improved solubility of all the heated samples, but more markedly so at pH 4.6 than 6.0 or 8.0 when heating was at 73 or 95°C (Fig. 2 and 3). On the other hand, ME was most effective for samples heated at pH 6.0 or 8.0, and had hardly any effect for samples heated at pH 4.6 (Fig. 2 and 3). Solubility of samples heated at 40% total solids content was more effectively regained by adding SDS/ME than samples heated at lower solids content (Fig. 4).

The inability to regain solubility in some samples may indicate importance of bonding forces other than hydrophobic forces and disulfide links. For example, the aggregation of β-lactoglobulin when heated at 90°C in the presence of calcium is believed to be initiated by an isoelectric aggregation; the dissolution of this type of precipitate can

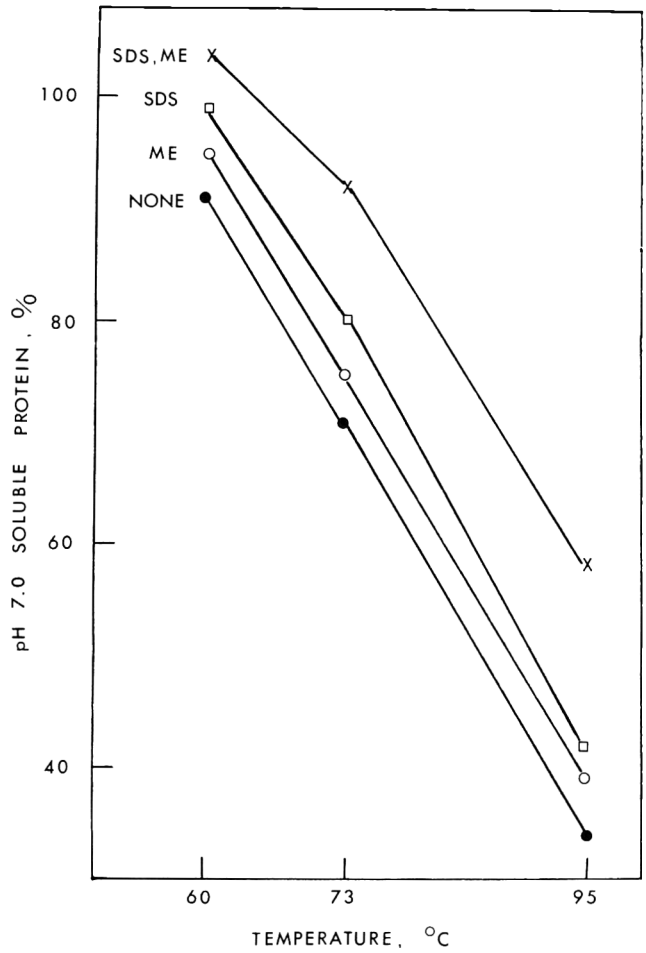

Fig. 1—Effect of temperature of heating on the pH 7.0 solubility of heated WPC, determined in the absence or presence of SDS and ME.

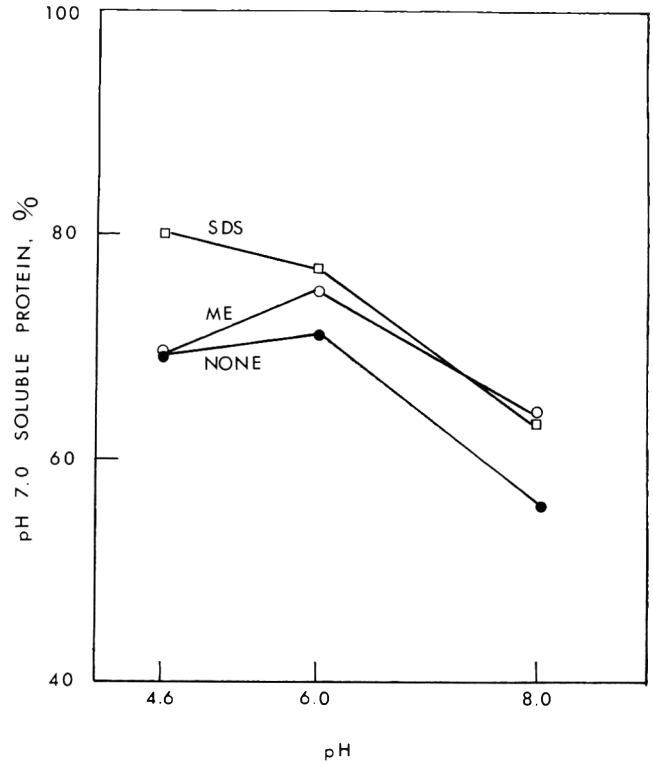

Fig. 2—Effect of pH during heating on the pH 7.0 solubility of heated WPC, determined in the absence or presence of SDS and ME.

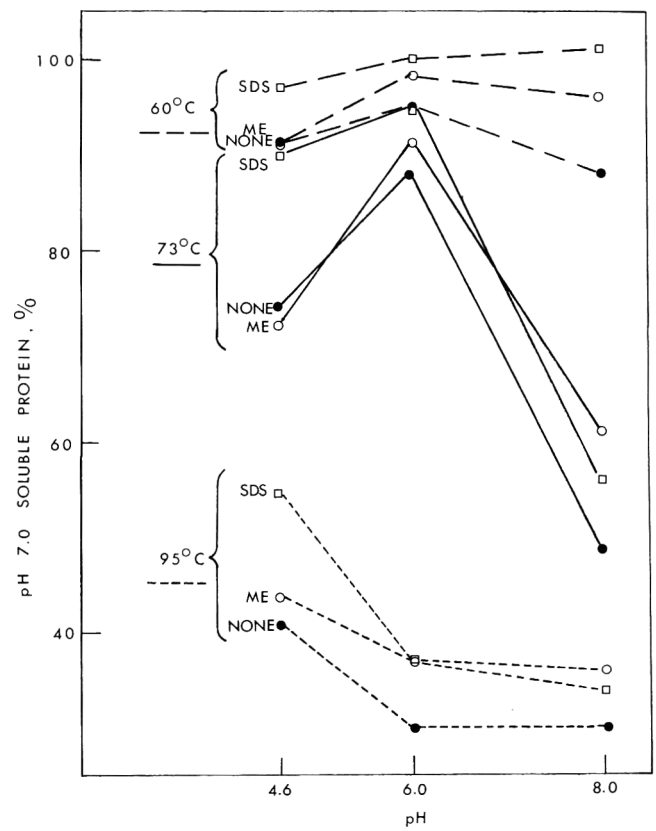

Fig. 3—Effect of interaction between pH and temperature of heating on the pH 7.0 solubility of heated WPC, determined in the absence or presence of SDS and ME.

be accomplished with urea only if a reducing agent like ME is added, suggesting that disulfide crosslinks as well as hydrogen bonds are formed after initiation by electrostatic forces (Zittle et al., 1957). The possibility of intermolecular covalent crosslinkages (e.g. isopeptide bonds) also cannot be excluded.

SH, SH + SS contents

The content of SH groups in heated samples was determined using Ellman's reagent in the presence of urea to expose "buried" groups. Analysis of variance indicated that pH ($P < 0.01$), temperature ($P < 0.01$) and time ($P < 0.05$) of heating significantly influenced SH content. As shown in Table 3, SH content was lower for samples heated at higher pH, for longer time or at higher temperature.

The total content of SH and SS groups was determined by using Ellman's reagent in the presence of urea and ME. Since SH + SS contents were not significantly different among heated samples or between heated samples and unheated control, it can be concluded that the decreases in SH content shown in Table 3 were attributable to corresponding increases in SS content in those heated samples.

Linear regression analyses were carried out to determine whether the solubility of the heated samples could be described as a function of their SH content. The results are shown in Table 4. The solubility at pH 4.6, as well as solubility at pH 4.6 in the presence of ME, could be significantly ($P < 0.01$) described as linear relationships with the SH content of the heated samples. The Y-intercept values ("a" in Table 4) for these two cases indicate that about 20% of the protein remains soluble at pH 4.6 when the SH content of the heated WPC is zero. This result is in agreement with other findings, such as that reported by Larson

and Rolleri (1955) using moving boundary electrophoresis, that approximately 20% of the protein remains undenatured after 30 min at 95°C. It is believed that the proteose peptones are the major proteins in this fraction; these proteose peptones generally have low cysteine/cystine content (McKenzie, 1971a). Insignificant relationships ($P > 0.05$) were found between pH 4.6 solubility determined in the presence of SDS or SDS and ME, and SH content. This may be explained by the low solubility even of the control when SDS was present. Significant relationships ($P < 0.01$) were obtained between pH 7.0 solubility and SH content regardless of whether or not SDS and/or ME were present for the solubility determination.

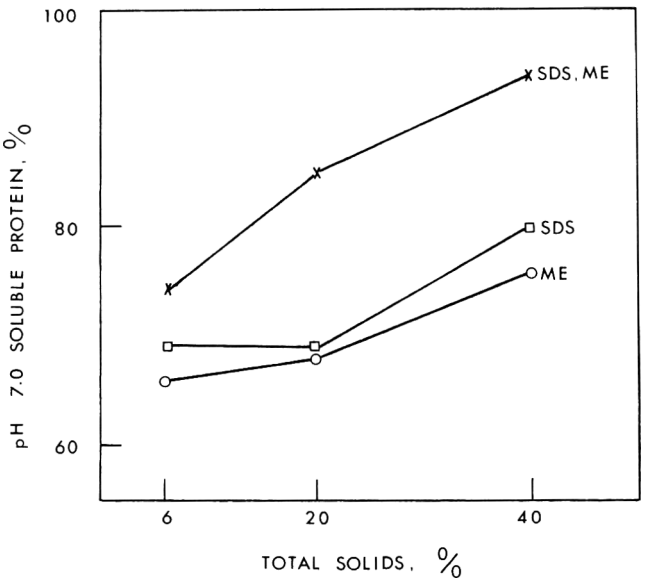

Fig. 4—Effect of % total solids during heating on the pH 7.0 solubility of heated WPC, determined in the presence of SDS and ME.

Table 3—Effects of pH, time and temperature of heating on the sulfhydryl group content of heated WPC

Heating conditions	Average μmoles SH/gram WPC (95% confidence limits = ±0.3)
Unheated control	7.0
pH 4.6	6.7
6.0	5.8
8.0	3.4
Time 15 min	5.7
30 min	5.1
45 min	4.7
Temperature 60°C	7.0
73°C	5.5
95°C	2.9

These results indicate that although disulfide bond formation was significantly affected by various parameters of heatng conditions, the loss of solubility in the samples could not be completely explained in terms of SS crosslinks. Since the solubility determined in the presence of ME to reduce SS crosslinks could still be described as a function of the SH content of the samples, it may be inferred that other heat-induced reactions are predominating to influence solubility. Although these other reactions may depend on or alternatively influence the SH-SS reactions, once they have occurred, the changes leading to loss of solubility may occur independently of the SH-SS balance and thus are irreversible even upon addition of ME. The slope ("b" value) of the solubility-SH relationship is only slightly affected by the presence of SDS and/or ME. However, the intercept ("a" value) is increased to 44.93 for pH 7.0 solubility determined in the presence of SDS and ME, inferring that some of the loss of solubility is reversible by reagents capable of breaking hydrophobic and disulfide bonds.

The results obtained here with WPC closely resemble observations noted previously on β-lactoglobulin by others. The relevance of disulfide bond formation on aggregation of β-lactoglobulin has been investigated by many workers (e.g. Sawyer, 1968; Briggs and Hull, 1945). It is generally agreed that both SH dependent and independent types of aggregation occur, and that even within the SH dependent type, the aggregates themselves are not formed necessarily by SS linkage, but may require a specific conformation which is dependent on the SH group reactivity.

Polyacrylamide gel electrophoresis

pH 4.6 and 7.0 supernatants of the 27 heated samples were subjected to PAGE after neutralization to pH 7. Calculation of peak areas by integration from densitometric scans of PAGE gels was carried out, and the relative proportion of each of the protein components remaining in the supernatant of heated samples was calculated by comparing the relative density of the component in the heated sample with that in the unheated control, a control being run with each gel. A typical densitometric scan is shown in Fig. 5.

Analysis of variance indicated that the factors pH, temperature, and $CaCl_2$ addition, and the interaction between pH and temperature were significant ($P < 0.01$) in

Table 4—Results of linear regression analyses of the relationships between pH 4.6 or pH 7.0 solubility and SH content of heated WPC samples[a]

Y	a	b	$s_{y.x}$	r
pH 4.6 solubility	16.65	10.46	17.68	0.80 (sig, P<0.01)
pH 4.6 solubility in presence of ME	20.80	8.49	12.26	0.84 (sig, P<0.01)
pH 4.6 solubility in presence of SDS	0.56	29.13	5.13	0.24 (not sig, P>0.05)
pH 4.6 solubility in presence of SDS and ME	0.11	50.03	18.79	0.01 (not sig, P>0.05)
pH 7.0 solubility	13.69	9.73	15.36	0.82 (sig, P<0.01)
pH 7.0 solubility in presence of ME	24.31	8.57	15.92	0.77 (sig, P<0.01)
pH 7.0 solubility in presence of SDS	17.30	10.63	13.87	0.87 (sig, P<0.01)
pH 7.0 solubility in presence of SDS and ME	44.93	7.44	15.71	0.73 (sig, P<0.01)

[a] Y = a + bX, where Y = solubility determined under conditions specified in the table; X = SH content of heated samples.

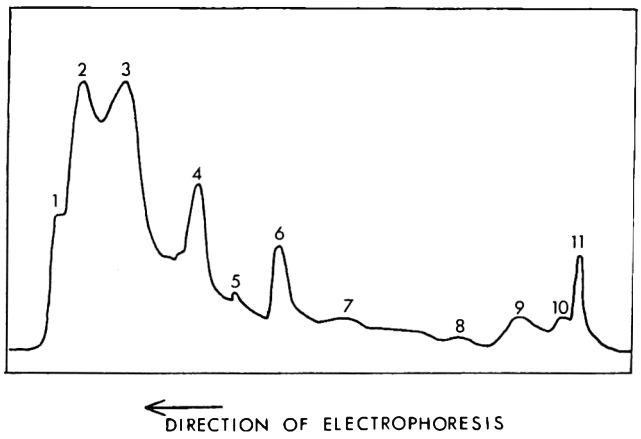

Fig. 5—Densitometric scan of PAGE electrophoretogram of WPC (unheated control). (1, 2, 3 = β-lactoglobulin; 4 = α-lactalbumin; 5, 7, 8 = proteose peptones; 6 = bovine serum albumin; 9, 10, 11 = immunoglobulins).

affecting the content of β-lactoglobulin remaining in the supernatant. pH ($P < 0.05$) and temperature ($P < 0.01$) were significant in affecting α-lactalbumin and bovine serum albumin contents remaining in the supernatant. Only temperature ($P < 0.01$) was significant in influencing immunoglobulin content. No significant changes ($P > 0.05$) were noted in the proteose peptone contents in heated samples versus unheated control.

Table 5 shows how the contents of the various protein fractions were affected by the various heating conditions. The contents are expressed as a percentage of the content in the corresponding unheated control; only those factors which were significantly influential at the 5% or 1% level are indicated.

From the overall average content of the protein fractions in the 27 heated samples, the general order of increasing heat resistance of the components in WPC was as follows: immunoglobulins, bovine serum albumin, β-lactoglobulin, α-lactalbumin, and proteose peptones. These trends for thermal resistance are similar to those reported by Larson and Rolleri (1955) for whey protein denaturation in milk, but the extent of denaturation in the present work is lower, presumably due to differences between milk and WPC. Hillier and Lyster (1979) reported greater thermostability of α-lactalbumin and β-lactoglobulin in cheese whey than in skim milk, particularly at temperatures below 95°C.

The temperature of heating affected significantly all the protein components, with the exception of the proteose peptones. Greatest losses of protein occurred at 95°C. For β-lactoglobulin and α-lactalbumin, no loss was measured at 60°C.

The effects of pH varied depending on the individual whey proteins. Whereas the greatest loss of both β-lactoglobulin and α-lactalbumin occurred at pH 8, that of bovine serum albumin occurred at pH 4.6. The latter may be explained by the isoelectric point of bovine serum albumin at pH 4.7; the absence of any substantial net charge at this pH would facilitate intermolecular interactions and aggregation. β-Lactoglobulin and α-lactalbumin have isoelectric pH values above 5. In these two proteins, the dependence of heat instability on pH may be a result of increasing SH reactivity with increasing pH, or increased calcium ion mediated aggregation of negatively charged molecules. In all three proteins, minimum loss appeared at pH 6. This is in agreement with the findings of Nielsen et al. (1973), who found minimum whey protein denaturation between pH 6 and 7, but conflict with the conclusions of Hillier et al. (1979) and Guy et al. (1967) of minimum denaturation at lower pH values.

Table 5—Effects of heating conditions on proteins of WPC, determined by PAGE

	% of unheated control[a]			
	β-lg	α-la	BSA	Ig
Unheated control	"100"	"100"	"100"	"100"
pH 4.6	76 ± 4	64 ± 10	40 ± 10	NS
6.0	78 ± 4	86 ± 10	68 ± 10	
8.0	59 ± 4	49 ± 10	64 ± 10	
Temp. 60°C	99 ± 4	105 ± 10	81 ± 10	82 ± 10
73°C	82 ± 4	66 ± 10	66 ± 10	38 ± 10
95°C	29 ± 4	30 ± 10	24 ± 10	20 ± 10
$CaCl_2$ added 0	65 ± 4	NS	NS	NS
0.015M	69 ± 4			
0.030M	59 ± 4			
Temp.-pH 60°C pH 4.6	93 ± 8	NS	NS	NS
60°C pH 6.0	104 ± 8			
60°C pH 8.0	102 ± 8			
73°C pH 4.6	95 ± 8			
73°C pH 6.0	104 ± 8			
73°C pH 8.0	57 ± 8			
95°C pH 4.6	42 ± 8			
95°C pH 6.0	27 ± 8			
95°C pH 8.0	18 ± 8			
Overall average of heated samples	65	69	57	46

[a] "±" indicates the 95% confidence limits; NS = not significant at $P>0.05$. β-lg = β-lactoglobulin; α-la = α-lactalbumin; BSA = bovine serum albumin; Ig = immunoglobulins. The content of proteose peptones was not significantly altered ($P>0.05$) and is not included in the table.

The interaction between pH and temperature significantly affected the β-lactoglobulin content. No significant insolubilization of this protein occurred after heating at 60°C, regardless of pH. At 73°C, decrease in pH 4.6 soluble β-lactoglobulin occured only at pH 8 heating. At 95°C, significant decreases occurred at all pH levels but was greatest at pH 8.

β-Lactoglobulin content was also significantly lower when 0.03M $CaCl_2$ was included during heating, presumably due to a counterbalance of negative charges on the protein upon binding of the positively charged Ca^{++} ions, resulting in less hindrance against intermolecular aggregation. Townend and Gyuricsek (1974) reported that calcium caused excellent precipitation of β-lactoglobulin at pH values above 5.5–5.7, whereas Hidalgo and Gamper (1977) reported that 0.03M $CaCl_2$ increased the amount of protein precipitated from WPC over the whole pH range of 2–12.

HPLC gel filtration

Fig. 6 shows the HPLC gel filtration chromatograms of four separate runs on the standard proteins bovine serum albumin, α-lactalbumin and β-lactoglobulin in pH 6 phosphate buffer with 0.025% SDS and 0.02% sodium azide, and β-lactoglobulin in the above buffer plus 0.5% 2-mercaptoethanol.

Integration of peak areas and comparison of peak retention times with a calibration plot of log molecular weight versus retention time for standard proteins allowed characterization of these peaks for later identification of the proteins in the WPC system. For bovine serum albumin, 90% of the total area of the chromatogram could be assigned to a retention time of 24.5 min, corresponding to the monomer molecular weight (mol. wt.) of this protein, 69,000; 9% of the area was attributed to a peak at 20.8 min, corresponding to the dimer mol. wt. of 138,000. Immunoglobulin G, mol. wt. 160,000, eluted at approximately 20.3 min (not shown in figure). For α-lactalbumin, 96% of the area was assigned to a peak at 26.7 min, corresponding to a trimer form, mol.

wt. 42,450. For β-lactoglobulin, 97% of the peak area was assigned to a retention time of 28.6 min, for the dimer, mol. wt. 36,000, and 2% to a retention time of 26.5 min, probably from contaminating α-lactalbumin. Upon inclusion of 0.5% ME for sample preparation, a major peak with retention time of 39–40 min appeared, corresponding to the monomer of β-lactoglobulin, with mol. wt. 18,000.

Fig. 7 shows the elution pattern of the unheated WPC control, in the absence and presence of ME. Aside from the major peaks at approximately 26 and 28 min, corresponding to α-lactalbumin and β-lactoglobulin, respectively, peaks were also noted at retention times of 21 min (probably immunoglobulins and bovine serum albumin), 24 min and 34 min (probably proteose peptones). In addition, a fairly large peak was noted at retention time of 14.5 min, which corresponds to mol. wt. greater than 200,000. This probably consists of a heterogeneous polymer formed by intermolecular bonds between the different whey proteins. Upon addition of ME, the most notable change was the appearance of a large peak at 39–40 min, which corresponds mainly to the monomers of β-lactoglobulin and α-lactalbumin. It is also notable that the % area ascribed to the peak at 14.5 min was decreased from 15% to about 5% of the total area upon ME inclusion, and that the baseline absorbance between 15 and 20 min was low, compared to a high absorbance when no ME was present. These results indicate that a large proportion of the high molecular weight aggregates (corresponding to the 14.5 min peak and the area of tailing from 15–20 min) are held by disulfide bonds; the size of these aggregates can be decreased by adding ME.

Fig. 8 shows a typical elution pattern of a heated sample. The major change upon heating of this sample was a reduction in the relative areas at 21, 26 and 28 min. In addition, an extra peak at 29.5 min and a broad shoulder from 30–35 min were observed, which may correspond to heterogeneous aggregates of β-lactoglobulin and α-lactalbumin. Upon inclusion of ME, the peak at 39–40 min appeared, as occurred in the case of the unheated control. In addition, the size of the peak at 29.5 min was decreased, indicating that this fraction is probably a mixed dimer or trimer held by disulfide bonds. However, unlike the unheated control, the size of the peak at 14.5 min was relatively unchanged by ME in the case of the heated sample. This implies that upon heating, the high mol. wt. aggregates are strengthened by additional intermolecular forces and no longer can be dissociated simply by reduction of SS bonds.

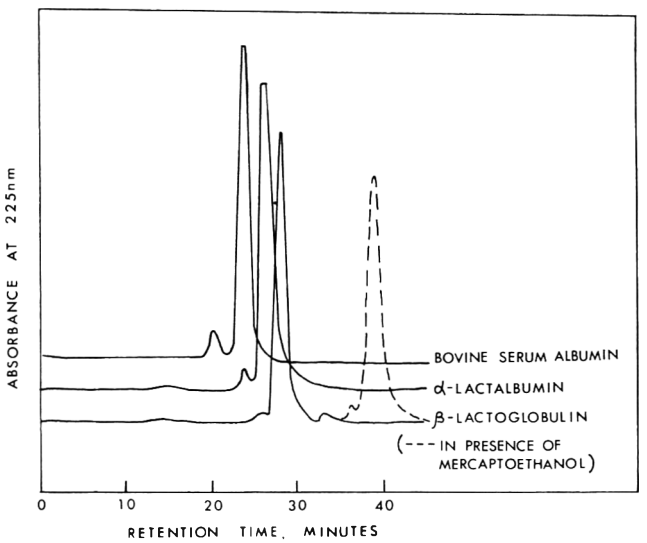

Fig. 6—HPLC elution patterns of protein standards on Bio Sil TSK columns (see text for conditions; the eluting buffer was 0.05M phosphate buffer (pH 6) with 0.025% SDS and 0.02% azide; 0.5% mercaptoethanol was also present for the β-lactoglobulin elution depicted with the dashed line).

Amino acid composition of "digestible" and "available" lysine contents

Amino acid analysis after acid hydrolysis of heated samples showed no significant changes in the amino acid composition after heating.

Samples were subjected to in vitro digestibility tests by successive incubation with the enzymes pepsin and pancreatin (pepsin pancreatin digestion or "PPD"). Analysis of the amino acid contents after precipitation of undigested protein and large peptides indicated some changes in the profile of essential amino acids released by PPD. Although the overall content of the essential amino acids released (μmole/ml) was not significantly affected ($P > 0.05$) by heating, the pattern of essential amino acids released was altered. In particular, the percentage of lysine released was significantly influenced ($P < 0.05$) by temperature at which

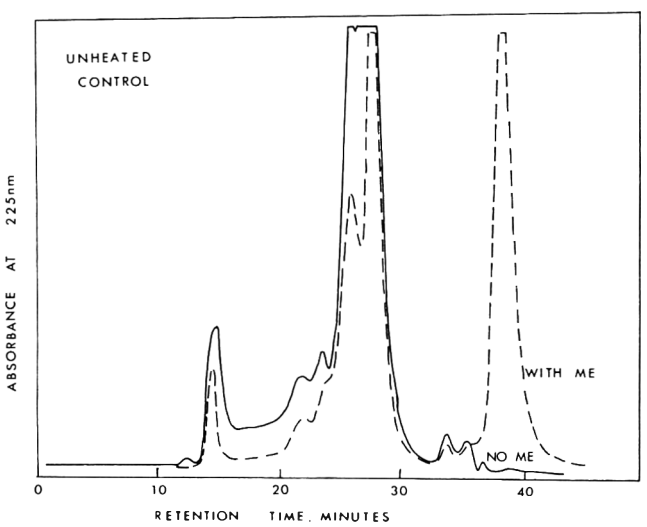

Fig. 7—HPLC elution patterns for unheated WPC control in 0.05M phosphate buffer in the absence (—) and presence (----) of 0.5% mercaptoethanol (ME).

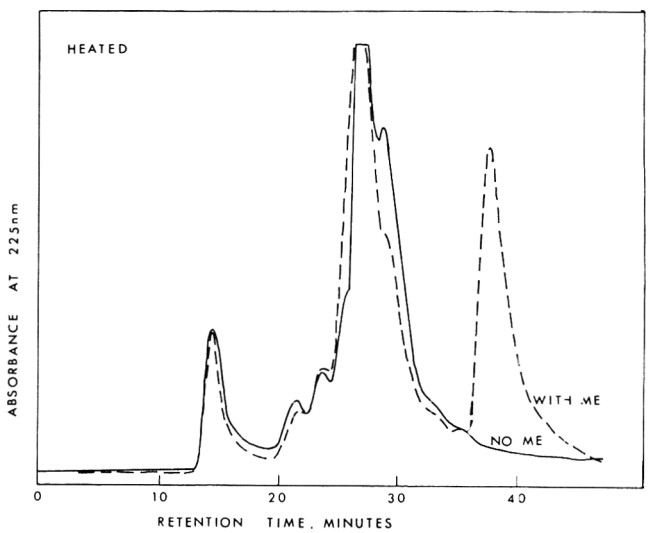

Fig. 8—HPLC elution patterns for heated WPC sample in 0.05M phosphate buffer (pH 6) in the absence (—) and presence (----) of 0.5% mercaptoethanol (ME). (Heating condition: pH 8.0, 6% total solids dispersion heated at 60°C for 45 minutes).

samples had been heated. Samples heated at 95°C showed on average about 15% lower PPD-lysine content than samples heated at 60°C. The PPD contents of valine, leucine, tyrosine + phenylalanine and histidine were significantly affected ($P < 0.05$) by pH at which samples had been heated. Samples heated at pH 6.0 had approximately 20% lower PPD-histidine than samples heated at pH 4.6 or 8.0; this may perhaps reflect a greater reactivity during heating of the histidine residues at a pH value close to the pK value of their imidazolium groups. No consistent trends appeared for the other residues, and their different extents of digestibility may be an indication of different extents of exposure to the proteases, perhaps due to conformational changes or intermolecular aggregate formation during heating.

Free epsilon amino group or "available" lysine content was determined by the dinitrobenzenesulfonate (DNBS) spectrophotometric method. Analysis of variance showed that the pH ($P < 0.01$) and temperature ($P < 0.05$) of heating were significant in the resulting content of available lysine in the heated samples. Samples heated at pH 8.0 possessed, on average, almost 30% lower available lysine content than samples heated at either pH 4.6 or 6.0, while samples heated at 95°C had, on average, about 15% lower available lysine content than those heated at 60°C.

The lysine residues of proteins are notorious for their reactivity upon heating. Possible reactions in which the epsilon amino group may participate include Maillard reaction with reducing sugars (such as lactose in WPC), crosslinking via lysinoalanine formation with dehydroalanine, or isopeptide formation with the amide groups of glutamine or asparagine residues. While the very early reactions in the Maillard reaction pathway are reversible, the later ones are irreversible. Isopeptides are cleaved by acid hydrolysis but not by hydrolysis with enzymes such as pepsin and pancreatin.

Since the overall composition of amino acids as determined by analysis of acid hydrolyzed samples was not significantly different from an unheated control, it may be assumed that the extent of irreversible Maillard reaction is relatively mild. The lower contents of both "digestible" and "available" lysine, and the relatively unchanged content of lysine determined after acid hydrolysis, suggest that some lysine ϵ-amino groups are bound, perhaps through isopeptide crosslinkage. These crosslinks may also have contributed to the observed decrease in solubility for heated samples.

Diffuse reflectance spectra

Diffuse reflectance spectra measurements were carried out on the finely ground powders of the 27 heated samples and the unheated control. Typical spectra are shown in Fig. 9. Heated samples generally showed somewhat lower absorbance values in the ultraviolet wavelength region than the unheated control. In the visible region, most heated samples showed higher absorbance than the control, particularly in the 400–500 nm region. These samples appeared to have a beige or tan color. However, several samples had a white color, and showed lower absorbance in the visible wavelength than the control. These samples had higher % Unreal Primary Y values (% lightness) than magnesium oxide and the unheated control, compared to lower % Unreal Primary Y values for samples which appeared beige or tan in color.

Samples were compared with respect to their absorbance at 420 nm relative to a reference baseline obtained with magnesium oxide ("ΔA_{420}"). The color of milk and milk products including whey solids can be monitored in this wavelength region, where the compounds formed during browning absorb light strongly (Tinkler et al., 1955).

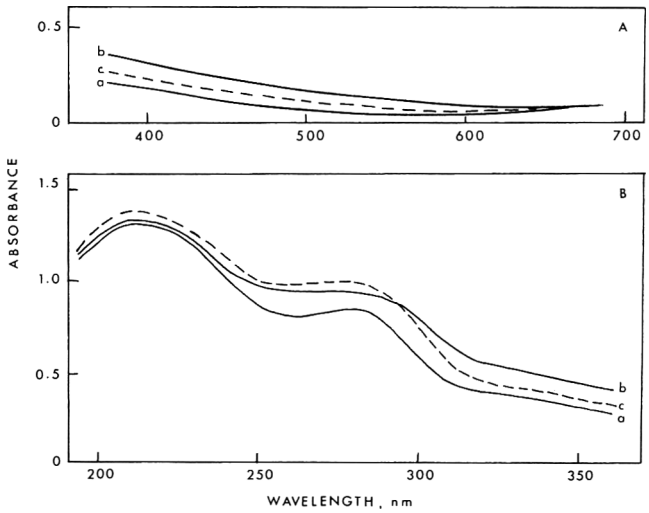

Fig. 9—Diffuse reflectance spectra in the visible (A) and ultraviolet (B) wavelength regions. (··· (c) = unheated control; — (a) or (b) = heated samples: (a) pH 4.6, 6% total solids dispersion with 0.015M $CaCl_2$ addition, heated at 95°C for 45 min; (b) pH 6.0, 6% total solids dispersion with 0.030M $CaCl_2$ addition, heated at 73°C for 45 min).

Table 6—Average ΔA_{420} values from diffuse reflectance spectra of heated WPC

Heating conditions	Average ΔA_{420} (95% confidence limits = ±0.01)
Unheated control	0.05
pH 4.6	0.07
6.0	0.09
8.0	0.11
Temp. 60°C	0.09
73°C	0.10
95°C	0.07

ΔA_{420} values ranged from 0.01–0.14 for heated powders, compared to 0.05 for the unheated control. Analysis of variance of the data indicated that pH ($P < 0.025$) and temperature ($P < 0.05$) of heating were significant factors in influencing ΔA_{420} of heated samples. Table 6 shows tht heating at pH 6.0 or 8.0 resulted in higher ΔA_{420} values than heating at pH 4.6. Heating at 60 or 73°C resulted in higher ΔA_{420} value than heating at 95°C. Some of the samples which had been heated at 95°C had lower ΔA_{420} values than the unheated control.

The overall darker color of the majority of the samples after heating indicates that it is likely that colored Maillard browning reaction products have been formed. The higher ΔA_{420} values in samples heated at higher pH supports this, since the Maillard reaction is favored at more alkaline pH values. However, instead of a darker color with increasing temperatures, as would be expected for the Maillard reaction, samples heated at 95°C were lighter in color. It is possible that extensive changes in molecular conformation and association/aggregation have occurred at high temperatures, resulting in physical changes including loss of solubility and patterns of light reflection quite different from the unheated control, giving a "whiter" appearance. Possible insolubilization of calcium salts may also have contributed to this white color.

The diffuse reflectance spectra thus suggest that while some colored products are formed presumably by Maillard reactions between protein and lactose, other processes which affect conformation and aggregation of the protein

Table 7—Average total hydroxymethylfurfural contents of heated WPC

Heating conditions	Avg total HMF (μmole/100g WPC) (95% confidence limits = ± 2)
Unheated control	23
pH 4.6	26
6.0	27
8.0	30
Temp. 60°C	25
73°C	27
95°C	30

molecules are predominant, particularly at high temperature.

Hydroxymethylfurfural and lactose contents

"Total" hydroxymethylfurfural (HMF) content was determined to detect "early symptoms of the browning reaction in milk products" (Keeney and Bassette, 1959). Free HMF as well as HMF from browning intermediates were included in this measurement. Almost all the heated samples had HMF content greater than that of the unheated control (23.5 μmoles/100g WPC). Analysis of variance of the data indicate that higher HMF contents ($P < 0.01$) were found in samples heated at pH 8.0 than at pH 4.6 or 6.0, and with increasing temperature of heating from 60 to 73 to 95°C (Table 7). However, even in these cases, the increase in HMF content was relatively small (highest HMF content was 33 μmole/100g WPC, compared to 23 μmole/100g for the unheated control and almost 200 μmole/100g for an unheated sample stored at 37°C, 75% relative humidity for 42 days).

The content of "free" lactose remaining after precipitation and removal of protein and fat was used to indicate the trends of physical binding or chemical reactions of lactose by heating. The unheated WPC powder contained approximately 43% lactose by this determination, while heated samples had "free" lactose content varying from 33–43%. Analysis of variance of the data indicated that only the level of % total solids of WPC dispersions during heating had a significant effect ($P < 0.05$) on the "free" lactose content. Samples heated as 6% dispersions had average % lactose content of 36%, compared to 41% for samples heated as 20 or 40% dispersions. This suggests that at the higher solids concentrations, lower extents of protein-lactose reactions may occur due to decreased exposure of reactive protein amino groups resulting from more extensive protein-protein association.

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An Improved Model for Food Thickness from non-Newtonian Fluid Mechanics in the Mouth

A. M. DICKIE and J. L. KOKINI

ABSTRACT

An improved model for food thickness has been developed which accounts for the transient viscoelastic behavior of food materials. This model, previously tested for spreadability, satisfactorily predicts subjective thickness as well. Subjective scores obtained through the use of ratio scales correlated well with calculated shear stress on the surface of the tongue. The best slope of thickness vs shear stress on the tongue was 0.74 with a correlation coefficient of 0.93. The final result provides a design equation for the thickness of foods from a rheological standpoint.

INTRODUCTION

THICKNESS is one of the most important textural attributes of fluid and semi-solid food materials. Statistical analysis of a group of 10 textural attributes relevant to the texture of fluid and semi-solid foods (Kokini et al., 1977) and an independent investigation using 75 words as descriptors for a set of 33 fluid foods (Szczesniak, 1979) showed that thickness was the single most important descriptor of fluid food texture. Other important textural attributes are smoothness and creaminess, but since they are not relevant to the purpose of this paper they are not discussed here.

It was also shown using a group of 16 fluid materials, consisting mostly of gum solution, that thickness is assessed by the shear force exerted on the tongue (Kokini et al., 1977). A model able to predict "thickness" was derived by solving material and momentum balances relevant to the geometry of the mouth. This model, successful in predicting "thickness," used the power-law model for non-Newtonian fluids. The power-law is only valid where the relaxation times of materials studied are much smaller than assessment times of thickness which are usually of the order of seconds (Shama and Sherman, 1973; Kokini et al., 1977).

Most food materials have been shown to have relatively long relaxation times (Kokini and Dickie, 1981; 1982) and an empirical model first introduced by Leider and Bird (1974) approximated the transient shear stress overshoots generated by most fluid and semi-solid food materials.

The Bird-Leider model was used to derive an improved model for predicting the shear stresses developed during the assessment of food spreadability (Kokini and Dickie, 1982). Sensory scores for spreadability, generated using ratio scales and a semi-trained panel of 17 panelists, correlated well with prediction of the model. This led to the conclusion that estimated shear stress on a knife can predict spreadability quite well.

This paper has two objectives: (1) To test the reproducibility that shear forces correlate well with sensory thickness for a group of common foods; and (2) To make the previously developed model for sensory thickness more realistic by accounting for the transient vicsoelastic rheology of common fluid and semi-solid food materials.

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MATERIALS & METHODS

A GROUP OF 15 FOODS used previously to study the spreadability of foods were also used here. They are White House apple butter, Hellmann's mayonnaise, Parkay Squeeze margarine, Heinz ketchup, Gulden's mustard, Land 'O Lake stick butter, Promise tub margarine, Parkay stick margarine, Breadstone's whipped butter, Temp Tee whipped cream cheese, Birdseye Cool Whip, Marshmallow Fluff, Golden Blossom honey, Skippy peanut butter and Betty Crocker canned frosting. Foods were freshly purchased before every test. Special precautions taken to minimize problems associated with use of several packages of the same food are discussed elsewhere (Kokini and Dickie, 1981). Sensory and instrumental measurements were conducted using these foods.

Sensory experiments

Samples of each food were placed in paper cups. Each type of food was assigned anumber and this number was written on the cup containing the food. They were then allowed to equilibrate at room temperature (24–27°C). Two types of sensory experiments were carried out for the purposes of this work. In the first experiments 20 Food Science students, ranging in age from 20–28, experienced in sensory analysis through a sensory analysis course were selected as panelists. Panelists were placed in private booths under moderate lighting. This setting reduced interpersonal biasing. The panelists in this first experiment were not given any prior definition of the subjective attribute "thickness" and were not asked nor allowed to have any discussion. They were simply given written instructions prior to the experiment.

One of the foods of medium "thickness" was assigned to be the standard and the rest of the samples, presented in random order, were rated relative to this standard (Stevens, 1975). Proper care was taken during the experiments to avoid sensory fatigue by allowing panelists to each unsalted crackers as they pleased. They were subsequently asked to wash their mouths thoroughly. Other special precautions as well as normalization and averaging of sensory scores have been described elsewhere (Kokini et al., 1977; Kokini and Dickie, 1982).

In the second type of sensory experiment with a completely different group, panelists were first given the opportunity to have a group discussion to define the meaning of the textural attribute "thickness" and to agree on a standardized testing procedure. They were also extensively instructed about magnitude estimation. The actual testing was similar to the first experiments. After the experiment was finished the panelists were allowed to have a second group discussion to compare their scores. All scores were written on a blackboard and each panelist had a chance to revise his/her score after the discussion. Comparison of the results obtained using these two radically different experiments is discussed in the results section.

Instrumental measurements

Instrumental measurements consisted of rheological measurements and techniques used to measure parameters of the mouth. Rheological measurements are discussed first.

Characterizing rheology. The rheological measurement relevant to the purposes of this study is measurement of shear stress development at inception of steady shear flow. At short times these experiments display the transient shear stresses which are portrayed by the material. At long enough times (of the order of several minutes) a steady state shear stress is reached. These measurements were conducted at 7 shear rates between 0.1 sec^{-1} and 100 sec^{-1}. The long time or steady-state behavior as well as the transient behavior was used to characterize the rheology of the foods studied. The use of the Bird-Leider equation (Leider and Bird, 1974) to characterize the transient rheology of the food materials studied necessitates the

measurement of primary normal stresses as well. Both shear and normal stresses were determined as a function of time using the cone and plate geometry of a Rheometrics Mechanical Spectrometer. A 2.5 cm diameter cone with an angle of 0.04 radians was used. A 0–100 g-cm transducer gave best results. When this transducer was overloaded a 0–2000g transducer was necessary. Experiments were carried out at 30.5°C as an arithmetic average of room and mouth temperature. The possible problems created by the selection of this temperature are discussed in the next section. Calculation of viscosities and primary normal stress coefficients using torque and normal force data as well as other aspects of rheological measurements have also been thoroughly discussed previously and need not be repeated here (Kokini and Dickie, 1981).

Measurements of parameters of the mouth. To estimate shear rates in the mouth, it is necessary to estimate the squeezing force of the tongue, the average velocity of the tongue, the effective radius of the tongue and the initial height of the food at the start of the assessment. Among these, measurement of the squeezing force of the tongue is most critical.

To make this measurement a balloon was attached to a syringe and was filled with water without expanding the balloon. A syringe needle provided resistance to flow. The balloon was calibrated by placing different weights on it and measuring different flow rates. Panelists were then asked to put the same balloon in their mouths and simulate the force of squeezing. The flow rate of water out of the balloon was used to estimate the force of squeezing.

The average velocity of the tongue was estimated by measuring the total displacement of the tongue during 5 sec. The effective radius of the tongue was estimated by obtaining the contact area between the tongue and a piece of paper. The initial height was measured by dividing the average volume of a spoonful by the average contact area of the tongue.

RESULTS & DISCUSSION

Reproduceability of sensory scores

Comparison of unstructured experiments with structured experiments. Comparison of normalized and averaged scores from the structured and unstructured experiments is shown in Fig. 1: the ordinate represents the structured experiments and the abcissa the unstructured experiments. It can be seen from this figure that the agreement between the two sets of scores is excellent with a slope of 0.95 and a correlation coefficient of 0.96. This clearly shows that the textural attribute "thickness" is very well understood by panelists and can be used as a very reliable descriptor of rheology related texture. This result is also very encouraging because it shows the consistency of different panels under radically different conditions. A similar reproduceability was found when a group of 16 foods mostly consisting of gum solutions and simple foods was used (Kokini et al., 1977).

Steady and transient rheology of semi-solid foods

The steady-state ($t \gtrsim 3$ min) apparent viscosity of all material studied followed power-law behavior which can be stated as follows:

$$\eta_{app} = m(\dot{\gamma})^{n-1} \quad (1)$$

where η_{app} is apparent viscosity in Pa·sec; m,n as power-law parameters; $\dot{\gamma}$ is shear rate in sec^{-1}. A log-log plot of η_{app} vs $\dot{\gamma}$ provides a slope equal to n–1 and an intercept equal to log m. Least squares estimates of m and n are shown in Table 1. m values range from 8.68 Pa·sec^n to 563.10 Pa·sec^n and n values range from 0.057–0.989. The coefficient of determination R^2 is 0.99 in all cases.

The primary normal stress coefficient is defined as:

$$\psi_1 = \frac{(\tau_{11} - \tau_{22})}{\dot{\gamma}^2} \quad (2)$$

where $\tau_{11} - \tau_{22}$ is first normal stress in Pa; $\dot{\gamma}$ is shear rate in sec^{-1}; ψ_1 is primary normal stress coefficient Pa·sec^2. It was also found to follow a power-law relationship of the following form:

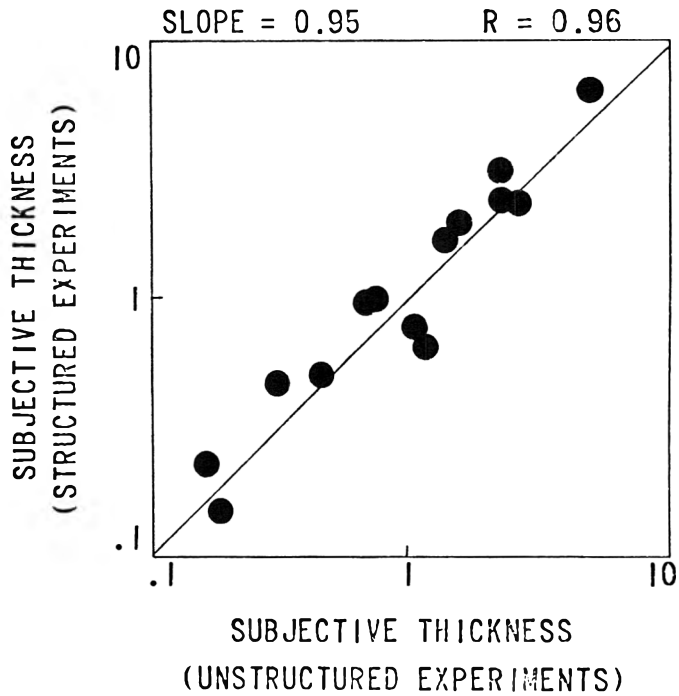

Fig. 1—A comparison of subjective thickness scores obtained from two independent experiments.

Table 1 — Rheological parameters of foods

	m (Pa sec^n)	n	R^2	m′ (Pa sec$^{n'}$)	n′	R^2	λ (Sec)
Apple butter	222.90	0.145	0.99	156.03	0.566	0.99	8.21×10^{-2}
Canned frosting	355.84	0.117	0.99	816.11	0.244	0.99	2.90×10^{0}
Honey	15.39	0.989	0.99	—	—	—	—
Ketchup	29.10	0.136	0.99	39.47	0.258	0.99	4.70×10^{-2}
Marshmallow cream	563.10	0.379	0.99	185.45	0.127	0.99	1.27×10^{3}
Mayonnaise	100.13	0.131	0.99	256.40	−0.048	0.99	2.51×10^{-1}
Mustard	35.05	0.196	0.99	65.69	0.136	0.99	2.90×10^{0}
Peanut butter	501.13	0.065	0.99	3785.00	0.175	0.99	1.86×10^{5}
Stick butter	199.29	0.085	0.99	3403.00	0.393	0.98	1.06×10^{3}
Stick margarine	297.58	0.074	0.99	3010.13	0.299	0.99	1.34×10^{3}
Squeeze margarine	8.68	0.124	0.99	15.70	0.168	0.99	9.93×10^{-2}
Tub margarine	106.68	0.077	0.99	177.20	0.358	0.99	5.16×10^{-1}
Whipped butter	312.30	0.057	0.99	110.76	0.476	0.99	1.61×10^{-2}
Whipped cream cheese	422.30	0.058	0.99	363.70	0.418	0.99	8.60×10^{-2}
Whipped desert topping	35.98	0.120	0.99	138.00	0.309	0.99	3.09×10^{1}

$$\psi_1 = m' (\dot{\gamma})^{n'-2} \quad (3)$$

where m′ and n′ are primary normal stress power-law parameters. A log-log plot of ψ_1 vs $\dot{\gamma}$ allows estimation of the slope equal to n′−2 and the intercept equal to log m′. These values are also shown in Table 1. m′ ranges from 15.70 Pa·sec$^{n'}$ to 3785.0 Pa·sec$^{n'}$; n′ ranges from −0.048 to 0.566. The R^2 in all cases is 0.99 except for stick butter where it is 0.98.

Table 2 — Force velocity and shear rate in the mouth

	Force (Newton)	Velocity (m/sec) (x 10^{-2})	$\dot{\gamma}$ predicted (sec^{-1})
Apple butter	0.735	1.84	9.2
Canned frosting	0.765	1.57	7.9
Honey	0.613	1.90	14.1
Ketchup	0.481	2.18	14.4
Marshmallow cream	1.18	1.45	5.1
Mayonnaise	0.726	1.95	9.8
Mustard	0.549	2.03	11.8
Peanut butter	1.33	1.31	6.6
Stick butter	0.431	1.66	8.3
Stick margarine	0.834	1.88	9.4
Squeeze margarine	0.343	2.48	36.5
Tub margarine	0.363	1.71	8.6
Whipped butter	0.392	1.72	8.6
Whipped cream cheese	1.31	1.83	9.1
Whipped dessert topping	0.510	1.90	11.5

$h_o = 2.0 \times 10^{-3}$ m
t = 0.5 sec
R = 2.0×10^{-2} m

Estimation of the shear rate in the mouth

Next the shear rate in the mouth can be estimated using (Kokini et al., 1977):

$$\dot{\gamma} = \frac{\bar{V}}{\left\{\frac{1}{h_o^{(n+1)/n}} + \left(\frac{F}{R^{n+3}}\frac{n+3}{2\pi m}\right)^{1/n}\frac{n+1}{2n+1}t\right\}^{-n/(n+1)}} \quad (4)$$

where h_o is the initial thickness of the sample in the mouth, m; R is effective radius of the tongue, m; t is assessment time, sec; F is force of the tongue, Newtons; $\bar{V}$ is average velocity of the tongue in m/sec.

Measured values for $\bar{V}$ and F are shown in Table 2 for all foods. $\bar{V}$ ranges from 1.31 X 10^{-2} m/sec to 2.48 m/sec and F ranges from 0.343 (Newtons) to 1.33 (Newtons). Average values for h_o, t and R are also shown in the same table. Consequently, $\dot{\gamma}$ calculated using all these parameters as well as the steady power-law parameters for the apparent viscosity, ranges from 5.09 sec^{-1} to 36.50 sec^{-1}. These values are in agreement with previous estimates of shear rates in the mouth (Shama and Sherman, 1973).

Transient rheology of semi-solid foods

As explained in the experimental section, shear stress was measured as a function of time at the estimated shear rate of the mouth for each particular food. To clearly show the variation of shear stress with time these results are plotted as the ratio of the instantaneous shear stress τ_{21} to the steady state shear stress τ_∞ vs time. Fig. 2 and 3 show

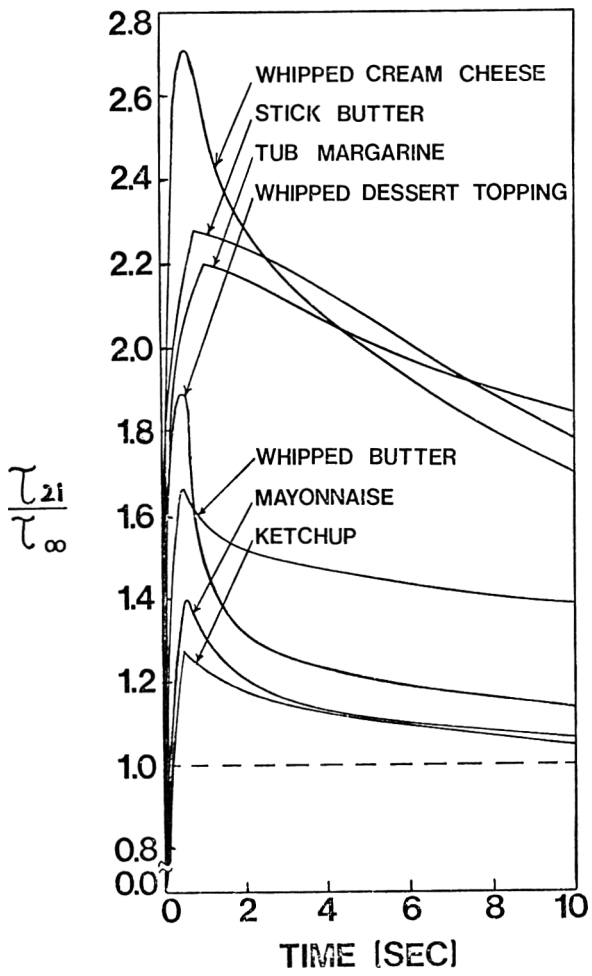

Fig. 2—Transient shear stress development for foods at shear rates of mouth.

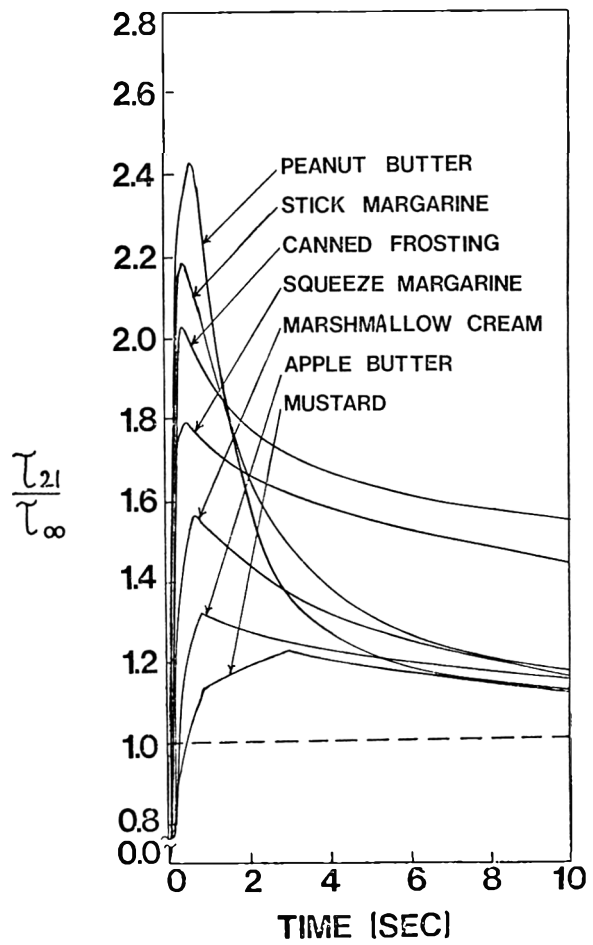

Fig. 3—Subjective thickness vs predicted τ_{max}.

Table 3 — Transient sheal stress parameters and τ_{max} predicted in the mouth

	$\dot{\gamma}_{pred.}$ (sec^{-1})	a	b	$\tau_{max,\ pred.}$ (Pascal)
Apple butter	9.21	1.04×10^{2}	2.17×10^{-1}	496.05
Canned frosting	7.87	4.05×10^{0}	3.54×10^{-1}	954.32
Honey	14.11	–	–	206.96
Ketchup	14.41	7.03×10^{1}	3.27×10^{-1}	58.66
Marshmallow cream	5.09	1.69×10^{-3}	6.40×10^{-1}	1740.16
Mayonnaise	9.75	1.85×10^{1}	2.67×10^{-1}	177.56
Mustard	11.76	1.58×10^{0}	1.33×10^{-1}	72.11
Peanut butter	6.55	4.50×10^{-5}	1.42×10^{0}	1430.99
Stick butter	8.30	1.92×10^{-2}	3.75×10^{-1}	626.56
Stick margarine	9.38	8.95×10^{-3}	4.47×10^{-1}	720.35
Squeeze margarine	36.50	9.47×10^{0}	1.04×10^{-1}	31.08
Tub margarine	8.57	3.65×10^{1}	3.50×10^{-1}	285.90
Whipped butter	8.58	1.27×10^{3}	3.21×10^{-1}	658.89
Whipped cream cheese	9.14	3.33×10^{2}	4.14×10^{-1}	1427.77
Whipped dessert topping	11.53	1.38×10^{-1}	4.14×10^{-1}	77.04

that τ_{21}/τ_{∞} range from 2.7–1.2. Therefore, steady state models such as the power-law model would be in error ranging from 20–270% when the materials studied develop such transient stresses.

These time-dependent shear stresses are simulated using the Bird-Leider equation.

$$\tau = m(\dot{\gamma})^{n}\ [1 + (b\dot{\gamma}t - 1)\exp(-t/an\lambda)] \qquad (5)$$

where τ is shear stress; m, n are limiting viscous power-law parameters; $\dot{\gamma}$ is imposed shear rate; t is time; a, b are adjustable parameters; λ is time constant.

The time constant is calculated using:

$$\lambda = \left(\frac{m'}{2m}\right)^{1/(n'-n)} \qquad (6)$$

a, b in Eq. (5) are estimated using a nonlinear least squares package from SAS. These values for each material are shown in Table 3.

A psychophysical model for thickness

To test the assumption that shear stress in the mouth is responsible for assessment of sensory thickness, a psychophysical model which relates sensory, thickness to shear stress in the mouth is used (Kokini et al., 1977).

$$(\text{thickness}) \propto (\text{shear stress on tongue})^{\beta} \qquad (7)$$

If the logarithms of both sides are taken, the slope of the line is equal to β. If β is equal to 1 there is a linear relationship between the sensory scores and the physical parameter associated with the sensory scores. The physical parameter associated with "thickness" is the shear stress generated in the mouth. The shear stress in the mouth can be estimated using the following equation:

$$\tau_{yx} = m\left\{\frac{\bar{V}}{\left\{\frac{1}{h_0^{(n+1)/n}} + \left(\frac{F}{R^{n+3}}\frac{n+3}{2\pi m}\right)^{1/n}\frac{n+1}{2n+1}t\right\}^{-n/(n+1)}}\right\}^{n} \left[1 + \left\{\frac{b\bar{V}t}{\left\{\frac{1}{h_0^{(n+1)/n}} + \left(\frac{F}{R^{n+3}}\frac{n+3}{2\pi m}\right)^{1/n}\frac{n+1}{2n+1}t\right\}^{-n/(n+1)}} - 1\right\}\exp(-t/an\lambda)\right] \qquad (8)$$

This equation relates the Bird-Leider equation to conditions in the mouth by replacing the shear rate in the Bird-Leider equation by the expression for the shear rate in the mouth shown in Eq. (4). Since thickness is assessed at short times (Kokini et al., 1977; Shama and Sherman, 1973) it is assumed that the peak force applied by the tongue is the force assessed as sensory thickness. This peak shear stress can be estimated by taking the derivative of Eq. (8) with respect to time and equating that to zero. The result is as follows:

$$\tau_{max} = m(\dot{\gamma})^{n}\ [1 + (an\lambda b\dot{\gamma})\exp(-1 - 1/b\dot{\gamma}an\lambda)] \qquad (9)$$

Eq. (9) has been used to estimate the maximum shear stress in the mouth. These results are shown in Table 3 τ_{max} ranges from 31.1 Pa to 1740.2 Pa. To be consistent with techniques of magnitude estimation (Stevens, 1975) calculated τ_{max} values are normalized by taking the geometric average of all τ_{max} values and then dividing each value by the geometric average. These final values are shown in Table 4. The first column in this table is normalized scores of sensory thickness and the second column is normalized scores of τ_{max}. A log-log plot of scores of normalized subjective "thickness" vs normalized τ_{max} would give the slope β (Eq. 7).

Table 4—Calculated τ_{max} vs subjective thickness

	Subjective thickness	Normalized $\tau_{max,\ pred.}$
Apple butter	1.11	1.52
Canned frosting	2.73	2.92
Honey	1.19	0.63
Ketchup	0.32	0.18
Marshmallow cream	2.32	5.32
Mayonnaise	0.76	0.54
Mustard	0.47	0.22
Peanut butter	5.34	4.37
Stick butter	1.40	1.91
Stick margarine	1.56	2.20
Squeeze margarine	0.18	0.10
Tub margarine	1.21	0.87
Whipped butter	0.71	2.01
Whipped cream cheese	2.34	4.36
Whipped dessert topping	0.16	0.24

A plot of normalized sensory thickness scores vs estimated shear stresses is shown in Fig. 4. The ordinate in this figure is scores of normalized thickness and the abcissa is normalized peak shear stresses. The best line through all points plotted on these two axes has a value of 0.74 and the correlation coefficient R is 0.93. When experimental peak shear stresses are used instead of the predicted peak shear stress, the slope of the line is equal to 0.73 and the correlation coefficient is equal to 0.90. The slope of 0.74 is lower than expected and lower than previous correlations of thickness vs shear stress.

As a comparison, steady state shear stress values vs thickness are shown in Fig. 5. The slope of the line in this figure is equal to 0.74 similar to the unsteady state model and the correlation coefficient equal to 0.89 is significantly

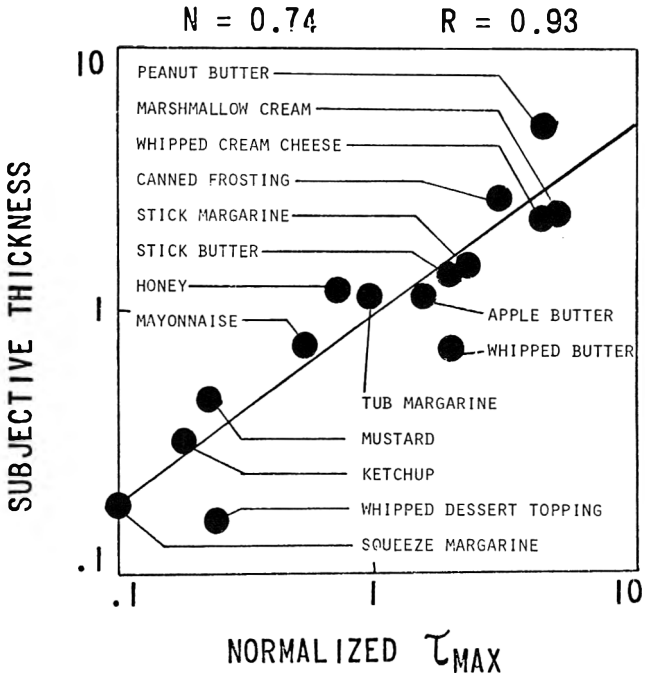

Fig. 4—Subjective thickness vs transient shear stress on the tongue.

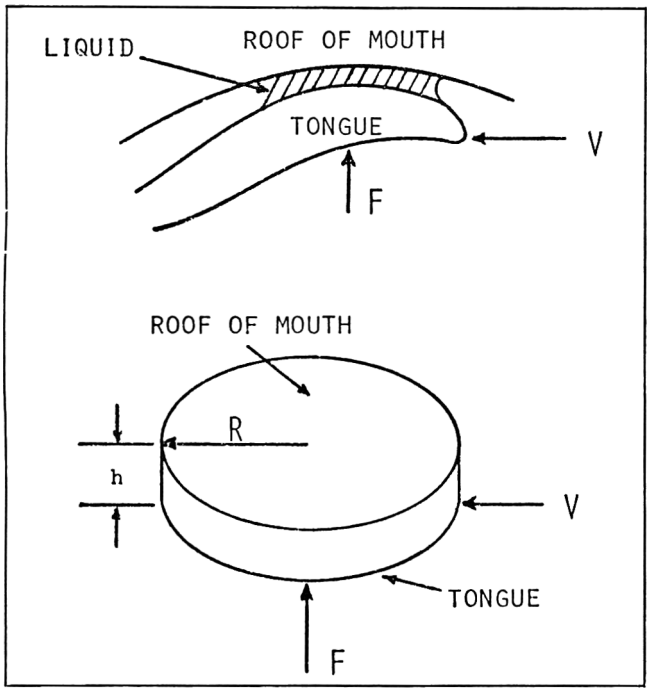

Fig. 6—A model geometry of the mouth.

lower. The comparison shows that the unsteady model does explain thickness significantly better. However, the slope obtained with either model could be improved.

There are several reasons for this result. First of all, the foods studied are highly temperature sensitive and predicting the exact temperature of the food is beyond the scope of this study. The temperature selected is an average temperature. The actual temperature is likely to deviate from food to food depending on its thermal diffusivity. Moreover, at short times instead of a single temperature throughout the food a temperature distribution is likely to be found. For example, assuming that one has perfect contact between

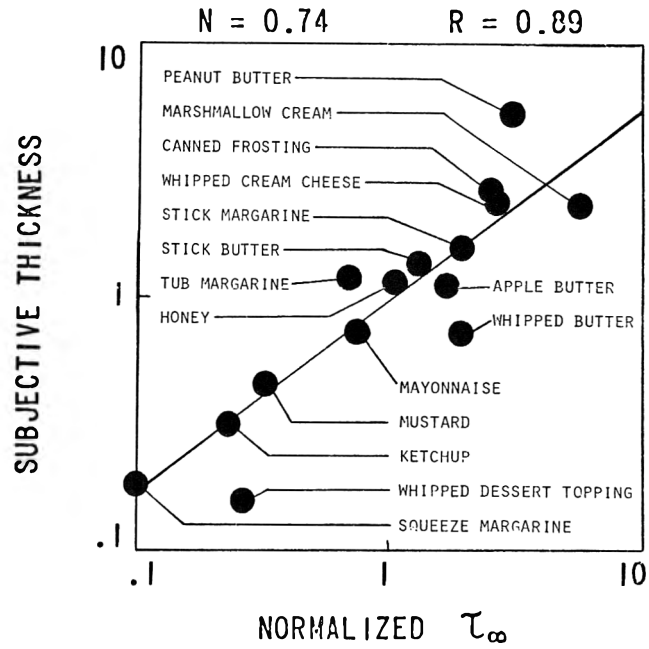

Fig. 5—Subjective thickness vs steady state shear stress on the tongue.

the food and the tongue, a first approximation for the temperature distribution can be obtained using:

$$\left(\frac{T - To}{T_1 - To}\right) = 1 - \text{erf}\,\frac{y}{\sqrt{4\alpha t}} \qquad (10)$$

where To is initial temperature of the material; T_1 is temperature of the tongue and the roof of the mouth; T is the temperature of the food at time t and distance y, where y = 0 is the contact point between the surface of the tongue and the food; α is thermal diffusivity; t is time; y is distance from surface of food.

Moreover, some of the foods such as butter and margarine might have reached their phase-transition temperature. In a separate study where it is attempted to further clarify the effect of such transitions a more realistic model for thickness is derived (Kokini and Cussler, 1982). Only the final result is given here for ice cream:

$$\text{Thickness} = \mu^{3/4}\, F^{1/4}\, V \left\{\frac{(1-\phi)\Delta Hj\rho}{K\Delta T\,\pi\, R^4}\,\frac{2}{3}\right\}^{1/4} \qquad (11)$$

where ϕ is the volume fraction of air in the ice-cream; ΔHj is the heat of fusion of the ice cream; μ is viscosity of ice cream melt; K is the thermal conductivity of melted ice cream; ΔT is temperature difference between the tongue and frozen ice cream; F is force of the tongue; V is velocity of the tongue; ρ is density of the ice cream; R is radius of tongue.

Clearly, the model presented in Eq. (7) does not account for heat effects, temperature changes and phase transitions in the mouth. All rheological measurements are carried out at a single temperature. This is probably one of the most serious limitations of our model.

Second the effect of saliva has not been considered here. Saliva would act as a lubricant and would tend to reduce the shear stress on the surface of the tongue in some cases and reduce the viscosity of some foods such as ketchup and apple butter. However, because assessments are made at short times, it is hoped that saliva does not cloud the results totally. The fact that the correlation coefficient is high substantiates this opinion.

Other limitations of the model come from the approximations involved. For example, the actual geometric model is shown in Fig. 6. This quite ideal stiuation assumes that

—Continued on page 65

Determination of Foaming Properties of Proteins by Conductivity Measurements

AKIO KATO, ATSUNOBU TAKAHASHI, NAOTOSHI MATSUDOMI, and KUNIHIKO KOBAYASHI

ABSTRACT

A new method was established to estimate the foaming properties of proteins from the conductivity of foams using a simple apparatus that consisted of a glass column with the conductivity cell. A close correlation was observed between the initial conductivity of foams and the foam volumes of heat-denatured ovalbumins or 11 native proteins, suggesting that the initial conductivity of foams can be used as a measure of foaming power. In addition, a close correlation was obtained between the foam stability determined from changes in the conductivity and foam volume with time of heat-denatured ovalbumins or 11 native proteins, suggesting that foam stability also can be estimated from changes in the conductivity of foams. The advantages of the conductivity measurements are to determine the foaming properties more simply and accurately than the currently used methods.

INTRODUCTION

FOAMING PROPERTIES are an important functionality of food proteins. Foaming properties include whippability and foamability, and both are used interchangeably by various researchers. These properties are elucidated by two factors, namely foaming power and foam stability. Foaming power is determined by measuring an increase in foam volume, upon the introduction of a gas into protein solution. Foam stability is determined by measuring the rate of liquid leakage from foam or the rate of a decrease in foam volume with time. However, methods and criteria used for determining foaming properties vary with investigators. In addition, a variety of factors, such as concentration, pH and temperature, and methods for foam production influence the foaming properties of proteins. Therefore, it is difficult to make quantitative comparisons between the foaming properties of various proteins, despite many investigations on the foaming properties of food proteins (Nakamura and Sato, 1964; Buckingham, 1970; Lawhon et al., 1972; Richert et al., 1974; Grunden et al., 1974; Groninger and Miller, 1975; Wang and Kinsella, 1976; Horiuchi and Fukushima, 1978). Thus, the need for developing standard procedures to measure foaming properties has been emphasized. Unfortunately, the foaming properties determined from foam volume do not necessarily reflect the quality factors of foams, such as the thickness and size. In addition, it is usually difficult to measure accurately a decrease in foam volume and the rate of fluid leakage from foam. Although the heights or volumes of foams apparently remain fixed, the thickness of foam film, the size of air cells and the quantity of fluid adsorbed to foams change gradually with time. Therefore, it is desirable to estimate the foaming properties by measuring the physical constant reflecting the condition of foams. Since foams consist of a number of bubbles separated by liquid and solid films, it may be possible to estimate foaming properties by measuring the conductivity of foams which contain conductors, such as the fluid adsorbed to the film of foams.

This paper describes a new method to determine the foaming properties of proteins from the conductivity of foams using a simple device that consists of a glass column with the conductivity cell.

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MATERIALS & METHODS

Preparation of protein samples

Ovalbumin was prepared from fresh egg white by sodium sulfate procedure and recrystallized five times. Lysozyme was prepared from fresh egg white by a direct crystallization method and recrystallized five times (Alderton and Fevold, 1946). Ovotransferrin was prepared from fresh egg white by the method of Azari and Baugh (1967). 7S globulin and 11S globulin were prepared from soybean by the method of Thanh et al. (1975). κ-Casein was prepared by the method of Zittle and Custer (1963). Serum albumin (bovine) and α-lactoalbumin were purchased from Sigman Chemical Co. (St. Louis, MO). Ribonuclease (bovine pancreas) was from P-L Biochemicals (Milwaukee, WI). β-Lactoglobulin (bovine) was from ICN Pharmaceuticals Inc. (Cleveland, OH). α-Chymotrypsin was from Miles Laboratories (U.K.).

Heat denaturation of ovalbumin

Five ml of 0.1% protein solutions in 0.1M phosphate buffer, pH 7.4, were heated in an incubator and the temperature was increased at the rate of 1°C per minute, from 30°C to 80°C. The heat-denatured protein solutions were immediately cooled to 20°C after the rise to a given temperature (50, 60, 70, 75 and 80°C) and then the foaming properties were immediately measured.

Measurement of conductivity of foams

Foaming properties were determined by measuring the specific electric conductivity (abbreviated as conductivity) of foams produced when air at a constant flow rate of 90 cm^3/min was introduced for 15 sec into 5 ml of 0.1% protein solution in 0.1M phosphate buffer, pH 7.4, in a glass column (2.4 x 30 cm) with a glass filter (G-4). A schematic diagram of conductivity measurements of foams is shown in Fig. 1. The conductivity of foams was measured using the electrode which had a cell constant of 1.0. The cell was fixed in a glass column at 1 cm interval in a distance of 2.4 cm from a glass filter, connecting with conductivity meter (Kyoto Electrics Industry Co., CM-07). The conductivity reading was recorded automatically using recorder connected with conductivity meter.

Determination of foaming power and foam stability

Foaming power was determined by measuring the conductivity of foam produced immediately after air was introduced into protein solution for 15 sec, that is, initial conductivity (C_i). Foam stability was represented as the foam stability index, $C_0 \cdot \Delta t/\Delta c$, where Δc is the change in conductivity, c, occurring during the time interval, Δt, and C_0 is the conductivity at 0 time obtained from the extrapolation of linear c vs t plot after 1 min. That is, foam stability index indicates the time of disappearance of foams. The method of calculation is shown in Fig. 2.

For comparison, foaming power was also determined by measuring the volume of foams immediately after the introduction of air into protein solution for 15 sec in a glass column under the same conditions as conductivity measurements. Foam stability was also determined from the following equation: foam stability = $V_0 \cdot \Delta t/\Delta V$, where ΔV is the change in the volume of foam, V, occurring during the time interval, Δt, and V_0 is the volume of foam at 0 time.

RESULTS & DISCUSSION

PRELIMINARY EXPERIMENTS were carried out to investigate the relationship between the conductivity of foams

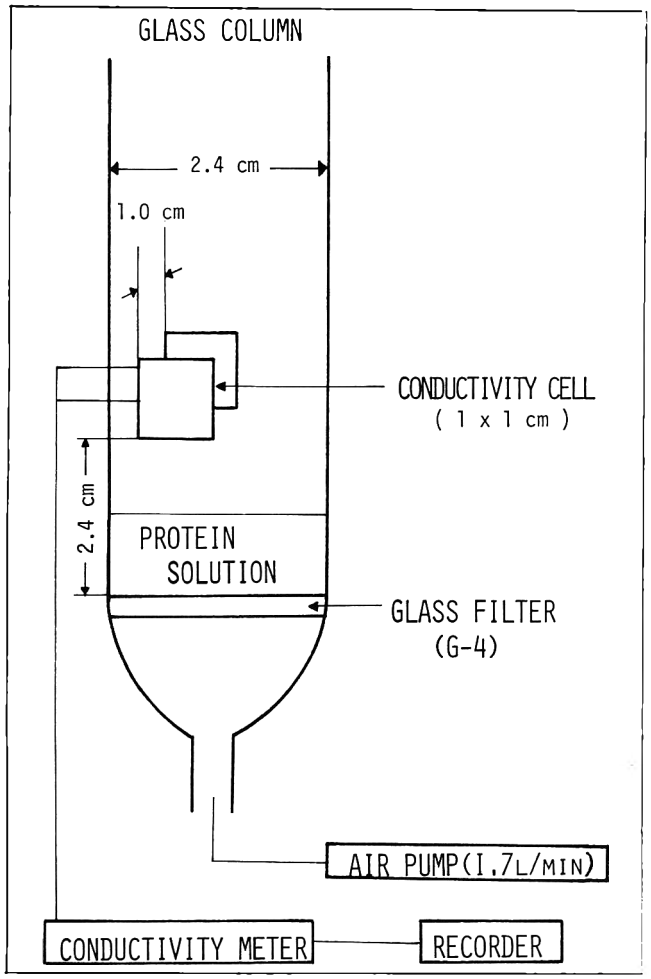

Fig. 1—Apparatus for the measurement of the conductivity of foams.

and foaming properties using heat-denatured ovalbumin. It was reported that the foaming power and foam stability of ovalbumin greatly increased with denaturation (Kato et al., 1981). As shown in Fig. 3, the conductivity of foams remarkably increased with heat denaturation of ovalbumin, suggesting that the foaming properties can be measured using the conductivity of foams. A rapid decrease in the conductivity of foams within 1 min after foam production may reflect the effusion of buffer solutions adsorbing to foams and a gradual decrease after 1 min may reflect the gradual degradation of foams. The effect of ionic strength in protein solutions on the conductivity of foams were investigated using 0.02M, 0.05M and 0.1M phosphate buffer, pH 7.4. The conductivity of foams was naturally affected by the ionic strength of buffer solutions. Since the optimum foaming properties were obtained in 0.1M phosphate buffer, the measurements of foaming properties were carried out in 0.1M phosphate buffer, pH 7.4.

As shown in Fig. 4, a close correlation was observed between the initial conductivity and the foam volume produced after aeration of heat-denatured ovalbumin. The correlation coefficient is 0.95 and is significant ($p < 0.01$). This result suggests that the initial conductivity of foams can be used as a measure of foaming power. Advantageously, the initial conductivity of foams increased in proportion to the denaturation of ovalbumin, while foam volume showed a slight scatter of increase with heat denaturation. In addition, big differences in the initial conductivity were observed between native and denatured ovalbumin, despite small differences in foam volume of ovalbumin during denaturation. Therefore, the measurement of conductivity seems to reflect more exactly the foaming power than that of foam volume.

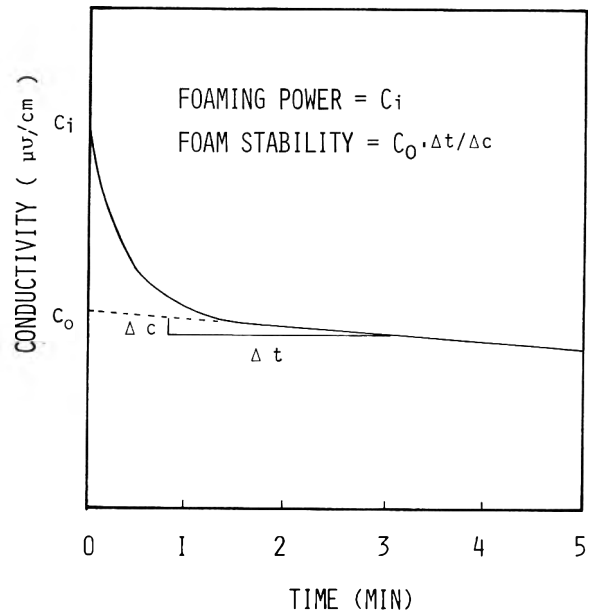

Fig. 2—Determination of foaming power and foam stability from the conductivity curve of foams with time.

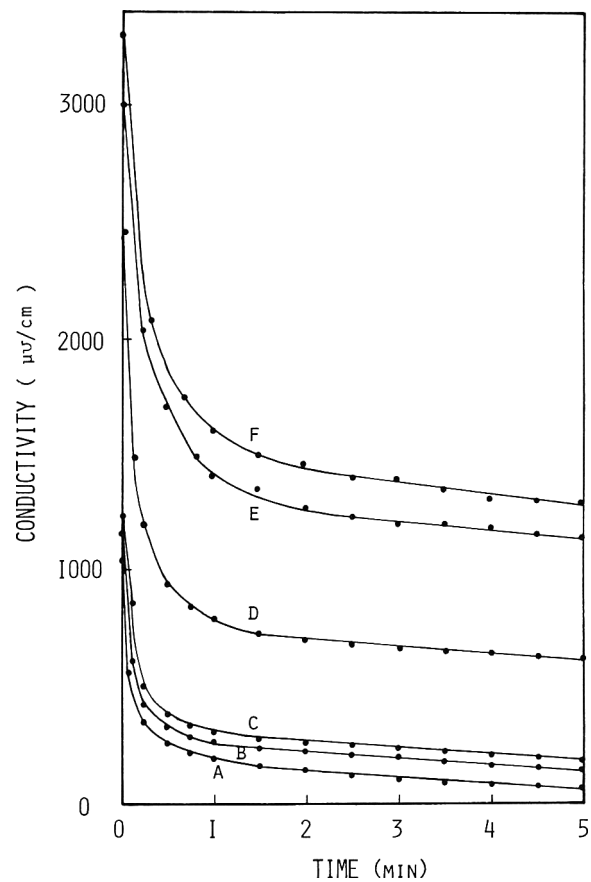

Fig. 3—Changes in the conductivity of foams produced from heat-denatured ovalbumin. (A) native ovalbumin; (B-F) denatured ovalbumin by heating at 50, 60, 70, 75, and 80°C, respectively.

The foam stability of heat-denatured ovalbumin was determined from the changes in conductivity using the curves in Fig. 3. A close correlation was observed between the foam stability index determined from changes in conductivity and the foam stability determined from changes in foam volume (Fig. 5). The correlation coefficient is 0.98

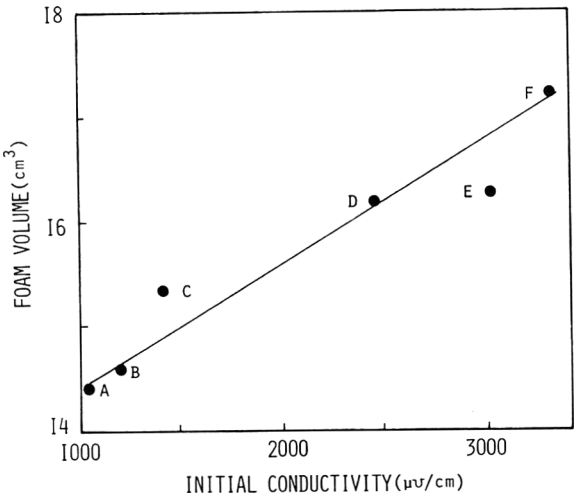

Fig. 4—Correlation of initial conductivity with initial foam volume of heat-denatured ovalbumin. (A-F) same as Fig. 3.

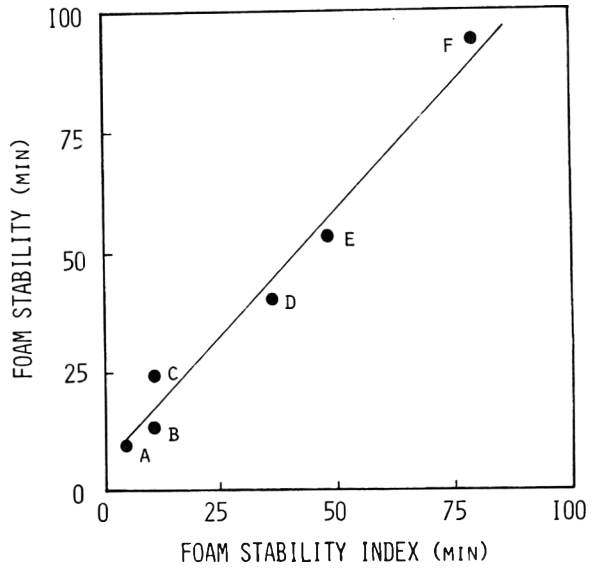

Fig. 5—Correlation of foam stability index determined from changes in conductivity with foam stability determined from changes in foam volume of heat-denatured ovalbumin. (A-F) same as Fig. 3.

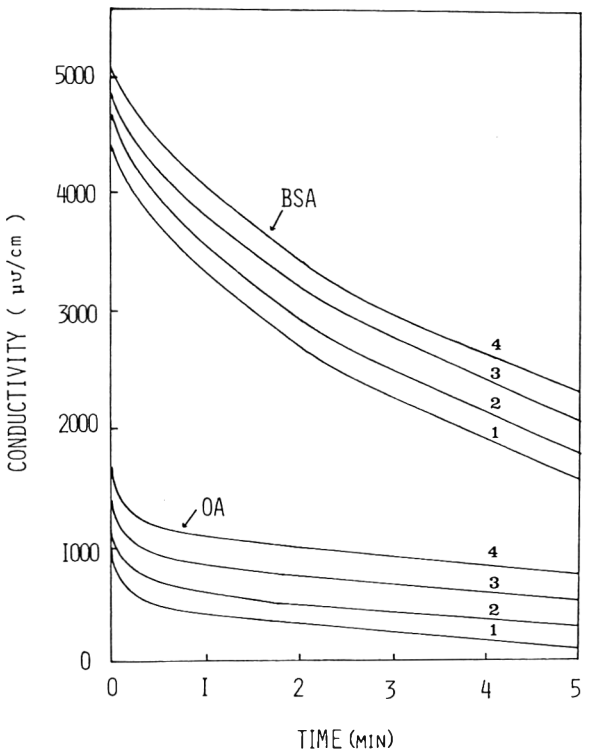

Fig. 6—Reproducibility of conductivity curves of ovalbumin and bovine serum albumin foams. BSA, bovine serum albumin; OA, ovalbumin; 1-4, four different measurements.

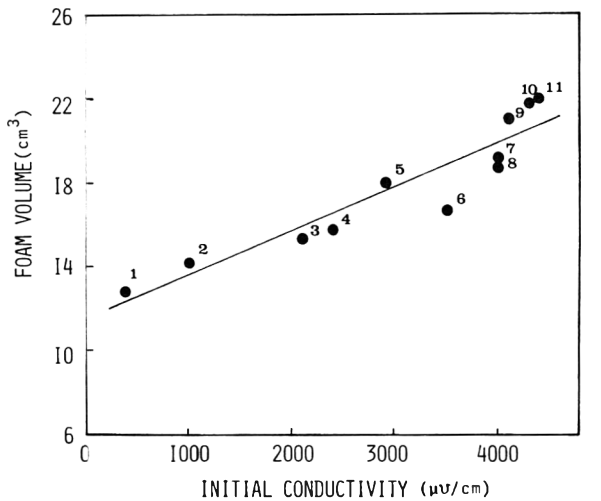

Fig. 7—Correlation of initial conductivity with initial foam volume of protein; 1, lysozyme; 2, ovalbumin; 3, 11S globulin; 4, ribonuclease; 5, 7S globulin; 6, κ-casein; 7, ovotransferrin; 8, α-chymotrypsin; 9, β-lactoglobulin; 10, α-lactoalbumin; 11, bovine serum albumin.

and is significant ($P < 0.01$). This result suggests that foam stability also can be estimated from changes in the conductivity of foams. The conductivity measurement is possible to determine more accurately and simply the foam stability than the currently used methods. The conductivity of foams can be measured continuously and automatically with time, while it is very difficult to measure accurately changes in foam volume with time because of uniformity of foams and steep decrease in foam volume, especially for poor foaming proteins. The reproducibility of conductivity curves is very rich. Fig. 6 shows four different conductivity curves of ovalbumin and bovine serum albumin. The zero points of conductivity meter were displaced each 200 $\mu\upsilon$/cm so that the curves can easily distinguish. As shown in the figure, the reproducbility of curves was markedly rich for each four different conductivity measurements.

In order to confirm the conclusion that the foaming power and the foam stability of protein solutions can be measured using the conductivity of foams, similar attempts were carried out using 11 native proteins to correlate the foaming properties determined from the conductivity with those from the foam volume. As shown in Fig. 7, a good correlation was obtained between the foaming power determined from the foam volume and the initial conductivity of 11 proteins, as well as the result of denatured ovalbumin. The correlation coefficient is 0.93 and is significant ($P < 0.01$). The foam stability also showed a good correlation between that determined from changes in the conductivity and the foam volume of proteins, as shown in Fig. 8. The correlation coefficient is 0.95 and is significant ($P < 0.01$).

These results support that the foaming properties of proteins can be measured accurately and simply by using the initial conductivity (foaming power) and the changes in the conductivity with time (foam stability index) of proteins. The conclusions described above were obtained in 0.1M phosphate buffer. Since the conductivity of foams is pro-

portional to the ionic strength of the buffer solution, the same buffer solutions should be used for comparison of the foaming properties of proteins. The same conclusions that foaming properties can be measured using the conductivity method were obtained, even in lower ionic strength buffers (0.02M and 0.05M), although the correlations with the foaming properties obtained from the foam volume were slightly bad, due to the poor uniformity of foams.

The principal advantages of the conductivity method can be summarized as follows: (1) Even differences in the foaming properties that can not be detected from changes in foam volume are observed, because the conductivity of foams reflects the internal conditions of foams; (2) the measurement of conductivity can be automatically carried out; and (3) high reproducibility of measurement is obtained.

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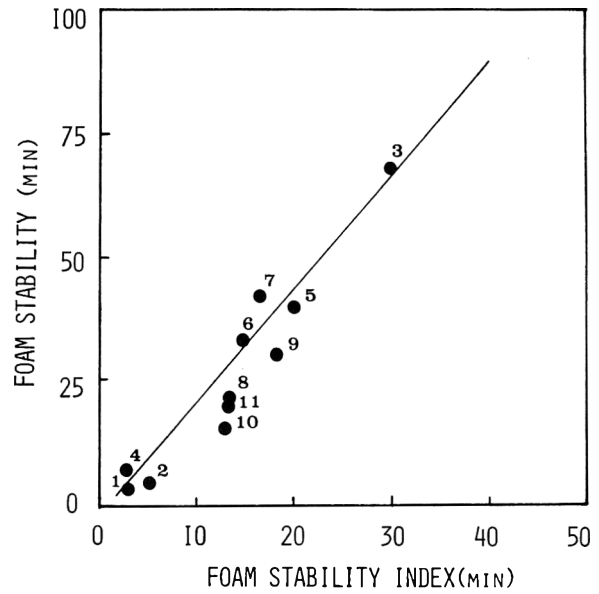

Fig. 8—Correlation of foam stability index determined from changes in conductivity with foam stability determined from changes in foam volume of proteins. 1 - 11, same as Fig. 7.

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MODEL FOR FOOD THICKNESS. . . From page 61

the thickness of the sample is larger than the asperities on the surface of the tongue and at the same time that the thickness is much smaller compared to the radius R. Clearly, both of these assumptions are only approximately correct. A more detailed discussion of other approximations is given elsewhere (Kokini and Dickie, 1982).

Despite all the limitations discussed above, it is shown that sensory thickness correlates well with shear stress on the tongue with the group of typical consumer foods studied. This result is quite comforting since it provides further proof that the physical situation in the mouth as approximated by two parallel plates is not totally unrealistic. Furthermore, a new model previously tested for spreadability provides a more realistic design equation which incorporates the transient viscoelastic rheology of food materials into material and momentum balances of the mouth.

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Relationships Among Cell Wall Constituents, Calcium and Texture During Cucumber Fermentation and Storage

HSIAO-CHIEN LIN TANG and R. F. McFEETERS

ABSTRACT

Demethylation of pectin was the major change in cell wall constituents which occurred during controlled fermentation of cucumbers. The average degree of polymerization of the pectin was 402 residues. This did not change until 6 months after brining. Galactose, xylose, glucose, mannose, and arabinose were present in order of decreasing abundance in the mesocarp cell walls. These noncellulosic neutral sugars did not change during cucumber fermentation and storage. Mesocarp firmness increased when pectin was demethylated, but the firmness subsequently decreased. Calcium chloride at 20 and 40 mM prevented firmness loss compared to fresh cucumbers during 11 months of storage.

INTRODUCTION

DESPITE THE IMPORTANCE of firmness to the quality of cucumber products, there have been only a few investigations of the pectin content and structure in cucumbers (Fabian and Johnson, 1938; Lampi et al., 1958; Fukushima and Yamazaki, 1978). The composition of neutral sugars in cucumber cell walls and the effect of brining on these sugars has not been investigated.

Calcium ions help prevent softening in fermented cucumber slices (Fleming et al., 1978). Buescher et al. (1979) found that $CaCl_2$ concentrations of 0.4% could be used to maintain cucumber firmness with low salt levels even when fungal polygalacturonase mixtures were added to brined cucumbers. The calcium firming effects observed in cucumbers are probably similar in nature to those observed during processing of other fruits and vegetables (Hoogzand and Doesburg, 1961; Van Buren, 1968; Collins and Wiley, 1963).

The objectives of this investigation were: (1) to observe changes in cell wall composition during controlled fermentation and storage of cucumbers and (2) to determine the effect of cell wall changes and $CaCl_2$ addition on the firmness of salt-stock cucumbers.

MATERIALS & METHODS

Fermentation and storage

Size no. 3 (3.8–5.1 cm diameter) 'Chipper' cucumbers were grown at the North Carolina State University Horticultural Science Research Farm. The fruit were hand-harvested and stored at 10°C, 95% relative humidity, for 4 days prior to brining. Duplicate 5-gal plastic pails of cucumbers with 0, 20 and 40 mM equilibrated concentrations of $CaCl_2$ were fermented using the controlled fermentation procedure of Etchells et al. (1973). Each pail was filled with 9.5 kg cucumbers and an equal weight of brine which contained 12.1% NaCl, 28 mM acetic acid and the appropriate amount of $CaCl_2 \cdot 2H_2O$. Four days after brining, when the salt concentration was 7.4%, 95g of sodium acetate trihydrate were added to each pail. The pails were inoculated with 10 ml of a 15-hr culture of *Lactobacillus plantarum* WSO grown in MRS broth (DeMan et al., 1960) with 4% NaCl added. The cucumbers were held at 27°C until sampling. The cucumbers were continuously purged with 25 ml/min N_2 for 1 month. One month after brining, the fruit were repacked into 1-gal jars such that the 50/50 cucumber/brine packout was maintained. The jars were tightly closed with minimum headspace to prevent subsequent growth of film yeasts or molds. At 1, 3 and 6 months after brining, a 1-gal jar from each of the six original 5-gal pails was analyzed. The firmness of cucumber mesocarp and endocarp was measured. Mesocarp tissue and brine samples from each jar were frozen for subsequent analysis. A texture evaluation of the $CaCl_2$-treated fruit only was done at 11 months.

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Effect of post-fermentation calcium addition

Two experiments were done to determine the effect on cucumber firmness of addition of calcium after fermentation. The first experiment was to add 40 mM $CaCl \cdot 2H_2O$ to duplicate, 1-gal jars of cucumbers from the 0 mM calcium pails 1 month after brining. The texture was analyzed 2 months later.

In the second experiment, cucumbers were fermented without calcium, as described above. Three months after brining, the cucumbers were cut into 0.476 cm thick slices and packed into 8-oz jars with an equal weight of brine. $CaCl_2 \cdot 2H_2O$ was added to the jars to give an equilibrated concentration of 40 mM. The firmness of slices from duplicate jars was analyzed weekly for 8 wk.

Polygalacturonase assay

Brine samples were dialyzed and tested for polygalacturonase activity according to Bell et al. (1955).

Firmness measurements

A single, 0.476 cm thick cucumber slice was cut from the center of each cucumber. A single punch was made with the Instron UMT in the mesocarp and endocarp of 15 slices with a 0.315 cm flat-tipped plunger. A 2-kg compression force transducer was used. The crosshead speed and chart drive speed on the Instron were 200 mm/min and 500 mm/min, respectively. The maximum penetration force expressed in newtons was the only parameter used for firmness measurement (Thompson et al., 1982).

Extraction and purification of pectic substances

The general extraction procedures for pectic substances suggested by Doesburg (1965) were used. The frozen mesocarp tissue, from one of the duplicate gallon jars at each calcium chloride level, was partially thawed and a representative 50g tissue sample was blended for 3 min in 5-fold (v/w) of 95% ethanol with a Tekmar homogenizer. The blended tissue was filtered through a Whatman no. 1 filter paper on a Buchner funnel. The residue was washed twice with 50 ml of 95% ethanol. The volume of the total filtered ethanol was measured. The alcohol insoluble solids (AIS) were collected, dried in a vacuum oven at 50°C to constant weight and stored in a desiccator until further extraction.

The AIS from 50g of tissue was extracted for 30 min consecutively with 100 ml of 0.05N HCl at 80°C and 0.5% EDTA solution at pH 6, 50°C. Each extraction step was repeated two to three times until a cooled aliquot of extract did not show pectin flocculation in two volumes of 95% ethanol. At the end of each extraction, the mixture was filtered. Residue from the last EDTA extraction was weighed and refrigerated. Pectic substances, solubilized by the extraction steps, were isolated by adding two volumes of 95% ethanol (v/v) to the extract. The pectic substances precipitated were collected on Whatman no. 1 filter paper. The precipitate was washed with 70% ethanol, 95% ethanol, and acetone. The pectic substances were dried in a vacuum oven at 40°C to a constant weight.

Quantitation and characterization of cucumber pectic substances

The colorimetric technique of Scott (1979) was used to measure

the uronic acid content of each pectin fraction. EDTA was found to interfere with the determination because samples developed a pink color. Therefore, the pectic substances from the EDTA extracts were precipitated with two volumes of 95% ethanol and then redissolved in water before measurement.

The degree of methylation of the pectic substances was measured by titration with poly-NN-dimethyldiallylammonium chloride (Cat-Floc) (Mizote et al., 1975) and the degree of polymerization was estimated viscometrically (Versteeg, 1979). The viscosity at pectin concentrations of 0.2, 0.15, 0.1 and 0.05% in the 0.1M tris-succinate buffer, pH 6.0, with 0.02M NaCl was measured in a no. 1 Ubbelohde viscometer. The intrinsic viscosity $[\eta]$ was determined by plotting the ratio $(\eta_{r-1})/C$ against C and extrapolating to 0 concentration (C = pectin concentration, η_r = viscosity relative to solvent). The formula $[\eta] = 1.4 \times 10^{-6} M^{1.34}$ was used to calculate the average molecular weight (Owens et al., 1946). The acid-soluble fraction was used for the molecular weight estimation of pectin from fresh cucumbers. The amount of EDTA-soluble pectin was too small to be isolated. Since this pectin was 62% methylated, a 184.4 monomer molecular weight was used to calculate the average degree of polymerization. The EDTA-soluble pectic substances were used for size estimation of fermented cucumbers. The amount of the acid-soluble fraction was too small to be isolated after fermentation. A monomer molecular weight of 176 Daltons was used to calculate degree of polyermization since methylation was not detected in the samples.

Cell wall preparation and analysis

Cell walls were isolated from 20 g samples of cucumber mesocarp tissue by the procedure of English et al. (1971). Dried cell walls were hydrolyzed with 2N trifluoroacetic acid (TFA) as described by Albersheim et al. (1967). After hydrolysis, the soluble portion was evaporated to dryness at 50°C under a stream of nitrogen. The dried sample was placed in a desiccator over KOH pellets for 24 hr to remove any residual trifluoroacetic acid. The sample was then dissolved in 0.5 ml of deionized water.

The neutral sugar composition of noncellulosic polysaccharides in the cell wall hydrolyzate was determined by HPLC using a Bio-Rad HPX87 heavy metal column at 73.6°C. Twenty μl of sample was injected and eluted with deionized water at a flow rate of 0.6 ml/min. Glucose, galactose, mannose, xylose, and arabinose were separated and estimated using an external standard calculation. Rhamnose was separated from galactose when standard samples were chromatographed. However, rhamnose, if present, was covered up by a large galactose peak in the cell wall samples.

Statistical analysis of data

Coefficients of variation and significant differences for the treatment means at $P \leq 0.05$ were calculated from analyses of variance for the firmness, pectin fractions and cell wall neutral sugar data.

RESULTS & DISCUSSION

Cucumber characteristics

A normal fermentation occurred in all pails. There was no evidence of bloating or growth of film yeasts or molds in any of the samples. The fruit were normal in odor and appearance. The brine pH was 3.4 and the lactic acid concentration averaged 1.5% during storage. Polygalacturonase activity was not detected in the brine.

Pectin and neutral sugar changes

The acid-soluble pectin decreased while the EDTA-soluble material increased during the first month after brining (Fig. 1). Little or no change occurred after the first month. There was no significant effect of calcium on the distribution of these fractions, so the data presented in Fig. 1 are the averages over the three calcium concentrations.

The acid-soluble fraction would be expected to contain pectin with a high methoxyl content. Analysis of this fraction from fresh cucumbers showed a 62% degree of methylation. This is very close to the 64.6% methylation reported for a Japanese cultivar (Fukushima, 1978). The acid-soluble pectic substances declined to such a low level by the 1-

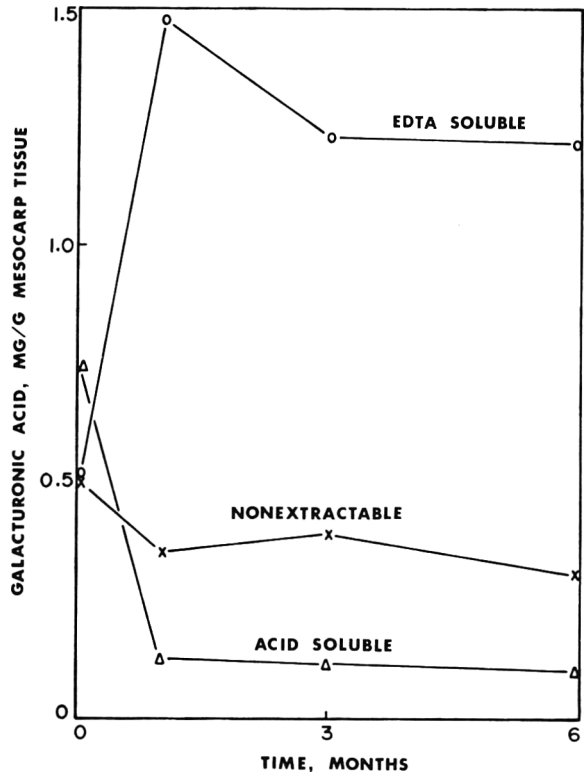

Fig. 1—Changes in pectin fractions during fermentation and storage of cucumbers. Results are the mean of samples over the three calcium levels. The coefficient of variation was 5% for the acid-soluble pectin, 7% for the EDTA-soluble pectin and 10% for the nonextractable pectin in the residue fraction.

month sampling period that they could not be isolated for further characterization. From 1 month onward, the EDTA-soluble fraction was isolated. It was found to have non-detectable levels of methoxyl groups at all three calcium levels, which indicated that complete or nearly complete demethylation of the pectin occurred during the first month of brining. Lampi et al. (1958) reported that conversion of the acid-soluble pectic substances to EDTA-soluble pectic material accompanied the softening of salt-stock cucumbers. However, the measured degree of methylation changed irregularly during softening. A different extraction sequence was used, but we do not know the reason for the apparent large differences in the methylation observed in this study compared to the earlier results. Fukushima and Yamazaki (1978) observed pectin demethylation in chill-injured cucumbers. However, the conversion to low methoxyl fractions did not appear to be as extensive as occurred in the brined fruit.

Neutral sugars in the cucumber cell wall

The neutral sugars from cucumber cell walls had not been previously analyzed, so it was possible that changes in neutral polysaccharides might be responsible for texture changes during brining and storage of the fruit. The amount of cell wall isolated by phosphate buffer extraction did not change as a result of fermentation or storage. The fruit contained 8.3 ± 0.6 mg/g fresh weight of cell walls. After hydrolysis with 2N trifluoroacetic acid, 13–16% of the cell wall was recovered as neutral sugars.

The yield of noncellulosic neutral sugars from isolated cell walls has been found to vary from 50% in suspension-cultured sycamore cells (Talmadge et al., 1973) to only 12% by weight of the cell walls from mung bean leaves (Nevins et al., 1967). In green tomatoes, the neutral sugar content was 29%. This declined to 20% in ripe tomatoes

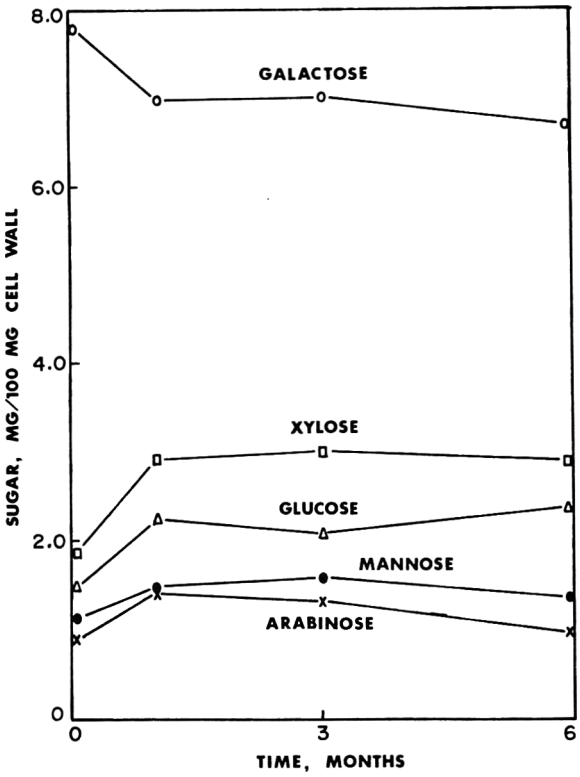

Fig. 2—Changes in cell wall neutral sugar content during brining and storage of cucumbers. Results are the means over the three calcium levels. The coefficients of variation for the individual sugars were galactose, 8%; xylose, 13%; glucose, 16%; mannose, 14%; and arabinose, 25%.

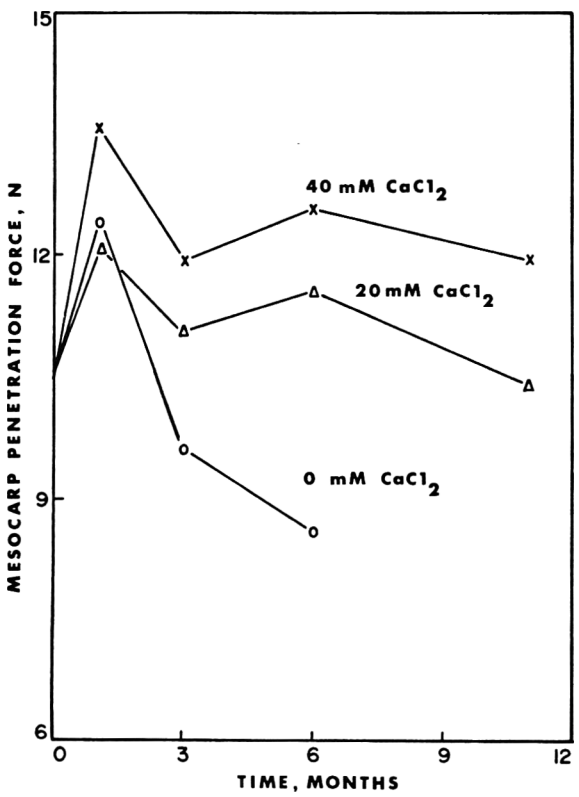

Fig. 3—Effect of $CaCl_2$ on the firmness of cucumber mesocarp tissue during fermentation and storage. The coefficient of variation for the firmness measurements was 16% without added calcium, 15% with 20 mM calcium and 12% with 40 mM calcium.

(Gross and Wallner, 1979). Therefore, cucumber cell walls have a total neutral sugar content in the lower range among the plant tissues which have been analyzed. Fukushima and Yamazaki (1978) found a rather high concentration of cellulose in cucumbers, so cellulose and pectin were probably the major cell wall components not recovered by the TFA hydrolysis.

The presence of calcium ions did not affect the amount of sugars isolated from the wall preparations. Therefore, Fig. 2 shows the mean sugar concentrations over the three calcium concentrations. Galactose, xylose, glucose, mannose, and arabinose were found in order of decreasing abundance. Galactose constituted about 50% of the total hydrolyzable neutral sugars present in the cucumber cell wall. Galactose has also been found to be the major sugar in the walls of apples and tomatoes.

Tomatoes and apples both have arabinose rather than xylose as the second most abundant sugar (Gross and Wallner, 1979; Bartley, 1976). In general, the neutral sugar composition of cucumbers appeared to be similar to that observed in other fruit.

Large decreases of neutral sugars, especially galactose, have been observed during ripening of apples (Knee, 1973; Tavakoli and Wiley, 1968; Bartley, 1976), tomatoes (Gross and Wallner, 1979) and Japanese pears (Yamaki et al., 1979). Wallner (1978) has suggested that removal of neutral sugars that serve as crosslinks could weaken the cell wall structure and contribute to firmness loss. In addition, removal of neutral sugar side chains from pectin could increase its susceptibility to degradation by polygalacturonase. However, Fig. 2 shows that there were not significant changes in neutral sugars during 6 months' storage. Fermentation appears to have little effect on the neutral sugar content of cucumber cell walls.

Effect of calcium on cucumber texture

Fig. 3 shows the firmness changes in the mesocarp tissue of cucumbers at three calcium concentrations during fermentation and storage. Cucumbers with and without added calcium increased in firmness during the first month after brining. This firming effect may be related to crosslinking or gelation of the pectic substances caused by demethylation and the presence of high sodium concentrations and also, calcium ions when $CaCl_2$ was added (Kohn and Sticzay, 1977). An increase in tissue firmness by the interaction of calcium ion and demethylated pectic substances has been observed in the processing of cauliflower (Hoogzand and Doesburg, 1961), snap beans (Van Buren, 1968), and tomatoes (Hsu et al., 1965).

There was a decrease in the firmness of all samples after 1 month. The cucumbers brined without $CaCl_2$ showed a 23% decline in firmness during the 1- to 3-month period and an additional 8% loss from 3 to 6 months (Fig. 3). This decrease could not be readily explained because neither the amount of pectin in the cell walls, the neutral sugars nor the degree of methylation changed significantly during the 1- to 6-month storage period.

Table 1 shows the average molecular weight and degree of polymerization of the major extractable pectin fraction

Table 1—Changes of the average molecular weight and average degree of polymerization of extracted pectic substances during fermentation and storage of cucumbers

Time (months)	Average molecular weight, Daltons	Average degree of polymerization
0	74,200	402
1	71,200	404
3	71,000	403
6	64,400	365

from cucumbers to which calcium was not added. We do not know whether this represents the molecular size of pectin as it exists in the cucumber cell wall. However, the data show that the degree of polymerization of the pectic substances extracted did not change for 3 months after brining. The molecular weight difference between the fresh cucumbers and the cucumbers 1 month after brining was the decrease that should occur if 62% of the galacturonic residues lost a methyl group. There was a 9.4% decrease in the degree of polymerization at 6 months compared to 3 months. This may, perhaps, explain the 8% decrease in mesocarp firmness during this period. However, since the size of pectin did not change during the 1- to 3-month storage period when a large decrease in firmness occurred, this loss of firmness remains to be explained.

The addition of calcium had a significant firming effect on mesocarp tissue. At 3 and 6 months, both 20 and 40 mM calcium chloride showed improved firmness compared to the control fruit without added calcium. Control fruit were not available at 11 months; however, the 20 mM calcium concentration maintained a mesocarp tissue firmness not significantly different from fresh cucumbers. Addition of 40 mM calcium resulted in the mesocarp remaining firmer than the fresh fruit. The endocarp tissue of the cucumbers was less firm and more variable than mesocarp tissue (Fig. 4). Thompson et al. (1982) observed this same result for fresh cucumbers. Due to the variability of the endocarp texture measurements, no significant firming effect of calcium on the endocarp was observed. The endocarp tissue did retain its structure in all treatments so that whole cucumber slices were obtained when the fruit were cut. The data on the firmness changes suggest that properly stored cucumbers could be held in 6% NaCl with 20 mM or more $CaCl_2$ for nearly a year at 27°C without significant loss of firmness compared to fresh fruit. With good tanks and proper control of brining, it may be possible to eliminate the addition of NaCl that is usually carried out after fermentation.

Buescher et al. (1981) found that calcium addition to brined cucumbers after a 2-wk exposure of the fruit to commercial polygalacturonases caused an initial firming response, but the firmness remained much less than that of cucumbers treated at brining with both calcium and polygalacturonase. The breakdown caused by polygalacturonase resulted in some irreversible texture loss.

Two experiments were done to evaluate the effect of calcium added to cucumbers after fermentation under good brining conditions when no detectable polygalacturonase activity was present. Addition of 40 mM $CaCl_2$ to whole cucumbers 1 month after brining prevented the large loss of firmness that occurred in cucumbers without added $CaCl_2$ during the 1- to 3-month storage period. At 1 month the penetration force for cucumbers brined without calcium was 12.4N. By 3 months it had declined to only 9.6N. When calcium was added at 1 month, the firmness decreased to 11.5N compared to 11.9N for fruit brined initially with 40 mM $CaCl_2$.

In the second experiment, fermented cucumbers were held for 3 months without calcium. Calcium was then added to the sliced cucumbers at a 40 mM concentration. Fig. 5 shows that a large increase in mesocarp firmness occurred in the first week after calcium addition, though the slices did not become as firm as when calcium was added at the time of brining. However, the improvement in firmness was temporary. During 8 weeks' storage, the firmness gradually declined until the slices were only slightly firmer than they had been before the addition of calcium. Additional work is required to fully understand the effects of post-brining calcium addition to cucumbers. These limited results indicate that, while later addition of calcium can provide some benefit, the addition of calcium at the time of initial brining is most likely to result in effective control of decreases in cucumber firmness. Buescher et al. (1981) reached the same conclusion based upon their experiments in which polygalacturonases were intentionally added to the brined cucumbers. *—Continued on next page*

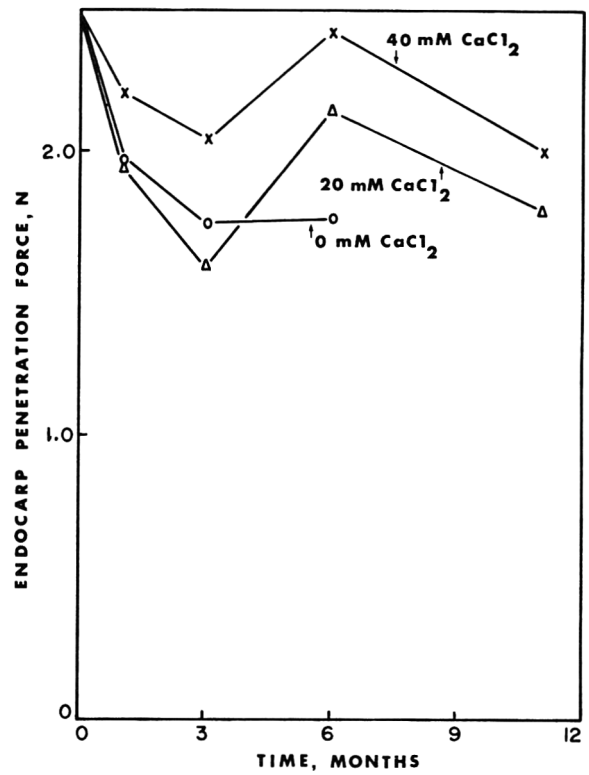

Fig. 4—Effect of $CaCl_2$ on the firmness of cucumber endocarp tissue during fermentation and storage. The coefficient of variation for the firmness measurements was 21% without calcium, 22% with 20 mM calcium and 19% with 40 mM calcium.

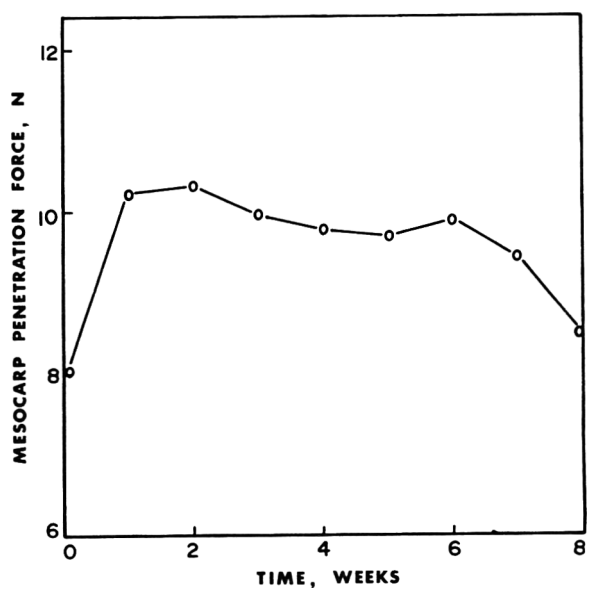

Fig. 5—Effect of 40 mM $CaCl_2$ added to cucumbers after 3 months' storage on the mesocarp firmness of slices. The coefficient of variation for the firmness measurements was 12%.

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HEAT-AGGREGATION OF WPC. . . From page 46

studies as to the effect of Ca^{++} on the heat denaturation of β-lactoglobulin are now in progress in this laboratory.

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Characterization of the Aroma of Raw Carrots (*Daucus carota L.*) With the Use of Factor Analyses

M. R. Mc LELLAN, J. N. CASH, and J. I. GRAY

ABSTRACT

Sensory evaluations of 20 aroma related characteristics derived using open discussion profile methods were made on 10 different carrot selections. Factor analysis was applied to the sensory evaluations to determine an orthogonal set of descriptors which were derived from the original set of data. Five factors, defining independent aroma characteristics of raw carrots, were determined to be earthy-organic aroma, basic raw carrot aroma, fruity-perfumy aroma, nonearthy aromatics, and piney aroma. A second application of factor analysis was utilized to identify components in the head space profile which might correlate with the five newly determined aroma descriptions.

INTRODUCTION

THE EVALUATION of quality attributes in raw carrots include aroma, taste and texture. In order to evaluate characteristics of appropriate attributes, each must be identified and understood. Those characteristics which explain the variation between cultivars could be of key importance in the overall perception and evaluation of quality.

In recent years, the aroma characteristics of carrots have been studied by various workers. Sabinene and myrecene were reported to give odors somewhat similar to the green tops of carrots (Buttery et al., 1968). Heatherbell et al. (1971) described the raw carrot aroma as predominately a strong carrot tops note with varying degrees of soft, sweet, pumpkin-like, and perfumey notes. And the compound, 2-methoxy-3-sec-butyl pyrazine was reported to contribute significantly to the overall aroma of carrots imparting a slightly sharp, raw, earthy, rooty character (Cronin and Stanton, 1976). A dimensional characterization of carrot-root oil aroma indicated prefered descriptors as aromatic, light, fragrent, sweet, soft, green and warm (Alabran et al., 1975). Martens et al. (1979) reported that the most salient features of carrots in terms of sensory input are sharp, bitter, aftertaste, green-grass, fruity, sweet, juiciness, crispness and chewing resistance. Additionally, they reported that correlations, canonical and simple, between chemical and physical variables on one side and sensory ones on the other, were low, although some were significant. Other researchers worked with indiscrete carrot flavor terms which did not strive to separate aroma from taste (Schreens and Hosfield, 1976; Simon et al., 1980).

It has been shown that only low correlations exist between sensory and physical data. Thus external criteria such as shape and weight, which are currently the most important basis for the assessment of quality, actually indicate very little about internal quality. The objectives of this work include the characterization of raw carrot aroma as determined by non-oral odor evaluations, as well as, a study of those aroma components which may help explain intervarietal variations. Additionally, correlations between headspace analysis and aroma components were studied for significance.

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MATERIALS & METHODS

CARROT CULTIVARS, Table 1, selected for this study were grown at the Michigan State University muck farm near Bath, MI. using standard cultural practices for organic soils (Anonymous, 1970). Carrot roots were stored under controlled conditions of temperature and relative humidity.

An open discussion profile panel, consisting of 14 experienced judges, was convened for the purpose of developing a list of possible raw carrot aroma descriptors for eventaul use with the Quantitative Descriptive Analysis (QDA) method. Of the total number of descriptors listed, those rejected were: stale, rancid, green, bitter, aromatic, and pungent. The following nine descriptors were used in the training-testing phase: sweet, carroty, perfumey, fruity, woody, musty, haylike, piney, and earthy. Additionally, an overall aroma characteristic was included bringing the total to ten.

Fifty gram sub-samples of raw carrot puree, prepared cold, were placed in refrigerated containers and sealed until ready for use by the QDA panelists. The QDA panel and data acquisition were handled as described by McLellan and Cash (1983). Panelists were asked to sniff each sample presented and rate each aroma category for two attributes: level of intensity and level of desirability. The measurement of intensity was a quantity measurement for the characteristic of interest. The second measurement, level of desirability, was more of an attitudinal measurement designed to evaluate how the panelists view the previously measured quantity.

A factor analysis applied to the panel results using the ten descriptors was performed using the Statistical Package for the Social Sciences (SPSS) on a Control Data Computer. Alpha factoring and Varimax criteria were used in the analysis. Further method and background information on this factor analytical technique is explained by Rummel (1967) and Cattell (1952).

Headspace analysis of samples for component identification and quantification was performed using a modified technique by Simon et al. (1980). The carrot puree was made under controlled low temperature conditions. Volatiles analysis was performed on a Hewlett Packard Model 5840A Research Gas Chromatograph equipped with a 25 m x 0.2 mm Carbowax 20M fused silica capillary column. The flame ionization detector was set at 400°C and the

Table 1—Carrot lines and cultivars utilized in the study of raw carrot aroma

Parental line No./Cultivar	Line no./Pedigree	Noted field trial comments (By Carrot Breeder)
Parent		
1413	MSU 1413	Bitter
1385	MSU 1385	Bitter - harsh
1383	MSU 1383	Perfumey - bland
5987	MSU 5987	Piney
107	MSU 107	Perfumey
6000	MSU 6000	High Sugar
Cultivars		
Spartan Sweet	MSU 5931 x 6000	Commercial
Spartan Fancy	MSU (5931 x 5986) 6000	Commercial
Gold Pak	Open-polinated	Commercial
Gosinoostrovakaja 13	—	USSR - Low Sugar

injector temperature was 195°C. Column temperature was programmed at 7°C/min from −30°C to 130°C with a final hold at 130°C for 5 min. Nitrogen makeup gas was adjusted to 12 ml/min and detector gases to 35 and 240 ml/min for hydrogen and air, respectively. Compund identification was done by mass spectrometry and standards.

RESULTS & DISCUSSION

THE OPEN DISCUSSION PANEL rejected two descriptors, green and aromatic, that have been previously noted as characteristic components of raw carrot odor (Alabran et al., 1975; Martens et al., 1979). However, the ten descriptors agreed upon by the panel were all previously reported as contributers to carrot aroma. Additionally, it might be surmised that the perfumy and fruity descriptors would measure the same odor quality as the descriptor, aromatic. Similarly, hay-like, piney and earthy, might be considered collectively as a possible synonym for the green odor.

The factor analysis indicated that 69.2% of the inter-varietal variation in the carrot cultivars are explained by five independent aroma components (Table 2). This is not to say that these five components are the key constituents of carrot aroma but rather that a major portion of the aroma variation and difference in the ten cultivars used in this study can be interpreted or explained through the five components. Table 3 shows the rotated orthogonal factor loadings used for the interpretation of the sensory analysis.

Factor 1

Twenty-six percent of the variation between the carrot cultivars was explained by the difference in Earthy-Organic Aroma. This aroma component is defined by the strong positive correlation of the following descriptors: woody, hay-like, earthy and musty.

Table 2—Five independent factors derived from the sensory study, defining aroma components used to account for inter-varietal variation.

	Aroma component	Percent Accounted for variation
[1]	Earthy-Organic Aroma	26.1
[2]	Basic Raw Carrot Aroma	18.2
[3]	Fruity-Perfumy Aroma	10.6
[4]	Aromatics (Non-earthy)	8.4
[5]	Piney Aroma	6.0

Factor 2

The second factor which appears to be the overall basic carrot aroma accounts for 18% of the explained variation between carrot cultivars and is defined by the descriptors carroty, overall aroma and sweet, each correlating positively with the factor. It is important to note that based upon these results the largest explainable portion of the inter-varietal variation, when dealing with aroma, is not associated with the basic raw carrot aroma (factor 2) but rather with, what might be termed a nonbasic carrot aroma, Earthy-Organic Aroma. This should not be considered an unreasonable finding since varietal variation could reasonably be due to nontypical, desirable or undesirable, odor fractions.

Factor 3

This factor is typified by the fruity and perfumy descriptors including that part of musty and earthy which could be considered similarly aromatic. This component differs from factor four by the inclusion of the earthy and musty aromatics and explains 10% of the inter-varietal variation.

Factor 4

Pleasant Aromatics (Non-earthy) describes this fourth factor which accounts for 8.4% of the inter-varietal variation. In this factor the earthy descriptor correlates negatively and perfumy, sweet and fruity correlate positively with the factor.

Factor 5

This is the only factor characterized by a single sensory descriptor by the panel, "Piney." This aroma component accounts for 6% of the inter-varietal variation. Although "Piney" stands an an independent aroma component in these results; it has not been noted as a particularly important contributor by other workers.

All of the factors, except for factor 2, are based on nonbasic or nontypical carrot odor descriptors. This may indicate inter-varietal variation is due more in part to off odors or characteristics not associated with the overall

—Continued on page 74

Table 3—Rotated factor loadings for the factor analysis of the sensory evaluation data using the Varimax Criteria system of rotation

Aroma variable		Menomics	Fact (1)	Fact (2)	Fact (3)	Fact (4)	Fact (5)
Carroty	I[a]	(Q-1)	−0.10991	0.84602	0.06197	−0.04853	0.00648
	D[b]	(Q-2)	−0.08480	0.78784	−0.04481	−0.03524	0.02829
Piney	I	(Q-3)	0.13644	−0.00018	0.33630	−0.25984	0.62041
	D	(Q-4)	0.28574	0.11693	0.06910	0.01790	0.56863
Sweet	I	(Q-5)	−0.10143	0.44588	0.45644	0.16541	−0.11116
	D	(Q-6)	−0.17622	0.56557	0.00534	0.41520	−0.17382
Woody	I	(Q-7)	0.53794	−0.10208	0.25962	−0.36007	0.30383
	D	(Q-8)	0.67103	0.00321	−0.05614	0.01811	0.30605
Hay-like	I	(Q-9)	0.57868	−0.07092	0.36490	−0.35559	0.19665
	D	(Q-10)	0.76021	0.00457	−0.02794	0.03288	0.28718
Fruity	I	(Q-11)	−0.03311	0.14284	0.64538	0.03098	0.16538
	D	(Q-12)	−0.15201	0.39105	−0.08448	0.51985	0.02521
Perfumey	I	(Q-13)	0.06778	−0.11356	0.75381	0.05756	0.13214
	D	(Q-14)	0.14861	−0.08968	0.22300	0.83278	−0.13519
Earthy	I	(Q-15)	0.50830	−0.09897	0.46912	−0.54486	−0.03198
	D	(Q-16)	0.64461	0.04959	−0.01158	−0.11956	0.02758
Musty	I	(Q-17)	0.49159	−0.07832	0.60691	−0.36695	−0.00796
	D	(Q-18)	0.70050	0.03160	0.09833	0.07307	−0.04370
Overall	I	(Q-19)	0.25791	0.73363	0.04909	0.10460	0.12432
	D	(Q-20)	0.38569	0.65466	−0.05054	0.03810	0.14475

[a] Intensity.
[b] Desirability.

Gas Chromatographic Determination of Butylated Hydroxyanisole, Butylated Hydroxytoluene and Tertiarybutyl Hydroquinone in Soybean Oil

DAVID B. MIN and DENISE SCHWEIZER

ABSTRACT

Oils containing different levels of the most common phenolic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiarybutyl hydroquinone (TBHQ) from 0–200 ppm were analyzed by a new gas chromatographic method. The antioxidants in oils were isolated by nitrogen gas purging, collected on Tenax GC coated with polymetaphenoxylene, and then separated and quantitated by gas chromatography. This method requires neither extraction of antioxidants from oil nor derivatization of antioxidants. The correlation coefficients (r) between gas chromatographic peak heights or peak area and their concentrations in oils were 0.99 for BHA, BHT, and TBHQ. This simple gas chromatographic method can determine as little as 10 ppm pf BHA, BHT, or TBHQ in oils in an hour.

INTRODUCTION

ANTIOXIDANTS are frequently added to foods to minimize the oxidation of fats and oils. The maximum legal antioxidants level allowed in most food products is 200 ppm of the oil content in foods (Code of Federal Regulations, 1980). The quantitative and qualitative determinations of antioxidants are very important for regulation, quality control and research in food products.

Angilin et al. (1956) isolated butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) from oil by steam distillation and then analyzed them by a colorimetric method. Sahsrabudhe (1964) extracted BHA and BHT in oil with organic solvents followed by thin-layer chromatography. Stoddard (1972), Jedrych and Karloweski (1979) and Austin and Wyatt (1980) reported gas chromatographic methods for determination of antioxidants in oils. Pokorny et al. (1972), Hammond (1978) and Doeden et al. (1979) reported liquid chromatographic methods for determination of antioxidants in oil.

Most of the reported procedures are time consuming, tedious, complex, and give generally poor reproducibility due to the multisteps required for sample preparation. This paper reports a simple gas chromatographic method which requires neither extraction nor derivatization of antioxidants for the determination of the phenolic antioxidants in oil.

MATERIALS & METHODS

Materials and sample preparation

Fresh deodorized soybean oil was obtained from Capital City Products, Columbus, OH. Tertiary butylhydroquinone (TBHQ) and BHA were obtained from Eastman Chemical Products, Inc. (Kingsport, TN), and BHT was obtained from UPO, Inc. (Des Plaines, IL). Soybean oils containing 0, 50, 100, 150 and 200 ppm (w/v) of BHA, BHT, and TBHQ were prepared.

Antioxidant isolation from oil

The preparation of the antioxidant isolation apparatus and procedures for the isolation of antioxidants from oil are essentially the same as for the isolation of flavor compounds previously described by Min (1981). The antioxidants in 1 ml of oil were isolated without any preliminary sample cleaning by nitrogen gas purging and were collected on Tenax GC coated with polymetaphenoxylene. The compounds were separated and quantitated by gas chromatography.

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Gas chromatography

A Hewlett Packard 5880A gas chromatograph with an electronic integrator and a flame ionization detector was used. A 10 ft x 1/8 inch stainless steel column packed with 80/100 mesh Tenax-GC coated with 10% polymetaphenoxylene (Applied Science Laboratories, State COllege, PA) was used. The program temperature used consisted of 2 min at 140°C followed by 6°C/min to 250°C for 15 min. The injector and detector temperatures were 270°C. The nitrogen flow was 40 ml/min.

RESULTS & DISCUSSION

A PRELIMINARY STUDY showed that the coefficient of variation for reproducibility of the isolation and separation of 100 ppm BHA in oil was less than 2.5% when six replicates were analyzed. Since no extraction and/or derivatization steps were required for analysis and the results showed good reproducibility of quantitation, an internal standard was not used in this study.

The gas chromatogram of oil containing 100 ppm each of BHA, BHT, and TBHQ is shown in Fig. 1. These three antioxidants were base-line separated. BHT and BHA gave symmetric GC peaks, but the GC peak of TBHQ was a little broad with slight tailing. The GC peak heights or peak areas of oils containing 0, 50, 100, 150, and 200 ppm each of BHA, BHT and TBHQ are shown in Table 1. The correlation coefficient (r) between BHA or BHT concentration and GC peak height in Table 1 is 0.99 and the r between TBHQ concentration and peak area in Table 1 is also 0.99. The GC peak area of TBHQ was used instead of peak height because peak area gave a better r than the peak height. This may be due to the skewed GC peak shape of TBHQ as shown in Fig. 1.

Five replicate samples of soybean oil containing 200 ppm BHA and 200 ppm BHT were analyzed and the concentrations of the antioxidants in oil were determined using

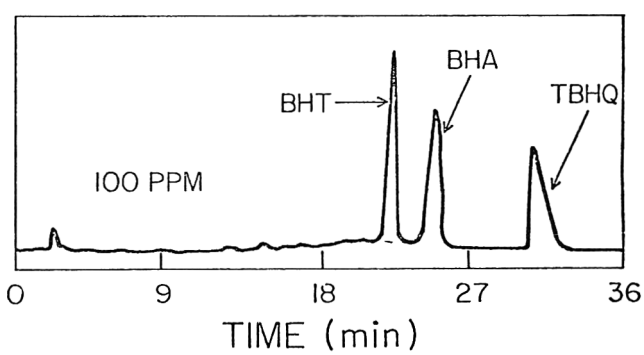

Fig. 1—Gas chromatogram of oil containing BHT, BHA, and TBHQ.

Table 1—Various contents (ppm) of BHA, BHT and TBHQ in soybean oil and gas chromatographic peak heights of BHA and BHT and gas chromatographic peak areas of TBHQ

BHA (ppm)	Peak height (mm)	BHT (ppm)	Peak height (mm)	TBHQ (ppm)	Peak area[a]
0	0	0	0	0	0
50	16.5	50	21.6	50	4.3
100	34.0	100	44.1	100	8.4
150	52.0	150	67.9	150	10.3
200	67.0	200	87.3	200	14.4

[a] Gas chromatographic peak area of TBHQ was calculated by an electronic calculator in HP 5880A gas chromatograph.

the standard calibration lines of the data in Table 1. BHA content ranged from 193–203 ppm; BHT ranged from 195–207 ppm. The coefficient of variation for 200 ppm BHA and 200 ppm BHT were 2.2% and 2.9%, respectively. The triplicate analyses of soybean oil containing 100 ppm TBHQ showed that TBHQ ranged from 98–106 ppm.

To determine the lowest limit of detection of BHA, BHT, and TBHQ in oil, studies were conducted with oils containing 0, 5, 10, 20, 30 and 50 ppm of BHA, BHT, and TBHQ. The results show that the method used can detect 10 ppm of these antioxidants in oil when the signal to noise ratio is around three.

The relatively low coefficient of variation for the reproducibility of determining antioxidants content indicates that antioxidants in soybean oil can be determined by the GC analysis method described without derivatization and extraction of antioxidants from oil.

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RAW CARROT AROMA. . . From page 72

carroty aroma. For quality evaluations, these results indicate that the quantity of these nonbasic carroty odors could be as important, or more important, in making varietal evaluations than the basic raw carrot odor itself.

Twenty-six volatiles in the headspace of the macerated raw carrot samples were studied for correlations with the five newly defined aroma components. Nine of the twenty-six volatiles were identified. These were predominately terpinoids. The pyrazine compound discussed by Cronin and Stanton (1976) was not among the nine. Generally, the relationship between the headspace measurements made in this experiment and the five new aroma definitions gave no significant or very low correlations. A number of factors may account for this result. The various volatiles measured may not have been important to the aroma; i.e., key volatiles may have been at too low levels to detect. The polymer absorbtion technique used may not have absorbed the components responsible for these particular aromas. Additionally, there may have existed too many uncontrollable variables in the raw material.

SUMMARY

A LIST of five independent aroma attributes have been defined which may help characterize inter-varietal variation based on aroma as detected nasally. These attributes should not be considered as describing the key components of the raw carrot aroma, but rather, describing those aroma attributes most likely to differ between varieties. Their use in evaluating varieties could prove to be a valuable tool. Although we did not find correlations with headspace volatiles significant; further work may identify particular chemical in the carrot that are responsible for the five aroma components.

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Presence of Nonprotein Trypsin Inhibitor in Soy and Winged Beans

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ABSTRACT

The total trypsin inhibitor was determined in extracts of 11 cultivars of soybeans and 11 strains of winged beans. The proteins in the extracts were precipitated by 16% trichloroacetic acid (w/v), and nonprotein trypsin inhibitor activities were determined in the protein-free supernatants. The nonprotein trypsin inhibitor activities varied between 10.32 ± 1.39 and 24.41 ± 1.04 units/mg defatted soybean samples and in winged beans ranged between 2.65 ± 0.62 and 6.79 ± 0.27 units/mg defatted samples. The nonprotein trypsin inhibitor composed 27–55% of the total trypsin inhibitor activity in the soybeans, and 5–14% of the total activity in winged beans.

INTRODUCTION

LEGUME SEEDS are an important component of animal and human diets. Soybean seeds contain antinutritional factors such as trypsin inhibitors (Liener and Kakade, 1969). The content of trypsin inhibitors and their variation among cultivars has been studied in faba bean (Wilson et al., 1972; Bhatty, 1979), lima beans (Haynes and Feeney, 1967), chick peas (Belew et al., 1975) and winged beans (Hildebrand et al., 1981). The antitryptic activity of winged bean and extracts is at least as high as that of soybeans (Sohonie and Bhandarker, 1954). Several soybean and winged bean trypsin inhibitors have been isolated, with molecular weights ranging from 8,000–24,000 and their biological and physicochemical properties have been studied (Rackis and Anderson, 1964; Eldridge et al., 1966; Yamamoto and Ikenaka, 1967; Frattali, 1969). Although the role of the inhibitors in the seeds is not understood, they are thought to be the cause of the pancreatic hypertrophy in rats and chicks ingesting raw soybeans (Bray, 1964; Kakade et al., 1972; Birk, 1974).

The objective of this study was to determine differences between two fractions having antitryptic activity in soybean and winged bean seeds; a protein fraction and a nonprotein, water-soluble fraction which has not been reported in the literature.

MATERIALS & METHODS

ELEVEN SOYBEAN CULTIVARS tested in this study were Beeson, Davis, Corsoy, Will, Bay, Essex, Amsoy, York, Williams, Miles, and Harcor. The eleven winged beans strains tested were SLS-1, SLS-6, SLS-11, SLS-37, SLS-41, UPS-31, UPS-46, UPS-47, UPS-53, UPS-139, and LBNC-3. Samples of soybean and winged bean seeds were ground in Wiley mill to pass through a 60 mesh screen and extracted with 10 volumes of petroleum ether at room temperature. Approximately 10g of the meal were accurately weighed and suspended in 150 ml water, and the pH of the suspension adjusted to 7.6. The samples were shaken for 1 hr, quantitatively transferred to a 200 ml volumetric flask, and the volume brought to the mark. The suspension was centrifuged. One portion of the crude supernatant was used for determining the total trypsin inhibitor (TTI) activity and another portion was used for determining the nonprotein trypsin inhibitor (NPTI) activity. To determine the TTI activity, 1.0 ml of supernatant was diluted to 50 ml with deionized water. A 0.4 ml aliquot was used for the assay of the trypsin inhibitor activity by the method of Kakade et al. (1969). Trypsin inhibitor activity was evaluated in terms of the inhibition of the meal on the action of trypsin on benzoyl-DL-arginine-p-nitroanilide (BAPA).

To determine the NPTI activity, 5.0 ml of 100% trichloroacetic acid (TCA w/v) was added to 30.0 ml of the crude supernatant to precipitate all the protein, the suspension was centrifuged and the pH of the supernatant was adjusted to 7.6 with 1.0N NaOH. The volume was brought to 50 ml. One ml of the solution was diluted to 50 ml with deionized water and 0.4 ml was used in the assay of the NPTI activity by the same method used to assay the TTI activity.

One trypsin unit is defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of the reaction mixture under the conditions defined herein. Trypsin inhibitor activity is defined as the number of trypsin units inhibited.

The nonenzymatic browning of free-protein extract and the same extract heated at 100°C was determined by the method of Fishwick and Zmarlicki (1970) with slight modification by Rhee and Rhee (1981). The results were expressed as absorbance at 380 nm x 100/g sample.

RESULTS & DISCUSSION

THE TTI AND NPTI ACTIVITIES for the eleven soybean cultivars are given in Table 1. The TTI varied among the different cultivars ranging from 36.74 ± 1.10 to 66.10 ± 1.44 TIU/mg meal. A large variation in antitryptic activity among different varieties of soybeans has previously been reported by Kakade et al., 1972.

When the proteins of the soybeans and winged beans were precipitated by TCA some of the antitryptic activity remained in the protein-free supernatant which was called non-protein trypsin inhibitor (NPTI). The NPTI activity varied among the different cultivars ranging between 10.32 ± 1.39 to 24.41 ± 1.04 TIU/mg meal (Table 1). It should be noted that there was no protein detected in the supernatant by folin-reagent of Lowry et al. (1951). There was no effect of TCA on the folin color test under our experimental conditions. The effect of TCA salt on the trypsin enzyme was tested, the salt had no effect on the enzyme activity. We also used ammonium sulphate to separate proteins from nonproteins and obtained similar results to those reported with TCA.

The TTI and NPTI activities for winged beans are given in Table 2. The TTI activities varied from 34.35 ± 0.47 to 78.33 ± 0.46 TIU/mg meal among the different strains of winged beans. The NPTI activity varied from 2.56 ± 0.62 to

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Table 1—Comparison of the percentage of nonprotein trypsin inhibitor (NPTI) activity among different cultivars of soybeans

Cultivars	TTI activity TUI/mg samples[a]	NPTI activity TUI/mg sample	% of NPTI in TTI
Beeson	45.00 ± 4.22	24.41 ± 1.04	54.31
Davis	36.74 ± 1.10	20.50 ± 0.82	55.80
Corsoy	45.6 ± 2.77	20.39 ± 1.17	44.56
Will	51.98 ± 2.10	18.59 ± 0.93	35.76
Bay	66.10 ± 1.45	18.25 ± 1.22	27.61
Essex	52.64 ± 1.58	18.19 ± 0.91	34.55
Amsoy	51.6 ± 3.29	20.83 ± 1.68	40.37
York	40.4 ± 1.21	14.95 ± 0.75	37.00
Williams	40.54 ± 1.62	14.82 ± 0.59	36.54
Miles	46.0 ± 3.52	13.31 ± 0.92	28.94
Harcor	48.7 ± 2.08	10.32 ± 1.39	21.20

[a] Trypsin units inhibited/mg fat-free soybean meal, data are reported as the mean of four replicates ± S.E.M.

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Table 2—Comparison of the percentage of nonprotein trypsin inhibitor (NPTI) activity among different selections of winged beans[a]

Cultivars	TTI activity TUI/mg sample[b]	NPTI activity TUI/mg sample[b]	% of NPTI in TTI
SLS-1	46.84 ± 0.37	3.64 ± 0.24	7.77
SLS-6	34.35 ± 0.47	4.98 ± 0.39	14.19
SLS-11	50.01 ± 0.45	2.98 ± 0.23	5.95
SLS-37	65.66 ± 0.74	5.48 ± 0.23	8.34
SLS-41	51.02 ± 0.17	6.08 ± 0.30	11.92
UPS-31	61.28 ± 0.22	4.03 ± 0.79	6.57
UPS-46	76.12 ± 0.54	2.56 ± 0.62	3.37
UPS-47	62.38 ± 0.33	4.33 ± 0.49	6.94
UPS-53	57.57 ± 0.41	3.32 ± 0.27	5.78
UPS-139	78.33 ± 0.46	3.53 ± 0.27	4.51
LBNC-3	55.24 ± 0.41	6.79 ± 0.27	12.30

[a] The winged bean seeds were given to us by Dr. W. Herath from the Univ. of Peradeniya, Sri Lanka. SLS are Sri Lanka selections; UPS are selections from the Univ. of Papua, New Guinea and LBNC are Indonesian selections.
[b] Trypsin units inhibited/mg fat-free winged bean meal, data are reported as the mean of four replicates ± S.E.M.

Table 3—Thermal effect on the NPTI activity[a]

Time of incubation (min)	Incubation at 60°C	Reflux at 100°C[b]	Browning index[c]
0	38.75	37.50	0.115
5	38.75	47.50	1.029
10	39.50	53.50	2.009
15	38.75	55.50	2.432
20	37.75	54.00	4.441
25	39.50	52.00	5.527
30	36.50	51.20	7.344
45	37.50	51.20	10.612
60	38.75	50.00	13.690

[a] Trypsin units inhibited/ml supernatant (solution had been treated with 30% TCA to precipitate the protein as described under the experimental).
[b] The samples were taken at time intervals as shown under time of incubation.
[c] Browning Index was expressed as absorbance at 380 nm x 100/g sample. Sample was taken at time intervals.

6.79 ± 0.27 TIU/mg meal (Table 2). Quantitative differences in the TTI activity of winged beans was reported by Hildebrand et al. (1981).

The amount of NPTI present in TTI of the soybeans varied between cultivars from 21.2–55.8% (Table 1), and in the winged beans from 3.4–14.2% (Table 2). The concentration of the NPTI in the soybeans is much higher and more significant than that in the winged beans. Gennis and Cantor (1976) isolated two trypsin inhibitors from black-eyed peas which were not affected by 10% TCA, and had molecular weights of about 10,000 Daltons. Sohonie and Bhandarkar (1954) reported that the acid extracted winged bean trypsin inhibitor was not affected by 1 hr boiling in water. They also found that winged bean seeds autoclaved for 30 min at 121°C still had 40% of the original trypsin inhibitor.

To determine the thermal effect on the NPTI, the protein-free supernatant was incubated at 60° or refluxed at 100°C. The samples were taken at time intervals as indicated in Table 3. These data indicated that there was no thermal effect on NPTI activity when the supernatant was incubated at 60°C. However, when the supernatant was refluxed at 100°C there was an increase of approximately 48% in the NPTI activity. There was an increase in browning index with the increase in incubation time (Table 3). The increase in the inhibitor activity may be due to the increase of the browning substances in the solution during the incubation period. Browning substances have some inhibitory action on the trypsin itself which may lead to an overestimate of the NPTI activity when the supernatant was fluxed at 100°C.

Browning substances, which had been formed during the boiling of a solution containing small peptides (peptides formed from the digestion of the soybean protein) and reducing sugar (glucose, galactose, fructose or lactose), had inhibitory effect on trypsin activity (unpublished data). Rhee and Rhee (1981) also observed decreased digestibility of the protein in vitro with an increase of browning intensity (Browning Index) when they used the multi-enzyme methods of Hsu et al. (1977). McNaughton et al. (1981) concluded that soybean meal color can predict overprocessing of soybean meal because the Maillard reaction (browning effect) continued even though all trypsin inhibitor had been destroyed.

Bowman (1946) reported on small trypsin inhibitor with molecular weight 8,000 – 10,000. Bowman inhibitor was extracted from soybean by 60% ethanol and was precipitated by 90% acetone. The NPTI reported in this study is different from that reported by Bowman, in which, NPTI was not precipitated by 90% acetone and was recovered quantitatively from the solution. Research is underway to determine the chemical properties of the NPTI.

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Temperature Response of Frozen Peas to Di-Thermal Storage Regimes

SUDHIR K. SASTRY and ARUN KILARA

ABSTRACT

A mathematical model was developed for prediction of temperature fluctuations in warehouse stacks of frozen peas subjected to di-thermal storage regimes. The model was tested by comparing its predictions with temperatures measured in frozen peas packed into a slab-shaped container. Satisfactory agreement was found between model and experiment. Other model predictions indicate that large container sizes and rapid fluctuation regimes result in the greatest thermal stability at interior locations in bulk-stored peas. Thermal properties of frozen peas were also determined as part of the experimental verification. The model can be used to evaluate quality changes in frozen products due to di-thermal storage and the energy needed to maintain a certain final quality.

INTRODUCTION

FREEZING is generally considered one of the best commercially available methods for the long term preservation of foods. Frozen foods degrade in nutritional value over long storage periods—yet large quantities of energy are used for prolonged low temperature preservation. The longer the storage period at a given temperature, the greater the energy consumption and the lower the quality of the end product. It is therefore desirable to evaluate storage practices for purposes of optimizing energy use and nutrient retention. One potential energy conservation alternative is the use of a di-thermal storage regime, i.e. periodic resetting of thermostats to a higher temperature. Numerous researchers such as Woodroof and Shelor (1947) and Fennema and Powrie (1964), indicate that temperature fluctuations produce adverse effects on food quality. Fennema (1977) notes that the rate of oxidation of ascorbic acid in frozen peas shows an unusually large temperature dependence in the range of $-18^{\circ}C$ to $-7^{\circ}C$. These results imply that fluctuating temperatures would create highly detrimental effects in such a temperature range.

Other studies, such as those of Hustrulid and Winter (1943), Boggs et al. (1960), Gortner et al. (1948), and Dietrich et al. (1957), indicate that temperature fluctuations have no significant effect on product quality. Ashby et al. (1979), indicate that di-thermal storage regimes resulted in a net energy savings without serious damage to food quality. In comparing results of the two groups of researchers, it is important to note that fluctuating air temperatures in frozen food storages, do not necessarily imply the same fluctuations within the food product. Well-packaged foods tend to be protected against extreme fluctuations. Results of Moleerantanond et al. (1981) show the damped effects of fluctuations within boxed beef products. These researchers found greater deterioration in the periphery of pallet-loads – where fluctuation effects were most severe – than at interior locations.

In evaluating the feasibility of storage practices, it is important to analyze the heat transfer within frozen foods. Dagerskog (1974) provides a simulation of time-temperature exposure of frozen food during handling in the distribution chain. Efforts in modeling of the effects of fluctuating temperatures have been primarily aimed at quality loss aspects, assuming the entire food product to fluctuate in temperature. Schwimmer et al. (1955) consider regular periodic temperature fluctuations, such as saw-toothed, square, and sine waves. Van Arsdel and Guadagni (1959) have developed the time-temperature tolerance method, involving graphical integration of irregular fluctuations. Singh (1976) has used a mathematical interpolating formula to determine quality changes.

A need exists for analyses of heat transfer in frozen foods exposed to di-thermal storage regimes. The exact nature of the air-temperature fluctuations resulting from thermostat resetting depends on the type and construction of the storage facility. Thus, analysis can only be performed for given temperature regimes.

This research involves an analysis and experiments on heat transfer in frozen peas exposed to arbitrary periodic temperature fluctuations. The peas are assumed to be in boxes of various sizes, with stacking patterns similar to those found in commercial warehouses. The influence of the packaging is not considered in this analysis; however, packaging can only be expected to have beneficial effects in terms of damping temperature fluctuations. The effects of box size, fluctuation period, and temperature range are considered. The data and experimental work are performed for frozen peas, but the analysis procedure can be used for other foods.

MATERIALS & METHODS

Derivation of formulas

The stacking pattern shown in Fig. 1 is typical of many warehouses, and is considered in the analysis. The width is, in general, much smaller than the length or height, resulting in an approximate unidimensional heat transfer problem. The problem of heat transfer in a frozen food product involves consideration of the latent heat effects due to partial melting and freezing over a range of temperatures. The problem can be reduced to one of ordinary conduction heat transfer with temperature-dependent thermophysical properties. This approach has been used by Bonacina et al. (1973) and Heldman (1974). The formulation for the problem of heat transfer in frozen foods exposed to arbitrary periodic temperature fluctuations is

$$\frac{\partial}{\partial x}\left(k(T)\frac{\partial T}{\partial x}\right) = \left(\rho(T)\frac{\partial h}{\partial T}\right)\frac{\partial T}{\partial t} \quad (1)$$

with boundary conditions

$$T(0,t) = T(L,t) = \sum_{n=1}^{\infty} b_n \sin\frac{(n\pi t)}{\tau} \quad (2)$$

$$\frac{\partial T(L/2,\, t)}{\partial x} = 0, \quad (3)$$

and the initial condition

$$T(x,0) = T_i(x) \quad (4)$$

The boundary condition (2) represents an arbitrary periodic temperature fluctuation expressed in the form of a Fourier sine

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series. Thermal resistances due to packaging and boundary layer are considered negligible in this analysis. The assumption of an infinite Biot number results in conservative prediction. Packaging and low air movement in commercial warehouses would result in improved thermal stability. The value of the coefficients b_n in condition (2) are determinable for specific situations. For the relatively small temperature ranges involved in storage situations, the values of the properties k, ρ, and dh/dT, may be approximated as constants, yielding the following modified form of Eq (1).

$$\frac{\partial^2 T}{\partial x^2} = \left(\frac{1}{\alpha_a}\right)\frac{\partial T}{\partial t} \tag{1a}$$

The parameter α_a is frequently referred to as the "apparent" thermal diffusivity, termed apparent due to the inclusion of latent heat terms.

For long time durations typical of frozen storage, the solution reduces to the steady periodic solution, as described by Arpaci (1966). Solution of (1a) subject to conditions (2), (3), and (4), involves the method of complex temperatures of which a supplementary problem is required as follows:

$$\frac{\partial^2 T^*}{\partial x^2} = \left(\frac{1}{\alpha_a}\right)\frac{\partial T^*}{\partial t} \tag{1b}$$

$$T^*(0,t) = T^*(L,t) = \sum_{n=1}^{\infty} b_n \cos\left(\frac{n\pi t}{\tau}\right) \tag{2b}$$

$$\frac{\partial T^*(L/2,t)}{\partial x} = 0 \tag{3b}$$

$$T^*(x,0) = T_i^*(x) \tag{4b}$$

Multiplying Eq (1a), (2), (3), and (4) by the complex constant i, and adding each to its corresponding supplementary Eq (1b), (2b), (3b), or (4b), results in the problem of complex temperatures, as follows:

$$\frac{\partial^2 \Psi}{\partial x^2} = \left(\frac{1}{\alpha_a}\right)\frac{\partial \Psi}{\partial t} \tag{1c}$$

$$\Psi(0,t) = \Psi(L,t) = \sum_{n=1}^{\infty} b_n \exp\left(\frac{in\pi t}{\tau}\right) \tag{2c}$$

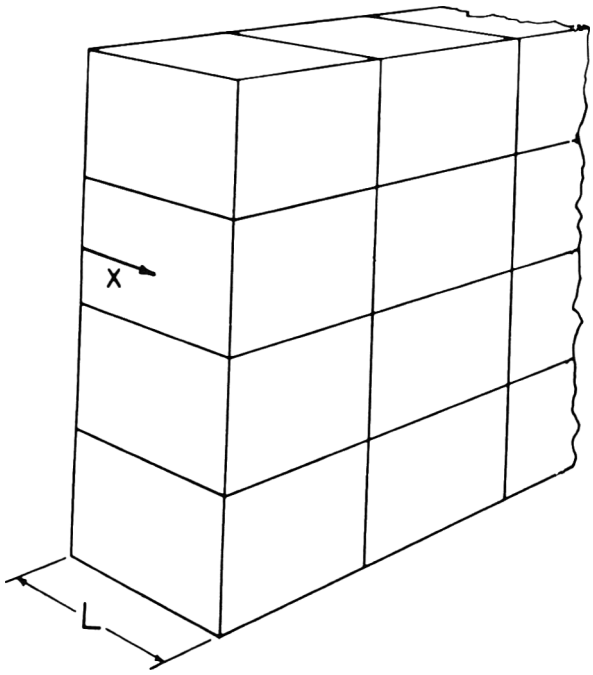

Fig. 1—Schematic of warehouse stacking pattern.

$$\frac{\partial \Psi(L/2,t)}{\partial x} = 0 \tag{3c}$$

$$\Psi(x,0) = \Psi i(x) \tag{4c}$$

where $\Psi = T^* + iT$.

Defining

$$\Psi(x,t) = \sum_{n=1}^{\infty} \Psi_n(x,t) \tag{5}$$

the problem (1c) through (4c) can be subdivided into an infinity of subproblems, the general form of which is

$$\frac{\partial^2 \Psi_n}{\partial x^2} = \left(\frac{1}{\alpha_a}\right)\frac{\partial \Psi_n}{\partial t} \tag{1d}$$

$$\Psi_n(0,t) = \Psi_n(L,t) = b_n \exp\left(\frac{in\pi t}{\tau}\right) \tag{2d}$$

$$\frac{\partial \Psi_n(L/2,t)}{\partial x} = 0 \tag{3d}$$

$$\Psi_n(x,0) = \Psi_{ni}(x) \tag{4d}$$

The steady periodic solution to each of the subproblems is obtained by the method outlined by Arpaci (1966). The solution to Eq (1c) through (4c) is the sum of the solutions of the infinity of subproblems, and the imaginary part of this solution gives the temperature distribution, as follows.

$$T(x,t) = \sum b_n \left(\frac{[1+\exp(\gamma_n L)\cos(\gamma_n L)]\,[\exp(\gamma_n x)\sin(\gamma_n x+\omega_n t)+ \exp(\gamma_n (L-x))\sin(\gamma_n (L-x)+\omega_n t)] - [\exp(\gamma_n L)\sin(\gamma_n L)]\,[\exp(\gamma_n x)\cos(\gamma_n x+\omega_n t)+\exp(\gamma_n (L-x))\cos(\gamma_n (L-x)+\omega_n t)]}{[1-\exp(\gamma n L)\cos(\gamma n L)]^2 + [\exp(\gamma n L)\sin(\gamma n L)]^2} \right) \tag{6}$$

where $\gamma_n = \left(\frac{n\pi}{2\tau\alpha_a}\right)^{1/2}$ (7)

$$\omega_n = \frac{n\pi}{\tau} \tag{8}$$

Eq (6) is used to calculate temperature distributions in stacks of frozen peas.

Determination of properties

Solution of the heat transfer problem requires determination of properties, ρ, k, and dh/dT for frozen peas under a variety of conditions. For a bulk-stored material, the value of thermal conductivity, k, depends on the bulk density, ρ, and temperature. The slope, dh/dT of the enthalpy-temperature curve, depends on the temperature. The following procedures were used.

The thermal conductivity, k, was determined by the conductivity probe method, described by Nix et al. (1967). The entire apparatus was placed in a freezer at test temperature. A sample of frozen peas was packed into the apparatus at the required bulk density and temperatures allowed to stabilize prior to the start of each test. Low power inputs – approximately 0.65 w/m – were used to minimize freezing and thawing near the heat source. Tests were conducted at four bulk densities (400, 450, 500, and 517 kg/m^3) and at three temperatures (−10, −19, and −29°C) with three replications for each condition.

Enthalpy of frozen peas was determined at various temperatures using the method of mixtures, described by Mohsenin (1980). A datum of −40°C was used. Pre-weighed samples of frozen peas at −40°C were mixed in a box calorimeter with known quantities of anhydrous ethyl alcohol at various temperatures. Equilibrium temperatures were measured and the following relation used for calculation of the enthalpy at any temperature.

$$h(T_e) - h(-40°C) = \frac{m_{al} c_{al} (T_i - T_e)}{m_p} \tag{9}$$

The results were used to develop an enthalpy-temperature curve for frozen peas, as shown in Fig. 2.

Experimental verification

Frozen peas were packed at a bulk density of 450 kg/m^3 into a specially constructed slab-shaped container of 28-gage galvanized steel with dimensions 0.505m x 0.23m x 0.051m. The dimensions were chosen so that (a) the slab could fit into a shelf of the test freezer, and (b) end effects in heat transfer could be minimized by making the thickness considerably smaller than the length and width. The container was sealed and placed in a freezer. Upon attainment of thermal equilibrium, the freezer temperatures were fluctuated between temperatures of −20°C and −11°C within a period of 24 hr.

Temperatures were measured at 11 points across the slab thickness, spaced apart at distances equal to 1/10th the thickness. To minimize end effects, all thermocouples were placed close to the center of the rectangle formed by the length and the width. Thus, one-dimensional heat flow as approximated.

Due to the difficulties involved in obtaining reliable surface-temperature measurements, an alternative approach was chosen. The air within the freezer was continuously agitated by two small blowers to ensure high Biot number values. Air temperatures were measured and assumed to correspond to surface temperatures.

Temperatures were measured every 20 min. The test was conducted over 2 wk, thereby allowing ample time for the steady periodic response to set in.

A single cycle of the surface temperature fluctuation regime is illustrated in Fig. 3. The curve was divided into four segments and regression curves fitted to each. The following curves were obtained:

Section I, $0 \text{ hr} < t \leq 5.5 \text{ hr}$

$$T = 0.529t - 13.4294 \quad (10)$$

Section II, $5.5 \text{ hr} < t \leq 6.5 \text{ hr}$

$$T = -11.3 \quad (11)$$

Section III, $6.5 \text{ hr} < t \leq 18.5 \text{ hr}$

$$T = -0.01314t^3 + 0.5684t^2 - 8.255t + 21.2901 \quad (12)$$

Section IV, $18.5 < t \leq 24 \text{ hr}$

$$T = 1.1713t - 41.7599 \quad (13)$$

All equations yielded correlation coefficients greater than 0.99. These relations were used in determination of Fourier sine series coefficients for the regime of Fig. 3. It must be noted that a simple Fourier sine series is not determinable for an asymmetric regime such as the one in Fig. 3. A Fourier sine series yields a pattern that alternates between the illustrated cycle and its mirror reflection about the horizontal axis. The problem was circumvented by considering the cycle to be one of 10 similar subcycles contained in a large cycle of period 240 hr. Fourier sine series for the curve then yield 10 consecutive, similar curves prior to a change to a mirror-reflection- type pattern. Ten cycles was found to be more than sufficient for the onset of a steady-periodic response.

Temperature predictions for different slab locations and various times in the cycle were obtained using Eq (6). Values of thermal properties used were those determined experimentally. The predictions were compared to experimental data for verification.

Computational procedure for other predictions

Predictions were made for several potential temperature fluctuation situations. Due to the wide variation in types of storage facilities, a temperature regime was chosen which permitted flexibility, and represented symmetric variation between two thermostat set points, as shown in Fig. 4. The equations describing the regime are as follows:

for zone I, $0 \leq t < (1-X_s)\frac{\tau}{4}$

$$T = \frac{2(T_h - T_\ell)}{(1-X_s)\tau} t + \frac{(T_h + T_\ell)}{2} \quad (14)$$

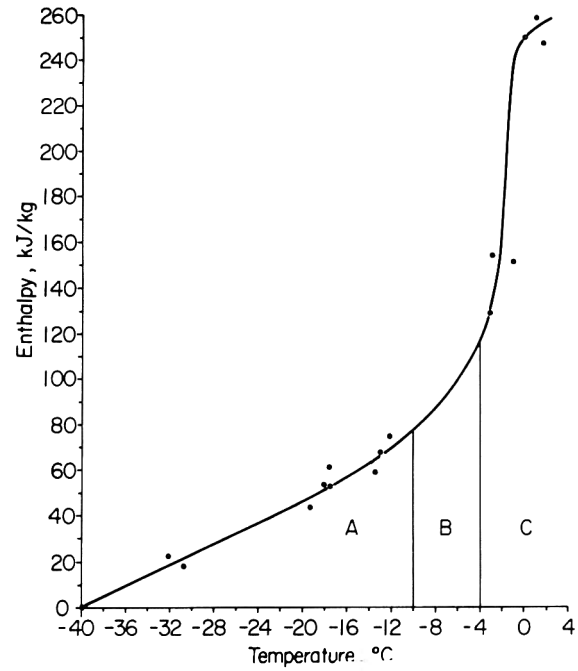

Fig. 2—Enthalpy-temperature curve for frozen peas showing zones A, B, and C used for computational purposes.

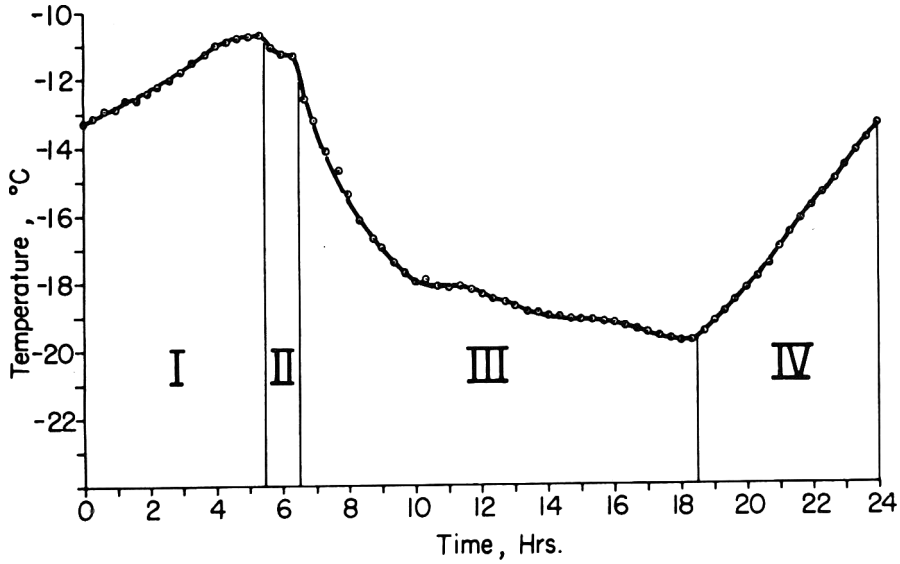

Fig. 3—Temperature fluctuation regime of freezer.

For zone II, $(1-X_s)\frac{\tau}{4} \leq t < (1+X_s)\frac{\tau}{4}$

$$T = T_h \tag{15}$$

For zone III, $(1+X_s)\frac{\tau}{4} \leq t < (3-X_s)\frac{\tau}{4}$

$$T = \frac{-2(T_h-T_\ell)}{(1-X_s)\tau} t + T_h \left[\frac{3}{2} + \frac{X_s}{(1-X_s)}\right] - T_\ell \left[\frac{1}{2} - \frac{X_s}{(1-X_s)}\right] \tag{16}$$

for zone IV, $(3-X_s)\frac{\tau}{4} \leq t < (3+X_s)\frac{\tau}{4}$

$$T = T_\ell \tag{17}$$

for zone V, $(3+X_s)\frac{\tau}{4} \leq t < \tau$

$$T = \frac{2(T_h-T_\ell)t}{(1-X_s)\tau} + T_\ell - \frac{(T_h-T_\ell)(3+X_s)}{2(1-X_s)} \tag{18}$$

The exact nature of the temperature regime may be varied by variation of any of four parameters: the high and low set points, T_h and T_ℓ, the period of oscillation, τ, or the fraction of total cycle time spent at the setpoints, X_s. The Fourier sine series of this temperature regime with the datum point at the mean temperature, yields the following expression for the coefficients, b_n,

$$b_n = \frac{4(T_h-T_\ell)}{(1-X_s)n^2\pi^2}\left(\sin[n\pi(1-X_s)/4] + \sin[n\pi(1+X_s)/4] - \sin[n\pi(3-X_s)/4 + \sin[n\pi(3+X_s)/4]\right) \tag{19}$$

Experimentally determined property values were used in each simulation. The enthalpy-temperature curve of Fig. 2 was divided into three adjacent zones, A, B, and C within which the slopes could be approximated as constant. The value of the slope for a given temperature regime was determined from the fractional amounts of each zone overlapped by the regime range.

Thus, if T_ℓ lay in zone A,

$$T_{\ell a} \leq T_\ell \leq T_{hA}$$

and T_h lay in zone B,

$$T_{hA} = T_{\ell B} \leq T_h \leq T_{hB}$$

the slope, dh/dT, was calculated as

$$\frac{dh}{dT} = \frac{X_A\left(\frac{dh}{dT}\right)_A + X_B\left(\frac{dh}{dT}\right)_B}{X_A + X_B} \tag{20}$$

where

$$X_A = \frac{T_{hA}-T_\ell}{T_{hA}-T_{\ell A}} \tag{21}$$

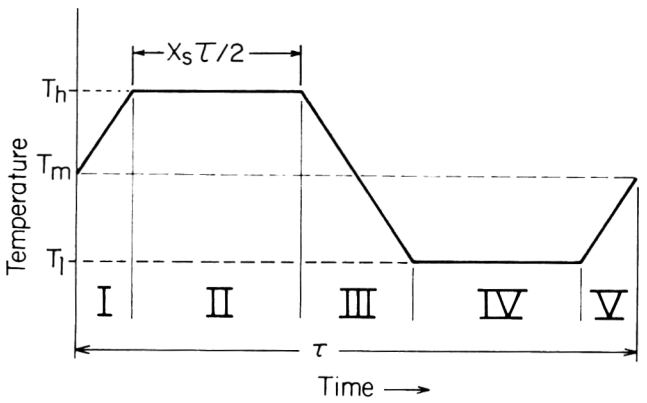

Fig. 4—Hypothetical temperature fluctuation regime.

and

$$X_B = \frac{T_h-T_{\ell B}}{T_{hB}-T_{\ell B}} \tag{22}$$

A FORTRAN program developed for computation of temperature distributions, was run for a wide range of conditions. Parameters varied were container size (widths of 0.1524, 0.3048, 0.6096, and 1.2192m); fluctuation period (3, 6, 12, 24, and 48 hr); and temperature range (−23°C to −13°C, −18°C to −8°C, and −13°C to −3°C). Amplitudes of oscillation were not varied since satisfactory indications of trends could be obtained from the large ranges chosen. The temperature range −13°C to −3°C was of academic interest, chosen to represent a zone of considerable phase-change.

RESULTS & DISCUSSION

Properties

Thermal conductivities of frozen peas, as affected by temperature and bulk density, are shown in Table 1. Comparison of treatment means by Students'-t tests are also shown, where values marked with the same symbol are significantly different at the 95% confidence level. The trend towards increasing thermal conductivity with increasing temperature is observable, although significant differences are not obtained in certain cases (such as readings at a bulk density of 450 kg/m^3). The only difference noted between two samples at different bulk densities but at the same temperature is that between the sample at −10°C, 400 kg/m^3, and that at −10°C, 517 kg/m^3. It must be noted that Hsieh et al. (1977) predict that thermal conductivity of frozen peas increase with decreasing temperature; however, their work relates to individual particles, hence the results are not conflicting. It was observed that the high-temperature samples showed a greater tendency to stock together and form clumps than those at lower temperature. This increased particle-to-particle contact may contribute both to the temperature trend and towards masking any bulk-density effects that may exist. Moisture content of all samples were in the range of 82 ± 1.33% (w.b.); thus variations are not explainable on this basis.

The enthalpy-temperature curve of frozen peas is illustrated in Fig. 2. The curve shows the sharply increasing slope similar to many food products indicating the zone where significant freezing and thawing takes place. The curve of Fig. 2 is divided into three zones, A, B, and C for use in prediction computations.

Experimental verification

A comparison of predicted and measured temperatures for the center of the slab is shown in Fig. 5 for the fluctuation cycle of Fig. 3. Due to the small size of the system, the center is close to the surface, and the magnitude of the fluctuations are large. The predicted temperature curve is observed to be slightly out of phase with the measured temperature; the experimental system showing a faster response to changes in temperature. Possible reasons for

Table 1—Thermal conductivities of frozen peas as affected by temperature and bulk density[a-e]

Bulk density (kg/m^3)	−29°C	−19°C	−10°C
	Mean thermal conductivity (w/m°C)		
400	0.0728[a]	0.0752[b]	0.1072[abcd]
450	0.0874	0.0991	0.1029
500	0.0809[ce]	0.0917	0.0970
517	0.0833[d]	0.0996	0.0933[ae]

[a-e] Values marked with the same symbol are significantly different from one another at a 95% confidence level, using a Students'-t test.

the deviation are errors in estimation of thermal properties, assumptions involved in experimentation and/or modeling, such as for the slab surface temperature, and the temperature dependence of the thermal properties of peas. The maximum lag observed over a 24-hr cycle is 1 hr. A plot (not shown) of measured temperatures against predicted temperatures yields a straight line of slope 1.05 and intercept 0.82, with a correlation coefficient of 0.9837. Thus the agreement between measured and predicted temperatures may be considered satisfactory.

Other predictions

Predictions obtained using the hypothetical cycle of Fig. 4 show three major effects due to (a) container size, (b) fluctuation period, and (c) temperature range over which fluctuation takes place.

Effects of container size are shown in Fig. 6 where temperatures are plotted against location in the container (from the surface to the center) for four selected times in the cycle. The temperature fluctuation for a box of width 1.2192m shows the stability of temperatures within the

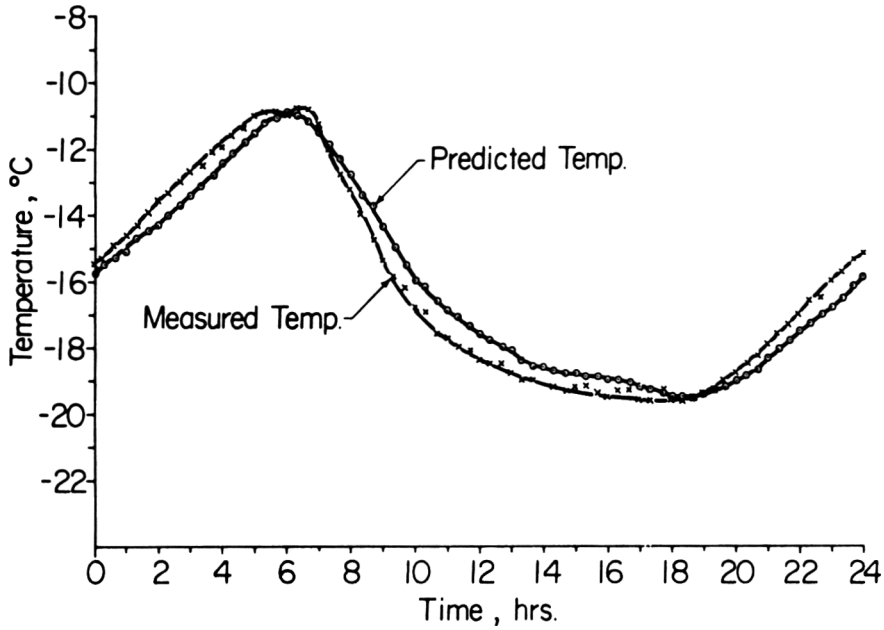

Fig. 5—Comparison of predicted and measured temperatures at the center of the slab.

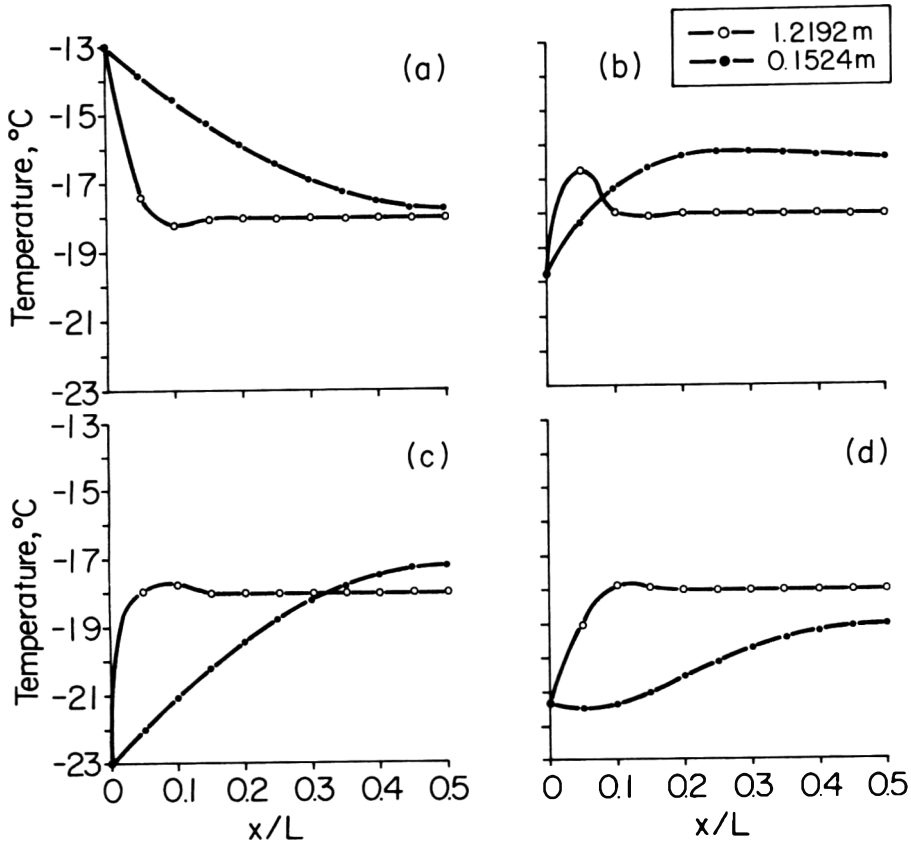

Fig. 6—Comparison of temperature distributions for box sizes of 1.2192m and 0.152m; fluctuation period of 24 hr: (a) at an initial time τ_0, (b) after 1/5 of the cycle period, (c) after 2/5 of the cycle period, and (d) after 3/5 of the cycle period.

stack despite the large surface temperature fluctuations. Temperature fluctuations are more readily transmitted into the box of width 0.1524m, where interior temperatures fluctuate considerably.

Effects of fluctuation period are shown in Fig. 7 for containers of the same size. Fluctuations of short periods result in more stable interior temperatures than do the slower fluctuations. With the rapid fluctuations, the temperature changes much faster than the system can respond.

Effects of temperature range are shown in Fig. 8. Fluctuations in the range $-13^{\circ}C$ to $-3^{\circ}C$ result in more stable interior temperatures than the range $-23^{\circ}C$ tc $-13^{\circ}C$. This effect is primarily due to the consicerable amount of thawing and freezing that occurs in the range $-13^{\circ}C$ to

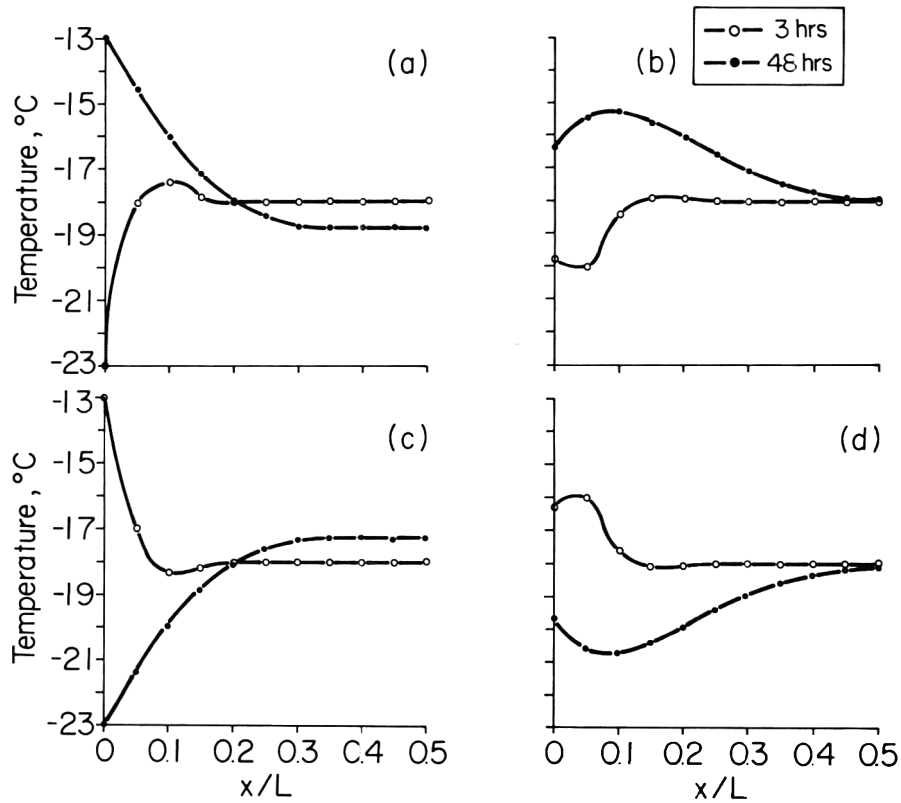

Fig. 7—Comparison of temperature distributions for fluctuation periods of 3 and 48 hr; box size of 0.3048m: (a) at an initial time τ_0, (b) after 3/10 of the cycle period, (c) after 1/2 of the cycle period, and (d) after 4/5 of the cycle period.

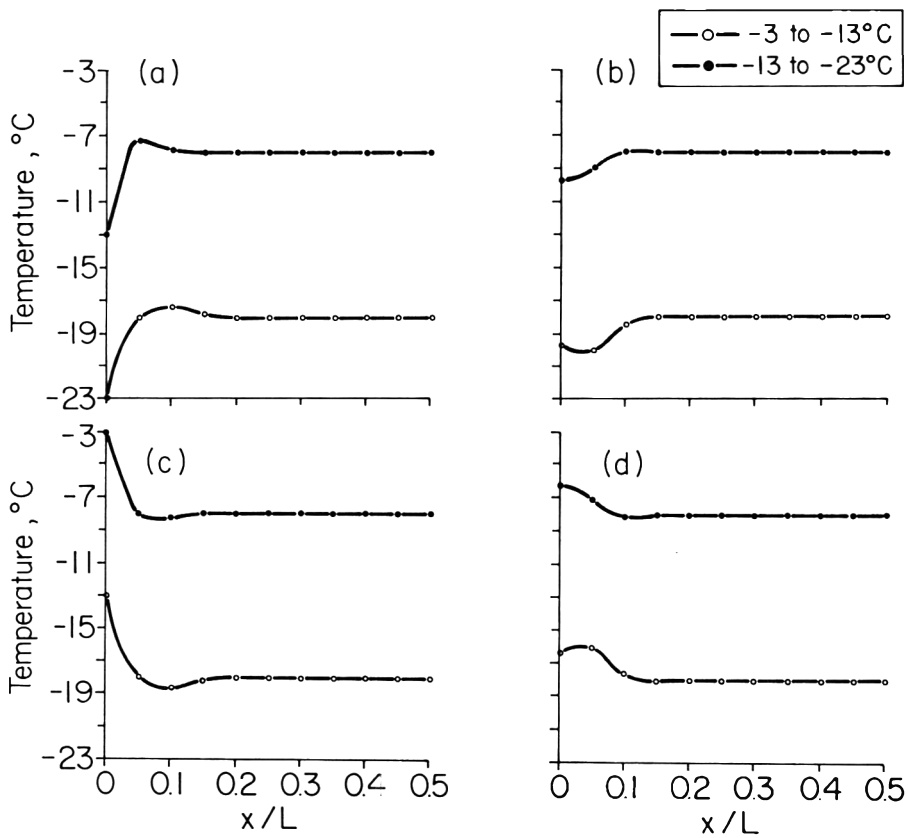

Fig. 8—Comparison of temperature distributions for fluctuation ranges of $-3^{\circ}C$ to $-13^{\circ}C$, and $-13^{\circ}C$ to $-23^{\circ}C$; fluctuation period of 12 hr; box size of 0.6096m: (a) at an initial time τ_0, (b) after 3/10 of the cycle period, (c) after 3/5 of the cycle period, and (d) after 4/5 of the cycle period.

$-3^{\circ}C$, resulting in a very large resistance to heat transfer in this range. Since frozen foods are generally not stored above a mean temperature of $-18^{\circ}C$, the higher temperature ranges are merely of academic interest. It is to be noted that, for symmetrical temperature fluctuation regimes such as that of Fig. 3, the temperature of the stick oscillates about the mean of the two extremes. Hence, it may not be desirable to use di-thermal regimes if the mean temperature is above the recommended value for the product in question.

The model may be used to predict product quality degradation in di-thermal storage. This would involve a combination of the model's time-temperature relationship with kinetic data relating quality degradation rate with temperature.

Feasibility of energy conservation

The results indicate that a combination of large box size and a rapid fluctuation regime would result in minimized fluctuations within the food product. However, the period of fluctuation depends to a large degree, on the construction of the warehouse and the external temperature. Further, rapid fluctuations are more likely to occur in poorly insulated warehouse; thus, it is doubtful if energy can be conserved based on the fluctuation period.

The box size and stacking pattern offer greater potential. Large stacks provide excellent protection for interior product locations. However, large fluctuations near the periphery would result in large localized quality deterioration. End effects are particularly important. As observed by Moleeratanond et al. (1981), deterioration at the periphery of pallet loads is greater than for interior locations. The protective effects of packaging materials are important, and must be evaluated. The feasibility of energy conservation depends on the tradeoff between energy savings and product losses at the periphery. A possible stacking pattern would involve boxes of high-quality peas at the center of a stack, with smaller boxes of lower quality peas at the exterior. Another possibility is the use of a "buffer" material for protection against fluctuation within stacks. Analyses of heat transfer into buildings can be used to determine energy requirements for di-thermal storage. Such analyses would utilize weather data for a given location in prediction of energy requirement. The model can then be used in evaluating quality loss and energy use in a variety of scenarios. It must be emphasized that further research is necessary prior to making recommendations regarding any particular practices; however, the potential for energy conservation clearly exists.

CONCLUSIONS

A MATHEMATICAL MODEL has been developed for prediction of temperature fluctuations in stacks of peas subjected to di-thermal storage regimes. The results indicate that potential exists for energy conservation with di-thermal storage. Further research planned involves kinetic modeling of quality parameters and nutritional factors, and incorporation of the effects of packaging into the model.

NOMENCLATURE

- b Fourier series coefficient
- c specific heat ($J/kg^{\circ}C$)
- h enthalpy (kJ/kg)
- k thermal conductivity ($w/m\ ^{\circ}C$)
- L slab thickness (m)
- m mass (kg)
- n Fourier series index
- t time (hr)
- T temperature ($^{\circ}C$)
- x length coordinate (m)
- X fraction

Greek letters

- α thermal diffusivity (m^2/s)
- ρ bulk density (kg/m^3)
- τ period of temperature fluctuation (hrs)

Subscripts

- a apparent
- A segment A of curve
- al alcohol
- B segment B of curve
- C segment C of curve
- e equilibrium
- h high
- i initial
- ℓ low
- m mean
- n Fourier series index
- p peas
- s set point

Superscript

- * supplementary

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Alpha-Chaconine and Alpha-Solanine Content of Potato Peels and Potato Peel Products

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ABSTRACT

Twelve samples of raw and cooked potato peels from commercial potato varieties were analyzed for their α-chaconine and α-solanine content by high-performance liquid chromatography (HPLC). Raw peels contained 1.30–56.67 mg/100g peel (wet weight) α-chaconine and 0.5–50.16 mg/100g peel (wet weight) α-solanine. Raw flesh from the same potatoes contained 0.02–2.32 mg/100g flesh (wet weight) α-chaconine and 0.01–2.18 mg/100g flesh (wet weight) of α-solanine. Peels were cooked by baking, frying and baking-frying. The two types of fried peels contained more α-chaconine (2.18–92.82 mg/100g cooked peel) and α-solanine (1.09–72.09 mg/100g cooked peel). Four commercial potato peel products – wedges, slices, fried peels and baked-fried peels – contained 3.60–13.71 mg α-chaconine/100g cooked product and 1.60–10.48 mg α-solanine/100g cooked product.

INTRODUCTION

POTATO GLYCOALKALOIDS are naturally occurring toxicants. They are comprised of a steroidal-like alkaloid nucleus to which one to four monosaccharides are attached. Commercial potato varieties contain primarily glycoalkaloids of the solanidine series which includes predominantly α-chaconine and α-solanine. Besides the aglycone solanidine, both compounds contain three monosaccharides. These are rhamnose (two moieties) and glucose for α-chaconine and glucose, galactose and rhamnose for α-solanine. Because of their known acute toxicity (Jellema et al., 1981; Willimott, 1933; McMillan and Thompson, 1979), their possible chronic toxicity (Mun et al., 1975; Keeler et al., 1975, 1976) and their characteristic bitter taste (Sinden et al., 1976; Filadelfi, 1980); glycoalkaloids have been under investigation for years.

Since glycoalkaloid poisoning has been demonstrated, new potato varieties must be checked for glycoalkaloid levels. If the concentration exceeds the guidelines of 20 mg glycoalkaloid/100g tuber (wet weight), the tubers will most likely not be released commercially. It is assumed that if potatoes being released meet this glycoalkaloid specification of 20 mg/100g that all potatoes and potato products purchased will be within this guideline. However, this is not always true since glycoalkaloid levels can increase during potato growth and after harvest, depending upon environmental conditions, cultural practices and postharvest handling procedures (Sinden and Webb, 1972). Also the latitude where the potatoes are grown can increase the amounts of glycoalkaloids (Sinden and Webb, 1974). Furthermore if the peels are used for processed products, then the glycoalkaloid levels may be extremely high.

Recently, there has been a significant increase in the consumption of potato peel products in restaurants and the development of new peel products for retail stores. Since studies concerned with the glycoalkaloid concentration of peels and peel products are nonexistent, with the exception of a few investigations done in the 1920's and 40's on raw peels of varieties not in production today (Maga, 1980), this investigation was undertaken to determine the glycoalkaloid content of raw and cooked potato peels from the most widely used commercial varieties along with peel products obtained from restaurants and retail markets.

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MATERIALS & METHODS

Samples

Peels were obtained from tubers that were purchased from local retail stores and growers. Commercial peel products were obtained from local supermarkets and restaurants.

Materials

α-Chaconine and α-solanine were extracted from potato blossoms using the method of Bushway et al. (1980) and purified using the semi-preparative HPLC procedure of Bushway and Storch (1982). Solvents used in the extraction and partition steps were ACS grade (Fisher Scientific Co., Fair Lawn, NJ) while those employed for the HPLC analyses of glycoalkaloids were HPLC grade (Fisher Scientific).

Methods

Preparation of sample. Potato peels with a thickness of 3.0–3.5 mm were extracted raw and cooked for glycoalkaloid analyses. The amount extracted for each type of peel was as follows: raw peels, 200g; fried peels, 200g of raw peels fried; baked peels, 125g; baked-fried peels, 125g baked peels fried. Ten tubers were used to obtain the peel sample for each extraction. Cooking conditions were as follows: baking, 218°C for 1 hr; frying, 177–182°C with vegetable oil for 3–4 min; baked-fried, baked as described above, oven dried at 100°C for 20 min, and deep fat fried for 2 min at 177–182°C.

Two hundred gram samples of commercial potato peel products were extracted. Frozen products were deep fat fried according to the instructions on the label before being extracted.

Extraction of glycoalkaloids. Samples were blended in a king-size Waring Blendor (3.8L capacity) with 550 ml of tetrahydrofuran-acetonitrile-water-acetic acid (49:20:30:1) for 10 min at 18,300 rpm, followed by vacuum filtration using a Buchner funnel fitted with Whatman #42 filter paper. The filtrate was brought to a final volume of 1 L.

Quantitative determination. A 100 ml aliquot (taken in duplicate) was placed in a 500 ml round-bottom flask and concentrated to 20 ml using a rotary evaporator. The rest of the method was the same as that developed for a previous study (Bushway and Ponnampalam, 1981) with one exception. A 100 ml aliquot of petroleum ether was employed instead of 50 ml. The final glycoalkaloid pellet was dissolved in 10 ml of tetrahydrofuran-acetonitrile-water (50:20:30). A 5 μl aliquot was injected into the HPLC operating under the conditions described in Bushway et al. (1979).

RESULTS & DISCUSSION

BECAUSE OF THE POPULARITY of fried, baked and baked-fried potato peels, glycoalkaloid levels in cooked peels was investigated. Twelve different potato peel samples (a list is shown in Table 1) were analyzed for their glycoalkaloid content. Eight of the peel samples were taken from seven different known varieties: Bel Rus (two samples), Kennebec, Green Mountain, Katahdin, Allagash Russet, Russet Burbank and Superiors while four samples were of unknown varieties. These seven specific varieties represent a large portion of the cultivars that are used for table stock and processed products throughout the U.S. The four

Table 1—α-Chaconine and α-solanine levels in baked peels of commercial potato varieties

Variety[a]	Range mg glycoalkaloid/100g of baked peel		
	α-Chaconine	α-Solanine	α-Chaconine + α-Solanine
Russet Burbank	31.47–45.33	17.67–27.17	49.14–72.50
Kennebec	63.47–66.50	50.13–53.30	103.60–109.80
Katahdin	21.33–57.45	9.33–28.82	30.66–80.27
Superior	17.60–27.06	8.00–11.73	25.60–38.79
Allagash Russet	12.80–20.65	8.53–12.09	21.33–32.74
Round White	10.72–15.86	4.00–7.54	14.72–23.40
Green Mountain	17.94–22.75	8.32–10.40	26.26–33.15
Round White	10.24–11.04	4.32–4.80	14.56–15.84
Bel Rus	4.96–8.96	2.40–5.46	7.36–14.42
Russet	1.60–6.37	0.80–4.16	2.40–10.53
Bel Rus	4.96–6.76	2.40–4.03	7.36–10.79
Russet	2.47–5.46	1.70–3.12	4.17–8.58

[a] All varieties analyzed three times in duplicate

Table 2—α-Chaconine and α-solanine levels in fried peels of commercial potato varieties

Variety[a]	Range mg glycoalkaloid/100g of fried peel		
	α-Chaconine	α-Solanine	α-Chaconine + α-Solanine
Russet Burbank	41.09–44.25	20.40–24.13	61.49–68.38
Kennebec	69.16–92.82	51.32–72.07	120.48–164.89
Katahdin	33.01–59.61	16.34–35.25	49.35–94.86
Superior	25.73–35.90	12.30–15.80	38.03–51.70
Allagash Russet	22.13–32.25	14.03–22.06	36.16–54.31
Round White	18.73–26.26	7.19–11.35	25.92–37.61
Green Mountain	24.73–31.52	10.37–14.55	25.10–46.07
Round White	11.55–16.20	4.80–6.15	16.35–22.35
Bel Rus	3.58–8.25	1.89–4.65	5.47–12.90
Russet	2.18–7.35	1.09–5.40	3.27–12.75
Bel Rus	6.42–13.18	3.36–6.91	9.78–20.09
Russet	4.46–5.34	2.52–4.01	6.98–9.35

[a] All varieties analyzed three times in duplicate

unknown peel samples were grouped into general categories such as Round Whites and Russets. Round Whites were probably Katahdins or Superiors while the Russets were most likely Bel Rus.

The α-chaconine, α-solanine and α-chaconine plus α-solanine content of these cooked peels are presented in Tables 1–3. Glycoalkaloid levels of the baked peels were lower (Table 1) than the fried (Table 2) or baked-fried (Table 3). Since it has been shown in a previous study that glycoalkaloids were heat stable (Bushway and Ponnampalam, 1981), this difference was not due to heat degradation of the glycoalkaloids, but was caused by greater moisture loss during frying than during baking. The glycoalkaloid content of raw peels (Table 4) further substantiates the concentrating effect of cooking on glycoalkaloids in the peels. Fried peels had the highest amount of α-chaconine and α-solanine, 69.16– 92.82 mg/100g cooked peel and 51.32–72.07 mg/100g cooked peel, respectively, while baked Russet peels contained the lowest amount of glycoalkaloids 1.60–6.37 mg α-chaconine/100g baked peel and 0.80–4.16 mg of α-solanine/100g baked peel. For all varieties except Kennebec the quantity of α-chaconine in peels was much greater than α-solanine. More research is necessary to determine if the different glycoalkaloids are associated with different degrees of toxicity. Preliminary data indicate that α-chaconine is more toxic than α-solanine (Pierro et al., 1977), but further work is needed in this area before definitive statements can be made.

Also presented in Tables 1–3 are the values for α-chaconine plus α-solanine content of these cooked peels. This value according to data reported by Sinden and Webb (1972) on potato tubers should represent at least 95–99% of the total glycoalkaloids present in these peels. Thus, the sum of the α-chaconine and α-solanine content should be a good representation of the total glycoalkaloid (TGA) content of these peels and peel products. New potato varieties being released in commercial markets have a TGA value below 20 mg glycoalkaloids/100g potato (wet weight) since higher values are considered unsafe for human consumption. The α-chaconine plus α-solanine range of these cooked peels (Table 1–3) indicate that seven baked, eight baked-fried and nine fried peels had TGA levels greater than 20 mg/100g cooked peel. In fact, seven samples – Russet Burbank, Kennebec, Katahdin, Superior, Allagash Russet, Green Mountain and Round White – contained extremely high levels of glycoalkaloids with Russet Burbank, Katahdin and Kennebec peels containing over 100 mg/100g peel. Bel Rus and Russets had the lowest glycoalkaloid concentration in the peels.

In order to demonstrate that the peel layer contained

Table 3—α-Chaconine and α-solanine levels in baked fried peels of commercial potato varieties

Variety[a]	Range mg glycoalkaloid/100g of fried peel		
	α-Chaconine	α-Solanine	α-Chaconine + α-Solanine
Russet Burbank	34.91–79.10	20.95–46.58	55.86–125.68
Kennebec	76.75–86.45	59.65–69.29	136.40–155.74
Katahdin	37.39–66.88	16.31–37.26	53.70–104.14
Superior	20.10–35.17	8.82–14.37	28.92–49.54
Allagash Russet	22.25–26.84	14.56–15.71	36.81–41.40
Round White	17.76–20.61	7.48–9.80	25.24–31.41
Green Mountain	22.32–27.58	10.82–13.52	34.14–41.10
Round White	15.56–16.22	6.00–6.80	21.56–23.02
Bel Rus	7.17–11.64	4.13–7.06	11.30–18.70
Russet	2.87–8.28	1.84–5.40	4.17–13.68
Bel Rus	7.17–8.78	4.13–5.23	11.30–14.03
Russet	3.21–7.11	2.21–4.05	5.42–11.06

[a] All varieties analyzed three times in duplicate

the majority of the glycoalkaloids in potato tubers, a study was conducted in which the raw peel layer and the remaining raw flesh were analyzed for glycoalkaloid levels. The results are given in Tables 4 and 5. The α-chaconine plus α-solanine in raw peels ranged from 1.80–106.83 mg/100g peel (wet weight) while the amounts in the flesh varied from 0.03–4.52 mg/100g peel (wet weight). Taking into account the weight of the peel region versus the weight of the flesh of a potato, the peel layer contains 84 to 96% of the glycoalkaloids in these potato varieties. Only two varieties, Russet Burbank and Kennebec, had TGA levels in the raw flesh greater than 1.0 mg/100g flesh (wet weight). These low glycoalkaloid levels in the flesh are further substantiated by the results from the analyses of frozen peeled products (Bushway and Ponnampalam, 1981).

Four types of commercial potato peel products – slices, wedges, fried peels, and baked-fried peels – were analyzed for their glycoalkaloid content (Table 6). Wedges and slices were frozen products and the sum of the α-chaconine and α-solanine levels for several different brands of these two types of products ranged from 6.55–12.05 mg/100g product. Although none of these samples approached the 20 mg/100g amount, they were much higher than one would find in peeled products like French fries (Bushway and Ponnampalam, 1981). The other two kinds of products were obtained from local restaurants – fried peels and baked-fried peels. Glycoalkaloid levels in the fried peels were similar to the frozen peel slices while the baked-fried peels had glycoalkaloid amounts that in some instances

Table 4—α-Chaconine and α-solanine levels in raw peels of commercial potato varieties

Variety[a]	Range mg glycoalkaloid/100g of fried peel		
	α-Chaconine	α-Solanine	α-Chaconine + α-Solanine
Russet Burbank	24.33–42.83	13.50–27.00	37.83–69.83
Kennebec	46.67–56.67	36.17–50.16	82.84–106.83
Katahdin	21.33–46.83	9.33–25.83	30.66–72.66
Superior	14.83–17.90	7.00–7.90	21.33–25.80
Allagash Russet	14.83–14.90	9.50–10.17	24.33–25.07
Round White	12.90–13.60	3.90–5.10	16.80–18.70
Green Mountain	14.40–17.50	6.70–8.00	21.70–25.50
Round White	8.60–12.20	3.40–5.80	12.00–18.00
Bel Rus	3.67–6.80	2.00–4.10	5.67–10.90
Russet	2.40–4.20	1.70–2.40	4.10–6.60
Bel Rus	4.20–5.50	2.40–3.10	6.60–8.60
Russet	1.30–4.90	0.50–3.60	1.80–8.50

[a] All varieties analyzed three times in duplicate

Table 5—α-Chaconine and α-solanine levels in raw flesh of commercial potato varieties

Variety[a]	Range mg glycoalkaloid/100g of fried peel		
	α-Chaconine	α-Solanine	α-Chaconine + α-Solanine
Russet Burbank	0.98–2.32	0.58–2.18	1.58–4.50
Kennebec	0.75–2.13	0.55–2.10	1.30–4.23
Katahdin	0.15–0.35	0.15–0.23	0.30–0.58
Superior	0.07–0.23	0.05–0.10	0.12–0.33
Allagash Russet	0.19–0.45	0.25–0.48	0.44–0.93
Round White	0.18–0.60	0.03–0.38	0.21–0.98
Green Mountain	0.05–0.30	0.05–0.18	0.10–0.48
Round White	0.02–0.06	0.01–0.11	0.03–0.17
Bel Rus	0.08–0.38	0.03–0.15	0.11–0.53
Russet	0.03–0.10	0.01–0.10	0.04–0.20
Bel Rus	0.08–0.38	0.03–0.15	0.11–0.53
Russet	0.05–0.30	0.05–0.18	0.10–0.48

[a] All varieties analyzed three times in duplicate

Table 6—α-Chaconine and α-solanine levels in commercial potato peel products

Product	No. Brands[a] Analyzed	Range mg glycoalkaloid/100g of product		
		α-Chaconine	α-Solanine	α-Chaconine + α-Solanine
Frozen Peel Wedges	6	4.30–7.40	3.30–4.65	7.60–12.05
Frozen Peel Slices	2	3.80–4.20	2.75–2.90	6.55–7.10
Restaurant Fried-Peels	3	3.60–4.00	1.60–2.30	5.20–6.30
Restaurant Baked-Fried Peels	3	6.79–13.71	5.31–10.48	12.01–24.19

[a] All brands analyzed in duplicate

surpassed the 20 mg/100g quantity. However, none of the commercial products contained the levels of glycoalkaloids that some of the varieties did in the cooked peel study. These differences could be accounted for by variation among varieties used in the commercial products or by how the tubers were grown or stored. Environmental factors or variety differences can cause changes in the glycoalkaloid content of tubers (Sinden and Webb, 1974; Maga, 1980).

CONCLUSION

IN VIEW of the known acute toxicity of potato glycoalkaloids and of the increased human consumption of potato peels, this paper provides information on the glycoalkaloid content of cooked potato peels and commercial potato peel products. Many of the cooked peel samples, especially cooking processes that cause the greatest moisture loss, had glycoalkaloid levels much greater (two to eight times) than the upper safety limit of 20 mg glycoalkaloid/100g potato established for use in releasing new potato varieties. These high glycoalkaloid concentrations could potentially cause glycoalkaloid poisoning. Therefore, in preparation of such peel products, it would be advisable to choose a variety with low glycoalkaloid concentration. This would guarantee low levels of glycoalkaloids unless the potatoes were mishandled during or after harvest. Furthermore this research also shows, as did others (Maga, 1980), that after peeling the first 3–3.5 mm from potatoes, the remainder contains very little glycoalkaloid. Therefore if varieties with good processing characteristics, but high glycoalkaloid levels (Lenape) are developed, it may be possible to use them for potato products that are peeled.

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A New Protein Band Appearing in the Electrophoretic Pattern of Egg White Heated at Below 60°C

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ABSTRACT

When egg white was heated to between 50° and 60°C, a new protein band appeared in its polyacrylamide gel electrophoretic pattern. The formation of the protein band in stored egg white was almost the same as that of fresh egg white. This protein band was shown to be induced by the interaction between macroglobulin and lysozyme during the egg white heating. The optimum temperature for its formation was between 54°C and 57°C. This band formation may be used as an indicator for heat treatment of egg white.

INTRODUCTION

THE PASTEURIZATION of egg white has posed a considerably greater problem because of its greater instability in the range of effective pasteurization. Any changes induced by heating must be studied in detail. To study the changes in protein induced by heating, electrophoretic analysis seems to be useful.

In previous research at this laboratory, Matsuda et al. (1981) showed that a new protein band appeared in the electrophoretic pattern of egg white heated below 60°C. The intensities of both conalbumin and macroglobulin in the electrophoretic pattern of egg white are known to decrease on heating above 60°C (Chang et al., 1970; Sato and Nakamura, 1977; Nakamura et al., 1979), but no explanation has been given about the protein band appearing in the electrophoretic pattern of egg white heated below 60°C.

When no additives are used prior to heating, the pasteurization temperature of commercially prepared egg white is usually below 60°C (Cunningham, 1977). In the pasteurization of food products, a simple test to check the efficiency of the pasteurization process is desirable. Thus, the above phenomenon might be applied as an indicator for heat treatment of egg white.

In the present study, both the characterization of this protein band and the environmental conditions affecting its formation were investigated.

MATERIALS & METHODS

Egg white and egg white proteins

Fresh egg white was prepared from 1- to 2-day old eggs produced by a strain of White Leghorn Layers and stored egg white was prepared from the shell eggs stored at 30 ± 1°C for 1 wk. The egg white was carefully homogenized without foaming. The pH of fresh and stored egg whites were 8.0 and 9.4, respectively; pH was adjusted with careful addition of small amounts of 0.5N NaOH or 0.5N HCl when necessary.

Macroglobulin was prepared from egg white according to the method of Donovan et al. (1969). Conalbumin (Lot C-0755) and ovalbumin (Lot A-5503) were purchased from Sigma Chemical Company. Lysozyme (Lot E-8301) was purchased from Seikagaku Kogyo Co. Ltd.

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Fractionation of egg white

Fractionation of egg white into albumin and globulin was carried out by the salting-out with $(NH_4)_2SO_4$ as described by Warner (1954). Briefly, an equal volume of saturated $(NH_4)_2SO_4$ was added to egg white and centrifugation (10,000 rpm, 30 min) was carried out to separate the precipitated globulin fraction from the supernatant, albumin fraction. Both fractions were used after dialysis against distilled water.

Heat treatment

Heat treatment of egg white was conducted by placing 1 ml of egg white in test tubes (1.2 x 14 cm), which were positioned in a rack and immersed in a controlled temperature water bath. The test tube was shaken gently for 30 sec, and then kept at definite temperatures for various periods of time. Each sample was cooled immediately after heat-treatment by placing the tubes in ice water.

Polyacrylamide gel electrophoresis

A vertical flat-sheet polyacrylamide gel electrophoretic method (Reid and Bieleski, 1968) was used. Gel sheets (0.1 x 13 x 13 cm) of 7.5% polyacrylamide and electrophoresis buffer of Tris-Glycine were prepared as described by Davis (1964). Electrophoresis was performed at room temperature with a constant current of 20mA for 3 hr using a discontinuous buffer system. The gel sheets were stained with a solution of 0.2% Coomassie Brilliant Blue R-250 in water/methanol/acetic acid (5:5:1, V/V/V) and destained by 7% acetic acid overnight. The relative intensity of the stained band on polyacrylamide gel was determined by scanning the gel sheets with a Shimadzu dual-wavelength chromatoscanner, Model CS-910.

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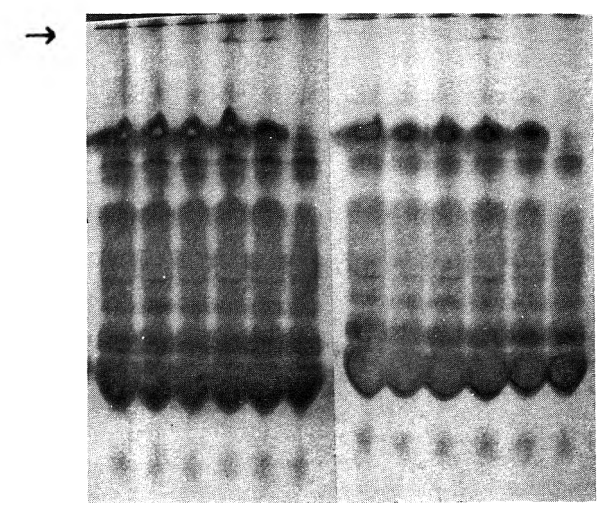

Fig. 1—Electrophoretic patterns of egg white heated for 2 min at various temperatures. (A) fresh egg white; (B) stored egg white (30°C for 1 wk) Arrow shows the position of a new protein band.

RESULTS

Some conditions affecting formation of a new protein band

Fig. 1 shows the electrophoretic patterns of both fresh and stored egg white which were heated for 2 min at various temperatures. In these figures a new protein band clearly appears in the electrophoretic patterns of both egg whites heated at 50°C for 2 min. The intensity of this protein band is further increased by heating to 55°C, but it disappeared when heated to 65°C. The temperature which causes the disappearance of this protein band is slightly higher in the fresh egg white than in the stored egg white. As the pH of the egg white increased during storage, this difference in the heating temperature between fresh and stored egg white might be caused by the difference in the pH of both egg whites.

To study the effect of pH on the disappearance of this protein band, the pH of fresh egg white was adjusted to a definite value and heated at 55°C or 60°C for 2 min. The results are shown in Fig. 2. At 60°C the band disappears

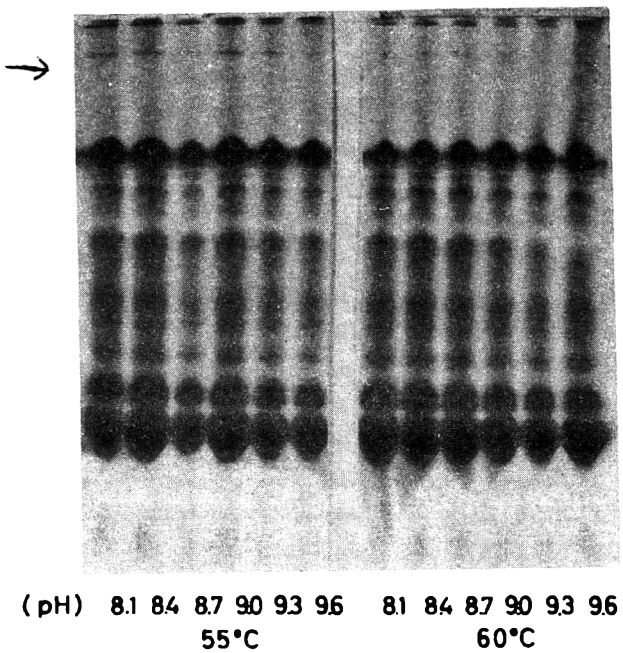

Fig. 2—Electrophoretic patterns of heated egg white with pH adjusted to definite values (8.1–9.6). Heating condition was 2 min at 55°C or 60°C. Adjustment of pH was carried out before heat treatment. Arrow shows the position of a new protein band.

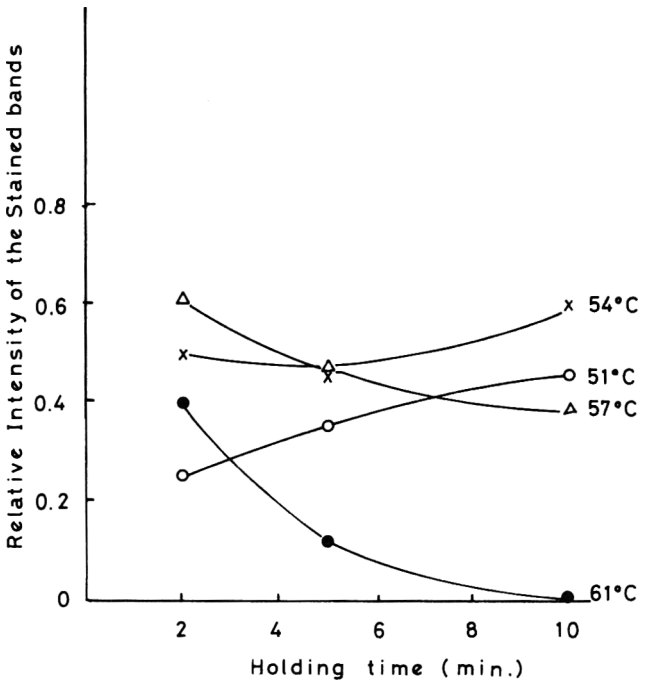

Fig. 4—Changes in the relative intensity of the stained bands as determined by densitometric tracing of electrophoretograms shown in Fig. 3. Relative intensity of the stained bands obtained in terms of the intensity of ovalbumin A_3 band.

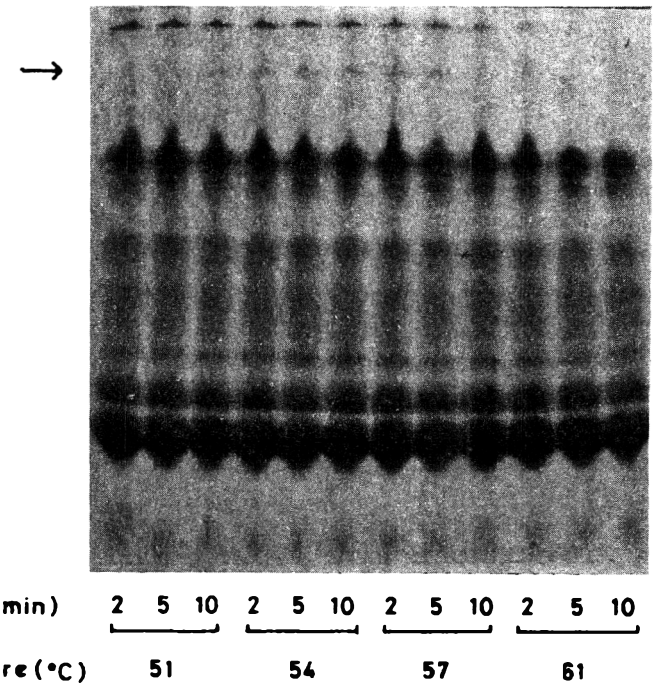

Fig. 3—Electrophoretic patterns of egg white heated for various periods (2, 5 and 10 min) at various temperatures (51°, 54° and 61°C). Arrow shows the position of a new protein band.

when the pH is above 9.0. High pH of stored egg white might affect the formation and disappearance of this protein band. When the pH of egg white was previously adjusted to the acidic pH region, this protein band did not appear in the electrophoretic pattern of heated egg white (Nakamura, unpublished work).

As shown in Fig. 3 and 4, the intensity of this protein band varied with heating conditions; the largest value was obtained by heating at 57°C for 2 min or 54°C for 10 min, and much smaller values were obtained by heating at 51°C for 2 min or 61°C for 5 min or longer. These results suggest that the optimum temperature for the formation of this protein band is between 54°C and 57°C. Adjustment of the pH of egg white after heating may be useful to regulate the appearance of this protein band; when the pH was adjusted to 4 or 10 after heating at 54°, 57° or 61°C, the intensity of the protein band decreased in the electrophoretic pattern of egg white heated at higher temperature (Fig. 5).

When the egg white heated at 55°C for 2 min was stored at 4°C for 1 wk, the intensity of the protein band was the same as that of the unstored one indicating that the protein band is relatively stable during storage of heated egg white. Electrophoretic patterns of heated egg white which contained small amounts of yolk are shown in Fig. 6. In this figure, a new protein band can be noted in the egg whites when the yolk amounted to less than 1/10 of egg white, although it appears to be fading with increased amounts of yolk.

Characterization of protein band

To investigate the kinds of protein contributing to the formation of the protein band, egg white was fractionated into globulin and albumin by salting-out with $(NH_4)_2SO_4$. The electrophoretic patterns of both fractions heated at various temperatures are shown in Fig. 7. This figure clearly shows that the protein band was formed from the globulin fraction of the egg white. Although many proteins are known to be present in the globulin fraction, macroglobulin is considered to be the most likely source of this protein band. Macroglobulin is known to consist of two subunits of equal weight and is dissociated easily by lowering its pH below 3.0 (Donovan et al., 1969). As the mobility of this protein band is faster than that of macroglobulin, the subunit dissociation of macroglobulin might occur by mild heat treatment. To verify this assumption, we prepared macroglobulin from fresh egg white, adjusted its pH to 3.0 and kept it overnight. In the electrophoretic pattern of the treated macroglobulin two protein bands were clearly noted: one is native macroglobulin and the other may be the dissociated subunit. The mobility of the latter protein band was almost the same as that of the new protein band noted in this work.

The electrophoretic patterns of macroglobulin heated at various temperatures are shown in Fig. 8. Although a rather broad band appeared on heating at almost the same temperature as that of egg white, it did not disappear with heating at 60°C or above. The heat-induced protein band of macroglobulin, however, became sharper when other constituent proteins of egg white were present (Fig. 9). This result indicates that some proteins may well interact with the subunit of macroglobulin dissociated by mild heating. Among the egg white proteins tested in the present study, the effect of lysozyme was the most significant.

When macroglobulin-lysozyme mixture was heated at various temperatures, a sharp protein band was formed at 50°C and disappeared at 64°C (Fig. 10). These temperatures, which caused both the appearance and disappearance of protein band in the electrophoretic pattern of macroglobulin-lysozyme solution, were almost the same as those

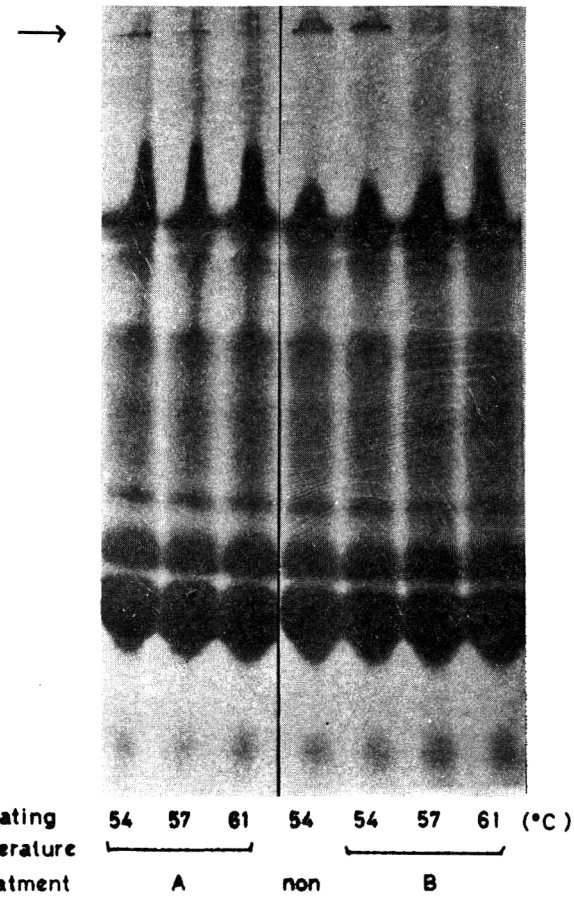

Fig. 5—Electrophoretic patterns of egg white with pH adjusted to 4.0 or 10.0. Heating conditions was 2 min at 54°, 57° or 61°C. Adjustment of pH was carried out after heat treatment. (A) pH adjusted to 4.0; (B) pH adjusted to 10.0. non = without pH adjustment. Arrow shows position of a new protein band.

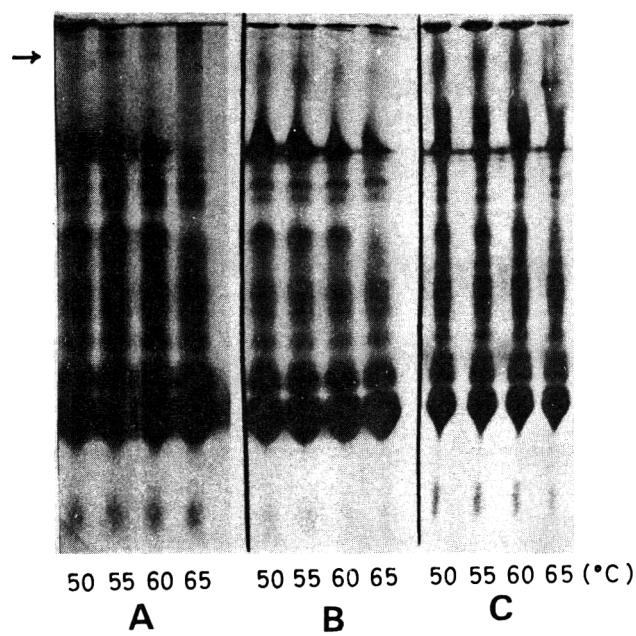

Fig. 6—Electrophoretic patterns of heated egg white which contained small amounts of yolk. Heating condition was 2 min at 50°, 55°, 60° and 65°. (A) Amount of yolk was 1/20 of egg white; (B) Amount of yolk was 1/10 of egg white; (C) Amount of yolk was 1/2 of egg white. Arrow shows position of a new protein band.

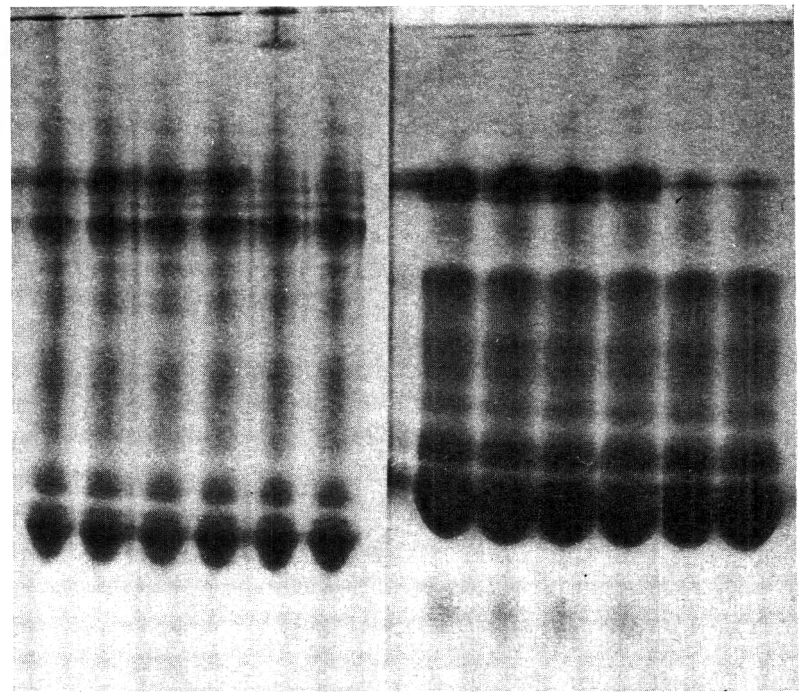

Fig. 7—Electrophoretic patterns of egg white fractions (pH 8) heated for 2 min at various temperatures (45–65°C). (A) Globulin fraction; (B) Albumin fraction. Arrow shows position of a new protein band.

used when the new protein band was obtained. Moreover, the intensity of the protein band in the electrophoretic pattern of macroglobulin-lysozyme mixture heated at 60°C was decreased when the pH of the solution was above 9.0 (Fig. 11). These results clearly show that the protein band appeared in the electrophoretic pattern of egg white heated at between 50° and 60°C is caused by the interaction between macroglobulin and lysozyme during heating of egg white. This was further ascertained by the experiment shown in Fig. 12. In this figure, a new protein band appeared at the same position as that of heated egg white when heated macroglobulin-lysozyme mixture was added to the unheated egg white. When heated macroglobulin-lysozyme mixture was added to the heated egg white, only one protein band appeared at the place where a new protein band was present in the electrophoretic pattern of heated egg white.

DISCUSSION

IN PREVIOUS STUDIES on heated egg white using polyacrylamide gel electrophoresis (Chang et al., 1970; Nakamura

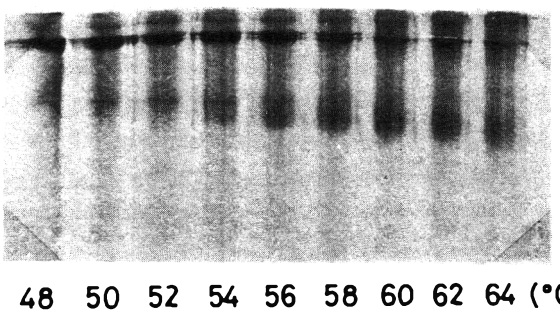

Fig. 8—Electrophoretic patterns of macroglobulin (pH 8) heated for 2 min at various temperatures (48–64°C).

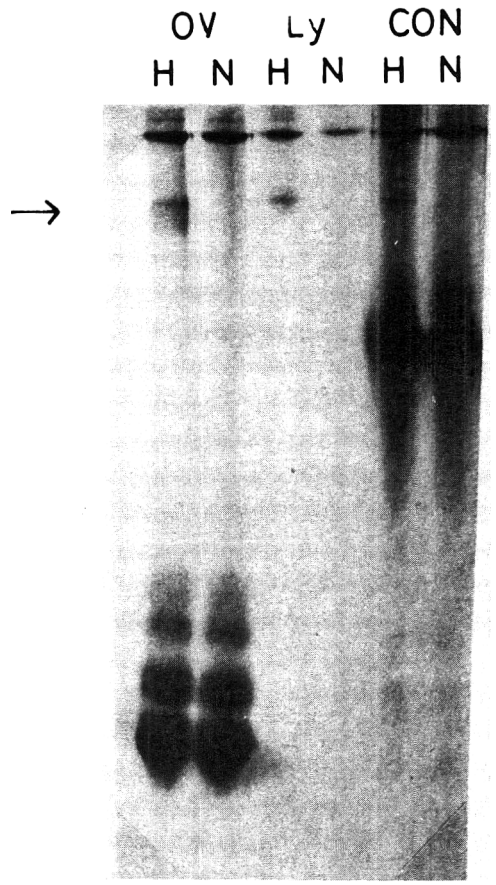

Fig. 9—Electrophoretic patterns of macroglobulin (pH 8) heated for 2 min at 55°C in the presence of ovalbumin (OV), lysozyme (Ly) or ccnalbumin (CON). N: Unheated solution; H: Heated solution. Arrow shows position of a new protein band.

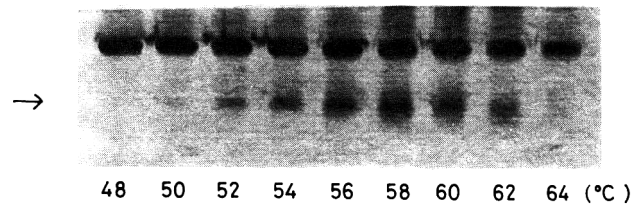

Fig. 10—Electrophoretic patterns of macroglobulin-lysozyme mixture (1:1, pH 8) heated for 2 min at various temperatures (48–64°C). Arrow shows position of a new protein band.

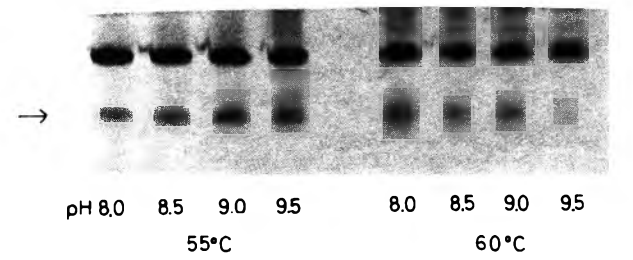

Fig. 11—Electrophoretic patterns of macroglobulin-lysozyme mixture (1:1) with different pH's heated for 2 min at 55°C or 60°C. Arrow shows position of a new protein band.

et al., 1979), no protein band was found in the same position cited in the present investigation. Since the heating conditions used in the former studies were almost the same as in the present one, the main reason why no protein band was found in the electrophoretic pattern of heated egg white seems to be the difference in the stain reagent; the former workers used Amido Black solution, whereas the present authors used Coomassie Brilliant Blue R-250, which is much more sensitive. Furthermore, the thin gel sheet used in the present experiment might be favorable for the detection of any protein band.

Since the band protein was relatively stable during the storage of the egg white after heating and the procedure of electrophoresis is very simple, one may use this band-formation phenomenon as an indicator for heat treatment of egg white. The conventional method is to measure the thermal inactivation of enzyme activities. For example, Monsey and Jones (1979) proposed the use of starch-degrading enzyme activity of egg white. The advantage of using polyacrylamide gel electrophoresis is the rather minimal effect of yolk contamination. The starch-degrading enzyme of Monsey and Jones (1979), on the other hand, was easily affected by very small amounts of yolk contamination, but the electrophoretic pattern of egg white was not affected by the yolk contamination when the yolk amounted to less than 1/10 of the egg white (Fig. 6).

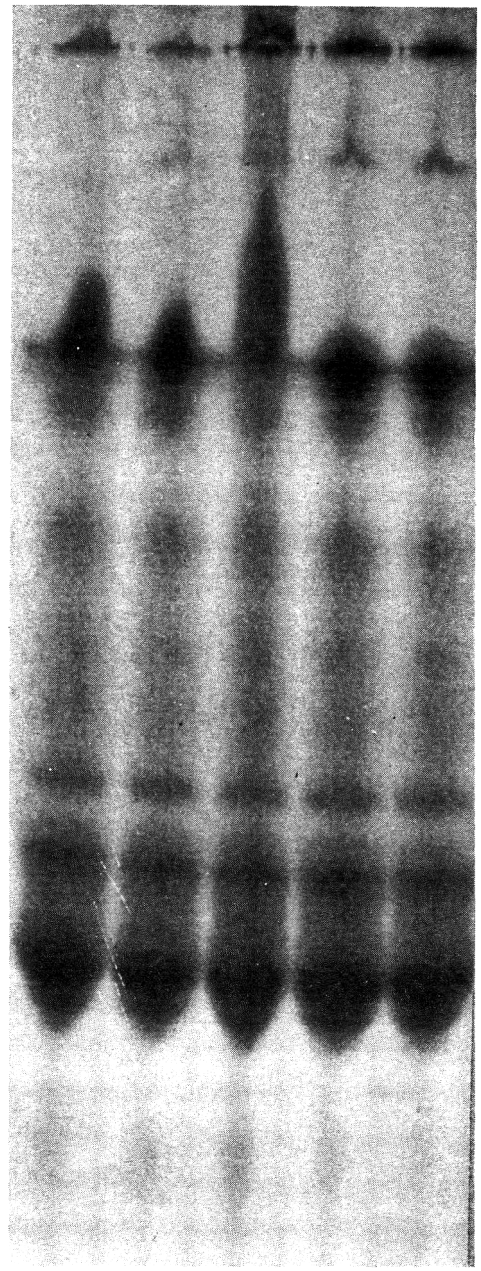

Fig. 12—Electrophoretic patterns of egg white, heated egg white and egg white with heated macroglobulin-lysozyme mixture. (A) Unheated egg white; (B) Egg white heated at 55°C for 2 min; (C) Egg white with heated macroglobulin (pH 8, 55°C for 2 min); (D) Egg white with heated macroglobulin-lysozyme mixture (1:1, pH 8, 55°C for 2 min). Arrow shows position of a new protein band.

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HPLC Determination of Riboflavin in Eggs and Dairy Products

S. H. ASHOOR, G. J. SEPERICH, W. C. MONTE, and J. WELTY

ABSTRACT

An isocratic HPLC method has been developed to determine riboflavin in eggs, whole milk, 2% fat milk, skim milk, dry milk, yogurt, cottage cheese, and cheddar cheese. The developed method involves acidification, centrifugation, and quantification of riboflavin in the supernatant with HPLC. The HPLC system consisted of a μ Bondapak C_{18} column, a solvent system of water-methanol-acetic acid (68:32:0.1 v/v), a flow rate of 1.0 ml/min, and a UV detector. The method is simple, rapid, sensitive, and specific for riboflavin. Recoveries of more than 90% were obtained in all samples analyzed.

INTRODUCTION

THE PRESENT ANALYTICAL METHODS for the determination of riboflavin include microbiological methods (Snell and Strong, 1939; Association of Vitamin Chemists, 1966; Bell, 1974); biological methods (Wagner et al., 1940); colorimetric methods (Effern, 1947); polagraphic methods (Breyer and Bregler, 1960); fluorometric methods (Woodrow et al., 1969; Roy et al., 1976; AOAC, 1980; Rashid and Potts, 1980), and high speed ion exchange chromatography (Williams et al., 1973).

Fluorometric methods are considered the most desirable because of their sensitivity, rapidity, and specificity. However, they require extra treatments to separate riboflavin from interfering fluorescent compounds. In the AOAC method (1980), samples are treated with $KMnO_4$ and H_2O_2 to remove background fluorescence. Such treatment however, may destroy riboflavin (Woodrow et al., 1969). Rashid and Potts (1980) treated milk samples with acidified lead acetate solution to separate extraneous protein and recommended avoiding washing glassware and cuvettes with detergents in order to minimize background fluorescence. Williams et al. (1973) succeeded in separating riboflavin and other water soluble vitamins with high speed ion exchange chromatography. Riboflavin was qualitatively detected in a standard solution with a UV detector at 254 nm but no quantitative results were given.

The objective of this study was to develop a sensitive and specific HPLC method for the quantitative determination of riboflavin in selected good sources of the vitamin such as eggs and dairy products.

MATERIALS & METHODS

Apparatus

A Waters Associates Liquid Chromatograph (Water Associates, Milford, MA) was used for HPLC analysis. The chromatograph was equipped with Model 6000 A pump, Model U6K injector, data module, and Gilson Model 222 detector (Gilson Medical Electronics, Middleton, WI). A Sorval Model RC-5 refrigerated centrifuge with rotor # SS-34 (DuPont Company, Newton, CT) was used for centrifugation.

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HPLC operating conditions

Column: 30 cm x 3.9 mm μ Bondapak C_{18} fitted with C_{18} Porasil B guard column (Waters Associates). Solvent system: water-methanol-acetic acid (68:32:0.1 v/v). Flow rate: 1 ml/min. Detector: 270 nm at 0.02 sensitivity.

Sample preparation

All samples used in this study were purchased from local stores and were prepared for HPLC analysis on the same day of purchase. All samples were protected from light during the entire preparation process.

Liquid milk samples. A volume of 10 ml of each was acidified to pH 3.0 with aqueous glacial acetic acid solution (1:1 v/v) added dropwise with constant stirring. The acidified milk sample was stirred gently for an additional 5 min then centrifuged at 15,000 rpm for 15 min. The supernatant was transferred to a 25 ml volumetric flask and the sediment was washed twice with 5 ml of 2% acetic acid solution. The washings were combined and centrifuged at 15,000 rpm for 15 min. The second supernatant was added to the first in the volumetric flask and the volume was made to mark with 2% acetic acid solution.

Solid and semi-solid dairy products. An appropriate weight of each (1g dry milk, 10g cottage cheese, 5g shredded cheddar cheese, and 7g yogurt) was first suspended in 15 ml of water-methanol solution (2:1 v/v) in a Virtis homogenizer for 2 min at low speed. The suspensions were acidified and centrifuged as indicated in liquid milk samples except that the sediments were washed with 2% acetic acid-methanol solution (2:1 v/v).

Egg sample. 10g of each of gently mixed whole egg, separated egg white and egg yolk were suspended with 25 ml of water-methanol solution (1:1 v/v). The suspensions were acidified and centrifuged as indicated above for in liquid milk samples. The sediments were washed twice with 10 ml of 2% acetic acid solution and the final volume of each supernatant was made to 50 ml.

Calibration curve

Seven riboflavin aqueous standard solutions were prepared by dissolving each of seven weights (0.840, 1.252, 2.068, 3.208, 4.080, 5.00, and 6.00 mg) of standard riboflavin (Sigma Chemical Company, St. Louis, MO) in 1 liter of distilled water. A Mettler Model H54 analytical balance (Mettler Instrument Corporation, Princeton, NJ) was used for weighing the riboflavin quantities. A volume of 25 μl of each standard solution was injected and the corresponding area units were obtained directly from the data module. The data module area units (average of two runs) were plotted against the corresponding ng of riboflavin injected.

Quantification of riboflavin in samples

An injection of 25 μl of freshly prepared riboflavin aqueous standard solution was used to calibrate the data module (external standard method). The calibration was checked at least three times before any sample was injected. A volume of 25 μl of each of the sample supernatants was then injected and the riboflavin content of the injection was obtained directly from the calibrated data module. An average of six determinations (two sample preparations, each injected three times) was used in calculating the riboflavin content of liquid samples (μg/ml), solid and semi-solid samples (μg/g).

Recovery study

Each of the samples analyzed was spiked with a known volume of a riboflavin standard solution containing an approximately equal amount of riboflavin to that of the sample. The spiked samples were prepared for HPLC analysis and their riboflavin content was determined as mentioned previously. The average amount of riboflavin

(average of two determinations) detected in the spiked samples was used in calculating percent recoveries.

Analysis of riboflavin by AOAC method

The riboflavin content of selected samples was also determined by the AOAC method in order to compare the results obtained from both HPLC and AOAC methods.

RESULTS & DISCUSSION

SEVERAL SOLVENT SYSTEMS of different compositions, water-acetonitrile, water-methanol, and water-methanol-acetic acid were tried for HPLC analysis of riboflavin. The solvent system used throughout this study, water-methanol-acetic acid (68:32:0.1 v/v), yielded better separation than the other solvent systems tried. The presence of acetic acid in the solvent system was essential for higher responses. The detector response at any given wavelength increased significantly as the acetic acid content of the solvent system increased and reached a maximum at an acetic acid content of 0.1%. The detector response to riboflavin was also tested at different wavelengths ranging from 250–290 nm. The maximum response was obtained at 270 nm.

Excellent linear responses over wide concentrations of riboflavin were obtained by the developed method as shown in the calibration curve (Fig. 1). As low as 10 ng of riboflavin could be detected reliably indicating the high sensitivity of the developed method.

Riboflavin was separated as a single peak with a retention time of 12.4 min in the chromatograms of egg samples (Fig. 2) and dairy products (Fig. 3). The identity of the riboflavin peak in each sample analyzed was confirmed by two methods. First, when samples were spiked or co-injected with a riboflavin standard solution, the only peak in the chromatogram which increased in area, was that at a retention time of 12.4 min. Second, when the solvent fraction under the peak with the retention time of 12.4 was collected and rechromatographed using solvents with different strengths (water-methanol, 60:40 v/v; water-acetonirile, 70:30 v/v), only one peak with a retention time matching that of the standard riboflavin was obtained. These results indicated that the riboflavin peak in all samples had no interfering compounds. They also indicated that the developed HPLC method was specific for the quantification of riboflavin in all samples analyzed.

The recoveries of riboflavin from the spiked samples and the riboflavin content of the egg and dairy product samples obtained by the developed HPLC method are presented in Table 1. The results show that the recovery of added riboflavin was more than 90% in all samples analyzed. Methanol was included in the suspensions of solid and semi-solid dairy products and eggs and in the washings of their sediments to improve recoveries. Heating eggs in a boiling water bath for periods of up to 10 min did not improve riboflavin content determined by the HPLC method.

The results obtained from riboflavin analysis in selected samples by the HPLC and AOAC methods are presented in Table 2. Riboflavin content obtained by the HPLC method was slightly lower than that obtained by the AOAC method. This is probably because of the absence of interfering substances and the higher specificity of the HPLC method. In general, the content of riboflavin in the samples analyzed by the developed HPLC method was in agreement with that found in the literature (Deatherage, 1975; Arlin, 1977; Osborne and Voogt, 1978; Rashid and Potts, 1980).

The results obtained from this study indicated that the developed HPLC method has several advantages and can be used to quantify riboflavin reliably in various sources. The developed method is specific to riboflavin which is quantified as a single peak with no interference. The method is sensitive and has a linearity of response over wide concentrations of riboflavin. It enjoys the simplicity and speed of analysis inherent in HPLC.

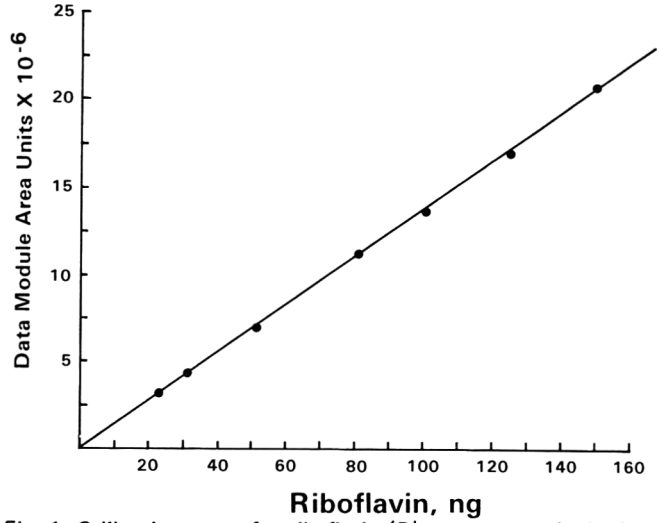

Fig. 1—Calibration curve for riboflavin (R) aqueous standard solutions.

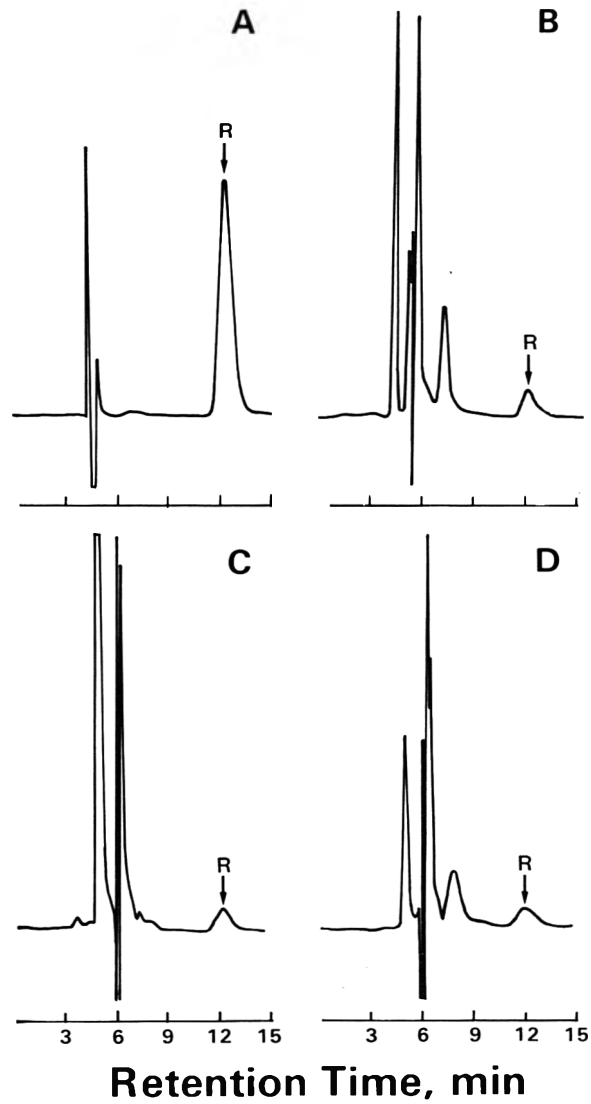

Fig. 2—Chromatograms of riboflavin (R) aqueous standard solution (A), whole egg (B), egg white (C) and egg yolk (D).

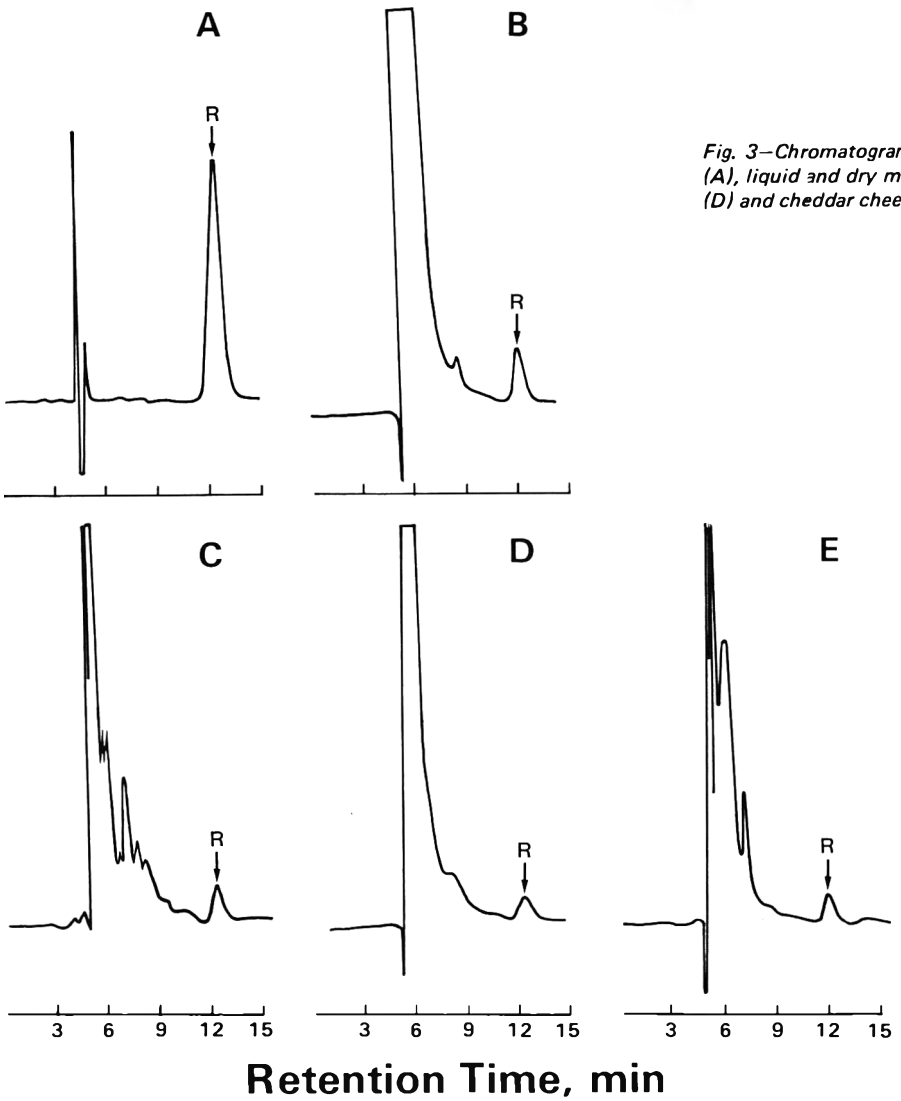

Fig. 3—Chromatograms of riboflavin (R) aqueous standard solution (A), liquid and dry milk samples (B), plain yogurt (C), cottage cheese (D) and cheddar cheese (E).

Table 1—Riboflavin analysis of eggs and dairy products

Sample	Riboflavin content[a] (μg/g)	C.V.%[b]	Recovery %[c]
whole egg	3.16 ± 0.21	6.65	91.3
egg white	2.92 ± 0.12	4.10	95.9
egg yold	3.48 ± 0.28	8.05	92.6
whole milk	1.11 ± 0.09[d]	8.11	105
2% fat milk	1.02 ± 0.01[d]	0.98	91.5
skim milk	1.01 ± 0.06[d]	5.94	93.5
Nonfat dry milk	15.0 ± 0.79	5.27	94.1
yogurt (plain)	1.17 ± 0.10	8.55	109
cottage cheese	0.56 ± 0.06	10.7	108
cheddar cheese	2.88 ± 0.24	8.33	91.3

[a] Average of six determinations
[b] Coefficient of variation
[c] Average of two determinations
[d] μg/ml

Table 2—Riboflavin analysis with HPLC and AOAC methods

Sample	Riboflavin content[a] HPLC[b]	AOAC[c]	C.V.%[d] HPLC	AOAC
whole egg	3.16 ± 0.21	3.28 ± 0.32	6.65	9.76
2% fat milk	1.02 ± 0.01	1.19 ± 0.08	0.98	6.72
yogurt (plain)	1.17 ± 0.1	1.22 ± 0.09	8.55	7.38
cheddar cheese	2.88 ± 0.24	2.83 ± 0.41	8.33	14.5

[a] μg/ml or μg/g in liquid or solid and semi-solid samples, respectively
[b] Average of six determinants
[c] Average of four determinants
[d] Coefficient of variation

The developed HPLC method may also be used to monitor the rate of riboflavin decomposition in eggs and dairy products upon exposure to heat or light during storage. In the early stages of this study an attempt was made to extract riboflavin from dry milk by blending with a large volume of methanol and the methanol extract was reduced in volume by heating under vacuum. When a volume of the reduced methanol extract was analyzed by HPLC two peaks with close retention times were obtained. One of the peaks was identified as that of riboflavin with a much smaller area than expected, and the second with a retention time slightly longer than that of riboflavin was not identified. The same results were obtained with a standard riboflavin solution treated similarly. It was concluded that the second peak was that of the decomposition product(s) of riboflavin resulting from heating during methanol evaporation. The use of the developed method to detect the decomposition product(s) or riboflavin in eggs and dairy products stored under various conditions is being investigated in our laboratories.

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Water Binding of Wheat Flour Doughs and Breads as Studied by Deuteron Relaxation

H. K. LEUNG, J. A. MAGNUSON, and B. L. BRUINSMA

ABSTRACT

Water mobility in wheat flour doughs and breads was investigated by deuteron relaxation using pulsed NMR. Water was replaced by deuterium oxide in dough and bread at different concentrations. Mixograms indicated that wheat flour associated more strongly with D_2O than it did with H_2O. Varying the D_2O:H_2O ratio of flour doughs had no effect on the longitudinal (T_1) or transverse (T_2) relaxation times of deuteron. Hard wheat and soft wheat flour doughs showed similar increases in T_1 and T_2 with increasing moisture content. Staling of bread crumb was accompanied by decreased T_1 and T_2, indicating an overall decrease in water mobility and increase in water binding. The decrease in water mobility of bread crumb with storage time was shown to be independent of reduction in moisture content.

INTRODUCTION

THERE IS CONSIDERABLE EVIDENCE that the physical states of water are closely related to the structural, physical, chemical and sensory properties of foods. The significance of water binding in foods has been discussed in several articles (Karel, 1975; Fennema, 1976; Labuza, 1977; Leung and Steinberg, 1979). It is generally agreed that a portion of the water in foods is "bound." The exact meaning of bound water remains somewhat controversial. The amount of so-called bound water in a food system appears to vary with the technique and the parameter measured by individual investigators. What is clear is that water interacts in various ways with solutes or solids in biological systems and these interactions can be extremely important in determining macroscopic physical properties.

Water comprises about 45% of a bread dough and about 35% of bread. It plays a vital role in dough development and mixing properties. However, it is still not clear how the physical state of the water changes as the mixture of water and flour is developed into a dough. Water also plays an important role in the keeping quality of bread. The major stability problem with bread is staling which affects 8% of the bread produced in the U.S. (Ponte, 1971). Despite extensive studies on bread staling (Willhoft, 1973; Zobel, 1973; Maga, 1975; Knightly, 1977), little attention has been given to the effect of water binding. Bread staling is generally associated with starch crystallization (Maga, 1975). However, it is not clear how the crystallization process affects the water-binding properties of bread. An early study suggested that bread staling was accompanied by a reduction in hydration capacity (Fuller, 1938). Bachrach and Briggs (1947) found a small but significant increase in water binding in bread during staling. However, Bushuk and Mehrota (1977b) showed a significant decrease in bound water content in bread crumb with storage time using differential thermal analysis. Knjaginciev (1970) discussed the possibility that free and bound water may alter the rate or extent of staling.

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Pulsed nuclear magnetic resonance (NMR) has been used extensively to study the properties of water in biological systems (Kuntz et al., 1977). Leung et al. (1976) examined water protons in food systems and showed that the longitudinal (T_1) and transverse (T_2) relaxation times are indicative of the water binding properties. Recently Leung et al. (1979) studied water in wheat flour doughs and identified two components with different T_2 values and different molecular mobility. The less mobile fraction was independent of flour strength and mixing time, whereas the more mobile fraction increased with moisture content. Due to the lack of correlation between water mobility and flour strength, they questioned the importance of water binding on the rheological properties of wheat flour doughs. This aspect was further investigated using deuterated water in the study reported here. Quadrupolar relaxation is the primary mechanism for deuteron relaxation and changes in hydration states of bread components might be directly reflected in the longitudinal (T_1) and transverse (T_2) relaxation times. If water were to interact strongly, the relaxation rates would be shortened. Rearrangements of hydrated states should lead to changes in relaxation times.

MATERIALS & METHODS

Preparation of flour doughs

The wheat flour used in this study has been described previously (Leung et al., 1979). Prior to dough preparation, the wheat flour was dried in a vacuum oven at 50°C for 48 hrs and stored in a desiccator. Doughs were mixed at room temperature in a National 10g Mixograph to optimum consistency (minimum mobility) using deuterium oxide (99.75% minimum, J.T. Baker Chemical Co.) or distilled water. Additional wheat gluten or wheat starch was added to the flour to study its effect on mobility of the dough. Immediately after mixing, duplicate dough samples were transferred to 10 mm NMR tubes and covered. The NMR measurement was performed within 2 hr after the samples were prepared. Moisture content of the dough was calculated from the dry flour weight and the amount of deuterim oxide added.

Bread baking

The wheat flour used in baking was a commercial straight grade baker's flour with a moisture content of 10.1% and a protein content of 11.7%. The flour was dried in a vacuum oven at 50°C for 48 hr, and stored in a desiccator until use. The bread baking formula included 8.6g dried wheat flour, 0.70g fresh baker's yeast (Fleischmann's Standard Brands), 0.6g sucrose, 0.4g nonfat dry milk, 0.3g vegetable shortening (Crisco), 0.03g malted barley flour (52 Dextrinizing Unit/g, 20°C), 0.15g sodium chloride, 0.36 mg potassium bromate, and varied amounts of distilled water and deuterium oxide. Deuterium oxide was added to the dough replacing 25, 50, 75, or 100% of water. Four times the quantity of the above ingredients were mixed to the point of maximum resistance with a 35g National Swanson-Working mixer, and the dough was divided into four equal portions by weight. The ten-gram baking method developed by Shogran et al. (1969) and the short-time baking system of Magoffin et al. (1977) were combined in this study. The doughs were fermented for 70 min at 30°C, punched, panned, proofed for 24 min, and baked at 218°C for 13 min.

Each loaf of bread was wrapped with two layers of aluminum foil and kept in air-tight glass containers. The bread was stored at 23°C for up to 10 days. Duplicate samples were removed from stor-

age at different time intervals for NMR, moisture, water activity and compression tests. Only bread crumb was used for the analyses.

Physical analyses of bread

Moisture content of wheat flour and bread crumb was determined by the AOAC (1975) vacuum oven method, except that the drying temperature and time were changed to 60°C and 48 hr, respectively. Equilibrium vapor pressure of H_2O and D_2O in bread at 21–25°C was determined by the vapor pressure manometer method according to Lewicki et al. (1978). These values were adjusted to 22°C assuming constant water activity of breads in the range of 21–25°C. Vapor pressure of pure D_2O was obtained from the data by Miles and Menzies (1936). Water activity of the breads containing 50% D_2O and 50% H_2O was calculated based on the assumption that H_2O and D_2O contributed 50% each to the total vapor pressure of water. Water activity values of duplicate samples were within 0.01.

Compression tests of bread crumbs were conducted with an Allo-Kramer Shear Press Model No. S-2HE and Recorder Indicator Model E-2EZ (Mackey et al., 1973). A 500 lb proving ring and flat plunger were used. Bread crumb samples of approximately 1.2 cm cubes were prepared with a sharp knife. Each sample was compressed to a height of 0.9 cm at a rate of 10 mm/min. The peak height was recorded as compressive force. The apparent elastic modulus of bread crumb was calculated using the following equation:

$$E = \frac{F/A}{\Delta L/L}$$

Where E is the modulus of elasticity, F is the compressive force, A is the surface area of the sample, ΔL is the change in sample height and L is the original height of the sample. Variation of E among replicate samples was about 20%.

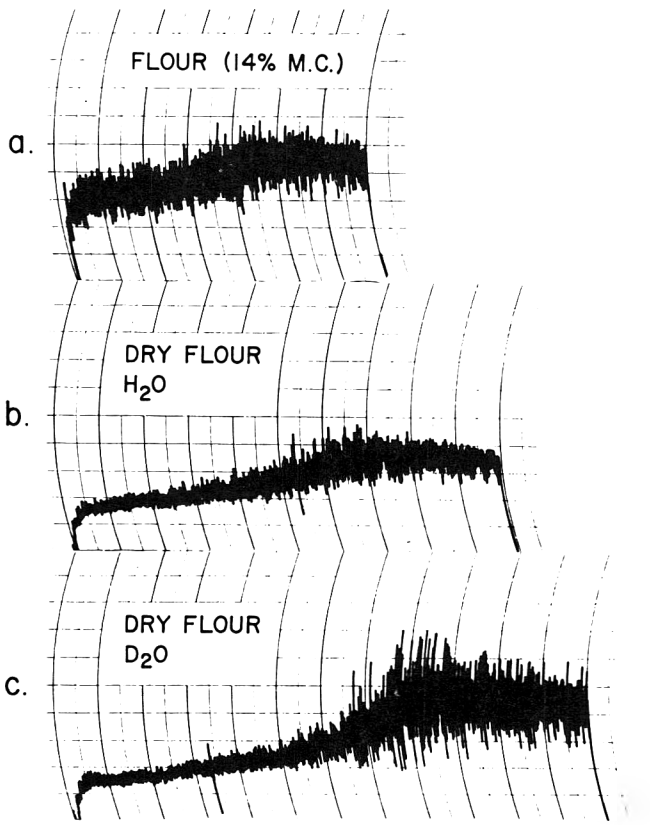

Fig. 1—Mixograms of hard wheat flour doughs: (a) flour (14% M.C.) and water at 68% absorption; (b) dry flour and water at 68% absorption; (c) dry flour and deuterium oxide at 70% absorption (14% moisture basis).

NMR study

The deuteron relaxation studies were carried out at 13.8 MHz using a Bruker WH-90/SXP NMR Spectrometer. The transverse relaxation time (T_2) was determined at 30°C using the Carr-Purcell-Meiboom-Gill Technique, whereas the longitudinal relaxation time (T_1) was determined using the null method (Farrar and Becker, 1971). Variation between duplicate samples was usually less than 5% for T_2 and less than 10% for T_1.

RESULTS & DISCUSSION

Effect of D_2O on flour dough

Some interesting changes of the mixing properties of wheat flour doughs were noted when H_2O was replaced by D_2O. The first mixogram in Fig. 1a shows the mixing characteristics of typical hard wheat flour at 68% water absorption (68g water per 100g flour, 14% moisture basis). Using dry flour instead of regular flour (14% moisture) caused an increase in mixing time and decreases in peak height and peak width (Fig. 1b). The decrease in peak height and width was an indication of overhydration, which may be due partly to some moisture uptake from the air during handling. When D_2O was added to dry flour at 70% absorption, both the mixing time and dough strength increased (Fig. 1c). This effect became more pronounced when 2% sodium chloride was added to the dough (Fig. 2). These mixograms indicate that wheat flour associated with D_2O more strongly than it did with H_2O. Hoseney (1979) also noted that wheat flour dough produced with D_2O was much stronger than that with H_2O. He attributed this effect to hydrogen bonding. According to Walrafen (1972), ΔH value for the disruptions of O-H · · · 0 and O-D · · · 0 units is about 2.5 Kcal/mole from 0–100°C. However, presence of macromolecules may have marked effects on the thermodynamic properties of water (Berendsen, 1975). It is very likely that hydrogen bonding plays an important role in the mixing properties of H_2O and D_2O flour doughs.

T_1 and T_2 of flour dough

The deuteron T_2 relaxation curves (Log signal vs time) of most flour dough samples show some slight curvature initially, indicating the existance of a small fraction of D_2O with short T_2. The fast relaxing component, however, was too small to be resolved. Therefore, only the predominant, long T_2 was reported. In our previous study with proton relaxation in flour dough, two distinct fractions of water with different T_2 values were observed (Leung et al., 1979). We were unable to resolve the two fractions of water in the case of deuterated flour dough, probably because of the different relaxation mechanisms for protons and deuterons.

Fig. 3 shows the changes of T_1 and T_2 of deuterated wheat flour dough with moisture (D_2O) content. As D_2O content of flour dough increased, T_1 and T_2 also increased, indicating a general increase in water mobility. Although hard wheat and soft wheat flour doughs have different viscoelastic and mixing properties, they showed similar T_1 and T_2 values in the moisture range of 0.5–0.9g D_2O/g solid. This observation confirmed our previous study with proton relaxation which indicated doughs made with different types of wheat flour have similar T_2 values (Leung et al., 1979). Thus water mobility as determined by proton and deuteron relaxation does not reflect the different rheological properties of soft and hard wheat flour doughs. It is very likely that mobility and conformation of the macromolecules are largely responsible for the different properties of doughs made of different types of wheat flours. This explanation is consistent with the findings of water mobility in gels. Woessner et al. (1970) found that the self diffusion coefficient of water in agar gel is essentially the same as that of bulk water. In an NMR study of agarose and carrageenan gels, Child et al. (1970) also concluded that the sol to gel transition is accompanied by distinct changes in

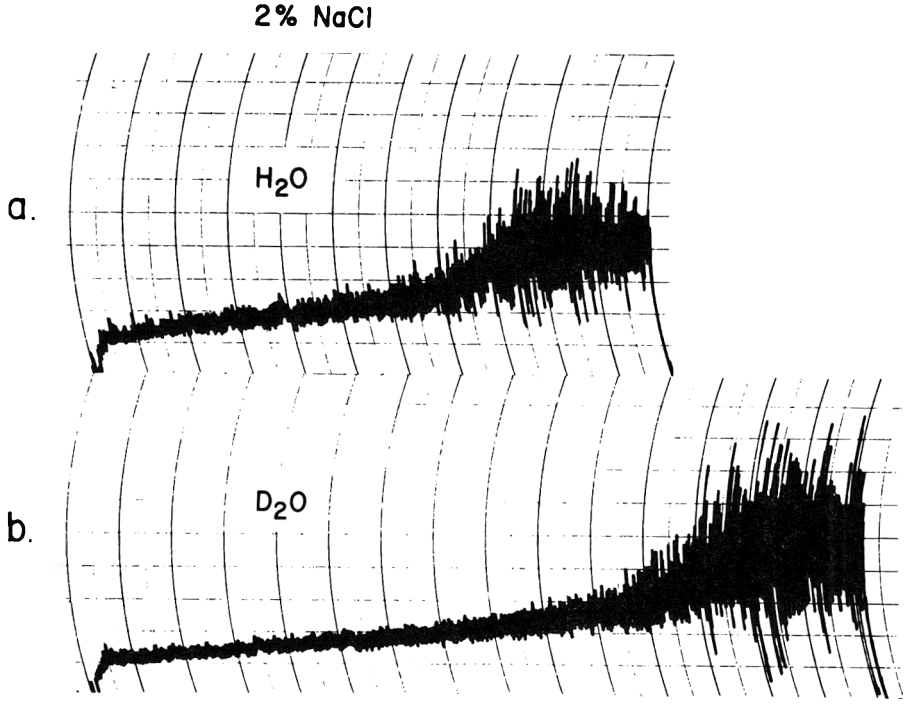

Fig. 2—Mixograms of hard wheat flour doughs containing dry flour, 2% sodium chloride and (a) water at 68% absorption; (b) deuterium oxide at 70% absorption (14% moisture basis).

polysaccharide conformation, whereas most water molecules remain in a highly mobile state.

Addition of up to 15% vital (wheat) gluten and 20% wheat starch to flour doughs changed the mixing properties but had little or no effect on T_1 and T_2. The different dough properties probably reflect changes in macromolecular conformation rather than water mobility. Varying the D_2O:H_2O ratio of flour doughs from 1:0 to 1:3.5 at constant moisture contents had no effect on deuteron T_1 and T_2. This indicates that the quadrupolar relaxation of deuteron was the dominant mechanism in the relaxation process.

Physical changes of bread during storage

Some physical changes of bread occurred soon after it was removed from the oven and cooled. Table 1 summarized the changes in water activity (a_w) and elastic modulus (E) of bread crumb during storage. Water activity of bread crumb decreased from day 0 to day 1, but showed little or no change thereafter. The decrease in a_w was due mainly to transfer of moisture from bread crumb to bread crust. Preliminary studies showed increases of moisture content in the crust and corresponding decreases in the crumb upon storage. Although breads made with H_2O had higher equilibrium vapor pressure at 22°C than those made with D_2O, they showed lower a_w since pure H_2O has a higher vapor pressure than pure D_2O. The a_w of regular breads (D_2O:H_2O = 0:100) was lower than the value (0.96) reported by Prior et al. (1977). The difference in a_w may be due to variations in bread samples, baking conditions and methods of determination.

Firmness of bread has been shown to correlate well with modulus of elasticity (Conford et al., 1964; Elton, 1969; Bashford and Hartung, 1976). For breads made with H_2O and/or D_2O, the elastic modulus of bread crumb increased markedly during the first two days of storage and then leveled off gradually. The moduli of elasticity obtained in this study are somewhat larger than those reported by Conford et al. (1964) and Elton (1969). For example, we found the elastic modulus of bread crumb after 2 days of storage to be 2.88 x 10^5 dynes/cm^2, whereas Conford et al. (1964) reported a value of 1.50 x 10^5 dynes/cm^2 for bread crumb stored for 2 days. On the other hand,

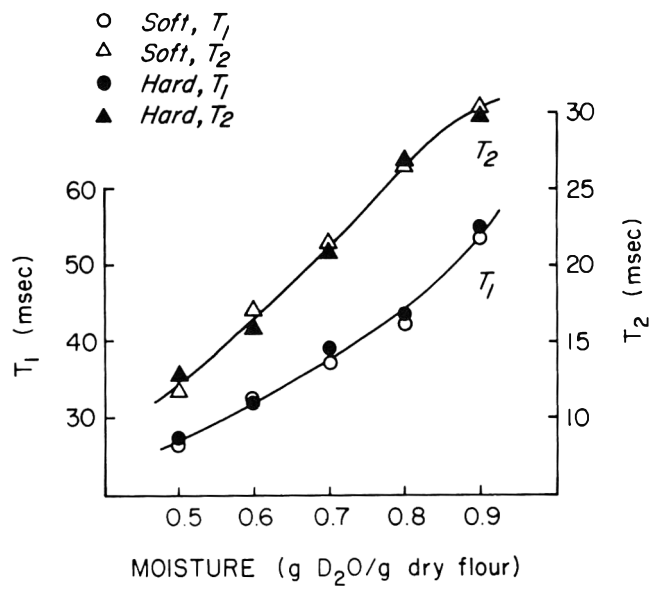

Fig. 3—Effect of moisture content on T_1 and T_2 of hard wheat and soft wheat flour doughs.

Babb (1965) found the elastic modulus of fresh white bread crumb to be 1.5 x 10^5 dynes/cm^2, higher than the value of 0.8 shown in Table 1. Bread with a crumb modulus of 2.00 x 10^5 dynes/cm^2 was considered by Elton (1969) to be quite stale based on taste panel rating. The different crumb modulus values obtained in the present study and those by previous workers may be due to variations in experimental conditions. Most staling studies are conducted with 100g or pound loaves, much larger in size than the 10g loaves used in this study. Also, Finney (1972) stated that caution should be used when comparing published results of elastic modulus of breads since many measurements are performed under other than small strain conditions.

Based on the data in Table 1, it is obvious that staling or firming of breads did occur during storage, especially the

first 2 days. It should be pointed out that water activity of bread crumb showed little or no change from day 1 to day 2 although the elastic modulus increased substantially. This observation indicates that water activity of bread crumb is insensitive to changes during staling. The initial decrease in water activity from day zero to day one was due largely to loss of moisture from bread crumb to crust (Willhoft, 1973; Bushuk and Mehrotra, 1977b).

NMR studies of bread

Two additional baking experiments were conducted to study the change in the physical state of water in bread during storage or staling. Changes in moisture content, longitudinal (T_1) and transverse (T_2) relaxation times of bread crumb upon storage are shown in Table 2. In trial (I), the breads prepared had lower moisture content than normal bread (0.5–0.6g water/g solid) and they were analyzed after 1, 6 and 10 days of storage. Moisture content of the breads increased with percentage of D_2O used. This may be partly due to the higher baking absorption of the D_2O bread dough as compared to regular dough. For instance the baking absorption of the bread doughs with D_2O:H_2O ratio of 50:50 and 100:0 were 66 and 68%, respectively. The small difference in baking absorption, however, could not account for the large difference in moisture content of the bread crumb. A likely explanation is that the interaction between bread dough and D_2O is stronger than that between bread dough and H_2O. As a result, more moisture was retained in bread after baking as percent D_2O increased. Similar results were obtained in trial (II) in which breads were prepared at the normal moisture contents.

In the first trial, little or no change in moisture content in bread crumb was observed after day 1 (Table 2). Transfer of moisture from crumb to crust probably occurred mainly during the first day of storage. Although moisture content in bread crumb remained relatively constant from day 1 to day 10, the deuteron T_1 and T_2 values changed considerably in breads made with D_2O:H_2O ratio of 50:50 or higher. For example, breads made with D_2O:H_2O of 75:25 showed only a decrease in moisture of 0.02g water/g solid from day 1 to day 6, but T_1 decreased from 12.7 to 9.0 msec, and T_2 from 6.3 to 3.7 msec during the same period. The decrease in nuclear relaxation times indicate an overall decrease of water mobility in bread during storage. Analysis of variance of the data showed no significant change in mean moisture content of bread crumb from day one to day six, but showed significant decreases in mean T_1 and T_2 values during the same period (95% confidence interval). Therefore the decrease in water mobility of bread crumb during storage was independent of changes in moisture content.

In trial (II), fresh breads contained normal moisture contents (47–57%). Changes in moisture contents, and T_1 and T_2 of bread crumbs were similar to those in trial one (Table 2). Most of the changes occurred during the first 2 days. Moisture contents of the crumb decreased by 0.05 to 0.12g water/g solid, while T_1 and T_2 were reduced by about one-half after two days of storage. The substantial decrease in water mobility as detected by T_1 and T_2 seem to agree with the large increase in elastic modulus which occurred during the first 2 days of storage (Table 1). The relaxation times of trial (II) were longer than those of trial (I), indicating that as moisture content increased, the amount of mobile or free water also increased. This is the expected result if the amount of water tightly associated with the bread crumb is constant.

Based on the data in trial (II), it appears that the reduced water mobility of bread crumb upon storage was accompanied by decreasing moisture content. However, a further examination of Table 2 indicates that much of the decrease in water mobility during storage was due to factors other than reduction in moisture content. The effect of moisture content on T_1 and T_2 of bread crumb can be estimated by correlating moisture content and deuteron relaxation times of fresh bread crumbs (day 1 for trial I and day 0 for trial II) made with different D_2O:H_2O ratios. A plot of T_1 versus moisture content gave a slope of 78.6 (msec/g water/g solid) with a correlation coefficient (r) of 0.995. T_2 was also highly correlated with moisture

Table 1–Effect of storage on some physical properties of H_2O and D_2O breads

D_2O:H_2O	Storage time (Days)	Equilibrium vapor pressure at 22°C (mm Hg)	Water activity	Elastic modulus (10^5 dynes/cm^2)
0:100	0	17.3	0.92	0.8
	1	16.7	0.89	1.9
	2	16.7	0.89	2.8
	3	16.5	0.88	2.6
	4	16.5	0.88	3.0
50:50	0	16.9	0.94	0.7
	1	16.6	0.92	1.9
	2	16.6	0.92	2.6
	3	16.6	0.92	3.0
	4	16.6	0.92	3.0
100:0	0	16.7	0.97	0.7
	1	16.3	0.95	1.4
	2	16.2	0.94	2.3
	3	16.2	0.94	2.8
	4	16.2	0.94	3.0

Table 2–Effect of storage on moisture content, longitudinal (T_1) and transverse (T_2) relaxation times of D_2O bread crumb

Trial no.	D_2O:H_2O	Moisture content (g water/g solid)			T_1 (msec)			T_2 (msec)		
		Day 1	Day 6	Day 10	Day 1	Day 6	Day 10	Day 1	Day 6	Day 10
(I)	25:75	0.30	0.30	0.30	7.2^a	6.5^b	7.2^a	3.0^a	2.5^b	2.7^b
	50:50	0.32	0.32	0.32	10.1^a	7.6^b	7.5^b	4.5^a	3.3^b	3.1^b
	75:25[a]	0.37^a	0.35^c	0.36^b	12.7^a	9.0^b	9.1^b	6.3^a	3.7^b	3.9^b
	100:0	0.38^b	0.35^b	0.35^b	13.4^a	9.4^b	9.1^b	6.2^a	3.8^b	3.5^b

Trial no.	D_2O:H_2O	Moisture content (g water/g solid)				T_1 (msec)				T_2 (msec)			
		Day 0	Day 2	Day 5	Day 8	Day 0	Day 2	Day 5	Day 8	Day 0	Day 2	Day 5	Day 8
(II)	25:75	0.47^a	0.42^b	0.41^{bc}	0.39^c	22.5^a	11.6^b	10.9^b	11.0^b	12.3^a	4.9^b	5.0^b	4.2^b
	50:50	0.52^a	0.40^b	0.38^{bc}	0.37^c	25.4^a	12.3^b	11.6^b	11.6^b	12.6^a	6.2^a	4.6^c	4.0^c
	75:25	0.54^a	0.45^b	0.41^c	0.40^c	26.1^a	12.3^b	11.6^b	12.0^b	13.6^a	5.6^b	4.6^c	4.2^c
	100:0	0.57^a	0.46^b	0.42^c	0.41^c	28.3^a	13.0^b	11.9^b	11.6^b	15.7^a	8.0^b	6.0^c	5.5^c

[abc]MC, T_1 and T_2 values in the same row with different letters are significantly different ($P < 0.05$).

content (r = 0.990, slope = 45.4). Thus a decrease of 0.79 msec in T_1 and a decrease of 0.45 msec in T_2 were expected when bread crumb moisture was reduced by 0.01g water/g solid. The marked decrease in T_1 and T_2 of bread crumb with storage time cannot be accounted for by the effect of moisture alone. For example, bread crumb with a D_2O:H_2O ratio of 25:75 showed a moisture loss of 0.05g water/g solid, a decrease of 10.9 msec in T_1 and a decrease of 7.4 msec in T_2 from day 0 to day 1. The decrease in moisture content would account for less than 40% of the reduction in relaxation times. Therefore, aging of bread resulted in decrease in water mobility not attributable to moisture loss.

Bread staling and water mobility

Why is firming of bread crumb or bread staling accompanied by decrease in water mobility? A possible explanation may be found in the physical changes occurring in starch during aging or staling of bread. It has been shown that gelatinized starch in bread has a tendency to revert from the amorphous state to the more stable, crystalline state during storage (Senti and Dimler, 1960). Wright (1971) demonstrated that bread crumb firmness increased with the increase in starch crystallinity during storage at 4° and 21°C. The amount of crystallinity was measured by the ratio of the B-type diffraction pattern level to that of the V-pattern which remained constant during storage. More recently, Sarko and Wu (1978) elucidated the structures of A-, B- and C-patterns of starch and amylose. The B-pattern amylose was shown to crystallize in a hexagonal unit cell with 36 water molecules per unit cell, corresponding to 25% water. Thus, a portion of the water in bread crumb during staling could become bound, or closely associated with, the B-amylose crystals, resulting in an overall decrease in water mobility as detected in the deuteron relaxation times. That either the percentage of immobile water increased or the mobility of that immobile fraction decreased further is clear from the NMR studies. The rearrangement of starch is certainly a possibility for explaining the results.

Elucidation of interactions influencing deuteron quadrupolar relaxation will require much further study. Fung (1977) has suggested that the shortened relaxation rates observed in heterogeneous systems can be accounted for by a small fraction of water molecules (deuterium oxide) hydrogen bonded to macromolecules. According to this, we have detected a change in the extent of hydrogen bonding, or water interaction, upon aging of bread.

The dough mixing experiments show clearly that hydrogen bonding is important in dough preparation and that differences are observed between water and deuterium oxide. The deuterium relaxation times for bread do not vary greatly with the ratio of D_2O:H_2O. If the bread were preferentially interacting with deuterated water, one might observe that at lower D_2O levels, a larger fraction of D_2O would be associated with bread components and the relaxation time would be shorter. As the percentage of D_2O were increased, for example, the amount of mobile D_2O would increase and the relaxation times would increase. The increases in relaxation time observed with increasing D_2O content (Fig. 3) most likely reflect greater moisture content rather than any preferential association of D_2O for flour dough components.

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Effect of Trans Fatty Acids on Protein Utilization and Serum Cholesterol

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ABSTRACT

Rats were fed PER diets containing various proportions of *cis* and *trans* fatty acids as triolein, trielaidin, soybean oil (SO) or hydrogenated SO at 7.8% by weight of the diet. Additional rats were fed SO or hydrogenated SO at 20.1% of the diet. The PER, although in the normal range for casein, was significantly ($P < 0.01$) lower in rats fed trielaidin. Food consumption and weight gains were slightly greater in *trans*-fed than *cis*-fed rats. The former had greater fecal fat excretion than the latter; serum cholesterol levels of the former were lower with the 7.8% but not the 20.1% fat diet.

INTRODUCTION

UNSATURATED FATTY ACIDS exist naturally in the *cis* rather than the *trans* configuration. Hydrogenation of unsaturated fatty acid-containing oils for margarines and shortenings produces both geometric and positional isomers, with *trans* monoenes with the double bond at position 9–12 from the carboxyl end predominating (Smith et al., 1978).

Weirauch et al. (1977) compiled data on the fatty acid composition of 84 brands of margarines and shortenings manufactured in the U.S. Data were obtained from manufacturers, FDA analyses, and reports in the literature since 1970. Total *trans* acids values in g/100g margarine for stick margarines ranged from 9.9–28.7, with a mean of 21.7. A Canadian study of 50 brands of margarine (Beare-Rogers et al., 1979) reported that the *trans* fatty acid content was usually 30–35%.

Studies which have quantified *trans, trans* dienes in margarine have found that they are usually not detectable in most samples; when detectable, except in rare cases, they accounted for less than 1.0% of the fatty acids (Heckers and Melcher, 1978; Sahasrabudhe and Kurian, 1979).

Since 1950, there has been a continuous increase in U.S. consumption of margarine from 6.1 pounds per capita to 11.7 pounds per capita in 1979 (U.S. Bureau of the Census, 1980). This dietary change has generated interest in the implications for health of increased consumption of *trans* fatty acids.

While it has been reported that composition of dietary fat has no effect on protein utilization as determined by PER assay (Hurt et al., 1975) and nitrogen balance (Naismith and Qureshi, 1962), several investigators have reached other conclusions (Kluszczynska, 1979; Krajčovičova and Dibak, 1979). Kluszczynska (1979) concluded that the kind and content of fat in the diet had a partial influence on net protein utilization in rats, particularly when the diet was poor in protein. When a 10% casein diet with 5, 10, 25, or 40% fat as margarine was fed to rats for 14 days, net protein utilization was greatly decreased in the animals fed the 40% margarine diet (Krajčovičova and Dibak, 1979). De et al. (1969) found no differences in PER values when hydrogenated fats were compared with natural fats in diets containing 8% fat and various levels and sources of protein; the ratio of liver fat to liver nitrogen, however, was higher with hydrogenated than with natural fats.

Naismith (1962) studied the effect of essential fatty acid (EFA) deficiency on protein utilization in rats fed an 18% casein diet. EFA-deficient rats, compared with control rats, had marked growth depression, increased nitrogen excretion in urine and feces, and an average 39% reduction in nitrogen retention.

Trans isomers of linoleic acid (*cis, cis*-18:2) lack EFA activity and, in fact, intensify EFA deficiency (Matson, 1960). Hill et al. (1979) have demonstrated that *trans* fatty acids in a margarine stock, present mainly as *trans* 18:1 (positional isomers), with no detectable *trans, trans* 18:2 also exacerbated the signs and symptoms of EFA deficiency in rats fed low-EFA diets.

The effects of *trans* fatty acids on serum cholesterol are also of particular interest because of the apparent relationship between serum cholesterol levels and development of atherosclerosis and the widespread use of special margarines for the purpose of modifying dietary fat intake (Anonymous, 1980).

In view of the above information on *trans* fatty acid and its dietary relevance this study was designed to determine if the *trans* isomer of oleic acid, elaidic acid, affects (a) protein utilization and (b) serum cholesterol when the diet is not deficient in essential fatty acid.

MATERIALS AND METHODS

Preparation of diets

The diet composition was based on the AOAC (1975) protein evaluation (PER) basal diet. Diets varied in level of fat, either 7.8% or 20.1% (Table 1), and source of fat (Table 2). The 7.8% level of

Table 1—Composition and calorie and nitrogen densities of the test diets

	7.8% Fat diets	20.1% Fat diets
Casein, ANRC[a], g/100g	11.10	12.82
Fat, g/100g	7.80	20.10
ICN Salt Mixture[b] XVIII, g/100g	4.76	4.76
ICN Vitamin Diet[b] Fortification Mixture, g/100g	2.20	2.20
Alphacel, g/100g	1.00	1.00
Water, distilled, g/100g	4.30	4.30
BHT, g/100g	0.005	0.005
Sucrose, g/100g	34.50	27.40
Cornstarch, g/100g	334.50	27.40
kcal/g	3.906	4.514
g N/kcal	0.0134	0.0164

[a]Humko Sheffield Chemical, (Lyndhurst, NJ)
[b]ICN Nutritional Biochemicals (Cleveland, OH)

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Table 2—Fat composition of diets

Diet[a]	% and Source of fat
1. TO	0.5% trilinolein and 7.3% triolein
2. TE	0.5% trilinolein and 7.3% trielaidin
3. SO	7.8% soybean oil
4. HSO	7.8% hydrogenated soybean oil (2.8% *trans* 18:1)
5. HFSO	20.1% soybean oil
6. HFHSO	20.1% hydrogenated soybean oil (7.2% *trans* 18:1)

[a] TO (triolein); TE (trielaidin); SO (soybean oil); HSO (hydrogenated soybean oil); HFSO (high-fat soybean oil); HFHSO (high-fat hydrogenated soybean oil).

Table 3—Fatty acid composition of soybean oil and hydrogenated soybean oil used in test diets

Fatty acid	Soybean oil (%)	Hydrogenated soybean oil (%)
16:0	10.5	12.5
18:0	5.5	14.2
cis 18:1	22.1	24.2
trans 18:1	–	35.6
cis,cis 18:2	61.9	13.5

fat conformed to the AOAC specification for PER studies. The 20.1% fat diet supplied 40% calories as fat as does the typical, high-fat American diet (Friend, 1976). The proportion of trilinolein included in the diets which had triolein or trielaidin as their primary fat source is based on a linoleic acid requirement for male rats of 1.3% of calories (NRC, 1978). The antioxidant BHT was included in all the diets at a recommended level (American Institute of Nutrition, 1980).

In total there were six experimental diets and they are expressed in the tables with the following abbreviations: (1) TO (triolein diet), (2) TE (trielaidin diet), (3) SO (soybean oil diet containing 7.8% fat), (4) HSO (hydrogenated soybean oil diet containing 7.8% fat), (5) HFSO (high-fat soybean oil diet containing 20.1% fat), and (6) HFHSO (high-fat hydrogenated soybean oil diet containing 20.1% fat).

A fat-free stabilization diet in which a 1:1 mixture of sucrose/cornstarch replaced the fat was fed for the 3 days prior to the initiation of the feeding study.

Trilinolein was prepared by the method of Wheeler et al. (1940). Trielaidin was prepared by the combined methods of Khan (1959) and Jart (1960). The following were commercially obtained: triolein (United States Biochemicals, Cleveland, OH), soybean oil and hydrogenated soybean oil (C&T Refinery, Inc., Charlotte, NC). Methyl esters were prepared (Bohles et al., 1976) and the fatty acids were identified and quantified in the soybean oil and hydrogenated soybean oil by gas chromatography (F&M Research Chromatograph Model 810, F & M Scientific Corp.) with a 20-foot by 1/8 inch stainless steel column packed with 15% OV-275 on 100/120 Chromosorb P AW-DMCS (Supelco, Inc., Bellefont, PA). The carrier gas was helium at a flow rate of 10 ml/minute. Column temperature was 220°C.

The fatty acid composition of the soybean oil and hydrogenated soybean oil used in the test diets is presented in Table 3. The gas chromatographic analysis indicated that the soybean oil contained no *trans* fatty acids; the hydrogenated soybean oil contained 35.6% *trans* fatty acids as elaidic acid.

Feeding experiment

Male, weanling CD outbred Swiss Albino rats (Charles River-Carworth, Wilmington, MA) were distributed into 6 groups of 10 animals in such a way that the range between the mean animal weights of the groups was minimized. Mean animal weights of the groups were 57.0–57.3g. Animals were housed individually and fed the experimental diets and water ad libitum. Food intakes and body weights were recorded three times/week for 28 days. Fecal pellets were collected and dried at 100°C.

After 28 days of the feeding study, the rats were sacrificed by a blow on the head. Blood samples were obtained by heart puncture. Blood was allowed to clot, then centrifuged at 2000 rpm for 10 min. Serum was recovered and stored at −20°C. Livers were removed and weighed, then stored at −20°C.

The carcasses (entire body except liver and kidneys) were stored at −20°C until they were removed to be homogenized. Frozen carcasses were autoclaved 2 hr at 15 psi, homogenized in a high speed blender, and stored at −20°C until they were analyzed.

Dried fecal pellets from pairs of rats, collected during the 28-day study period, were pooled, ground, dried to constant weight, and stored in a desiccator.

Protein and nitrogen

The percentage nitrogen of carcass, liver and feces was determined in triplicate by the macro-Kjeldahl method (AOAC, 1975). Crude protein in liver and carcass was calculated as N x 6.25.

Total lipid

Lipid was extracted from the carcass, liver, and dried fecal samples with chloroform and methanol (Bligh and Dyer, 1959) and quantified by the lipid charring method of Kritchevsky et al. (1973).

Serum cholesterol

Serum cholesterol was determined on a BioDynamics Auto-Analyzer II using the bmc Autoflo Cholesterol kit.

Statistical analyses

Food consumption, body weight, PER, and serum cholesterol data for groups 1–4 were analyzed on a Burroughs 7700 computer with Fortran program MUTIRANGE for analysis of variance and Duncan's multiple range test.

The t-test (Duncan et al., 1977) was used for analysis of fecal fat and fecal nitrogen data. Comparisons were made of group 1 with group 2 and group 3 with group 4.

All of the data for high-fat groups 5 and 6 were analyzed by the t-test.

—Continued on next page

Table 4—Food consumption, weight gain, PER and CER of rats[a] fed two levels of fat from various sources

Group/Diet[b]	Food consumption, g	Weight gain, g	PER[c]	CER[d]
1. TO	428.5±11.3a	142.2±3.8a	2.99±0.09a	0.085
2. TE	474.5±14.4b	150.8±6.5ab	2.85±0.05b	0.081
3. SO	414.5±13	139.9±5.2a	3.04±0.07a	0.086
4. HSO	475.3±10.4b	161.5±4.0b	3.06±0.02a	0.087
5. HFSO	384.9±14.0NS[e]	149.3±7.7NS	3.02±0.07NS	0.086
6. HFHSO	395.7±9.1	152.4±8.1	2.99±0.11	0.085

[a] Mean + SEM for 10 rats. Means in a column (groups 1-4) not followed by a common letter are significantly different ($P < 0.05$, food consumption and weight gain data; $P < 0.01$, PER data).
[b] TO triolein); TE (trielaidin); SO (soybean oil); HSO (hydrogenated soybean oil); HFSO (high-fat soybean oil); HFHSO (high-fat hydrogenated soybean oil).
[c] PER (Protein Efficiency Ratio) = Weight gain/Protein intake.
[d] CER (Calorie Efficiency Ratio) = Weight gain/kcal intake. No statistical analysis performed.
[e] No significant difference between groups 5 and 6 according to the t-test.

Table 5—Fecal fat and nitrogen of rats fed two levels of fat from various sources[a]

Group/ diet[b]	Total wt of dried fecal pellets,g	Total fat of fecal pellets,g	% Fat of fecal pellets	Apparent fat digestibility[c]	Total N of fecal pellets	% N of fecal pellets	Apparent N digestibility[d]
1. TO	17.3	0.860±0.169**	4.96±0.29**	97.4	0.4987±0.0584NS	3.00±0.08**	98.2
2. TE	28.5	2.341±0.299	8.21±0.90	93.7	0.5500±0.0247	1.94±0.05	98.2
3. SO	25.1	1.461±0.129**	5.82±0.25**	95.5	0.7724±0.0316**	2.90±0.25**	97.1
4. HSO	37.7	3.460±0.298	9.17±0.64	90.7	0.8700±0.0204	2.32±0.06	97.1
5. HFSO	23.5	1.952±0.278NS	8.31±0.20NS	97.5	0.6801±0.0741NS	2.94±0.07**	97.6
6. HFHSO	29.6	2.846±0.420	9.60±0.89	96.4	0.6196±0.0483	2.11±0.04	97.9

[a] Mean ± SEM, N = 5.
[b] TO (triolein); TE (trielaidin); SO (soybean oil); HSO (hydrogenated soybean oil); HFSO (high-fat soybean oil); HFHSC (high-fat hydrogenated soybean oil).
[c] Apparent fat digestibility = Fat intake − Fecal fat + Fat intake x 100. No statistical analysis performed.
[d] Apparent N digestibility = N intake − fecal N + N intake x 100. No statistical analysis performed.
**Significant difference between groups 1 and 2, 3 and 4, or 5 and 6 ($P < 0.01$).
NS No significant difference between groups 1 and 2, 3 and 4, or 5 and 6 ($P > 0.05$).

RESULTS & DISCUSSION

Food consumption

The rats fed *trans* fatty acids (groups 2, 4, 6) had greater mean food consumptions than rats fed *cis* fatty acids (groups 1, 3, 5) (Table 4). The food consumptions of groups 2 and 4 were statistically the same and were significantly ($P < 0.05$) greater than the food consumptions of groups 1 and 3. The food consumption of group 5 did not differ significantly from that of group 6.

Weight gain

The increased food consumptions of rats fed *trans* fatty acids were accompanied by consistently greater weight gains (Table 4). However, this difference was significant ($P < 0.05$) only between groups 3 and 4, and 1 and 4, with group 4 significantly greater in both cases. Groups 5 and 6 were not significantly ($P > 0.05$) different from each other in weight gain.

PER

PER values are shown in Table 4. The greater food consumptions and greater weight gains of *trans*-fed animals, for the most part, equalized the PER's among the groups. This was not the case, however, for group 2 which had a significantly ($P < 0.01$) lower PER than groups 1, 3 and 4. The diet of group 2 had the highest proportion of elaidic acid relative to other fatty acids. Groups 1 and 2 had the same ratio of dietary nonessential to essential fatty acids, approximately 15:1. The *cis* or *trans* isomer was the only difference between these groups. The other groups fed *trans* fatty acid diets (groups 4 and 6) also had other fatty acids, such as oleic, stearic, and palmitic acids, in their diets. The ratio of nonessential to essential fatty acids in *trans*-fed groups 4 and 6 was approximately 6:1. As well as a more varied mix of fatty acids, these groups had a greater proportion of essential fatty acid in their diets.

Although significantly ($P < 0.05$) lower than that of groups 1, 3 and 4, the PER of group 2 was more than 90% of the PER values of the 3 other groups. The PER values of all the groups, including group 2 were well within the acceptable range of values for casein reported by Jansen (1962).

Only in group 2 did the Calorie Efficiency Ratio (CER) vary to any extent from that of the other groups (Table 4).

Table 6—Percentages[a] *of protein and fat in livers and serum cholesterol of rats fed two levels of fat from various sources*

Group/Diet[b]	% Liver protein	% Liver fat	Serum cholesterol (mg/dl)
1. TO	20.2±0.6a	4.5±0.3a	110±4a
2. TE	20.2±0.5a	4.5±0.2a	83±5b
3. SO	19.7±0.6a	4.5±0.4a	103±6ac
4. HSO	21.3±0.6a	4.1±0.3a	88±8bc
5. HFSO	20.4±0.6NS[c]	6.1±0.4**	96±6NS
6. HFHSO	20.8±0.9	4.7±0.2	101±8

[a] Mean ± SEM for 10 rats. Means in a column (groups 1-4) not followed by a common letter are significantly different ($P < 0.05$, liver data; $P < 0.01$, serum cholesterol).
[b] TO (triolein); TE (trielaidin); SO (soybean oil); HSO (hydrogenated soybean oil); HFSO (high-fat soybean oil); HFHSO (high-fat hydrogenated soybean oil).
[c] No significant difference between groups 5 and 6 according to the t-test.
**Highly significant ($P < 0.01$) difference between groups 5 and 6 according to the t-test.

Fecal fat and nitrogen

At the lower level of dietary fat (7.8%), the *trans* fatty acid-fed animals had greater fecal fat excretion, both as percentage fat of fecal matter and total amount of fat excreted (Table 5). The amount of fecal fat and percentage fecal fat of group 2 was significantly ($P < 0.01$) greater than that of Group 1, and that of group 4 was significantly ($P < 0.01$) greater than that of group 3. With the high-fat diets, rats in group 6 excreted a higher percentage and total quantity of fat than rats in group 5, but the differences were not significant ($P > 0.05$). The groups which had greater fat excretion were also those which had greater food intakes. Therefore fat excretion expressed as a percentage of fat intake (apparent fat digestibility) is also presented in Table 5. Apparent fat digestibility was lowest for group 4 (90.7); the value for group 2 (93.7) was also considerably lower than that for groups 1 and 3.

It may be that the lower PER of the trielaidin-fed animals (group 2) was due to poor absorption of fat resulting in a lower energy yield from the diet. The fact that hydrogenated soybean oil-fed rats, particularly those in group 4, also had lower apparent fat digestibility values than *cis* fatty acid-fed rats, but comparable CER values suggests that poor fat digestibility alone does not fully explain the PER obtained with trielaidin-fed rats.

All the groups fed *trans* fatty acids (1, 3, 5) had significantly ($P < 0.01$) higher percentages of fecal nitrogen than their *cis*-fed counterparts (2, 4, 6). However, because *trans*-fed animals had greater food intakes and greater quantities of fecal matter, when nitrogen excretion was expressed as a percentage of nitrogen intake (apparent nitrogen digestibility), differences between groups 1 and 2, 3 and 4, and 5 and 6 were not evident (Table 5).

Carcass fat and protein

Carcass fat as a percentage of body weight (excluding liver and kidneys) did not vary significantly ($P>0.05$)

among the groups. The mean percentage carcass fat for the 6 groups was 7.5% (range 7.3 – 7.8%). The percentage carcass protein also showed no significant differences among the groups. The mean percentage carcass protein for the 6 groups was 20.0% (range 19.5 – 20.4%).

Liver fat and protein

Groups 1 through 4 exhibited no significant differences in percentage liver fat (Table 6). At the higher level of fat, group 5 had a significantly ($P<0.01$) higher level of liver fat than group 6. This difference was not evident at the lower level of dietary fat in diets 1 through 4, but appeared when the level of dietary fat was increased.

The results are at variance with those of De et al. (1969) who reported higher ratios of liver fat to liver nitrogen for hydrogenated fat-fed rats compared to natural oil-fed rats when the dietary fat level was 8% and the dietary protein level was 15% casein.

Morris et al. (1965) observed that rats fed a threonine deficient diet which causes development of fatty livers, had lower levels of liver fat when hydrogenated, rather than natural corn oil, was used in the diet.

As in the carcasses, there were no significant differences among any of the groups in percentage protein of liver.

Serum cholesterol

Serum cholesterol values are presented in Table 6. The *trans* fatty acid-fed rats had lower serum cholesterol values than the *cis* fatty acid-fed rats when the fat level in the diet was 7.8%. In the rats fed 20.1% fat diets, no difference in serum cholesterol level between *cis*- and *trans*-fed animals was observed. Group 2 had a significantly ($P<0.01$) lower serum cholesterol value than groups 1 and 3. Group 4 also had a significantly ($P<0.01$) lower serum cholesterol value than group 1. Groups 5 and 6 were not significantly ($P>0.05$) different from each other in serum cholesterol value.

Moore et al. (1980) fed male rats for nine months 15% fat diets in which the fat was supplied by various hydrogenated fat mixtures containing *trans* fatty acids, corn oil, or lard. At the end of the study, free and total serum cholesterol levels were lower in the *trans* fatty acid-fed animals than the corn oil- or lard-fed animals. Such an effect of *trans* fatty acids was apparent in our study after a 28-day feeding study.

In other animal studies, a hypercholesteremic effect of *trans* fatty acids was found when high fat, high cholesterol diets were fed to rabbits (Weigensberg and McMillan, 1964) and rats (Rand and Quackenbush, 1965). However, *trans* fatty acid-fed rabbits fed cholesterol-free diets had serum cholesterol levels comparable to *cis* fatty acid-fed rabbits (Shrock and Conner, 1975).

The rats which had lower serum cholesterol levels (groups 2 and 4) were also those which eliminated greater quantities of fecal fat. This suggests that the hypocholesteremic effect of the *trans* fatty acids was related to poorer absorption of *trans* fatty acids and/or promotion of endogenous cholesterol elimination in the feces by *trans* fatty acids. Lin et al. (1955) reported that trielaidin prevented absorption of dietary cholesterol to a greater extent than triolein. It is possible, therefore, that unabsorbed trielaidin or elaidic acid in the gut also prevents the reabsorption of endogenous cholesterol.

CONCLUSIONS

DIETARY *trans* fatty acids as a high proportion of the total fatty acid content of the diet had an adverse effect on PER. When diets, in which all the nonessential fatty acid was supplied by triolein or trielaidin, were fed to rats, replacement of triolein with trielaidin resulted in a significantly lower PER. Diets, in which *trans* fatty-acid containing hydrogenated soybean oil was the fat source at fat levels of 7.8% and 20.1%, produced PER values no different from those obtained with all *cis* fatty acid soybean oil diets. Because the proportion of *trans* fatty acids in the normal diet would more closely resemble that of the hydrogenated soybean oil diets than the trielaidin diet, the results of this study support the conclusion that normal dietary levels of *trans* fatty acids have no adverse effect on protein utilization.

It was also demonstrated that *trans* fatty acid-fed rats had lower serum chlesterol levels and greater fecal fat elimination. However, it is not clear if the lower serum cholesterol levels are a result of the greater fat excretion.

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—Continued on page 110

Chemical Characterization and Edibility of the Oil Extracted from *Citrullus colocynthis* Seeds

W. N. SAWAYA, N. J. DAGHIR, and P. KHAN

ABSTRACT

The characteristics and composition of the crude oil extracted from *Citrullus colocynthis* seeds were examined. Data obtained for the iodine value, saponification number, Reichert-Meissl and others compare well with other edible oils. Thin-layer chromatography in conjunction with gas-liquid chromatography-mass spectrometry revealed over 75% degree of unsaturation major among which were linoleic, 50.6% and oleic acids, 25%. Upon storage, the oil showed relatively low peroxide values that were within the acceptable limits set for other vegetable oils. Animal experimentation trials on the oil using chicks as the experimental animals did not show any toxicity effects. Based on these results, *Citrullus colocynthis* oil might be of some potential use for animal feed and/or human consumption.

INTRODUCTION

Citrullus colocynthis belongs to the *Cucurbitaceae* or squash family which produces seeds rich in oil and protein. It is believed that the plant is native to Africa and the Middle East and is probably an ancestral type of the watermelon (Curtis, 1969). It is a long-lived perennial and grows wild in deserts under extreme xerophitic conditions. Young fruits are fleshy, mottled with dark-green and usually turn yellow when ripe (Tackholm, 1974). They are extremely bitter in taste and are full of smooth and shiny seeds which are thought to be rich in oil and protein.

Several cucurbit oils are currently used for cooking in countries other than the United States (Curtis, 1946, 1948; Girgis and Said, 1968). Data on the utilization of various cucurbit seeds is extensive in the literature including seeds of *Cucurbita foetidissima* Curtis, 1946; Bemis et al., 1967; Shahani et al., 1951; Hensarling et al., 1973; Jacks et al., 1972; Bemis et al., 1975), *C. digitata* and *C. palmitata* (Ault et al., 1947; Bolley et at., 1958). *Apadanthera undulata* (Bemis et al., 1967) and *C. peppo* (Curtis, 1948; Jacks et al., 1972; Alekseeva, 1950). However, incomplete and sometimes controversial data are reported on *Citrullus colocynthis* seed oil.

In the present investigation, an attempt was made to study the chemical characterization and suitability of the oil extracted from the seeds of *Citrullus colocynthis* plants grown in the Kingdom of Saudi Arabia for animal feed and/or human consumption.

MATERIALS & METHODS

Preparation of the oil

Wild *Citrullus colocynthis* gourds were collected from the vicinity of Riyadh city, Saudi Arabia. The dried fruits were crushed by hand and the seeds separated. The seeds were then ground into powder by a Wiley mill (Model 4, Philadelphia, PA) using 2.0 mm sieve. 120g of the ground seeds were extracted for 36 hr with hexane in a soxhlet extractor. After extraction of the oil the solvent was evaporated under reduced pressure by using a rotary evaporator. The oil obtained was kept under nitrogen in sealed aluminum foil-wrapped bottles under refrigeration for further analyses.

Chemical analysis of oil

All chemical analyses including acid value, iodine number, saponification value, Hehner value, Reichert-Meissl number, and unsaponifiable matter were determined according to procedures outlined in AOAC (1980). Refractive index was determined by an Abbe refractometer with temperature adjustment (American Optical, Model 10450).

Isolation and identification of fatty acids

The extracted oil was dissolved in chloroform and subjected to analytical and preparative thin-layer chromatography (TLC) on silica gel GF plates (0.5 mm and 1 mm respectively) with petroleum ether-diethyl ether-acetic acid (75:24.5:0.5, v/v) as solvent system. The components were visualized under ultraviolet light after spraying the plates with a 0.1% ethanolic solution of 2',7'-dichlorofluorescein and were identified by comparing their Rf values with those of known standards. For the preparation of the methyl esters, 2g of the oil was treated with 0.5N methanolic NaOH and refluxed with 15 ml of a 14% solution of BF_3 in methanol and 5 ml benzene for 12–16 min AOAC (1980). After extraction with chloroform or ethyl ether, the extract was dried with anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The methyl esters were separated by thin-layer chromatography on silica gel. Qualitative analyses of the methyl esters were performed with a gas-liquid chromatograph (GLC) equipped with a hydrogen flame ionization detector using a 0.3 x 170 cm glass column packed with 5% SP-2340, 75% cyanopropyl (Supelco, Bellefone, PA) on gas Chrom Q, 80-100 mesh (Applied Science Lab, State College, PA). Injections were made at 140°C with temperature increase of 15°C/min with helium (20 ml/min) used as the carrier gas. Identification of the different peaks was done by comparing their retention time with those of authentic standards and peak areas were integrated by a computing integrator. The gas chromatograph was attached to a Perkin-Elmer-Hitachi RMU6D mass spectrometer with a Biemann separator interphase and the esters were identified by comparison of mass spectra of individual components recorded with 70 ev ionizing voltage with those of known standards and on the basis of parent ion and GLC retention time. Spectra were recorded at the ascending and descending slopes of the chromatographic peak in addition to the top of the peak.

Storage experiment

To test for the level of oxidative rancidity upon storage of the oil under different conditions of light and temperature, samples of the oil were put in 50 ml tightly capped vials and were divided into two groups The first group consisted of two batches that were stored under indoor natural lighting conditions at 25°C and 32°C. The second group consisted of three batches of samples which were all wrapped in aluminum foil and stored at 7°C (refrigeration temperature), 25°C and 32°C and thus were considered to be stored under dark conditions. Samples from the different batches of each group were analyzed periodically for their peroxide values at zero time and at one month intervals for a period of six months according to AOAC (1980).

Animal experiment

Feeding trials for the detection of any toxicity in the oil were done on one day-old chicks. The semi-purified diets shown in Table 1 and patterned after Scott et al. (1976) were used in the feeding experiment. *C. colocynthis* oil was compared to corn oil by feeding

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Table 1—Composition of diets used (% of diet)

Ingredients	1	2	3	4
Isolated soybean protein	25.00	25.00	25.00	25.00
DL-Methionine	0.60	0.60	0.60	0.60
Glycine	0.40	0.40	0.40	0.40
Corn oil	5.00	10.00	–	–
Citrullus colocynthis oil	–	–	5.00	10.00
Glucose	59.17	54.17	59.17	54.17
Cellulose	3.00	3.00	3.00	3.00
Vitamin premix[a]	1.20	1.20	1.20	1.20
Mineral premix[a]	5.63	5.63	5.63	5.63

[a] The vitamin and mineral premix supplied the same levels of nutrients as those recommended by Scott et al. (1976) for semi-purified chick diets.

each of these oils at levels of 5 and 10% of the diet. The extra oil in the 10% diets was added at the expense of glucose. The diets were identical in all other respects. A completely randomized design was employed and broiler-type day-old chicks were used. Three groups of five chicks each were assigned to each treatment. Chicks were reared in a thermostatically-controlled six-deck battery brooder, and feed and water were provided ad libitum. The experimental period was from day-old to 20 days of age. Chick weights and feed consumed were recorded weekly and at the end of the experiment. Data were analyzed statistically by analysis of variance (Snedecor and Cochran, 1976).

RESULTS & DISCUSSION

Characteristics of crude oil

The yield of oil extracted from *Citrullus colocynthis* whole seeds was found to be 24.86% and 26.1% on fresh and dry weight basis respectively. This is comparable to the yield of other oil seeds such as sunflower and safflower and is somewhat higher than that of cotton seeds and soybean (Swern, 1979). However, the yield of oil obtained here was much less than that obtained by Singh and Yadava, (1978) in India, who reported a fat content in *Citrullus colocynthis* seeds that ranged between 30.12–35.66% with a mean of 34.38%. The freshly extracted oil was dark yellow in color with a greenish tint and had a mild odor and flavor. No extremely bitter taste in the oil was detected as was reported by Darwish-Sayed et al. (1973) on the oil of *C. colocynthis* in Egypt.

Physical and chemical characteristics of the oil are shown in Table 2. The oil had a relatively high iodine value thus reflecting a high degree of unsaturation, a fair acid value and average values for the refractive index, unsaponifiable matter, Hehner number and Reichert-Meissl number when compared to other commonly consumed vegetable oils (Swern, 1979).

TLC analysis of the crude oil showed that the major constituents were mainly the triglycerides (Rf = 0.74). Free fatty acids (Rf = 0.37), phospholipids (Rf = 0.22) and sterols (Rf = 0.16) were also detected in addition to other minor unidentified constituents. GLC analyses of the fatty acid methyl esters in conjunction with GLC-MS showed that the degree of unsaturation was over 75%. Linoleic acid was found to be the dominant fatty acid, 50.6%, followed by oleic, 25%, palmitic, 13.5%, stearic, 10.5% and myristic acid, 0.4% (Table 3). Neither fatty acids of chain length greater than C_{18} nor linolenic acid were detected under these conditions. These results are completely different from those reported by Darwish-Sayed et al. (1973) in Egypt in their work on the *C colocynthis* seed lipids. The GLC analysis results of the fatty acids by those workers were done only qualitatively with no quantitation of the fatty acid contents. However, from the GLC chromatogram presented by them on the fatty acid composition of the oil, myristic acid represented the major peak followed by oleic, with linoleic acid coming third or fourth. Moreover, methyl

Table 2—Physical and chemical characteristics of crude oil of C. colycylnthis

Assay	Value
Refractive index, n	1.4655
Iodine number, Wijis	123.90
Saponificati number	202.45
Hehner value	73.35
Acid value, as percent oleic	2.75
Unsaponifiable matter, %	2.15
Reichert-Meissl value	1.95

Table 3—Fatty acid composition of crude oil of Citrullus colocynthis seeds[a]

Fatty Acid	g/100g
C_{14}	0.4
$C_{16:0}$	13.5
$C_{18:0}$	10.5
$C_{18:1}$	25.0
$C_{18:2}$	50.6

[a] Analysis by GC-MS.

myrestoleate and methyl palmitoleate were also detected. On the other hand, results reported by Bishay and Gomaa (1976) in their study on the oils of some medicinal plants including *C. colocynthis*, indicated a linoleic acid content of 48.01%, oleic, 19.59%, palmitic, 18.12% and stearic 12.10%. Palmitoleic was detected at levels of 2.15%. Although these results are closer to the results obtained in this study, yet they still show some significant variation which probably could be due to varietal differences. Based on the results we obtained, the fatty acid composition of *C. colocynthis* oil showed that it falls in the linoleic-oleic acid oils category and is similar to several other vegetable oils. The linoleic acid content was higher than that of cotton seed and close to that of sunflower, soybean and corn (Anonymous, 1961). Therefore the *Citrullus* oil, like some other cucurbit seed oils, might have a good potential use as a cooking oil in spite of the fact that the degree of unsaturation as well as its linoleic acid content are less than those of other known cucurbits seed oil such as *cucurbita foetidissima* which is reported to have over 86% degree of unsaturation and 61–63% linoleic acid content (Bemis et al., 1975; Khoury et al., 1982). The absence of linolenic acid from the *Citrullus* oil might contribute positively to the stability of the oil upon storage.

Oxidative rancidity

Peroxide values of the oil samples after 6 months of storage under various conditions of light and temperature are presented in Fig. 1. As expected (Swern, 1979), both temperature and light had an adverse effect on the stability of the oil in terms of the formation of oxidative rancidity as evident from the higher peroxide values. Oil samples stored at a temperature of 32°C under both dark and indoor light conditions showed higher peroxide values (5.5 and 7.6 meg/kg, respectively) than those stored at the lower temperature of 25°C (4.4 and 6.6 meg/kg), with the lowest values obtained for the oil samples stored at 7°C (1.0 meg/kg). However, at the end of the 5 months period of storage, no increase in the peroxide values was observed at 25°C for both samples stored in the dark and under indoor light conditions. However a continuous increase was observed at 32°C in both groups of oil samples from zero time up to the 6 months period of storage with an abrupt rise in the peroxide value of samples stored at 7°C. Likewise the effect of storage on the oil samples stored under indoor light conditions in comparison to those stored in the dark

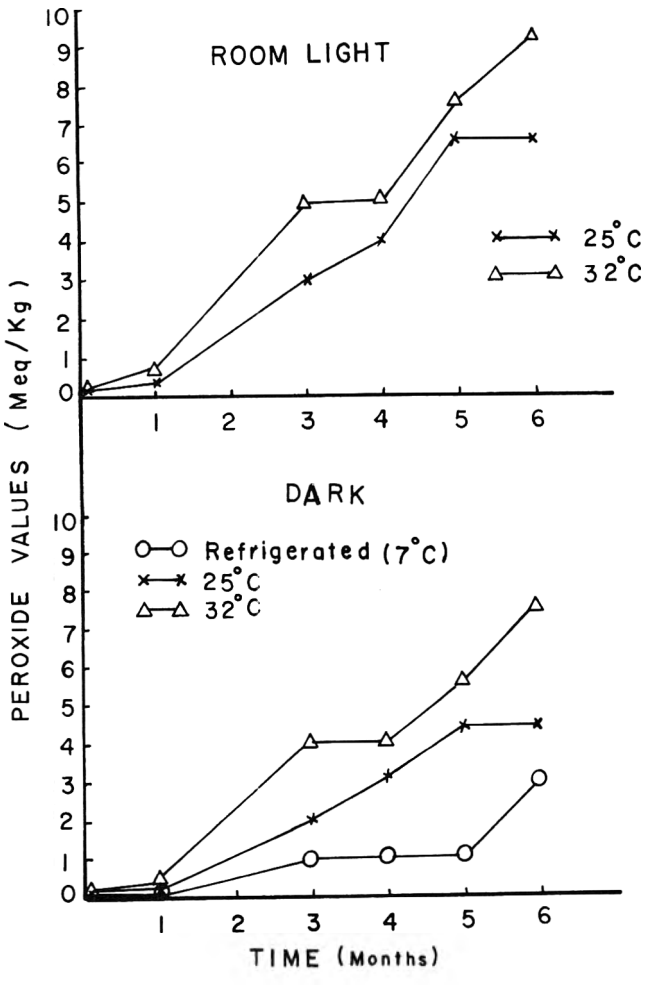

Fig. 1—Peroxide values of the oil under different storage conditions.

showed that the peroxide value was higher in the samples stored under indoor light conditios than in those stored in the dark. Since most consumable vegetable oils are stored either at room temperature, 20–25°C or even lower, and usually in opaque containers, then the peroxide values obtained here for both groups, whether they were stored under indoor light conditions or in the dark, are still below the recommended standards of the Codex Alimentarius (1970) for all the edible vegetable oils and hence can be considered relatively stable upon storage for at least several months.

Animal experiment

Results of the animal feeding trials to investigate the edibility of the oils are shown in Table 4. Feeding *C. colocynthis* oil at 5 and 10% of the diet had no significant effect on body weight, feed consumption or feed efficiency as compared to feeding corn oil at the same levels. Groups receiving 10% of either oil had slightly lower weights than those receiving 5% of the oil as a result of reduced feed consumption, but differences in body weights were not statistically significant ($P > 0.05$). Feed consumption of birds receiving 10% of either oil was also significantly ($P < 0.05$) lower than those receiving 5%. This was expected since these diets were higher in energy and birds usually eat to satisfy their energy requirements. The differences in feed efficiency between the 5 and 10% oil diets were also significant ($P < 0.05$) since raising the fat level of the diet always improves the efficiency of its utilization.

The above results indicate that the nutritional value of

Table 4—Results of feeding experiment (0–20 days of age)[a]

Treatment	Body wt. (g/bird)	Feed Consumed (g/bird)	Feed/Gain
Corn oil, 5%	337 ± 7.2	446 ± 9.8[a]	1.32 ± 0.014[a]
Corn oil, 10%	319 ± 10.8	411 ± 14.3[b]	1.29 ± 0.002[b]
Citrullus colocynthis oil, 5%	338 ± 3.4	446 ± 5.7[a]	1.32 ± 0.006[a]
Citrullus colocynthis oil, 10%	317 ± 3.7	404 ± 5.6[b]	1.27 ± 0.010[b]

[a] Means ± S.E. Those with different superscripts are significantly different at the 5% level of probability.

C. colocynthis oil is not different from that of corn oil when fed to growing chicks up to 3 wk of age.

CONCLUSIONS

THE EVIDENCE PRESENTED in this study shows that *C. colocynthis* oil, which comprised 26% of the whole seeds inicated that *Citrullus* oil might be of certain potential use for human and/or animal consumption. However, long-term (life-term) studies with rats or with other experimental animals will still be needed. Further studies are also still needed for the breeding and selection of the best yielding varieties that are suitable for cultivation on scientific lines. Furthermore, the meal left after the extraction of the oil, might be a good source of protein but more studies are required to evaluate its nutritional quality. Thus, *C. colocynthis* presents an example of an untapped food source that has the potential of being adapted to arid and semi-arid lands. Being resistant to drought and high temperatures, it might then be of great potential as an oil seed crop in many desert areas of the world which are relatively or completely nonproductive. In countries where the major constraint in agriculture is the availability and quality of water and where the priority of cropping is directed toward staple food crops like in Saudi Arabia, this plant can be considered a potential food crop for the production of edible oil.

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Iranian Flat Breads: Relative Bioavailability of Zinc

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ABSTRACT

The effect of flour extraction rate, type and length of fermentation and baking conditions on relative bioavailability of zinc of five distinctly different Iranian flat breads and their unfermented doughs, was determined using weanling rats fed low (5.5 ppm) levels of dietary zinc. When breads rather than unfermented doughs were used for preparation of diets, with one exception, bioavailability of Zn significantly ($P < 0.05$) improved. Rats on the barbari-bread-based diet showed the highest weight gain and femur zinc content. There was no significant difference in weight gain among rats on dough-based diets. High correlation of weight gain and femur zinc with feed intake and low or no correlation with fiber or Zn:phytate molar ratio of the diets was observed.

INTRODUCTION

OCCURRENCE of suboptimal zinc nutrition among village dwellers of Iran is reported. For example, 48 of 59 school children examined in a village by Mahloudji et al. (1975) had low plasma zinc concentrations associated with delayed sexual maturity and retarded growth. Ronaghy et al. (1974) reported comparably lower plasma zinc in 35 or 49 village boys in a more prosperous rural area, a finding shared by 16% of the adults in the vicinity.

Wheat is the major crop and bread and other cereal products provide as much as 50–90% of total caloric and protein intakes of the Iranian people (Faridi and Finney, 1980). Iranian diets have long been suspected of having a causative role in zinc deficiency. Maleki (1972) reported that the intakes of zinc, iron, calcium, magnesium, and phosphorous exceeded recommended dietary allowances by a considerable margin but average daily caloric intake was low. Reinhold (1972) and Reinhold et al. (1974, 1975, 1976) linked the development of zinc deficiency to the high consumption of large amounts of bread made from wheat and whole meals of nearly total extraction rate, usually without leavening or effective fermentation. In a malnutrition case study in Iran, Caughey (1973) reported that while many factors might be responsible, the leading cause was a deficiency in protein intake and linked the occurrence of zinc deficiency to hypoalbuminaemia.

There are five breads popular in Iran and their traditional preparation methods and physical characteristics differ significantly (Faridi et al., 1982). Iranian breads are mainly produced from soft white wheat flours of high extraction levels compared to western-type breads. These flat breads also differ considerably in the extent and type of fermentation given (yeast raised, sour starter and unleavened) and time and temperature at which they are baked. Higher flour extraction rate and inadequate fermentation will produce breads that are relatively high in phytic acid and fiber. The bioavailability of zinc in cereals depends on the presence or absence of certain dietary (and nondietary) factors that either enhance or hinder zinc absorption. Particularly fiber and phytate are believed to adversely affect the bioavailability of zinc (Reinhold et al., 1975; Franz et al., 1980).

The objective of this study was to investigate the influence of flour extraction rate, type and length of fermentation, and baking conditions on relative bioavailability of zinc of five distinctly different Iranian flat breads using rats as a test model.

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MATERIALS & METHODS

THE FIVE TEST BREADS and their corresponding unfermented doughs (immediately after mixing) were prepared according to the formulae and procedures shown in Table 1 and detailed elsewhere (Faridi et al., 1982). Breads were air-dried at room temperature and doughs were freeze-dried, finely ground and stored (3°C) in airtight plastic bags.

Samples of breads and doughs were analyzed for protein, moisture, and ash by AACC (1962) methods. Neutral detergent fiber (NDF) was measured by AOAC (1973) methods, and modified by Robertson and Van Soest (1977) to include pre-digestion of starch and protein by 5 ml of 2% solution of bacterial alpha-amylase which also contains proteases and functions for a limited time at boiling temperature, digesting both starch and protein that might clog the filter. Phytic acid was measured by the method of Thompson and Erdman (1982). Zinc and calcium were determined following dry ashing by atomic absorption spectrophotometer (Rowe, 1973).

The composition of the control and the test diets is listed in Table 2. Composition of the protein-oil-vitamin-mineral-blend used for preparation of the test diets is shown in Table 3. That blend contributed 0.5 ppm zinc to the diet. Zinc was provided in the control diet as zinc sulfate and in the test diets it came from the breads and doughs. because of the low zinc content of barbari bread (and dough), zinc levels of all diets (including control) were adjusted to 5.5 ppm. The protein content was not adjusted. Diets provided adequate amounts of protein (20–23%) and calories (360–380 cal/100g).

Weanling male, Sprague-Dawley rats, averaging about 42g initially, were housed individually in mesh-bottom stainless steel cages. Diets and deionized water were offered (10 rats per diet) ad libitum for 3 wk. Body weight gain, and diet intake records were kept on individual rats. After 3 wk, rats were sacrificed using carbon dioxide and femurs were removed and frozen for later analysis.

Femurs were prepared for Zn analysis according to the method described by Turnland and Margen (1979). Statistical analysis of feed intake, weight gain, femur dry weight and femur zinc content were analyzed by analysis of variance (2 x 5 factorial, completely randomized design) as described by Steel and Torrie (1980).

RESULTS & DISCUSSION

ZINC CONCENTRATIONS and NDF of breads (and doughs) increased with flour extraction rate. Measurable NDF was apparently increased during baking of all breads (Table 1). This may be the result of formation of fiber-like substances produced by non-enzymatic browning and measured as lignin. Van Soest and Robertson (1977) called attention to the generation of materials measured as lignin during the toasting of bread.

Phytate content of unfermented dough increased with flour extraction rate except for sangak dough to which sourdough (pH 3.85) was added (20% on flour basis). Pringle and Moran (1942) showed that as pH of the dough

Table 1—Formulation and composition of five Iranian flat breads

	Breads				
	Barbari	Lavash	Taftoon	Sangak	Village
	78[a]	82	84	87	87
Formula					
Wheat flour (g)	100	100	100	100	100
Yeast (g)	1	0.5	0.5	0.125	–
Sourdough (g)	–	–	–	20	–
Salt (g)	2	2	1	1	1
Date syrup (g)	–	–	1.5	–	–
Water (ml)	60	45	60	85	48
Baking parameters					
Fermentation time (min)	155	90	60	120	90
Baking Temp (°F)	500	630	600	520	410
Baking Time (min)	12.0	1.3	2.5	5.0	3.0
Composition[b]					
Moisture (%)	9.2 (2.8)[c]	8.2 (3.4)	11.8 (2.8)	10.5 (6.3)	11.9 (3.1)
Protein (N x 5.7, %)	8.0 (8.7)	8.4 (9.0)	8.4 (9.2)	8.8 (9.2)	8.9 (9.7)
Ash (%)	1.30(1.34)	1.38(1.42)	1.42(1.52)	1.48(1.61)	2.08(2.12)
NDF (%)[d]	0.56(0.5)	1.39(1.21)	2.09(1.95)	3.56(3.32)	6.41(5.88)
Phytic acid (mg/100g)	41 (105)	81.4 (134)	105 (157)	41 (134)	308 (448)
Zinc (ppm)	7.32(7.55)	9.44(9.93)	10.47(11.41)	12.17(12.93)	17.16(18.68)
Ca (mg/100g)	17.1 (17.5)	19.7 (20.0)	23.40(23.90)	27.00(27.70)	30.90(31.20)

[a] Extraction rate of flour used to produce bread and dough.
[b] Data presented as "composition" in this table are for freeze-dried doughs and air-dried breads.
[c] Values within parentheses refer to doughs.
[d] Neutral detergent fiber

Table 2—Rat diet formulae for zinc study (1 kg)

	DIETS										
	Barbari		Lavash		Taftoon		Sangak		Village		
Ingredients	Dough	Bread	Dough	Bread	Dough	Bread	Dough	Bread	Dough	Bread	Control
Protein-oil-minera -vitamin blend (g)[a]	326	326	326	326	326	326	326	326	326	326	326
Dough (g)[b]	662	–	503.6	–	438.2	–	387.2	–	267.6	–	–
Bread (g)[b]	–	670.4	–	529.6	–	477.6	–	410.8	–	291.4	–
Glucose hydrate (g)[c]	12	3.6	170.4	144.4	235.8	196.4	286.8	263.2	406.4	382.6	674
Zinc as $ZnSO_4$ (ppm)	–	–	–	–	–	–	–	–	–	–	5
NDF (%)[d]	0.33	0.38	0.61	0.74	0.85	1.00	1.28	1.46	1.57	1.87	–
Phytic acid (mg/100g)	69.51	27.63	67.48	43.11	68.80	50.15	51.88	16.84	119.88	89.75	–
Phytic acid:Zinc molar ratio	1.38:1	0.55:1	1.38:1	0.85:1	1.36:1	0.99:1	1.03:1	0.33:1	2.73:1	1.78:1	–

[a] Detailed in Table 3
[b] Bread or dough was added to the level that provided 5 ppm zinc in the diet.
[c] Glucose hydrate was added to make the diet to 1 kg.
[d] Neutral detergent fiber

Table 3—Protein-oil-vitamin-mineral blend ingredients used for preparation of the diets

Ingredients	g/326g Blend
Dried egg white	200
Corn oil	50
Vitamin B + K mix[a]	20
Vitamin A, D, E, in oil mixture[b]	10
Choline bitartrate mix[c]	10
Macro mineral mix[d]	35
Micro mineral mix (zinc free)[e]	1

[a] Vitamin mixes contained mg/326g blend; thiamin·HCl, 30.7; riboflavin, 61.4; niacinamide, 377.61; Ca, d-pantothenate, 187.27; pyridoxine·HCl, 61.4; folic acid, 12.28; biotin, 24.56; vitamin B-12 (0.1% Trit), 125.87; menadione, 3.07.
[b] mg/326g blend: vitamin A acetate (500,000 U/g), 61.4; ergocalciferol (40,000,000 U/g), 0.921; DL α-tocopherol (250 U/g), 921.
[c] 1.8g choline bitartrate in 10g mix.
[d] As g/326g blend: $CaCO_3$, 23.12; Ca_2HPO_3, 36.04; Na_2HPO_3, 20.69; KCl, 23.27; $MgSO_4$, 7.34.
[e] As mg/326g blend: $MnSO_4$, 494.27; $CuSO_4$, 42.98; Ferric citrate, 967.05; KIO_3, 3.07; NaF, 6.14; $VaSO_4$, 12.28; $C_6H_9CrO_6$, 15.35; $C_6H_9CoO_6$, 12.28; $C_4H_6NIO_4$, 12.28; $MoNa_2O_4$, 9.21; BrNa, 3.07; Na_2O_3Se, 0.614; $B_4Na_2O_7$, 73.99; $SnSO_4$, 0.614.

decreased, phytate destruction increased. Phytate destruction during fermentation and baking was most significant for sangak bread (sour starter) followed by barbari, lavash and taftoon (all yeast raised) then village (nonleavened).

Among diets, NDF content of village bread diet was the highest (1.87%) and the barbari dough diet was the lowest (0.33%) (Table 2). Village dough diet contained the highest concentration of phytic acid (120 mg/100g) and sangak bread diet contained the least (16.84 mg/100g). When phytic acid:zinc molar ratio was calculated only village dough diet had a ratio more than 2:1.

Weight gain, feed intake, feed efficiency, femur weight and zinc content of rats consuming five Iranian breads and their unfermented dough diets are shown in Table 4. Among breads and dough-based diets, rats consumed significantly higher amounts of barbari and taftoon breads and subsequently achieved greater weight gain, femur dry weight and zinc content. A high correlation between feed intake and weight gain and femur content was observed (0.96 and 0.87, respectively). It was surprising to see that the

Table 4—Weight gain, feed intake, feed conversion, femur weights and zinc content in animals consuming five Iranian breads and their unfermented dough diets (10 rats per diet)[a,b]

Diets		Weight gain	Feed intake	Feed efficiency[c]	Femur		
					Dry weight mg	Zinc Content μg/femur[d]	Zinc Content μg/g femur[d]
Barbari (78)[f]	Dough	74 ± 6d[e]	117 ± 17c	2.39 ± 0.04a	198 ± 13b,c	11 ± 1.2d	55.5 ± 2.4c
	Bread	112 ± 8a	235 ± 15a	2.10 ± 0.02a	231 ± 14a	16.7 ± 0.9a	72.3 ± 1.5a
Lavash (82)	Dough	76 ± 8d	156 ± 11d	2.05 ± 0.07a	188 ± 16c,d	8.3 ± 1.0e	44.0 ± 1.6d
	Bread	89 ± 6b,c	183 ± 13c	2.06 ± 0.01a	206 ± 14b	13 ± 1.9b	63.1 ± 5.0b
Taftoon (84)	Dough	75 ± 15d	170 ± 20c	2.27 ± 0.20a	192 ± 15c,d	8.5 ± 0.6e	44.3 ± 1.3d
	Bread	95 ± 10b	203 ± 13b	2.14 ± 0.09a	215 ± 18b	13.4 ± 1.1b	62.3 ± 1.1b
Sangak (87)	Dough	70 ± 15d	156 ± 18d	2.23 ± 0.23a	188 ± 12c,d	8.4 ± 0.9e	44.7 ± 2.0d
	Bread	75 ± 15d	170 ± 18c	2.27 ± 0.22a	191 ± 18c	11.9 ± 0.8c,d	62.3 ± 1.7b
Village (97)	Dough	68 ± 8d	151 ± 11d	2.22 ± 0.10a	183 ± 10d	7.9 ± 1.2e	43.2 ± 4.2d
	Bread	80 ± 12c	179 ± 10c	2.24 ± 0.22a	205 ± 11b	12.2 ± 1.0c	59.5 ± 1.7d
Control		81 ± 7c	170 ± 17c	2.10 ± 0.03a	199 ± 11b,c	10.5 ± 0.9d	52.7 ± 1.5c

[a] Mean ± SD.
[b] All diets provided 5.5 ppm zinc.
[c] Grams food consumed per gram weight gain.
[d] Dry Basis.
[e] Means without a common letter in their superscripts are significantly different ($P < 0.05$).
[f] Values in parenthesis indicate extraction rate of flours used to produce breads and doughs.
[g] Zinc was added as $ZnSO_4$.

differences in feed efficiency of bread- and dough-based diets were not significant.

Animals on bread-based diets showed significantly ($P < 0.05$) higher weight gain than those on the corresponding dough-based diets (except for sangak bread). Among bread-diets barbari induced the highest weight gain followed by taftoon ($P < 0.05$). There was no significant difference in weight gain among rats on dough-diets indicating that fiber and/or phytate content at the levels employed in this experiment had no significant impact on zinc bioavailability. However, different methods of fermentation (yeast-raised, sourdough, or unleavened) and time and temperature of oven baking improved palatability and feed intake and consequently the weight gain. Rats on village bread diet (phytate:Zn molar ratio 1.78:1) showed higher growth than those on sangak bread diet (phytate:Zn molar ratio 0.33:1). The lower phytic acid content of the western white wheat flour used in this investigation might be one of the factors responsible for higher zinc bioavailability compared to Reinhold's findings (1971). The phytic acid content of village bread we prepared (0.45%) was significantly less than that used in previous studies in Iran (more than 0.8%) (Reinhold, 1972; Ter-Sarkisian et al., 1974). Nahapetian and Bassiri (1976) reported large variations in Iranian wheat phytate content due to environmental conditions under which wheat is grown, so it may be expected that significant variations of phytate concentrations are observed. There are many reasons to expect the fiber content would also vary from year to year within and among varieties of wheat.

Animals on bread-based diets showed significantly higher ($P < 0.05$) femur zinc content than those on the corresponding dough-based diets. Among breads, barbari-diet induced the highest ($P < 0.05$) femur zinc content. There was no significant difference among rats on diets based on lavash and taftoon breads and among those on sangak and village breads. Between dough-based diets, barbari was the highest ($P < 0.05$) and differences among the others were not significant. Correlations between femur zinc content with fiber or Zn:phytate molar ratio were 0.67 and 0.68, respectively.

When breads rather than unfermented doughs were used for preparation of diets, with one exception bioavailability of Zn significantly ($P < 0.05$) improved. One explanation is that fermentation and/or baking improved palatability and induced higher feed intake. Another reason is that more zinc was available due to phytate hydrolysis during fermentation; however, no strong correlation was found in this study. In addition, protein changes that occur during bread making processes, particularly oven baking, might prevent the complexing of phytate, protein and Zn.

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Dietary Fibers in Muffins

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ABSTRACT

Product acceptability of muffins prepared with 25% (w/w) of the flour substituted with α-cellulose, corn bran, oat hulls, rice bran, soy bran, a commercial wheat bran, and an AACC wheat bran was evaluated. Muffins with corn and both wheat brans were the only acceptable products. The oat hull muffin had such an objectionably gritty mouthfeel that oat hulls cannot be considered a feasible fiber additive in baked products. Flavor and mouthfeel were the leading characteristics in the product. Particle size was the only physical or chemical characteristic which correlated to the sensory results. There was a significant ($p=0.001$) correlation ($r=0.74$) between overall acceptability on a sliding line scale and the FACT scale.

INTRODUCTION

THE IMPORTANCE of dietary fiber in the diet was reported by Burkitt et al. (1972) and Painter and Burkitt (1971) who delineated possible "fiber deficiency diseases" in Western or developed societies. The advantages of supplementation with fiber were recognized by commercial interests and many products have been "improved" by the addition of dietary fiber. Baked products have been used as fiber carriers by substituting some of the flour with fiber sources. Cellulose products and various wheat brans have been incorporated successfully in bread (Pomeranz et al., 1977), layer cakes (Zabik et al., 1977; Springsteen et al., 1977), and cookies (Vratanina and Zabik, 1978; Goreyzca and Zabik, 1980). These studies and the findings of Shafer and Zabik (1978) showed that the various fiber sources may alter properties of the food products including appearance, flavor, texture, and mouthfeel. The purpose of this investigation was to incorporate seven different dietary fiber sources in muffins, examine the products' consumer acceptance and determine possible correlations among sensory, physical and chemical characteristics.

MATERIALS & METHODS

Fiber sources and preparation

The seven different dietary fiber sources used in this study included α-cellulose (ICN Pharmaceuticals, Inc.), corn bran (The Quaker Oats Co.), oat hulls (National Oats Co., Inc.), rice bran (Rivera Foods, Inc.), soy bran (ADM Co.), a commercial wheat bran (Gooch Milling & Elevator Co.), and an AACC hard red spring wheat bran.

With the exception of α-cellulose, all fiber sources were ground with a Wiley mill Model No. 2. Particle size of fiber sources was determined by placing a 20g sample of the material subsequently used for muffin preparation on the largest sieve of the following numbered series of stainless steel U.S. Standard Sieves: 10, 20, 30, 40, and 60 (mesh sizes, 9, 20, 28, 35, and 60, respectively), fitted with a pan and a cover. The nested sieves were shaken for 3 min; the retained material on each sieve was weighed and expressed as a percent of the original sample weight.

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For computer analysis of the particle size data, it was necessary to obtain one value for the particle size of each fiber source. U.S. Standard Sieves, 20, 30, 40, 60, and <60, were given values of 5, 4, 3, 2, and 1, respectively. These values were then multiplied by the percent of sample retained on the various sieves and summed to yield one value (particle size index) (Table 1).

Preliminary sensory evaluation using a 12-member trained panel determined that the highest fiber level at which all seven fiber sources were found to be acceptable was a 25% (w/w) substitution of fiber source for flour. All muffins used for this study were prepared according to a formulation using this 3:1 ratio (Table 2).

Three technicians were trained in muffin preparation and produced all muffins used in the study. The dry and liquid ingredients were first mixed separately and then combined according to the muffin method of mixing (Campbell et al., 1979). A fork was used to combine the ingredients, mixing only enough to moisten the dry ingredients but not enough to produce a smooth batter (16 stirs). The resulting lumpy batter was divided evenly into standard size 12 count, teflon-coated muffin tins. The muffins were baked at 218°C (425°F) on the center rack in homestyle ovens (General Electric Model No. 750S2WH) for 20 min. After cooling for 5 min, the muffins were removed from the tins and cooled on racks. The cooled muffins were sealed in polyethylene bags and frozen (−17°C to −11°C) for a minimum of 3 wk.

Proximate analyses

The fiber sources and muffins were analyzed for moisture, protein (micro-Kjeldahl), crude fat (hexane extraction), and ash using AOAC standard methods (AOAC, 1970). Nonfiber carbohydrate was determined by difference on a dry weight basis {100 − (protein + fat + ash + enzymatic neutral detergent fiber)}.

Two dietary fiber determinations were used in order to emphasize the effect of methodology on the quantitative identification of this material. The AACC Method 32-20 (1978) is a neutral detergent method with an amylase digestion, referred to here as ENDF for Enzymatic Neutral Detergent Fiber. The other dietary fiber determination used was the Enzymatic Indigestible Residue (EIR) which employs pepsin and pancreatin digestions, (Hellendoorn et al., 1975).

All analyses were replicated two or more times, and mean scores of all replications were calculated.

Sensory evaluation

Frozen muffins were sliced in half and placed cut side down on clear plastic film on the glass plate of a Xerox copy machine, Model

Table 1—Sample calculation of particle size index using AACC bran

U.S. Std. Sieve and Mesh Size	Sample retained on U.S. Std. sieves, %		Corresponding values		Particle size index
#20 20 mesh	0.5	X	5	=	2.5
#30 28 mesh	25.2	X	4	=	100.8
#40 35 mesh	47.0	X	3	=	141.0
#60 60 mesh	16.2	X	2	=	32.4
<#60 <60 mesh	11.1	X	1	=	11.1
					287.8

5400. These photocopies were then evaluated by a three member trained panel as to muffin shape, grain, and other visual characteristics.

Room temperature muffins were coded and served in randomly assigned order to a 100-member untrained panel. Each panelist received a total of seven muffins to evaluate. Sensory evaluation was conducted under ASTM guidelines for sensory testing (ASTM, 1968) using two different acceptability methods. A nine-point line scale was used to evaluate the following seven acceptability characteristics: color, aroma, general appearance, texture, mouthfeel, flavor, and overall acceptability. The scale consisted of horizontal lines 18 cm long with the word unacceptable written above one end and the word excellent above the other. The panelist placed a slash on each line according to how much he/she liked or disliked the muffins. These slashes were then converted to numerical values from 1 to 9. Nine was the highest acceptability score. The other method used was the Food Action Rating (FACT) scale (Schutz, 1965). The FACT scale consisted of nine statements reflecting how a person might feel about eating the food product. The panelist checked the one statement which best represented his/her attitude toward each muffin type. Each statement corresponded to a numerical score of 1 to 9, with 9 being the highest acceptability score. One score for each characteristic was obtained for each muffin type by each judge using this method.

All panel members were students, faculty or staff in the Department of Nutrition and Food Science and School of Home Economics. Panelists' ages ranged from 20 to 58 with approximately 60% being female. All were given explicit instructions in use of the line scale. The majority of the panelists (approximately 90%) had used this scale previously. All panelists were familiar with muffins and their preferred characteristics.

Statistical analyses

The results of the sensory evaluation and the physical and chemical analyses were statistically analyzed using the *Statistical Package for the Social Sciences* (SPSS) for analysis of variance and Pearson's correlations (Nie et al., 1975). Means were compared by the Least Significant Difference (LSD) Test (Little and Hills, 1978).

Table 2—Muffin formulation

Dry ingredients:	225 g all-purpose flour
	140 g sugar
	75 g fiber source
	12 g baking powder
	4 g salt
Liquid ingredients:	270 ml reconstituted nonfat dry milk
	60 ml safflower oil
	50 ml egg

Table 3—Particle size of dietary fiber sources (as % of sample retained on U.S. Standard Sieves)

Fiber source	#20 20 mesh	#30 28 mesh	#40 35 mesh	#60 60 mesh	<#60 <60 mesh
AACC bran	0.5	25.2	47.0	16.2	11.1
Wheat bran	3.6	29.7	33.9	11.8	21.0
Oat hulls	0.5	2.6	29.2	20.3	47.4
Soy bran	1.5	6.1	13.8	14.8	63.8
Corn bran	—	1.0	11.2	39.1	48.7
α-Cellulose	—	4.1	11.3	8.8	75.8
Rice bran	0.5	2.0	3.1	12.7	81.7

RESULTS & DISCUSSION

Particle size

Particle size data of the fiber sources as they were incorporated into the muffins are shown in Table 3. The two wheat brans (commercial and AACC sources) were the only fiber sources which did not have the largest percentage of particles less than 60 mesh in size. The magnitude of this difference is better shown in Table 4. The particle size indices of the two wheat brans were almost 100 points greater than the next largest particle size index of the oat hulls.

Differences in particle size between fiber sources and the diversity of particle size within each fiber can be explained by the preparation (grinding and recombination) of the fiber sources for incorporation into muffins. During grinding in the Wiley mill, some particles were forced through the sieves in an elongated fashion and therefore actually had larger particle sizes than the holes in the sieves. Also, the particles which would not pass through the sieves were recombined with the sieve portion to avoid separation by grinding as the complete fiber was desired for muffin preparation. The proportion of sample passed to that retained by the sieves varied between fiber sources.

The differences in grinding ability between the fiber sources may be due to differences reflected in ash content (see Table 5) and/or to the original particle size as received from the suppliers.

Proximate analyses

The results of the proximate analyses on the fiber sources are given in Table 4. Determination of dietary fiber levels was made by two established analyses, ENDF and EIR, rather than by a combination of the two methods, in order to provide data comparable to previous studies and commercial analyses, and to avoid the introduction of an additional untested procedure. While highly purified α-cellulose showed a fiber content of 98% by ENDF and 100% by EIR, in all other fibers the ENDF method yielded consistently lower values than the less stringent and more physiologically modeled EIR analysis. This point is also

Table 4—Particle size index of dietary fiber sources

Fiber source	Particle size index
AACC bran	288
Wheat bran	283
Cat hulls	189
Soy bran	167
Corn bran	165
α-Cellulose	144
Rice bran	127

Table 5—Proximate analyses of dietary fiber sources (as % on dry weight basis)

Fiber source	Moisture	Protein[a]	Fat	Nonfiber carbohydrate[b]	Ash	ENDF	EIR
α-Cellulose	5.0	0.0	tr	0.0	0.3	100.0	98.0
Corn bran	7.7	5.0	0.5	17.7	0.5	76.3	86.4
Oat hulls	6.0	5.2	1.2	12.4	7.0	74.3	79.1
Rice bran	9.5	17.8	2.5	43.5	13.2	23.0	36.0
Soy bran	7.8	10.7	1.3	21.9	4.2	61.9	74.0
Wheat bran	8.7	16.4	3.3	28.9	7.0	44.5	52.0
AACC bran	7.6	20.1	4.2	23.6	7.1	45.0	51.4

[a] Protein = Nitrogen x 6.25
[b] Nonfiber carbohydrate = 100 — (Protein + Fat + Ash + ENDF)

Table 6—Dietary fiber content of fiber muffins (on dry weight basis)

Muffin type	ENDF %	ENDF per muffin, g[a]	EIR %	EIR per muffin, g[b]
α-Cellulose	15.3	7.3	17.2	8.2
Corn bran	10.2	4.5	15.0	6.6
Oat hulls	9.0	3.6	14.9	5.9
Rice bran	9.0	3.9	12.9	5.6
Soy bran	2.7	1.3	8.5	4.1
Wheat bran	2.9	1.4	10.0	4.7
AACC bran	4.1	1.8	10.3	4.6

[a] ENDF per muffin, g = % ENDF x dry weight of one muffin (g)
[b] EIR per muffin, g = % EIR x dry weight of one muffin (g)

Table 7—Order of acceptability and comparison of means[a] (with standard errors) of fiber muffins

Muffin type	Line scale	FACT scale
Wheat bran	$6.38^{a} \pm 0.184$	$6.22^{a} \pm 0.141$
AACC bran	$6.31^{a} \pm 0.180$	$6.11^{a} \pm 0.173$
Corn bran	$5.79^{b} \pm 0.198$	$5.47^{b} \pm 0.207$
α-Cellulose	$4.73^{c} \pm 0.215$	$4.93^{c} \pm 0.201$
Soy bran	$4.50^{c} \pm 0.178$	$4.37^{d} \pm 0.172$
Rice bran	$4.39^{c} \pm 0.199$	$4.09^{d} \pm 0.190$
Oat hulls	$4.37^{c} \pm 0.185$	$3.99^{d} \pm 0.190$

[a] Means with the same superscript cannot be judged significantly different by the LSD Test.

Table 8—Comparison of means[a] of the acceptability characteristics of fiber muffins

Muffin type	Flavor	Mouthfeel	Texture	Appearance	Aroma	Color	Overall acceptability
Wheat bran	6.38^{a}	6.14^{a}	6.17^{a}	6.50^{a}	6.06^{a}	6.39^{a}	6.38^{a}
AACC bran	6.32^{a}	6.08^{a}	6.16^{a}	6.54^{a}	6.12^{a}	6.54^{a}	6.31^{a}
Corn bran	5.47^{b}	5.28^{b}	5.64^{b}	$6.14^{a,b}$	6.39^{a}	$6.05^{a,b}$	5.79^{b}
α-Cellulose	4.90^{c}	4.74^{c}	5.05^{c}	4.59^{c}	4.54^{c}	4.50^{c}	4.73^{c}
Soy bran	4.17^{d}	4.78^{c}	5.13^{c}	4.91^{c}	$4.86^{b,c}$	4.93^{c}	4.50^{c}
Rice bran	3.76^{d}	$5.00^{b,c}$	5.49^{c}	4.93^{c}	4.52^{c}	4.95^{c}	4.39^{c}
Oat hulls	3.96^{d}	4.18^{d}	5.05^{c}	5.84^{b}	5.18^{b}	5.79^{b}	4.37^{c}

[a] Means with the same superscript cannot be judged significantly different by the LSD Test.

true, without exception, for the dietary fiber contents of the muffins as shown in Table 6.

The fiber sources other than α-cellulose contained significant amounts of other components. Rice bran, for example, had large amounts of protein, non-fiber carbohydrate, and ash. The dietary fiber content of rice bran was therefore very low, 23% or 36%, depending on the method used.

The differences in the results provided by these methods and the range of values for different fiber sources found in the literature emphasize the variability of the sources and the need to continue efforts to achieve improved measurement of dietary fiber.

Sensory evaluation and statistical analyses

The order of acceptability of the muffins as determined by analysis of the overall acceptability characteristics is given in Table 7. The two wheat brans (commercial and AACC source) were the most acceptable muffins and were not significantly different by the LSD Test. The corn bran muffin was judged significantly different from all others as the third most acceptable product. The acceptability of these three products may be due to the panelists' familiarity with wheat and corn brans. Consumers are accustomed to wheat bran in various baked products and corn bran, particularly as cornmeal, in corn bread products. The α-cellulose muffin was judged significantly different in the FACT method only.

The acceptability of the muffins is clarified in Table 8 which compares the means of the individual acceptability characteristics of the various muffins. Muffins made with wheat, AACC and corn brans were judged to be acceptable on all characteristics evaluated. (An acceptable mean was arbitrarily set at ≥ 5.0 as the panelists were instructed that any rating at midpoint or above would be considered acceptable.) These results are in agreement with the findings of Shafer and Zabik (1978) who prepared acceptable layer cakes with 30% (w/w) of the flour substituted with corn and various wheat brans. They also found that oat and soy bran substitution produced unacceptable cakes due to their poor flavor.

The data reported in Table 8 indicate that the oat hull muffin was judged significantly different from all others as having the least acceptable mouthfeel, an objectionable quality of oat hulls also noted in a study by Pomeranz et al. (1977). They stated that the undesirable and unacceptable grittiness (presumably from the silica) and splinter-like particles of oat hulls would exclude their use in breadmaking.

Table 9—Correlations of flavor, mouthfeel, texture, appearance, aroma and color

Acceptability characteristics[a]	Line scale, overall acceptability rating	FACT scale rating
Flavor	0.88*	0.71*
Mouthfeel	0.74*	0.58*
Texture	0.68*	0.48*
Appearance	0.66*	0.48*
Aroma	0.65*	0.46*
Color	0.61*	0.45*

[a] Mean score for all muffins
* Significance: $p = 0.00001$

All muffins were judged acceptable for texture. Visual evaluation of the general shape and air spacing of all muffins, as recorded by the photocopies, was judged good to excellent. Variance between muffins with the same fiber source was judged to be minimal.

Appearance, aroma and color correlated least with the overall acceptability characteristic indicated in Table 9. Aroma may have been difficult to judge in the room temperature muffins.

The similar results of the two sensory evaluation methods are emphasized in Table 9. The correlation coefficients for the FACT scale were consistently lower than those of the line scale. All correlations were highly significant ($p = 0.00001$). Flavor and mouthfeel were the most strongly correlated to the overall acceptability characteristic ($r = 0.88$ and $r = 0.74$), respectively).

The pairing of the means of each acceptability characteristic with the corresponding particle size index indicated a positive correlation in every case. Correlation coefficients and their significance were calculated and the mean scores are shown in Table 10. There were no other significant correlations between the sensory results and the physical and chemical analyses.

In summary, muffins prepared with 25% (w/w) wheat and corn brans were found to be acceptable products as judged by 100 panelists using both a line scale and the

—Continued on page 118

Gas-Particle Heat Transfer Coefficient for the Fluidization of Different Shaped Foods

A. VAZQUEZ and A. CALVELO

ABSTRACT

Gas-particle heat transfer coefficients for the fluidization of diced potatoes and potato strips were measured in a batch fluidized bed in which wet particles were dried under constant rate period conditions. Thus, the surface temperature of particles was equal to the wet-bulb temperature of the air. The minimum fluidization voidage and velocity were also measured as well as the bed expansion characteristics. Results are expressed through Colburn's factor, as a function of a modified Reynolds number and an Archimedes number, allowing for the correlation of data for different shaped foods (potato cubes and strips, peas, etc.) into a single equation.

INTRODUCTION

THE FREEZING of granular foods as "individually quick frozen" (IQF) products increases continuously through the use of fluidized bed freezers. Even though a wide variety of products can be processed by this method, peas, diced potatoes and potato strips (or French fries) involve the most important production volume.

The design of fluidized bed freezers requires information on solid fluidization conditions, on gas-particle heat transfer coefficients which must be used with freezing time models for individual particles, and on residence times of solids in the continuous bed.

Residence time predictions for fluidized pea beds have already been obtained in a previous work (Vázquez and Calvelo, 1982) and several models for freezing time calculations of particles are described in the literature (Nagaoka et al., 1955; Cleland and Earle, 1979a, b; De Michelis and Calvelo, 1982). However, these models require the knowledge of gas-particle heat transfer coefficients as well as its dependence on the fluidizing conditions and the properties of the system.

The heat transfer coefficient for fluidized beds depends on the density, size and shape of particles, as well as on the minimum fluidization voidage. It is also related to the thermal properties and the superficial velocity of air.

Many studies on gas-particle heat transfer coefficients have been published although important discrepancies exist among the predictions made by different authors. Moreover, few publications deal with shallow beds and big particle sizes.

The heat transfer coefficient in fluidized pea beds was experimentally determined in a previous work (Vázquez and Calvelo, 1980) showing satisfactory agreement with the correlations proposed by Bradshaw and Myers, 1963 and by Chang and Wen, 1966 for particles of several materials. The particles' density effect was considered through the Archimides number, extending the results to spherical shaped products in general.

However, for particles with shapes other than spherical, the fluidizing conditions change and a different behavior is detected from the heat transfer view point. The effect of particle shape upon fluidization has been dealt with in literature through the adoption of an effective particle diameter in order to consider the influence of size and shape (Richardson and Zaki, 1954, Beránek, 1960, Kmieć, 1976; Rowe, 1978).

As far as potato strips and dices are concerned, some fluid dynamic aspects have been studied (Mc Lain and Mc Kain, 1979) for the case of hydraulic transportation. However, information on heat transfer coefficients is not available for aggregative fluidization of foods and a generalization of the existing correlations for spheres is required for different-sized dices and strips.

In the present paper, the gas-particle heat transfer coefficient was experimentally measured in a batch fluidized bed under similar conditions to those existing in industrial processing equipments.

The experimental method used was the same as applied in a previous work performed with peas (Vázquez and Calvelo, 1980) where wet particles were maintained in the constant rate period of drying and a pseudo-steady state was achieved where the heat transferred to the particle evaporated an equivalent quantity of water.

The minimum fluidization conditions (voidage and velocity) and the bed expansion in terms of the superficial velocity were also measured for the different particles studied.

MATERIALS & METHODS

Bed fluid dynamics

The gas-particle heat transfer calculation in fluidized beds requires information not only on the heat transfer coefficient but also on the bed voidage for different superficial velocities of air. Consequently, the initial studies were carried out on bed fluid dynamics which besides ensuring a correct fluidization, allowed for a minimum fluidization voidage and velocity as well as a voidage for different flow rates to be evaluated.

A batch fluidized bed with a 0.24m x 0.30m section and glassed side walls was used for the fluidization experiments. Air was supplied at room temperature by a centrifugal blower that could yield a maximum volumetric flow rate of 70 m^3/min and a discharge pressure of 28 mm of water. The air distribution grid was a perforated stainless steel plate with 30% of free area and 1.25 mm diameter holes displayed in a triangular arrangement. This type of grid showed a satisfactory behavior in a previous work (Vázquez and Calvelo, 1980).

By using baffles and grids a pressure chamber was formed below the air distributor which improved the uniformity of the air flow inside the bed.

The superficial air velocity was measured by a velometer located on the upper part of the bed, while pressure drop was determined by an open manometer with four static pressure inlets connected each one to a side of the bed below the air distributor. This device smoothed fluctuations due to the aggregative fluidization.

Initially, the pressure drop through the grid was measured as a function of the superficial air velocity. Subsequently the pressure drop (grid-bed) for several heights of bed and different superficial air velocities were measured. The bed pressure drop was evaluated as a difference.

The expanded bed height was also determined by direct observation through the glassed bed walls.

The fluid-dynamic behavior was experimentally studied with both wooden and fresh potato particles. The wooden particles were

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used to analyze the expansion properties of dry materials detected in a previous paper as different from the wet particle behavior.

The solid density ρ_s was measured by using picnometry or by calculating the volume in the case of perfect geometrical shapes. The bed porosity ϵ was evaluated from the weight of solids m_s and the bed height H as:

$$\epsilon = 1 - (m_s/\rho_s\, S\, H) \qquad (1)$$

where S is the bed section.

The particles tested were 1.53 cm wooden cubes, 0.89 cm and 1.04 cm diced potatoes and 0.92 x 0.92 x 5.3 cm potato strips.

Gas-particle heat transfer coefficient

The experimental method used for evaluating the gas-particle heat transfer coefficient was based on the steady state heat transfer from the air to the wet particle and the consequent release of latent heat through the evaporation of water. Thus, the surface temperature of particles, undergoing a drying process within the constant rate drying period, will be equal to the wet-bulb temperature of the air (Vázquez and Calvelo, 1980).

The initial period of the fluidized bed operation was discarded in order to avoid transient phenomena as well as water removed mechanically from particles. Once this period is finished (1 min approximately) a steady state condition is reached, where the temperature of solids remains constant and air flows through the bed while moving along the corresponding adiabatic line on the psychrometric chart. The bed walls were thermally insulated to accomplish this condition.

The steady state behavior ends when the constant-rate drying period of solids is finished. The steady state period depends on the material to dry as well as on the velocity, temperature and relative humidity of the flowing air. The end of this period was detected by an increase in the exit-air dry bulb temperature. For the case of diced potatoes and potato strips, the steady state period was approximately 3 min long.

Cubes when fluidized, show a natural tendency to arrange one over the other lining up around the bed walls (Richardson and Zaki, 1954). This effect was avoided by periodic mixing of the nearby-wall cubes during runs.

The bed used was the same already described but laterally insulated with expanded polystyrene. The wet and dry bulb temperatures of the inlet and outlet air was measured by means of cooper-constantan thermocouples connected to a multichannel potentiometric recorder. The agreement between the wet-bulb temperatures of the inlet and outlet air gave evidence that the adiabatic performance was fulfilled and also indicated that particles behaved under the constant rate drying condition.

Prior to each run, the potato particles were kept under water at the wet-bulb temperature in order to shorten the transient initial period. The runs for heat transfer coefficients evaluation were performed with 0.89 cm potato cubes and with 0.92 x 0.92 x 5.3 cm potato strips.

Heat transfer coefficients, h, were calculated from the following equation:

$$h = \frac{G_o\, C_p\, D_p}{6\,(1 - \epsilon_{mf})H_{mf}} \ln \frac{(T_s - T_1)}{(T_s - T_2)} \qquad (2)$$

where T_s is the surface temperature of particles (supposed equal to wet-bulb temperature of the air), T_1 and T_2 are the dry-bulb temperatures of inlet and outlet air respectively, H_{mf} is the bed height under minimum fluidization conditions, and G_o the superficial air mass flux. Perfect mixing for the solids and plug flows for the gaseous phase were assumed. (Vázquez and Calvelo, 1980).

The effective diameter D_p for nonspherical particles was calculated as:

$$D_p = D_e\, \psi \qquad (3)$$

Where D_e is the equivalent diameter [diameter of a sphere with the same volume as the particle: $D_e = (6\, V_p/\pi)^{1/3}$ and ψ is the sphericity factor, the ratio of the surface area of a sphere to the surface area of a nonspherical particle with the same volume ($\psi = \pi\, D_e^2/A_p$). Hence:

$$D_p = 6\, V_p/A_p \qquad (4)$$

RESULTS & DISCUSSION

Bed fluid-dynamics

Fig. 1 shows the pressure drop per unit bed height for potato strips and diced potatoes of different sizes.

Full lines in the fixed bed region of Fig. 1 corresponds to Ergun's predictions (Kunii and Levenspiel, 1969) according to:

$$\frac{\Delta p}{(1 - \epsilon_m)H_m} = \frac{150\mu\,(1 - \epsilon_m)}{D_p^2\, \epsilon_m^3} v_o + \frac{1.75\,\rho}{D_p\, \epsilon_m^3} v_o^2 \qquad (5)$$

The properties of air used in the calculations were ρ = 1.213 kg/m^3 and $\mu = 1.813 \times 10^{-5}$ kg/ms.

The minimum fluidization superficial velocities were obtained in each case by extending the horizontal line of the fluidized bed region until intersection with Ergun's equation. The horizontal line in Fig. 1 corresponds to:

$$\Delta p = (\rho_s - \rho)\,(1 - \epsilon)\, H\, g \qquad (6)$$

Fig. 1 also shows that the experimental pressure drop at minimum fluidization velocity is smaller than that predicted by Ergun's equation with the fixed bed porosity ϵ_m. This discrepancy is attributed to the rearrangement of the bed into a looser structure just before fluidization, with a minimum fluidization voidage ϵ_{mf} higher than the fixed bed value.

This effect is shown in Fig. 2 where the bed expansion is

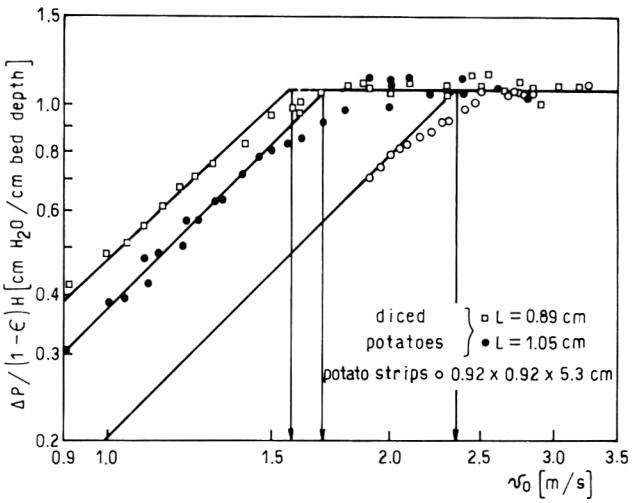

Fig. 1—Pressure drop as a function of superficial velocity.

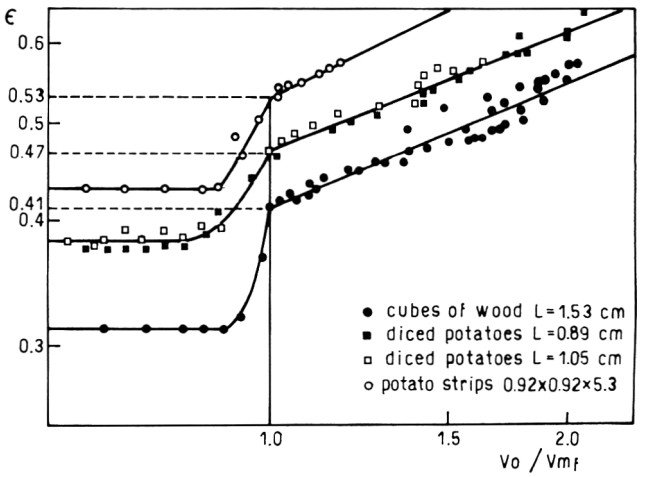

Fig. 2—Fluidized bed voidage as a function of superficial velocity.

Table 1—Fluidization characteristics of different particles

Material	$\rho_s \times 10^{-3}$ (kg/m^3)	$D_e \times 10^2$ (m^2)	ψ	ϵ_m	ϵ_{mf}	n	v_{mf} (m/s)
Cubes of wood L = 1.53 cm	1.00	1.90	0.806	0.31	0.41	0.438	1.45
Diced potatoes L = 1.04 cm	1.07	1.29	0.806	0.38	0.47	0.400	1.70
Diced potatoes L = 0.89 cm	1.08	1.10	0.806	0.38	0.47	0.400	1.57
Potato strips 0.92 x 0.92 x 5.3 cm	1.07	2.17	0.634	0.43	0.53	0.469	2.36

Table 2—Gas particle heat transfer coefficients for diced potatoes L = 0.89 cm

v_o m/s	$H_{mf}10^2$ m	$\bar{h}$ W/m^2°C	Ar 10^{-7}	J_H	Re′ 10^{-3}	$J_H Ar^{-0.179}$ 10^3
2.5	3.5	167	2.64	0.0441	3.32	2.07
2.7	3.5-5.0	184	2.74	0.0456	3.62	2.13
2.8	3.5-6.0	163	2.74	0.0375	4.01	1.75
3.0	3.0-9.0	183	2.73	0.0395	4.42	1.84
3.2	3.5-6.0	186	2.73	0.0378	4.92	1.76
3.4	3.0-6.0	177	2.68	0.0342	5.36	1.60
3.5	3.5-6.5	169	2.73	0.0309	5.76	1.44
3.6	3.0	173	2.77	0.0314	6.09	1.46
3.7	3.5-6.0	189	2.76	0.0329	6.36	1.53
3.8	3.5-6.5	175	2.76	0.0296	6.68	1.38
3.9	3.5	188	2.74	0.0311	6.95	1.45
4.1	3.5	180	2.70	0.0292	7.31	1.37
4.3	4.0	178	2.69	0.0279	7.98	1.30

plotted as a function of the superficial velocity v_o and in Table 1 which compares ϵ_{mf} with ϵ_m for different fluidized particles.

Results from Fig. 2 were correlated similarly to those obtained for peas (Vázquez and Calvelo, 1980) through the following empirical equation:

$$\epsilon = \epsilon_{mf} \left(\frac{v_o}{v_{mf}}\right)^n \qquad (7)$$

where n = 0.40 and n = 0.44 are the exponents for potato and wooden cubes respectively. For potato strips n = 0.47. These values are comparable with that previously obtained for peas (n = 0.44) and were obtained from a least square correlation of experimental data through the logarithmic form of Eq. (7).

Moreover, Fig. 2 and Table 1 show that the minimum fluidization voidage ϵ_{mf} for wet materials (potato cubes) is greater than for dry materials (wooden cubes). This effect was also detected in a previous work for peas and was attributed to a looser bed arrangement favored by moisture in particles.

Gas-particle heat transfer coefficient

Table 2 shows the experimental values of gas particle heat transfer coefficients for diced potatoes under superficial air velocities ranging from 2.55 m/s to 4.25 m/s and bed heights under minimum fluidization conditions from 3 cm to 9 cm. Average operating temperatures changed between 15°C and 29°C. Greatest data scatter among runs performed at the same superficial velocity were less than 12%. No definite tendency was observed due to different bed heights.

Similar results are presented in Table 3 for fluidized potato strips under superficial air velocities changing from 2.8 m/s to 3.8 m/s and variations in the bed height from 3 cm to 6 cm.

Table 3—Gas particle heat transfer coefficients for potato strips 0.92 x 0.92 x 5.3 cm

v_o m/s	$H_{mf}10^2$ m	$\bar{h}$ W/m^2°C	Ar 10^{-7}	J_H	Re′ 10^{-3}	$J_H Ar^{-0.179}$ 10^3
2.8	3.5-5.5	163	9.56	0.0391	5.86	1.46
3.0	3.0	174	9.07	0.0396	6.39	1.49
3.2	2.5	167	9.50	0.0351	7.44	1.31
3.4	2.5-3.0	175	9.76	0.0344	8.44	1.28
3.6	3.0-6.0	163	9.17	0.0309	9.04	1.16
3.8	3.0	171	9.32	0.0306	10.20	1.15
4.3	6.0	160	9.68	0.0259	13.66	0.93

Both Tables show that heat transfer coefficients for potato strips are smaller than for cubes and these in turn are smaller than those obtained for peas (Vázquez and Calvelo, 1980).

This fact is consistent with Bradshaw and Myers' (1963) results who have found smaller values with decreasing sphericity.

The heat transfer coefficients in Tables 2 and 3 show a small dependence on the fluidization superficial velocity in agreement with results obtained for peas and other materials at Re′ > 1000 (Bradshaw and Myers, 1963; Chang and Wen, 1966).

The experimental values of heat transfer coefficients obtained for potato cubes and strips, as well as those reported in a previous paper (Vázquez and Calvelo, 1980) for peas and spherical alumina pellets were correlated according to the following equation:

$$J_H = K\, Re'^a\, Ar^b \qquad (8)$$

where $J_H = Nu\, Re^{-1} Pr^{-1/3}$ and $Re' = \rho\, D_p\, v_o/\mu(1-\epsilon)$. The Archimides number ($Ar = D_p^3\, g\, \rho(\rho_s - \rho)/\mu^2$) was included to extend the relationship to products with different densitites.

By applying the least square method to the logarithmic form of Eq. (8) the coefficients were calculated from the experimental data as K = 0.204; a = −0.563, and b = 0.179.

Eq. (8) is plotted in Fig. 3 together with the correlated experimental values. The correlation coefficient for the ordinate of Fig. 3 ($J_H/Ar^{0,179}$) was r = 0.986.

It must be remarked that experimental values for peas were up to 10% higher than Eq. (8) predictions, while in the case of alumina pellets a maximum overestimation of the same order was detected.

The exponent found for the Archimides number is comparable to that proposed by Chang and Wen (1966) for different fluidizing materials (b = 0.198).

Moreover, the modified Reynolds' effect is in close agreement with values proposed by other author (Bradshaw and Myers, 1963; Chang and Wen, 1966).

In order to provide a fast evaluation of the gas-particle heat transfer coefficient under conditions similar to those prevailing in fluidized bed freezers, Fig. 4 shows the predictions of the model (Eq. (7) and (8)) for peas, potato cubes

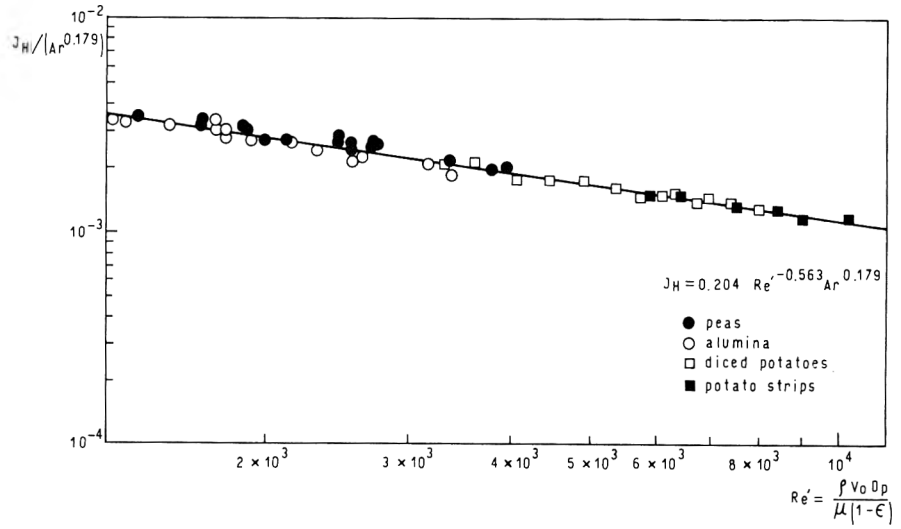

Fig. 3—Correlation of data on gas particle heat transfer coefficient for different shaped foods.

and strips at different operating temperatures of air.

It must be mentioned that the maximum shown for potato strips at $v_o/v_{mf} = 1.29$ can also be found for peas and diced potatoes at $v_o/v_{mf} = 2.92$ and $v_o/v_{mf} = 2.39$, respectively. This maximum is attributed to the combined effects of superficial velocity and bed expansion on the interstitial air velocity which affects the heat transfer coefficient.

CONCLUSIONS

(1) The analysis of bed expansion for fluidized cubes shows a different minimum fluidization voidage for wet and dry materials. A similar effect was detected for spheres in a previous paper.

(2) Bed voidage is related to the superficial air velocity by means of an empirical correlation. Such information is important when extending the heat transfer correlation to solids of different densities (different minimum fluidization velocities).

(3) Ergun's equation provides satisfactory pressure drop predictions in the fixed bed region by considering particle shape in terms of an effective diameter.

(4) However, the minimum fluidization voidage does not agree with the fixed bed porosity because of a rearrangement of particles into a looser structure before fluidization.

(5) The heat transfer coefficients showed a small dependence on superificial velocity and were independent of the bed height. By using the Colburn's factor, the modified Reynolds number and the Archimides number, a single equation is provided as a correlation of previous results for peas and spherical alumina pellets as well as of present data for potato cubes and strips.

(6) The correlation of data for different shaped-particles shows the effective diameter is a satisfactory characteristic length.

(7) A maximum value of the heat transfer coefficient in terms of the superficial velocity is detected as a consequence of a simultaneous bed expansion which decreases the interstitial air velocity.

(8) For a fast and simple use in designing continuous fluidized-bed freezers, heat transfer coefficients for different particles and operating conditions are provided.

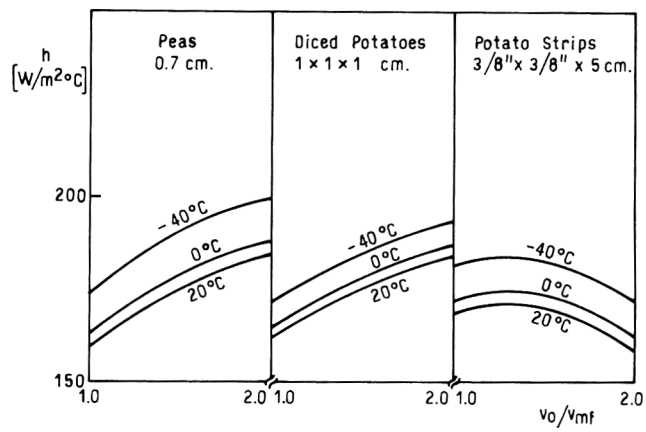

Fig. 4—Gas particle heat transfer coefficient dependence with the superficial velocity and the temperature of air.

LIST OF SYMBOLS

a = parameter defined in Eq. (8)
A_p = particle surface area, m^2
Ar = Archimides number, $Ar = D_p^3\, g\, \rho(\rho_s - \rho)/\mu^2$
b = parameter defined in Eq. (8)
C_p = air specific heat, J/kg°C
D_e = particle equivalent diameter, m^2
D_p = effective diameter, m^2
g = gravity acceleration, m/s^2
G_o = air superficial mass flux, kg/m^2s
h = gas particle heat transfer coefficient, $W/m^2{}^\circ C$
J_H = Colburn's factor for heat transfer
k = air thermal conductivity, W/m°C
K = coefficient in Eq. (8)
H = bed height, m
H_m = fixed bed height, m
H_{mf} = minimum fluidization bed height, m
m_s = total mass of solids in the bed, kg
n = parameter defined in Eq. (7)
Nu = Nusselt number
Pr = Prandtl number
Re = Reynolds number
Re' = modified Reynolds number
S = bed section, m^2
T = air temperature, °C
T_1, T_2 = inlet and outlet air temperature, °C
T_s = solid temperature, °C
v_o = air superficial velocity, m/s
v_{mf} = minimum fluidization superficial velocity, m/s
V_p = particle volume, m^3

Greek symbols

ϵ = bed voidage
ϵ_m = fixed bed voidage
ϵ_{mf} = minimum fluidization bed voidage

μ = air viscosity, kg/m s
ρ = air density, kg/m^3
ρ_s = solid density, kg/m^3
ψ = sphericity

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DIETARY FIBERS IN MUFFINS. . . From page 113

Table 10—Correlations of acceptability characteristics with particle size index[a]

Acceptability characteristics	Coefficient (r)	Significance (ρ)
Appearance	0.84	0.019
Flavor	0.82	0.024
Overall acceptability (Line scale)	0.81	0.026
Texture	0.80	0.030
FACT Scale	0.79	0.035
Mouthfeel	0.77	0.042

[a] Acceptability characteristic score for each muffin type was paired with its corresponding particle size index to determine correlation. Figures expressed here are mean scores.

FACT scale. Oat bran muffins had an objectionable gritty mouthfeel. Particle size was the only physical or chemical characteristic which correlated to the sensory results. The similar sensory results and the strong correlation of the FACT scale with overall acceptability on the sliding line scale (r=0.74 and p=0.001) supported the findings of Schutz (1965) that the FACT scale can be considered a very useful tool in assessing product acceptability when employed alone or in conjunction with another method which evaluates specific characteristics.

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Reference to a company and/or product is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Effects of Non-meat Protein Products on Properties of Fat-Batters and Mortadella Sausage

L. W. HAND, R. N. TERRELL and G. C. SMITH

ABSTRACT

Fat-batters made from pork fatback, hot water, salt and each of four different nonmeat protein products were manufactured and subsequently used in making mortadella sausages. Fat-batters made with sodium caseinate (SC) had the highest ($P < 0.05$) percentage of total weight loss upon heating but the percentage of the entity as free liquid oil was lowest ($P < 0.05$) among all comparisons. Cooked mortadella made with fat-batters containing SC had less ($P < 0.05$) processing shrinkage than those made with isolated soy protein. Mortadella sausages made with diced pork fatback were more desirable ($P < 0.05$) in overall appearance, and contained pieces of diced fat that sustained less ($P < 0.05$) rendering, than sausages made with fat-batters extended with protein products.

INTRODUCTION

MAJOR RESEARCH on nonmeat protein products in the United States has emphasized their use to replace portions of lean skeletal meat in formulated products (Terrell and Staniec, 1975; Kalin, 1979; Terrell et al., 1979; Hand et al., 1981). These studies reported that as increasing amounts of skeletal meat were replaced, such properties as texture, binding strength and overall palatability decreased.

Fat is an essential component of formulated meat products (frankfurters, ground beef, restructured meats) because it improves tenderness, juiciness and overall palatability. Thus, in a two-component system of fat and lean meat, fat may be equally as important as lean meat for desirable effects. For example, when such products are made to contain extremely low fat contents (less than 10–15%) these products may be tough unless enough moisture is added to achieve a degree of tenderness that would otherwise be associated with higher fat contents. Although the United States is an exporter of animal fats, future changes in breeding and feeding regimens to produce less-fat carcasses may reduce domestic supplies of animal fat to such an extent that (1) means for extending fat will be developed or (2) laws regarding added-water limits for U.S. processed meat products may have to be altered in order to formulate products with optimal tenderness, juiciness and overall palatability.

Although Schut (1978) reported that in Europe pork fat is emulsified in water by using sodium caseinate or by using other protein products (isolated soy) with other types of animal fats, this practice is not widely used in the U.S. These emulsified fat-batters may be directly incorporated into finely comminuted sausages like frankfurters or they may be chilled and diced into discrete particle sizes to create a desired visual effect in mortadella sausages. Therefore, technologies for extending animal fats may be important for future use in manufacturing processed meat products. Accordingly, the aim of this research was to determine effects of nonmeat protein products on properties of fat-batters for use in making mortadella sausages.

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MATERIALS & METHODS

FAT-BATTERS (quadruplicate 4.5 kg batches) were made to contain pork fatback, hot water, salt and four protein products (16 total batches). The following described protein products by name, minimum contents of protein and source were used: vital wheat gluten (VWG), 80%, Industrial Grain Products, Ltd.; isolated soy protein (ISP), 90%, Ralston Purina, Inc.; soy protein concentrate (SPC), 73%, Griffith Laboratories, Inc., and; sodium caseinate (SC), 90%, De Melkindustrie Veghel. Fat-batter formulations are shown in Table 1 and these batters were subsequently used in 16 batches (4 replications of 1.36 kg batches) of mortadella sausage.

Pork fatback was initially chopped (8 min, 3-blade nonvacuum bowl cutter) with salt and 30% of the total water followed by addition of dry protein products and the remaining hot water (71.1°C). After chopping protein-extended batters were discharged, stuffed (pneumatic piston) into No. 4 fibrous casings and chilled at 3.3°C for 48 hr.

A heat stability test (Carpenter and Saffle, 1964) has been used to indicate sausage batter stability (resistance to stress during stuffing and heating) and a modification of their procedure was used for this purpose. Two 100g samples were removed from the center of each chilled fat-batter and used as follows: (1) duplicate 100g samples of protein-extended fat-batters were placed on wire-mesh screens which covered the top of 200 ml beakers; (2) beakers were placed in a preheated gas oven (170°C) and heated to an endpoint temperature of 60°C as measured with thermocouples; (3) weight loss was recorded and liquid material that passed through the wire mesh was transferred to a 50 ml conical tube and centrifuged (2,500 rpm, 5 min at 60°C); and (4) phases of separation were recorded (ml) and reported as a percentage of the total ml of weight loss. Phases of separation are described as follows: (1) free liquid oil, the top phase that was a clear liquid oil at 60°C; (2) water, the second phase as measured from the top of the tubes; (3) particulate matter, the insoluble fraction located just below the water phase and probably contained insoluble proteins; and (4) encapsulated fat, that phase

Table 1—Formulations for making fat-batters and mortadella sausages

Ingredient (%)	Protein-product in fat batter[a]				Mortadella[b]
	Vital wheat gluten	Isolated soy protein	Soy protein conc	Sodium caseinate	
Pork fatback	43.1	43.8	42.5	43.8	–
Hot water	43.1	43.8	42.5	43.8	–
Protein-product	12.4	11.0	13.6	11.0	–
Salt	1.4	1.4	1.4	1.4	–
Lean beef	–	–	–	–	22.8
Pork trim	–	–	–	–	45.5
Ice/water	–	–	–	–	21.8
Seasonings/curing ingredients[c]	–	–	–	–	9.9
TOTAL	100.0	100.0	100.0	100.0	100.0

[a] Formulated to contain equivalent protein contents of 9.9%; made as 4.54 kg batches.
[b] Formulated to contain approximately 30% fat including addition of appropriate amounts of fat-batter.
[c] Seasonings/curing ingredients (4.10% nonfat dry milk, 2.0% salt, 1.36% corn syrup solids, 1.10% spices, 1.02% dextrose, 0.08% onion powder, 156 ppm sodium nitrite and 550 ppm sodium erythorbate).

of caseinate-treated samples that was a semi-solid mass located at the bottom of tubes after centrifugation.

In addition to heating stability tests, additional slices (four at 3.18 mm thickness) were removed from the outer portions of chilled fat-batters and used for color and sliceability determinations. These properties were evaluated by an 8-member panel using the following scales: visual color (unstructured 10 cm line-scale), 10 = light, 0 = dark; sliceability, 10 = not crumbly, 1 = extremely crumbly. Slicing was done with a Rival electric food slicer, Model No. 11101E13. Panelists were trained during eight separate sessions to evaluate these slices for visual color – that of white similar to pork fatback as being most desirable – and for the degree of shattering after slicing – that which did not crumble or fall apart as being most desirable.

The remaining portions of these chilled fat-batters were frozen (–34°C, 2 wk) and used to manufacture mortadella sausages. Mortadella sausages were formulated with a lean mix of 67% beef and 33% pork. After 10 min of chopping (meat, salt, water, seasonings), this lean mix was removed, placed in pans and diced portions (6.35 × 12.7 mm cubes) of protein-extended fat-batters or pork fatback were added and the composite was mixed by hand. These sausages were stuffed (pneumatic piston) into No. 4 fibrous casings and cooked/smoked (6-7 hr cycle in a commercial process oven, Alkar-DEC) to achieve an internal product temperature of 66.7°C as measured with thermocouples. Mortadellas were removed from the process oven, cold-showered (15 min) and chilled overnight in a 3.3°C cooler. Determinations of pH (duplicates) were made by grinding raw and cooked mortadella samples twice through a plate with 3.2 cm holes and blending a 10g sample with 100 ml of distilled-deionized water.

The same eight-member panel that evaluated fat-batters also evaluated slices of mortadella sausage for the following traits: definition of diced fat pieces, 8 = extremely discrete, 1 = extremely nondiscrete; degree of breakdown of diced fat pieces, 10 = 0%, 0 = 100%; visual color of diced fat pieces (unstructured 10 cm line-scale), 10 cm = light, 0 cm = dark; overall appearance of mortadella sausages, 8 = extremely desirable, 1 = extremely undesirable. Since fat-batters were diced and added to mortadella sausages which were fully cooked (66.7°C), the degree to which these fat-batter pieces retained their discrete geometric shape (particle-size definition) and whether or not the fat-batter pieces were stable and did not break down into fat pockets (degree of breakdown) were considered to be important traits of cooked mortadella sausages. Additional slices of mortadellas were used for palatability tests according to the following scales: cohesion, 3 = extremely cohesive, 1 = extremely non-cohesive; off-flavor, 8 = no off-flavor, 1 = extreme off-flavor; and overall palatability, 8 = extremely desirable, 1 = extremely undesirable. Palatability tests were conducted with duplicate slices served at room temperature and under red lighting. A reference standard of mortadella made with only diced pork fatback was also served at the beginning of each panel session.

Data were analyzed by analysis of variance (Steel and Torrie, 1960) and means were separated by use of the Duncan (1955) multiple range test.

Table 2–Mean values and standard deviations (parenthetical values) for processing shrinkage, and pH of fat-batters containing different protein-products and of mortadella sausages made with these fat-batters

	Fat-batters		Mortadella sausages		
Protein-product in fat-batter[a]	Processing shrinkage[b] (%)	pH	Processing shrinkage (%)	Raw batter pH	Cooked batter pH
Vital wheat gluten	30.9[c] (6.15)	6.2[d] (0.13)	7.61[cd] (0.41)	6.12[d] (0.08)	6.32[c] (0.03)
Isolated soy protein	28.2[c] (6.03)	6.6[c] (0.20)	8.11[c] (1.07)	6.20[cd] (0.07)	6.34[c] (0.04)
Soy protein concentrate	24.1[c] (2.72)	6.4[cd] (0.20)	7.43[cd] (0.63)	6.15[d] (0.09)	6.32[c] (0.06)
Sodium caseinate	25.3[c] (4.74)	6.6[c] (0.19)	6.63[d] (0.58)	6.26[c] (0.04)	7.13[c] (0.04)

[a] Fat-batters were formulated to contain equivalent contents of protein.
[b] Chopped weight minus stuffed weight divided by chopped weight.
[c,d] Means in the same column bearing a common superscript letter are not different (P > 0.05).

RESULTS & DISCUSSION

MEAN VALUES for processing shrinkage and pH of fat-batters and of mortadella sausages made with these fat-batters are shown in Table 2. There were no differences ($P > 0.05$) among fat-batters for processing shrinkage but those made with VWG had slightly more shrinkage than those made with SC or SPC. pH values were highest for fat-batters made with SC or ISP and were lowest for those made with VWG ($P < 0.05$). Cursory observations of fat-batters made with VWG indicated that this protein product did not form a homogeneous, creamy-textured and stable fat-batter. Mortadella sausages made with SC had higher ($P < 0.05$) raw batter pH values than those for mortadellas made with VWG or SPC. Among cooked sausage batters, pH values were not different but these values were numerically higher than those for raw batters made with VWG or SC.

Data from heating stability tests are shown in Table 3. Fat-batters made with SC had the highest percentage of total weight loss ($P < 0.05$). Values for percentages of free liquid oil were lowest ($P < 0.05$) for fat-batters made with SC and water loss was greatest for fat-batters made with VWG. Fat-batters made with SC had a two-phase separation. This second phase of separation for SC was defined as encapsulated fat and this phase represented a substantial portion of total weight loss for SC extended fat-batters. Although not different ($P > 0.05$) from each other, fat-batters made with VWG or ISP had higher percentages of particulate matter than those made with SPC or SC. Heating stability data suggest that although SC-batters may lose considerable weight during heating (first column in Table 3) this protein product retains liquid fat and water within its matrix after heating and centrifugation as evidenced by the encapsulated fat-phase. Such values as particulate matter, encapsulated fat and water may indicate the potential ability of various nonmeat protein products to hold fat and/or water after heating. Thus, a measure of fat binding or water binding may be obtained for various conditions of heating.

Although binding of fat and water are important measurements of potential functionality in mortadella sausages, visual color and sliceability are also important. Fat-batters made with SC were ($P < 0.05$) lightest in color and were the least ($P < 0.05$) crumbly when sliced (Table 3). Fat-batters made with SPC were darker ($P < 0.05$) in color but were less ($P < 0.05$) crumbly during slicing than fat-batters made with VWG or ISP. The plasticity of fat-batters made with SC and their extremely light color suggest that SC may be the protein additive of choice for making fat-batters for use in mortadella sausages.

Mean values for visual appearance of diced fat-batters in mortadella sausages and for palatability traits of mortadella sausages made with fat-batters containing different protein-products or made with pork fatback are presented in Table 4. Diced pieces of fat-batters suspended in the lean matrix of cooked mortadella sausages were evaluated for degree of breakdown (rendering of fat), particle-size definition and color. Mortadella sausages made with pork fatback had ($P < 0.05$) less breakdown of diced fat pieces, more discrete fat pieces, lighter-colored diced fat pieces and more desirable overall appearance than sausages made with protein extended fat-batters. Mortadella sausages made with fat-batters containing SPC had less breakdown of diced fat pieces, more discrete fat pieces, darker-colored diced fat pieces and more desirable overall appearance than mortadella made with fat-batters that contained other protein-products ($P < 0.05$). Mortadella sausages made with fat-batters containing SC had the most extensive breakdown of diced fat pieces, the lightest-colored fat pieces and the least desirable overall appearance among sausages made

Table 3—Mean values and standard deviations (parenthetical values) for heat-stability traits and visual traits of fat-batters containing different protein-products

Protein-product in fat-batter[a]	Fat-batter characteristic						
	Heat-stability traits					Visual traits	
	Total weight loss (%)[b]	Free liquid oil (%)[c]	Water (%)[c]	Particulate matter (%)[c]	Encapsulated fat (%)[c]	Color[d]	Sliceability[e]
Vital wheat gluten	51.7[g] (6.60)	85.5[f] (10.46)	11.2[f] (7.02)	4.0[f] (3.18)	0.0[g] (—)	5.8[g] (1.96)	2.8[h] (1.92)
Isolated soy protein	37.6[h] (6.17)	96.4[f] (1.68)	0.1[g] (0.38)	1.4[fg] (1.28)	0.0[g] (—)	6.1[g] (1.48)	2.6[h] (1.92)
Soy protein concentrate	20.3[i] (3.23)	98.1[f] (2.57)	0.0[g] (—)	0.0[g] (—)	0.0[g] (—)	3.9[h] (1.39)	6.8[g] (1.73)
Sodium caseinate (control)	87.3[f] (7.04)	34.8[g] (24.02)	0.0[g] (—)	0.0[g] (—)	64.3[f] (24.4)	9.3[f] (0.73)	9.7[f] (0.82)

[a] Fat-batters were formulated to contain equivalent contents of protein.
[b] Total weight loss percentage was computed as the difference between original sample weight and sample weight after heating at 107°C to an endpoint temperature of 60°C.
[c] Liquid collected in determining total weight loss was centrifuged for 5 min at 2,500 rpm and phases (free liquid oil, water, particulate matter and encapsulated fat) were quantitated as percentages of total ml weight loss.
[d] Color was evaluated by use of an unstructured 10 cm line-scale; 10 = light, 0 = dark.
[e] Sliceability was visually evaluated by use of a 10-point rating scale; 10 = not crumbly, 1 = extremely crumbly.
[fghi] Means in the same column bearing a common superscript letter are not different (P > 0.05).

Table 4—Mean values and standard deviations (parenthetical values) for visual appearance of diced fat in mortadella sausages and for palatability traits of mortadella sausages made with fat-batters containing different protein-products or made with pork fatback

Protein-product in fat-batter or fat in mortadella sausage	Mortadella sausage characteristic						
	Appearance of diced pieces[a]			Overall appearance of sausage[c]	Cooked sausage palatability[a]		
	Degree of breakdown	Particle-size definition	Visual color		Cohesion	Off-flavor	Overall palatability
Vital wheat gluten	4.9[f] (1.85)	4.4[g] (1.00)	5.4[f] (1.73)	3.2[f] (1.08)	4.6[e] (1.76)	4.6[e] (2.19)	4.5[e] (1.84)
Isolated soy protein	4.6[f] (2.94)	2.6[h] (0.83)	4.6[g] (2.24)	2.6[f] (1.19)	4.2[ef] (1.88)	4.2[e] (1.94)	3.7[ef] (1.62)
Soy protein concentrate	8.8[e] (1.03)	6.4[e] (0.77)	2.8[h] (1.54)	5.8[e] (0.97)	3.7[f] (1.52)	4.0[e] (2.25)	3.3[f] (1.95)
Sodium caseinate	3.6[g] (1.33)	5.4[f] (1.34)	6.6[e] (1.92)	2.1[g] (1.06)	4.3[ef] (2.11)	4.4[e] (2.25)	3.1[f] (2.07)
Pork fatback	9.7[d] (0.40)	7.6[d] (0.43)	9.6[d] (0.47)	7.3[d] (0.86)	7.2[d] (1.10)	7.0[d] (1.28)	6.7[d] (1.38)

[a] Degree of breakdown, 10 = 0%, 0 = 100%; particle-size definition, 8 = extremely discrete, 1 = extremely non-discrete; visual color, 10 = light, 0 = dark (unstructured 10cm line scale).
[b] Cohesion, 8 = extremely cohesive, 1 = extremely non-cohesive; off-flavor, 8 = no off-flavor, 1 = extremem off-flavor; overall palatability, 8 = extremely desirable, 1 = extremely undesirable.
[c] 8 = extremely desirable, 1 = extremely undesirable.
[defgh] Means in the same column bearing a common superscript letter are not different (P 0.05).

with protein-extended fat-batters ($P < 0.05$).

These data (Table 4) suggest that it is difficult to simulate use of solid pieces of pork fatback in mortadella sausages by extending pork fat mixed with water and protein-products without having some detrimental effect on visual properties. It is possible that these detrimental effects can be overcome, to some extent, by reducing initial piece-sizes (making them smaller than 6.35 X 12.7 mm cubed) or by using protein-extended fat-batters in finely comminuted sausages, like frankfurters or bologna, which do not require piece-size definition of fat-batters as a finished product characteristic. Schut (1978) outlined process steps for using SC fat-batters in the latter products.

Mean palatability ratings for mortadella sausages made with fat-batters or with pork fatback are also shown in Table 4. Sausages made with pork fatback were more cohesive ($P < 0.05$), had less off-flavor ($P < 0.05$) and were more palatable ($P < 0.05$) than sausages made with protein-extended fat-batters. Among mortadella sausages made with fat-batters containing protein-products, those made with fat-batters containing SPC were less cohesive ($P < 0.05$) than those made with VWG fat-batters and, although not significant ($P > 0.05$) those made with SPC had slightly more off-flavor and were less palatable ($P < 0.05$) than those made with VWG. Off-flavor did not differ among mortadella sausages made with protein extended fat-batters but these values were lower ($P < 0.05$) than those for sausages made with diced pork fatback. Mortadella sausages containing VWG or ISP fat-batters did not differ ($P > 0.05$) in overall palatability but overall palatability ratings for those made with VWG were higher ($P < 0.05$) than those for sausages made with SPC or SC extended fat-batters. These data suggest that differences in palatability among mortadella sausages made with protein-extended fat-batters were not very large. Thus, physical properties (color, particle-size-definition and breakdown) of protein-extended fat-batters may be the most important factor in the selection of protein additives for use in making diced fat-batters to be used in mortadella sausages.

Regardless of the protein-product used to extend fat-batters for inclusion as diced pieces in mortadella sausages, detrimental effects on visual and palatability traits resulted when compared with use of only diced pork fatback. Cooking to an internal product temperature lower than 66.7°C may affect the stability of the matrix of fat-batters, but this was not determined in this study. Because SC appears to bind fat and retain this fat after heating, it may be the protein-product of choice when directly incorpo-

—Continued on page 124

Effects of Reduction or Replacement of Sodium Chloride on Growth of Micrococcus, Moraxella and Lactobacillus Inoculated Ground Pork

R. N. TERRELL, M. QUINTANILLA, C. VANDERZANT, and F. A. GARDNER

ABSTRACT

Three replications each of ground pork inoculated with either a *Moraxella*, *Micrococcus*, or a *Lactobacillus* species were used to determine effects of reduction or replacement of NaCl on viable counts of these bacteria. Type or level of chloride salt did not affect ($P > 0.05$) *Micrococcus* counts. Likewise, *Moraxella* counts were not affected ($P > 0.05$) by type or level of chloride salt. However, with *Lactobacillus* as inoculum, reducing NaCl by 50% slightly increased these counts. Data suggest that reduction or replacement of NaCl influenced the development of the *Lactobacillus* culture but had no significant effect on the growth characteristics of the other two bacterial cultures.

INTRODUCTION

SALT INTAKE, as influenced by dietary patterns, and its association with hypertension and related diseases, is of utmost concern to consumers and to public health policy (USDA-HEW, 1980). Daily intakes of sodium in the U.S. are estimated to range from 3,900–4,700 mg (10–12g NaCl) per person. This intake is about 20–25 times greater than that of the minimum daily adult requirement (IFT, 1980). Based on the estimate that 10–12g of salt per person per day is presently consumed in the United States, approximately 25–30% is discretionary, that is, added during home or institutional preparation or at the time of consumption, while 40–60% is added during commercial processing and is considered to be nondiscretionary (IFT, 1980). It is this nondiscretionary salt intake that is receiving most attention by industry and regulatory agencies.

Scientific concerns about the 40–60% of daily sodium intake estimated to result from commercial processing center on levels of salt required for product safety and stability, and to a lesser extent on amounts of salt required for product functionality and acceptability. The function of salt in food preservation (Ingram and Kitchell, 1967) and its functionality and sensory acceptance in processed meats has been reviewed (Olson and Terrell, 1981; Terrell and Brown, 1981; Terrell and Olson, 1981).

Concepts of either reducing levels of salt (NaCl) added to processed meats or replacing part or all of this NaCl with other types of chloride salts have been investigated (Hand et al., 1982a, b, c,; Terrell and Olson, 1981; Terrell et al., 1981, 1982). In those studies, use of magnesium chloride ($MgCl_2$) or calcium chloride ($CaCl_2$) decreased pH values of linked pork sausage. After 12 days of retail case storage, pork sausages made with $CaCl_2$ had the lowest aerobic plate count, those made with $MgCl_2$ or potassium chloride (KCl) had the highest aerobic counts and addition of any chloride salt, including NaCl, decreased aerobic plate counts ($P < 0.05$) when compared with controls (no chloride salts added). Functional properties of beef muscle (pH and water holding capacity) are also affected by type of chloride salt (Terrell et al., 1981). In that study, the highest pH value was obtained when NaCl or KCl was added; the lowest pH value was obtained when $MgCl_2$ was used and during cooking, the least amount of juice loss was associated with use of either $MgCl_2$ or NaCl. Other processed meat products such as hams, frankfurters and pork roasts have also been studied to determine effects of reductions or replacements of NaCl on processing and sensory properties (Hand et al., 1982a, b, c).

In addition to these effects on functional properties, reduction or replacement of sodium in processed meats may also influence microbial growth. The present study provides information on the effects of reducing (50%) or replacing (100%) NaCl with either KCl or $MgCl_2$ on the growth of *Micrococcus*, *Moraxella* and *Lactobacillus* cultures in inoculated ground pork.

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MATERIALS & METHODS

Microbiological procedures

The *Micrococcus*, *Moraxella* and *Lactobacillus* cultures used for inoculation of ground pork were the predominant isolates from a commercial pork sausage (pre-rigor muscle, chub packaged). They were maintained on tryptic soy agar (TSA, Difco) slants at 25°C. Inocula of *Micrococcus* or *Moraxella* for the ground pork were prepared by placing a loopful from a 24-hr TSA slant into a tube with 10 ml sterile tryptic soy broth (TSB, Difco); for the *Lactobacillus* sp. MRS broth (Difco) was used. Broth cultures were incubated overnight at 25°C. Inocula were added to the ground pork (approx. 1 ml per kg) to give an initial cell concentration of about 10^5 (*Micrococcus*, *Moraxella*) and 10^7 (*Lactobacillus*) per gram of ground pork. The inoculum was distributed thoroughly through the pork by hand using sterile gloves.

At each sampling interval, triplicate samples (10g each) were placed in separate Stomacher bags with 90 ml of sterile 0.1% peptone broth (Difco) and macerated for 1 min in a Stomacher 400. Plating was done by spreading 0.1-ml portions of appropriate dilutions (0.1% peptone broth) onto prepoured plates of TSA. Plates were incubated for 3 days at 25°C. Moisture and NaCl contents (AOAC, 1975) were determined (on 0-day samples only) and used to calculate brine content (salt in the aqueous phase of meat). Brine contents were 5.00% for samples with 2.50% NaCl and 2.71% for samples prepared with 1.25% NaCl.

Meat samples

Nine Hampshire X Yorkshire crossbred barrows (live weight, 97–106 kg) were conventionally slaughtered and dressed in the Texas A&M University Meats Laboratory. Three animals were used for each trial involving a single bacterial species. After splitting the carcass, the wholesale shoulder was removed from the left side (1 hr postmortem). The shoulder was separated into a picnic shoulder and a Boston butt. The exterior portion of the Boston butt was burned thoroughly with a gas flame to destroy microorganisms at the surface of the cut. The burned exterior portion of the cut was then removed with sterile instruments. The interior portion of the sample was cut, aseptically with sterile scalpels, into pieces (5 cm cubes) and ground in a sterile grinder (Model H, General Co., Walden, NY).

After inoculation, portions (900g) of the ground pork were assigned to each of the following treatments: No salt added (Control); NaCl (2.50% or 1.25%); KCl (3.19% or 1.60%); and $MgCl_2$ (1.36% or 0.67%). In addition, non-inoculated samples with no

added chloride salts were prepared to check for possible microbial contamination. Reduction (50%) or replacement (100%) of NaCl with either KCl or $MgCl_2$ was based on ionic strengths equivalent to that of 2.50% or 1.25% NaCl, respectively. For each trial involving one bacterial species, 72 samples (100g each) were placed in 18-oz polyethylene pouches (Whirl-pak, Nasco, Fort Atkinson, Wisconsin). Both inoculated and control samples were stored at 5°C for 0, 5 or 10 days. Ground pork inoculated with a bacterial species was subjected to all of the experimental treatments and packaged on the same day. The individual cultures were inoculated into ground pork on separate days.

Analysis

Data were subjected to analysis of variance (Steel and Torrie, 1960). When interactions were significant ($P < 0.01$) the interaction mean square was used to test main effects. Means were separated by use of a multiple range test (Duncan, 1955).

RESULTS

ANOVA mean squares of *Micrococcus*, *Moraxella* or *Lactobacillus* counts (log_{10}) of ground pork incoulated separately with each of these microorganisms and treated with various chloride salts are shown in Table 1. An evaluation of main effects and main effect interactions indicate that *Micrococcus* or *Moraxella* counts were not affected ($P > 0.05$) by reducing (50%) or by replacing (100%) NaCl with equivalent ionic strengths of KCl or $MgCl_2$. Storage periods (0, 5 or 10 days) did not affect ($P > 0.05$) *Micrococcus* counts. However, *Moraxella* counts were affected by storage period ($P < 0.01$). *Lactobacillus* counts were affected ($P < 0.01$) by chloride salt treatment, by storage period and by the interaction of treatment and storage period ($P < 0.01$).

Means for *Micrococcus* counts according to storage period and chloride salt treatment are shown in Table 2. Within each storage period, a 50% reduction of NaCl or a 100 or 50% replacement of this salt with KCl or $MgCl_2$ did not ($P > 0.05$) affect counts of *Micrococcus*. Increasing storage periods from 0 through 10 days also did not ($P > 0.05$) affect these counts for any chloride salt treatment, including controls.

Moraxella counts, arranged according to storage period and chloride salt treatment, are shown in Table 3. Neither storage period nor chloride salt treatment affected these counts, except for those of ground pork made with 1.60% KCl, in which case, counts at 10 days of storage were lower than those at 0 or 5 days of storage.

Means for *Lactobacillus* counts, arranged according to storage period and chloride salt treatment, are shown in Table 4. Within each storage period counts were not affected ($P > 0.05$) by chloride salt treatment except for 0-day samples made with 1.36% $MgCl_2$. In that treatment, *Lactobacillus* counts were higher ($P < 0.05$) for samples made with 1.36% $MgCl_2$ than for all other salt-substitute treatments and the controls. In contrast with data presented in Tables 2 and 3, *Lactobacillus* counts were lower at 0 days of storage and were higher at 5 or 10 days of storage ($P < 0.05$) regardless of chloride salt treatment.

DISCUSSION

REDUCTION of current levels of NaCl, as opposed to replacement of NaCl with KCl, appears to be a viable option for reducing the sodium content of processed meats without detrimentally affecting sensory and functional properties (Terrell, 1982). Also, the addition of sodium or potassium phosphates (0.5%) for certain sausage products has been recently approved (USDA-FSIS, 1982) and that approval was based, in part, upon the fact that these phosphates may compensate for the possible loss of functionality when levels of NaCl are reduced in such products. However, the latter two reports do not address microbiological implica-

Table 1 — ANOVA mean squares of Micrococcus, Moraxella or Lactobacillus counts (log_{10}) of ground pork inoculated separately with each of these microorganisms and treated with various chloride salts

Source	Degrees of freedom	Micrococcus MS	Moraxella MS	Lactobacillus[a] MS
Treatment[b]	6	0.0332	0.0776	0.7641**
Storage period[c]	2	0.0640	0.6757**	47.2316**
Treatment x storage perod	12	0.0308	0.0523	0.2782**
Error	42	0.1273	0.0740	0.1067
TOTAL	62			

[a] The interaction was significant ($P < 0.01$), therefore the interaction mean square was used to test main effects.
[b] Reduction (50%) or replacement (100%) of sodium chloride with either potassium chloride or magnesium chloride based on ionic strengths equivalent to that of either 2.50% or 1.25% of sodium chloride, respectively.
[c] Stored in Whirl-pak bags (not under vacuum) at 5°C for 0, 5 or 10 days.
**($P < 0.01$)

Table 2 — Means for Micrococcus counts (log_{10}) of inoculated pork according to storage period and chloride salt treatment

Chloride salt treatment	Days of storage at 5°C: 0 (A)	5 (B)	10 (C)	Order of means[b]
Control	5.51[a]	5.54[a]	5.58[a]	C B A
NaCl 2.50%	5.67[a]	5.44[a]	5.27[a]	A B C
1.25%	5.44[a]	5.58[a]	5.60[a]	C B A
KCl 3.19%	5.67[a]	5.50[a]	5.38[a]	A B C
1.60%	5.63[a]	5.57[a]	5.54[a]	A B C
$MgCl_2$ 1.36%	5.71[a]	5.67[a]	5.51[a]	A B C
0.67%	5.64[a]	5.63[a]	5.61[a]	A B C

[a] Means in a column bearing a common letter superscript are not different ($P > 0.05$). n = 3.
[b] Means within a chloride salt treatment, underscored by a common line are not different ($P > 0.05$).

Table 3 — Means for Moraxella counts (log_{10}) of inoculated pork according to storage period and chloride salt treatment

Chloride salt treatment	Days of storage at 5°C: 0 (A)	5 (B)	10 (C)	Order of means[b]
Control	5.13[a]	5.07[a]	5.06[a]	A B C
NaCl 2.50%	5.12[a]	4.89[a]	4.81[a]	A B C
1.25%	5.19[a]	5.01[a]	4.81[a]	A B C
KCl 3.19%	5.03[a]	4.89[a]	4.71[a]	A B C
1.60%	5.16[a]	5.07[a]	4.61[a]	A B C
$MgCl_2$ 1.36%	5.12[a]	5.39[a]	4.79[a]	B A C
0.67%	5.18[a]	5.32[a]	4.84[a]	B A C

[a] Means in a column bearing a common letter superscript are not different ($P > 0.05$). n = 3.
[b] Means within a chloride salt treatment, underscored by a common line are not different ($P > 0.05$).

Table 4 — Means for Lactobacillus counts (log_{10}) of inoculated ground pork according to storage period and chloride salt treatment

Chloride salt treatment	Days of storage at 5°C 0 (A)	5 (B)	10 (C)	Order of means[c]
Control	5.81[b]	8.40[a]	8.37[a]	B C A
NaCl 2.50%	5.63[b]	8.49[a]	8.62[a]	C B A
1.25%	6.00[b]	8.89[a]	8.95[a]	C B A
KCl 3.19%	5.70[b]	8.29[a]	8.53[a]	C B A
1.60%	5.94[b]	8.85[a]	8.43[a]	B C A
$MgCl_2$ 1.36%	7.27[a]	9.14[a]	8.58[a]	B C A
0.67%	5.93[b]	8.67[a]	8.69[a]	C B A

[a,b] Means in a column bearing a common letter superscript are not different ($P > 0.05$). n = 3.
[c] Means within each chloride salt treatment underscored by a common line are not different ($P > 0.05$).

tions that may affect stability when such reductions in NaCl content or addition of phosphates are made. In the present study, reduction of NaCl by 50% had little or no effect on *Micrococcus* or *Moraxella* counts but slightly increased *Lactobacillus* counts.

In the present study, effects of changes in chloride salts on microbial counts in ground pork were studied with three individual organisms. One limitation to this approach is that it excludes potential microbial interactive phenomena. The slightly higher but not significantly different counts, for *Lactobacillus*, in samples with 1.36% $MgCl_2$ as compared to those for samples with 2.50% NaCl or no added NaCl could have resulted from minor changes in pH or from a direct effect of $MgCl_2$ on the *Lactobacillus* species.

Although differences were not significant, when NaCl was reduced 50%, *Lactobacillus* counts were slightly higher at each storage period when compared to treatments in which NaCl levels were not reduced. In fermented sausages, higher *Lactobacillus* counts may reduce fermentation time when 50% less NaCl is used, but effects on properties like flavor, color, water activity and survival of *Trichinella spiralis* may also be affected by this same reduction in NaCl.

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FAT-BATTERS/NONMEAT PROTEINS/SAUSAGE . . . From page 121

rating fat-batters into finely comminuted sausages like frankfurters, but other protein products (SPC, VWG and ISP) may provide acceptable overall appearance when the fat-batters are to be diced and used in mortadella sausages. However, in mortadella sausages, overall palatability may be less detrimentally affected when VWG is used rather than SPC or ISP. It may also be that no single protein-product will provide both desirable visual and palatability traits, rather a combination of such protein-products may lessen these detrimental affects. Additional research to determine factors affecting the physical structure of fat-batters as well as the concept of extending animal fats via use of non-meat protein-products should be considered.

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Efficacy of Hydrogen Peroxide as a Bactericide in Poultry Chiller Water

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ABSTRACT

Hydrogen peroxide (H_2O_2) as a bactericide in poultry chiller water reduced aerobic organisms by 95–99.5% with 6,600 ppm or higher H_2O_2, and *E. coli* by 97–99.9% with 5,300 ppm or higher. Even higher concentrations were required for similar bacterial reductions on carcasses; aerobic organisms on carcasses were reduced by 94% with 11,000 ppm and *E. coli* were reduced by 80% with 12,000 ppm. However, the reaction of H_2O_2 with catalase from the blood resulted in a bleached and bloated carcass which would be commercially undesirable for fresh or frozen retail sales, but may not be objectionable when used for deboned meat.

INTRODUCTION

CHLORINE has been regarded as a choice bactericide and is used frequently in bird chiller water to reduce cross-contamination of poultry carcasses during immersion chilling. Based on prior use, FDA approved chlorine for GRAS (Generally Regarded As Safe) status. Recently, however, concerns have been expressed about the possible formation of chlororganic compounds during bactericidal treatment of processing waters with chlorine (Cumming, 1975; Cunningham and Lawrence, 1977). The use of chlorine dioxide as a suitable substitute for chlorine has been reported (Lillard, 1979). Chlorine dioxide had the same bactericidal effect as chlorine when used at only one-seventh the concentration of chlorine, because it does not combine as readily as chlorine with all forms of organic matter. However, the use of chlorine dioxide in poultry processing plants has been suspended by the USDA Food Safety and Inspection Service pending GRAS approval by FDA. Because obtaining GRAS approval is a lengthy procedure, this study was undertaken to investigate the use of hydrogen peroxide (H_2O_2), which is GRAS, as an alternative bactericide to chlorine in bird chiller water.

MATERIALS & METHODS

Chilling poultry

Commercial immersion chilling of broilers was simulated in the laboratory. Chill water from the overflow and thirty processed broiler carcasses from the exit end of the chiller were obtained from a commercial broiler processing plant. A suitable container was used in which carcasses were immersed and agitated in the chiller water. Proportions of chill water to carcasses, and residence time of carcass in chiller were the same as in the commercial plant: 1 gal. chill water per carcass, and 30 min carcass residence time in chiller water. All samples were transported to the laboratory on ice and testing was started within 30 min of sample pick-up.

Bactericidal treatment

Various levels of Food Grade H_2O_2 (FMC) were added to the water from the commercial chiller (1,100–12,000 ppm). The quantitative potassium permanganate test was used to determine that the solution contained 44.8% H_2O_2 (FMC, 1982). Chiller water was tested for residual H_2O_2 after 0, 15, and 30 min, when carcasses were introduced. EM Quant peroxide test strips (E. Merck, Darmstadt, Germany) were used for the detection and semi-quantification of residual peroxide.

Microbiological tests

Duplicate chill water samples were taken before and after addition of H_2O_2 and appropriate dilutions made in 1% peptone.

Ten grams of breast skin from carcasses were blended (Osterizer with mini jars) in 1% peptone for 1 min and appropriate decimal dilutions made starting with 1:10. One side of breast skin was sampled before chilling; the other side after chilling.

Total aerobic counts and *Escherichia coli* levels (Most Probable Number technique) were determined by standard methods (APHA, 1976). Plate counts (in duplicate) were incubated at 20°C for 72 hr and inoculated EC medium tubes at 45.5°C for 24 hr. Confirmations for *E. coli* were performed according to standard methods (APHA, 1976).

Water pickup and carcass appearance

In order to determine if excision of breast skin prior to chilling had an effect on water pickup of the birds, weight gains were determined on control birds with intact breasts chilled without H_2O_2 treatment of water (4 birds), birds with intact breasts chilled after H_2O_2 treatment of water (4 birds), and birds with breast tissue excised and chilled after H_2O_2 treatment of water (10 birds). All 18 birds were held at refrigeration temperatures for 19 hours, then weighed again to determine weight losses. Effects on appearance of carcasses as related to use of H_2O_2 were determined by two experienced observers.

RESULTS & DISCUSSION

THE ADDITION of 6,600–12,000 ppm H_2O_2 to chiller water reduced aerobic counts in water by 95–99.5%, and addition of 5,300–12,000 ppm reduced *E. coli* in water by 97 to >99.9% (Table 1). This indicated that Enterobacteriaceae may be more sensitive to this bactericide than other organisms.

Reductions in aerobic organisms and *E. coli* on carcasses were accomplished with 9,200–12,000 ppm H_2O_2 (Table

Table 1–Mean percentage reduction in total aerobic and Escherichia coli counts in poultry chill water resulting from addition of hydrogen peroxide (H_2O_2) to the water

H_2O_2 added ppm	Mean percent reduction in counts	
	Total aerobic[a]	*E. coli*[b]
1,100	12.4 a[c]	20.7 a
1,600	17.0 a	57.1 a
2,100	39.7 b	49.5 a
2,600	33.9 b	40.5 a
5,300	64.3 c	97.4 b
6,600	94.9 d	>99.9 b
7,900	97.1 d	>99.9 b
9,200	99.1 d	>99.9 b
11,000	98.4 d	>99.9 b
12,000	99.5 d	>99.9 b

[a] Mean $\log_{10}$ total aerobic count per ml before addition of H_2O_2 - 4.2 (range 3.1–4.6).
[b] Mean $\log_{10}$ E. coli count per ml before addition of H_2O_2 - 3.4 (range 2.7–4.2)
[c] Means within a column followed by the same letter are not significantly different at the 5% level according to analysis of variance and the multiple range test (Duncan, 1955).

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Table 2—Mean percentage reduction in total aerobic and Escherichia coli counts on poultry skin resulting from addition of hydrogen peroxide (H_2O_2) to the chill water

H_2O_2 added ppm	Mean percent reduction in counts	
	Total aerobic[a]	*E. coli*[b]
9,200	47.5 a[c]	37.9 a
11,000	93.5 a	45.5 a
12,000	77.5 a	79.8 a

[a] Mean $\log_{10}$ total aerobic count per g before addition of H_2O_2 - 4.2 (range 2.7—5.1).
[b] Mean $\log_{10}$ E. coli count per g before addition of H_2O_2 - 2.9 (range 1.4—4.0).
[c] Means within a column followed by the same letter are not significantly different at the 5% level according to analysis of variance and the multiple range test (Duncan, 1955).

2). The lower percentage reduction attained indicates that bacteria on carcasses apparently were less accessible than those in the water to the bactericidal action of H_2O_2.

Reduction of bacterial levels in water occurred at H_2O_2 concentrations which resulted in detectable levels in chill water up to 15 and 30 min. At 5,300 ppm H_2O_2 and above there was 15 to >30 ppm residual H_2O_2 after 30 min of carcass immersion, which resulted in a significant ($P<0.05$) reduction in bacterial numbers.

Kotula (1966), using a swab method, found significant differences in counts obtained on sampling different sites on the same side of the carcass but not between similar sites on the two sides. Cox et al. (1976) found no difference in counts when sampling different sites or different sides of the carcass by the swab method. Klinger et al. (1981), using a skin removal and stomacher homogenizing method, found no significant differences in plate counts among five different sampling sites on carcasses. Therefore, sampling by blending skin from one side of the breast prior to and the other side after chilling would not introduce an uncontrolled variable into the microbiological results. However, the effect of excised breast tissue on water pickup by the carcass required investigation. Comparisons made of the mean weight gains and losses of broiler carcasses with excised breast tissue or with breast tissue intact, chilled in H_2O_2-treated water, and control carcasses with breast tissue intact and chilled without addition of H_2O_2 to the chill water (Table 3), showed that carcasses chilled in H_2O_2-treated water had greater weight gain than the controls, but that excising of breast skin did not consistently affect water pickup.

Carcasses chilled in water to which H_2O_2 was added had a bloated appearance. After chilling, the carcass appeared significantly larger, the skin was rubbery and bleached, and there appeared to be gas and water accumulation under the skin. Since water pickup alone did not explain the undesirable appearance of the carcasses, it was concluded that H_2O_2 probably reacted with catalase from carcass tissues and cells (particularly erythrocytes) to produce water and oxygen gas, resulting in a bloated appearance.

Addition of H_2O_2 to commercial chill water also resulted in a strong visible foaming and bleaching effect, again, probably due to the reaction of H_2O_2 with catalase from the blood.

After carcasses were held for 19 hr, their average weight loss exceeded their average water pickup weight gain (Table 3). This probably occurred because the carcasses used had been previously immersion chilled, thus had picked up water during commercial processing. For carcasses chilled in H_2O_2-treated water, the bloated appearance declined after the 19-hr holding period, the bleached appearance virtually disappeared, but the rubbert texture of the skin remained.

Table 3—Effect of hydrogen peroxide (H_2O_2) treatments on water pickup by chilled broiler carcasses

Treatment	Number of carcasses	Mean wt gain after chilling	Mean wt loss 19 hr after chilling
Breast skin intact; no H_2O_2	4	1.3%	2.7%
Breast skin intact; approx. 10,000 ppm H_2O_2	4	3.0%	3.1%
Breast skin excised; approx. 11,000 ppm H_2O_2	10	2.0%	5.5%

It was concluded that H_2O_2 is an effective bactericide in poultry chiller water, but its undesirable effect on the appearance and skin texture of broiler carcasses renders this bactericide commercially unfeasible under present processing procedures for fresh market retailing. Carcass appearance and skin texture are not significant in deboned poultry meat. Further studies are planned to determine if the use of H_2O_2 in chill water has an adverse effect on sensory qualitites of deboned poultry meat.

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Moisture Sorption Isotherms for Bacon Slices

R. P. KONSTANCE, J. C. CRAIG, JR., and C. C. PANZER

ABSTRACT

There is substantial evidence that controlled dehydration of bacon products to a water activity of 0.92 or below should allow control of spore outgrowth and toxin production by *Clostridium botulinum* and permit reduction of added nitrite levels. This study presents sorption data necessary for the development of a practical dehydration technique for bacon. Variations in fat/lean ratio, storage temperature, sorption mode, and drying method result in changes to the moisture sorption isotherms. Of these parameters only fat/lean variability of bacon significantly affects the isotherms at water activity levels above 0.90. Empirical equations for the isotherms were derived and used to establish differences in the isotherms due to variation in the parameters studied.

INTRODUCTION

THE STATE OF WATER in foods has a direct effect on their quality and stability through its effects on chemical (Labuza, 1980) and enzymatic (Rockland, 1957; Acker, 1963) reactions. For this reason, control of water activity (a_w) has been an area of concentrated research. In traditional processing, a_w is reduced by freezing, addition of ionic or nonionic solutes, or drying. In bacon, although curing additives (salt, sugars, nitrite, spices) reduce a_w, *Clostridium botulinum* spore outgrowth is retarded principally through the action of sodium nitrite (Benedict, 1980). Recent proposed reductions in the level of added sodium nitrite to reduce the formation of carcinogenic nitrosamines in fried bacon products have produced concern about possible botulism outbreaks and led to research on alternative methods of preservation. One such alternative is to reduce the a_w of bacon by drying. An a_w reduction to 0.92 or lower presumably provides ample assurance that the outgrowth and toxin production of *Clostridium botulinum* will be inhibited (Anon, 1975).

Ongoing studies of low-temperature air drying of bacon slices indicate that dehydration to an a_w = 0.92 can be accomplished with no substantial change in product quality. The extent of the moisture reduction required is, however, dependent on a number of processing and storage parameters. The purpose of this study is to identify the role of these parameters in the sorption isotherms of bacon slices. Leistner and Rödel (1975) indicated that fat content indirectly influences a_w. In bacon products where fat content is substantial and highly variable, it is important to fully understand the role that changes in composition play on product stability. Wolf et al. (1973) showed that changes in storage temperature markedly affected the isotherms for raw chicken. Kapsalis (1980) discussed hysteresis as related to food products and Wolf et al. (1972) showed the effects of sorption mode on the isotherms for freeze-dried pork, apples, and rice. The sorption data presented for bacon slices include the effects of these parameters as well as drying method over an a_w range 0.10–0.96. Using this range of data, empirical equations were developed that predict the major portion of the isotherm. The empirical relationships were then used to determine statistical differences due to the parameters under investigation. These relationships also provide a meaningful tool for the development and comparison of isotherms for other salted cured meats.

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MATERIALS & METHODS

BACON USED in this study was purchased locally as fully processed slabs. The bacon was sliced to about a 0.3-cm thickness, with slice order being maintained. Alternate slices were analyzed for fat content, and the others were dried and used for a_w and moisture determinations. The average fat content of the two surrounding slices was used as the fat estimate of the dried slice. Chopped rather than ground samples were used for the fat analyses because fat loss due to grinding was found to be significant. Bacon pieces, about 3-cm square, were soaked in petroleum ether for 18 hr and then extracted for 5 hr by Soxhlet extraction.

Bacon slices used for the desorption studies were either freeze-, air-, or microwave-dried. Samples to be freeze-dried were frozen at −18°C and dried in a Virtis Model 10-145 MR-BA. A desiccated-air dryer (CGS Laboratory) was used for air-drying bacon. Samples were dried at 10°C dry-bulb temperature and 10% relative humidity with air velocity at 360 ft/min. The microwave unit was a Cober continuous dryer with through-flow heated air at 38°C. Piece temperature was monitored, and severe overheating was prevented through intermittent application of the 2-kw power.

Water activity measurements for the desorption isotherms were determined with a hygrometer/sensor system from the American Instrument Co. (Model LI 53054). A set of eight narrow-range sensors, each with a 15% RH span, were used. After each sample measurement, the sensors were calibrated against various saturated salt solutions of known relative humidity. Water activity values for these solutions were reported by Greenspan (1977). Sensor repeatability was ±1.7% RH, which agrees with the findings of Stoloff (1978) for this equipment.

Sample holders for the a_w measurements were ½-pint mason jars with lid assemblies modified for sensor attachment. These mason jars were used to minimize headspace volume and decrease equilibration time. Samples were maintained at constant temperature (±0.1°C).

Bacon slices used for the adsorption tests were freeze-dried until completely dry, placed in vacuum desiccators, and allowed to equilibrate with selected saturated salt solutions of known relative humidities. Weights were monitored daily until constant weight was reached. All slices used for a_w determination were subsequently analyzed for moisture content by the standard vacuum oven method (AOAC, 1970).

RESULTS & DISCUSSION

A MAJOR CONCERN in the development of a safe bacon product through dehydration is the possible difference in a_w between the fat and lean portions of the bacon immediately after drying. Slices dried to various degrees of moisture content were manually separated into fat and lean, and each component was analyzed for a_w within 30 min of drying. The a_w (lean) was plotted against a_w (fat), and the regression line was forced through the origin (Fig. 1). The resultant slope was not significantly different (P = 0.01) from 1.0, as indicated by t-test. Thus, the a_w values of the two components were considered equal under the conditions of the study. *–Continued on next page*

Since variations in the fat/lean ratio of bacon are quite large, examining the effects of fat content on a_w was of prime importance. Desorption isotherms (25°C) for freeze-dried bacon slices at 10, 50, and 60% fat content are presented in Fig. 2. Fat levels of 50 and 60% are typical in bacon, whereas levels as low as 10% are only found on end slices. Generally for the isotherms studied, at constant a_w, the quantity of water sorbed increased with decreasing fat content. Superficially this finding appears to be in contrast to that of Iglesias and Chirife (1977), who detailed the effect of fat content on the sorption data for air-dried minced beef. Using a fat-add-back method (fat and connective tissue removed from samples and then added back at desired levels after drying), they determined that the isotherms were not significantly affected by fat content. Their sorption data for the minced beef study was plotted on a fat-free, dry basis. In the present study, differences in bacon isotherms with fat in situ were still evident when data were plotted on a fat-free, dry basis (Fig. 3). However, when moisture reduction was plotted against $1 - a_w$, a fat-independent relationship was indicated (Fig. 4).

Regression analysis was performed on the data at each fat level, and compared according to Neter and Wasserman (1974). In this method a sum-of-error square (SSE) is determined by first pooling all the water activity data and then comparing the SSE to that obtained considering each fat level independently. The SSE values for both models were compared by an F-test. In all cases differences were not statistically significant and indicated that the relationship was not fat dependent. The practical consequence is that by use of this fat independent relationship it is possible to

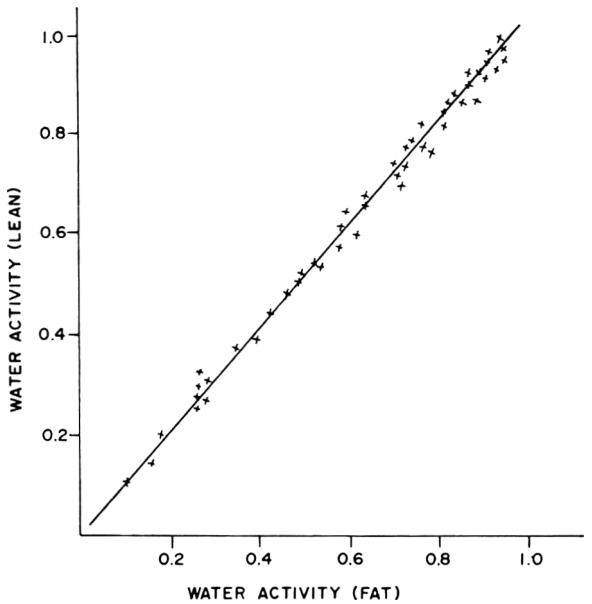

Fig. 1—Comparison of the a_w values of the fat and lean portions of bacon.

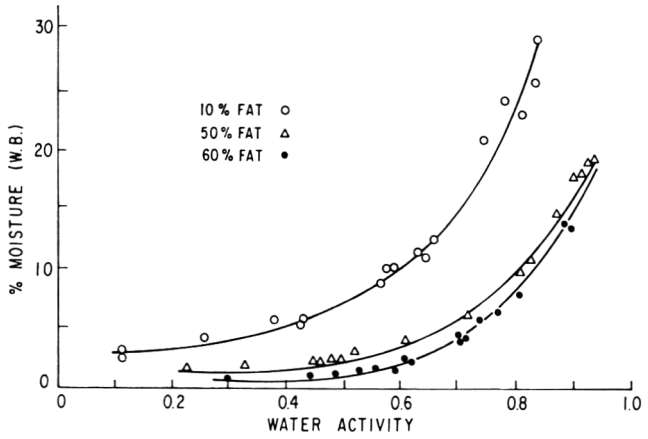

Fig. 2—Effect of fat content of bacon desorption isotherm (25°C).

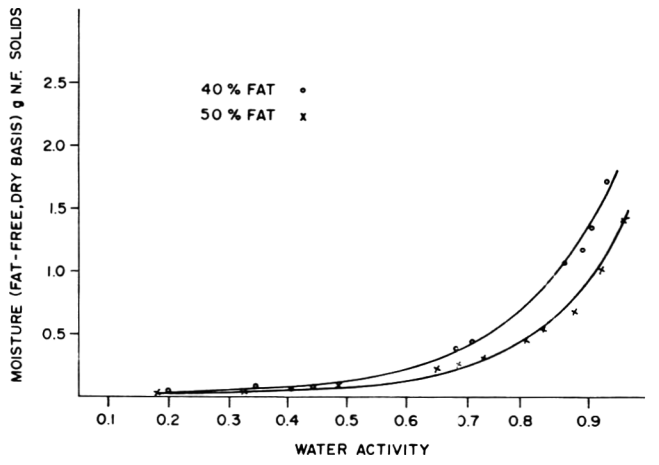

Fig. 3—Bacon desorption isotherm 25°C (fat-free, dry basis).

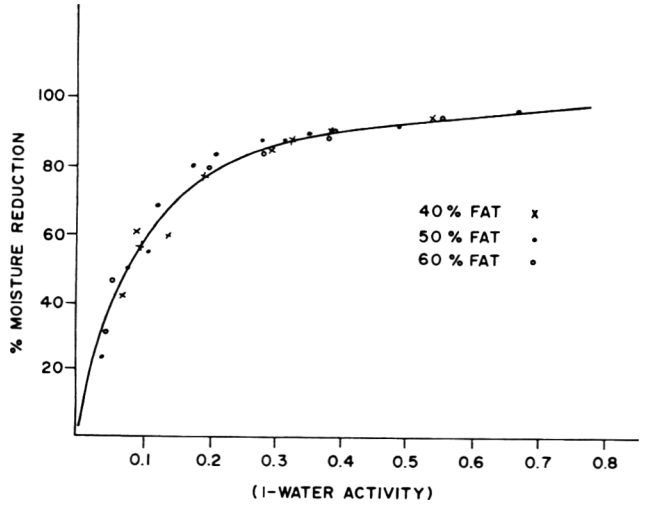

Fig. 4—Moisture reduction, water activity relationship exhibiting fat independence.

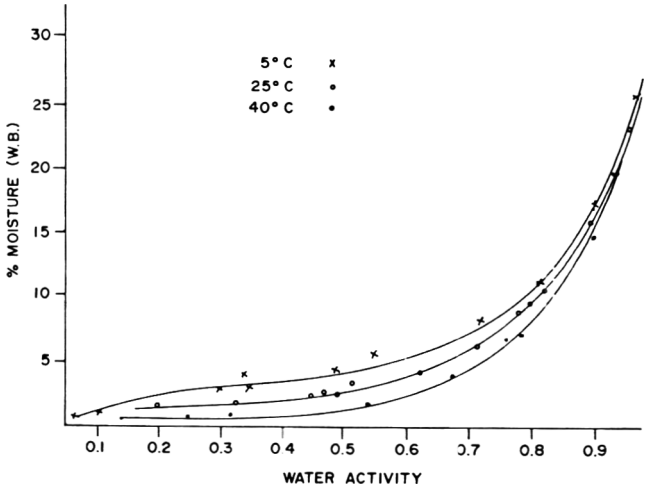

Fig. 5—Effect of storage temperature on bacon desorption isotherm (50% fat).

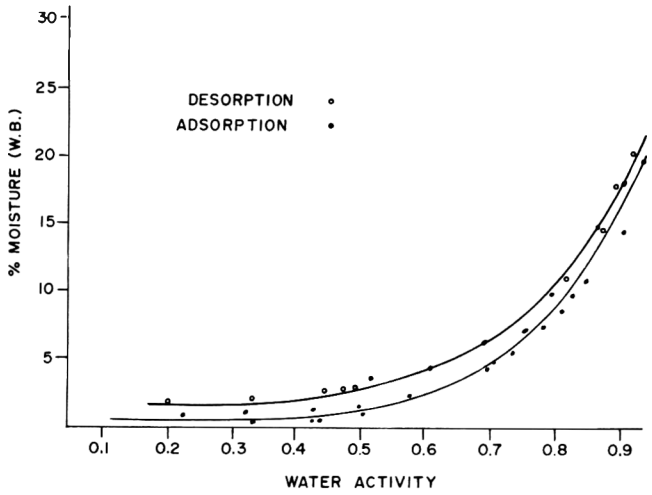

Fig. 6—Effect of sorption mode on bacon desorption isotherm (25°C, 50% fat).

predict the moisture reduction required for product stability, given only the initial moisture concentration. For example, in order to achieve an a_w = 0.92, approximately 60% of the water must be removed.

Desorption isotherms for freeze-dried bacon slices at different temperatures showed that, typically, the quantity of sorbed water at a given relative humidity increased as temperature decreased (Fig. 5). Temperature effects were more apparent at a_w values of less then 0.90. The stability of bacon dried to an a_w of 0.92 would not be affected by storage temperature.

The effects of sorption mode (adsorption vs desorption) are shown in Fig. 6. As with most foods, bacon slices at a given a_w had a higher moisture content in the desorption mode. The differences in moisture content indicate the hysteresis effects and agree with the findings of Wolf et al. (1972), for freeze-dried pork. Hysteresis begins in the capillary condensation region and continues to the regions of lower a_w. Although differences in the sorption isotherms were evident, sorption mode, like storage temperature, would not affect the stability of bacon dried to an a_w of 0.92.

To determine the significance of the differences between isotherms, an effort to fit the data to known theoretical relationships was attempted. Use of the Brunauer et al. (1938), Langmuir (1918), and Harkins and Jura (1944) relationships resulted in poor correlation. Prediction was adequate only at relatively low a_w levels ($a_w \leq 0.6$). An empirical expression to describe the moisture-water activity relationship was then developed by the simple curvilinear regression analysis of the bacon sorption data. The equation [Eq (1)] showed an excellent fit for all bacon isotherms for $0.1 < a_w < 0.95$

$$\text{Moisture (W.B.)} = ae^{b(a_w)} \qquad (1)$$

where "a" and "b" are regression coefficients and moisture is expressed on a wet basis (g H_2O/g original wet product). Fig. 7 is typical of the agreement between experimental and predicted values. Coefficients for the isotherm equations are shown in Table 1. The isotherms were then compared by the regression comparison method shown previously. The results, when submitted to an F-test, showed that the differences in the isotherms due to changes in composition (Table 2), temperature (Table 3), and sorption mode (Table 4) were highly significant.

Multiple linear regression techniques were used to develop a relationship which included the variables a_w, moisture content, and fat content. The equations for this multiple correlation are shown in Table 5. Correlation for the isotherm

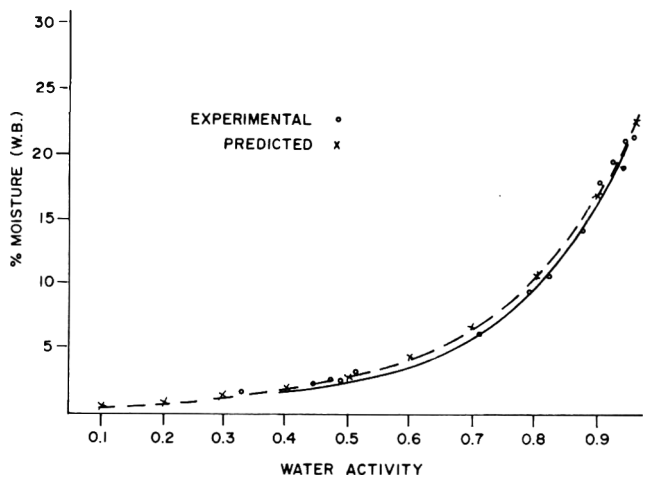

Fig. 7—Experimental vs predicted values bacon desorption isotherm (25°C, 50% fat).

Table 1—Coefficients in equations for freeze-dried bacon isotherms general form: Moisture = $ae^{b(a_w)}$

Isotherm	a	b	r
Desorption:			
5° C 30% fat	0.00760	4.5342	0.834
5° C 40% fat	0.00877	3.4532	0.999
5° C 50% fat	0.00493	3.9578	0.989
25° C 10% fat	0.01990	2.8140	0.989
25° C 40% fat	0.00199	5.2488	0.993
25° C 50% fat	0.00386	4.2252	0.987
25° C 60% fat	0.00087	5.5941	0.984
40° C 50% fat	0.00336	4.1163	0.995
40° C 60% fat	0.00349	3.6048	0.991
Adsorption:			
25° C 30% fat	0.00841	3.7003	0.954
25° C 40% fat	0.0056	3.9305	0.992
25° C 50% fat	0.0021	4.8186	0.990
25° C 60% fat	0.00189	4.7185	0.976

Table 2—Isotherm differences due to variation in fat composition

Isotherm comparisons			
Composition effects			
Temp Temp	Comparisons Comparisons	Sorption mode[a]	Fcal[b]
5° C	30% vs 50% fat	D	5.91*
5° C	40% vs 50% fat	D	6.57*
25° C	10% vs 40% fat	D	219.28**
25° C	40% vs 50% fat	D	74.50**
25° C	50% vs 60% fat	D	54.70**
40° C	50% vs 60% fat	D	48.33**
25° C	30% vs 40% fat	A	65.32**
25° C	40% vs 50% fat	A	60.23**
25° C	50% vs 60% fat	A	32.21**

[a] D = Desorption mode; A = Adsorption mode
[b] Fcal = Calculated F values
* 95% significance level
** 99% significance level

Table 3—Isotherm differences due to variation in temperature

Isotherm comparisons			
Temperature effects			
Composition	Comparisons	Sorption mode[a]	Fcal[b]
50% fat	5° C vs 25° C	D	145.23**
50% fat	25° C vs 40° C	D	19.11**

[a] D = Desorption
[b] Fcal Calculated F values
** 99% significance level

Table 4—Isotherm differences due to variation in sorption mode

Isotherm comparisons			
Sorption mode effects			
Temperature	Comparisons	Composition	Fcal[a]
25°C	Adsorption vs desorption	40%	55.92**
25°C	Adsorption vs desorption	50%	3.21*
25°C	Adsorption vs desorption	60%	30.90**

[a] Fcal Calculated F values
* 95% significance level
** 99% significance level

Table 5—Multiple regression equation for isotherm of freeze-dried bacon Ln [−Ln(1−a_w)] = Co + C_1 fat + C_2 Ln moist + C_3 fat x Ln moist

	Co	C_1	C_2	C_3	R[a]
5°C desorption	−2.531	10.381	0.437	1.101	0.867
25°C desorption	−3.133	3.990	1.171	0.909	0.958
40°C desorption	−1.641	8.456	0.314	1.106	0.880
25°C adsorption	2.415	−1.150	1.363	−1.428	0.967

[a] R = Coefficient of correlation; C = Regression coefficients

at 25°C was considerably better than that at either 5°C or 40°C.

The effects of drying method on the desorption isotherm are shown in Fig. 8. Correlation of the data for the microwave-dried product was poor due to significant melting of fat. Since adequate empirical prediction of the isotherm is a prerequisite for statistical comparison, the isotherm for the microwave-dried product was not evaluated statistically. The regression curves for the isotherms of freeze- and air-dried bacon differed significantly, indicating that additional sorption data will have to be developed for use in air drying studies. Ongoing studies in low-temperature air drying, prompted by the findings in the air-dried isotherm, indicate that sufficient water activity reduction can be accomplished in less than 2 hr with no melting of fat and no significant changes in product quality.

CONCLUSIONS

OF THE PARAMETERS investigated, composition appears to have had the most significant effect on the moisture sorption for bacon. For sufficient a_w reduction in a bacon product with unknown or widely varied fat/lean ratios, the product must be dried as indicated by the isotherm established for high fat content. An adequate prediction of fat or moisture content would permit minimal drying times to achieve safe a_w levels. The effects of storage temperature and sorption mode on the bacon isotherm are significant only for a_w values below 0.90, and would be unimportant in a processing scheme designed for a water activity reduction to 0.92. The isotherms reported here indicate that low-temperature air-drying may be a viable dehydration technique.

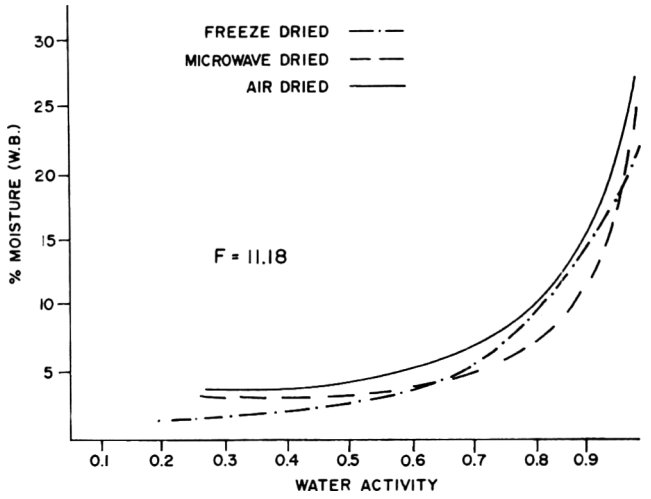

Fig. 8—Effect of drying method on bacon desorption isotherm (25°C, 50% fat).

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

Effects of Electrical Stimulation, Aging, and Blade Tenderization on Hot-Boned Beef Psoas major and Triceps brachii Muscles

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ABSTRACT

Forty-six steer carcasses were used to evaluate shear force values (SFV) for triceps brachii (TB) and psoas major (PM) muscles from sides assigned to three treatments: (1) chilled at 2–4°C for 48 hr (C); (2) hot boned 2 hr postmortem (HB); and (3) electrically stimulated 1 hr postmortem and hot boned 2 hr postmortem (ESHB). Some steaks were cut and frozen immediately after muscle excision or after 6 days of aging, and some were blade tenderized. HB and ESHB steaks had equal or smaller SFV relative to C after aging the TB and PM muscles; however, this was not true when TB steaks were cut after muscle excision. Electrical stimulation or blade tenderization did not improve HB.

INTRODUCTION

HOT-BONING (HB) research that has produced beef steaks and roasts that are equal or superior in tenderness to control counterparts, has included elevated temperature conditioning of hot-boned subprimals (Schmidt and Gilbert, 1970) or carcasses before HB (Kastner et al., 1973, 1976; Kastner and Russell, 1975; Falk et al., 1975; Will et al., 1976). Additionally, elevated temperature conditioning of hot-boned subprimals in conjunction with aging at conventional refrigeration temperatures has proved to be a successful combination (Follett et al., 1974; Dransfield et al., 1976; Taylor et al., 1980). Limited research has relied primarily on the aging of hot-boned cuts at conventional refrigeration temperatures to alleviate potential tenderness problems associated with pre-rigor excision. Even though Schmidt and Keman (1974) did use aging (1°C until 8 days postmortem), they hot boned subprimals at approximately 1 hr postmortem and conditioned the subprimals at 7°C for 4 hr before aging at 1°C.

Many of the more recent HB studies have incorporated electrical stimulation (ES) before HB (Gilbert and Davey, 1976; Gilbert et al., 1977; Seideman et al., 1979; Kastner et al., 1980; Nichols and Cross, 1980; Smulders et al., 1981) to speed the onset of rigor mortis. By using ES, those authors generally have been able to eliminate or reduce carcass or subprimal conditioning and aging times used in those studies not involving ES.

This research was primarily designed to evaluate HB of the triceps brachii (TB) and psoas major (PM) muscles plus a conventional aging period representative of the minimum time between slaughter and marketing of commercially centralized processed subprimal cuts. On the contingency that HB plus aging might result in a less tender product, ES, blade tenderization, and combinations of these treatments were superimposed on the HB and aging treatment. Processors may also be interested in fabricating subprimals and freezing resultant steaks after HB or ES plus HB; therefore, these treatments were also evaluated. Frozen steaks may be cooked from the frozen or thawed state; therefore, both methods were studied.

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MATERIALS & METHODS

FORTY-SIX CROSSBRED STEERS were obtained from the R.L. Hruska, U.S. Meat Animal Research Center at Clay Center, Nebraska. Steers were slaughtered in four groups at the Kansas State University Meat Laboratory. Average slaughter weights were 430, 522, 505, and 591 kg, respectively, for slaughter groups I to IV. Average USDA quality grades were average Good for slaughter groups I and III and high Good for slaughter groups II and IV. Slaughter groups I and III consisted of animals of medium or large size body types, respectively, finished on an accelerated feeding regimen while slaughter groups II and IV also consisted of animals of medium or large size body types, respectively, but were finished on a conventional feeding regimen. Those body types and feeding regimens are further described by Kastner et al. (1980) and Lyon (1981).

Cattle were stunned, bled, skinned, eviscerated, and split into sides. Kidney fat was removed from all sides during the dressing procedure. The right side of each carcass was electrically stimulated at 1 hr postmortem and hot boned at 2 hr postmortem (ESHB). The electrical stimulus was applied for 2 min: with 500 volts (V) and 60 Hertz (Hz) of alternating current with approximately 1.0 amp (A) continuously delivered through the carcass. One stainless steel probe was inserted on the inside of the rear leg approximately 8 cm below the attachment of the Achilles tendon and one laterally along the humerus.

The left side of each carcass was assigned to one of two treatments: conventional processing which involved chilling at 2–4°C until 48 hr postmortem (C) or hot boning at 2 hr postmortem (HB) (Fig. 1).

The TB, long head, and PM muscles were excised (ESHB and HB at 2 hr; C at 48 hr postmortem) and two steaks (2.5 cm) were removed from each. These steaks were frozen immediately at −26°C until evaluated. The remaining muscle portions were vacuum packaged at 23–27 in. of Hg in B 620 Cryovac® bags and aged at 1–5°C in cardboard boxes until 6 days postmortem.

Temperature data were collected for the TB, long head, at 2, 4, 6, 8, and 24 hr postmortem by inserting a thermometer into the center of the muscle. Samples for pH determination were obtained from the PM and TB, lateral head, at 1, 2, 4, 6, 8, and 24 hr postmortem. Muscle samples were removed from the TB with a 1.27 cm coring device, and thin strips were excised from the anterior end

LEFT SIDE	RIGHT SIDE
CONTROL - (C) SIDES CHILLED AT 2-4°C UNTIL 48 HR POSTMORTEM N = 23	ELECTRICALLY STIMULATED AND HOT BONED - (ESHB) ELECTRICALLY STIMULATED AT 1 HR POSTMORTEM AND HOT BONED AT 2 HR POSTMORTEM N = 46
HOT BONED - (HB) HOT BONED AT 2 HR POSTMORTEM N = 23	

Fig. 1—Assignment of treatments to carcass sides.

of the PM. A 1- to 2-g muscle sample was blended with 10 ml of 5 mM NaIAc in 150 mM KCl (Bendall, 1973).

At 6 days postmortem, one steak (2.5 cm) was cut from each muscle, wrapped in freezer paper, and stored at −26°C until evaluated. The remaining muscle portion was passed once through a Ross blade tenderizer. An additional steak (2.5 cm) was removed, wrapped in freezer paper, and stored at −26°C until evaluated.

One steak removed immediately after muscle excision was cooked from the frozen state. All exterior fat was removed from the frozen steak and weights were recorded before and after cooking to determine percentage weight loss. Other steaks were trimmed of exterior fat while frozen, weighed, then thawed at 2°C for 15–16 hr prior to cooking. Steaks were reweighed before and after cooking to obtain percentage thaw, cooking, and combined losses. Percentage thaw loss was calculated by dividing the thaw loss weight by the frozen steak weight x 100. Percentage cooking loss was calculated by dividing the cooking loss weight by the thawed steak weight x 100. Percentage combined loss was calculated by dividing the weight lost during thawing and cooking by the frozen steak weight x 100.

Steaks were cooked according to AMSA (1978) guidelines in a 163°C gas oven to an internal temperature of 70°C. Temperatures were recorded using thermocouples inserted into the center of each steak. After the steaks had cooled at room temperature for 2 hr, shear samples were removed perpendicular to the steak surface by using a drill press equipped with a 1.27-cm diameter coring device (Kastner and Henrickson, 1969). Six cores were obtained per steak and each core was sheared once with a Warner-Bratzler shearing apparatus.

Statistical analysis

The experimental design was a split-split plot design. Time of slaughter was confounded with the various body type and feeding regimen combinations. Note that for every C or HB treatment, two ESHB treatmens were observed (Fig. 1). Therefore, the tables show two ESHB means, the one paired with C and the other paired with HB.

An analysis of variance was performed and where there was a significant (α=0.05) slaughter group by treatment interaction, means within each slaughter group were compared. Where there was no significant interaction, treatment means were averaged over slaughter groups and compared. Additional details of the statistical analysis are outlined by Lyon (1981).

RESULTS & DISCUSSION

Temperature and pH

Postmortem pH and temperature declines of the TB muscle are shown in Figs. 2 and 3, respectively. Carcass ES increased the rate of pH decline in the TB muscle, compared with the C and HB treatments. A pH of 6.0 was reached approximately 5 hr postmortem in the ESHB TB, as opposed to more than 8 hr in the C and HB muscles. Bendall et al. (1976) reported that ES beef TB muscles reached pH 6.0 in 2.5 hr when they used 700V, 25 Hz for 2 min administered at 45 min postmortem. The HB TB pH at 8 hr was 6.0, while the C TB was 6.2. This slight increase in the rate of pH decline might have been due to the influence of pre-rigor excision or the higher temperature of the HB muscle at 2, 3, and 4 hr postmortem (2.2, 1.6, 1.1°C higher, respectively, Fig. 3). The influence of temperature was noted by Newbold and Harris (1972).

The postmortem pH decline of the PM muscle is shown in Fig. 4. The ESHB PM muscle reached pH 6.0 at 2 hr postmortem; therefore, electrical stimulation appeared to slightly accelerate the rate of postmortem pH decline in the PM. It should be noted that the 1 hr postmortem PM pH values for all treatments were relatively low (6.1–6.2). Davey et al. (1976) reported an accelerated pH decline in the PM when they used 3600V, 16 Hz, 2A delivered 30 min postmortem for 30 sec to 10 min. However, McCollum and Henrickson (1977) found no significant differences in pH decline between ES and nonstimulated PM (stimulation time 1 hr postmortem, for 30 min duration, using 300V at 400 Hz).

Comparisons of shear force values for C, HB, and ESHB treatments

Steaks removed upon muscle excision then frozen. Bendall and Rhodes (1976) reported that rapid cooling of muscles to 2°C can be started without danger of cold shortening as soon as pH 6.0 is reached, and rapid freezing can be initiated without danger of thaw shortening at pH 5.7. Shear force values (SFV) from steaks frozen prerigor and cooked from the frozen state should reflect cold shortening and thaw rigor shortening because of the rapid thawing associated with cooking from the frozen state (Perry, 1950). However, thaw rigor can be avoided by thawing slowly so that the structures remaining frozen provide sufficient restraint to prevent thaw shortening (Chrystall, 1976; Marsh et al., 1968).

In this study, the HB and ESHB TB steaks excised at 2 hr postmortem were placed in the freezer no later than 3 hr postmortem (pH values were at 6.5 and 6.3, respectively, Fig. 2). These steaks were frozen within 60 min after being placed in the freezer. HB and ESHB TB steaks treated in

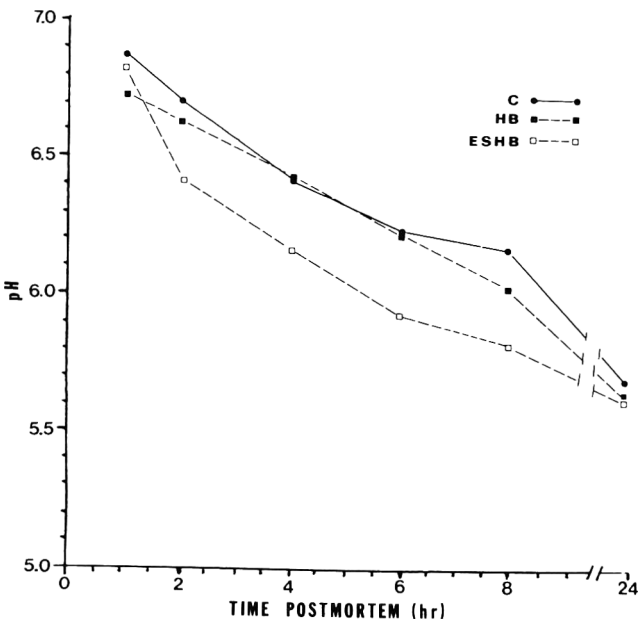

Fig. 2—Postmortem pH declines for the triceps brachii by carcass treatment.

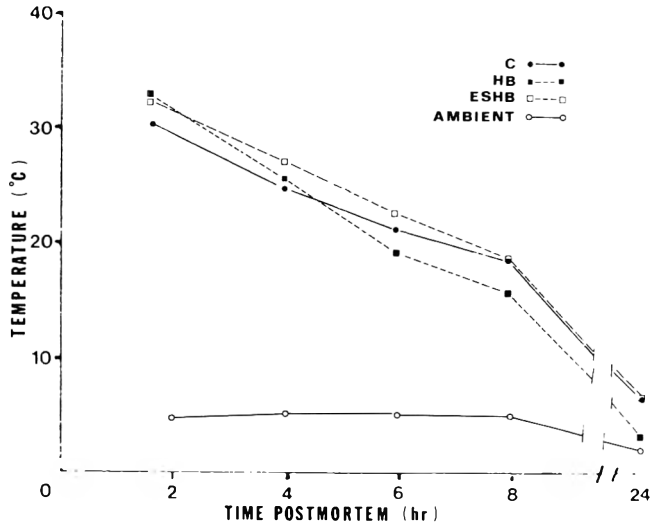

Fig. 3—Postmortem temperature declines for the triceps brachii by carcass treatment.

that manner and cooked from the frozen state had higher SFV (were less tender) than C counterparts excised at 48 hr postmortem and cooked from the frozen state. Differences were significant ($P<0.05$) in 6 of 8 comparisons (Table 1). Those HB and ESHB TB steaks removed 2 hr postmortem and thawed before cooking also had higher SFV than C counterparts; however, differences were generally of less magnitude than in steaks cooked frozen and were only of statistical significance in 3 of 8 comparisons (Table 1). Cold shortening could have had an influence on HB and ESHB TB steaks in both cases (cooking from frozen or thawed state), whereas the thawing procedure may have reduced any effect of thaw rigor shortening. C TB muscles chilled in the carcass did not reach 10°C within 10 hr postmortem or before pH 6.0 was attained (Fig. 2 and 3); therefore, cold shortening should not have been a problem with C TB samples (Locker and Hagyard, 1963; Davey and Gilbert, 1974; Chrystall, 1976).

When comparing HB to ESHB, all SFV mean comparisons for the TB msucle were similar (Table 1). That indicated that even though ES accelerated pH decline of the TB muscle (Fig. 2), steaks from ESHB TB muscles were still frozen in a pre-rigor condition (pH 6.3 at 3 hr postmortem) as were HB counterparts (pH 6.5 at 3 hr postmortem).

HB and ESHB PM steaks excised at 2 hr postmortem were at pH 5.92 and 5.86, respectively (Fig. 4) when placed in the freezer (3 hr postmortem). Those steaks cooked frozen had similar ($P>0.05$) SFV to C counterparts, whereas ESHB SFV were lower ($P<0.05$) than HB SFV (Table 1). This indicates that ES may have accelerated pH decline enough to minimize any effects of thaw rigor. Some degree of thaw rigor may have occurred in the HB steaks cooked from the frozen state, because HB and ESHB comparisons made for those steaks thawed prior to cooking revealed HB and ESHB steak SFV to be equal (Table 1). HB and ESHB steaks cooked thawed were more tender than C possibly because HB and ESHB steaks were frozen pre-rigor, and thawed slowly, such that the frozen structure restrained contraction relative to C. This effect may have been accented by our method of pH sampling, which may have allowed the C PM muscle to contract more than in an

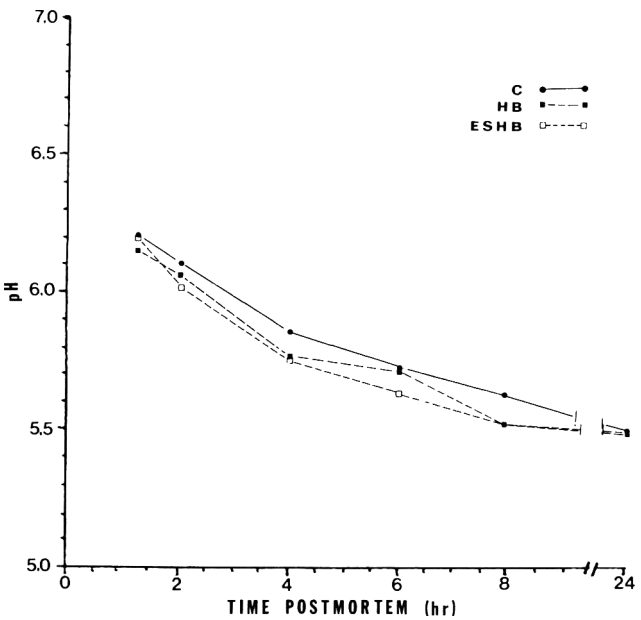

Fig. 4—Postmortem pH declines for the psoas major by carcass treatment.

Table 1—Triceps brachii and psoas major Warner-Bratzler shear force (kg) mean comparisons for carcass treatments by steak treatments

Slaughter group	Carcass treatment	Steak treatment: Removed upon muscle excision[a] cooked frozen	Removed upon muscle excision[a] cooked thawed	Aged[b], non-BT	Aged[b], BT
		Triceps brachii - comparisons within slaughter group			
I	C	3.43	2.83[f]	3.57	2.75
	HB	3.75	3.95	4.18	4.02
	ESHB[c]	4.05	4.81	3.81	4.03
	ESHB[d]	3.80	4.33	4.00	3.64
II	C	3.45[ef]	3.40	2.92	2.79
	HB	6.60	4.80	3.35	3.73
	ESHB[c]	6.03	4.82	3.57	3.27
	ESHB[d]	5.32	4.22	3.43	3.61
III	C	3.69[ef]	3.78[ef]	3.70	2.59
	HB	6.18	5.77	4.11	3.36
	ESHB[c]	5.72	6.03	4.04	3.21
	ESHB[d]	5.10	5.39	3.83	3.15
IV	C	4.21[ef]	3.63	3.40	2.62
	HB	7.09	4.18	3.93	3.47
	ESHB[c]	7.48	4.74	3.51	2.49
	ESHB[d]	7.32	4.23	3.77	3.22
		Psoas major - comparisons pooled over slaughter groups			
	C	3.10	3.20[ef]	2.88[ef]	2.77[ef]
	HB	3.40[g]	2.63	2.33	1.98
	ESHB[c]	2.81	2.68	2.33	2.36
	ESHB[d]	3.01	2.68	2.34	2.08

[a] HB and ESHB steaks cut at 2 hr postmortem then frozen; C steaks cut at 48 hr postmortem then frozen
[b] Muscles aged until 6 days postmortem, nonblade tenderized (non-BT) or blade tenderized (BT)
[c] ESHB paired with C
[d] ESHB paired with HB
[e] C vs HB mean comparisons differ ($P < 0.05$) for triceps brachii (within slaughter group and steak treatment) and psoas major (by steak treatment)
[f] C vs ESHB mean comparisons differ ($P < 0.05$) for triceps brachii (within slaughter group and steak treatment) and psoas major (by steak treatment)
[g] HB vs ESHB mean comparisons within steak treatment for psoas major differ ($P < 0.05$).

Table 2—Triceps brachii and psoas major Warner-Bratzler shear force (kg) mean comparisons for selected carcass treatments and steak treatments

Slaughter group	Carcass and steak treatment: C Aged[a], non-BT	HB Aged[a], BT	ESHB[b] Aged[a], BT	ESHB[c] Aged[a], BT
Triceps brachii — comparisons within slaughter group[d]				
I	3.56	4.02	4.03	3.64
II	2.92	3.73	3.27	3.61
III	3.70	3.36	3.21	3.15
IV	3.40	3.47	2.49	3.22
Psoas major — comparisons pooled over slaughter groups[e]				
	2.88	1.98	2.36	2.08

[a] Muscles aged until 6 days postmortem, nonblade tenderized (non-BT) or blade tenderized (BT)
[b] ESHB paired with C
[c] ESHB paired with HB
[d] All C vs HB and C vs ESHB mean comparisons within slaughter group for triceps brachii do not differ ($P > 0.05$)
[e] C vs HB and C vs ESHB mean comparisons for psoas major differ ($P < 0.05$)

unsampled side where the PM is restrained. The PM muscle can be removed 2 hr postmortem, with or without prior ES without adversely affecting tenderness. Even though steaks from the PM muscle might have suffered from effects of pre-rigor excision, cold shortening or thaw rigor did not appear to be of practical significance, particularly when considering the magnitude of the SFV.

Steaks aged 6 days postmortem, nonblade tenderized (non-BT). Those steaks from TB muscles aged at 1–5°C until 6 days postmortem had statistically equal SFV regardless of treatment (Table 1). These results agree with results of Schmidt and Keman (1974) who reported similar SFV for HB (1 hr postmortem) and cold-boned (48 hr postmortem) steaks (longissimus, psoas major, and gluteus medius) and roasts (semitendinosus, semimembranosus, biceps femoris, and quadriceps femoris) aged at 1°C until 8 days postmortem. Aging HB and ESHB TB muscles eliminated shear force differences when compared to C. Aging HB and ESHB TB muscles in a box at higher initial temperatures (Fig. 3) than C for the first 4 hr postmortem may have helped ensure those results. ES did not appear to improve the HB technique for steaks subsequently aged because all HB versus ESHB comparisons were nonsignificant (Table 1).

ESHB and HB PM steaks from muscles aged 6 days postmortem had lower SFV than did C steaks (Table 1). HB and ESHB PM steak SFV were similar ($P>0.05$), indicating that ES did not improve the HB technique. In that kidney fat was removed from C sides, possibly the rate of temperature decline was faster in the unprotected PM muscle on the C side than in the HB and ESHB PM muscle chilled in a box. A faster chilling rate may have led to some degree of cold shortening. Cold shortening should not be extensive in the PM muscle stretched in the normally suspended carcass (Herring et al., 1965); however, our method of pH sampling may have allowed relatively more contraction than in an unsampled side. Additionally, slower chilling of HB and ESHB muscles in a box could have accelerated the conditioning process relative to C counterparts chilled on the carcass. No temperature data were collected on the PM muscle to the substantiate these theories, but the C TB chilled slightly faster than did the boxed HB and ESHB TB muscles during the first 4 hr postmortem (Fig. 3).

Steaks aged 6 days postmortem, blade tenderized (BT). SFV for BT TB and PM steaks are reported in Table 1. Mean values were generally lowered by BT relative to non-BT; however, the relative SFV differences between carcass treatments remained similar ($P>0.05$). This indicates that the influence of BT on SFV was not affected by carcass treatment.

Steaks from BT HB and ESHB muscles aged until 6 days postmortem were compared with non-BT C counterparts aged 6 days postmortem to determine if BT would result in a more tender product. TB results (Table 2) indicate that steak SFV comparisons were statistically equal. Even though BT generally lowered SFV for all carcass treatments (Table 1), the change was not great enough to create a difference ($P<0.05$) between HB and ESHB BT steaks and C non-BT TB steaks. SFV for aged BT HB and ESHB PM steaks were smaller than the C non-BT aged steaks (Table 2). However, non-BT HB and ESHB steaks also had lower SFV than C steaks (Table 1) so BT was not necessarily needed to improve the tenderness of the PM as this muscle is inherently of acceptable tenderness.

Thaw, cooking,and combined losses

Because of the lengthy nature of thaw, cooking, and combined loss data, this information is not presented in tabular form. However, Lyon (1981) presented a detailed tabular summary of these data.

HB and ESHB TB steaks removed 2 hr postmortem and cooked after thawing had lower ($P<0.05$) thaw losses than did C steaks. Because the HB and ESHB TB steaks were frozen in a pre-rigor condition (within 3 hr postmortem), the pH and thus water-holding capacity were higher than for C steaks which had reached their ultimate pH at the time of freezing (48 hr postmortem). TB steaks from muscles aged until 6 days postmortem (nontenderized) had similar ($P>0.05$) thaw loss, regardless of treatment, indicating that the water-holding capacities had equalized during the aging period. Cooking and combined losses for these steaks were also similar, regardless of the carcass treatment.

Cooking data for the PM indicated that thaw, cooking, and combined losses for C, HB, and ESHB treatments were generally statistically equal regardless of steak treatment.

Both HB and ESHB BT TB steaks had significantly higher percentage thaw losses than did C counterparts, but percentage cooking and combined loss means were equal for all carcass treatment comparisons. Furthermore, PM percentage thaw, cooking, and combined loss means were similar for all carcass treatment comparisons regardless of the steak treatment. Comparing HB and ESHB BT steaks with C non-BT steaks revealed that the BT steaks had significantly higher thaw losses than those non-BT steaks; however, cooking and combined losses were similar ($P>0.05$). This was true for both TB and PM steaks. Other research generally agrees that drip and cooking losses are similar for BT and non-BT steaks (Bowling et al., 1976; Savell et al., 1977; Seideman et al., 1977; Tatum et al., 1978; Smith et al., 1979; Raccach and Henrickson, 1979).

CONCLUSIONS

HOT BONING the PM at 2 hr postmortem was successful without an aging period, but generally the same was not true for the TB. Nonetheless, processors could partially hot bone the carcass by removing the kidney fat and PM at 2 hr postmortem from otherwise conventionally processed carcasses without adversely affecting PM SFV. This was true even though the PM was frozen immediately and not aged, and regardless whether or not electrical stimulation was used. However, this practice should be further evaluated considering the influence of PM hot boning on the longissimus muscle of the remaining conventionally chilled and processed carcass.

Hot boning at 2 hr postmortem combined with 6 days of vacuum aging resulted in SFV and cooking characteristics for the TB and PM steaks that were equal or superior to

that of C counterparts. If it takes 6 days or longer to market unfrozen subprimal cuts, both the TB and PM muscles could be successfully hot boned at 2 hr postmortem. Electrical stimulation was not needed to HB the TB and PM when they were aged until 6 days postmortem. However, other muscles may not respond as those in this study. Even though BT generally lowered SFV of both the TB and PM muscles, it also did not improve the HB technique.

Additional research is required to determine if the spectrum of subprimal cuts obtained from beef carcasses can be successfully hot boned when conventional aging is used. The utility of ES in these investigations as well as the definition of optimum ES conditions for hot boning requires further study. Earlier HB times than that used in this study also deserve consideration along with determining the need for ES to decrease the time between slaughter and HB.

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Biochemical and Quality Characteristics of Ovine Muscles as Affected by Electrical Stimulation, Hot Boning, and Mode of Chilling

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ABSTRACT

The combined effects of electrical stimulation and carcass holding temperature were evaluated on some biochemical and quality characteristics of intact and hot-boned ovine muscles. Twenty-four lamb sides were randomly assigned to four treatments. Electrical stimulation was performed within 15 min postmortem (350 V with 10 Hz) for 4 min. Electrically stimulated and slowly chilled (5 hr at 14 ± 2°C) sides significantly exhibited more rapid pH decline in the longissimus dorsi (LD) muscle, less cold shortening in the semitendinosus (ST) muscle and greater tenderness in both LD and ST muscles than sides chilled at 2°C. None of the treatments had any effect on cooking loss in ST and LD muscles, lean color of LD muscle during a 4-day retail display, and solubility of different protein fractions as well as the swelling factor of the stroma protein of LD muscles.

INTRODUCTION

HIGH TEMPERATURE CONDITIONING of the carcass as a method of preventing or reducing cold shortening and the accompanying toughness of meat has received considerable interest. It is generally accepted that cold shortening will cause muscle toughness when lamb (March and Leet, 1966; Marsh et al., 1968; McCrae et al., 1971) and beef (Locker and Hagyard, 1963) carcasses are chilled or frozen in the pre-rigor state. One approach used to prevent muscle toughening is to hold the carcass at 14–20°C until pre-rigor changes in the muscle are near completion since minimum shortening occurs at this temperature range (Locker and Hagyard, 1963). In the case of lamb, at least a 16-hr holding period is required (McCrae et al., 1971). Cold shortening can be minimized by delaying the exposure of the carcass to cold temperatures until the muscle pH has reached a value below 6.0 and approximately 50% of the adenosine triphosphate (ATP) has been depleted (Bendall, 1975).

A carcass conditioning period may introduce an undesirable delay in processing. However, this problem can be resolved by electrical stimulation of the carcass which ensures a fast drop in pH and a rapid depletion of muscle ATP (Carse, 1973; Locker et al., 1975; Bendall et al., 1976; Davey et al., 1976a, b; McCollum and Henrickson, 1977; Shaw and Walker, 1977; Savell et al., 1977; Bouton et al., 1978; Chrystall and Devine, 1978; Will et al., 1979; Elgasim et al., 1981; Whiting et al., 1981). Even though electrical stimulation has been adopted, little information is available regarding its combined effect with the mode of chilling. Recently, Rashid (1982) has found that the pH of the Longissimus dorsi (LD) and semitendinosus (ST) muscles reached nearly 6.0 after 4 hr postmortem when lamb sides were electrically stimulated at 350 volts (V), 10 pulses (Hz) and 20% duty cycle (DC). Some of the biochemical-biophysical changes which take place may be related to meat quality. Hence, the aim of this study was to investigate the combined effect of electrical stimulation and slow chilling of lamb carcasses at 14 ± 2°C for 5 hr postmortem on some biochemical and quality characteristics of specific ovine muscles.

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MATERIALS & METHODS

Animal and experimental design

Twelve Suffolk wether lambs (hot dressed carcass weight ranged from 21–29 kg) were slaughtered according to commercial practices (in the Abattoir of the Meat Science Laboratory, Okalhoma State University), skinned, eviscerated and divided into sides. The two sides within each carcass were randomly assigned to two different treatments and a balanced incomplete block design, block size 2, was used. Accordingly, a total of 12 sides, selected at random, were electrically stimulated (ES) while 12 sides were kept as unstimulated (US) or control. In each case, 6 sides, at random, received a rapid chilling (RC) treatment and the other 6 sides were subjected to slow chilling (SC) as shown in Table 1.

Electrical stimulation

The sides were electrically stimulated within 15 min postmortem using a direct current with a square wave pulse for 4 min. Since a previous study (Rashid, 1982) had shown that electrical stimulation using 350 V with 10 Hz (20% DC) resulted in the highest rate of glycolysis as compared to some other combinations of different voltages and frequencies, these stimulation parameters were used in the present experiment. The electrical current was applied by two wires each terminated with a clamp. One clamp was attached to the neck region at the level of the 5th and 6th cervical vertebrae as the negative charge and the other clamp was attached to the achilles tendon (near its muscular attachment) as the positive charge to complete the circuit.

Muscle sampling procedure

Two muscles, namely the LD and ST muscles were used to study the changes in some biochemical and quality characteristics. The ST muscle was hot boned from both the ES and US sides immediately after electrical stimulation and the LD muscles remained attached to the skeleton. The extent of cold shortening and cooking loss on hot-boned ST muscle were determined at 24 hr postmortem. Postmortem pH and temperature changes were monitored on intact LD muscles for 24 hr postmortem. Thereafter, fresh samples were taken to measure the lean color, protein solubility, and cooking loss. The shear force value for both LD and ST muscles was determined at 48 hr postmortem.

Muscle pH. Sample cores (1.27 cm in diameter) were taken from intact LD muscles (at levels of 9th and 13th thoracic vertebrae) at 0, 2, 4, 6, 8 and 24 hr postmortem and 1.5-g samples (taken from the center of the cores) were immediately homogenized with 15 ml of 0.005M sodium iodoactetat (Nichols and Cross, 1980) for 30 sec using a Brinkman Polytron homogenizer. The pH of the slurry was measured with a Digital Corning-130 pH meter.

Muscle temperature. The changes in the internal temperature of the LD muscles were measured with a temperature probe, (Koch Model 1364), at the same time intervals as for the pH measurements.

Muscle shortening. The ST msucle was divided longitudinally into two strips of approximately equal weight and length. The initial length of each strip was marked by inserting straight pins in either end. The strips were placed in deep trays, covered with Handi-W food wrap film (Dow Chemical Company, Midland, MI) to guard against evaporation and subjected, as appropriate, to either rapid or slow chilling as described in Table 1. Then the final length of each strip was measured to calculate the percent shortening.

Cooking loss. Chops from the LD (3.8 cm in diameter) at the level of 1st and 3rd lumbar vertebrae and ST strips were cooked to an internal temperature of 70°C in a convection oven (Blodgett Co.,

Table 1—Treatment description

Treatment	Number of sides	Description
1. Electrical Stimulation + Slow Chilling (ES + SC)	6	The sides were electrically stimulated and held at 14±2°C for 5 hours (slow chilling) before being subjected to a chilling temperature (2°C) for subsequent 19 hours.
2. Electrical Stimulation + Rapid Chilling (ES + RC)	6	The sides were electrically stimulated and immediately subjected to a rapid chilling temperature (2°C) for 24 hours.
3. Unstimulated + Slow Chilling (US + SC)	6	The sides were unstimulatec (control) and treated as in treatment 1.
4. Unstimulated + Rapid Chilling (US + RC)	6	The sides were unstimulatec (control) and held as in treatment 2.

Inc.). The heat penetration rate was monitored by a copper constantan thermocouple and a recording thermometer assembly (Honeywell Co., Electronik 15). Cooking losses were derived from difference between weight of each chop or strip before and after cooking and expressed as a percentage of raw weight.

Shear force value. The cooked chops or strips were wrapped in a Handi-W food wrap film and placed in a cooler at 2°C for 24 hr to provide equalized firmness to insure uniform cores (Kastner and Henrickson, 1969). Three cores (1.27 cm in diameter) were taken from the LD chops (lateral, dorsal and medial) and two cores (1.27 cm in diameter) were obtained from the ST strips parallel to the direction of fibers using a coring device with an electrical drill. Two shear readings were recorded from each core at right angles to the muscle fiber using a Warner-Bratzler cell on the Instron Universal Testing Machine (Instron Corp., Model 1132). The drive and chart speeds were calibrated at 10 cm/min.

Lean color. Boneless loin chops were cut from the sides at the level of the 3rd and 6th lumbar vertebrae and allowed to bloom for 45 min. They were then placed on a plastic foam tray, wrapped in oxygen permeable commercial type film and placed in a retail case at 2°C under 70 ft-c fluorescent light for 4 days. HunterLab L, a, and b values which indicated respectively the lightness, redness and yellowness were measured at 24-hr intervals using HunterLab Tristimulus Colorimeter Model D25 L-9. The ratio of redness to yellowness (a/b) was also calculated.

Protein solubility. The solubility of different protein fractions was determined according to the procedure of Asghar and Yeates (1974) with modifications. Triplicate samples, 2-g each, from homogeneous minced LD muscle were extracted sequentially with different buffer systems. The sarcoplasmic proteins were extracted with 2% glycerol solution (Scopes, 1968). The residue was extracted with 0.3M NaCl unbuffered solution to dissolve myofibrillar protein and then with 0.6M KI in 0.1M phosphate buffer to extract the remaining myofibrillar proteins. All extractions and centrifugations were performed at 2°C. The resulting residue after washing thoroughly with deionized water was extracted with chloroform-methanol (3:1, v/v) to remove lipid fractions. Thereafter, the residue was extracted with 0.1M lactic acid to estimate the acid soluble protein. Finally, the remaining residue was again washed with deionized water, dried at 105°C overnight, and designated as acid-insoluble stromal proteins. The swelling factor was also estimated according to the procedure of Asghar and Yeates (1974). The protein content in different extracts was measured by biuret reaction and the $A_{540\,nm}$ was determined using a Gilford 240 Spectrophotometer (Gornall et al., 1949).

Statistical analysis

The data were subjected to analysis of variance using a balanced incomplete block design, block size 2. The F-test was used to determine if significant differences occurred among treatments. Means were compared by Duncan Multiple Range Test at the 5% level of significance (Steel and Torrie, 1960).

RESULTS & DISCUSSION

pH and temperature decline

Both the electrical stimulation and the chilling methods had marked influence on muscle postmortem glycolysis (Fig. 1). Stimulated sides, whether rapid or slow chilled, had a significantly ($P < 0.05$) lower pH than the respective control muscles at 2, 4, 6 and 8 hr postmortem. This is in agreement with various researchers who have shown that

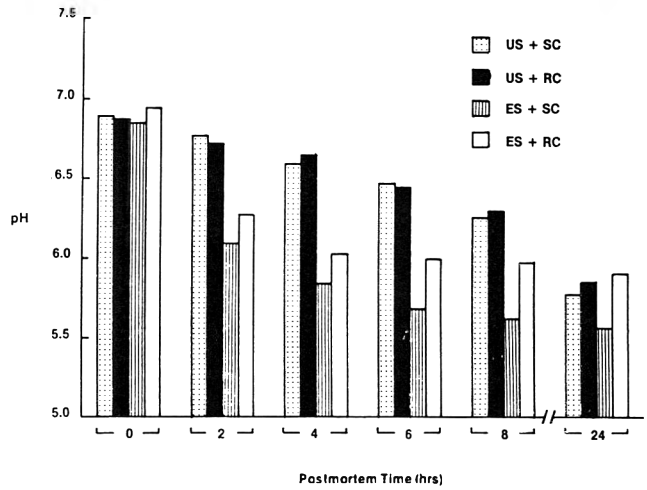

Fig. 1—Postmortem pH decline for LD muscle as affected by electrical stimulation and mode of chilling.

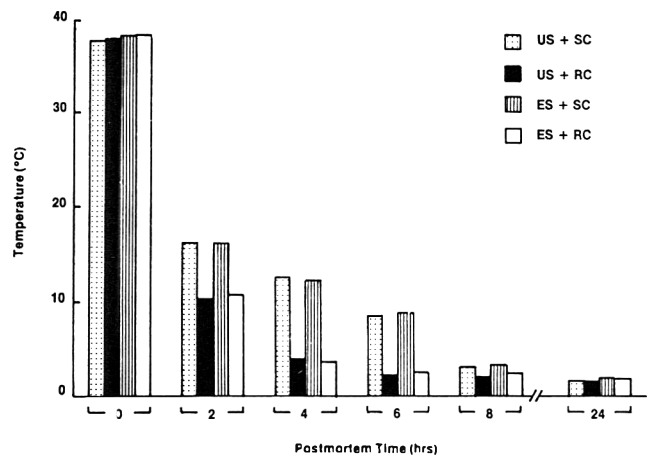

Fig. 2—Postmortem temperature decline for LD muscle as affected by electrical stimulation and mode of chilling.

electrical stimulation accelerates the rate of postmortem glycolysis (Carse, 1973; Bendall et al., 1976; Davey et al., 1976a; McCollum and Henrickson, 1977; Chrystall and Devine, 1978; Will et al., 1978; Whiting et al., 1981). However, muscle from electrically stimulated slow chilled sides (ES + SC) experienced a greater pH decline ($P < 0.05$) than the rapid chilled (ES + RC) sides. On the other hand, postmortem pH decline in the unstimulated sides whether slow or rapid chilled (US + SC) and (US + RC) was almost identical.

The internal temperature of the LD muscle at 2, 4 and 6 hr postmortem of stimulated (ES + RC) and unstimulated (US + RC) sides which were rapidly chilled was lower than the stimulated (ES + SC) and unstimulated (US + SC) sides which were slow chilled (Fig. 2). However, there was no

Table 2—Muscle shortening (%), shear force (kg) and cooking loss (%) values for ST and LD muscles as affected by electrical stimulation and mode of chilling

Treatment[a]	Muscle shortening (%)[b]	Shear force, (kg)[c]		Cooking loss (%)[b]	
	ST	ST	LD	ST	LD
ES + SC	10.6a	5.0a	4.1a	13.2a	19.7a
ES + RC	13.1ab	5.6b	4.0a	16.1a	18.9a
US + SC	15.7b	6.3c	5.1b	16.3a	19.7a
US + RC	19.6c	6.4c	5.4b	14.3a	19.0a
S.D. of Adj. Mean[d]	0.99	0.13	0.13	0.52	0.50

[a] See Table 1 for treatment.
[b] Each muscle shortening and cooking loss value is averaged from 12 samples in both ST and LD muscles.
[c] Each shear force value is averaged from 48 samples for ST muscle and from 72 samples for LD muscles.
[d] Means within a column followed by different letters are significantly different ($P < 0.05$).

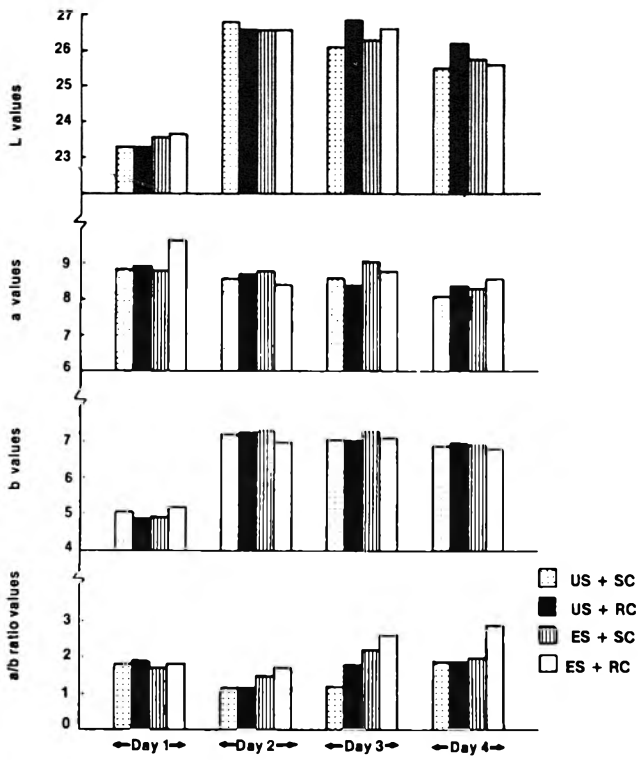

Fig. 3—HunterLab L, a, and b color values and a/b color ratio for LD muscle at day 1 to day 4 as affected by electrical and mode of chilling.

significant variation in temperature decline between the stimulated and control sides for any given chilling procedures (rapid or slow). Hence, differences in the rate of pH decline for the LD muscle between stimulated and control cannot be ascribed to difference in carcass temperature decline as assumed by Bendall (1980). Activation of glycolytic enzymes by electrical stimulation may be one of the causative factors accounting for the rapid drop in pH in electrically stimulated carcasses. This explanation is supported by Clarke et al. (1980) who found that phosphofructokinase, aldolase, glyceraldehyde 3-phosphate dehydrogenase and pyruvatekinase bound to actin filaments in electrically stimulated muscles and accelerated glycolytic rate. Whether or not electrical stimulation increased the glycolytic enzymes activities per se in muscle has not been completely defined.

Muscle shortening

Both electrical stimulation and chilling treatments significantly reduced cold shortening in the hot boned ST muscle (Table 2). The muscle from stimulated and slow chilled sides (ES + SC) had significantly less ($P < 0.05$) shortening than the control groups. However, there was no significant difference ($P > 0.05$) in the percent of shortening of the ST strips from electrically stimulated sides, whether they were rapid or slow chilled. On the other hand ST strips from control sides shortened significantly more when rapidly chilled than when slow chilled. The present study shows that ES reduced the percent of muscle shortening. Rapid depletion of the energy rich phosphate compounds (adenosine triphosphate and phosphocreatine) which determine the degree of muscle fiber shortening during chilling or freezing of carcasses may be attributed to ES (Asghar and Henrickson, 1982). Many researchers have reported that electrical stimulation accelerates musculature ATP depletion (Bowling et al., 1978; Will et al., 1979; Whiting et al., 1981). This study supports previous reports in that, by reducing the time required for muscles to reach pH 6.0 (through the application of ES), and by holding carcasses for about 5 hr at 14 ± 2°C, the extent of cold shortening was reduced as compared to carcasses conventionally chilled at 2°C.

Shear force and cooking loss

Electrical stimulation significantly ($P < 0.05$) decreased shear force value as compared to those from the control regardless of the postmortem chilling procedure for both ST and LD muscles (Table 2). Most investigators have shown that electrical stimulation of carcasses produced a tenderizing effect on the musculature (Carse, 1973; Chrystall and Hagyard, 1976; Davey et al., 1976b; Grusby et al., 1976; Ray et al., 1978; Stiffler et al., 1978; Cross, 1979; Nilsson et al., 1979; Savell et al., 1979; Smith et al., 1979; Rilery et al., 1980b; Bouton et al., 1980; Taylor and Marshall, 1980; McKeith et al., 1981). However, the shear force value of the ST muscle (hot boned from electrically stimulated sides) was significantly ($P < 0.05$) less when it was slow chilled as compared to rapid-chilled; whereas the electrically stimulated sides of intact LD muscle did not show significant differences in shear values between modes of chilling. Several explanations have been given by different researchers to account for improvements in tenderness from electrical stimulation. They include: (a) prevention of cold shortening (Bendall et al.,1976; Davey et al. 1976a; Gilbert et al., 1976; Walker et al., 1977; Boutor et al., 1980); (b) increase in autolytic enzyme activity (Sorinmade et al., 1978; Dutson et al., 1980); and (c) physical disruption of muscle fiber (Savell et al., 1978a; George et al., 1980).

With respect to cooking loss, the data indicated no significant differences ($P > 0.05$) in either ST and LD muscles as affected by electrical stimulation and chilling temperature (Table 2). This is in agreement with Riley et al. (1980b) and Thompson (1981). However, Savell et al. (1978b) noted a high cooking loss from electrically stimulated meat These studies are not directly comparable as different stimulation techniques and conditions were used.

Lean color

The lean color measurements using HunterLab L, a, and

Table 3—Solubility of different protein fractions in LD muscle as affected by electrical stimulation and mode of chilling

Protein fraction (%)	Treatment[a]				S.D. of Adj Mean[e]
	ES + SC	ES + RC	US + SC	US + RC	
Sarcoplasmic	3.54a	3.94a	4.11a	3.81a	0.29
Myofibrillar[b]	4.46b	4.22b	4.40b	4.42b	0.19
Myofibrillar[c]	7.13c	7.04c	6.91c	7.00c	0.18
Total Myofibrillar	11.59d	11.26d	11.31d	11.42d	0.19
Intracellular Protein	15.13e	15.20e	15.42e	15.23e	0.35
Acid-Soluble Protein	1.19f	1.25f	1.19f	1.22f	0.13
Acid-Insoluble Stromal Protein	2.77g	2.55g	2.47g	2.63g	0.45
Extracellular Protein	3.96h	3.80h	3.66h	3.85h	0.39
Total Protein	19.09i	19.00i	19.08i	19.08i	0.03
Swelling factor[d]	59.88j	56.24j	60.24j	59.65j	0.06

[a] See Table 1 for treatment.
[b] Extracted with 0.3M NaCl in unbuffered solution.
[c] Extracted with 0.6M KI in 0.1M phosphate buffer.
[d] Swelling factor = Weight of the sample (drained)/Dry weight of sample.
[e] Means within each row followed by the same letter are not significantly different ($P > 0.05$).

b values and the a/b color ratio of LD loin chops at 24 hr intervals for 4 days are shown in Fig. 3. The treatment X day interaction exhibited no influence ($P > 0.05$) on L, a, and b color values and a/b color ratio. The data also indicated no significant differences ($P > 0.05$) in the objective HunterLab color values of meat among all treatments. This was contrary to the finding of Riley et al. (1980a) who showed by subjective evaluation that electrical stimulation improved muscle color, decreased surface discoloration, and improved overall appearance of boneless loin chops from lambs during 4 days of display. Most of the studies, based on panel evaluation, have found the meat from stimulated carcasses generally to be brighter (Smith et al., 1977, 1979; Savell et al., 1978a, b, 1979) with a more youthful lean color (McKeith et al., 1981) than that from unstimulated carcasses. However, several workers agreed that electrical stimulation did not improve lean color (Grusby et al., 1976; Nichols and Cross, 1980).

Protein solubility

Neither electrical stimulation nor chilling rate had any significant effect ($P > 0.05$) on the solubility of the sarcoplasmic protein fraction as compared to the control. This is in disagreement with George et al. (1980) who have concluded that slow cooling of electrically stimulated carcasses causes denaturation and precipitation of sarcoplasmic proteins onto the myofibrils. If such a deposition occurs, it should be reflected in decreased sarcoplasmic protein solubility. No change was noted in the solubility of myofibrillar proteins extracted sequentially with unbuffered 0.3M NaCl solution followed by 0.6M KI in 0.1M phosphate buffer. It is generally thought that presence of the phosphate ions in the buffer dissociates the actomyosin complex and probably increases the solubility of the myofibrillar protein (Mihalyi and Rowe, 1966). In view of this proposition, the myofibrillar proteins were first extracted with unbuffered 0.3M NaCl solution to see whether or not actomyosin complex formed to a different degree as a result of the different treatments applied to the carcass sides. From the solubility test with unbuffered 0.3M NaCl solution, it seems that the different treatment had no significant effect on the extent of actomyosin formation. Similarily, the total percentage of myofibrillar protein and the intracellular protein were also not significantly different ($P > 0.05$) among treatments. These observations agree with those of McKeith et al. (1980) who found no measurable differences in the solubility of the myofibrillar protein of muscle from electrically stimulated and unstimulated steer carcasses. Acid-soluble protein (freshly synthesized collagen) and acid-insoluble stromal proteins (biologically mature collagen and some elastin) were not significantly affected by electrical stimulation and carcass chilling (Table 3). The swelling factor which is used as an indicator of changes in the extent of cross-linkage of collagen (Asghar and Yeates, 1974, 1979) was also not affected by electrical stimulation. On the other hand, Judge et al. (1980) found no increase in the solubility of the perimysial collagen from electrically stimulated muscle, but their data on differential scanning calorimetery showed a significant decrease (0.6°C) in the thermal stability of the perimysial collagen of the L. dorsi muscle from electrically stimulated carcasses as compared to that from the control. As a matter of fact, very limited information is available on the influence of electrical stimulation of carcasses on the connective tissue (extracellular) proteins, and more information is needed.

CONCLUSIONS

THIS STUDY has shown that the combined effect of electrical stimulation and mode of chilling profoundly affects some biochemical, biophysical and quality characteristics of ovine muscles. The sides which were electrically stimulated and slowly chilled (holding the carcass sides for 5 hr at 14 ± 2°C) exhibited more rapid pH decline, less cold shortening and greater tenderness than those which were subjected to other treatments. However, the lean color during a 4-day retail display and the solubility of different protein fractions showed no improvement by either electrical stimulation or carcass holding temperature.

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Clostridium botulinum Ionizing Radiation D-Value Determination Using a Micro Food Sample System

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ABSTRACT

A miniaturized system for rapid and accurate irradiation D-value determinations was evaluated. The test units consisted of pork, ham or chicken substrates inoculated with a mixed suspension of *Clostridium botulinum* spores, sealed inside polypropylene cryotubes at vacuum levels of 250, 125 and 50 torr. Samples were irradiated with ^{60}Co gamma rays at −30 ± 10°C up to a dose of 1.8 Mrads. D-values were in close agreement with historical determination for the substrates and conditions tested. The D-value estimates encompass a standard error of 0.025 Mrad at $p < 0.05$. Replicate experiments conducted on different days demonstrated a high degree of reproducibility.

INTRODUCTION

TRADITIONAL IRRADIATION D-value estimates for FDA process approval have been commonly measured with inoculated packs. These studies take a year or more to complete and present potential hazards due to the relatively large amount of toxic material that spoil in cans. Small change in process parameters, such as vacuum, ingredient amounts, and irradiation temperature necessitate repeated studies. The inoculated pack methods also normally generate only two data points per test, complicating the statistical prediction of D-value point estimates (Ross, 1977). The buffer suspension methods for bacterial spore D-value determinations give better data confidence (Anellis et al., 1977). However, the relation of the determined values to a true food system is questionable due to the different microenvironments. Despite the limitation, the suspension methods provide valuable information on the survivor curve shape (lags, slopes, tails, etc.) which is essential to constructing process estimates.

Snyder (1969) developed a prototype microsystem to correct some of the deficiencies of the traditional approaches to irradiation D-value determinations. The system uses 2-g meat slices inoculated with spores of *Clostridium botulinum* as test units. substrates of beef, pork chicken and ham were tested in this miniaturized system at precisely controlled environmental conditions. The samples were sealed in glass tubes and irradiated under vacuum levels ranging from 10–760 torr and at temperatures from −196°C to ambient. Although this system provided admirable control of test irradiation conditions, the glass tubes were cumbersome to seal and open, and spore recovery by simple glass rod mastication was time-consuming.

The objective of this study was to integrate the best features of these historical approaches to irradiation D-value determinations with a new sample-handling and spore recovery methodology. The result of this synthesis was to be an accurate, convenient, and reproducible alternative to the inoculated pack method for radiation D-value determination. This new system should provide future irradiation processors with a D-value determination method that will allow them to rapidly assess how changing additives, process temperature, and vacuum level affect the dose estimate for safe food processing.

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MATERIALS & METHODS

Test unit preparation

Samples of chicken, pork and ham were obtained from the U.S. Army Natick Research and Development Command. Chicken and pork were injected with a solution to give a final product concentration of 0.75% sodium chloride and 0.3% sodium chloride and 0.3% sodium tripolyphosphate. In addition, pork also contained 0.1% pepper. Ham samples contained 100 ppm nitrate, 1–2 ppm nitrite, 2.75% sodium chloride and 6.7% fat. Test units were prepared by inoculating 1 x 1.5-cm substrate slices with 4 μl of a mixed spore suspension of *Clostridium botulinum* to give approximately 1×10^6 spores per substrate slice. Because the slice was so small and the inoculum was absorbed almost immediately, no attempt was made to spread the inoculum. The spore suspension contained equal numbers of strains 33a, 62A, 36A, 77A, 12285A, 9B, 40B, 41B, 53B and 67B, cultured using biphasic methods (Anellis et al., 1972a).

The inoculated substrates were sealed inside cryotubes (NUNC, Model 1077) at vacuum levels of 250, 125 and 50 torr, using a device constructed for this purpose. A sealant (3M #3762) was applied to the cap juncture of each cryotube to maintain vacuum integrity. Samples were stored at −30°C until use.

Sample irradiation

Samples were irradiated at the U.S. Army Natick Research & Development Laboratories using a cobalt 60 gamma source. D-value curves were produced for each substrate-vacuum level combination at an irradiation temperature of −30 ± 10°C. Four replicate test units were exposed at each of nine dose increments (0.2 Mrad) to produce survivor curves. Each substrate type (chicken, pork, ham) was tested at the three vacuum levels to evaluate substrate effects, vacuum effects and interaction effects on the *Clostridium botulinum* spore irradiation D-value estimates.

Recovery methods

After irraditation, samples were thawed at room temperature. Substrate slices were removed from the cryotubes and processed in 10.0 ml of 1% DAXAD 23 (Grace Co.) using a glass tissue homogenizer. Each meat slice was homogenized to produce a uniform suspension under aseptic conditions. Aliquots of the spore homogenate were diluted in DAXAD 23 in preparation for plating. Surviving spores were quantified using pour plate techniques (Gilliand et al., 1976).

A yeast extract recovery medium (Augustin and Pflug, 1976) used to optimize spore recovery was prepared from the following components: Yeast extract (BBL), 10.0g; Starch (Mallinckrodt), 1.0g; K_2HPO_4 (Mallinckrodt), 2.0g; Agar (Difco), 15.0g; and Distilled water, 1,000 ml. This medium was dispensed in 300-ml volumes and autoclaved at 110°C for 15 min. Immediately before use, the following sterile solutions were added to each flask: 10% Sodium thioglycolate, 3.75 ml; 40% Dextrose, 3.75 ml; and 4% Sodium bicarbonate, 7.5 ml.

Two different dilutions of substrate homogenate were used to make duplicate plate sets, enhancing the probability of obtaining CFU counts between 30 and 300 per plate. Replication at each data point also facilitated the calculation of confidence intervals (CI) for each point of the survivor curve, as well as the overall-D-value-point estimates for each test condition. *–Continued on next page*

Data analysis

For each test condition, a radiation death survivor curve was constructed by plotting the $\log_{10}$ of the number of survivors on the ordinate against the radiation dose on the absicissa. A least squares regression line was fitted through the data points, excluding the 0 dose data (Draper and Smith, 1966). The classic semilogarithmic survivor curve equation was used:

$$\log N = -U/D + \log N_o$$

where N_o = Number of spores surviving at dose 0; U = radiation dose (Mrad); D = the decimal reduction dose (D-value) of spores at predetermined environmental test conditions (i.e., temperature, vacuum, substrate type); N = number of spores surviving at dose U.

By definition, the slope of the survivor curve is tan θ, where:

$$\tan \theta = \Delta y/\Delta x$$

The radiation D-value is subsequently interpreted as the negative reciprocal of the regression line slope for each curve.

Given that the experimental design provides replicate points at each dose increment of the curve:

$$\text{slope } \hat{K} = \frac{\Sigma U_i (\log N_i) - \frac{(\Sigma U_i)(\Sigma \log N_i)}{n}}{\Sigma U_i^2 - \frac{\Sigma (U_i)^2}{n}}$$

where n = replicates; U_i = doses; N_i = number of survivors at U_i.

An estimate of the variance of the individual data points about the regression line ($S^2\epsilon$) is obtained by the formula:

$$S^2_\epsilon = \frac{\Sigma(\log N_i - \log \hat{N}_i)^2}{n-2}$$

where $\log N_i$ is an observation at U_i and $\hat{N}_i$ is the regression estimate.

An estimate of the variance of a particular point on the regression line is derived from:

$$s^2_{\log \hat{N}_j} = \frac{1}{n} + \frac{(U_j - \bar{U})^2}{\Sigma(U_i - \bar{U})^2} s^2_\epsilon$$

where $\bar{U} = \frac{\Sigma U_i}{n}$ and U_j = a specific value of u. Thus $s^2_{\log \hat{N}_j}$ is the variance estimate of the value of $\log \hat{N}_j$ on the regression line at dose U_j.

The CI for the regression line are expressed as:

$$\log \hat{N}_j \pm 2 s_{\log \hat{N}_j}$$

The variance of a predicted new observation is, therefore:

$$s^2 \text{ new observation} = s^2_{\log \hat{N}_j} + s^2_\epsilon$$

Finally, the variance estimates of the slope $s^2_{\hat{K}} = \frac{s^2_\epsilon}{\Sigma(U_i - \bar{U})^2}$ and y intercept is: $s^2_{\log \hat{N}_{Y_o}} = s^2_\epsilon \frac{(\Sigma U_i^2/n)}{\Sigma(U_i - \bar{U})^2}$ -

One measure of the goodness of fit $\hat{p}^2$ of a data set to the regression line is:

$$\hat{p}^2 = \frac{\hat{K}^2 \Sigma(U_i - \bar{U})^2}{\Sigma(\log N_i - \log \frac{\Sigma N_i}{n})^2}$$

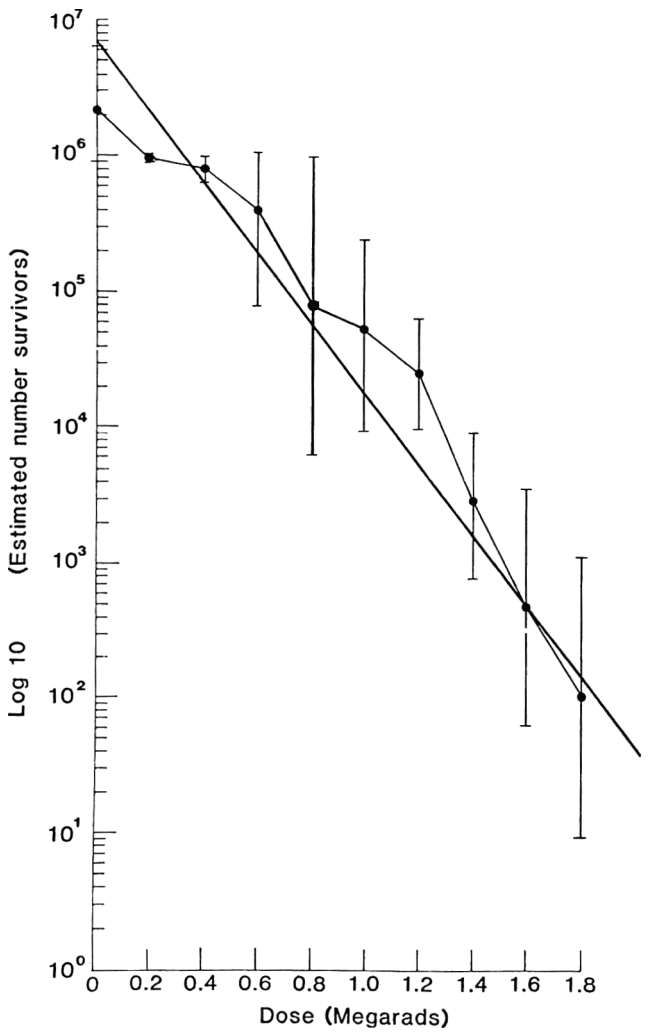

Fig. 1—Pork irradiated at a vacuum level of 250 torr and −30°C ± 10°C (RK0248A).

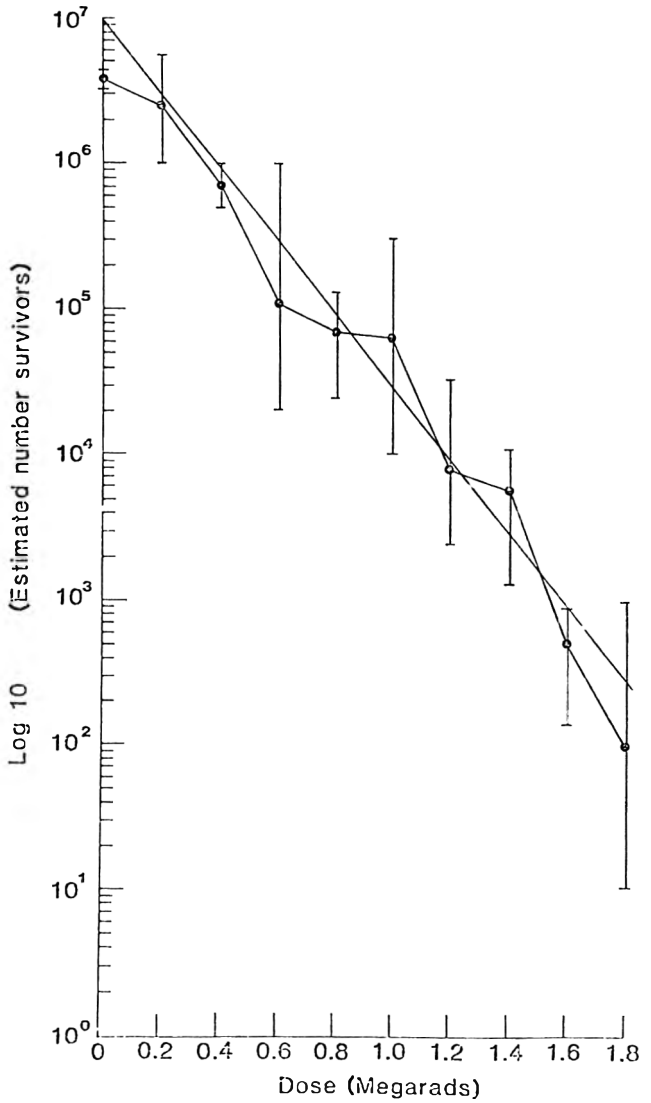

Fig. 2—Pork irradiated at a vacuum level of 250 torr and −30°C ± 10°C (RK0244A).

Table 1—Log survivor data for pork irradiated at 250 torr and −30°C ± 10°C, experiment RK0248A

X Value	No. of replicates	Y Value	Std Dev	Upper CI	Lower CI
0	3	6.38	0.009	6.42	6.34
0.2	3	6.27	0.065	6.55	5.99
0.4	3	5.72	0.050	5.94	5.50
0.6	3	5.10	0.203	5.95	4.20
0.8	3	4.70	0.099	5.12	4.28
1.0	6	4.64	0.259	5.31	3.98
1.2	7	3.80	0.218	4.38	3.27
1.4	7	3.58	0.177	4.02	3.15
1.6	7	2.51	0.144	2.86	2.16
1.8	7	1.99	0.394	2.95	1.03

Table 3—Log survivor data for pork irradiated at 250 torr and −30°C ± 10°C, experiment RK0244A

X Value	No. of replicates	Y Value	Std Dev	Upper CI	Lower CI
0	3	6.23	0.086	6.60	5.86
0.2	3	5.91	0.003	5.92	5.90
0.4	3	5.79	0.029	5.91	5.66
0.6	3	5.41	0.142	6.02	4.80
0.8	3	4.81	0.268	5.96	3.65
1.0	7	4.54	0.294	5.26	3.83
1.2	7	4.27	0.148	4.63	3.91
1.4	7	3.29	0.238	3.87	2.71
1.6	7	2.53	0.352	3.39	1.67
1.8	7	2.05	0.433	3.11	0.99

RESULTS

TYPICAL EXPERIMENTAL RESULTS for a duplicate set of D-value experiments using irradiated substrate samples are presented in Fig. 1 and 2. In both cases, samples of pork were irradiated at −30 ± 10°C, using a vacuum of 250 torr. The survivor curves include point estimates for each dose increment, as well as 95% CI for each dose administered. Each data point represents an average of four replicate test units exposed to the same dose. In addition to each curve, a statistical analysis of the experiment is also included to document error sources and produce a 95% CI for each D-value-point estimate obtained from survivor recovery procedures (Tables 1–4).

These figures show that a high degree of reproducibility is possible using this system, even though samples were processed by different personnel and the experiments conducted four days apart. The D-values in this case were identical, and calculated 95% confidence D-value intervals were close, indicating variance stability.

The results of an experimental series designed to validate this method for irradiation D-value estimation are presented in Table 5 and 6. This summary documents the test conditions and illustrates the effect of process parameter changes on the radiation resistivity of botulinum spores inoculated onto substrates.

The results of an experiment to test the effect of holding samples after irradiation and prior to recovery are shown in Table 6. The results are very close to those in Table 5.

The experimental designs and data output formats follow those described in Fig. 1 and 2 and Tables 1–4.

Table 2—Summary statistics for pork irradiated at 250 torr and −30°C ± 10°C, experiment RK0248A

D-value	= 0.38	95% Ci =	0.35 0.41
Slope	= 2.63	95% CI =	−2.83 −2.43
Intercept	= 6.92	95% CI =	6.67 7.17
X Mean	= 1.17		
Y Mean	= 3.83		

ANOVA DATA

Source	Degrees of freedom	Sum of squares	Mean squares	F	P
Mean	1	676.10	676.10	12646.33	0
Treatment	8	75.77	9.47	177.15	0
Regression	1	73.26	73.25	1370.25	0
Deviation	7	2.51	0.36	6.71	0
Error	37	1.98	0.05		
Total	46	753.64			

Intercept ratio = 1.08

Table 4—Summary statistics for pork irradiated at 250 torr and −30°C ± 10°C, experiment RK0248A

D-value	= 0.38	95% CI =	0.35 0.42
Slope	= −2.61	95% CI =	−2.84 −2.38
Intercept	= 6.94	95% CI =	6.64 7.23
X Mean	= 1.17		
Y Mean	= 3.88		

ANOVA DATA

Source	Degrees of freedom	Sum of squares	Mean squares	F	P
Mean	1	708.76	708.76	6851.38	0
Treatment	8	75.74	9.47	118.23	0
Regression	1	72.43	72.43	904.58	0
Deviation	7	3.30	0.47	5.89	0
Error	38	3.04	0.08		
Total	47	787.54			

Intercept ratio = 1.11

DISCUSSION

THE DATA SHOW two principal results. The test system possesses the attribute of reproducibility. Replicate experiments using identical process parameters produced equivalent D-value estimates at the 95% CI as shown in Table 5.

The second result relates to the precision of the test system in producing D-value estimates for irradiated botulinum spores under various simulated process conditions. The data tabulated for the validated experimental series (Table 5) show that the 95% CI for D-value estimates encompass a standard error of less than 0.025 Mrad. This

Table 5—Summary of D-value estimates for radiation kinetic death curves of C. botulinum spores inoculated onto meat substrates

	Substrate type								
	Chicken			Ham			Pork		
Torr	Experiment no.	D-value (Mrad)	95% CI Range	Experiment no.	D-value (Mrad)	95% CI Range	Experiment no.	D-value (Mrad)	95% CI Range
250	RK0158A	0.38	0.35–0.41	RK0232A	0.34	0.32–0.36	RK0244A	0.38	0.35–0.42
	RK0164A	0.38	0.36–0.41	RK0262A	0.32	0.30–0.36	RK0248A	0.38	0.36–0.41
125	RK0218A	0.38	0.35–0.41	RK0234B	0.39	0.36–0.42	RK0226B	0.38	0.34–0.42
50	RK0162A	0.41	0.37–0.43	RK0235A	0.37	0.35–0.40	RK0253A	0.38	0.36–0.41
	RK0168A	0.44	0.41–0.46	RK0267A	0.36	0.33–0.39	RK0253A	0.38	0.36–0.41

error margin is quite small for such a system, less than 10% of the D-value estimate. Using this criterion in conjunction with the ANOVA tables, the test system exhibits good precision. In addition, the regression line model gives a close approximation to the curve shapes produced, as shown by the correlation coefficient calculation, ρ, of 0.9 or higher.

The process calculations projected in Tables 5 and 6 also look reasonable in terms of related historical work (Table 7). Annellis et al. (1976) reported on an inoculated pack study that gave a 12-D process for ham ranging from 2.57–3.87 Mrad, depending upon the model used for statistical analysis. Results from this study are somewhat higher (3.98–4.32 Mrad). Depending upon the vacuum level used. This is reasonable because in this study recovery was after only 1 wk and it is possible there could have been some injured spore repair which would not be evident in an inoculated pack. If anything, this system would provide a more conservative D-value than an inoculated pack.

Table 6—D-value estimates for samples held 1 wk at 25°C before recovery after irradiation at −30°C at a vacuum level of 250 torr

Substrate type	D-value (Mrad)	95% CI Range
Chicken	0.38	0.35–0.42
Ham	0.34	0.31–0.37
Pork	0.36	0.32 – 0.40

Table 7—Radiation resistance of anaerobic bacteria

Organism	Spore type	D-value (Mrad)	Test substrate	Test temp. (°C)	Reference
Clostridium botulinum	A	0.224–0.344	M/15 PO_4 Buffer (pH 7)	–	Anellis and Koch (1962)
	B	0.120–0.349	M/15 PO_4 Buffer (pH 7)	–	Anellis and Koch (1962)
	A	0.188–0.221	Bacon	–	Anellis et al. (1964)
	A	0.212–0.338	–	–	Anellis and Rowley (1968)
	B	0.256–0.334	–	–	Anellis and Rowley (1968)
	A&B	0.070–0.331	Codfish Cake	–	Anellis et al. (1972b)
	A&B	0.098–0.262	Corned Beef	–	Anellis et al. (1972b)
	A&B	0.070–0.184	Pork Sausage	–	Anellis et al. (1972b)
	A&B mixture	0.25 –0.36	Beef	−30±10	Anellis et al. (1975)
	A&B	0.18 –0.38	M/15 PO_4 Buffer (pH 7)	–	Denny et al. (1959)
	B	0.325–0.369	Chicken	–	Grecz et al. (1965)
	B	0.160–0.207	Bacon	–	Grecz et al. (1965)
	A	0.121–0.237	Beef	95°	Grecz (1966)
	A	0.32 –0.387	Beef	65°	Grecz (1966)
	A	0.316–0.363	Beef	24°	Grecz (1966)
	A	0.34 –0.41	Beef	0°	Grecz (1966)
	A	0.590–0.712	Beef	−196°	Grecz (1966)
	A	0.34	Buffer	0°	Grecz (1966)
	A&B	0.197–0.226	Chopped Ham	–	Greenberg et al. (1965)
	A	0.12 –0.14	Water	–	Roberts and Ingram (1965)
	B	0.11	Water	–	Roberts and Ingram (1965)
	D	0.22	Water	–	Roberts and Ingram (1965)
	F	0.25	Water	–	Roberts and Ingram (1965)
	A&B mixture	0.30 –0.35	Chicken Parts	27°	Schmidt and Nank (1960)
	A&B mixture	0.30 –0.34	Beef Steak	27°	Schmidt and Nank (1960)
	A&B mixture	0.27 –0.31	Pork Loins	27°	Schmidt and Nank (1960)
	A&B mixture	0.17 –0.19	Green Beans	27°	Schmidt and Nank (1960)
	A&B mixture	0.31 –0.35	Corn in Brine	27°	Schmidt and Nank (1960)
	A	0.279	Beef Stew	22°	Schmidt et al. (1960)
	B	0.238	Beef Stew	22°	Schmidt et al. (1960)
	A	0.277–0.416	Pork Loin	–	Anellis et al. (1969)
	B	0.122–0.418	Pork Loin	–	Anellis et al. (1969)
	A	0.40	Beef	5°	Kempe et al. (1954)
	B	0.34 –0.40	Beef	5°	Kempe and Graikoski (1962)
	A&B mixture	0.40 –0.68	Beef	−29°	Wheaton et al. (1961)
	E	0.129–0.134	M/15 PO_4 Buffer (pH 7)	–	Grecz et al. (1965)
	E	0.08 –0.16	Water	–	Roberts and Ingram (1965)
	E	0.122–0.144	Beef Stew	–	Schmidt et al. (1960)
	Vegetative Cells	0.25	–	–	Grecz (1966)
Clostridium perfringens	–	0.132–0.345	M/15 PO_4 Buffer (pH 7)	−30°	Clifford and Anellis (1975)
	–	0.30	Cooked Meat Broth	–	Midura et al. (1965)
	–	0.23 –0.25	M/15 PO_4 Buffer (pH 7)	–	Midura et al. (1965)
	–	0.12 –0.20	Water	–	Roberts (1968)
	–	0.12 –0.34	Water	21–23°	Roberts (1968)
Clostridium sporogenes	3679	0.202–0.215	Water	–	Anellis and Koch (1962)
	3679	0.22	Water	–	Roberts and Ingram (1965)
	–	0.16	Water	–	Roberts and Ingram (1965)
Clostridium tetani	–	0.24	Water	–	Roberts and Ingram (1965)
Clostridium putrefaciens	–	0.18	–	28–30°	Roberts and Derrick (1975)

Estimates for 12-D pork processes (Rowley et al., 1974) ranged from 3.9–4.8 Mrads, whereas data from this study range from 3.8–4.8. While some experimental results, shown in Table 7, are difficult to compare, as early work did not document the process conditions such as vacuum and temperature, the data do show the sensitivity of D-values to process conditions such as temperature and substrate, and the need to precisely determine D-values each time process conditions are changed.

CONCLUSIONS

THIS STUDY has shown that the miniaturized system using cryotubes to test radiation resistivity of *Clostridium botulinum* spores under simulated process conditions, is precise. The advantages of small size, capability for multiple replications at each dose, simple recovery methods and rapid generation of full survivor curve profiles demonstrate significant improvements compared to historical inoculated pack D-value methodologies. The system also demonstrates the ability to detect the effect of small process parameter changes on the radiation resitivity of botulinum spores inoculated into food samples.

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Effect of Processing Variables on the Microbial, Physical and Sensory Characteristics of Pork Sausage

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ABSTRACT

A randomized block design with a 4 x 3 x 5 factorial arrangement of treatments involving four fat levels, three grinding systems and five storage periods was utilized to determine the effects of processing variables on microbial, physical and sensory characteristics of pork sausage. In general, microbial numbers decreased as the level of fat increased and increased with reduced particle size and time in storage. The surface color became lighter and less red with increasing levels of fat and time in storage. Higher cook yields (%) were associated with higher degrees of maceration while lower yields were observed for sausage manufactured to contain high levels of fat (40 and 45%). Length of storage had the greatest effect on the desirable sensory characteristics of the product. An optimum processing system was postulated from the results of the study.

INTRODUCTION

PORK SAUSAGE is a very popular meat item in American homes and restaurants. Throughout the years, pork sausage has been one of the leading pork items to be served by the institutional food industry. The overall quality of this product is affected by method of processing, length of storage and various formulated ingredients. Acton (1972) reported decreased cook losses and increased binding strength with higher levels of tissue maceration. Reagan et al. (1981) observed that length of time in storage was related to cooking yields. Longer periods of storage resulted in higher yields and moisture content. The effects of various processing treatments on the functional properties of pork products have been reported by Theno et al. (1976) and Siegel et al. (1976). The purpose of this study was to determine the effects of different processing variables utilized in the production of pork sausage on the microbial, textural and sensory characteristics of the final product. An optimal processing system is proposed, based upon the results.

MATERIALS & METHODS

A RANDOMIZED BLOCK DESIGN with a 4 x 3 x 5 factorial arrangement of treatments involving four fat levels, three grinding treatments and five storage periods was utilized. Fresh, unfrozen sausage raw materials were obtained from two, average quality market hogs each weighing approximately 130 kg. The carcasses were deboned approximately 24 hr postmortem and the meat was coarsely ground through a 13 mm plate using a Hobart grinder (Model #4046) and mixed thoroughly (3 min) prior to sampling for fat determinations using the modified Babcock procedure. The coarsely ground lean meat was divided into four batches of approximately 24 kg each. Fresh pork fatback, coarsely ground, was added in order to obtain the desired fat levels (30, 35, 40 and 45%). Each batch was mixed for approximately 2 min with the appropriate amounts of fresh pork sausage seasoning (Formula 64T, A. C. Legg Packing Co., Inc., salt = 1.6%) in a Butcher Boy Meat mixer to distribute the added fat and seasoning. The commercial seasoning used contained no antioxidants. After mixing, each batch was sub-divided into three additional batches (8 kg each) and randomly assigned to one of three different grinding treatments (G_1 = 13 mm plate + 6.5 mm plate; G_2 = 13 mm plate + 3.3 mm plate; G_3 = 13 mm plate + 6.5 mm plate + 3.3 mm plate). After grinding, each subbatch was stuffed into 0.5 kg commercial opaque sausage bags (E. G. Luber Engineering & Sales Co.), tied with string and placed in a 0°C cooler for storage.

The five storage periods used in the study were 0, 7, 14, 21 and 28 days. At the end of each storage period, samples from each subbatch were obtained for external and internal color determinations, microbial numbers, TBA analysis, shear test, texture measurements, cook loss tests and sensory panel evaluations. Color and microbiological numbers were determined immediately after sampling. The remaining samples were placed in a −18°C freezer for approximately two months prior to completing the remaining analyses.

Chemical analyses

Moisture, fat and protein were determined on the day of manufacturing (day 0) according to AOAC (1970).

Microbiological assays

Microbial samples representative of each grinding treatment and fat level were obtained at the end of each storage period. External surfaces of the sample packages were disinfected by rinsing with a 70% ethanol solution prior to removing the packaging film and both end sections of each sample. The remaining portion of the sample was mixed thoroughly by hand using a sterile polyethylene glove. After mixing, an 11-g sample was placed in a sterile blender jar containing 99 ml of 0.85% NaCl solution and blended at high speed for 30 sec. Appropriate dilutions were transferred to plates of standard plate count agar and incubated at 30°C for 48 hr. Total plate counts (TPC) were reported as $\log_{10}$ organisms/gram of sample.

TBA tests

TBA values were determined by a modification of the Distillation method of Tarladgis et al. (1960) in which a 10-g meat sample was homogenized with 50 ml of distilled water in a Virtis Homogenizer (model 23) for 2 min. The homogenate was then transferred to a Kjeldahl flask to which 47.5 ml of distilled water and 2.5 ml of 3.99N HCl (final pH = 1.5) were added. The mixture was boiled until about 50 ml of distillate was collected. Five ml of distillate were added to 5 ml of 0.02M 2-thiobarbituric acid (TBA) aqueous solution, heated in a boiling water bath for 38 min, and then cooled in tap water for 10 min. The percentage transmittance of the pink solution was determined by a Bausch & Lomb Spectronic 20 at 538 nm, then converted into absorbance [OD = 2 − log (% transmittance)]. The OD was converted to mg of malonaldehyde per 1000 grams of sample by multiplying by a factor of 7.8. All analyses were performed in triplicate.

Color measurements

Color measurements were determined for the external and internal surfaces of the samples using a Macbeth's colorimeter (Model MC-1010S). These data were reported in the 1976 L*a*b* system, in which L* (lightness), a* (redness) and a*/b* (red-yellow ratio) were used to correlate color changes to other properties of the pork sausage. For external color measurement, a 5 x 10 cm^2 section of the packaging film was removed from the surface of the sample. A cross-section of sample was used for internal color measurement. The meat samples were covered with polyethylene film during all of the color measurements.

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Cooking methods and cooking yields

The samples were removed from a −18°C freezer and sliced into patties, 13 mm thick, placed into polyethylene bags and stored in a −18°C freezer for a period of 30 days during which time cook loss and sensory evaluation tests were performed. The frozen patties were cooked in preheated (150°C) electric fry pans (Sunbeam, Model 7-30, with 1550W, 120V) for 7 min on one side, then turned and cooked for an additional 5 min. After cooking, the patties were blotted with tissue paper to absorb the excessive juice.

Percentage cook yield was calculated using the following equation:

$$\% \text{ Cook Yield} = \frac{\text{Final wt of sample}}{\text{Initial wt of sample}} \times 100$$

Texture measurements

An Instron Universal Testing Machine (Model 1130) was used to measure the required penetration and compression stress of the cooked patties. After cooking, the samples were held at room temperature (approximately 25°C) for 1 hr prior to performing either the penetration or the compression test. Stress values were calcuated and reported as Newton/cm^2.

Penetration tests. A cylindrical die (0.8 cm in diameter) and a cross-head load cell (45.4 kg) were used to determine the force required to penetrate the cooked patties. The cross-head and chart speed were 25.4 cm/min and 50.4 cm/min, respectively. Values were calculated by dividing the required force by the area of the die. The reported values are means of three determinations/patty and three replications for each sample.

Compression tests. A stainless steel compression cage with a plate 6.4 cm in diameter was used for compression tests. The cross-head and chart speed were 5.08 cm/min and 50.8 cm/min, respectively. Samples were cut into cores 2.14 cm in diameter and 1.3 cm high. The force required to compress the sample (the force at the bioyield point on the force-deformation curve) was recorded and calculated into the compression stress. Three cores were evaluated for each patty and three replications were performed for each sample.

Sensory evaluation

A sensory panel, composed of 30 untrained panelists from the faculty, staff and students of the Food Science Department was utilized. Sensory panel samples were prepared according to the cooking procedures previously described. Samples for each sensory session were selected at random. After cooking, the samples were cut into quarters and each panelist was served four samples per session with water and unsalted crackers. Panelists were asked to evaluate each patty using an eight point descriptive scale for amount of greasiness (8 = none; 1 = abundant); cohesiveness (8 = extremely cohesive; 1 = extremely uncohesive); juiciness (8 = extremely juicy; 1 = extremely dry); particle size (8 = extremely fine; 1 = extremely coarse); firmness (8 = extremely firm; 1 = extremely soft); flavor acceptance (8 = extremely acceptable; 1 = extremely unacceptable) and overall satisfaction (8 = extremely acceptable; 1 = extremely unacceptable).

Statistical analyses

Analysis of variance was performed according to the Statistical Analysis System of Barr and Goodnight (1976). Duncan's multiple range test (Duncan, 1955) was used to indicate significant differences between particular variables. The ANOVA was run as a randomized block design with a factorial arrangement of the treatments (fat level, grind size, storage time). Interactions between main effects were found to be nonsignificant and were therefore deleted from the model.

RESULTS & DISCUSSION

Chemical analyses

Overall mean values for the chemical composition of the four batches of pork sausage (Table 1) indicate that the desired levels of fat were achieved as outlined in the experimental design.

Microbial growth

Microbial growth was significantly affected by fat level, grinding system and length of storage (Table 2). Microbial numbers decreased as percent fat increased. The effect of fat concentration on microbial numbers may be due to the inability of bacteria to utilize lipids as a source of nutrients. It has also been shown that the free fatty acid content of meat is increased by hydrolysis of fat (Acton, 1979). Free fatty acids, glycerol esters and certain glycerides have been found to exhibit antimicrobial properties (Conley and Kabara, 1973). These findings suggest the need for additional studies to determine the relationship of fat level to the presence and action of lipid-type inhibitors in pork sausage.

Significant statistical differences in microbial growth were associated with the different grinding systems; however, these differences may not actually be very meaningful as the numerical differences were less than one log. Reduced particle size of the product was associated with greater numbers of microorganisms (Table 2). The higher microbial counts may be due to a greater surface area and/or the release of nutrients during the grinding process. The incorporation of oxygen, temperature rise due to grinding and the homogeneous distribution of microorganisms during grinding also promote microbial growth. Microbial numbers increased significantly with time in storage.

—Continued on next page

Table 1—Mean values for percentage fat, moisture and protein of pork sausage stratified by levels of formulated fat

Formulated fat level (%)	Fat (%)	Moisture (%)	Protein (%)
30	30.3	51.8	15.0
35	34.8	48.3	14.2
40	40.0	44.0	13.2
45	44.8	40.4	12.1

Table 2—Overall mean values for microbial numbers and TBA values of pork sausage manufactured with varying fat levels and grinding systems and stored for various periods of time

Treatment variables	Microbial Numbers (TPC/gram)[a]	TBA Values (mg malonaldehyde per kg)
Fat level (%)		
30	7.5237[c]	0.19[c]
35	7.3304[d]	0.19[c]
40	7.1173[e]	0.18[c]
45	6.8573[f]	0.22[d]
Grinding systems[b]		
G_1	7.1239[c]	0.19[c]
G_2	7.2227[d]	0.19[c]
G_3	7.4183[e]	0.19[c]
Storage time (days)		
0	4.2304[c]	0.13[c]
7	4.5682[d]	0.13[c]
14	5.5911[d]	0.19[d]
21	6.4624[e]	0.23[e]
28	7.9542[f]	0.29[f]

[a] Total plate counts (TPC) reported as $\log_{10}$ numbers/g.
[b] Grinding systems used for manufacturing: G_1 = 13 mm plate + 6.5 mm plate; G_2 = 13 mm plate + 3.3 mm plate; G_3 = 13 mm plate + 6.5 mm plate + 3.3 mm plate.
[c,d,e,f] Mean values in the same column within treatment variables bearing unlike superscripts differ significantly ($P < 0.05$).

Table 3—Mean values for color measurements of external and internal surfaces of pork sausage manufactured with varying fat levels and grinding systems and stored for various periods of time

	Color Measurements[a]					
	External			Internal		
Treatment variables	L*	a*	a*/b*	L*	a*	a*/b*
Fat level (%)						
30	59.8[c]	8.0[c]	49.0[c]	61.0[c]	11.8[c]	76.6[c]
35	62.3[d]	7.2[d]	43.8[d]	60.3[c]	11.4[c]	72.1[d]
40	64.7[e]	6.3[e]	37.9[e]	64.5[d]	10.5[d]	64.7[e]
45	65.5[f]	6.3[e]	38.2[e]	65.7[d]	9.7[e]	61.8[f]
Grinding systems[b]						
G_1	63.0[c]	6.9[c]	42.3[c]	61.4[c]	11.4[c]	69.8[c]
G_2	62.9[c]	7.0[c]	43.3[d]	63.3[c]	10.8[d]	67.8[c]
G_3	63.5[d]	7.0[c]	41.0[e]	63.9[c]	10.3[e]	69.0[c]
Storage period (day)						
0	60.7[c]	11.0[c]	66.8[c]	61.4[c]	12.0[c]	79.6[c]
7	62.2[d]	8.6[d]	51.2[d]	62.1[c]	11.2[d]	71.9[d]
14	63.2[e]	6.9[e]	42.2[e]	63.1[c]	10.7[e]	69.0[d]
21	63.9[f]	4.4[f]	27.7[f]	63.8[c]	10.2[f]	63.2[e]
28	65.6[g]	3.8[g]	24.2[g]	64.1[c]	9.6[g]	60.2[e]

[a] L* (lightness), a* (redness), a*/b* (% of red-yellow ratio) are based on the 1976 CIE-L*a*b* color system.
[b] Grinding systems used for manufacturing: G_1 = 13 mm plate + 6.5 mm plate; G_2 = 13 mm plate + 3.3 mm plate; G_3 = 13 mm plate + 6.5 mm plate + 3.3 mm plate.
[c,d,e,f,g] Mean values in the same column within treatment variables bearing unlike superscripts differ significantly ($P < 0.05$).

Table 4—Mean values for percentage cook yield compression and penetration stress values of cooked pork sausage manufactured with varying fat levels and grinding systems and stored for various periods of time

Treatment variables	Cooking yield (%)	Compression stress (Newton/cm^2)	Penetration stress (Newton/cm^2)
Fat level (%)			
30	68.1[b]	35.7[b]	13.5[b]
35	68.1[b]	36.3[b]	12.9[c]
40	64.2[c]	35.2[c]	12.5[d]
45	60.5[d]	34.2[d]	12.2[d]
Grinding systems[a]			
G_1	64.4[b]	35.6[b]	13.1[b]
G_2	65.3[c]	35.4[b]	12.7[bc]
G_3	66.0[d]	35.4[b]	12.5[c]
Storage time (day)			
0	64.5[b]	39.7[b]	16.5[b]
7	65.8[c]	39.0[c]	13.7[c]
14	64.3[b]	35.8[d]	12.3[d]
21	63.9[b]	33.7[e]	11.2[e]
28	67.5[d]	28.7[f]	10.2[f]

[a] Grinding systems used for manufacturing: G_1 = 13 mm plate + 6.5 mm plate; G_2 = 13 mm plate + 3.3 mm plate; G_3 = 13 mm plate + 6.5 mm plate + 3.3 mm plate.
[b,c,d,e,f] Mean values in the same column within treatment variables bearing unlike superscripts differ significantly ($P < 0.05$).

TBA test

TBA values were significantly affected by fat level and storage time (Table 2). Fresh pork sausage prepared with a 45% level of fat exhibited the highest TBA values. The TBA values increased significantly with each seven day increment of storage after day seven of storage.

Color measurements

The effects of fat level, grinding system and length of storage on the color characteristics of both the external and internal surfaces of fresh pork sausage are shown in Table 3. Values for lightness (L*) of the external surface increased significantly as percentage fat increased, but values for redness (a*) and the red-yellow ratio (a*/b*) decreased ($P < 0.05$). No significant differences in these values were noted beyond the 40% level of fat. L* values for the internal surface increased ($P < 0.05$) as the fat levels increased from 35% to 40 and 45%. Values for a* significantly decreased as the fat levels increased beyond the 35% level of fat. The values for a*/b* significantly decreased as fat levels increased Since the samples with lower fat levels had a higher content of lean meat and a higher concentration of myoglobin, higher values for a* and a*/b* and a lower value of L* would be expected.

Grinding did not significantly affect values for a* (redness) of the external surface or L* (lightness) and a*/b* (% of red-yellow ratio) of the internal surfaces. However, external values for lightness (L*) significantly increased, and internal values for redness (a*) decreased significantly as the particle size of the sausage was reduced. These differences may be the result of increased oxidation of oxymyoglobin and myoglobin due to the increased surface area of the smaller particles.

Length of storage was significantly related to values for L*, a* and a*/b* on the external surface of the packaged product. Values for redness (a*) and red-yellow ratio (a*/b*) decreased while L* values increased with time in storage, indicating that myoglobin or oxymyoglobin pigments were rapidly oxidized to the metmyoglobin state. Redness (a*) values for the internal surface area also decreased with increased time in storage; however, due to lesser amounts of available oxygen, the rate of change was slower than the rate observed for the external surfaces.

Cook yields and textural measurements

The effects of percentage fat, grinding system and storage time on the cook yields (%), compression and penetration stress are reported in Table 4. Pork sausage formulated to contain either 30 or 35% fat exhibited the highest values for cook yield. Cook yields increased as the particle size decreased ($P < 0.05$). The grinding treatment G_1 (13 mm plate + 6.5 mm plate, coarse grind) exhibited the lowest yields, while grinding treatment G_3 (13 mm plate + 6.5 mm plate + 3.3 mm plate, find grind) had the highest yields. This observation may have been due to an increased availability of myofibrillar protein in samples with a smaller particle size. The myofibrillar protein functions in binding

Table 5—Mean values for various sensory traits of cooked, pork sausage manufactured with varying fat levels and grinding systems and stored for various periods of time

Treatment Variables	Cohesion	Juiciness	Greasiness	Firmness	Particle size	Flavor acceptance	Overall satisfaction
Fat level (%)							
30	5.8[c]	5.1[c]	5.0[c]	5.7[c]	4.7[c]	6.0[c]	5.9[c]
35	5.7[d]	5.2[c]	5.0[c]	5.6[cd]	4.6[c]	6.0[c]	6.0[c]
40	5.5[d]	5.5[d]	5.0[c]	5.3[d]	4.8[c]	6.1[c]	6.0[c]
45	5.4[d]	5.4[d]	5.0[c]	5.1[d]	4.8[d]	6.1[c]	6.0[c]
Grinding systems[b]							
G_1	5.5[c]	5.4[c]	5.0[c]	5.3[c]	4.7[c]	6.0[c]	6.0[c]
G_2	5.8[d]	5.3[c]	5.1[c]	5.6[c]	4.8[d]	6.0[c]	6.0[c]
G_3	5.6[cd]	5.3[c]	5.0[c]	5.3[c]	5.0[e]	6.1[c]	6.0[c]
Storage time (day)							
0	5.6[c]	5.3[c]	5.2[c]	5.1[c]	4.7[c]	6.5[c]	6.5[c]
7	5.8[d]	5.5[c]	5.2[c]	5.6[c]	4.6[c]	6.6[c]	6.5[c]
14	5.8[d]	5.3[c]	5.0[c]	5.6[c]	4.8[cd]	6.4[c]	6.3[d]
21	5.4[c]	5.3[c]	4.9[c]	5.5[c]	4.9[d]	5.8[d]	5.9[e]
28	5.4[c]	5.0[d]	4.7[c]	5.3[c]	4.6[c]	4.9[e]	4.8[f]

[a] Means based on 8 point rating scales where: 8 = extremely cohesive, juicy, not greasy, firm, fine or acceptable; 1= extremely uncohesive, dry, greasy, soft, coarse or unacceptable.
[b] Grinding systems used for manufacturing: G_1 = 13 mm plate + 6.5 mm plate; G_2 = 13 mm plate + 3.3 mm plate; G_3 = 13 mm plate + 6.5 mm plate + 3.3 mm plate.
[c,d,e,f,g]Mean values in the same column within treatment variables bearing unlike superscripts differ significantly ($P < 0.05$).

water and fat thereby reducing the cooking losses (Acton, 1972; Chesney et al., 1978; Maesso et al., 1970; Popenhagen and Mandigo, 1978).

In general, samples prepared with the higher levels of fat (40 and 45%) had lower compression and penetration values. No significant differences in compression stress values were noted for the two lower fat levels (30 and 35%); however, as percentage fat was increased above the 35% level, a significant decrease in the compression stress values were observed indicating some loss in the binding properties of the product. This loss in functional properties was also indicated by the decreased penetration stress values.

Penetration stress values were significantly decreased as reduction of the particle size increased. Reagan et al. (1975) reported significant correlations between degree of muscle fragmentation and Warner-Bratzler shear values.

Length of time in storage significantly influenced both the penetration and the compression stress values of the cooked, pork sausage. As time in storage increased, penetration and the compression stress values decreased.

Sensory studies

All of the observed sensory traits, with the exception of greasiness, were affected ($P < 0.05$) by one or more of the treatment variables (Table 5). Samples prepared using the G_2 (13 mm plate + 3.3 mm plate) grinding system exhibited higher ($P < 0.05$) cohesive ratings than those prepared with the coarse (G_1) grinding system. Juiciness ratings were highest for those samples formulated to the 40 and 45% level of fat while these ratings were observed to decrease ($P < 0.05$) after 21 days of storage. Cross et al. (1980) reported that higher fat levels were significantly related to increased juiciness ratings in ground beef.

Pork sausage manufactured to contain 40 or 45% levels of fat were rated less firm than samples containing 30% fat ($P < 0.05$). It is interesting to note that sensory ratings for firmness were not affected by either method grind or length of time in storage. Ratings for particle size were influenced ($P < 0.05$) by fat levels and grinding systems; however, these differences were not reflected in the ratings for flavor acceptance or overall satisfaction. In general, ratings for flavor acceptance and overall satisfaction were highly acceptable up to 21 days. Ratings for both these traits were marginal in acceptance after 28 days of storage which suggests that length of storage may be a greater limiting factor in the production of fresh pork sausage than level of fat or method of grinding.

CONCLUSIONS

VALUES for microbial numbers, color characteristics, textural measurements, cook yields (%) and sensory traits (with the exception of greasiness) were significantly affected by the treatment variables fat level, method of grind and length of storage. These results suggest that a highly acceptable fresh pork sausage could be manufactured by formulating the product to a 35% fat level, then finely grind (13 mm plate + 6.5 mm plate + 3.3 mm plate) and by limiting storage to 21 days or less. This optimal system would minimize microbial numbers while maintaining a desirable product color and maximizing high cooking yields and sensory characteristics.

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—Continued on page 162

Processing Properties of Pork as Affected by Electrical Stimulation, Post-Slaughter Chilling and Muscle Group

R. L. SWASDEE, R. N. TERRELL, T. R. DUTSON, D. D. CRENWELGE, and G. C. SMITH

ABSTRACT

Left sides of 30 slaughtered pigs were electrically stimulated (ES); right sides were not stimulated (NES). Sides were placed in a conventional cooler (24 hr) or blast freezer (3 hr) or showered with a brine solution (15.8% salt, −5.6°C for 3 hr). Twenty-four hr postmortem, picnic shoulders were removed, dissected into four muscle groups and determinations made for certain processing properties. ES decreased ($P < 0.05$) pH values for predominantly white muscles, increased ($P < 0.05$) juice loss during cooking for shank muscles and decreased ($P < 0.05$) percentages of salt-soluble protein (SSP) for both predominantly red and shank muscles. Other muscle properties were not affected by ES. Rapid initial chilling did not affect ($P > 0.05$) processing properties of muscle groups.

INTRODUCTION

ALTHOUGH electrical stimulation (ES) is known to improve palatability and other properties of beef (Carse, 1973; Savell et al., 1978; Smith et al., 1980), effects of ES on pork have not been extensively reported. Crenwelge et al. (1979) reported that ES sides of pork produced softer muscles with more muscle separation than controls (not electrically stimulated, NES). Rapid chilling generally improved muscle color and firmness when compared to that of conventional chilling, and sensory properties of loin chops and hams were not affected by ES. Johnson et al. (1982) reported that ES of pork carcasses had minimal effects on weight loss of wholesale cuts and on palatability of loin chops. In their study effects of ES on muscle firmness were dependent upon pre-slaughter fasting periods and whether or not pigs were normal or stress-susceptible.

Processing properties, such as pH, salt-soluble protein percentage (SSP) and water-holding capacity (WHC) of ES beef clod muscles, have been characterized by Terrell et al. (1981; 1982a, b). In those studies it was reported that ES may increase percentages of SSP but, when ES beef was used to make frankfurters, effect of ES vs NES on properties of the cooked product were minimal and very inconsistent. Inconsistency in processing properties of meat from ES carcasses was also reported by Whiting et al. (1981) who used ES lamb to make frankfurters.

Seventy percent of the pork carcass may be used for processed product (cured and/or smoked primal cuts and sausages). Therefore, effects of ES on processing properties of porcine muscle should be of interest to the industry. Objectives of the present study were to determine effects of ES, post-slaughter chilling rate and muscle group on processing and other properties of porcine muscles.

MATERIALS & METHODS

THIRTY PIGS (99.8–108.9 kg live wt) were slaughtered, five each on six different days. Left sides were ES, 35 min postmortem; right sides were NES and served as controls. Stimulation was accomplished with a Koch-Britton 350 Stimulator. Twenty impulses (2.0 sec on, 1.0 sec off, 500 volts, 60 Hz, 2.5 amps) were delivered during a 1 min. period. As shown in Table 1, these sides were assigned (randomized incomplete block design) to the following chilling treatments: (I) conventional cooler (0–2°C), 24 hr; (II) air blast freezer (−34°C), 3 hr; and (III) refrigerated (−5.6°C) brine shower (15.8% salt), 3 hr. For treatments II and III, 3 hr of rapid initial chilling (blast freezer or brine shower) was used and then those sides were placed in the conventional cooler used to store carcasses in treatment I. Twenty-four hr postmortem (internal ham temperatures were about 3°C for all three treatments), picnic shoulders were removed and dissected to create four groups of muscles, shank (flexor and extensor groups) and three groups based on visual color, as follows: Predominantly white-caput mediale, caput lateral and tricipitus caput longum; intermediate-supraspinatus and teres major; predominantly red - infraspinatus and serratus ventralis. Muscle groups were placed in polyethylene bags, sealed and frozen (−34°C). All four muscle groups from the conventionally chilled sides (ES and NES) were used to determine effects of ES, on processing properties. Two muscle groups (predominantly white and shank) were used to determine effects of method of chilling on processing properties.

Muscle groups were thawed (0–2°C overnight), ground twice (plate with 4.8 mm holes), mixed (thaw fluids reincorporated), and stored (1–3 da at 0–2°C) for subsequent analysis. The following procedures were used to determine processing properties: pH (Acton et al., 1972); expressible juice loss [Terrell et al. (1981) modification of the Grau and Hamm (1953) procedure]; juice loss during cooking [Terrell et al. (1981) modification of the Wierbicki et al. (1957) procedure]; proximate composition (AOAC, 1975); and salt-soluble protein (Saffle and Galbreath, 1964). Approximately 4 months were required from the time muscles were initially frozen until completion of analyses.

Data were analyzed by analysis of variance (Steel and Torrie, 1960) and means were separated by use of a multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

ANALYSIS OF VARIANCE for main effects (ES; muscle group) is shown in Table 2. ES affected ($P < 0.05$) pH, juice loss during cooking, moisture and fat The interaction of these two main effects did not ($P > 0.05$) affect any of the processing properties. Within muscle groups ES decreased ($P < 0.05$) pH values for predominantly white muscles, increased ($P < 0.05$) juice loss during cooking for shank muscles and decreased ($P < 0.05$) percentages of SSP for

Table 1 – Experimental design for sides of pork according to chilling and electrical stimulation treatments

No. of animals	Chilling treatment (number of sides)[a]					
	Conventional (0–2°C, 24 hr)		Blast (−34°C, 3 hr)		Erine (−5.6°C, 3hr)	
	ES	NES	ES	NES	ES	NES
5	5			5		
5		5	5			
5	5					5
5		5			5	
5			5			5
5				5	5	

[a] Five pigs slaughtered on each of six days.

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Table 2 – Means for processing properties of porcine muscle groups[a] according to electrical stimulation treatment (ES or NES)

Property	Predominantly white			Intermediate			Predominantly red			Shank		
	ES	NES	Overall[b] $\bar{X}$	ES	NES	Overall[b] $\bar{X}$	ES	NES	Overall[b] $\bar{X}$	ES	NES	Overall[b] $\bar{X}$
pH	5.49	5.60	5.54[c]	5.63	5.68	5.65[d]	5.62	5.67	5.64[d]	5.78	5.87	5.82[e]
Expressible juice loss (%)	42.42	42.76	42.59[c]	42.39	42.13	42.26[c]	40.98	39.98	40.46[c]	44.14	41.86	42.94[c]
Juice loss during cooking (%)	38.11	40.23	39.17[c]	38.86	36.13	37.57[c]	32.41	31.79	32.12[d]	28.56	23.10	25.97[e]
Moisture (%)	73.06	72.19	72.62[c]	73.25	73.12	73.19[c]	72.34	70.81	71.57[d]	72.87	72.71	72.79[c]
Fat (%)	6.34	7.03	6.69[c]	6.42	6.86	6.64[c]	7.82	9.42	8.62[d]	7.11	6.96	7.04[c]
Total protein (%)	17.97	17.33	17.65[c]	17.20	16.90	17.05[cd]	16.85	17.02	16.94[d]	17.45	17.74	17.75[c]
Salt-soluble protein(%)	24.58	26.67	25.62[c]	24.18	26.63	25.40[c]	23.58	27.33	25.46[c]	23.28	27.53	25.41[c]

[a] Both sides of each pork carcass were chilled conventionally (24 hr @ 0—2°C). Predominantly white = caput medialе, caput lateral tricipitis, caput longum; Intermediate = supraspinatus, teres major; Predominantly red = infraspinatus, serratus ventralis; Shank = flexor and extensor muscle groups. Means within a muscle group, underscored by a common line, are not different ($P > 0.05$).
[b] Means regardless of ES or NES.
[cde] Means within the same row, bearing a common superscript letter are not different ($P > 0.05$).

predominantly red and shank muscles. With the exception of moisture and fat (predominantly red group), no other properties were affected by ES.

Although pH and SSP values were lower for all muscle groups from sides that were ES these values were significantly different ($P < 0.05$) for only predominantly white (pH) and predominantly red and shank muscles (SSP). In pork, when the rate of postmortem glycolysis is rapidly increased (pH values below 6.0 at 1 hr postmortem) myofibrillar proteins are denatured and a pale, soft muscle condition results (Bendall and Wismer-Pedersen, 1962; Kastenschmidt et al., 1964; Sayre et al., 1964; Penney, 1969). In our study, differences in SSP for the predominantly red and shank muscles, may support observations by Crenwelge et al. (1979) that picnic shoulders from these same pigs (after ES) had softer muscles and a greater incidence of muscle separation than NES controls.

Overall means for muscle groups, regardless of ES, are also shown in Table 2. Shank muscles had the highest pH value and lowest percentage of juice loss during cooking ($P < 0.05$). There were no differences ($P > 0.05$) among the four muscle groups for expressible juice loss or SSP. Although total protein contents were lower ($P < 0.05$) for the predominantly red muscle group as compared to the shank and predominantly white groups, differences in SSP percentages between these same groups were not apparent. Bouton et al. (1980) and Houlier et al. (1980) reported that properties of individual beef muscles may vary in their response to ES and Forrest and Briskey (1967) reported that certain porcine muscle properties also varied in their response to ES. Data from Table 2 suggest that regardless of ES, properties of predominantly red groups of porcine muscles differ more than properties of predominantly white and intermediate groups.

Rapid initial chilling of pork carcasses by use of blast freezers or liquid nitrogen has been done commercially (Ellis, 1980) and was detrimental to properties of bacon. In that study (Ellis, 1980), the integrity of fat and connective tissue was altered by rapid chilling to such an extent that bacon sustained extreme shattering during slicing. In our study, there were no differences ($P > 0.05$) in processing properties due to rates of chilling but two muscle groups used in this treatment (predominantly white and shank) had slightly more expressible juice loss and juice loss during cooking when rapidly chilled by blast freezing or brine showering than when chilled conventionally. Thus, rapid chilling may tend to decrease water holding capacity (WHC) of these two muscle groups.

ES does not appear to enhance visual properties of pork muscles as it does for beef and it may also decrease percentages of SSP. For these reasons we do not see any apparent advantages for using ES with pork but possible effects on WHC need further confirmation.

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–Continued on page 162

Effect of Nitrite and Salt on the Color, Flavor and Overall Acceptability of Ham

D. A. FROEHLICH, E. A. GULLETT, and W. R. USBORNE

ABSTRACT

Effect of nitrite and salt on color, flavor and overall accpetability of ham was examined. *Semitendinosus* pork muscles were cured with four levels of nitrite (0, 50, 100 and 150 ppm) and three levels of salt (0, 1 and 2%). Nitrite content decreased after processing and salt remained constant. The Semitendinosus muscle became darker, redder and more intensely colored as measured on the Hunterlab colorimeter. Also the increased redness was detected by panelists as nitrite increased. Cured meat flavor intensity increased with increasing salt and nitrite, with salt having the greater effect. Panelists' perceptions of saltiness and sweetness were most affected by level of salt. The sample containing 50 ppm nitrite was acceptable to untrained panel.

INTRODUCTION

Some serious concerns regarding the use and potential hazards of nitrite have developed (MacNeil and Mast, 1973; Rubin, 1977; Sebranek, 1979), resulting in recent research being directed towards reducing nitrite levels or searching for nitrite substitutes.

Before nitrite levels can be greatly lowered or eliminated in favor of alternatives, a better understanding of the role of nitrite in cured meat is needed. Nitrite's contribution to flavor has been reported in cured pork (MacDougall et al., 1975), ham (Barnett et al., 1965), smoked and nonsmoked frankfurters (Wasserman and Talley, 1972), cooked pork emulsions (Hadden et al., 1975), thuringer sausage (Dethmers et al., 1975) and corned beef briskets (Schults et al., 1977). In contrast, nitrite has been reported by other researchers to have little effect on cured meat flavor (Simon et al., 1973; Skjevlkvaale and Tjaberg, 1974; Wassermann et al., 1977). Other research has indicated that salt is the most important factor in cured meat flavor (Mottram and Rhodes, 1973; Green and Price, 1975; Kimoto et al., 1976). The development of the characteristic pink color of cured meat must also be considered in lowering nitrite levels or in looking for adequate nitrite replacements. The reaction of nitrite with the meat pigment to produce the typical cured meat color is well documented (MacDougall et al., 1975; Rubin, 1977; Cassens et al., 1979).

The purpose of this study was to gain a better understanding of the effect of nitrite and salt on color development, cured meat flavor development and overall acceptability of the muscle from the pork leg.

MATERIALS & METHODS

Ham preparation

Semitendinosus pork muscles obtained from a local packing house were cured with combinations of four levels of nitrite (0, 50, 100, and 150 ppm) and three levels of salt (0, 1 and 2%) for a total of 12 treatments. Muscles were pumped to 110% of initial weight with the assigned cure treatment, using a 16-hole, single-needle brine pump. Following injection, muscles were placed into rectangular cast-aluminum ham presses, along with a corresponding cover pickle and kept at 5.5°C for three days to allow for good cure distribution. For baking.the cover pickles were drained off each muscle and spring-loaded lids were placed on the ham presses. The muscles were baked in an electric oven at 163°C following a time schedule of 7.25 min per 100g pumped weight to achieve an internal temperature of 77 ± 2°C. Cooled cooked muscles were removed from the presses and wrapped tightly in Saran wrap and aluminum foil and kept at 5.5°C until evaluated; these will be referred to as hams for the remainder of the paper.

Chemical analysis

Nitrite and salt determinations were carried out following the methods outlined by AOAC (1975). Determinations were carried out in duplicate for each ham sample.

Instrumental color measurement

A Hunterlab D25A Color Difference Meter was used to obtain color measurements for each ham. The instrument was standardized with a white tile (L = 92.3, 'a' = −0.7, 'b' = −0.9) and a 2.5 cm specimen area. Readings were taken from four different areas on 2 mm thick hasm slices and mean results were expressed as L, 'a', 'b', hue angle ($\tan^{-1}$ b/a) (Little, 1975) and chroma $[a^2 + b^2)^{1/2}]$ values.

Sensory evaluation

Trained panel. Training of the eight member panel took place over an 8-wk period and involved panel discussions centered around the characterization of typical cured meat flavor, ballot construction and test sample evaluation. Preliminary sessions involved comparisons between uncured pork samples and ham samples available in the market.

The discussions centered around determining what constituted a typical cured meat flavor, and to what extent the intensity of this cured meat flavor varied between samples. During these preliminary training sessions, the combination of salty and sweet taste perceptions were identified by the panelists as typifying a cured meat flavor. Training progressed to using pork loaves containing increasing levels of nitrite and salt, followed by hams containing various levels of nitrite and salt. These sessions proved beneficial to the panelists in the transition from pork loaf evaluation to ham evaluation. During training, panel means for intensity of cured meat flavor for the pork loaves were determined and the treatment with the mean nearest the midpoint of the unstructured scale was selected as the reference sample for cured meat flavor (0 ppm nitrite, 2% salt).

Final ham evaluation took place over a 2-wk period, with a set of 13 hams (12 treatments plus reference) being processed for each week, resulting in two replications. Nine evaluation sessions were held per week, in order that all 12 treatments would be evaluated three times. Each session involved evaluation under both incandescent and red lighting. A split-plot design was used and treatment and lightings were assigned randomly to each panelist in each session.

Panelists evaluated one reference sample and four coded samples at one time. Ham samples were sliced to a 2 mm thickness, cut into 4 cm x 2 cm pieces and presented at room temperature. Samples were evaluated for cured meat flavor intensity under both incandescent and red lighting, using the descriptive analysis with scaling method (Larmond, 1977). Samples were scored by placing a mark on an unstructured 15 cm line. Anchor points 1.5 cm from each end of the line were identified to establish extremes of cured meat flavor. A reference sample was used for flavor and was anchored at 8.0 cm. In addition, the judges ranked the four samples for increasing redness and rated the intensity of the flavor characteristics, saltiness

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and sweetness, on a 0 (not present) to 3 (intense) scale, as developed by the judges during training sessions.

Untrained panel. An untrained panel consisting of 120 students, faculty, and staff at the University evaluated the hams over a 3-day period (40 per day). Three ham treatments were selected for evaluation based on the results of the trained panel: 150 ppm nitrite and 2% salt, 50 ppm nitrite and 2% salt, 0 ppm nitrite and 2% salt. Each panelist evaluated the ham for degree of liking for color, flavor and overall acceptability under incandescent lighting using the descriptive analysis with scaling method. The scales were anchored at 0, 7.5 and 15 cm with dislike very much, neither like nor dislike and like very much, respectively. In addition, a yes or no purchase intent question was asked for each sample.

Statistical analysis

Instrumental color measurement, and saltiness/sweetness intensity data obtained from the trained panel, and data from the untrained panel were analyzed for nitrite and salt effect using a randomized complete block design, while intensity of cured meat flavor data was analyzed using a split plot design due to the additional lighting effect. Tukey's test was used to judge the significance of the difference between the treatment means. An analysis of variance was carried out on both instrumental and sensory data to determine if the 7-day time span had an effect on the results obtained. The differences between the first and the third set of data obtained were calculated for each treatment and submitted to analysis of variance (Steel and Torrie, 1960). Cochran's Q test determined the effect of nitrite on the purchase intent of the untrained panel (Siegel, 1956).

RESULTS & DISCUSSION

Chemical analysis

Nitrite analysis. A decrease in nitrite levels, ranging from 22–34%, occurred between the time of cure injection and ham baking. This decrease agreed with Kolari and Aunan (1972), who estimated the loss of nitrite to range from 20–50%. During ham processing, nitrite reacted with certain meat components and further depletion in nitrite level resulted. Nitrite depletion due to processing was in the range 70–79% for the hams which was in agreement with the estimated range of 50–80% documented in other studies (Johnston et al., 1969; Greenberg, 1972). The loss of nitrite, although large initially, was very gradual during the sensory evaluation testing period (about 2% between the 2nd and 4th day after baking).

Salt determination. All salt levels were close to initial levels and remained stable over the week of sensory evaluation.

Instrumental color measurement

Analysis of variance indicated a singificant effect due to nitrite, with no effect due to salt or nitrite-salt interaction. Color values for the four nitrite treatments are presented in Table 1. Hunter L values, which measured the lightness of a sample, indicated an initial increase between 0 ppm and 50 ppm nitrite followed by a decrease in lightness with increasing nitrite. There was a significant increase in 'a' values wih each increase in nitrite level indicating a significant increase in redness of the samples. Hunter 'b' values decreased with an increase in nitrite; however, the significance was due to the presence or absence of nitrite and not the level. A decrease in 'b' values indicated a loss of yellowness in samples. Hue values decreased significantly with an increase in nitrite, which indicated a shift in hue toward redness due to an increase in nitrite. The chroma or intensity of color of the hams increased with increasing nitrite levels. There was a significant increase in color intensity from the 50 ppm to 100 ppm and 100 ppm to 150 ppm nitrite ham samples but no significant difference between 0 ppm and 50 ppm nitrite ham samples. Others have reported a similar effect of nitrite on development of pink color (Schults et al., 1977; Kemp et al., 1974). No significant change in treatment values during the week of testing was found.

Sensory evaluation

Trained panel. Data obtained from the ranking of amount of pink color in the ham samples were transformed into percentage of correct responses following a ranking order based on the Hunter 'a' values (Table 4). Results showed the ease with which the panelists were able to detect an increase in pink color intensity with increasing nitrite levels. Increasing nitrite levels in ham by 50 ppm increments had a significant visual effect.

Mean scores for cured meat flavor obtained from the trained panel are given in Table 2. Both nitrite and salt had a significant effect on the intensity of cured meat flavor. However, the role of salt in developing cured meat flavor appeared to be of greater importance than that of nitrite, as seen by the much larger MS value obtained for salt than for nitrite from the analysis of variance (Table 3). The intensity of cured meat flavor increased significantly over all three salt levels.

The effect of nitrite was much greater at the lower levels than at the higher levels of nitrite, in that the flavor was not significantly different between 100 ppm and 150 ppm

Table 1—Mean Hunter values for nitrite levels in ham samples

Hunter values	Nitrite level (ppm) 0	50	100	150
L	56.4b[a]	60.0a	55.3b	49.4c
'a'	3.9d	8.9c	11.0b	13.3a
'b'	11.1a	8.6b	8.4b	8.0b
hue ($\tan^{-1}$ b/a)[b]	70.8a	44.0b	37.3c	31.0d
chroma $(a^2 + b^2)^{1/2}$	11.8a	12.4a	13.8b	15.5c

[a] Any two means in the same row followed by the same letter are not significantly different at the 5% level.
[b] Little (1975)

Table 2—Mean values[a] for intensity of cured meat flavor for trained panel scores (maximum score = 15)

Sensory characteristic	Ham treatment: Nitrite (ppm) 0	50	100	150	Salt (percent) 0	1	2
Intensity of cured meat flavor	5.3c	6.2b	7.1a	7.3a	2.5c	7.0b	9.8a

[a] Any two means in the same row followed by the same letter are not significantly different at the 5% level.

Table 3—Analysis of variance results for cured meat flavor intensity from trained panel

Source of variation	df	MS flavor
Nitrite	3	9.92**
Salt	2	216.04**
Nitrite x salt	6	0.34
Error	11	0.01

**significant at the 1% level

Table 4—Percentage of correct responses by trained panelsist on the ranking of samples on amount of pink color

Nitrite level	Hunter 'a'	Rank order	Correct responses (%)
0	3.9	4	100
50	8.9	3	95
100	11.0	2	93
150	13.3	1	94

Table 5—Mean values[a] for saltiness and sweetness perceptions from the trained panel (maximum score = 3)

Salt level %	Mean panel scores	
	Saltiness	Sweetness
0	0.17a	0.77a
1	1.58b	1.26b
2	2.37c	1.46c

[a] Any two means in the same columns followed by the same letter are not significantly different at the 5% level

Table 6—Mean values[a] for color desirability, flavor desirability and overall acceptability from the untrained panel (maximum score = 15)

Sensory characteristics	Nitrite level (ppm)		
	0	50	150
Color desirability	2.9b	11.1a	10.4a
Flavor desirability	6.5b	9.7a	9.9a
Overall acceptability	5.2b	10.0a	10.0a

[a] Any two means in the same row followed by the same letter are not significantly different at the 5% level.

Table 7—Percentage yes/no responses by untrained panel to purchase question

Group[a]	Response	Percentage of Responses Nitrite Level (ppm)		
		0	50	150
1, 2 & 3	Yes	25	72.5	76
	No	75	27.5	24
1	Yes	20	80	80
	No	80	20	20
2	Yes	35	60	77.5
	No	65	40	22.5
3	Yes	20	77.5	70
	No	80	22.5	30

[a] Group refers to the 3 days of testing

nitrite ham samples, but was significant between the lower levels (Table 2). The importance of salt in cured meat flavor development was in agreement with other research results (Mottram and Rhodes, 1973; Greene and Price, 1975; Kimoto et al., 1976). However, results from this study, indicating the less important flavor contribution of nitrite as compared to salt, were in contrast to the results of other studies, from which researchers concluded that nitrite was the cure ingredient which contributed most and was essential to cured meat flavor (Dethmers et al., 1975; Hadden et al., 1975; Kemp et al., 1974). The lighting conditions did not have a significant effect on the panelists' flavor perception, which indicated that the training sessions were successful in eliminating other factors, such as color, from having an influence on the panelists' rating of cured meat flavor.

The perception of saltiness and sweetness in ham was greatly influenced by the salt level (Table 5). As expected, perceived saltiness increased with increasing salt levels. At higher levels of salt there was also a more intense perceived sweetness. Salt appeared to have a synergistic effect on sweetness perception in the ham samples.

Analysis of variance for the effect of time indicated no significant change in cured meat flavor intensity ratings over the one week period of testing.

Untrained panel. Mean treatment values for color desirability, flavor desirability and overall acceptability are given in Table 6. A significant decrease in color desirability was found between the samples with nitrite and those without nitrite. The mere presence of a pink color rather than the intensity of pink appeared to be the important factor influencing the panelists' ratings.

The desirability of flavor increased with increasing nitrite levels; however the only significant difference occurred between samples with and without nitrite. The small but significant difference in flavor intensity between 50 ppm and 150 ppm nitrite hams perceived by the trained panel did not influence the flavor desirability ratings of the untrained panel. However, it must be remembered that intensity of cured meat flavor as measured by the trained panel and flavor desirability as measured by the untrained panel are not necessarily the same. The fact that the untrained panelists rated the 0 ppm nitrite sample significantly lower than the other two samples might be due, in part, to the lack of the characteristic pink color of the 0 ppm nitrite sample.

Overall acceptability was also affected by the presence or absence of nitrite in the cure and not by nitrite levels. The ham samples without nitrite were disliked, while the other two ham samples containing nitrite were liked.

Results from the purchase intent question indicated that the panelists' intent to buy was influenced by the presence of nitrite as indicated by the significance obtained from the Cochran's Q test (Q = 64.69, 1% sig). The difference in response to 50 ppm and 150 ppm nitrite ham samples was relatively small in comparison to that of responses to samples with and without nitrite (Table 7). This suggested that consumers might be as likely to purchase 50 ppm as 150 ppm nitrite ham but that they might not purchase 0 ppm nitrite ham.

CONCLUSIONS

BOTH NITRITE AND SALT were found to contribute to the "typical" characteristics of cured hams. The main contribution of nitrite was the development of pink color which was considered desirable by the panelists. However increasing salt, at the levels tested, had a greater effect on the intensity of cured meat flavor, than did increasing the level of nitrite. This difference in the importance of nitrite as reported by others (Dethmers et al., 1975; Hadden et al., 1975; Kemp et al., 1974) may be attributed to differences in the way the sensory testing was conducted. Salt was also shown to affect the perception of the sweetness component as well as saltiness in the cured meat flavor. These components were considered important in characterizing cured meat flavor and warrant further investigation.

The untrained or consumer panel found no significant difference between the 50 ppm and 150 ppm nitrite with 2 percent salt ham samples, which suggests that from the standpoint of sensory acceptability, greatly reduced levels of nitrite are possible. Both color and flavor are important sensory qualities affecting overall acceptability, and therefore must be considered in combination with each other.

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—Continued on page 171

Screening Test for Histamine in Fish

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ABSTRACT

A rapid screening method is described that can detect histamine in fish. The method uses a two-step sequential enzyme system. First, diamine oxidase catalyzes the breakdown of histamine with formation of hydrogen peroxide. Hydrogen peroxide is then detected by formation of crystal violet from the leuco base in the presence of peroxidase. The two reactions can be combined and peroxidase can be used to control the tightness of the screening. The method can be used for raw or heat-processed fish.

INTRODUCTION

THREE FACTORS contribute to the relatively high frequency of scombroid poisoning from raw fish: (1) the speed with which toxic levels of histamine are formed (Frank et al., 1981); (2) the difficulty of detecting this type of spoilage by appearance or odor (Lerke et al., 1978); and (3) the broad range of spoilage among individual fish in a lot. In tropical fisheries, where the body temperature of metabolically active fish like tuna can be considerably higher than the ambient temperature of water (27–29.5°C) and where cooling facilities are inadequate or nonexistent, histamine in the flesh has been shown to rise to toxicologically significant levels within 12 hr at 37°C (Frank et al., 1981). Since odor and appearance do not reliably indicate this type of spoilage, consumers and food processing personnel often fail to be alerted and intoxications may occur (Boyer et al., 1956; Redmond et al., 1974; Reddi et al., 1980; Anon., 1980). Furthermore, there is no quick way to objectively detect histamine in fish. The best available method at present, the AOAC fluorometric assay (AOAC, 1980), is a relatively lengthy procedure. There is a need, therefore, for a rapid and simple semiquantitative test that will identify histamine-containing fish. Preferably, the reading of such a test should be by subjective means and not require instrumentation. If possible, the readings should be either positive or negative with no intermediate gradations.

We report on the development of an enzyme-based test that meets most of these requirements.

Enzymes are specific and efficient catalysts that are increasingly being used in biochemical analysis either singly or in sequence (Townshend, 1981).

In the procedure described, histaminase (diamine oxidase) acts on histamine, forming hydrogen peroxide which is then broken down by peroxidase with simultaneous formation of crystal violet from the oxidation of its leuco form, as reported by Mottola et al. (1970). The procedure can be used to screen large numbers of fish for histamine.

MATERIALS & METHODS

Materials

Fish preparations. Aqueous (1:1) extracts of tuna canned experimentally at varying stages of decomposition and 1:1 aqueous homogenates of raw tuna flesh from frozen fish containing varying amounts of histamine were prepared. No additional histamine was added. The desired concentration of histamine was achieved by analyzing several preparations, combining them in appropriate ratios and reanalyzing the mixture. The AOAC fluorometric method was used (AOAC, 1980). The materials were distributed among small containers and kept frozen until needed. Homogenates were used within 2 hr of thawing; extracts were used within 8 hr.

Reagents and buffer. Aqueous solutions of histamine dihydrochloride (Sigma Chemical Co., H7250) were used in preliminary tests. Diamine oxidase (DAO) and horseradish peroxidase (HRP) (Sigma Chemical Co., 7876 and P8250, respectively) were dissolved in distilled water and stored at 4.5°C. Leuco crystal violet (LCV) (Aldrich Chemical Co., 21,921-5) was dissolved in 0.5% HCl, protected from light, and refrigerated. The buffer was a mixture of 0.15M solutions of KH_2PO_4 and NA_2HPO_4.

Methods

Test procedure. The sample, either a free liquid or absorbed onto paper, 1 ml buffer, and 0.5 ml each of DAO and HRP, were placed into a polystyrene test tube (Falcon #2017). After mixing, 0.1 ml LCV solution was added; the appearance of color was observed either visually or with a Bausch & Lomb Spectronic 88 at 596 nm.

Optimization. Convenient volumes of reagents and other conditions that resulted in color formation in the presence of histamine were selected from preliminary tests. Optimum conditions were determined by testing the effect of the following components of the reaction mixture: buffer pH 6.0, 6.4, 6.8 and 7.2; buffer concentration 0.1–1.0 mg/ml; DAO concentration 1.0–10.0 mg/ml; HRP concentration 0.01, 0.1, 1.0, 2.5 and 5.0 mg/ml; and LCV concentration 0.005, 0.01, 0.05, 0.1, 0.5, 0.75 and 1.0 mg/ml. The objective was maximum sensitivity as determined by colorimetry after 20 min. Two extracts of canned tuna containing 0.1 and 6.0 mg% histamine were the test material. Once determined, the optimum conditions were tested on a series of extracts of canned tuna containing 0 (<0.05), 0.1, 1.0, 3.0 and 6.0 mg% histamine. Readings were made visually and by colorimetry every 5 min over a 30-min span.

Collection and introduction of sample material. Initially, test solutions were introduced by pipette. However, for fish testing purposes a liquid sample obtained by absorption on substrate was needed. We tested cotton-tipped applicators, filter paper strips (Gelman 51291) and ½-in. filter paper disks (Schleicher & Schuell 740E). Cotton-tipped applicators were tested on aqueous solutions of histamine hydrochloride and on tuna extracts by placing a saturated applicator into the reagent solution. Filter paper strips cut to 50-mm lengths were tested on whole raw fish under field conditions in a tuna cannery by inserting the strips to a depth of 10 mm into a cut in the dorsal area of the fish. After 5 min, the strips were removed and dropped into reaction tubes. Appearance of color was observed visually, and the results were compared with the histamine level in about 100g of flesh surrounding the test site. The flesh sample was excised, homogenized and analyzed by the AOAC method. The fish were from a group of 45 thawed skipjack tunas (*Katsuwonus pelamis*) known to contain some fish with high levels of histamine. Filter paper disks were tested in a similar manner on raw fish homogenates of known histamine concentration.

Control of sensitivity. To determine which reagents could be used to depress sensitivity, the effects of various concentrations of DAO, HRP and LCV on color development were tested. Measurement was by colorimetry at 5-min intervals over a span of 30 min and by visual estimation. Sample material consisted of canned tuna extracts containing 0(<0.05), 0.1, 1.0, 3.0 and 6.0 mg% of histamine.

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Performance on raw fish homogenates was evaluated by placing duplicate filter paper disks on the surface of the homogenates.

Possible interference by cadaverine was tested by applying the procedure to aqueous solutions of cadaverine (Sigma #C0500) in the range of 0.25-10 ppm of the free base and visually observing the appearance of color.

RESULTS & DISCUSSION

Test materials and standards

Only fish preparations could be used as standards. The aqueous solutions of histamine hydrochloride, though useful in the preliminary stages of the work, could not be used as standards because the blue of the solutions differed in hue from that given by fish preparations. The raw homogenates were found to be unstable; their histamine content tended to increase, presumably because of ongoing bacterial activity. For reliable results, they had to be used within 2 hr of thawing and analyzed for histamine before and after use. The extracts of canned tuna provided stable, convenient and suitable standards, showing the same blue as the homogenates of raw fish flesh.

Conditions for maximum sensitivity

The conditions for maximum color formation were: phosphate buffer, 0.15M, pH 6.8; DAO, 5.0 mg/ml; HRP, 0.1 mg/ml, and LCV, 0.5 mg/ml.

A series of extracts of canned tuna tested under these optimum conditions showed that the test can differentiate samples containing varying amounts of histamine, but only by colorimetry (Fig. 1). To the naked eye, the color intensity of a 3.0-mg% and a 6.0-mg% sample was indistinguishable.

Collection and handling of sample material from raw fish

Commercial cotton-tipped applicators produced false positive reactions. Filter paper strips proved more promising and were tested under field conditions to judge their performance and to detect any operational problems that might arise. The procedure proved to be quite rapid; it took two persons 30 min to test 45 fish. However, although convenient to use, the filter paper strips caused serious errors: The two high fish in the group (with 20 and 30 mg% histamine, respectively) were easily detected, but in some cases the test readings correlated poorly with the analytical results from the corresponding flesh samples.

Two factors were responsible for the discrepancies: (1) The fish varied markedly in free liquid. After 5 min, the level of saturation among the strips ranged from 1–5 cm, resulting in large variations in samples size. (2) Whole tuna fish that has undergone partial histamine spoilage has been shown to have an extremely uneven distribution of histamine (Lerke et al., 1978). Similar uneven distribution of histamine is encountered in mahi-mahi (Lerke, unpublished data). It is more than likely that the sample picked up by absorbent strip in one spot will have a different histamine content than one taken 1 or 2 cm away (Lerke et al., 1978). Consequently, one should not look for close correlation of the results from a sampling strip (or disk) and those from the analysis of a homogenate, even if the homogenate is from the immediately surrounding flesh. But one should expect good correlation when both test and analysis are done on a homogeneous preparation (extract or homogenate).

The error due to uneven distribution of histamine cannot be eliminated and the area of highest histamine concentration will be missed in some of the fish. In practice this should not matter since the number of fish samples will be large. Frank et al. (1981) have shown the highest concentration of histamine to be in the nape, therefore sampling from that area will reduce the risk of missing fish containing high amounts of histamine.

The error due to variability in sample size was reduced by using a smaller amount of absorbent material, so that all samples became saturated quickly. Suitable devices are ½-in. filter paper disks of the type used for the assay of antibiotics or absorbent strips cut to an equivalent area (133 mm^2). The filter paper disks proved preferable and were used in subsequent work. Variability in O.D. readings between duplicate disk samples decreased to less than 2% of the mean, compared with as much as 100% with the absorbent strips used initially.

Control of sensitivity

Although varying the concentration of DAO and LCV shifted the color reading along the O.D. scale, it did so to a similar degree for all samples, retaining the small subjective color differences at varying levels of intensity.

However, increasing concentrations of peroxidase brought the various samples, one by one, below the level of visible color (Fig. 2).

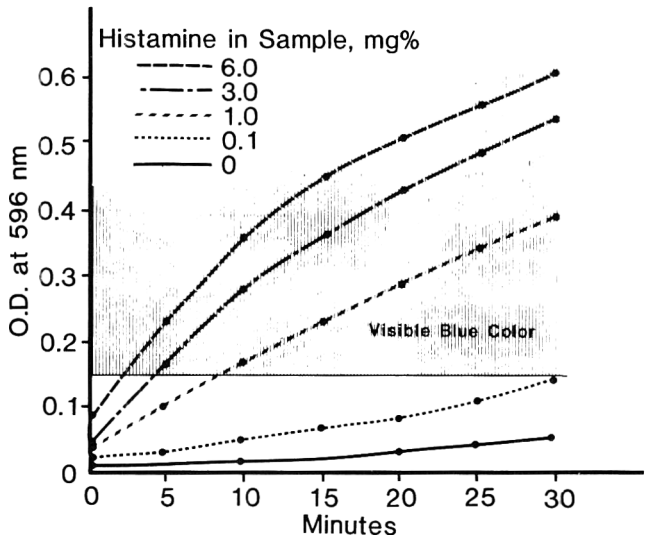

Fig. 1—Appearance of color in reagent mixture after enzymatic breakdown of histamine in aqueous extracts of canned tuna. DAO, 5.0 mg/ml; LCV, 0.5 mg/ml and HRP 0.1 mg/ml (maximum sensitivity). For n = 6 the coefficient of variation ranged from 0.061 at O.D. = 0.2 to 0.033 at O.D. = 0.5.

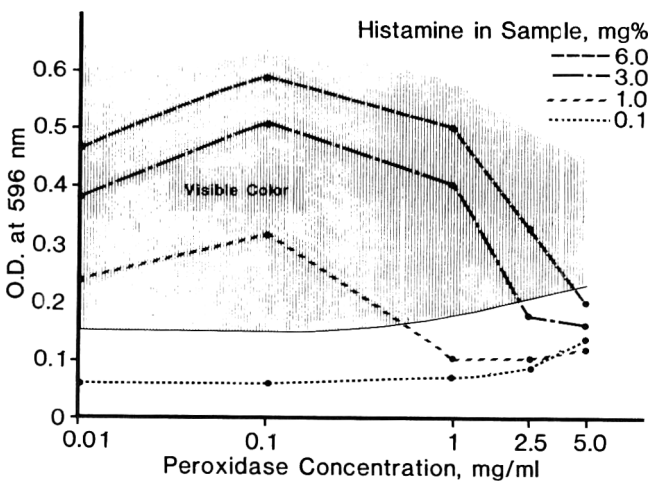

Fig. 2—Effect of peroxidase concentration on color intensity as measured 25 min after start of enzymatic breakdown of histamine.

Table 1—O.D. at 596 nm and visual color reaction in homogenates of raw tuna containing varying amounts of histamine[a]

mg% Histamine in sample	Peroxidase mg/ml			
	1.0		1.5	
	O.D.	Color[b]	O.D.	Color
0.5	0.15	v l p	0.08	n c
3.0	0.36	p	0.15	n c
6.0	0.52	d p	0.27	l p
10.0	0.71	d p	0.45	d p

[a] Readings taken at 25 min
[b] p = purple; d,l,vl = dark, light, very light; n c = no color

With HRP concentration of 0.1 mg/ml, the 0.1 mg% sample remained colorless whereas the 1.0, 3.0 and 6.0 mg% samples showed visible color. When HRP concentration was increased to 1.0 mg/ml, the 1.0 mg% sample no longer showed color. With 2.5 mg/ml HRP, only the highest (6.0 mg%) sample showed color. (The observed slight shift between O.D. and visible blue is due to the brownish appearance of HRP solutions above 1 mg/ml). These findings indicate that the sensitivity of the test can be adjusted to detect only samples (or fish) containing more than a given level of histamine. The threshold of detection selected would depend on the particular objective of the screening.

Performance of the test on raw fish homogenates

The effect of HRP concentration on the sensitivity of the test was confirmed on homogenates of raw tuna flesh. Representative results (Table 1) show how the test could be used in practice. For example, if the selected threshold of detection was 6 mg% histamine, 1.5 mg/ml HRP would be used. All samples containing up to 6 mg% histamine would then be either colorless, or, when approaching 6 mg%, light purple. A true purple would indicate a histamine concentration of about 8 mg%. If it were desired to detect fish with as little as 3 mg% histamine, the HRP concentration to use would be 1.0 mg/ml. Although intermediate color gradations are unavoidable, appropriate standards should provide adequate accuracy.

Table 1 shows that with 1.5 mg/ml HRP, the 3.0 mg% histamine sample remains colorless, a fact not borne out by the data in Fig. 2. However, with smaller increments of HRP concentration between 1.0 and 2.5 mg/ml, it was determined that the greatest depressant effect of HRP on sensitivity occurred between 1.0 and 1.5 mg/ml. Thus, 25-min O.D. readings with 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml HRP were, respectively, 0.40, 0.36, 0.16, 0.16 and 0.14. If a 1.5 mg/ml HRP concentration had been included in Fig. 2, the sample containing 3.0 mg% histamine would already be outside the zone of visible color (O.D. 0.16) and in agreement with the data in Table 1.

These results indicate that the test should work on whole raw fish. However, as with any test of this type, it is important that the sampling plan be designed to minimize the error arising from the heterogeneous nature of the material sampled.

The easy availability of tuna made it the material of choice. However, we determined that the test can detect histamine in mahi-mahi as well and probably in other species, such as mackerel, that undergo histamine spoilage.

Specificity

We have shown only an associative relationship between histamine in fish and positive test results. It is well known that besides histamine, DAO also attacks a number of amines that may be present in fresh and decomposing proteinaceous material. The amines spermine and spermidine tend to decrease with spoilage (Mietz and Karmas, 1977) and could therefore account for false positive reactions on histamine-free fish. We did not encounter such reactions since no color was ever obtained with fresh tuna material low in histamine. Putrescine, and to a much larger extent, cadaverine increase with spoilage and may thus add to positive reactions with histamine.

Aqueous solutions of cadaverine tested with 1.0 mg/ml HRP produced no color in any concentration up to 10 ppm. At the highest sensitivity (0.1 mg/ml HRP), a very faint blue appeared in the 10 ppm sample. In histamine-type spoilage, this amount of cadaverine would only be expected when tuna approaches the area of nonacceptability of 10-20 mg% histamine (U.S. FDA, 1981). The test described gives a strong positive response at much lower levels of histamine, where cadaverine, being present in correspondingly lower amounts, would not interfere. At times tuna spoils under conditions that lead to little or no histamine formation. In such cases, significant levels (10 ppm or more) of cadaverine have consistently been found (Staruszkiewicz, pers. comm.). Testing of one such lot of decomposed canned tuna (highest histamine level 0.12 mg%) did not result in visible color. While not definitive, these findings make it likely that cadaverine, and probably other amines, would not significantly influence the results of the test. However, the proposed screening procedure would also fail to detect fish that had undergone a non-histamine type of spoilage.

Except for a relatively minor degree of gradualness in color response to small increments of histamine concentration, the procedure described meets all the requirements set forth at the beginning of this report.

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Effects of Elevated CO_2 Atmosphere on Storage of Freshwater Crayfish (*Pacifastacus leniusculus*)

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ABSTRACT

The effects of elevated CO_2 levels on the keeping quality of cooked, freshwater crayfish was investigated. Experiments were conducted using an enriched atmosphere of 80% CO_2:20% air compared to air storage at 4°C. Chemical and microbial changes were correlated with sensory panel evaluations of flavor, odor and texture. After 28 days of storage, the concentrations of ammonia and trimethylamine and total plate counts were lower in crayfish stored under carbon dioxide as compared to samples stored in air. The sensory panel found no significant difference between the odors of the samples stored in CO_2 atmosphere for 21 days and fresh cooked crayfish, whereas samples stored in air were found to have significantly more fishy flavor and odor after 14 days of storage.

INTRODUCTION

FRESHWATER CRAYFISHES are found virtually in every North American aquatic ecosystem. Significant quantities of the animals are harvested for food. The crayfish producing states in the U.S. are Louisiana, California, Wisconsin, Oregon, and Washington (Huner, 1978). There is a large aquaculture industry in Louisiana for crayfish, and over 80% of the crayfish produced are consumed locally (Avault, 1972; Lovell, 1968). The California crayfish, *Pacifastacus leniusculus*, is making an important contribution to the food industry as a valuable export item to European countries (Nolfi, 1980; Dehlendorf, 1981; McGriff, 1981). The people of Sweden in recent years have imported crayfish since a fungal disease largely eliminated local supplies. Brood stock of *Pacifastacus* of the northwest region of the U.S., which closely resemble the species native to northern Europe, has been sent from the U.S. to help crayfish aquaculture there (Abrahamsson, 1972).

In response to the market in Sweden, a new commercial fishery began in 1970 in California (Nicola, 1971). Presently, annual landings exceed 200,000 kg (McGriff, 1981). The crayfish are found in the Sacramento-San Joaquin Delta area (formed by the confluence of the Sacramento River and San Joaquin River) in the central valley of California. It is probably the most heavily exploited wild population of crayfish in the State.

The signal crayfish, *Pacifastacus*, is particularly large and lobster-like when compared to other North American crayfish. Their mean total length ranges from 99–110 mm and the mean weight varies from 33–46g (Nicola, 1971). Over the years, commercial movement of live crayfish by air freight from California to Europe greatly stimulated the fishery development. Recently, however the bulk of crayfish are shipped frozen. Whole live crayfish are cooked, packaged in beer brine flavored with dill spices, vacuum sealed, pasteurized and frozen (Dehlendorf, 1981). The market is seasonal and there is great demand for high quality product. The use of modern preservation methods should enable more profitable utilization of the crayfish as well as insuring consumer acceptance of a quality product.

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The use of low temperatures is important in protecting certain qualitites of seafood. The temperature not only affects the rate of spoilage reactions, which involves both bacteria and autolytic enzymes, but also affects the rate at which bacteria multiply in the food (Ronsivalli and Charm, 1975; Shewan, 1962; Farber and Lerke, 1961). Since many spoilage bacteria require oxygen, the spoilage rate in seafood may be a function of oxygen availability under storage condition. The combination of low temperature and various gases replacing air in the storage atmosphere has proven effective in reducing the growth of the normal aerobic spoilage bacteria (Wolfe, 1980; Kramer et al., 1980). Studies have also shown that fish stored in CO_2-enriched atmosphere have a longer shelf life as compared to similar samples stored under air at refrigeration temperature (Brown et al., 1980; Parkin et al., 1982). The effects of low temperature carbon dioxide modified atmosphere storage on the spoilage of fish are similar to meat and poultry. Microorganisms that are least affected by CO_2 will thrive and predominate; they cause less noticeable sensory changes in food. (Ogrydziak and Brown, 1982; Souder, 1980; Silliker et al., 1977).

When compared to frozen storage, modified atmosphere refrigerated storage of seafoods seems to offer considerable potential for cost savings. However, research is lacking on modified atmosphere storage of freshwater shellfish products. The purpose of this study was to compare the quality changes of cooked cray-fish stored in 80% CO_2 (balance air) or in the air at refrigerated temperature. The changes in shelf life of the product were determined by microbial, chemical and sensory analyses for storage periods of up to one month.

MATERIALS & METHODS

Storage quality of cooked crayfish

The crayfish, *Pacifastacus leniusculus*, used for the experiment were purchased from a local commercial crayfish processor. They were delivered live daily to the processor by fisherman who harvested the animals in traps in the Delta during the months of July and August, 1981. The samples for CO_2 atmosphere storage (twenty-three kg of live crayfish per batch), were placed in a steam heated kettle of boiling water and, following a return to boil, they were scalded for 6 min. The cooked crayfish were then cooled promptly with ice cold water. The crates of cooked crayfish were then topped with slush-ice and immediately transported to the Institute of Marine Resources laboratory at the Univ. of California, Davis.

Freshly cooked and cooled crayfish were divided into 2 lots. They were evenly distributed on racks and placed into two polyvinylchloride cylinders previously described by Brown et al. (1980). A vacuum was formed in the tightly sealed cylinder by evacuation to 500 mm Hg (ca 20 in. Hg) for 1–2 min followed by flushing CO_2 gas into the cylinder to provide a final atmosphere of 80% CO_2:20% air. The other cylinder received the same evacuation process but the vacuum was replaced by air with no addition of CO_2. The time lapsed between top-icing and placement of crayfish was less than two hours. Both cylinders were stored at a temperature of 4 ± 0.2°C. The gas mixtures in each cylinder were analyzed once a week after sampling using a Carle Model 8000 Basic Gas Chromatograph with Porapak and Molecular sieve columns.

Periodically, samples of crayfish were removed from each cylinder for analyses, the animals taken randomly from each storage rack. The schedule was planned so that samples for chemical analyses

were removed in conjunction with those used for sensory evaluation studies. Samples for bacteriological analysis were removed more frequently. Following removal of samples, the respective cylinders were again evacuated and the vacuum replaced with 80% CO_2:20% air mixture or 100% air.

Bacteriological measurements

Total bacterial plate counts (TPC) on the cooked crayfish were done as follows: At each sampling time (0, 3, 7, 10, 14, 21 and 28 days), six to eight whole cooked crayfish were removed from the cylinders for examination. The flesh in the cephalothorax and abdomen (tailmeat) was removed aseptically from the shell and placed in a sterile blender jar and ground (2 min) to a slurry. Fifty grams of the ground samples were aseptically transferred into a sterile 0.1% peptone-water solution (450 ml) and homogenized. Serial dilutions in 0.1% peptone water were prepared for pour plates from the homogenate. Total aerobic counts were made using tryptone peptone agar as suggested by Liston and Matches (1976) and modified by Souder (1980). Plates were poured in triplicate for each dilution and the plates were incubated at 20°C for 2–5 days.

Chemical measurements

pH. A Beckman combination electrode was used to measure pH of the crayfish. Slurries prepared from grinding the flesh in a blender jar were used.

NH_3. Ammonia was assayed using an Orion ammonia specific-ion electrode connected to a Corning Model 12 pH meter following the procedure described by Ward et al. (1979). Twenty-five grams of ground crayfish flesh were added to 50 ml of perchloric acid (0.6N). The extract was neutralized with KOH to pH 6.5–7.0 and 10 ml of sample was used for ammonia determination. The electrode was inserted into the solution after the addition of 1.0 ml of NaOH (10 M) and dilution to 100 ml with water. The sample solution was stirred with a magnetic stirrer while millivolt readings were taken. Readings were translated into ammonia concentration from a standard curve prepared in conjunction with each test run.

Trimethylamine (TMA). Analyses were done using the TMA-specific electrode (Orion) and methods described by Chang et al. (1976). The internal filling solution for the electrode was 0.01M TMA-HCl: 0.05M KCl solution. The electrode potential readings of the neutralized perchloric acid extracts of crayfish samples were converted into trimethylamine concentrations using a standard curve.

Thiobarbituric acid (TBA). Values for lipid oxidation were obtained by the procedure described by Yu and Sinnhuber (1957). Five g of ground crayfish flesh were used and the samples were refluxed for 1 hr. Absorbance readings were taken at 535 nm of the final reaction mixtures on a Bausch and Lomb Spectronic-20.

Sensory evaluation

Samples of cooked crayfish from CO_2 atmosphere and air storage were examined at 7 day intervals to detect sensory changes during storage. Multiple paired comparison sensory tests were used in which control samples (freshly cooked crayfish) were used as the reference, and the treatment samples were compared to the reference. The sensory panels were composed of graduate students, staff and faculty members who were moderately experienced in sensory evaluation of seafood products.

The cooked and peeled crayfish (deveined tailmeat only) were evaluated at 7, 14, 21 days of storage. The questionnaire was a reference scoring method using a 10 cm unstructured category scale. The attributes examined were flavor (<5 cm = less fishy when compared to reference, 5 cm = same as reference, >5 cm = more fishy than reference), texture (<5 cm = less grainy when compared to reference, 5 cm = same as reference, >5 cm = more grainy than reference), and odor (<5 cm = less fishy when compared to reference, 5 cm = same as reference, >5 cm = more fishy than reference). Each judge evaluated a set of 7 samples for odor which was then followed by another set of 7 samples for flavor and texture. Each set of samples contained a reference, 2 carbon dioxide stored samples (CO_2), 2 air stored samples (air), and 2 freshly cooked samples (control). They were randomly arranged for the presentation to 22 judges during the hours of 11 am to 2 pm. Three-way analysis of variance (samples, panelists, and storage time) was used to analyze each sensory attribute, and significance was determined using Duncan's Multiple Range test (Amerine et al., 1965).

RESULTS & DISCUSSION

Microbial analysis

In crayfish, the greatest residual microbial load should be in the gut/intestinal region. Microbial flora are confined to the digestive tract and the reason for cooking the crayfish is to reduce the chance of interior muscle and tailmeat being contaminated by microbes. Cooking is also important because it inactivates endogenous enzymes. The results of the microbial analysis of this study are presented in Fig. 1. Our data demonstrate that CO_2 storage has the potential of extending the shelf life of cooked crayfish to 3 wk. Bacterial counts were significantly lower in samples held in CO_2 atmospheres as compared to samples stored in air ($p = 0.05$).

Lovell (1968) found that, in correlating sensory tests of cooked crayfish to bacterial number, samples having aerobic plate counts of 10^7 cells per g of tailmeat were considered spoiled. Samples with bacterial counts exceeding 10^8 cells/g sample were determined unfit for consumption. This study showed that even after four weeks of storage the total aerobic count remained low in CO_2 stored crayfish samples, while crayfish stored in air exceeded 10^7 cells/g sample after 14 days of storage, and increased significantly after that ($p = 0.05$).

This project did not include the classification of microbial flora. The spoilage bacteria of crayfish were characterized by Cox and Lovell (1973). They found that the greatest number of "rapid spoilers" in peeled tails of crayfish stored at low temperatures belonged to the genus *Pseudomonas* with *Achromobacter* being second.

Tissue pH

The results of the pH measurements are presented in Fig. 2. There is a consistent decrease in pH values in samples stored in modified atmosphere and it is significantly lower than the air stored samples ($p = 0.05$). Studies have shown that pH determination cannot be used as a reliable index of

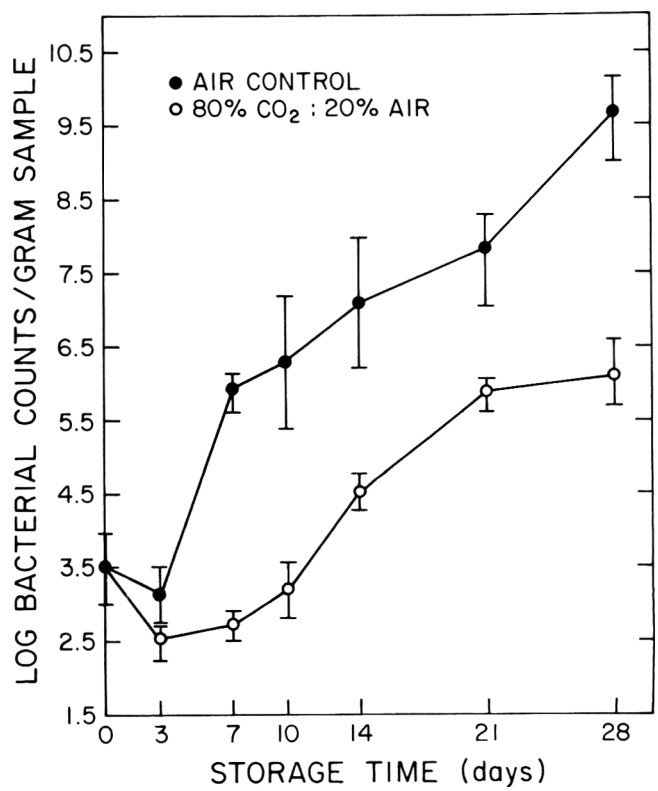

Fig. 1—Total plate counts. Bars denote the range of counts.

the state of freshness or of the onset of spoilage of seafood (Borgstrom, 1965; Reary and Shewan, 1949). However, the change of pH reflects bacterial activity. Parkin and Brown (1982) suggested that one possible factor in the carbon dioxide inhibition of bacterial growth is through lowering the pH of the product due to formation of carbonic acid. Also the increase of lactic acid bacteria may be inhibitory to the growth of spoilage bacteria that cause fishy odor.

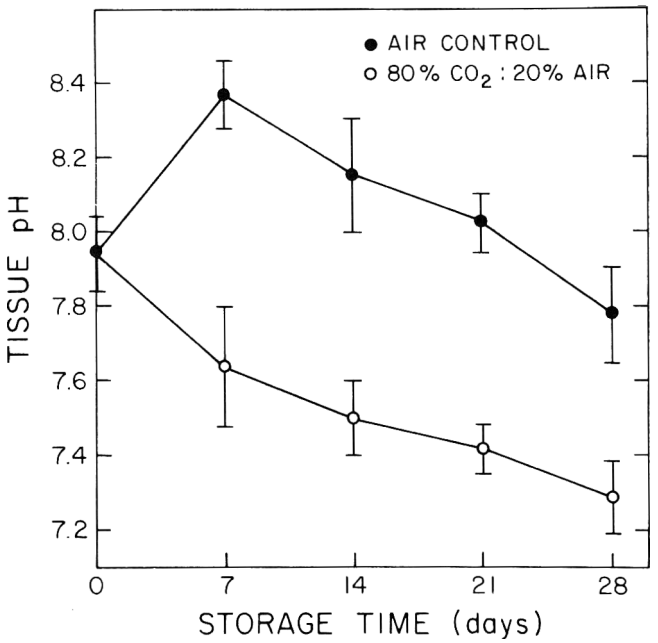

Fig. 2—pH values. Bars denote standard deviations.

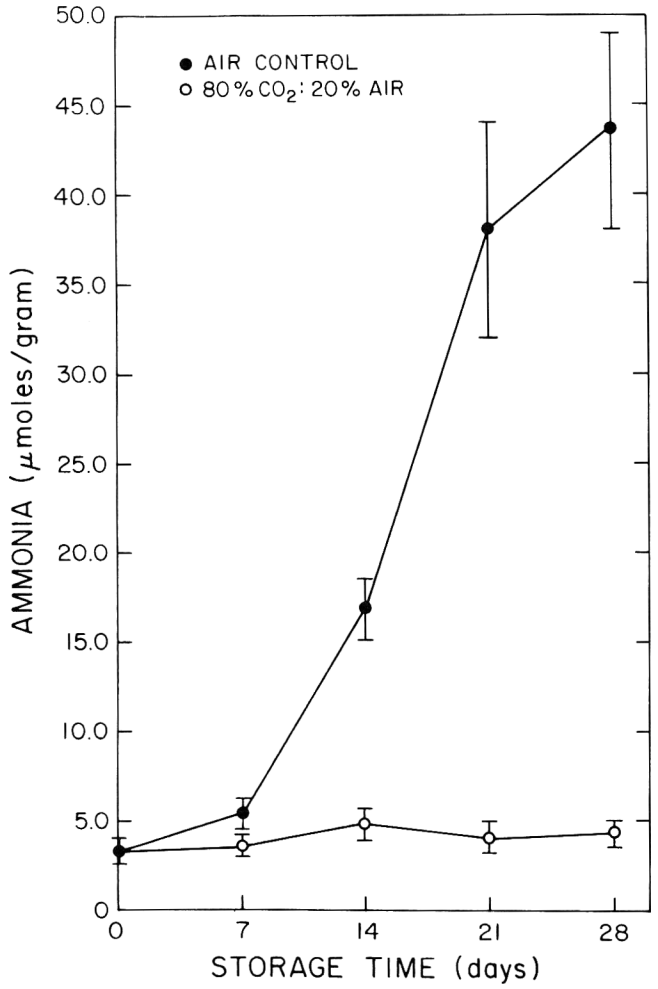

Fig. 3—Ammonia contents. Bars denote standard deviations.

The pH in air stored crayfish samples rose from 7.94 to 8.36 during the first week of refrigerated storage; in some seafood, increases in pH are usually observed with advanced bacterial spoilage, presumably due to the production of basic amines.

Ammonia

The results of ammonia determination in crayfish tissue are shown in Fig. 3. It is well recognized that ammonia poduction is due to bacterial breakdown of proteins and peptides in the muscle tissues of fish and shellfish during extended periods of storage. The accumulation of ammonia is particularly noticeable in shellfish and it imparts an unacceptable characteristic off flavor and odor in the stored product.

In this study, very low levels of NH_3 were present in the crayfish samples stored in CO_2. There was no significant difference ($p = 0.05$) among samples stored in CO_2 for 28 days and fresh cooked samples. However, after a week of storage in air, there is a significantly rapid accumulation of ammonia. This trend appears to follow the bacterial growth pattern shown in Fig. 1.

Cobb et al. (1973) found that shrimp containing from 11–20 mg NH_3-N/100g sample were of acceptable quality. Somaatmadja et al. (1961) found that catfish stored at low temperature began to have a bad odor when NH_3-N levels reached 20–30 mg/100g, whereas the fresh samples contained 7–11 mg NH_3-N/100g. We found that the ammonia concentration remained fairly low and constant during the 4 wk storage in CO_2 from 3.75–4.45 μmoles/g (equivalent to 6.26–7.4 mg NH_3-N/100g sample). After 2 wk storage in air, however, it reached 16.9 μmoles/g sample (28.2 mg NH_3-N/100g sample), and as noted in sensory tests, crayfish stored in air were evaluated as significantly more fishy in flavor and odor when compared to fresh cooked samples. From these results, as well as the microbiological data, analysis for ammonia concentration may provide an adequate quality index for shellfish products stored under ordinary refrigeration, but not for samples stored under CO_2 enriched atmosphere.

Trimethylamine

Results of trimethylamine (TMA) determinations are presented in Fig. 4. The spoilage pattern of fresh seafood generally shows a trend of an increase in TMA concentration, which closely parallels the bacterial population. Thus, TMA analysis is often used as an index in assessing the shelf life and keeping quality of seafood products. The accumulation of TMA in crayfish was low as compared to NH_3 concentration, both in CO_2 and air storage. However, the values obtained in this study on freshwater crayfish are in agreement with the spoilage pattern of shrimp. Frieger and Friloux (1954) found that less than 2 mg TMA-N/100g shrimp was considered acceptable. Flores and Crawford (1973) determined that raw shrimp were shown to increase from 0.24 to 1.6 mg TMA-N/100g shrimp after 8 days of storage on ice. Cooked shrimp meat was shown to be considerably lower but appeared to follow the same general pattern of development. Our data showed that there were no significant differences among samples stored for 7 days in air and samples stored for 14 and 21 days in CO_2. The values ranged from 0.79–1.34 μmoles/g crayfish which is equivalent to 1.11–1.87 mg TMA-N/100g sample.

The TMA values were low, probably due to the fact that the crayfish is a partially cooked product and the tissue

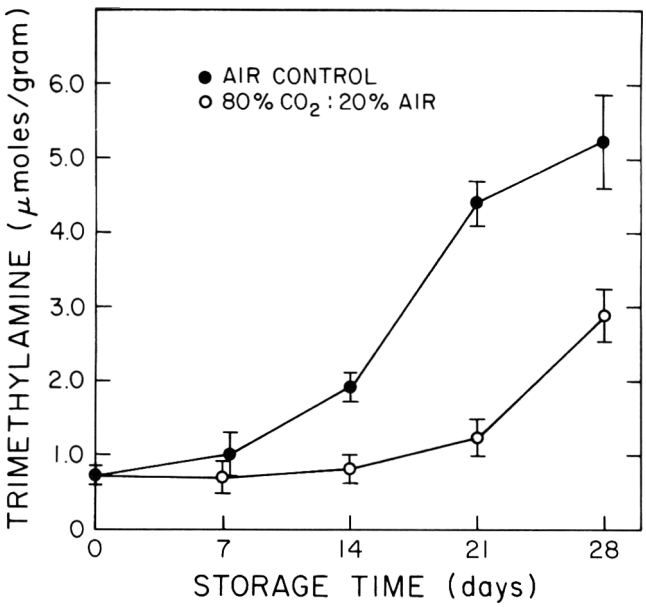

Fig. 4—Trimethylamine contents. Bars denote standard deviations.

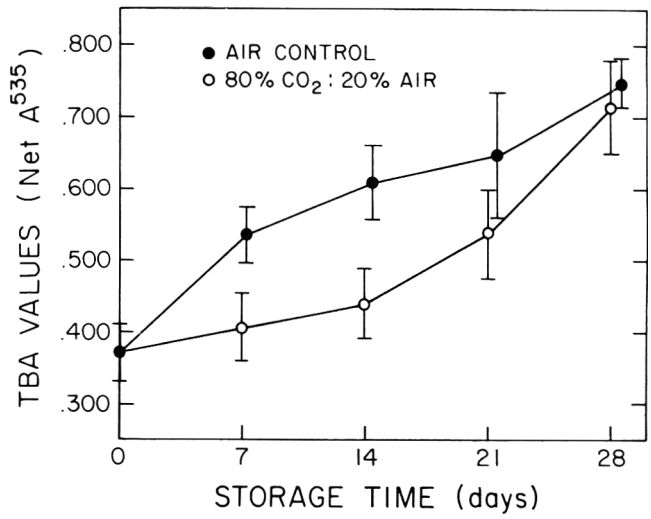

Fig. 5—TBA values. Bars denote standard deviations.

and/or bacterial enzymes had been destroyed in the scalding process. However, this may still be an useful quality index for assessing the quality of crayfish. As shown in the results, carbon dioxide samples were significantly different from air samples at 3 and 4 wk of storage ($p = 0.05$). As expected, the TMA concentration was consistent with bacterial plate counts.

Thiobarbituric acid

Thiobarbituric acid values are presented in Fig. 5. Rancidity in seafood develops from the oxidation of polyunsaturated fatty acids. The 2-thiobarbituric acid method is used to determine rancidity where malonaldehyde is a breakdown product of lipid oxidation. Fat spoilage has received less attention than bacterial spoilage in foods. However, rancidity particularly affects the acceptability and shelf life of certain seafoods during extended storage at low temperature. In this study, the TBA values were not significantly different between the fresh cooked crayfish and the 14 day CO_2 samples, but differed significantly in the samples stored in air atmosphere. However, TBA values were not significantly different between 21 day CO_2 samples and 14 day air samples. There was a consistent increase in TBA values in both treatments even though the values were low; the test signifies that CO_2 has little effect on the oxidation of lipid in the crayfish samples after 2 wk of storage. At this time the judges were not able to detect the flavor of rancidity.

Sensory evaluation

Fresh seafood is generally considered to have little odor. As seafood is stored, an odor develops that is often characterized as being "fishy." The product continues to deteriorate, ultimately having what is often described as an intense and putrid odor. Sensory methods are frequently applied in estimating the quality of seafood, and correlated to the microbiological data and chemical analyses. The results of sensory tests are presented in Table 1. At the 1% level of significance, there was no significant difference between the flavor in the 7 and 14 day CO_2 samples and the 7 day air sample when compared to the fresh cooked control. However, these samples' flavor differs significantly from the day 14 air stored samples. The judges commented that the air samples were fishy, bad, and putrid, while the CO_2 samples

Table 1—Mean sensory scores for flavor, texture and odor differences among storage treatment groups and fresh samples of cooked crayfish[a,b]

Sensory attributes	Storage time (days)	Control (fresh)	Treatment Group CO_2	Treatment Group Air
Flavor	7	5.02 a	5.02 a	5.80 a
	14	5.00 a	4.94 a	6.19 b
Texture	7	5.34 ab	5.78 bc	5.13 a
	14	5.10 a	6.19 c	5.53 ab
Odor	7	4.48 a	4.61 a	4.36 a
	14	4.47 a	4.77 a	6.51 b
	21	4.63 a	4.51 a	7.06 b

[a] Mean scores of combined columns and rows for each sensory attribute followed by a common letter are not significantly different at the 1% level.

[b] Sensory scale based on a 10-point unstructured category scale, (n = 44). Flavor (<5 = less fishy when compared to reference, 5 = same as reference, >5 = more fishy than reference); Texture (<5 = less grainy when compared to reference, 5 = same as reference, >5 = more grainy than reference); Odor (<5 = less fishy when compared to reference, 5 = same as reference, >5 = more fishy than reference)

were noted as not fishy but with a somewhat aromatic flavor.

Tests of fresh vs. fishy odor were conducted for 3 weeks on both treatment groups compared to a fresh cooked sample. When the sensory odor data were compared, the CO_2 = stored samples were not significantly different from the fresh samples. After 14 days of storage in air, the crayfish had a significantly stronger fishy odor. The sensory test on smell also correlated well with the increase in ammonia concentration and made the odor of these air samples unsatisfactory.

As to the texture of the crayfish, graininess was detected in the CO_2 samples and was found to be significantly different from the fresh and air stored samples. However, the panelists found samples from both treatments not to have as good a texture as the controls. Even though the air stored samples were not grainy, some judges commented that they were not as moist.

The problem encountered with the graininess of the CO_2 samples was due to crystal formation in the tailmeat served to the panels. The crystals examined were somewhat similar to struvite crystals found in certain seafoods following processing (Brogstrom, 1965). This problem seemed to be eliminated by subjecting the crayfish to a mild steam treatment before consuming; however additional studies are warranted.

—Continued on next page

CONCLUSIONS

THERE IS considerable potential for growth in the crayfish industry in the U.S. This research has shown that elevated levels of CO_2 will significantly extend the keeping quality of cooked crayfish up to 3 wk, based on chemical, microbiological, and sensory data. No major problems were encountered, although a slight degree of graininess was noted in some tail-meat from animals stored in CO_2; additional research is needed on this point. The use of high CO_2 modified or controlled atmospheres to improve the shelf life of cooked crustacenas should receive more attention in the future, since this technology may well result in reduced storage costs (compared to freezing) while providing a premium product that closely resembles fresh cooked product. Future work should include evaluation of post-treatment effects of storage in CO_2, i.e. do products removed to air from brief periods of storage in CO_2 atmospheres have shelf lives longer than ordinary fresh crayfish?

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Effect of Washing Treatment on Quality of Minced Mullet Flesh

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ABSTRACT

Washing treatments were evaluated for quality improvement of minced mullet. Thiobarbituric acid (TBA) number generally decreased with increased washing temperature from 5–35°C and increased at higher temperatures. At 35°C, a wash water pH of 6.0 or above resulted in decreased TBA number. Response surface analysis indicated maximum whiteness index, minimum TBA number, and minimum cooking loss with washing at 29–33°C for up to 10 min with a water to flesh ratio from 23–26. Highest springiness was indicated with washing ratio of 25 to 30 at 28–31°C The simultaneously optimized washing conditions for highest minced mullet quality were 31.4°C for 7.5 min at a ratio of 24.

INTRODUCTION

INCREASED USES of mechanically separated fish flesh in restructured fish products requires that more attention be given to quality changes which can occur during processing and storage of the fish product. Practical experience (Tanikawa, 1971) and research results (Miyanchi et al., 1973; Lee and Toledo, 1977) have indicated that washing mechanically deboned fish flesh can improve the quality and functional characteristics of minced tissue.

Miyauchi et al. (1975) suspended minced fish flesh in ice water and then drained off the excess. In a 12-month storage test, their results indicated significantly higher flavor and color characteristics in the washed minced muscle when compared to the unwashed product. Lee and Toledo (1977) suspended flesh in 15°C water and later filtered and squeezed out the excess water. They reported that leaching did not prevent the increase in oxidative rancidity or thiobarbituric acid (TBA) values associated with refrigerated deboned flesh. However, TBA values were significantly lower than in unleached flesh and washing improved product quality significantly in frozen fish stored for an extended period of time. Chao (1979) reported that washing minced fish at various temperatures for 10 min resulted in differing degrees of quality. The most effective water temperature in terms of high minced fish quality (color and texture), low oxidative rancidity, and increased water-holding capacity was 35°C. In our preliminary experiment, however, changing the level of washing water resulted in changes in the optimum washing temperature.

Since little data on the effects of washing on the quality of minced fish have been reported, the purpose of this study was to determine the optimum combination of the levels of three processing factors (washing temperatures, washing ratio of water volume to sample weight, and washing time) on minced fish quality.

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MATERIALS & METHODS

Materials

Mullet (*Mugil cephalus*) was purchased from Placida, FL, in Dec., 1978, and transported on ice to Gainesville where they were stored at −34°C until used (about 4 months). Prior to each experiment, frozen fish were thawed at 2°C for 18 hr. Food grade sodium tripolyphosphate (TPP) was purchased from the Food Machinery Corp. (FMC, Philadelphia, PA). Monosodium glutamate, sugar, and corn starch were obtained from a local market.

Methods

Fish processing. Deboning of previously scaled, gutted and filleted fish was performed with a Baader 694 Separator (Nordischer Maschinenbau, Lubek, Germany). Fish samples for the studies were immediately weighed (50g per replicate) after deboning. Each sample was washed in a beaker, and stirred (60 rpm) with a Univex Mixer (Model 1222, Universal Industries, Canton, MA). The washed samples were filtered through four layers of cheesecloth. Quality analyses were performed immediately following washing.

Quality analyses. The method reported by Yu and Sinnhuber (1967) was employed for TBA value with modification in sample size and preparation. Washed samples were stored at 2°C for 6 days before TBA measurements. One gram of sample was blended with 2 ml of cold deionized water for 30 sec. Duplicate aliquots of homogenate were used for determination of TBA number. Data were expressed as mg malonaldehyde per kg sample.

Percent cooking loss was used as an indicator of water-holding capacity (WHC). In preliminary studies, washed flesh samples were mixed with 100, 150, and 200% (w/w) tap water while 3.5% (w/v) salt was dissolved in each of mixtures and blended for 30 sec. The homogenate was poured into a weighed Erlenmeyer flask and sealed with aluminum foil, weighed again, and cooked in a 90°C water bath for 1 hr. The percent cooking loss was calculated as the difference between the raw weight and cooked weight of sample, divided by the raw weight and multiplied by 100. Measurements were done in duplicate.

The color difference of samples was measured with a Hunter Color Difference Meter based on a white standard Tile (L = +92.8, a = −1.1, and b = +0.4) (Hunter, 1942). Fifty grams of washed sample were put into a sample cup with an optically clean bottom and pushed tightly by hand. The color of the sample was described as L (lightness), a ("+", red; "−", green), and b ("+", yellow; "−", blue). The color changes were also explained in terms of white index (W.I.) (Hunter, 1975). The following equation was used to calculate W.I.:

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

Japanese style fish pastes were made from the washed samples. The proportions of minced fish, seasoning materials, and adhesive agent were minced fish, 910g; salt, 27.4g; sodium glutamate, 1.87g; sugar, 41.25g; corn starch, 28.63g; sodium polyphosphate, 2.02g; water 433.4g. The drained washed sample, seasoning materials, and adhesive agent were chopped in a silent cutter. After grinding for 4 min, the fish meat was weighed into 100g samples and piled on thin wooden plates (3 x 6 cm). The pieces of shaped fish pastes were placed in a steaming basket and steamed in the open air. The cooking was completed when the internal temperature of the fish paste reached 75°C. Steamed "kamaboko" were put in a 2°C cooler, and texture measurements were done the following day.

A compression test to determine texture profile was conducted using an Instron Model TM with load cell CCTM and a chart speed of 10 cm/min. Application of force to the sample was in two cycles traveling 0.5 cm for each cycle. Samples were cut from the center part inot 1 x 1 x 1 cm^3. Three measurements were obtained from

Table 1—Actual and experimental combinations of the three independent variables and the corresponding values of the four dependent variables.

Independent variables						Dependent variables			
Washing temp (°C) X_1	Washing time (min) X_2	Washing ratio X_3	Coded levels x_1	x_2	x_3	Springiness (mm)	TBA no.	Cooking loss %	Whiteness index
26.0	2.8	18.0	−1	−1	−1	1.83	29.31	29.50	50.36
40.0	2.8	18.0	1	−1	−1	1.73	39.32	19.40	48.16
26.0	8.2	18.0	−1	1	−1	1.85	25.16	25.70	50.72
40.0	8.2	18.0	1	1	−1	1.67	40.81	27.10	49.69
26.0	2.8	27.0	−1	−1	1	1.86	29.82	21.40	50.09
40.0	2.8	27.0	1	−1	1	1.77	32.20	24.00	50.61
26.0	8.2	27.0	−1	1	1	1.88	22.01	19.60	50.36
40.0	8.2	27.0	1	1	1	1.66	40.02	25.10	50.42
21.2	5.5	22.5	−1.682	0	C	1.81	33.00	24.20	29.31
44.8	5.5	22.5	1.682	0	C	1.37	51.59	30.60	50.67
33.0	1.0	22.5	0	−1.682	C	1.85	20.35	20.90	48.75
33.0	10.0	22.5	0	1.682	C	1.92	20.53	18.90	52.70
33.0	5.5	14.9	0	0	−1.682	1.88	23.85	23.00	50.19
33.0	5.5	30.1	0	0	1.682	1.90	20.16	21.20	50.86
33.0	5.5	22.5	0	0	0	1.89	21.72	18.50	50.84
33.0	5.5	22.5	0	0	0	1.88	21.21	18.60	50.93
33.0	5.5	22.5	0	0	0	1.87	21.55	16.80	50.98

each 100g sample. Data for the response springiness (the rate which a deformed material returns to its undeformed condition after the deforming force is removed) was reported in mm.

Individual effects of temperature and pH. In preliminary studies the effects of temperature and pH were evaluated. Mixtures of flesh and water were equilibrated and held at 5, 25, 35, and 45°C in separate water baths for 10.0 min at a ratio of water to flesh of 20:1 (w/v). Various buffers (0.1M) were used to investigate pH effects. Citrate buffer was used at pH 3, 4, and 5; phosphate buffer was used for pH 6, 7, and 8 and glycine-NaOH buffer was used to obtain pH 9 and 10. The washing ratio, time and temperature were kept constant at 20:1, 10 min, and 35°C, respectively. After washing, the appearance of the washed sample was recorded and wash water pH was measured. Data were analyzed by regression techniques.

Combined effects of washing treatment parameters

Combined effects of washing treatment parameters on the fish quality were examined using response surface techniques. A central composite experimental design (Cochran and Cox, 1957) was used to define the different combinations of the washing treatments. The quadratic polynomial equation that was fitted to the data collected is:

$$Y = \beta_0 + \beta_{1x1} + \beta_{2x2} + \beta_{3x3} + \beta_{11x1^2} + \beta_{22x2^2} + \beta_{33x3} + \beta_{12x1x2} + \beta_{13x1x3} + \beta_{23x2x3} + e$$

where x_1, x_2, and x_3 are the coded levels of the three controllable variables and y represents the responsive variable. The β_i coefficient (i = 1, 2, and 3) are measures of the linear effects for the coded x_i variables; the β_{ii} coefficients are measures of quadratic or curvilinear effects, and the β_{ij} coefficient represents a measure of the dependency (interaction effect) between x_i and x_j. After estimates of the coefficients β_i, β_{ii}, and β_{ij} have been calculated using the data values, the estimates are substituted in the model so that predicted values of the response can be obtained throughout the experimental region. Contours of constant predicted response values were calculated and plotted by fixing the value of one factor and solving the equation for combinations of the other two factors. Also, by changing the value of the fixed factor and solving the equation at the new level, the effect of the fixed factor was estimated.

Simultaneous optimization of the four responses (springiness, TBA number, cooking loss and whiteness index) was performed by determining the optimum operating conditions for the set of controllable variables (X_1, X_2, and X_3) which influence all of the response variables concurrently. The observations on the four response variables were obtained for each setting of X_1, X_2, and X_3. Each response variable under consideration was assumed to be dependent on the same set of controllable variables and each response was fitted to the second-degree polynomial regression model within the experimental region. Predictions were not extrapolated beyond the region of experimentation. In general, the method has three characteristics: (1) it detects any linear dependencies in the multiresponse data; (2) it considers variance imbalance and intercorrelations among the responses; (3) it safeguards against any sizable variation in the estimated optimum (Khuri and Conlon, 1981).

Table 2—Combinations of the three independent variables on dependent variables

Independent variables						Dependent variable
Washing temp (°C) X_1	pH X_2	Washing time X_3	Coded levels x_1	x_2	x_3	TBA no.
26.0	6.1	2.8	−1	−1	−1	31.59
40.0	6.1	2.8	1	−1	−1	40.41
26.0	6.7	2.8	−1	1	−1	24.61
40.0	6.7	2.8	1	1	−1	26.32
26.0	6.1	8.2	−1	−1	1	22.98
40.0	6.1	8.2	1	−1	1	38.49
26.0	6.7	8.2	−1	1	1	17.67
40.0	6.7	8.2	1	1	1	35.16
21.2	6.4	5.5	−1.682	0	0	23.28
44.8	6.4	5.5	1.682	0	0	33.94
33.0	6.9	5.5	0	−1.682	0	25.17
33.0	6.9	5.5	0	1.682	0	19.34
33.0	6.4	1.0	0	0	−1.682	22.82
33.0	6.4	10.0	0	0	1.682	20.42
33.0	6.4	5.5	0	0	0	20.81
33.0	6.4	5.5	0	0	0	19.50
33.0	6.4	5.5	0	0	0	21.54

Data were analyzed using the Statistical Analysis System (SAS) program package for fitting the regression models and for performing the analysis of variance calculations. Surface contours were also calculated and generated by computer.

Effect of washing temperature, washing time and washing ratios. Five levels of each of the three were chosen for study. Seventeen combination (including three replicates of the center point) were chosen and performed in random order. The actual values of the three factors and the coded values for the variables are shown in Table 1. In this investigation, washing temperature was designated as X_1, washing time as X_2, and washing ratio as X_3.

Effect of pH, washing temperature and washing time. In the second phase of studying the washing treatment effects, pH, washing temperature and washing ratio, were chosen for their effect on TBA number. The actual values of these three independent variables are shown in Table 2. Washing temperature was coded as x_1, pH as x_2, and washing time as x_3. The washing ratio was kept constant at 20:1.

RESULTS & DISCUSSION

Individual effects of washing temperature and pH

According to the preliminary study, when the ratio of tap water to fish flesh was fixed at 20:1, TBA number decreased significantly as the washing temperature increased from 5° to 35°C ($p = 0.05$), and then increased significantly ($p = 0.05$) as the temperature increased to 45°C (Fig. 1). The washed sample had approximately 65% lower TBA value than did the unwashed sample. The results of temperature effects on TBA number are in agreement with the report of Chao (1979). The effect of washing temperature on improving other quality parameters (water-holding capacity, springiness and color) generally followed the same trend.

With washing temperature set at 35°C, washing time at 10 min, and the washing ratio at 20:1, TBA number of refrigerated minced fish increased as pH of washing buffer increased from 3 to 5, but decreased as the pH of the buffer increased from 5 to 10 (Fig. 2). The washed samples appeared: (1) gel-like and pale in color at pH 3; (2) contracted at pH 4–5; (3) normal but slightly juicy at pH 6–7; (4) gel-like but dark brown at pH 8–10. The change in TBA number appears to be strongly related to the effect of pH on muscle protein solubility. The muscle protein extractability has been shown to increase significantly as pH values are increased from 5 to 6 (Helander, 1957) or to a higher pH (Ebashi and Ebashi, 1964; Ravesi and Anderson, 1969). When a buffer with a pH close to the isoelectric point of actomyosin (pH 5) was used to wash the minced flesh, the muscle protein became extensively aggregated and less hemeprotein was washed out. Therefore, greater development of lipid oxidation was detected in the minced fish flesh.

Table 3—Analysis of variance summaries for four dependent variables

Dependent variables	Code	R^2	F	Probability
Springiness	Y_1	0.946	13.53	0.0012
TBA number[a]	Y_2	0.959	18.33	0.0005
Cooking loss[b]	Y_3	0.879	5.66	0.0162
Whiteness index	Y_4	0.664	1.54	0.2923

[a]Samples were washed at different teperature, ratio, and time.
[b]Water-holding capacity

Combined effects of washing treatment parameters

Effects of washing temperature, washing time and washing ratio. Summaries of the analysis of variance tables for each of the dependent variables are shown in Table 3, with their corresponding coefficients of determination (R^2) (Mendenhall, 1975). The larger the value of R^2 (approaching 1), the better the empirical model fits the actual data. If a value of R^2 is small (near 0), indications are that for the independent variables considered, the model used did not explain enough of the variation in the response behavior. Thus we would not likely use the model to obtain predictions of the respective dependent variable response.

A low R^2 value was calculated for the whiteness index of color. As shown in Table 3, the corresponding F value was only 1.54 with a significance level of 0.2923. Thus, the model was not considered to adequately represent the whiteness surface for prediction purposes. One possible explanation of the low R^2 is material alteration. This may be due to denaturation and discoloration of fish protein duing frozen storage at −30°C (Sikorski et al., 1975; Dyer and Dingle, 1961).

The R^2 values for springiness, TBA number, and cooking loss were all high enough to enable predictions of values of these responses to be made with confidence ($p = 0.01$).

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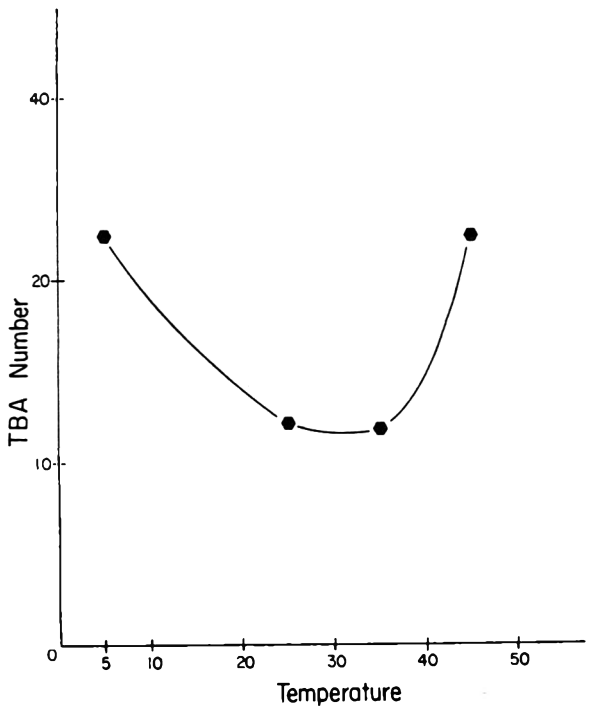

Fig. 1—Effect of washing temperature on development of oxidative rancidity (TBA number) in washed minced fish flesh during 6 days of refrigerated storage (TBA number for unwashed sample was 36.35) ($Y = 28.462 - 1.317X + 0.026X^2$; Y = TBA number, X = washing temperature (°C).

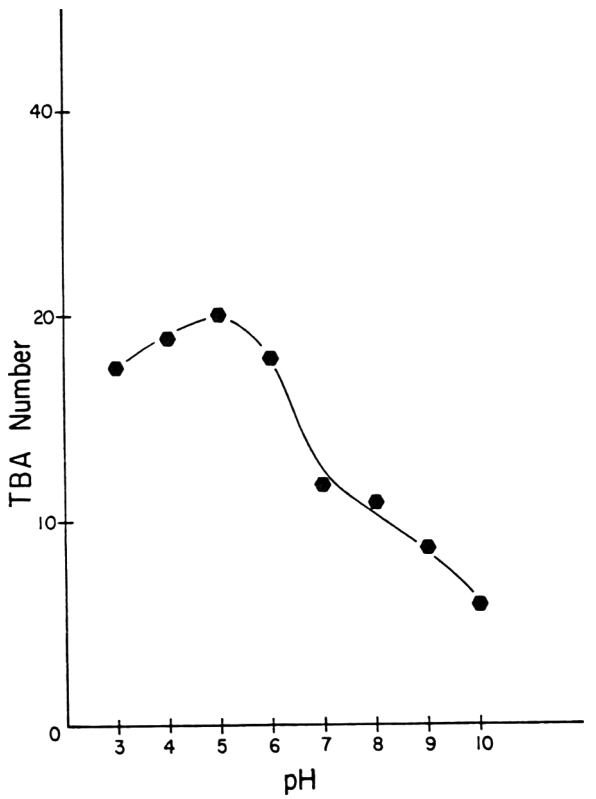

Fig. 2—Effect of pH on the change of TBA number of washed sample during 6 days refrigerated storage (TBA number of unwashed sample was 36.29) ($Y = 15.55 - 3.21X - 0.30X^2 + 0.13X^3$; Y = TBA number, X = pH).

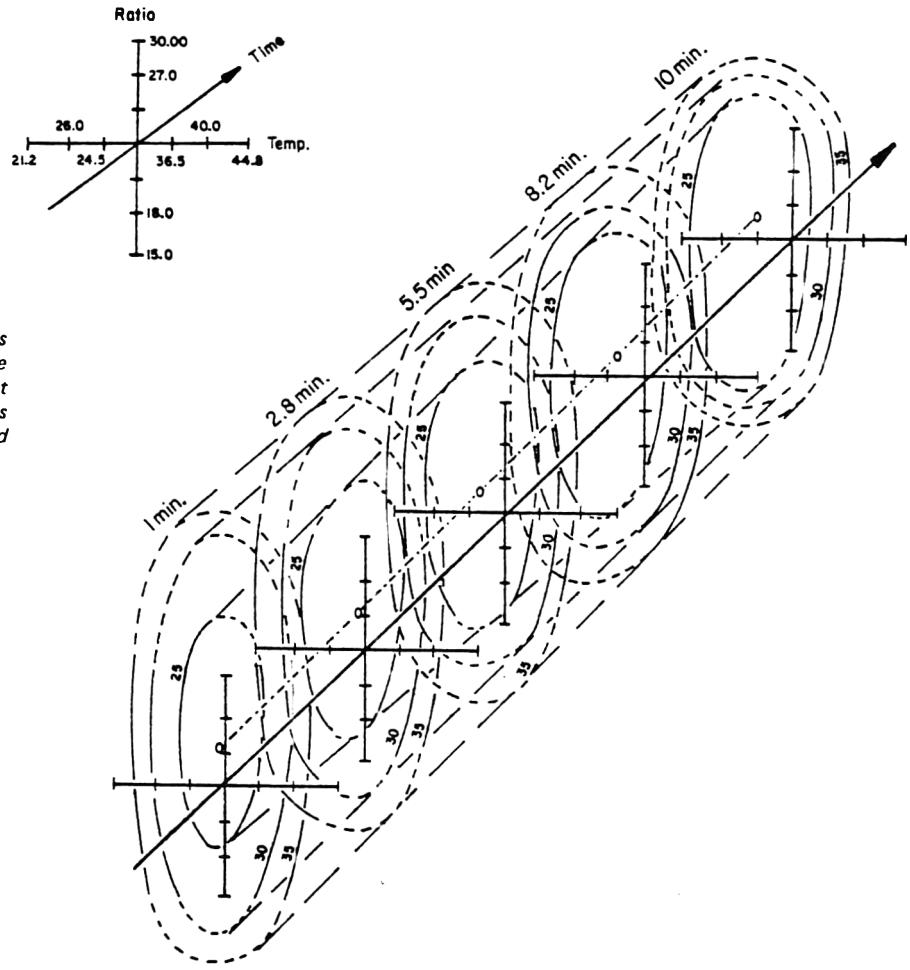

Fig. 3—Respone surface contours for the effects of washing temperature and ratio on the change of TBA number of minced flesh of mullet washed at various washing times during 6 days refrigerated storage (TBA number for unwashed sample was 41.87).

Coefficient estimates for the four regression models and the results of significance tests on the coefficients are shown in Table 4. According to the significance tests on the estimates, washing temperature seems to be the primary factor influencing springiness, TBA number, and cooking loss. The quadratic coefficient (β_{11}) of washing temperature was significantly ($p = 0.01$) different from zero with the responses springiness, TBA number, and cooking loss meaning each response behaved in a curvilinear fashion with increasing temperature values.

Representative contours are shown in Fig. 3 for effects of washing temperature and ratio on TBA number at five fixed levels of washing time to demonstrate the three-dimensionality of the surface. The geometrical surface for each TBA number is depicted as a cylinder.

The washing temperature and ratio axes are in the plane of the paper and the washing time axis passes through the plane of the paper. Long dashed lines represent the longitudinal cylindrical surfaces and short dashed lines curve around the cylinder circumference. A long and short dashed line connects the center points of the elliptical contours at each of the five times. For each two dimensional plot, the solid contour lines represent the estimated TBA number. For example, any point on the 25 contour line refers to a combination of temperature and ratio which will give an estimated TBA values of 25 at a fixed level of time. A general trend observed with increasing washing time was the shift of the contours downward and to the left of the central axis. As the washing time increased, an optimum condition could be maintained only by decreasing the temperature and ratio. Further increased washing time caused the contours to shift out of the region considered in the statistical design. Thus fewer combinations were possible for a TBA response of 25 or better. General trends for temperature and ratio can also be seen in Fig. 3. For both temperature and ratio, as the level of each is increased the estimated TBA response decreased to a minimum and then increased. This trend for temperature effect was expected, since an increase in washing temperature up to and above 35°C will wash out more soluble constituents (Tseo et al., 1982). The results of this study indicated that washed samples stored under refrigeration had 65% lower TBA values compared to that of unwashed samples. Lee and Toledo (1977) indicated that washing flesh with 15°C water improved product quality when the product was frozen for an extended period but had no major advantage when stored under refrigeration, and they had about 30% decrease in oxidative rancidity development via washing.

Response surface contours for washing temperature and ratio effects on cooking loss, springiness, and whiteness

Table 4—Regression coefficient estimates for five dependent variables

Coefficient[a]	Springiness	TBA no.	Cooking loss	Whiteness index
0 (intercept)	1.8800*	21.26**	17.93**	50.94**
1	−0.0974**	5.66**	0.74	−0.03
2	−0.0009	−0.17	−0.01	0.63**
3	0.0091	−1.23	−1.07	0.27
11	−0.1028**	8.17**	3.45**	−0.40
22	0.0014	0.44	0.80	−0.14
33	0.0032	1.00	1.58*	−0.21
12	−0.0263	2.66*	1.80*	0.09
13	−0.0038	−0.66	2.10*	0.48
23	−0.0063	0.33	−0.58	−0.23

[a]1 = washing temperature, 2 = washing time, 3 = washing ratio.
*Significant at 0.05 level
**Significant at 0.01 level

Table 5—Simultaneous optimization in response surface experiments

	Response[a]	Simultaneous	Individual
Max	y_1	1.91	1.93
	y_4	51.36	51.63
Min	y_2	17.88	17.07
	y_3	19.75	19.07
Location of max. or min	x_1	−0.227 (31.4°C)	
	x_2	0.733 (7.5 min)	
	x_3	0.354 (24.0)	

[a] y_1 = springiness; y_2 = TBA number; y_3 = cooking loss; y_4 = whiteness index.
[b] x_1 = easning temperature; x_2 = washing time; x_3 = washing ratio.

Table 6—Regression coefficient estimates for the dependent variable TBA number

Coefficient[a]	Coefficient estimate
0 (intercept)	20.28**
1	4.50**
2	−2.89*
3	−0.93
11	4.00*
22	1.75
33	1.53
12	−0.64
13	2.81
23	1.55

index were also drawn but are not included here. Rather, we shall present the estimated response equations that were used to generate the contours and mention briefly how the figures appeared to us. For each of the five washing times examined, cooking loss decreased as levels of temperature and ratio approached their control values (33°C and 22.5:1 respectively). The decreased cooking loss was maintained with increasing wash times only by decreasing temperature and ratio. For springiness there were many optimum combinations. At the shorter washing times (up to 2.8 min) a high value (1.92) of springiness was observed at an approximate temperature of 30°C and wash ratio of 28:1. Lower springiness values were observed with lower ratios at other temperatures. With longer washing times (≥8.2 min), high springiness values were observed in the temperature range 26–30°C with lower ratios (in the range 22:1–28:1). Apparently, as washing ratio and time increased, a higher ratio of myofibrillar protein to sarcoplasmic protein was attained (Tseo et al., 1982) resulting in high springiness. It is well established that myosin and actomyosin are essential among the myofibrillar proteins for producing firm product texture (Samajima et al., 1969; Nakayama and Sato, 1971). Samajima et al. (1969) further demonstrated that a whole intact myosin molecule is needed for the best performance in gel formation. Okada et al. (1973) cited both the extractability of myofibrillar proteins and the formation of a network of myofibrillar proteins during cooking as being important to the gel texture.

For whiteness index, as washing time increased, the optimum whiteness values could be maintained only by decreasing the washing temperature and ratio. Generally, when washing temperature and ratio were increased, whiteness index increased to a maximum and then decreased. Table 4 shows that whiteness index changes were significantly influenced by washing time ($p = 0.01$). According to Hamm (1966), increasing temperature up to 40°C increases globular protein solubility. Whiteness index of this study probably increased with increasing temperature because more heme proteins were washed out (Tseo et al., 1982). But as temperature increased further, protein solubility decreased due to denaturation. An increase in dark color with temperature and time was expected.

The optimized values of four responses studied simultaneously are shown in Table 5. The optimal values for the four responses studied individually are also listed and similar to those obtained when optimized simultaneously. The simultaneously optimized condition for washing temperature, washing time, and washing ratio are 31.4°C, 7.5 minutes, and 24.0, respectively (Table 5). It should be noted, however, that after washing minced mullet flesh under optimum conditions it should be soaked in ice water immediately to reduce the temperature and prevent increased microbial growth.

Effect of pH, washing temperature and washing time. A regression equation was fitted to measure effects of pH, washing temperature and washing time on TBA number. Coefficient estimates for the regression model and the results of significance tests on the coefficients are shown in Table 6. Washing temperature (β_1) had a significant curvilinear effect ($p = 0.05$), and pH had a significant linear influence ($p = 0.05$) on TBA number. A value of $R^2 = 0.836$ was obtained but since the test for regression was only slightly significant ($p = 0.041$), the model was not used for prediction purposes.

Data presented indicate optimized washing parameters for processing minced mullet flesh and the effects of altering these washing parameters on flesh quality have been assessed. The efficacy of washing as a commercial process is dependent upon strict control of these variables.

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Moisture Loss in Tray-Packed Fresh Fish During Eight Days Storage at 2°C

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ABSTRACT

Effects of moisture loss in tray-packed fresh fillets of Flounder (*Pogonias cromis*), Red Snapper (*Litjanus blackfordii*), Croaker (*Micropogon undulatus*) and Ocean Perch (*Sebastes marinus*) were studied. The fish, purchased from seafood suppliers and processed into fillets at their prospective plants, were packaged in plastic foam trays lined with absorbent pads, overwrapped, and subsequently stored at 2°C (35.6°F) for 8 days. Gross and net weights decreased whereas the percent drip increased for all fish during storage. Except for croaker, the total moisture content remained virtually unchanged for all fillets during storage. The acceptability of all fillets decreased as the Trimethylamine-Nitrogen concentrations increased.

INTRODUCTION

THE SHELF-LIFE of tray-packed fresh fish stored at 1–3°C (34–38°F) is approximately 3 days (Murray et al., 1971). It has been well documented that fish is a highly perishable food (Frazier, 1967). In general, when caught at sea, fish are packed quickly into crushed ice, and iced again as they travel from the sea to the consumer's table. For most inland markets, much of the fish is frozen and distributed in the frozen state. However, an increasing percentage of fish is being distributed fresh in todays markets due to consumer demand for "fresh fish." Consequently, more attention is being given to improving the shelf-life and quality of fresh fish.

A second area which has been given very little attention is the effect(s) of moisture loss on tray-packed fresh fish during retail storage. Moisture loss from the flesh of fresh fish is due primarily to the reduction of the moisture-binding capacity of the protein, as the acidity of the muscle is increased by lactic acid formation (Borgstrom, 1968).

This study was undertaken to determine the effects of moisture loss from the flesh of tray packed fresh fish fillets. Experiments were designed to investigate the effect(s) of moisture loss on the gross weight and the net weight of fresh fish fillets stored at 2°C (35.6°F) for 8 days.

MATERIALS & METHODS

Sample preparation

Fresh fish. The Red Snapper, Flounder, and Croaker (Table 1) were purchased from a retail seafood supplier, the Seafood Center (Jacksonville, FL). The fish were scaled, eviscerated, headed, and filleted by personnel of the Seafood Center. The skin was left intact on each fillet. The fillets were rinsed in a stream of cold tap water, and then placed in a large draining pan for approximately 5 min. The fillets were then packaged in 8S plastic foam trays (Cryovac) that had been lined with absorbent pads, and then overwrapped with an oxygen permeable film (Goodyear, Prime Wrap). The packaged fillets were transported to ABC in plastic foam coolers which contained frozen "blue ice" cool packs. Upon arrival at ABC, the individual packages of fish were placed in cold storage 2°C (35.6°F). Initial net weights and gross weights were recorded for each package. Each package was marekd with a code number.

The Ocean Perch (Table 1) was purchased from Bay State Lobster Company, Inc. (Boston, MA). The fillets were received packed in large plastic sealed containers which were shipped in an insulated chest containing frozen "blue ice" packs. Upon arrival at ABC, the fillets were packaged in 8S plastic foam trays that had been lined with absorbent pads, and then overwrapped with an oxygen permeable film (Goodyear, Prime Wrap). The packaged fish was stored at 2°C (35.6°F). Initial net weights were recorded for each package. Each package was marked with a code number. An attempt was made to package the fish as close to 1 lb as possible.

Percent yields were also recorded for the fish after processing (Table 2).

Sample evaluation

The fresh fish were evaluated through 8 days storage at 2°C (35.6°F). Four subsamples were evaluated for each of the four fish studied to determine gross weight (weight of fish and packaging material), net weight (weight of fish fillets), drip (weight of residual water collected in the tray during storage as a result of purge/weeping in the fish flesh), and total percent moisture. Subjective and objective measurements of the fresh fish were employed to assess the acceptability of the fillets during storage. Sensory evaluations were performed on fillets that had been baked for 10 min at 232°C (450°F) (optimum cooking conditions were determined by ABC) and served warm without condiments. The sensory panel scores represented mean ratings of 19–20 consumer panelists, rating the products on a 9-point hedonic scale where 9 = Like extremely and 1 = Dislike extremely for color, texture, flavor, odor and general acceptance (Peryman and Pilgrim, 1957). Taste panels were discontinued when the fillets were noted to have a pronounced off-odor. The objective evaluations involved analysis of the fillets for Trimethylamine-Nitrogen (TMA-N) concentrations (AOAC, 1975).

Statistical analyses were performed on the data using the Analysis of Variance and the General Linear Regression procedures (Snedecor and Cochran, 1967). Predicted values were used to construct the graphs. All analyses were performed at the 5% level of significance.

RESULTS & DISCUSSION

Flounder (Fig. 1)

The data demonstrated losses in net and gross weight during storage. The loss in gross weight remained less than

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Table 1—Sample identification

Fish	Scientific name	Habitat and location	Age of fish at time of processing (days)
Flounder	*Pogonias cromis*	Salt water, Southeast area – North Carolina in the Capes	2
Red Snapper	*Lutjanus blackfordii*	Salt water, Southeast area – Mayport	3
Croaker	*Micropogon undulatus*	Salt water, Southeast area – North Carolina in the Capes	2
Ocean Perch	*Sebastes marinus*	Salt water, Atlantic Ocean – Boston, Massachusetts	2

Table 2–Yields for fresh fish after processing

Fresh	Purchased whole weight (lb)	Fillet weight after process (lb)	Yield (%)
Red Snapper	70.0	39.45	56.36
Flounder	80.0	31.46	39.33
Croaker	83.0	35.56	42.84
Ocean Perch	80.0[a]	40.00	50.00

[a] Values are approximations based on percent yields by suppliers.

Table 3–Sensory scores and trimethylamine-nitrogen concentrations for tray-packed fresh flounder fillets stored at 2°C (35.6°F)

Days of storage	Panel responses					TMA-N (mg/100g)
	Color	Texture	Flavor	Odor	General acceptance	
1	7.0[a]	6.0	6.3	7.1	6.3	1.53
4	7.6	6.5	6.4	6.9	6.1	25.13
5	7.0	–	–	1.0	–	29.20

[a] Sensory scores measured on a 9 point hedonic scale; 9 = Like extremely; 1 = Dislike extremely.

Table 4–Sensory scores and trimethylamine-nitrogen concentrations for tray packed fresh red snapper fillets stored at 2°C (35.6°F)

Day of storage	Panel responses					TMA-N (mg/100g)
	Color	Texture	Flavor	Odor	General acceptance	
1	7.2[a]	7.1	6.9	7.2	7.2	1.87
3	7.0	6.9	6.7	6.6	6.6	2.96
4	7.0	–	–	3.0	–	–
6	7.0	–	–	1.0	–	16.8

[a] Sensory scores measured on a 9-point hedonic scale; 9 = like extremely; 1 = dislike extremely.

1% during storage. A positive relationship was revealed between the net weight and drip; the drip increased as the loss in net weight increased. A correlation coefficient of 0.99 was recorded as an expression of the relationship between the net weight and drip. A 1% increase in total moisture content was demonstrated for the fillets during storage.

The fillets were rated acceptable in color, texture, flavor, odor and general acceptance through 4 days storage (Table 3). No significant differences ($P>0.05$) were revealed in color, texture, flavor, odor and general acceptance through 4 days. The odor of the fillets was rated significantly low ($P<0.05$) after 5 days storage which resulted in discontinuation of the sensory evaluation. An extreme ammonia odor was detected. The TMA-N concentrations increased significantly ($P<0.05$) during storage. The concentrations were significantly higher ($P<0.05$) after 4 and 5 days storage when compared to the initial concentration of 1.53 mg/100g.

Red Snapper (Fig. 2)

The data demonstrated losses in net and gross weight, and total moisture content during storage. The loss in gross weight and total moisture content remained less than 1% during storage. A positive relationship was revealed between the net weight and drip; the drip increased as the loss in net weight increased. A correlation coefficient of 0.99 was recorded as an expression of the relationship between the net weight and drip.

The fillets were rated acceptable in color, texture, flavor, odor and general acceptance through 3 days storage (Table 4). No significant differences ($P>0.05$) were revealed in color, texture, flavor, odor and general acceptance through

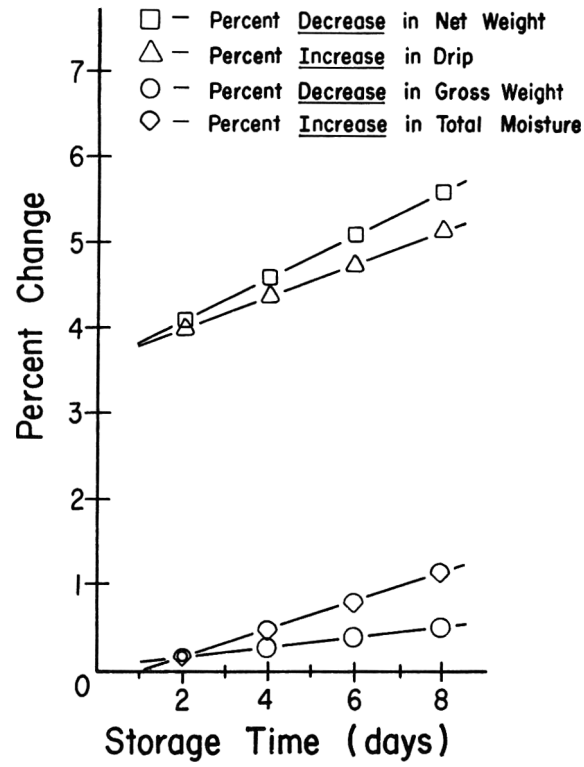

Fig. 1–Percent change in tray-packed fresh flounder fillets stored 8 days at 2°C.

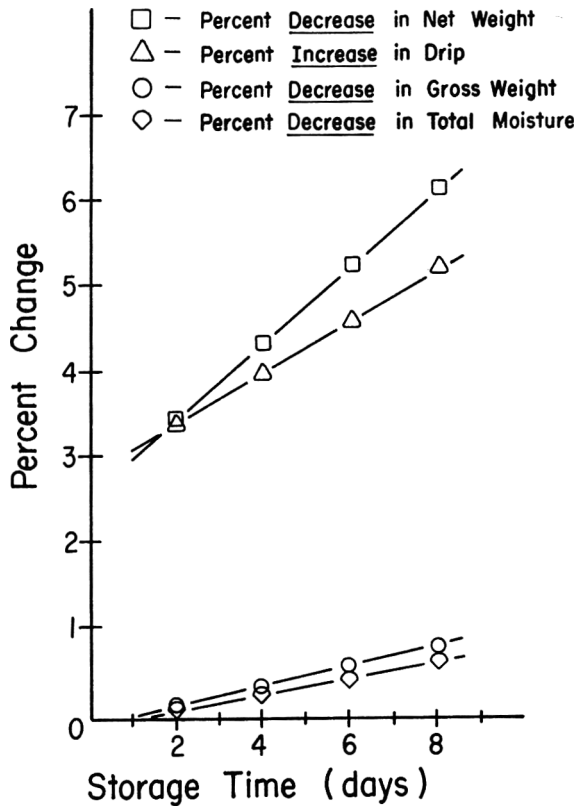

Fig. 2–Percent change in tray-packed fresh red snapper fillets stored 8 days at 2°C.

3 days storage. The odor of the fillets was rated significantly lower ($P<0.05$) after 4 and 6 days storage, which resulted in discontinuation of the sensory evaluation. Ten percent of the panelists detected a "strong fish odor," and 5% detected a "slight ammonia odor." The TMA-N concentra-

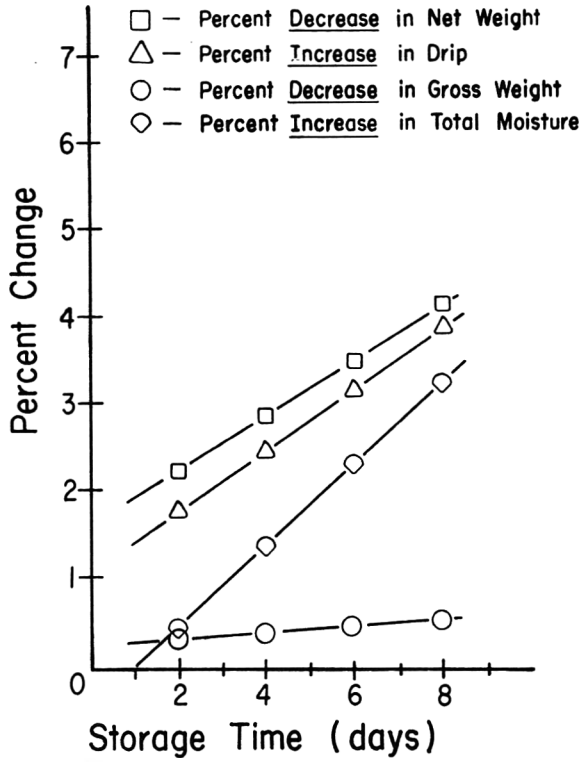

Fig. 3—Percent change in tray-packed fresh croaker fillets stored 8 days at 2°C.

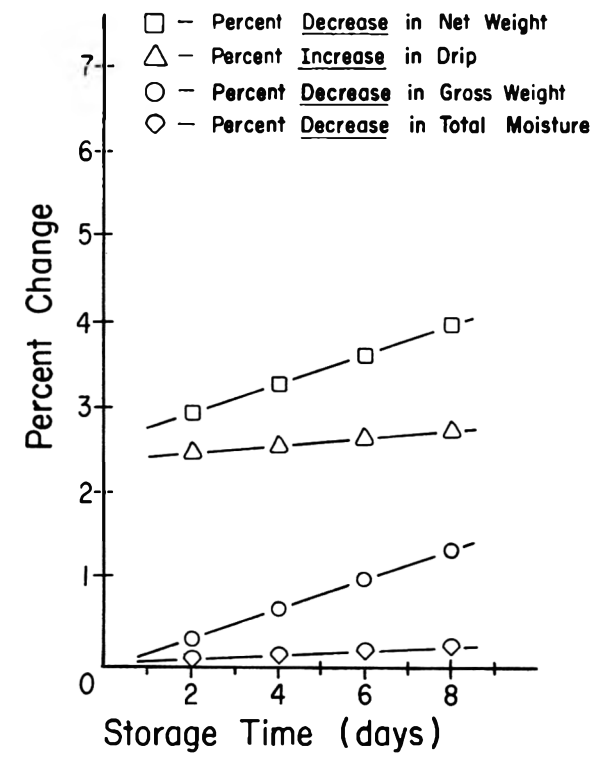

Fig. 4—Percent change in tray-packed fresh ocean perch fillets stored 8 days at 2°C.

tions increased significantly ($P<0.05$) after 6 days storage when compared to initial and 72 hr TMA-N concentrations. No significant increase in ($P>0.05$) TMA-N concentrations was revealed after 72 hr storage when compared to the initial TMA-N concentration of 1.87 mg/100g.

Croaker (Fig. 3)

The data demonstrated losses in net and gross weight during storage. The loss in gross weight remained less than 1% during storage. A positive relationship was revealed between the net weight and drip; the drip increased as the loss in net weight increased. A correlation coefficient of 0.99 was recorded as an expression of the relationship between the net weight and drip. A 3.25% increase in total moisture content was demonstrated for the fillets after 8 days storage.

The fillets were rated acceptable in color, texture, flavor and odor through 5 days storage (Table 5). No significant differences ($P>0.05$) were revealed in color, texture, odor, flavor or general acceptance through 5 days. The general acceptance of the product declined after 5 days storage which was evident by the "dislike slightly" rating (i.e., score = 4.1). Sensory evaluations were discontinued after 5 days because of a "strong fishy flavor" and a "slight ammonia odor." The TMA-N concentrations increased significantly ($P<0.05$) during storage. The TMA-N concentrations were significantly higher ($P<0.05$) after 4 and 5 days storage when compared to the initial concetration of 0.55 mg/100g.

Table 5—Sensory scores and trimethylamine-nitrogen concentrations for fresh tray packed croaker fillets stored at 2°C (35.6°F)

Day of storage	Panel responses					TMA-N (mg/100g)
	Color	Texture	Flavor	Odor	General acceptance	
1	6.7[a]	6.5	5.4	6.0	5.3	0.55
4	6.8	6.7	6.1	5.6	5.6	24.98
5	6.5	6.2	4.5	5.4	4.1	39.68

[a] Sensory scores measured on a 9-point hedonic scale; 9 = like extremely; 1 = dislike extremely.

Table 6—Sensory scores and trimethylamine-nitrogen concentrations for fresh tray packed ocean perch fillets stored at 2°C (35.6°F)

Day of storage	Panel responses					TMA-N (mg/100g)
	Color	Texture	Flavor	Odor	General acceptance	
1	6.3[a]	6.7	6.1	6.2	5.8	4.43
2	7.5	7.4	5.9	6.3	6.0	7.93
3	7.0	—	—	5.0	—	—
5	6.0	—	—	4.0	—	38.80

[a] Sensory scores measured on a 9-point hedonic scale; 9 = like extremely; 1 = dislike extremely.

Ocean Perch (Fig. 4)

The data demonstrated losses in net and gross weight, and total moisture content during storage. The loss in gross weight remained less than 2%, and the loss in total moisture remained less than 0.5% during storage. A positive relationship was revealed between the net weight and drip; the drip increased as the loss in net weight increased. A correlation coefficient of 0.99 was recorded as an expression of the relationship between the net weight and drip.

The fillets were rated acceptable in color, texture, flavor, odor and general acceptance through 2 days (Table 6). No significant differences ($P>0.05$) were revealed in color, texture, flavor, odor and general acceptance through 2 days. The sensory evaluations were discontinued on day 3 because of an intense ammonia odor. The TMA-N concentrations increased significantly ($P<0.05$) after 2 and 5 days storage when compared to the initial concentration of 4.43 mg/100g.

Species comparison (Table 7)

Gross weight. The loss in gross weight was significantly higher ($P<0.05$) for the ocean perch fillets when compared

to the flounder, red snapper and croaker fillets. No significant ($P>0.05$) weight loss was revealed between the flounder, red snapper and croaker fillets.

Net weight. The flounder and red snapper fillets lost significantly ($P<0.05$) more weight than the ocean perch and croaker fillets. The ocean perch fillets lost significantly ($P<0.05$) more weight than the croaker fillets. No significant ($P>0.05$) weight loss was revealed between the flounder and red snapper fillets.

Drip. The flounder and the red snapper fillets had significantly higher ($P<0.05$) drip than the ocean perch and the croaker fillets. No significant differences ($P>0.05$) in drip were revealed between the flounder and the red snapper fillets nor between the ocean perch and the croaker fillets.

Total moisture. The croaker fillets were significantly higher ($P<0.05$) in total moisture when compared to the flounder, ocean perch and red snapper fillets. No significant differences ($P>0.05$) were revealed between the flounder, ocean perch and red snapper fillets. The data demonstrated increases in total moisture content for the croaker and flounder fillets, and decreases in total moisture content for the red snapper and ocean perch fillets. The increase in moisture content for the flounder and croaker fillets could be largely attributed to the variability of total moisture from fillet to fillet.

Net weight vs drip. No significant differences ($P>0.05$) were revealed between the percent change in net weight and drip for the flounder fillets over 8 days storage. The percent change in net weight was significantly higher ($P<0.05$) than the percent change in drip for the red snapper, croaker and ocean perch fillets over 8 days storage.

Based on this study it can be concluded that all species of fish studied resulted in decreases in net and gross weight, and increases in drip during 8 days storage at 2°C (35.6°F). The increase in drip after 24 hr storage suggested that the dry absorbent pad used to line each of the plastic foam trays might have produced an osmotic reaction with the wet fish flesh. The data demonstrated that moisture loss in the tray-packed fresh fish fillets was due primarily to drip. A maximum of 1.38% weight (i.e., gross weight of ocean perch) was lost after 8 days storage due to vapor transmission through the package.

Table 7—Mean values[a] for percent change[b] in tray-packed fresh fish over eight days storage at 2°C (35.6°F)

Fish	Gross wt	Net wt	Drip	Total moisture
Flounder	0.32 (—)	4.74 (—)	4.48 (+)	0.57 (+)
Red Snapper	0.43 (—)	4.63 (—)	4.22 (+)	0.32 (—)
Croaker	0.39 (—)	3.01 (—)	2.65 (+)	1.61 (+)
Ocean Perch	0.74 (—)	3.40 (—)	2.60 (+)	0.11 (—)

[a] + = increase; — = decrease.
[b] Each value represents the average of eight measurements recorded over 8 days.

The TMA-N concentrations for all fillets increased significantly during storage. The TMA-N concentrations provided adequate objective measurements for assessing the acceptability of the fresh fish fillets as has been documented by several workers (Dyer, 1945). A trend was observed for all fillets wherein the TMA-N concentrations increased as the "odor" scores decreased.

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EFFECT OF NITRATE & SALT ON HAM. . . From page 154

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Storage Stability of Intermediate Moisture Mullet Roe

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ABSTRACT

A storage stability study was performed on intermediate moisture roe (a_w = 0.84, salt content = 4%). Samples were stored at various temperatures for up to 1 month. Microbial analyses indicated that bacteria could grow from 5–25°C. Fungi grew at 15° and 25°C while their growth was inhibited at 5°C; however, a lag phase was detected at 15°C. TBA values increased linearly during storage. Microbial analyses, chemical determination of rancidity and sensory evaluations showed that the product was still acceptable after 30 days storage at 5°, 15° or 25°C.

INTRODUCTION

APPROXIMATELY 30 million pounds of mullet are harvested annually in Florida which accounts for more than 20% of the finfish landed in the state (Pierce, 1975). However, mullet has only a limited market value as compared to other commercially valuable fish species. Recently, foreign countries have purchased large quantities of mullet roe from Florida during the November to January spawning season (Cato et al., 1976) increasing the price of mullet roe. Mullet roe is very popular in certain Asian countries where it is processed into a dried and cured product. Generally, an open sun-drying process is used (Hsu and Deng, 1980). An improved drying process using a mechanical drier was developed by Hsu et al. (1980). Mullet roe required 149 hr drying with the improved method to reach water activity 0.85. During the processing period, there could be considerable microbial growth. In addition, the high lipid content (18%) of the finished product (Hsu et al., 1980), could cause flavor changes during processing and subsequent storage. The objective of this research was to determine the quality changes during processing and storage of this product.

MATERIALS & METHODS

Materials

Mullet (*Mugil cephalus*) were purchased in Placida, Florida, at the end of November (spawning season), 1976. The fish were transported on ice to Gainesville, Florida, where the fish roe, encased in an ovarian membrane, were separated carefully from the fish without rupturing the membrane. Extraneous material, as well as the blood in the surface vessels, was either washed or squeezed out. The cleaned roe were then tied individually at the ends. Three or five roe were placed in a plastic bag and frozen at −30°C. The desired number of the frozen roe was thawed for 1 day at 5°C before processing in each experiment.

Sample preparation

Samples for the storage studies were prepared following the procedure of Hsu et al. (1980). The thawed roe were dry salted with 15% salt for 8.4 hr and then pressed with overlapped trays. The roe were desalted for 6 hr with twice the amount of water and dehydrated for 149 hr at 30.5°C, 74% RH, and 150 ft/min air velocity. If 10% potassium sorbate solution was sprayed (a Jet-Pak sprayer) on the roe, it was done after desalting and before drying. The finished products were individually sealed in polyethylene bags.

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Storage stability

For microbiological studies, the products with and without potassium sorbate treatment were stored at 5°, 15°, and 25°C for 1 month. Total plate counts and fungi were determined on the products during storage. Samples were taken at random for the determinations. Samples stored at 45°C were used in addition to 5° and 25°C for TBA tests. Multiple regression analysis was used to analyze the data. Predicted values of the TBA number, total plate count, and fungi counts were calculated from the models by computer using the SAS program package.

Water activity for the products held at 5° and 15°C were determined during storage.

Chemical analysis

Thiobarbituric acid (TBA) analysis was performed by the method of Yu and Sinnhuber (1967) with modification in sample preparation. A 15-g sample was blended with 30 ml of cold deionized water and a 2.5 ml aliquot of the roe homogenate was then used for TBA analysis.

Water was determined with an electronic hygrometer. Sample preparation was according to Hsu and Deng (1980).

Potassium sorbate content was determined by the procedure of Melnick and Luckmann (1954). A finely minced 6-g samples was used.

Microbiological assays

Microbiological analyses were performed according to the procedure outlined in the *Bacteriological Analytical Manual for Foods* (BAM) (AOAC, 1976). Analyses were done in duplicate for each sample.

Twenty-five g samples were removed from the mullet roe using a sterile spoon and knife. The samples were homogenized for 2 min with 225g of sterile peptone buffer in a sterile Waring Blendor jar at approximately 8000 rpm. Serial dilutions were made by aseptically transferring 11 ml of the homogenate into 99 ml of buffer. The pour plate technique with 1 ml aliquots of each dilution was used for all assays. Colonies were counted with a Quebec Darkfield Colony Counter.

Total plate count. The standard plate count was obtained using Plate Count agar with 0.5% salt and incubation at 20°C for 5 days. The data were averaged and expressed as aerobic plate count per gram at 20°C (APC/g).

Fungi count. The fungi count was obtained using Plate Count agar with 0.5% salt and 2 ml of antibiotics (500 mg chloramephenicol and 500 ml chlortetracycline HCl in 100 ml sterile phosphate buffer) per 100 ml agar. Plates were incubated at 20°C for 5 days before counting. The averaged data were expressed as yeast and mold colony forming units per gram at 20°C (CFU/g) (Koburger, 1976).

Sensory evaluation

Color and flavor panel evaluation were not conducted because of a shortage of mullet roe and difficulty of finding experienced oriental panelists. Thus, color of the intermediate moisture mullet roe during storage was examined by the authors and photographed to assist in evaluation of the color change. Flavor of the roe was evaluated by the authors after 30 days of storage.

Table 1—Effect of processing on TBA number in mullet roe

Sample	TBA number[a]
Raw material	3.25
After salting	3.36
After desalting	3.45
After dehydration (final product)	3.39

[a] Average of two determinations

Table 2—Multiple regression coefficients for dependent variables (TBA number)

Model Term	Regression coefficient
Constant	3.5773
X_1[a]	0.0450**
X_2[b]	−0.0398**
X_1^2	−0.0004
X_2^2	0.0011
X_1X_2	0.0015*

[a] X_1 = storage time
[b] X_2 = storage temperature
R^2 of the regression model is 0.89 at 0.01
* Significant at 0.05
** Significant at 0.01

Table 3—Regression coefficients for dependent variable (TBA number)

Model term	Regression coefficient
Constant	5.2809
X_1	−0.2176**
X_2	0.01633**
X_1X_2	−0.1891**
X_2^2	−0.0015**
$X_1X_2^2$	0.0025

X_2 = location
X_2 = storage temperature

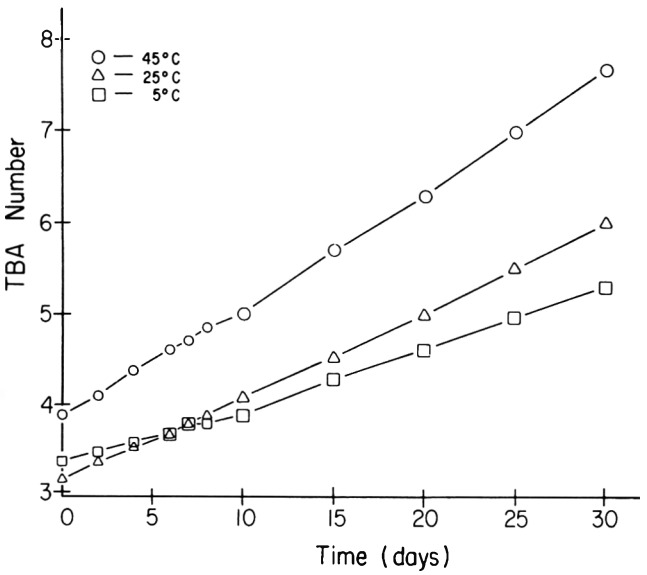

Fig. 1—Effect of various storage temperatures on predicted TBA number in intermediate moisture mullet roe.

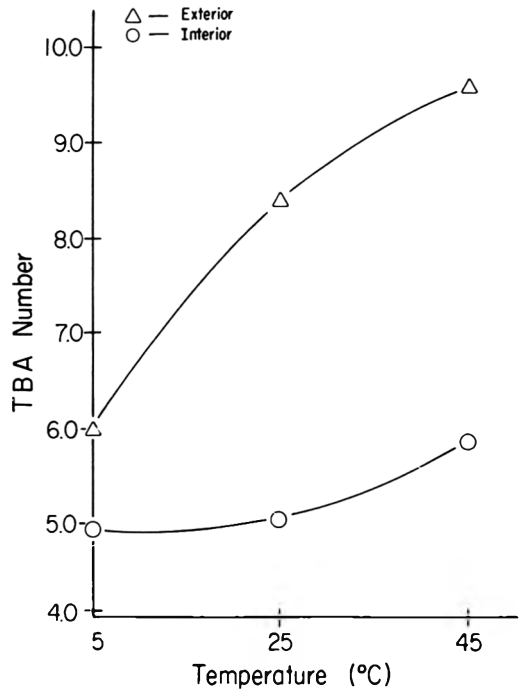

Fig. 2—Effect of locations on predicted TBA number of intermediate moisture mullet roe stored at various temperatures.

RESULTS & DISCUSSION

TBA analysis

Processing steps. TBA values of mullet roe in each processing step are shown in Table 1. The TBA values of mullet roe did not change during processing.

Storage stability. When intermediate moisture mullet roe was stored at the three different temperatures, both storage time and temperature significantly (p 0.01) affected the TBA value (Table 2). This was expected, since high temperatures accelerate the oxidation rate and food quality decreases with a longer storage period. An interaction between time and temperature was also present in the analysis of the TBA number (Table 2). This interaction is explained by noticing the difference in the effect of storage time on the predicted TBA values at different temperatures (Fig. 1). At 45°C, the TBA value increases more rapidly than at the two lower temperatures. After 30 days, the TBA value at 45°C is approximately twice the initial TBA number at 45°C. It appeared that storage temperatures between 5 and 25°C for up to 10 days storage had no effect on TBA values.

When compared to mullet flesh (Deng et al., 1977), TBA values of the roe were not high even after 30 days of storage at 45°C. Large amounts of saturated fatty acids present in the roe (Iyengor and Schlenk, 1967) and the possible lack of potent oxidation catalysts, such as heme proteins (Fischer and Deng, 1977), might explain the low TBA values.

Location effect. TBA values of surface and interior portions of intermediate moisture mullet roe after 30 days storage at 5, 25 and 45°C were also determined (Fig. 2). TBA values are highly affected by location (Table 3). The exterior had a higher TBA value than the interior, due to the surface area being exposed to air, thus accelerating oxidation.

Microbiological analyses

Microbiological counts of mullet roe in each processing step are shown in Table 4. The thawed raw mullet roe had a low APC and few fungi. This may be due to bacterial counts in frozen raw foods generally decreasing with time due to their sensitivity to freezing. Since the roe had been kept in the freezer for almost 6 months, the results were expected. APC of the thawed roe increased by approximately one log cycle during salting. The desalted roe and three times more APC than the salted roe. The bacterial counts, however, increased rapidly during the initial stage of dehydration (from 10^2 to 10^7). This might be due to the increased moisture content after desalting and a favorable growth temperature (30.5°C). During the initial drying stage, APC increased sharply and this increase cannot be attributed to moisture loss alone. There is only a 20–25% decrease in moisture during the total process (Hsu and Deng, 1980) and this would not account for the 5 log increase observed during the drying period. After 72 hr drying, aerobic growth rate decreased. Water activity in the roe also decreased as drying time increased, and may account for the decreased

rate of bacterial growth. The roe that had been dried for 149 hr had an APC of 21 x 10^7, which was two times greater than roe after 72 hr of drying.

Fungi were first detected on the roe after 72 hr drying and continued to increase with time. This was expected since the roe was exposed to airborne contaminants during the drying period.

The regression models for APC and fungal counts during storage possessed R^2 values of 0.91 and 0.87, respectively.

Table 4—Effect of processing on microbiological counts[a] in intermediate moisture mellet roe

Processing steps	APC/g[b]	CFU/g[c]
Raw mullet roe after thawed in cooler (4°C)	19.0 X 10	0
After salting	26.0 X 10^2	0
After desalting	77.5 X 10^2	0
72 hr dehydration	11.1 X 10^7	78.0 X 10
149 hr dehydration (final product)	20.8 X 10^7	51.5 X 10^2

[a] Average of two determinations
[b] Aerobic plate counts
[c] Fungal counts

Table 5—Multiple regression coefficients for APC[a] and CFU[b]

Model term[c]	APC[d]	CFU[e]
Constant	8.2675	3.4079
X_1	0.1855**	−0.2553**
X_2	−0.0103	−0.3901
X_3	0.0193	0.0810**
X_1X_2	0.0158**	0.3381**
X_1X_3	−0.0539**	0.0812**
X_2X_3	0.0062	0.0162
$X_1X_2X_3$	−0.0087*	−0.0232
X_2^2	−0.0009	0.0114
$X_1X_2^2$	0.0007	−0.0150
$X_3X_2^2$	−0.0001	−0.0009**
X_3^2	−0.0005	−0.0040
$X_1X_3^2$	−0.0014*	−0.0021
$X_2X_3^2$	−0.0004	−0.0004
$X_1X_2^2X_3$	0.0003	0.0021
$X_1X_2X_3^2$	0.0006	0.0004
$X_1X_2^2X_3^2$	0.0000	−0.0001

[a] APC — aerobic plate count
[b] CFU — fungi counts
[c] X_1 = potassium sorbate
X_2 = storage time
X_3 = storage temperature
[d] R^2 of APC model is 0.91
[e] R^2 of CFU model is 0.87

Regression coefficients of the mathematical models are presented in Table 5. APC's were highly affected by potassium sorbate treatment, (X_1) and interactions between potassium sorbate treatment and storage temperature (X_1X_3 and $X_1X_3^2$) and potassium sorbate treatment and storage time (X_1X_2). The three factor interaction between potassium sorbate treatment, storage time and storage temperature ($X_1X_2X_3$) also was present on bacterial count. The growth of fungi was highly influenced (p 0.01) by the potassium sorbate (X_1) treatment and storage temperature (X_3). Interactions between potassium sorbate treatment and storage time (X_1X_2) as well as between storage time and storage temperature ($X_2^2X_3$) were also present (p 0.01) with respect to fungal growth.

Fig. 3 shows the plot of $\log_{10}$ bacterial counts of samples without potassium sorbate treatment. Bacteria grew at all temperatures from 5–25°C. This increase in $\log_{10}$ bacterial count at a water activity level of about 0.84 (Fig. 7) was not expected. It was reported that most bacterial growth ceases as the water activity level reaches 0.85 or lower (Brockmann, 1973). The unexpected bacterial growth in the intermediate moisture mullet roe during storage might have been due to the condensed water vapor on the roe surface. As mentioned in the experimental section, the roe were kept in polyethylene plastic bags during storage. The environment surrounding the mullet roe inside the bag might often have a higher relative humidity than 0.84 which would help to condense some water vapor on the roe surface. Since the roe were kept in the bag, the condensed water would not readily vaporize due to limited air flow even when the relative humidity of the environment was lower than 84%. The aerobic bacteria, therefore, continued to grow on the roe surface, although at a slower rate as compared to foods with high water activity. The higher the storage temperature, the greater was the bacterial growth rate. At the lower temperature (5°C), a lag phase of up to 10 days was detected. No lag phase was observed at the higher temperatures. At both 15 and 25°C, APC increases steadily with upward and downward concave curves indicating that the initial flora was predominately mesophilic in nature.

Aerobic counts in samples treated with potassium sorbate decreased at all temperatures during storage, with the rate of decrease, being greater at the high temperature, 25°C, (Fig. 4). Aerobic counts at 25°C decreased rapidly during the first 3 wk whereas those at the lower temperatures decreased most rapidly after 2 wk. This probably was due to the greater activity of the sorbate on the more physiologically active cells at the higher temperature. A lag phase

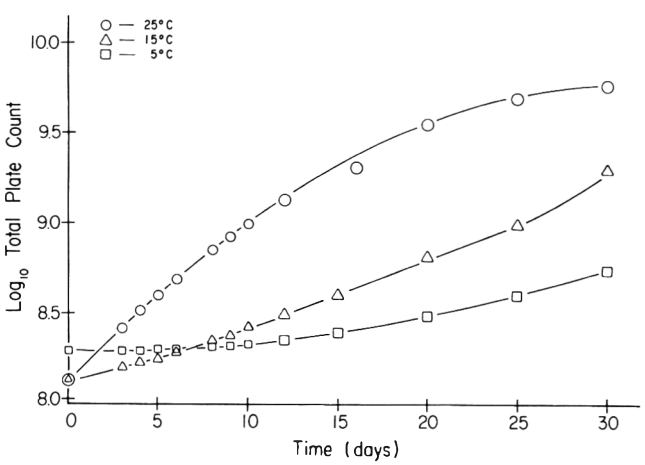

Fig. 3—Effect of various storage temperatures on $\log_{10}$ aerobic counts in intermediate moisture mullet roe without potassium sorbate treatment.

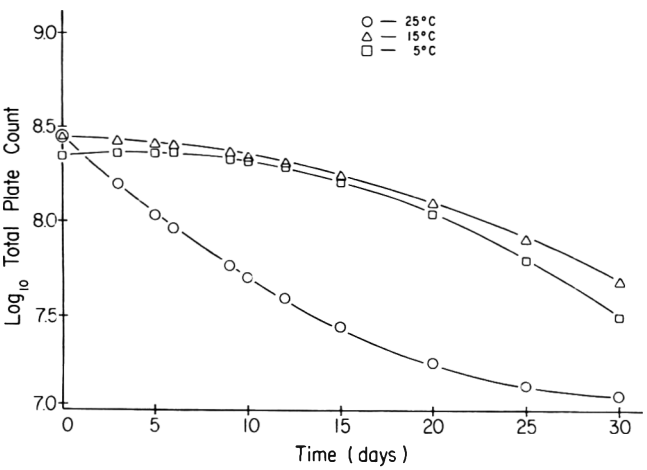

Fig. 4—Effect of various storage temperatures on $\log_{10}$ aerobic counts in intermediate moisture mullet roe treated with potassium sorbate.

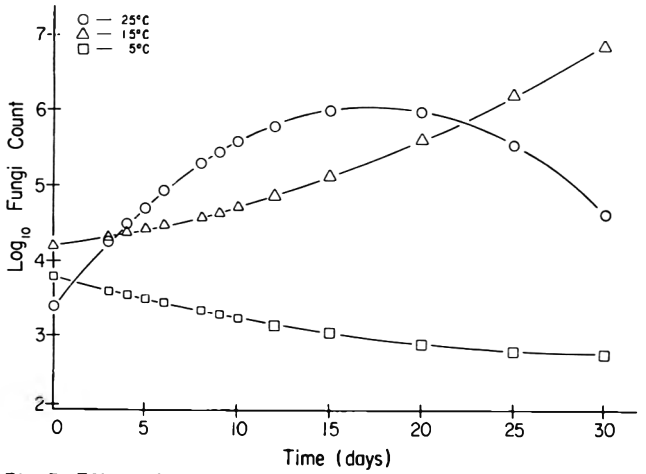

Fig. 5—Effect of various storage temperatures on log_{10} fungi counts in intermediate moisture mullet roe without potassium sorbate treatment.

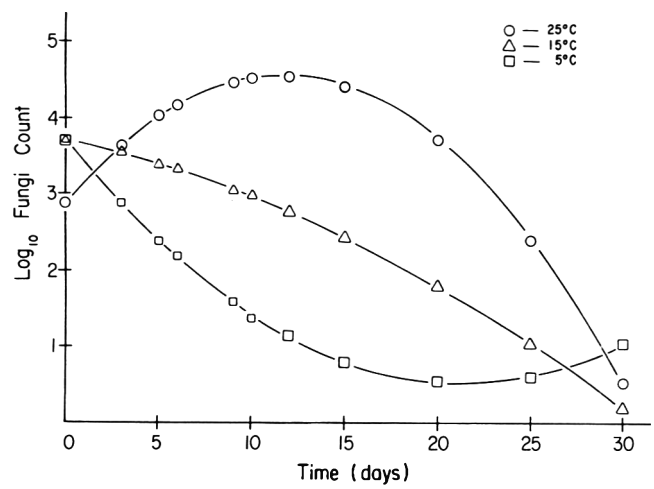

Fig. 6—Effect of various storage temperatures on log_{10} fungi counts in intermediate moisture mullet roe treated with potassium sorbate.

was detected at 5°C for up to 10 days storage. Although no lag phase was observed at 15°C, a very slow rate of APC decrease was found during the first 2 wk.

Log_{10} fungal growth in samples without potassium sorbate treatment are plotted in Fig. 5. Fungi grew rapidly at 25°C up to 15 days and then started to decrease after 20 days. Fungi increased gradually at 15°C but decreased slowly at 5°C. In samples treated with potassium sorbate, fungal growth decreased at 15°C and at a faster rate at 5°C (Fig. 6). At 25°C, fungi increased rapidly during the first 2 wk after which the counts decreased during the later storage periods. The critical storage time at 25°C for shifting of the growth trend is different for samples without potassium sorbate treatment which was 15 days (Fig. 5), and samples treated with potassium sorbate which was 10 days.

The results clearly indicated that potassium sorbate affected both fungal and bacterial growth in the intermediate moisture mullet roe. The effect of potassium sorbate on bacteria growth was greater than expected. It was also found that potassium sorbate was more effective in controlling the growth of yeast than that of mold. This observation was based on staining and visual scanning of the colonies developing on the plates. However, potassium sorbate concentration in the roe (0.024%) was too low to completely inhibit all the fungi present during the storage period.

Other quality factors

The color samples stored at 5°C did not change markedly after 30 days storage. But the samples stored at the other temperatures darkened with increasing temperature (45°C, 25°C, 15°C). The rate of quality change from golden brown to a dark color increased as temperatures increased from 15 to 45°C during storage. Samples stored at 15°C were dark brown in color within 30 days storage; while samples stored at 25°C turned dark after 10 days storage. At 45°C, samples became dark in color after only 5 days storage. The increase in color with temperature was expected. The browning of the product was due to the Maillard Reaction, an interaction between amino groups and reducing sugars (Lee, 1975). The rate at which browning occurs increases markedly with an increase in temperature. The rate of browning also depends on the moisture content or water activity of the material. The rate of browning reaches a maximum at some intermediate moisture content and water activity level, often in the range of about 15–20% moisture and 0.7–0.85 water activity (Van Arsdel, 1973; Labuza et al., 1970). The intermediate moisture mullet roe maintained a constant water activity level around 0.84 during storage

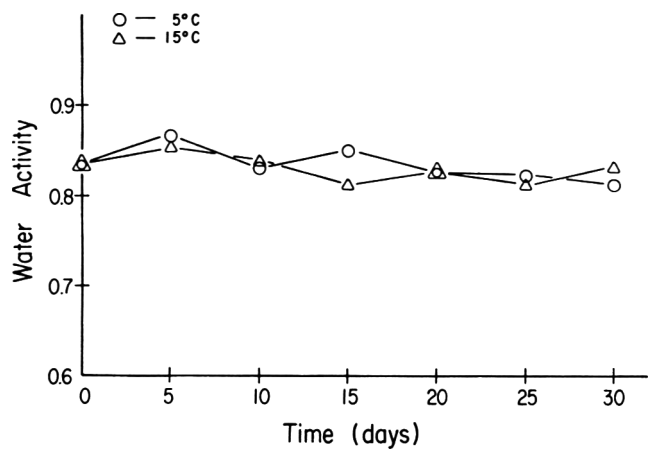

Fig. 7—Water activity of intermediate moisture mullet roe stored at 5°C and 15°C.

(Fig. 7) which is close to the maximum activity range of the browning reaction. Lipid oxidation was probably another factor that attributed to the color change. Oxidation end products, i.e. aldehydes, can interact with amino groups and form brown pigments (Lee, 1975). This was confirmed by the findings that the external roe layer, which were exposed to the oxygen environment, had a darker color than the interior roe. The thickness of the dark layer (which indicates the degree of lipid oxidation) increased as the storage temperature increased and was closely related to the change in TBA value.

Flavor of the roe stored at 5°C (with and without potassium sorbate), 15° and 25°C (with potassium sorbate) for 30 days was still acceptable, which indicates that the level of oxidative rancidity and microbial growth were not high enough to seriously affect the roe quality. This would indicate that it is possible to prepare intermediate moisture mullet roe based on the method of Hsu et al. (1980).

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—Continued on page 196

Control of Heat Induced Oxidative Rancidity in Refrigerated Shark and Mackerel

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ABSTRACT

Oxidative rancidity in heated shark and mackerel was determined by the thiobarbituric acid (TBA) test and sensory evaluations. Control measures used were 1% and 2% hot water extract of yellow onion peel and 1% onion juice. TBA values of heated shark and mackerel were significantly affected by storage periods and experimental treatments ($P<0.05$). A 2% yellow onion peel hot water extract was the most effective means of reducing rancidity in both shark and mackerel tissue. Fatty acid analyses showed that both tissues contained oleic acid (18:1ω9); mackerel contained a higher percentage of palmitic acid (16:0) than shark. Decosahexaenoic acid (22:6ω3) was present in larger amounts in mackerel than in shark.

INTRODUCTION

FOOD FROM THE SEA has been an important renewable source of protein for man since ancient times. In modern history the need for protein is growing at a rate greater than that of protein production. Lands for livestock production are limited and pressure is increasing to obtain protein from the sea to feed a rapidly expanding world population. A potential source of valuable protein exists in the sea. Turning to underutilized species of fish might aid in sustaining the world populace.

Two species of fish which have not achieved widespread popularity are mackerel and shark. Mackerel is marketed in the canned form to a small degree, but shark meat is virtually unused in any form in most American dietaries. Both contain high quality protein as well as other nutrients and might improve nutritional status, particularly in developing countries. Shark, in addition, feed on many already shrinking marine species and cause considerable damage to fishing gear. Therefore it seems inevitable that fishing efforts should be directed toward them.

The purchase of fish, either in the raw or precooked state has increased recently as Americans have become more concerned about the consumption of fat and polyunsaturated fatty acids. However, one critical problem with heated seafood products has been that their storage life, whether in a refrigerator or freezer, has been shortened by the problem of oxidative rancidity, a deteriorative mechanism known to be a problem in many types of precooked meats (Younathan et al., 1980; Deng, 1978). Control of rancidity might improve sensory acceptability of both mackerel and shark, thus increasing consumption.

Oxidative rancidity leads to off odors and flavors in cooked meats (Tims and Watts, 1958; Pearson et al., 1977; Sweet, 1973). Both freshly cooked meats or those stored in the freezer for several months are susceptible (Younathan and Watts, 1959, 1960; Caldironi and Bazan, 1982). Intramuscular phospholipids have been shown to be the most rapidly oxidized lipid component in cooked meat (Igene and Pearson, 1979). The tendency of phospholipids to oxidize very rapidly may be related to their polyunsaturated fatty acid content. Oxidation proceeds at a very rapid rate following tissue heating. Heme proteins are denatured, there is general disorientation and destruction of cellular structure, permitting intimate mixing of cellular constituents. The reaction probably is catalyzed by both heme and nonheme iron (Igene and Pearson, 1979; Reineccius, 1979).

Synthetic compounds such as butylated hydroxyanisole, butylated hydroxy-toluene, and propyl gallate are commonly used antioxidants in the food industry. These antioxidants are not available to the general public. Therefore this investigation focused on the use of natural food products as antioxidants. Onions were chosen because they often are used as an added ingredient in many cooked foods. The purpose of the study was to determine the effectiveness of onion juice and water extracts of onion peel in controlling oxidative rancidity in heated shark and mackerel after refrigerator storage.

MATERIALS & METHODS

Source of fish

Frozen whole mackerel and shark fillets were purchased in one lot from a seafood market in New Orleans. Previous histories of the fish were unknown.

Preparation of samples

The fish were defrosted at refrigerator temperature (4°C) overnight. Mackerel were filleted and deboned; one fish was used for each experiemental treatment in aliquots of 500g each. A large shark fillet was divided lengthwise into four portions of 600g each. The antioxidant to be tested was brushed on the fillet before cooking and when half-cooked. Samples were baked on a rack in a 177°C oven until the flesh flaked easily, which required about 20 min. After cooking, the meat was flaked with a fork into a fine texture, mixed to obtain a homogenous mass, and divided into three portions. Mackerel skins were removed prior to mixing. One portion was assayed on the day of heating, the other two were placed in air tight glass containers (nonsterile), stored at 4°C, then assayed after 1 and 3 days of storage. Prior to testing, samples were reheated for 10 min in a 149°C oven. A fresh reference sample was prepared for each sensory test. The procedure was repeated for five consecutive weeks.

Experimental treatments for the fillets consisted of adding 1% onion juice or 1% or 2% hot water extract of yellow onion peel (6 ml or 12 ml added to 600g fish). Onions were purchased in one lot for the five replications. Onion juice was prepared in a food press. Hot water extract of onion peel was prepared by blending 20g peel with 100 ml distilled water in a Waring Blendor for 2 min, boiling for 5 min, then filtering (Pratt and Watts, 1964).

Sensory tests

An experienced panel of seven members scored samples of fish for the presence of a warmed-over or rancid odor by checking an appropriate description on a score card. Descriptive terms used were: absent, very slight, slight, moderate, strong and very strong. Numerical values ranging from 0 (absent) to 6 (very strong) were assigned to each rating for obtaining average panel scores. Samples were evaluated in a room away from the preparation area. A control sample and 3 treated samples, along with a freshly cooked reference sample were presented to the panel on the day of heating, and again after 1 and 3 days of storage.

Thiobarbituric acid tests (TBA)

Immediately after removal of fish from the oven, four 10-g

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samples were weighed and placed in clean distillation flasks. TBA values were determined by the method of Tarladgis et al. (1960).

Fatty acid profile

The percentage fatty acid composition in shark and mackerel tissue were evaluated for comparative purposes. A mixture of chloroform:methanol (2:1, v/v) containing 0.02% butylated hydroxytoluene (BHT) was used to extract fish lipids. Analysis was performed using a Hewlett Packard Gas Chromatograph 5830A, with a 3.6 m stainless steel column packed with 10% silar 10C, Gas Chrom Q_1 100/120 mesh. Temperatures at the injection post, the column, and hydrogen flame ionization detector were 250°C, 200°C, and 300°C, respectively. Since the retention time of BHT was similar to that of myristic acid under these experimental conditions, myristic acid could not be quantitated. Individual fatty acid methyl esters, prepared according to the method of Morrion and Smith (1964), were identified by comparison of retention times (Hwang and Kinsella, 1979; Hwang and Carroll, 1980) of a standard mixture of fatty acid methyl esters (Nu Chek Prep, Elysian, Minn.) and the equivalent chain length method (Hofsteller et al., 1965), using one replication.

Statistical analyses

Analysis of variance using a randomized block design was employed to compare the effect of storage and antioxidant treatments for both TBA values and panel scores. Blocking was done on weeks where each week represented a complete replication. Appropriate sets of orthogonal comparison and linear correlation also were completed.

RESULTS & DISCUSSION

Influence of antioxidants on TBA values

Thiobarbituric acid values for heated mackerel and shark were significantly affected by storage periods and antioxidant treatments ($P<0.01$) as shown in Table 1. Orthogonal comparisons indicated that highly significant differences existed among the control and other treatments ($P<0.01$). TBA values for antioxidant treated shark and mackerel were consistently lower than the control. Samples treated with an onion peel-hot water extract, whether at the 1% or 2% level, had lower TBA values than those treated with onion juice.

All samples showed increased TBA values with storage. Although initial values were low, a rapid increase occurred during the first day of storage, with a slower rate between day 1 and day 3. When comparing TBA values of 0 day storage with those of 1 and 3 days storage, a significant difference was found ($P<0.05$). These results are in agreement with data of other investigators who reported an increase in TBA values in heated, stored meats as beef, turkey, pork, or chicken (Younathan et al., 1980; Keller and Kinsella, 1973; Johnson et al., 1974). Shark tissue had lower TBA values than mackerel.

The interaction effect between treatment and storage period was significant ($P<0.05$) for shark tissue. TBA values increased with storage ($P<0.05$). The presence of flavonoids, ascorbic acid and sulfur compounds in the extract probably contributed to the antioxidant effect of onion extract.

Influence of antioxidants on panel scores

There was a highly significant difference ($P<0.01$) in panel scores among the treatments applied to both mackerel and shark. Table 2 indicates that the control received higher (less desirable) panel scores than antioxidant treated samples. Highly significant differences ($P<0.01$) were found among panel scores for storage periods.

Panelists in this study seemed to detect a stronger rancid odor in mackerel than in shark. Differences in the proportion of fat in the two tissues, no doubt, contributed to this casual observation.

—Continued on next page

Table 1—Effects of treatments and days storage on TBA values (mg malonaldehyde per 1000g tissue) of heated mackerel[a] and shark[c]

	Mackerel				Shark			
	Storage days				Storage days			
Treatment	0	1	3	Average[b]	0	1	3	Average[d]
Control	2.70	4.74	6.03	4.49	0.28	1.21	2.14	1.21
1% onion peel extract	1.68	3.28	4.47	3.14	0.35	0.77	0.97	0.70
2% onion peel extract	1.42	2.85	4.09	2.79	0.23	0.38	0.59	0.40
1% onion juice	2.04	4.13	5.08	3.75	0.33	1.03	1.95	1.10
Average[b,d]	1.96	3.75	4.92		0.30	0.85	1.41	

[a] Values are mean of five samples.
[b] Treatment and storage days were highly significant ($P < 0.01$). Control vs antioxidants (onion peel extract and onion juice) was significant ($P < 0.05$). Treatment x storage days interaction was not significant ($P > 0.05$).
[c] Values are mean of ten samples.
[d] Treatment and storage days were highly significant ($P < 0.01$). Control vs. antioxidants (onion peel extract and onion juice) was highly significant ($P < 0.01$). Onion peel extract vs 1% onion juice was highly significant ($P < 0.01$). Treatment x storage days interaction was significant ($P < 0.05$).

Table 2—Effects of treatments and days storage on panel scores[a] for heated mackerel and shark

	Mackerel				Shark			
	Storage days				Storage days			
Treatment	0	1	3	Average[b]	0	1	3	Average[c]
Control	1.23	1.91	2.51	1.88	0.37	0.57	1.09	0.68
1% onion peel extract	0.66	1.00	1.68	1;11	0.26	0.49	0.57	0.44
2% onion peel extract	0.43	0.83	1.51	0.92	0.43	0.49	0.46	0.46
1% onion juice	1.06	1.54	2.17	1.59	0.17	0.34	0.43	0.31
Average[b,c]	0.85	1.32	1.97		0.31	0.47	0.64	

[a] 0, absent to 6, very strong
[b] Treatment differences were highly significant ($P < 0.01$). Interaction effect between panel scores and treatments was not significant ($P > 0.05$). Storage days x panel scores interaction was not significant ($P > 0.05$).
[c] Treatment and storage days were highly significant ($P < 0.01$). Treatment and storage days interaction was not significant ($P > 0.05$).

Table 3—Fatty acid composition of mackerel meat

Fatty acids	Cephalic %	Medial %	Caudal %
16:0	31.35	28.18	29.54
16:1ω7[a]	7.26	6.47	6.39
18:0	7.58	11.01	9.97
18:1ω9	32.15	32.03	31.41
18:2ω6	1.17	1.47	1.21
18:3ω6	1.07	1.34	1.24
18:3ω3	0.52	0.53	0.54
20:2ω6	0.71	0.81	0.72
20:3ω6	0.18	0.40	0.11
20:4ω6	1.64	1.49	1.30
20:5ω3	3.02	3.30	3.37
22:0	0.30	0.09	0.06
22:4ω6	0.14	0.24	0.23
22:5ω6	9.84	0.83	0.78
22:5ω3	0.84	1.22	1.09
22:6ω3	11.49	10.57	12.07
% of polyunsaturated fatty acid	21.65	22.20	22.12

[a] Fatty acids denoted by their carbon chain length: number of double bonds and ω indicates location of the first double bond from the methyl end.

Table 4—Fatty acid composition of shark meat

Fatty acids	Cephalic %	Medial %	Caudal %
16:0	13.60	14.59	14.47
16:1ω7[a]	7.25	8.69	7.76
18:0	10.62	11.03	12.16
18:1ω9	37.75	34.53	26.24
18:2ω6	3.34	3.47	8.59
18:3ω6	1.12	0.81	1.78
18:3ω3	0.62	0.59	—
20:3ω9	5.90	5.45	5.41
20:4ω6	2.47	2.70	3.53
20:5ω3	4.16	4.14	5.01
22:0	3.74	3.15	3.09
22:4ω6	0.76	0.95	1.05
22:5ω6	0.65	1.04	0.92
22:5ω3	2.25	2.21	2.53
22:6ω3	5.73	6.62	7.50
% of polyunsaturated fatty acid	27.0	27.98	36.32

[a] Fatty acids denoted by their carbon chain length: number of double bonds and ω indicates location of the first double bond from the methyl end.

Fatty acid analyses of shark and mackerel

In general, the percent of polyunsaturated fatty acids in the cephalic, medial, and caudal portions of shark and mackerel are similar for each species (Tables 3 and 4). Values reported for oleic acid (18:1ω9) content in both tissues may have been influenced by the elimination of myristic acid. Mackerel showed a higher percentage of palmitic acid (16:0) than did shark. The fatty acid with the same retention time as eicosatrienoic acid (20:3ω9) was present in shark but not in mackerel. Decosahexaenoic acid (22:6ω3) was present in higher amounts in mackerel than shark. Total fat, as well as polyunsaturated fatty acid content of shark and mackerel, may be useful in planning dietaries for cardiovascular patients. Studies are underway to determine total fat content of these tissues.

CONCLUSION

SHARK AND MACKEREL are potential palatable sources of protein which can be used to help alleviate hunger among the world populace. In addition, they contain a high percentage of polyunsaturated fatty acids. Oxidative rancidity occurred in heated tissues, especially after storage. Onion extracts rich in flavonoids, ascorbic acid and sulfur compounds were successful control measures for rancidity.

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Evaluation of Harp Seal Gastric Protease as a Rennet Substitute for Cheddar Cheese

K. SHAMSUZZAMAN and N. F. HAARD

ABSTRACT

A crude preparation of gastric proteases from Harp Seal (*Pagophilus groenlandicus*) was found to coagulate milk over a wider pH range than porcine pepsin and had a higher ratio of milk clotting to proteolytic activity with hemoglobin at pH 1.8. Cheddar cheese prepared with seal gastric protease (SGP) gave significantly higher sensory scores than cheese made with calf rennet. Chemical analysis of the cheeses revealed a lower concentration of citrate-HCl soluble nitrogen and less free and peptide-bound amino acids in SGP cheese than in the cheeses made with calf rennet and *Mucor miehei* protease.

INTRODUCTION

THERE HAS BEEN a continued interest in the search for rennet substitutes ever since a rennet shortage was anticipated in the 1960's because of a decline in the number of calves slaughtered and an increase in demand for cheese (deKoning, 1978). In 1974, two-thirds of the cheese manufacturing in the United States utilized rennet substitutes obtained from sources such as *Mucor miehei, Endothia parasitica* or *Mucor pusilus* (Huang and Dooley, 1976). Rennet substitutes of microbial origin have been accepted by the industry, although they have not been totally satisfactory (Ernstrom, 1974). A limitation of microbial rennets may be the relatively broad specificity of the enzyme(s) present in the rennet. Plant proteases have been employed experimentally as rennet substitutes and appear to give rise to a softer curd than calf rennet (Oosthuizen and Scott Blair, 1963). Swine pepsin is utilized as a rennet substitute but its ability to clot milk diminishes rapidly above pH 6.5 (Ernstrom, 1961). Residual swine pepsin activity has not been detected in Cheddar cheese curd following pressing, whereas about 5% of calf chymosin activity survives the manufacturing process (Holmes, 1973). Limited proteolysis by the clotting enzyme apparently contributes to desirable aged cheese flavor. Bovine pepsin will clot milk up to pH 6.9 (Fox, 1969) although it gives rise to differences in texture and flavor (Emmons et al., 1976: Stanley and Emmons, 1977). Chicken pepsin has also been employed as a rennet substitute (Green, 1972; Gordin and Rosenthal, 1978); however, Cheddar cheese prepared with this enzyme can have intense off-flavors. The purpose of this study was to evaluate the suitablity of gastric proteases from a marine mammal (*Pagophilus groenlandicus*) as a rennet substitute.

MATERIALS & METHODS

STOMACHS from 2 wk-old seal pups were collected by Fisheries and Oceans officers during the annual seal hunt off the coast of Newfoundland. Frozen stomachs were shipped to St. John's and on arrival were thawed, cleaned and subsequently frozen and stored at −20°C prior to extraction of gastric proteases. Calf chymosin and porcine pepsin were obtained from Sigma Chemical Co. (St. Louis, MO). *Mucor miehei* enzyme (fromase 100) was obtained from G.B. Fermentations, Ind. (IL). Hansen's buttermilk culture and rennet were obtained from Horan-Lally Co. (Mississauga, Ontario).

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Isolation of gastric proteases

The procedure of Qadri et al. (1962), with some modification, was employed to isolate gastric proteases from seal stomach. The frozen stomachs were lyophilized, powdered in a Waring Blendor and extracted with 10% acetic acid (20 ml:lg) by stirring at 4°C for 12 hr. The slurry was adjusted to pH 5.8 with sodium hydroxide (1N) and was centrifuged at 40,000 x *g* for 30 min at 4°C. The supernatant was collected and concentrated approximately 10-fold with a PM 10 membrane in an Amicon ultrafiltration apparatus. This preparation (SGP) was employed for enzyme characterization and Cheddar cheese production.

Milk clotting assay

A milk clotting assay was performed at 37°C as described by Foltmann (1970) at a milk sample volume of 1.0 ml in 10 x 75 mm borosilicate glass tubes. Milk clotting units were as defined by Berridge (1945).

Hydrolysis of hemoglobin

The method of Bohak (1970) with minor modification was used to measure enzyme catalyzed hydrolysis of hemoglobin. Hemoglobin (Type 111, Sigma Chem., Co., St. Louis, MO) was prepared for assay by dialyzing a 7.5% solution against 50 volumes (x 3) of 0.06N HCl at 4°C and lyophilization. A 2% solution of prepared hemoglobin in 0.06N HCl (8.75 ml) was incubated with 1.25 ml of enzyme solution at 37°C. After various time intervals, 1.25 ml samples were withdrawn and mixed with 2.5 ml of 5% trichloroacetic acid (TCA), incubated at 4°C for 30 min, centrifuged at approximately 3,000 x *g* for 30 min and the A 280 nm of the supernatant was recorded.

Hydrolysis of casein

Purified casein (Sigma Chemical Co., St. Louis, MO) was solubilized by the method of Foltmann (1959) and was adjusted with HCl to pH 6.1. A 10 ml solution of 2% casein containing 0.5 ml of enzyme preparation was incubated at 37°C. At various time intervals, 1.0 ml aliquots were mixed with 1.0 ml TCA (10%). The mixture was cooled at 4°C for 30 min and centrifuged at approximately 3,000 x *g* for 30 min. The A 280 nm of the supernatant fraction was recorded. A control was prepared by adding enzyme to the casein solution after addition of TCA.

Cheddar cheese preparation

One lot of Cheddar cheese was prepared with each type of coagulant using 8L of whole milk following the method of Kosikowski (1978). The milk was clotted using equivalent clotting units of SGP, commercial rennet and fromase 100. The cheese blocks (approx. 1 lb) were vacuum packed in polythene bags and ripened at 8°C in a Hotpack temperature humidity chamber for 7 months.

Analysis of cheese and whey

The water content of cheese was determined by the distillation method (AOAC, 1970). Citrate-HCl soluble nitrogen was determined by the method of Vakaleris and Price (1959). Amino acid analysis of citrate-HCl extracts of cheese was performed with a Beckman model 121 MB amino acid analyzer using the methods described in Beckman bulletin 121 M-TB-013. To determine free amino acids, the citrate-HCl extract was mixed with 4 volumes of 20% sulphosalicylic acid to precipitate protein prior to analysis.

—Continued on next page

Total and nonprotein nitrogen in whey was determined following the method of Rowland (1938).

Sensory evaluation of cheese

Cheeses were evaluated by preference test (Larmond, 1977) at the taste panel room in the Dept. of Fisheries and Oceans, St. John's. The cheeses employed for sensory evaluation were aged for 7 months at 8°C. Thirty untrained panelists were provided with number-coded cheese samples (3x3x2 cm), and general information about Cheddar cheese. Panelists were asked to rate the samples as to overall preference on a numerical scale ranging from 9 (like extremely) to 1 (dislike extremely). The data were evaluated by analysis of variance (Larmond, 1977).

RESULTS & DISCUSSION

Characterization of seal gastric protease

Unlike porcine pepsin, SGP clotted milk rapidly at pH values between 6.3 and 6.6 (Fig. 1). Pepsin differs from calf chymosin in its inability to clot milk at high pH (Ernstrom, 1961; Gordin and Rosenthal, 1978). In this respect, the SGP preparation has some similarity with chymosin. The inability of SGP to clot milk above pH 6.6 may be a limitation since some Cheddar cheese is prepared at pH 6.6 or slightly lower. A miscalculation in milk ripening on the part of the cheesemaker may lead to difficulties when milk is clotted at this pH.

The influence of enzyme dilution on clotting time is shown in Fig. 2. The clotting potency of SGP decreased more rapidly as the dilution was increased. Gordin and Rosenthal (1978) observed that chicken pepsin, like SGP, exhibits a greater increase in clotting time on dilution than does calf chymosin.

The ratio of milk clotting activity to hemoglobin hydrolysis activity is summarized in Table 1. This ratio is often used to assess the suitability of enzymes as rennet substitutes (Green, 1972). These data indicate SGP has intermediate proteolytic specificity relative to calf chymosin and swine pepsin at pH 1.8 with hemoglobin as substrate. The ratio of clotting activity to hydrolysis of casein at pH 6.1 was also examined. The results differ from those obtained when the assay was carried out at pH 1.8 (hemoglobin) in that SGP exhibited a lower ratio of clotting activity to protein hydrolysis than porcine pepsin. However, it should be pointed out that the reaction of casein hydrolysis catalyzed by porcine pepsin declined after 60 min while the reactions catalyzed by calf chymosin and SGP were linear for the 2 hr time period of the experiment (Fig. 3). The data in Table 1 is based on the initial velocities of the reaction. Accordingly, the data indicate SGP is more active in casein hydrolysis at pH 6.1 than equivalent clotting units of porcine pepsin or calf chymosin.

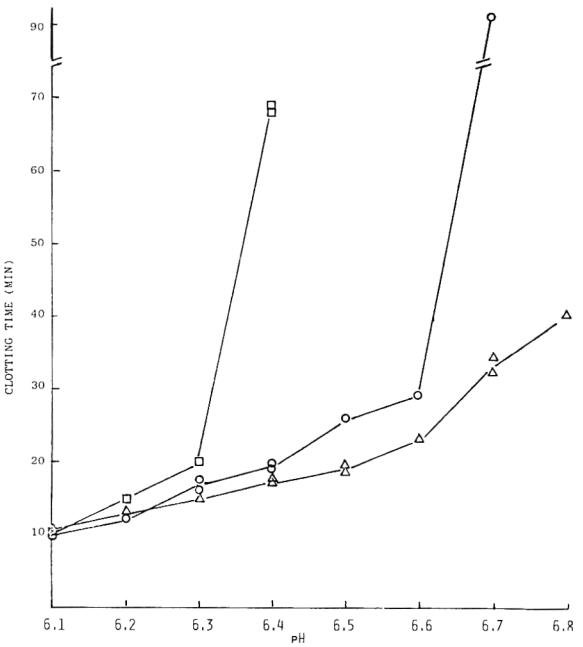

Fig. 1—Influence of pH on milk clotting times by the enzymes. Enzyme concentrations were adjusted to have equal milk clotting times at pH 6.1. The milk pH was adjusted with 1M NaOH or HCl. Duplicate determinations were made at each pH value. Porcine pepsin □, seal gastric protease ○ and calf chymosin △.

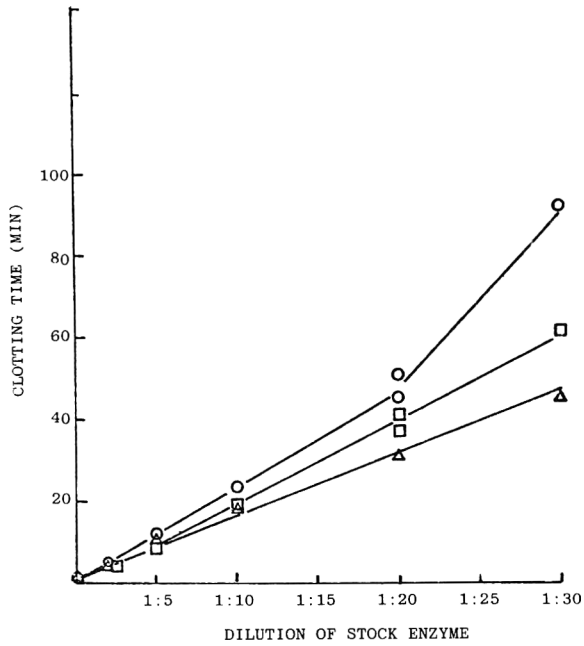

Fig. 2—Effect of dilution on the milk clotting times of the enzymes. A stock solution containing 0.1 clotting unit of the respective enzymes in 50 µl of 20 mM sodium acetate at pH 5.8 was diluted with the same buffer as indicated and 50 µl aliquots of the diluted enzymes utilized for clotting milk samples in duplicate at 37°C. Porcine pepsin □, SGP ○, and calf chymosin △.

Cheddar cheese

The yields of cheese prepared using *Mucor miehei* enzyme, SGP and calf rennet were similar (Table 2). This is consistent with the similar recovery of nonprotein and total nitrogen in the wheys and cheese (Tables 2 and 3). The greater hydrolytic activity of SGP on casein at 37°C and pH 6.1 did not seem to result in greater degradation of proteins

Table 1—Ratio of clotting activity to protein hydrolysis

Enzyme	Substrate[a] Hemoglobin CU/PU	Casein CU/PU
Calf Chymosin	0.170	1.61
Porcine Pepsin	0.005	0.39
SGP	0.025	0.26

[a] Pepsin unit (PU) and clotting unit (CU) are as defined by Anson (1938) and Berridge (1945) respectively. Data are averages of two trials and representative of additional trials.

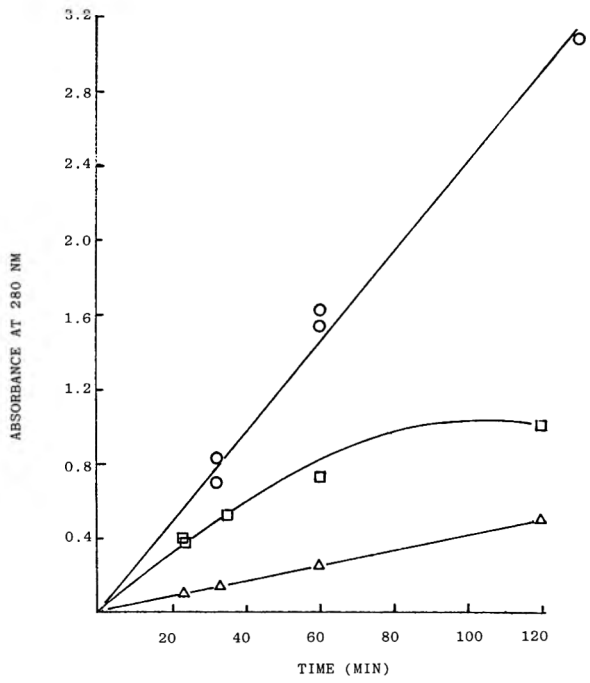

Fig. 3—Time course of Casein hydrolysis at pH 6.1. Ten ml of a 2% casein solution was incubated with 0.5 ml of each of the three enzymes at 37°C. At intervals 1 ml samples were withdrawn in duplicate and amount of hydrolysis product determined as described in the text. Porcine pepsin □, SGP ○, chymosin △.

in aged cheese. As shown in Table 4, the citrate-HCl extract obtained from SGP cheese after aging had lower concentration of materials absorbing at 274.5 nm than those from cheeses prepared with calf rennet or *Mucor* enzyme. Similarly, the free and peptide amino acid contents were lowest in SGP cheese (Table 4). Although SGP hydrolyzes casein faster than does chymosin at 37°C (Fig. 3) it may be less active under conditions of cheese preparation and aging at 8°C. It is also possible that proteolytic activity of bacterial origin is differentially affected. Previous studies have shown

Table 2—Comparison of the yields of Cheddar cheeses

Clotting enzyme	Yield of cheese (g/100 ml milk)		Moisture[a]	%N in whey (as protein)[a]	
	Total wt basis[b]	Dry wt basis	(%)	Total	Nonprotein
Seal gastric protease	9.961	6.149	38.26	0.7847	0.3298
Calf Rennet	10.040	6.184	38.41	0.6954	0.3209
Mucro miehei enzyme	10.000	6.134	38.66	0.8396	0.3247

[a] Data are averages of duplicate determinations.
[b] Actual weight without correction for moisture difference.

Table 3—Proximate analyses of aged Cheddar cheeses

Clotting enzyme	Percent composition[a]		
	Fat	Moisture	Protein[b]
Calf Rennet	30.97 (31.18)	38.41	25.19 (25.36)
Seal Gastric proteases	30.92 (31.05)	38.26	25.23 (25.34)
Mucor miehei enzyme	31.06 (31.39)	38.66	25.02 (25.29)

[a] Data are mean values of duplicate determinations; values in parentheses are normalized for 38% moisture content.
[b] Calculated by multiplying Kjeldahl N by a factor of 6.38.

Table 4—Analysis of citrate-HCl soluble N in experimental Cheddar cheeses

Clotting enzyme	Citrate-HCl soluble N (A274.5 nm)	Amino acids (μ moles/g cheese)	
		Free	Total
SGP	1.430	32.3	220.2
Calf Rennet.	1.623	38.9	269.2
Mucor miehei enzyme	1.665	35.1	315.4

[a] Cheeses were vacuum packed in polythene bag and aged for 7 months at 8°C; data are averages of duplicate determinations.

that the flavor intensity of Cheddar cheeses positively correlates with free amino acid content and the content of tyrosine and soluble peptides containing tyrosine (Vakaleris and Price, 1959). While the concentration of free tyrosine was almost equal in the three cheeses, concentrations of total amino acids both free and in peptides were lowest in aged SGP cheese (Table 5). Nevertheless the sensory scores for SGP cheese were significantly higher than for the cheese prepared with calf rennet at 5% level (Table 6). Some panelists indicated that SGP cheese had a stronger Cheddar flavor than the other cheeses. The stronger Cheddar flavor cannot be accounted for on the basis of protein degradation products and other parameters such as the quality of protein degradation products, active –SH groups (Singh and Kristoffersen, 1969), methyl sulphide (Manning, 1978) or fatty acids (Schormüller, 1968) are probably a better indication of flavor. In view of the high milk clotting activity of SGP at various milk pH's, high ratio of milk clotting to proteolytic activity and production of good flavored Cheddar cheese it is concluded that SGP is promising as a rennet substitute for making cheese by conventional methods which involve ripening of milk to pH lower than 6.6 before renneting. SGP may also be used to curdle milk at a higher pH by using a higher concentration of the enzyme.

Recent studies at this laboratory have shown that stomach extracts from adult harp seals have properties similar to those from two week old pups and would probably be equally suitable for making cheese. The total annual catch of pups and adult harp seals in Newfoundland is about 91,000 taking an average of the data for the years 1976–79. By following a modified extraction method the estimated total yield of coagulant from these seal stomachs would be sufficient to clot about 31 million liters of milk.

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Table 5—Amino acid composition of citrate-HCl extracts from Cheddar cheeses

Amino acid	Calf rennet		*Mucor miehei*		SPG	
	Free (μ mole/g)	Total (μ mole/g)	Free (μ mole/g)	Total (μ mole/g)	Free (μ mole/g)	Total (μ mole/g)
Cysteic acid	0.9	0.5	0.8	0.3	1.4	0.3
Glycerophosphoethanol amine	1.5	0.0	1.3	0.0	1.1	0.0
Aspartic acid	1.6	22.3	0.9	23.2	1.5	16.1
Methionine sulphone	0.0	0.0	0.0	trace	0.0	0.0
Threonine	0.6	9.8	0.8	12.0	0.9	6.8
Serine	1.2	17.9	1.7	20.4	0.5	13.2
Asparagine	2.5	*	2.4	*	1.9	*
Glutamic acid	4.4	56.8	4.3	61.2	4.2	44.3
Glutamine	1.4	*	1.8	*	2.2	*
Proline	0.7	30.4	0.8	38.3	0.4	23.0
Glycine	0.9	8.9	0.6	8.6	0.8	6.5
Alanine	1.0	9.1	1.0	8.5	1.5	6.2
Citruline	0.2	0.0	0.1	trace	trace	0.0
Valine	2.6	1.3	2.8	23.1	1.3	22.1
Half cystine	trace	0.3	trace	0.4	trace	0.2
Methionine	1.0	4.8	1.4	6.1	1.2	3.6
Cystathionine	trace	0.1	0.0	0.1	0.0	0.0
Isolencine	0.5	16.0	0.5	17.5	0.4	11.3
Leucine	7.7	26.4	4.2	28.4	6.1	20.5
Tyrosine	1.4	9.2	1.5	9.9	1.4	6.5
Phenylaline	4.3	11.3	3.8	13.1	2.3	8.2
γ-Aminobutyric acid	0.1	0.0	0.0	0.0	0.1	0.0
Tryptophan	0.1	0.0	0.1	0.0	0.1	0.0
Ornithine	0.3	0.2	0.4	0.3	0.1	0.1
Lysine	1.2	24.2	0.9	24.6	1.2	16.6
Histidine	0.2	7.9	0.2	8.4	0.2	5.8
Arginine	2.6	9.5	1.2	9.1	1.9	7.1
Taurine	0.0	2.3	0.0	1.8	0.0	1.7
β-Alanine	0.0	0.0	1.5	0.0	0.0	0.0
TOTAL	38.9	269.2	35.1	315.4	32.3	220.1

* Asparagine and glutamine are converted to corresponding acid during acid hydrolysis.

Table 6—Sensory preference score for experimental Cheddar cheese

Clotting enzyme	Mean score[a]
Calf Rennet	5.67a
SGP	6.83b
Mucro miehei enzyme	5.90ab

[a] Values bearing different letters differ significantly ($P < 0.05$); 9 = Like extremely, 1 = Dislike extremely.

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Examination of Sugars, Sugar Alcohols, and Artificial Sweeteners as Substitutes for Sucrose in Strawberry Jam. Product Development

LEA HYVÖNEN and RAIJA TÖRMÄ

ABSTRACT

Preparation of acceptable low sugar jams and replacement of sucrose by other sweeteners in jam were studied. Strawberry jam was sweetened with sucrose, fructose, high fructose syrup (HFS), xylitol, sorbitol, lactose, saccharin, cyclamate, or with combinations of these. It was technologically possible to prepare jams with lower amounts of sucrose than currently used and still attain an acceptable product. In addition, sucrose can be replaced in strawberry jam by other sweeteners or by combinations of sweeteners. The attainment of a suitable texture may be more difficult in xylitol and sorbitol jams than in jams with other sweeteners. The use of maltodextrin as bulking agent in jam is limited by the abnormal appearance and taste it gives to the product.

INTRODUCTION

THE DIETARY AWARENESS of consumers has brought demands for the reduction of the sugar content of industrially prepared foods and the replacement of sucrose by other sweeteners. Sweetening is almost the only function of sweeteners in drinks. Low-calorie drinks of good quality could be prepared by taking advantage of the combinations of noncaloric and carbohydrate sweeteners (Hyvönen et al., 1978a, b).

Sugars in solution exert an osmotic pressure which manifests itself in a reducing water activity as the concentration of sugar rises. In jam manufacture the principal spoilage organisms are the yeasts and moulds, so it is necessary to reduce the water activity to at least 0.8. This means that a 60–65% sugar (sucrose, invert sugar) content is needed in jam to prevent microbial spoilage (Salunkhe et al., 1963; Glicksman and Farkas, 1966; Nicol, 1980). The use of preservatives makes it possible to reduce the sugar content without risking a reduction in the keeping quality.

Sweetening, gel formation and color retention are the other roles of added sugar in jam. The present study was an attempt to investigate these functions of the new sweeteners in strawberry jam.

MATERIALS & METHODS

DEEP-FROZEN STRAWBERRIES (Senga Sengana) were used in the preparation of the jams. The alternative sweeteners comprised: 99% pure sucrose, 99.5% fructose, 99.8% xylitol, 96% sorbitol (Finnish Sugar Co., Helsinki, Finland); high fructose syrup containing 41–43% fructose, 52–55% glucose, 3–6% maltose (Hämeen Peruna Oy, Jokioinen, Finland); maltodextrin containing 2% glucose, 7% maltose, 6% trisaccharides and 80% higher saccharides (Hämeen Peruna Oy, Jokioinen, Finland); 99.0% pure lactose (Valio, Finnish Co-operative Dairies Association, Helsinki, Finland); saccharin, Ph. Nord., and cyclamate, DAK-63 (Apodan, Kastrup, Denmark). LM-Pectin Genu 102 AS (A/S Kobenhavns Pektinfabrik), sodium benzoate (Merck), potassium sorbate (Merck), citric acid (Merck), and $CaCl_2 \cdot 2H_2O$ (Merck) were used as additives.

The test jams were sweetened with various sweeteners or mixtures of sweeteners as shown in Table 1. The table gives the content of added sweeteners as a percentage of the jam dry matter. Maltodextrin was regarded as a sweetener although its actual role was a bulking agent.

The test jams were cooked in batches of 1 kg in open steel kettles. The ingredients and additives that were unchanged in all the jams with the same amount of soluble solids (s.s. level) are stated in the basic recipes (Table 2).

The frozen strawberries were defrosted 1 day before cooking. About 2/3 of the sweeteners was poured on to the berries and the dishes were covered with a plastic film. On the following day water and preservatives were added and the mixture was heated to boiling and allowed to boil for 10 min.

Pectin was mixed with the rest of the sweetener and added to the kettle, with the exception of maltodextrin jams, to which the pectin was added in water. Citric acid was also added at this stage. The mixture was allowed to boil for a further 5 min to ensure complete dissolution of pectin. Synthetic sweeteners dissolved in water were added at the end of cooking.

Soluble solids were determined before pouring the hot jam into 0.5 liter glass jars.

Physical measurements

pH Measurement. The pH of the jams was measured with a Radiometer, pH meter (PHM 61) at room temperature.

Color reflectance measurement. The color of the jams was measured with a Hunterlab Model D 25 Color and Color Difference Meter in Ø 90 mm x height 12 mm glass Petri dishes. The dish

Table 1—Sweetener(s) added to the test jams as percentage of dry matter

Jam No	Sweetener[a]							
	Xyl	Sorb	Fru	HFS	Lac	MD	Cycl	Sacch
1	40							
2		40						
3			40					
4				40				
5	20	20						
6	20		20					
7	20			20				
8	30				10			
9	20					20	0.3	
10	20					20		0.03
11	10					30	0.5	
12	10					30		0.05
13						40	0.8	
14						40		0.08
15	30							
16		30						
17			30					
18				30				
19	15	15						
20	15		15					
21	15			15				
22	20				10			
23	15					15	0.2	
24	15					15		0.02
25	8					22	0.5	
26	8					22		0.05
27						30	0.6	
28						30		0.06

[a] Xyl = Xylitol, Sorb = Sorbitol, Fru = Fructose, HFS = High Fructose Syrup, Lac = Lactose, MD = Maltodextrin, Cycl = Cyclamate, Sacch = Saccharin

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was filled leaving no air bubbles between the cover and the jam. Readings were taken from the L (lightness), a (redness) and b (blueness) scales. The instrument was standardized on the white standard (L = 92.5, a = −1.0 and b = −0.6).

Texture measurements. The spreadability of the jams was measured with a Bostwick Consistometer at room temperature. The distance (cm) of jam flow in 60 seconds was recorded. The jam from each jar was measured three times.

Measurement of soluble solids as °Brix. The total soluble solids of the jams were measured with an Abbe Contenta Refractometer. The differences between the °Brix values and the percentage concentration of sugars other than sucrose and of sugar alcohols were taken into account when the total soluble solids were measured. The revision was based on the percentage vs °Brix values determined. The possible effect of organic acids on the °Brix values was not taken into consideration.

Sensory evaluation

The panel consisted of ten members of the staff of the University laboratories. The judges had previous experience in the sensory evaluation of foods. The color, taste, texture and preference of the jams were evaluated immediately after preparation and after 3, 6 and 10 months of storage.

The scale used in scoring color and taste was as follows: 5 = excellent, 4 = good, 3 = average, 2 = fair, 1 = bad. The scale for scoring texture was: 5(2) = too hard, 4(1) = hard, 3(0) = suitable, 2(−1) = soft, and 1(−2) = too soft. The transformed scores given in parenthesis were used in the analysis of variance. The hedonic scale of preference was: 6 = very pleasant, 5 = pleasant, 4 = mildly pleasant, 3 = mildly unpleasant, 2 = unpleasant, 1 = very unpleasant.

The jam samples were served in randomized order at room temperature. A sample was a spoonful of jam on a white, three digit coded plate. Tap water was provided for oral rinsing and the panel was asked not to swallow the samples. At each session 3–5 jams were evaluated, one of which was always the sucrose jam. The jams of the two levels of soluble solids were evaluated as separate series.

The sensory evaluation data were analysed by two-way analysis of variance and the multiple range test was used to determine differences between jams in relation to the characteristic evaluated. The mean values of two replicate taste sessions were used in the analysis of variance.

Table 2—Basic jam recipes for one kilogram batches

Ingredient or additive	40% soluble solids	30% soluble solids
Strawberry	400 g	400 g
Sweetener as dry matter	400 g	300 g
LM-Pectin		
Citric acid 50% w/v sol.	10 ml	8 ml
Sodium benzoate 20% w/v sol.	3.5 ml	3.5 ml
Potassium sorbate 20% w/v sol.	2.5 ml	2.5 ml
Water		

Table 3—LM-pectin and citric acid added for suitable texture and pH in the different test jams prepared with various sweeteners and sweetener combinations

Sweetener(s)	Added LM-pectin g/kg jam	Added citric acid (50% w/v) ml	pH
40% Suc	7	10	3.20
40% Xyl	11	10	3.35
40% Sorb	13	10	3.30
40% Fru	7	10	3.25
40% HFS	9	10	3.20
20% Xyl + 20% Sorb	15	10	3.30
20% Xyl + 20% Fru	12	10	3.30
20% Xyl + 20% HFS	12	10	3.30
30% Xyl + 10% Lac	12	10	3.30
20% Xyl + 20% MD + 0.3% Cycl	8	10	3.30
20% Xyl + 20% MD + 0.003% Sacch	7	10	3.30
30% Suc	7	8	3.30
30% Xyl	15	8	3.30
30% Sorb	19	8	3.40
30% Fru	11	8	3.30
30% HFS	7	8	3.25
15% Xyl + 15% Sorb	15	8	3.30
15% Xyl + 15% Fru	10	8	3.30
15% Xyl + 15% HFS	10	8	3.25
20% Xyl + 10% Lac	10	8	3.30
15% Xyl + 15% MD + 0.2% Cycl	7	8	3.30
15% Xyl + 15% MD + 0.02% Sacch	7	8	3.30

Modification of recipe

In order to prepare a soft, spreading type jam, which is known to be acceptable to Finnish consumers (Törmä 1978), a test series was prepared in which the pH and the amount of LM-pectin were varied. At the 40% soluble solids level, jam with 7g of LM-pectin per kilo and pH 3.2 was considered best. In jams with 30% soluble solids the amount of pectin needed was the same but the most suitable pH was higher (3.3). Less acid could be added to the less sweet jam.

The amount of pectin needed for suitable texture and the quantity of 50% citric acid needed for adjustment of the proper pH of each test jam determined experimentally on the basis of selection from several varieties (Table 3).

During these test series the jams containing 22% and 30% maltodextrin were observed to solidify during cooling and then turn turbid and pink. These jams were omitted from the final scheme. Although pH 3.2 could not be achieved in all the test jams when the maximum amount of 0.5% citric acid permitted by Finnish food legislation was added, only this quantity was used at the 40% sweetener level.

When xylitol, sorbitol and xylitol-sorbitol jams were prepared, more pectin had to be added than to the other jams in order to achieve an acceptable texture (Table 3). In sensory evaluations these xylitol, sorbitol and xylitol-sorbitol jams were regarded as less pleasant because of their sticky mouthfeel. Reducing the amount of pectin needed was investigated by adding Ca^{++} ions as $CaCl_2 \cdot 2H_2O$, using 0.05–0.25 g/kg of jam. Added pectin vaired from 7–15 g/kg of jam. The best combinations of LM-pectin and $CaCl_2 \cdot 2H_2O$ proved to be as follows:

Table 4—L, a and b values for the color of freshly prepared test jams

Sweetener(s) in the jam	Color[b] L	s_x	a	s_x	b	s_x
40% Xyl[a]	6.8	0.0	43.3	0.0	−4.15	0.07
40% Xyl + Ca	6.8	0.4	40.8	3.1	−3.80	0.53
40% Sorb[a]	8.5	0.3	37.6	1.1	−2.35	0.07
40% Sorb + Ca	7.5	0.4	42.5	2.4	−1.70	1.04
40% Fru	7.9	3.2	39.2	1.1	−2.70	0.26
40% HFS	9.3	0.4	34.4	0.7	−1.70	0.32
20% Xyl + 20% Sorb[a]	9.3	0.1	33.4	0.9	−2.15	0.07
20% Xyl + 20% Sorb + Ca	8.5	0.0	37.2	0.4	−2.00	0.21
20% Xyl + 20% Fru	8.8	0.8	35.7	2.1	−2.40	0.78
20% Xyl + 20% HFS	9.6	0.1	34.4	0.5	−1.40	0.13
30% Xyl + 10% Lac	10.2	1.3	34.1	2.4	−0.80	0.90
20% Xyl + 20% MD + Cycl	6.4	0.9	41.8	3.4	−5.60	1.12
20% Xyl + 20% MD + Sacch	6.7	0.3	39.0	1.0	−5.10	0.43
40% Suc	8.6	0.9	40.4	2.5	−0.80	1.32
30% Xyl[a]	9.2	0.1	34.3	0.7	−1.80	0.0
30% Xyl + Ca	8.6	0.3	35.4	3.3	−1.30	0.25
30% Sorb[a]	10.0	0.0	32.3	0.2	−1.40	0.0
30% Sorb + Ca	9.6	0.1	33.5	1.4	−0.15	0.75
30% Fru	10.4	1.3	30.5	4.0	−1.40	0.33
30% HFS	9.4	0.7	35.2	0.4	−0.80	0.83
15% Xyl + 15% Sorb[a]	9.9	0.1	32.5	0.4	−1.40	0.0
15% Xyl + 15% Sorb + Ca	10.8	0.2	32.5	0.3	−0.60	0.0
15% Xyl + 15% Fru	9.6	1.0	35.0	2.6	−1.20	0.61
15% Xyl + 15% HFS	10.7	0.6	32.5	0.7	−0.40	0.46
20% Xyl + 10% Lac	9.0	0.5	45.8	2.0	−1.80	0.24
15% Xyl + 15% MD + Cycl	6.2	0.7	43.2	2.1	−5.80	1.37
15% Xyl + 15% MD + Sacch	7.4	0.2	37.3	0.9	−4.10	0.64
30% Suc	9.1	0.4	36.8	2.0	−0.70	0.62

[a] Measurements only during the first evaluation time (three readings)
[b] s_x = standard deviation

40% Xyl + 7g LM-pectin + 0.15g $CaCl_2 \cdot 2H_2O$
40% Sorb + 7g LM-pectin + 0.2g $CaCl_2 \cdot 2H_2O$
20% Xyl + 20% Sorb + 7g LM-pectin + 0.2g $CaCl_2 \cdot 2H_2O$
30% Xyl + 10g LM-pectin + 0.1g $CaCl_2 \cdot 2H_2O$
30% Sorb + 12g LM-pectin + 0.1g $CaCl_2 \cdot 2H_2O$
15% Xyl + 15% Sorb + 11g LM-pectin + 0.1g $CaCl_2 \cdot 2H_2O$

A more pleasant jam was produced by the addition of Ca^{++}, and therefore the xylitol, sorbitol and xylitol-sorbitol jams without Ca^{++} addition were rejected at the second evaluation.

RESULTS & DISCUSSION

Physical measurements

The colors of the freshly prepared test jams are given in Table 4. There were only slight differences in lightness (L) and redness (a) at the two soluble solids levels. However, the blueness (b) of xylitol-maltodextrin jams at both soluble solids levels and xylitol jam at the 40% soluble solids level differed clearly from the others.

The pH values, soluble solids and texture measurements are given in Table 5.

The effect of the sweetener used on the pH of the jam was slight. The pH varied from 3.2–3.3 at the 40% s.s. level and from 3.2–3.4 at the 30% s.s. level. Xylitol and sorbitol jams had a higher pH and sucrose and HFS jams a lower pH.

The soluble solids of the jams with 40% added sweetener amounted to 45%. When the amount of added sweetener was 30%, the soluble solids were about 35% because of the natural sugar of the strawberries.

Although the texture of the test jams was regarded as conventional in preliminary sensory tests, texture measurements by consistometer showed significant differences between the jams (Table 5). Xylitol and sorbitol jams were very soft and spreadable. Kawabata et al. (1976) noted exceptional behavior of xylitol and sorbitol in LM-pectin jellies. The jelly strength of LM-pectin jellies sweetened with sorbitol and xylitol did not increase with increased concentration of sugar alcohols.

Xylitol and sorbitol jams with added Ca were harder than without. Surprisingly, the xylitol-sorbitol jams without Ca addition were harder than those with Ca addition.

Sensory evaluation

Table 6 shows the average scores given for color, taste, texture and preference in the sensory evaluation of the freshly prepared jams by ten judges, mostly during two replicate sessions.

Color

At the 30% s.s. level the color of the xylitol-maltodextrin jams was judged to be significantly ($P < 0.01$) inferior to that of other jams. The xylitol-maltodextrin jams were judged to be dark. The color of the strawberries was almost black. There was no significant difference in color between the other jams.

At the 40% s.s. level the color of the xylitol-maltodextrin, xylitol-lactose and sorbitol jams was regarded as average. The color of the other jams was considered good (Table 6).

Taste

At the 30% s.s. level the taste of the sorbitol, xylitol-maltodextrin-cyclamate, xylitol-maltodextrin-saccharin and xylitol-lactose jams was judged to be significantly ($P < 0.01$) inferior to the other jams.

At the 40% s.s. level the taste of sucrose, xylitol and xylitol-fructose jams was regarded as significantly ($P < 0.01$) better than the taste of other varieties, which were

—Continued on page 192

Table 5—Spreading in 1 minute[a], pH, °Brix, and soluble solids (s.s.) of the freshly prepared test jams

Sweetener(s) of the jam	Texture spreading cm/1 min	s_x	pH	°Brix	s.s. %
40% Xyl[b]	12.7d	1.7	3.3	43.8	46.0
40% Xyl + Ca	11.4c	1.7	3.3	43.0	45.0
40% Sorb[b]	13.5d	0.4	3.3	44.2	45.2
40% Sorb + Ca	10.8c	1.0	3.3	43.6	44.6
40% Fru	10.3c	1.5	3.2	45.0	45.4
40% HFS	5.0a	1.5	3.2	45.2	45.7
20% Xyl + 20% Sorb[b]	8.6b	1.1	3.3	43.1	44.6
20% Xyl + 20% Sorb + Ca	10.0b	1.5	3.3	43.7	45.3
20% Xyl + 20% Fru	6.2a	0.9	3.3	43.8	46.0
20% Xyl + 20% HFS	5.9a	1.4	3.3	43.4	45.4
30% Xyl + 10% Lac	4.7a	1.8	3.3	43.7	45.8
20% Xyl + 20% MD + Cycl	4.6a	0.8	3.3	45.4	45.4
20% Xyl + 20% MD + Sacch	4.7a	1.3	3.3	45.4	45.4
40% Suc	5.4a	1.1	3.2	45.5	45.4
30% Xyl[b]	13.7ä	1.0	3.4	34.3	35.8
30% Xyl + Ca	9.0y	0.9	3.4	34.0	35.5
30% Sorb[b]	15.1ä	0.8	3.4	34.1	34.6
30% Sorb + Ca	10.6y	1.8	3.4	33.8	34.4
30% Fru	7.0x	0.8	3.3	35.2	35.5
30% HFS	7.5x	0.8	3.2	34.0	34.2
15% Xyl + 15% Sorb[b]	8.0x	0.4	3.4	34.4	35.5
15% Xyl + 15% Sorb + Ca	11.3z	1.3	3.4	33.9	35.0
15% Xyl + 15% Fru	10.5y	1.4	3.3	33.5	34.6
15% Xyl + 15% HFS	9.1y	0.7	3.3	34.2	35.1
20% Xyl + 10% Lac	8.9y	1.6	3.5	33.7	35.1
15% Xyl + 15% MD + Cycl	8.9y	0.7	3.4	35.3	35.3
15% Xyl + 15% MD + Sacch	9.7y	1.1	3.4	34.6	34.6

[a] Mean values marked with the same letter not significantly different ($P < 0.01$)
[b] Measurements only during the first evaluation time
s_x = standard deviation

Table 6—Average scores of ten judges for color, taste, texture and preference of the freshly prepared test jams

Sweetener(s) of the jam	Scores[b] Color	Taste	Texture	Preference
40% Xyl[a]	3.60b	3.70b	−0.90	3.90a
40% Xyl + Ca	3.85b	3.55b	−0.45	4.15b
40% Sorb[a]	3.30a	2.90a	−1.0	3.20a
40% Sorb + Ca	4.30b	2.90a	−0.40	3.40a
40% Fru	3.95b	3.45a	−0.40	3.90a
40% HFS	3.50b	2.60a	+0.80	3.05a
20% Xyl + 20% Sorb[a]	4.10b	3.50a	+0.40	4.30b
20% Xyl + 20% Sorb + Ca	4.30b	3.50a	−0.80	4.10b
20% Xyl + 20% Fru	4.05b	3.85b	+0.40	4.45b
20% Xyl + 20% HFS	3.55b	3.25a	+0.55	3.80a
30% Xyl + 10% Lac	3.45a	3.10a	+0.85	3.75a
20% Xyl + 20% MD + Cycl	2.90a	3.0a	+0.45	3.45a
20% Xyl + 20% MD + Sacch	2.70a	2.85a	+0.65	3.40a
40% Suc	4.0b	3.89b	+0.32	4.31b
30% Xyl[a]	4.10y	3.30y	−0.50y	3.60y
30% Xyl + Ca	4.05y	3.50y	0y	4.35y
30% Sorb[a]	4.10y	2.30x	−0.90x	2.60x
30% Sorb + Ca	4.05y	2.35x	−0.55y	3.55y
30% Fru	3.70y	3.45y	+0.30y	3.90y
30% HFS	3.85y	3.15y	−0.05y	3.75y
15% Xyl + 15% Sorb[a]	3.60y	3.20y	+0.20y	3.50x
15% Xyl + 15% Sorb + Ca	4.0y	3.60y	−0.50y	4.40y
15% Xyl + 15% Fru	4.0y	3.55y	−0.45y	4.0y
15% Xyl + 15% HFS	3.75y	3.55y	+0.10y	3.90y
20% Xyl + 10% Lac	3.85y	3.05x	−0.10y	3.45x
15% Xyl + 15% MD + Cycl	2.25x	2.90x	−0.20y	3.15x
15% Xyl + 15% MD + Sacch	2.40x	3.0x	−0.35y	3.25x
30% Suc	3.81y	3.64y	+0.06y	4.22y

[a] Judged only in one session, n = 10
[b] Mean values marked with the same letter not significantly different ($P < 0.01$)

Examination of Sugars, Sugar Alcohols, and Artificial Sweeteners as Substitutes for Sucrose in Strawberry Jam. Keeping Quality Tests

LEA HYVÖNEN and RAIJA TÖRMÄ

ABSTRACT

The keeping quality of the low sugar strawberry jams was tested during 10 months' storage at room and refrigerator temperature (5°C) according to physical color and texture measurements, and by sensory analysis. In general sorbitol and xylitol jams and many of the jams containing some percentage of xylitol kept either better than or as well as the conventional sucrose jam. The color, taste and preference of HFS and fructose jams deteriorated sooner than those of most other jams during storage. When xylitol was used with fructose and HFS the changes in the characteristics studied were retarded. During storage the xylitol-maltodextrin jams became crystallized and were unfit for sensory evaluation.

INTRODUCTION

THE KEEPING QUALITY of the low-sugar jams prepared with various alternative sweeteners was studied over a period of 10 months. Half of the samples were stored at room temperature and the other half in cold storage at 5°C to investigate the effect of temperature on the quality characteristics of the jams. Physical color and texture measurements and sensory evaluations were made immediately after preparation and after 3, 6 and 10 months.

MATERIALS & METHODS

USING THE RECIPES given in Hyvönen and Törmä (1982), 7 kg batches of the test jams were prepared for storage, with the exception that three 7 kg batches of sucrose jam were prepared for reference at both 30% and 40% soluble solids (s.s.) levels.

Color and texture measurements and sensory analysis of the jams were carried out as described in Hyvönen and Törmä (1982).

RESULTS & DISCUSSION

Color by Hunterlab

The L, a and b values for the color of the freshly prepared strawberry jams were almost identical with the values noted during the product development period (Hyvönen and Törmä, 1982). During 10 months both at either room temperature or in cold storage the L values for color rose slightly (Fig. 1); in other words, the jams lost some of their color. The a and b values, on the other hand, changed markedly: a decreased from approx. 40 to 10 and b increased from approx. −2 to +9, i.e., from blueness to yellowness (Fig. 2 and 3). These changes occurred during the first 6 months, after which no changes of any significance were noted. The a values for color after 6 and 10 months were somewhat higher in the jams stored at 5°C than in those stored at room temperature.

Color by sensory analysis

The average scores for color of the freshly prepared and stored strawberry jams are given in Fig. 4. The color of the freshly prepared jams was regarded as at least average in all cases, with the exception of the xylitol-maltodextrin jams, the color which was regarded as fair.

After 3 months' storage at room temperature the xylitol-maltodextrin-cyclamate and HFS jams were judged to be significantly inferior to the others at the 30% s.s. level. At the 40% s.s. level the color of sorbitol, xylitol and xylitol-sorbitol jams was judged to be significantly superior ($P < 0.01$) to the others. The color of the xylitol-maltodextrin and HFS jams was considered to be only fair, but that of xylitol-fructose, xylitol-HFS, xylitol-lactose, sucrose and fructose jams was regarded as average.

There were no significant differences in the color of the 30% s.s. level test jams stored for 3 months at 5°C. Jams containing maltodextrin were excluded because of crystallization of the maltodextrin at 5°C. At the 40% s.s. level the color of fructose, sucrose, xylitol, xylitol-sorbitol and sorbitol jams was ranked superior ($P < 0.01$) to the others. However, the color of the others was regarded as not lower than average.

After 6 months' storage at room temperature the color of the xylitol-maltodextrin-cyclamate, fructose and HFS jams was judged to be only fair and significantly inferior ($P < 0.01$) to the others at the 30% s.s. level. The color of the same jams stored at 5°C was mostly regarded as good. The average score for the color of fructose jam was somewhat lower. Xylitol-maltodextrin jams were not evaluated because of crystallization of the maltodextrin.

At the 40% s.s. level the color of the xylitol-sorbitol jam stored at room temperature was rated superior ($P < 0.01$) to that of other jams. The color of sorbitol, xylitol and sucrose jams was evaluated as average, that of fructose, xylitol-fructose, xylitol-lactose and xylitol-HFS jams as fair, and the color of HFS and xylitol-maltodextrin jams as poor.

At 5°C the color of sorbitol, xylitol, xylitol-sorbitol and sucrose jams preserved for 6 months as good and significantly superior ($P < 0.01$) to the color of the other jams, which rated only average. The color of HFS jam was given the lowest score.

After 10 months' storage at room temperature the color of xylitol-HFS, xylitol-sorbitol and xylitol-lactose jams was judged to be average and superior to the color of the other jams at the 30% s.s. level. The color of the others was rated as either fair or poor. At 5°C the same jams retained their color as at least average. The color score of fructose and HFS jams was average and significantly lower ($P < 0.05$) than the good scores of the other jams.

At the 40% s.s. level the color scores of xylitol and sorbitol jams stored at room temperature were significantly higher ($P < 0.05$) than those of the other jams. The color of sucrose, xylitol-lactose, xylitol-HFS and xylitol-sorbital was regarded as average. Color scores for xylitol-fructose, HFS and fructose jams were fair. Storage at 5°C retained the color of xylitol-sorbitol and xylitol jams as good and significantly superior ($P < 0.05$) to that of the other jams. The color scores for sorbitol, sucrose, xylitol-HFS and fructose jams also remained good and those for xylitol-fructose, HFS and xylitol-lactose jams were average.

Consequently, the color of the test jams after 10 months' storage at 5°C would not cause rejection of any jam, ex-

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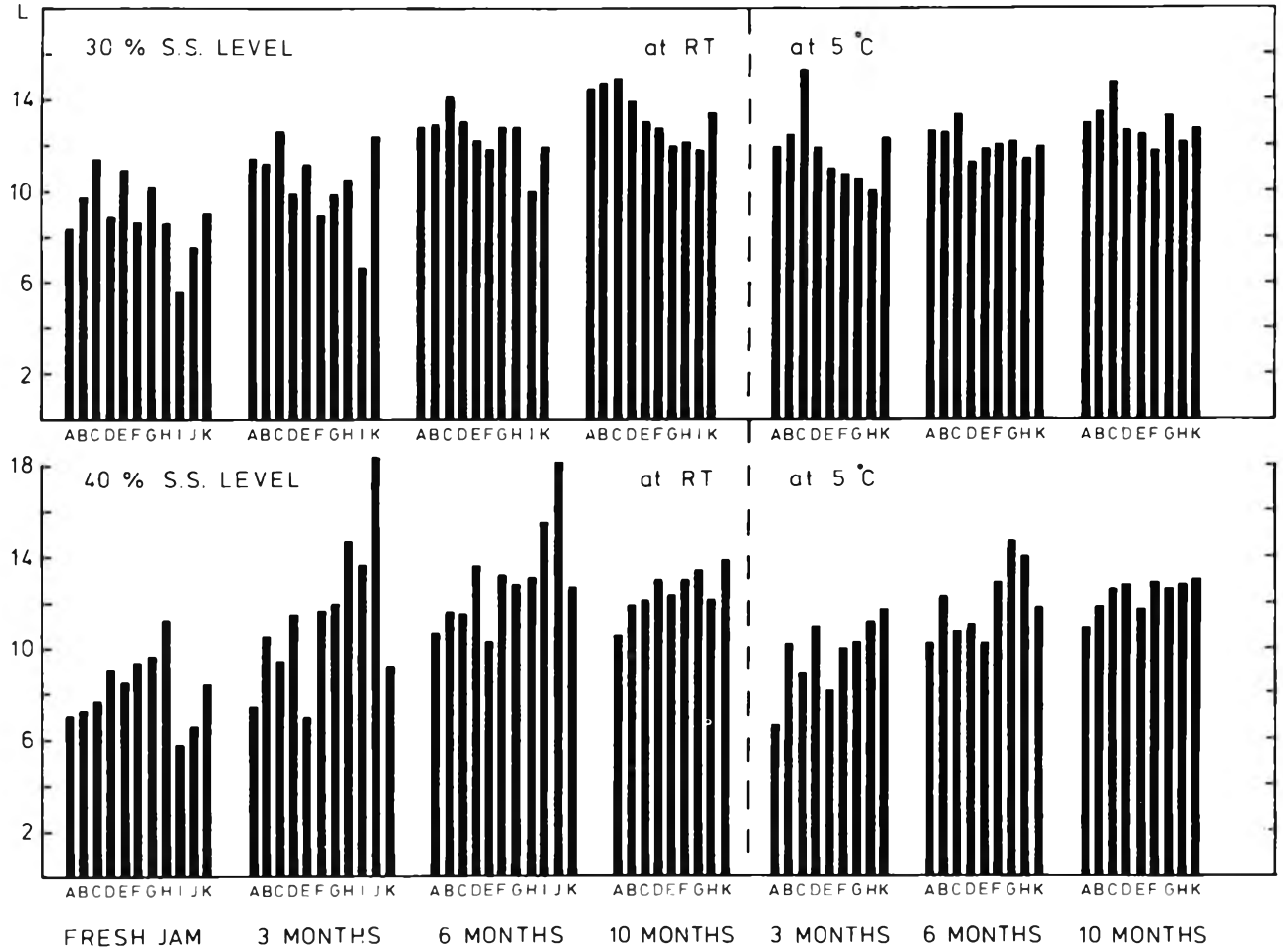

Fig. 1—L values for color of freshly prepared and stored strawberry jams measured by Hunterlab Color and Color Difference Meter. A = Xyl jam, B = Sorb jam, C = Fru jam, D = HFS jam, E = Xyl-Sorb jam, F = Xyl-Fru jam, G = Xyl-HFS jam, H = Xyl-Lac jam, I = Xyl-MD-Cycl jam, J = Xyl-MD-Sacch jam and K = Suc jam.

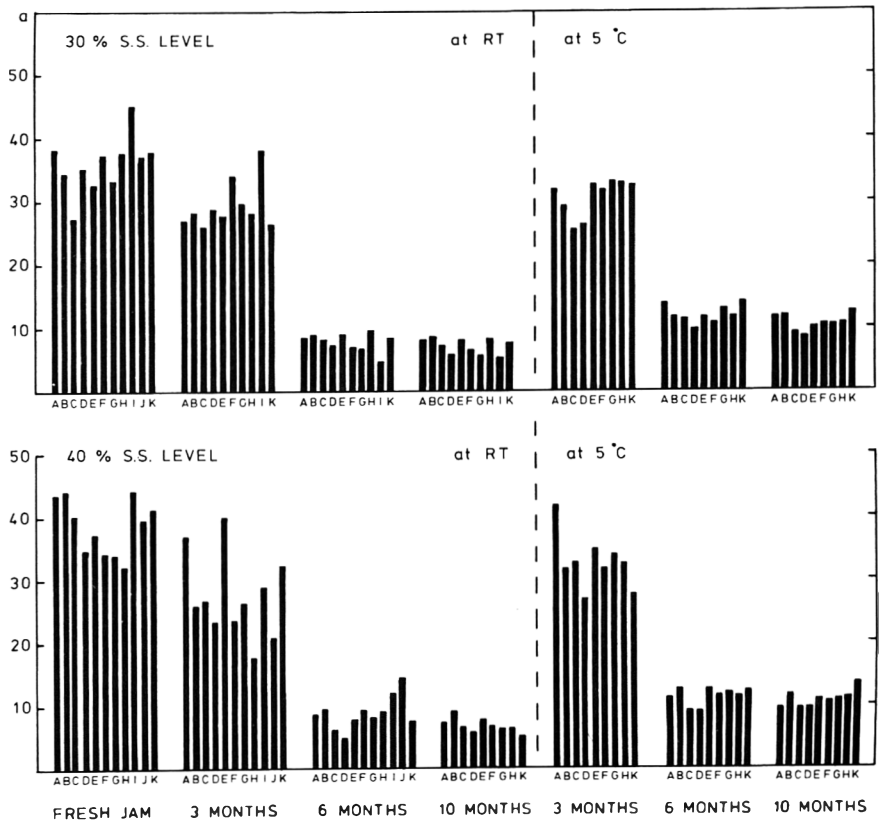

Fig. 2—a Values for color of freshly prepared and stored strawberry jams measured by Hunterlab Color and Color Difference Meter. See Fig. 1 for abbreviations.

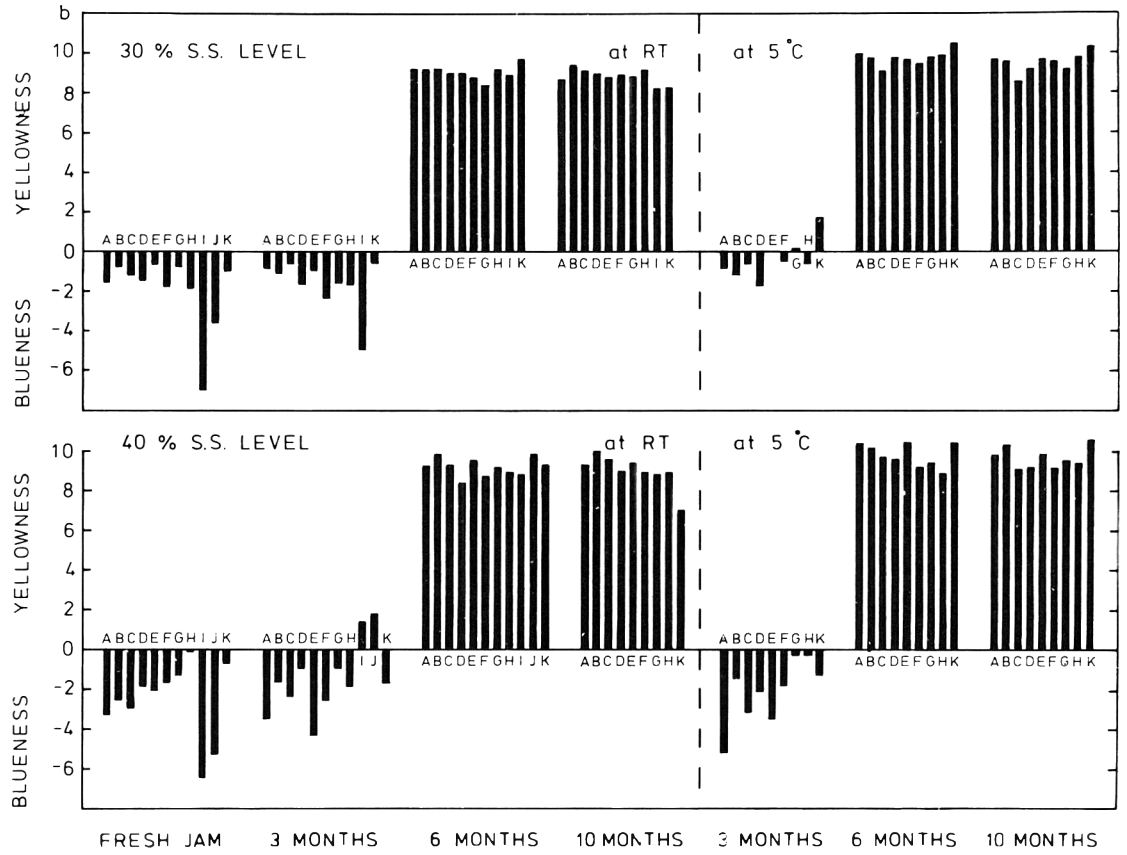

Fig. 3—b Values for color of freshly prepared and stored strawberry jams measured by Hunterlab Color and Color Difference Meter. See Fig. 1 for abbreviations.

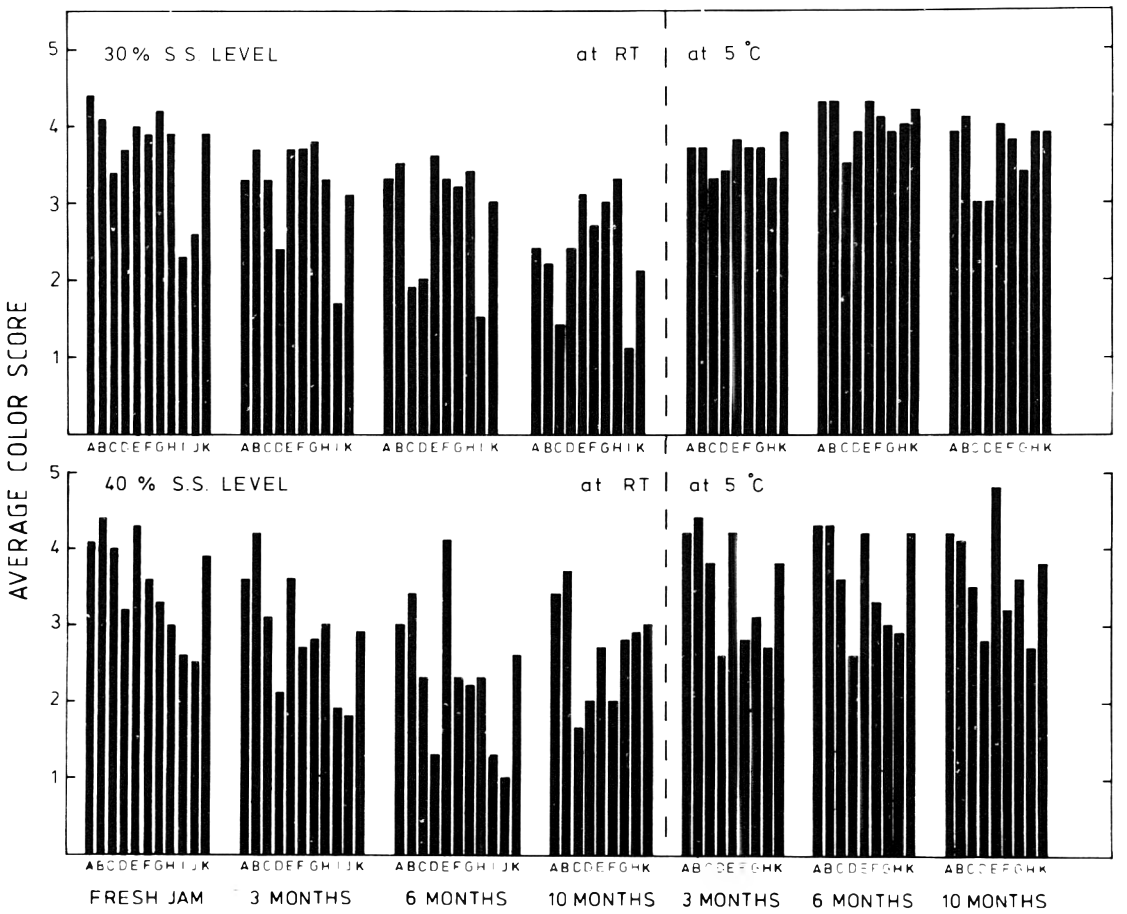

Fig. 4—Average scores for color of freshly prepared and stored strawberry jams. See Fig. 1 for abbreviations.

cept for xylitol-maltodextrin jams, which were rejected after 3 months' storage. The color of the jams containing maltodextrin was only fair immediately after preparation. The color of HFS jam was only fair as early as after 3 months at room temperature. At the 30% s.s. level, the color of fructose jam was also rated as fair after 6 months at room temperature. After 10 months at room temperature it was rated as poor at the 30% s.s. level and fair at the 40% s.s. level, as was also the color of HFS and xylitol-fructose jams at that s.s. level.

Taste

All the jams were acceptable by taste and were rated as average or better at the beginning of the keeping quality study (Fig. 5).

After 3 months' storage at room temperature the HFS jam was considered significantly inferior ($P < 0.01$) to the other jams at the 30% s.s. level. The taste of xylitol-HFS, xylitol, xylitol-sorbitol and xylitol-fructose jams was rated as good. At the 40% s.s. level xylitol-maltodextrin-cyclamate was rated as only fair and significantly inferior to xylitol-lactose ($P < 0.05$), xylitol-fructose ($P < 0.05$), sucrose ($P < 0.05$), xylitol-sorbitol ($P < 0.05$), sorbitol ($P < 0.01$) and fructose ($P < 0.01$).

At the 30% s.s. level, storage at 5°C did not preserve the taste of HFS jam any better than storage at room temperature, and it was rated significantly lower ($P < 0.01$) than all the other jams. The taste of xylitol-sorbitol, sucrose, xylitol-fructose and xylitol jams was rated as good. At the 40% s.s. level there were no significant differences between the tastes of the test jams after storage for 3 months at 5°C.

After 6 months' storage at room temperature the taste of xylitol-maltodextrin-cyclamate, fructose and HFS jams was regarded as fair. The taste of the other jams was rated as average at the 30% s.s. level. At the 40% s.s. level the taste of xylitol-maltodextrin-cyclamate, xylitol-maltodextrin-saccharin and HFS jams was rated as bad and significantly inferior ($P < 0.01$) to the other jams. Xylitol-sorbitol jam was judged to be superior to the others, which were rated as average.

After 6 months' storage at 5°C the HFS jam at the 40% s.s. level differed from the others by taste, being significantly inferior ($P < 0.01$). The taste of the other jams was rated at least average. Xylitol-sorbitol, sucrose and xylitol jams were rated as good. At the 30% s.s. level the taste of HFS, xylitol-fructose and xylitol-HFS jams was rated as fair, sorbitol, xylitol-lactose and fructose jams as average, and xylitol-sorbitol, sucrose and xylitol jams as good.

After 10 months at room temperature the taste of xylitol-maltodextrin-cyclamate jam was classed as poor, and HFS, sorbitol and sucrose jams were rated as only fair at the 30% s.s. level. The taste of xylitol-sorbitol, xylitol, fructose, xylitol-lactose, xylitol-HFS and xylitol-fructose jams was still regarded as average. After 10 months' storage at 5°C the HFS jam was rated lowest, as only fair. The taste of sucrose, xylitol-fructose and xylitol jams was still regarded as good, and xylitol-lactose, xylitol-sorbitol, xylitol-HFS, fructose and sorbitol jams were rated as average.

At the 40% s.s. level the taste of HFS, xylitol-HFS and fructose jams stored for 10 months at room temperature was judged to be fair and significantly inferior ($P < 0.05$) to the other jams, which were regarded as average. When stored at 5°C, no significant differences were noted in the taste of the test jams. *—Text continued on page 191*

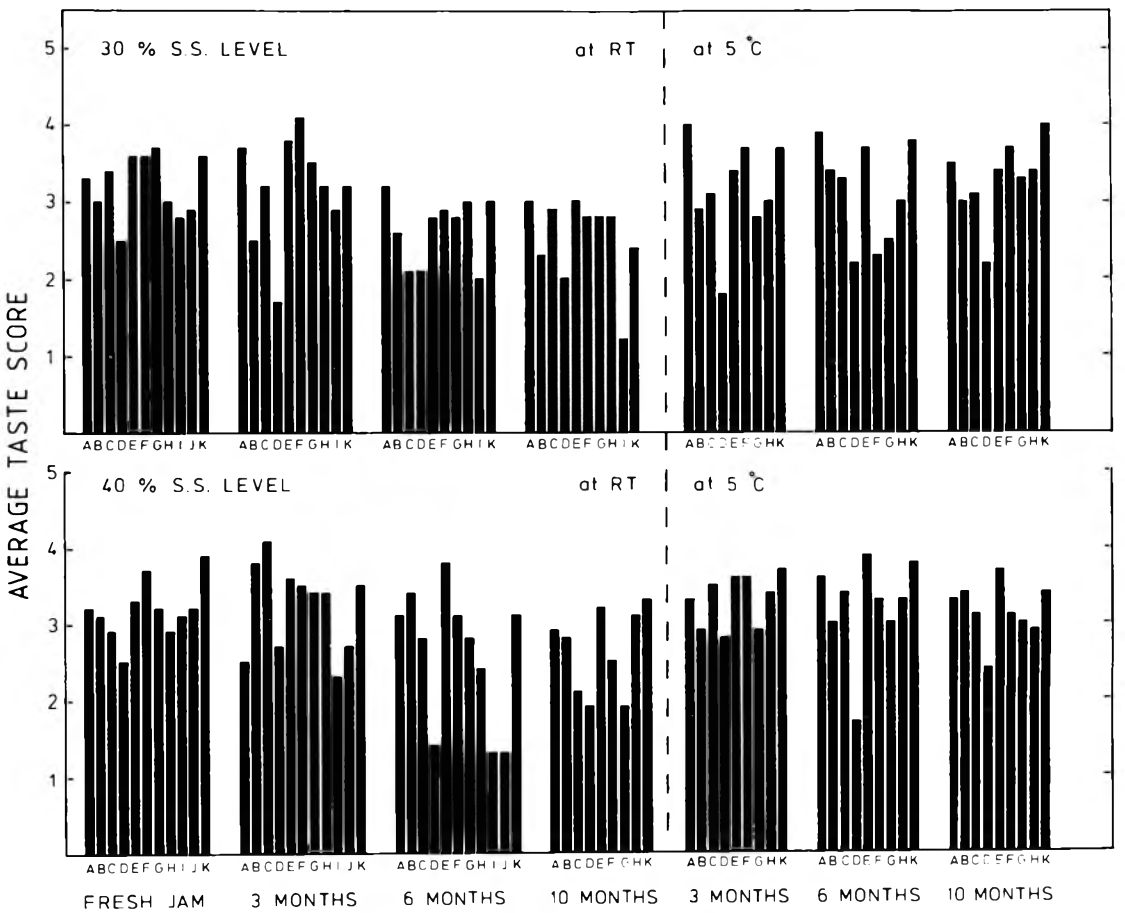

Fig. 5—Average scores for taste of freshly prepared and stored strawberry jams. See Fig. 1 for abbreviations.

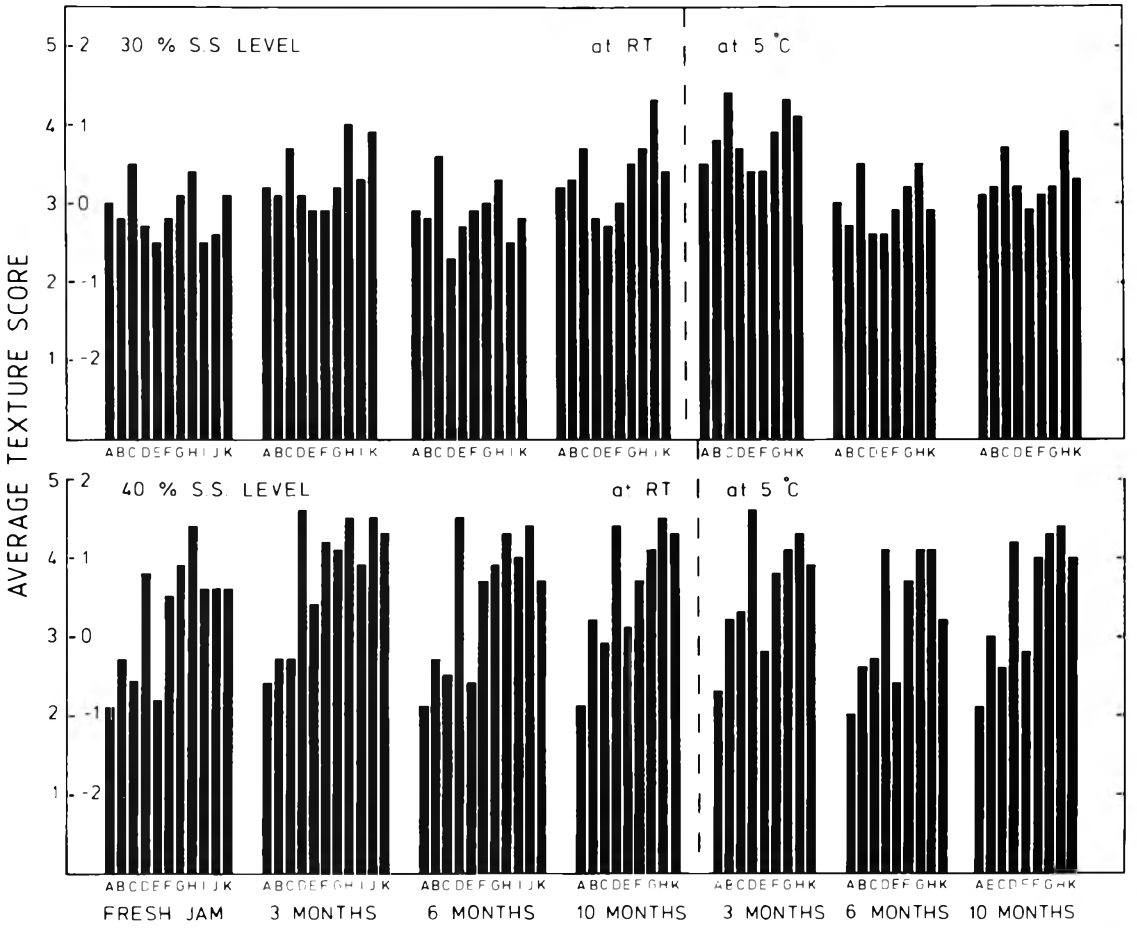

Fig. 6—Average scores for texture of freshly prepared and stored strawberry jams. See Fig. 1 for abbreviations.

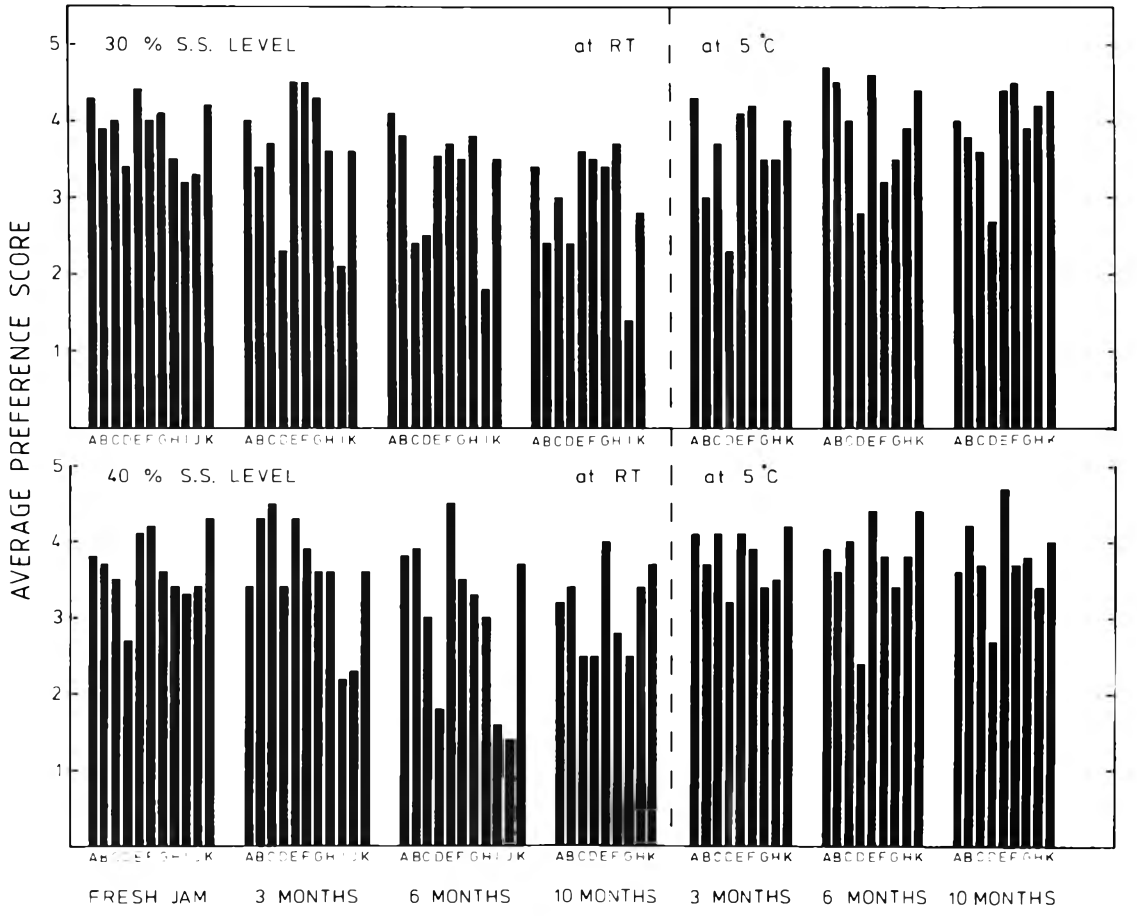

Fig. 7—Average scores for preference of freshly prepared and stored strawberry jams. See Fig. 1 for abbreviations.

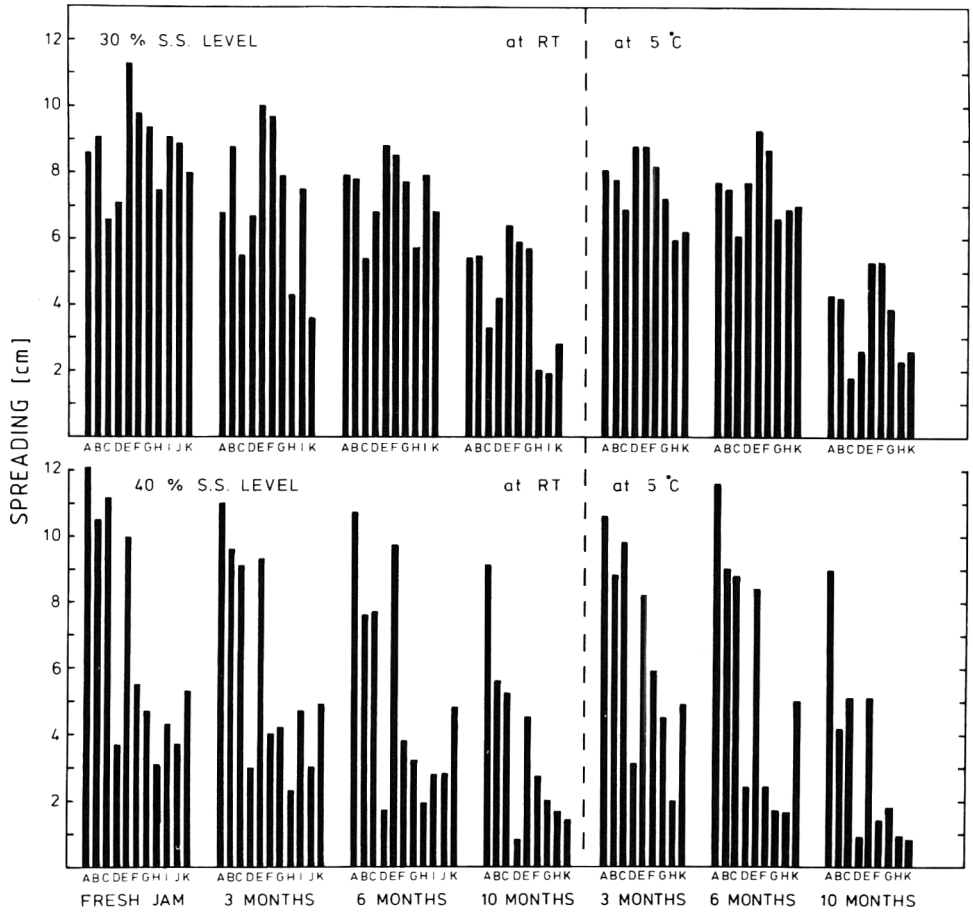

Fig. 8—Spreading of freshly prepared and stored strawberry jams measured with Bostwick consistometer. See Fig. 1 for abbreviations.

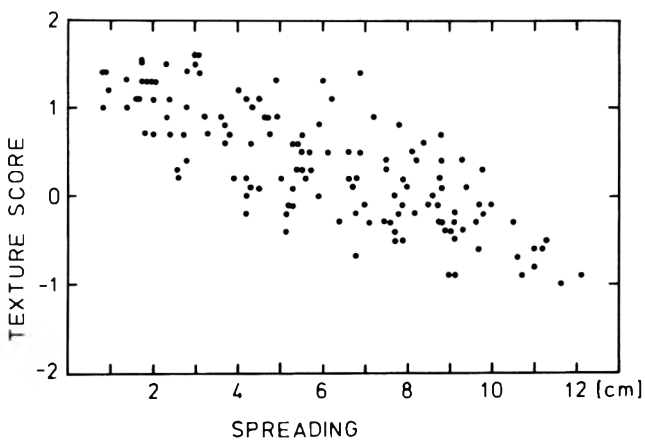

Fig. 9—Texture scores vs spreading (cm).

In the light of the sensory evaluations, the taste of jams containing maltodextrin and of HFS jams may be one reason for their rejection.

Texture

The texture of the jams measured with a Bostwick Consistometer changed during storage (Fig. 8). In general the spreading distance became shorter with time, indicating hardening of the jams. This was not clearly seen in the texture scores given in the sensory evaluation (Fig. 6).

Texture scores vs spreading distances (Fig. 9) show that the texture was evaluated as suitable when the distance of spreading was 4–10 cm. On the other hand, one jam which flowed 7 cm was rated as suitable and another as hard. Consequently, the judges regarded jams with greatly differing spreading quality as suitable in texture, although opinions on the hardness of the jams with the same spreading distance differed.

The textures of the various test jams at the 30% s.s. level were closer to each other than those of the jams at the 40% s.s. level. At the 40% s.s. level xylitol, sorbitol, xylitol-sorbitol and fructose jams spread distinctly more easily, while sucrose, HFS, xylitol-fructose, xylitol-HFS, xylitol-lactose and xylitol-maltodextrin jams were harder (Fig. 8). Since none of the jams was judged to be absolutely too hard or too soft, minor variations can be regarded as insignificant and as unlikely to cause rejection.

Preference

The preference scores for the jams (Fig. 7) at the 30% s.s. level remained as mildly pleasant when the jams were stored at 5°C. HFS jam was an exception. It was regarded as unpleasant after only 3 months' storage at 5°C. When stored at room temperature the xylitol-maltodextrin and HFS jams were considered unpleasant after 3 months, and after 6 months' storage fructose jam was also regarded as unpleasant. After 10 months' storage at room temperature the fructose and sucrose jams were rated as mildly unpleasant, HFS and sorbitol jams as unpleasant, and xylitol-maltodextrin-cyclamate jams as very unpleasant. Xylitol-lactose, xylitol-sorbitol and xylitol-fructose jams were rated as mildly pleasant after 10 months at room temperature when the s.s. level was 30%. *—Continued on next page*

At the 40% s.s. level the HFS jam was judged to be mildly unpleasant immediately after preparation. After 3 months' storage at room temperature xylitol-maltodextrin jams were considered unpleasant, and after 6 months they were regarded as very unpleasant and HFS jam as unpleasant. Fructose, xylitol-lactose and xylitol-HFS jams were rated as mildly unpleasant. After 10 months' storage at room temperature only sucrose and xylitol-sorbitol jams were regarded as mildly pleasant, the other jams being considered mildly unpleasant.

The preference for all the jams stored at 5°C was generally rated higher than for jams stored at room temperatures. HFS jams was an exception. It was rated as (mildly) unpleasant during the entire storage period. The other jams were regarded as mildly pleasant or better even after 10 months' storage at 5°C.

Kawabata et al. (1976) have also reported that the preference for corn syrup jelly was ranked significantly inferior to that for glucose, fructose, sucrose, sorbit and malbit jellies at an equivalent sweetness. Kossoy (1967) recommended certain sweetener combinations for jams, jellies and preserves of good quality, if prepared from corn syrups of various D.E. in order to avoid the tendency to crystallization and flavor deterioration. In the present study the combination of xylitol and HFS proved to be better than pure HFS in strawberry jam. Results to the contrary have been reported by Andres (1978), who claimed that HFCS improved the flavor retention of jellies, jams and preserves.

Although the sensory data in this study are comparable only at the same s.s. level, the same storage temperature and storage period, the lower temperature and the higher s.s. level seemed to protect the color of the strawberry jam. The taste and preference scores within the same s.s. level also tended to be somewhat higher when the jam was stored at refrigerator temperature. Storage of the fructose jam at 5°C improved the keeping quality markedly. Jams that contained maltodextrin could not be stored at 5°C because of crystallization. In the case of HFS jam, however, storage at 5°C did not improve the storage stability, whereas mixing HFS with xylitol did.

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SUGAR SUBSTITUTES IN STRAWBERRY JAM. . . From page 185

regarded as average (Table 6). Sucrose jam was rated the best in terms of taste at both soluble solids levels, but the difference was significant only in comparison with the lowest scored jams. Consequently, sucrose could be replaced in strawberry jam by many other sugars and sugar alcohols, at least on the basis of taste.

Texture

No very significant ($P < 0.01$) difference were recorded between the different jams at the 40% soluble solids level (Table 6). Only the sorbitol jam was judged to be very significantly ($P < 0.01$) softer than the others at the 30% soluble solids level.

The apparent contradiction between the physical (Table 3) and sensory (Table 6) texture measurements is due to the fact that in the sensory analysis the judges were asked whether they regarded the texture as suitable (not too hard or too soft) and not how hard they considered it, whereas the consistometer measurements demonstrated the spreadability of the jams through the hardness of the jam. Therefore the "suitable" jam texture by sensory analysis vary significantly as measured by the consistometer.

The optimum jam texture was achieved in the xylitol jams with added Ca. The texture of the sucrose jams was also regarded as close to the optimum.

The correlation coefficient between sensory scores of texture and spreading length as an indicator of the texture was −0.94 at the 40% s.s. level and −0.89 at the 30% s.s. level. This showed that the jams evaluated as soft flowed a long way in the consistometer.

Preference

The sorbitol jam without added Ca, xylitol-maltodextrin jams, xylitol-lactose and xylitol-sorbitol jams were judged to be significantly less pleasant than the other jams at the 30% s.s. level (Table 6).

At the 40% s.s. level the xylitol jam with added Ca, xylitol-sorbitol, xylitol-sorbitol with added Ca, xylitol-fructose and sucrose jams were significantly ($P < 0.01$) more preferable than the other varieties (Table 6).

Preference for a jam can be regarded as a general hedonic characteristic of the jam on which all the above separate characteristics – color, taste and texture – have an effect. The correlation coefficient between taste and preference was 0.87 and between color and preference 0.57 at the 40% s.s. level. These correlation coefficients at the 30% s.s. level were 0.83 and 0.37. Consequently taste had a much greater effect on preference than color had.

CONCLUSIONS

A low sugar jam with good taste, color and texture can be prepared using 30 or 40% added sucrose. Xylitol with added Ca, xylitol-sorbitol with added Ca, xylitol-fructose and xylitol-HFS proved to be good substitutes for sucrose in this type of jam. Maltodextrin proved to be an unsuitable bulking agent for low calorie strawberry jam.

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Forced Air Drying of Partially Freeze-Dried Compressed Carrot Bars

E. R. SCHADLE, E. E. BURNS, and L. J. TALLEY

ABSTRACT

The quality of compressed carrot bars produced by combining freeze drying with air drying was investigated. Quality parameters measured were color, texture, rehydration ratio, carotene, ascorbic acid, alphatocopherol, and sensory acceptance. It was found that a high quality compressed carrot bar could be obtained by freeze drying to 20–40% moisture, equilibrating with microwave energy, compressing, then air drying at 60°C. The scanning electron microscope proved useful in delineating reasons for differences in texture and rehydration.

INTRODUCTION

FREEZE DRYING has found increasing application for foods where convenience and weight reduction are the overriding concern such as military rations, space foods, and foods for campers (Longan, 1973).

Freeze drying reduces weight with little reduction in volume. Compression of freeze-dried foods can give a high degree of volume reduction, allowing for more compact packaging and shipment.

An analysis of the energy requirements for air drying, drum drying and freeze drying has shown the energy required to remove one kilogram of water is lower for air drying and drum drying than for freeze drying (Flink, 1977).

Freeze-dried foods, with adequate plasticity before compression, have been found to provide a compressed food with good rehydration and textural properties (MacKenzie and Luyet, 1969). Wisakowsky (1977) found microwave radiation to be an excellent means of plasticization. This was attributed to the rapidity of heating and resultant uniformity of water distribution. Optimum carrot quality was obtained by plasticizing at 50% moisture, 40 second microwave, 500 psi compression force, and 20 second dwell time.

DellaMonica and McDowell (1969) found that carrots prepared by three drying methods: air drying, freeze drying and explosion puff-drying had little difference in beta-carotene after processing, with the freeze dried carrots being somewhat higher. Panalaks and Murray (1970) stated that the retention of carotene after processing may be due to the presence of natural antioxidants such as tocopherol.

Harris et al. (1950) reported that the tocopherol content of carrots was almost 100% in the alpha form. The alpha-tocopherol content of fresh carrot roots reported in the literature varies from 0.11 mg/100g (Bunnell et al., 1965) to 0.50 mg/100g (Booth and Bradford, 1963).

Rapid drying retains greater amounts of ascorbic acid than does slow drying. Generally, the vitamin C content of vegetable tissue is greatly reduced during a slow sun drying process. Dehydration, especially spray drying and freeze drying, reduces these losses (Salunkhe and Bolin, 1973).

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Logically, better retention of nutrients will be found where the lowest dehydration temperature and shortest period of time are employed. Freeze drying allows the lowest possible temperatures used in dehydration; the vacuum used during the process should minimize oxidative processes.

This study considered the quality of freeze-dried and compressed carrots prepared in combinations of freeze drying and hot air drying.

MATERIALS & METHODS

Raw materials

Fresh carrots of the Imperator 58 variety were utilized. A portion was used for fresh product analysis. The remainder was sliced into 3/8 inch slices, water blanched to a negative catalase-peroxidase endpoint, and frozen at −30°C until further processing.

Processing

One group of slices was freeze-dried to a moisture content of 3% and plasticized in the manner of Rushing (1974), then compressed and refreeze-dried to remove the moisture of plasticization. A second sample was treated in the same manner except the moisture of plasticization was removed by air drying. Another sample was dried entirely in a circulating hot air cabinet drier.

Additional samples were freeze-dried to moisture levels of 50, 40, 30, 20, and 10%, plasticized by microwave at 500 watts power, compressed into bars 3 x 1 x ½ inch in the manner presented by Wisakowsky (1977) and dried in the cabinet drier at 60°C to a final moisture level of 5%. Temperatures higher than 60°C resulted in severe quality degradation due to excessive nonenzymatic browning.

Rehydration

Carrot bars were rehydrated in a water bath at 61°C, drained and weighed at 10, 20 30 and 60 min of rehydration. Rehydration ratios were calculated by dividing the rehydrated by the initial dry weight of the sample.

Texture

Texture was measured on 50g samples of rehydrated carrots utilizing an Allo-Kramer Shear Press. The 13 blade CS-2 thin blade shear compression cell with a 500 lb test ring was used. Peak shear force was recorded in pounds.

Color

Color was measured with a Gardner Automatic Color Difference Meter Model XL-10A, calibrated with the Gardner color standard for sweet potatoes. Color was reported on a 1:2 blend of product with distilled water placed to a depth of 3 cm in an optically correct cup. Measurement was in terms of Gardner color values "L", "a," and "b."

Carotene

Carotene analysis was done by the procedure outlined in Methods of Vitamin Assay (Anonymous, 1966). Extracts of alpha-carotene were read at 447 nm, and beta-carotene were read at 436 nm on a Bausch and Lomb Spectronic 20 Spectrophotometer.

Ascorbic acid

The 2,6-dichlorophenolindophenol visual titration method as described in Methods of Vitamin Assay (Anonymous, 1966) was used for ascorbic acid determination. *–Continued on next page*

Carrots were rehydrated with 6% HPO_3 to yield a homogeneous slurry. Prior to blending, nitrogen was bubbled into the blender bowl for 30 sec to lessen the possibility of oxidation.

Alpha-Tocopherol

Alpha-tocopherol was determined by the GLC method of Hartman (1977) following extraction and saponification by the method of AOAC (1975).

Scanning electron microscopy

Histological examination of the carrot tissue was accomplished with a JSM-U3 scanning electron microscope manufactured by the Japanese Electron Optical Laboratory. Samples were coated with pure gold in an argon atmosphere to prevent surface charging of the sample material.

Sensory evaluation

A trained sensory panel composed of six members utilized a nine-point scale ranging from "extremely poor" to "excellent" for scoring color, odor, flavor, texture, and appearance of rehydrated samples.

Experimental design was of a balance block type. Trained judges were utilized in a Scalar Difference method. Carrots freeze-dried and compressed in the manner presented by Rushing (1974) were used as a control.

Panelists were trained for four weeks prior to testing. Fresh carrot samples were used to define the ideal for color, odor, flavor, texture, and appearance.

Testing was done in an open area of the laboratory under daylight conditions. All samples were stored at −18°C in sealed containers prior to rehydration. Samples were rehydrated prior to testing and allowed to cool to room temperature. Samples were coded with 3 digit numbers generated randomly by a computer. Panelists were instructed not to swallow the samples and to rinse their mouths with distilled water between samples.

Data were subsequently analyzed by analysis of variance. A 95% confidence level was chosen to test for significance.

Statistical interpretation

The data from the subjective and objective tests were examined by analysis of variance to determine differences between drying treatments. Duncan's multiple range tests were used to separate the means of the quality attributes. Analyses were based on three replicates per treatment.

RESULTS & DISCUSSION

Gardner color

No significant differences were found for Gardner color values between drying treatments or between drying treatments and control. The optimum air drying temperature was 60°C. Considerable browning was noticed at temperatures in excess of 60°C.

Ascorbic acid

The results of the ascorbic acid analysis are presented in Table 1. The values for ascorbic acid in the dried carrots ranged from 15.97 mg/100g for the totally air-dried sample to 33.39 mg/100g for the totally freeze-dried sample. Drying was shown to have a highly significant effect on ascorbic acid concentration (Pr>F=0.0001).

In general, carrot samples which underwent longer periods of air drying had less retention of ascorbate. This was apparently due to irreversible oxidation of the ascorbic acid in the presence of heated air. The dried samples were significantly lower in ascorbate than the fresh carrots. Much of this loss was ascribed to leaching during the blanching process.

Alpha-Tocopherol

The range for alpha-tocopherol content was from 0.04 mg/100g in the totally air dried carrots to 3.45 mg/100g in the totally freeze-dried sample (Table 1). Drying treatment was shown to have a highly significant effect on alpha-tocopherol content (Pr>F=0.0001).

Although alpha-tocopherol is heat stable, it is susceptable to oxidation. As with other lipid soluble materials that undergo oxidation, alpha-tocopherol is particularly sensitive to oxidation in materials with a low water activity. Csallany et al. (1970) found that upon heating in air at 60°C the oxidation products formed by alpha-tocopherol were a dimer, a trimer, alpha-tocopherol quinone and numerous other unidentified oxidation products.

The totally freeze-dried sample retained alpha-tocopherol because the drying process is carried out in a vacuum. After drying, the carrot bars were immediately vacuum packed in light proof containers and stored at −30°C, which also deterred deterioration of the tocopherol. However, the air-dried samples all experienced some loss of alpha-tocopherol. The loss appeared to be related to the amount of time the samples were exposed to air drying.

Carotene

The mean values for alpha, beta, and total carotene are shown in Table 2. For all three values, the totally freeze-dried carrots showed the highest concentration: alpha-carotene 15.66 mg/100g, beta-carotene 54.71 mg/100g, and total carotene 70.37 mg/100g. The totally air-dried sample had the lowest values of the drying treatments: alpha-carotene 6.67 mg/100g, beta-carotene 27.50 mg/100g, and total carotene 34.16 mg/100g. All three were shown to be highly significantly affected by drying treatment: alpha-carotene (Pr>F=0.0001), beta carotene (Pr>F=0.0001), and total carotene (Pr>F=0.0001).

In general, the carrot samples which were air dried for the least time had higher carotene concentrations. Dehydrated foods are most susceptible to loss of vitamin A and provitamin A because of their propensity to undergo

Table 1—Effect of drying treatment on ascorbic acid and alpha-tocopherol of dehydrated carrots (mg/100g dry weight basis)[a]

Treatment (% Moisture)	Ascorbic acid	Alpha-tocopherol
Fresh	85.28	3.41
Totally freeze-dried	33.39 a	3.45 a
Totally air dried	15.97 d	0.04 f
Freeze-dried (3%), Mist plasticized (10%), Air dried	32.76 a	2.98 b
Freeze-dried (10%), Air dried	27.71 b	1.42 c
Freeze-dreid (20%), Air dried	16.78 cd	1.13 d
Freeze-dried (30%), Air dried	16.38 cd	1.10 d
Freeze-dried (40%), Air dried	20.38 c	0.96 d
Freeze-dried (50%), Air dried	17.49 cd	0.55 e

[a] Means within columns followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 2—Effect of drying treatment on carotene content of dehydrated carrots (mg/100g dry weight basis)[a]

Treatment (% Moisture)	Alpha-carotene	Beta-carotene	Total carotene
Fresh	14.14	52.06	66.20
Totally freeze-dreid	15.66 a	54.71 a	70.37 a
Totally air dried	6.67 e	27.50 f	34.16 f
Freeze-dried (3%), Mist plasticized (10%), Air dried	10.61 d	40.47 e	51.08 e
Freeze-dried (10%), Air dried	12.81 b	49.40 b	62.21 b
Freeze-dried (20%), Air dried	11.73 c	44.49 d	56.22 d
Freeze-dried (30%), Air dried	11.42 cd	47.22 c	58.68 c
Freeze-dried (40%), Air dried	11.02 cd	44.89 d	55.91 d
Freeze-dried (50%), Air dried	10.52 d	40.23 c	50.81 e

[a] Means within columns followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

oxidation. The destruction of provitamin A in processed and stored foods can follow a variety of pathways depending on reaction conditions. In the absence of oxygen, there are a number of possible thermal transformations, particularly cis-trans isomerization. This has been shown in both cooked and canned vegetables (Tannenbaum, 1976).

Rehydration

In theory, optimum rehydration of a food product would replace the same amount of moisture loss during dehydration. By calculating rehydration ratios, one can determine the capacity of a dried food to absorb water. Rehydration ratios vary with different food products because of compositional differences.

For example, carrots used in this study contained 89% moisture. The totally freeze-dried carrots were dried to a moisture content of 3%. Thus, a 14-g samples which rehydrated to 100g would have an ideal rehydration ratio of 7.14. The samples which underwent final drying by air had a finished moisture level of 5%. These samples would have an ideal rehydration ratio of 6.25.

The mean values for the rehydration ratios of the various drying treatments are shown in Table 3. In all cases, drying treatment highly significantly affected rehydration ratios (Pr>F=0.0001).

Table 3—Effect of drying treatment on rehydration ratio for dehydrated carrots[a]

Treatment (% Moisture)	Rehydration ratio			
	10 min	20 min	30 min	60 min
Totally freeze-dried	1.94 cd	2.68 cde	3.28 cd	4.86 d
Totally air dried	1.75 d	2.11 e	2.63 d	3.20 e
Freeze-dreid (3%), Mist plasticized (10%), Air dried	1.70 d	2.21 ed	3.06 cd	6.05 bc
Freeze-dried (10%), Air dried	4.78 b	6.07 b	6.34 b	6.61 b
Freeze-dried (20%), Air dried	2.47 c	3.33 c	4.23 c	6.03 bc
Freeze-dried (30%), Air dried	2.06 cd	2.53 cde	3.15 cd	5.13 cd
Freeze-dried (40%), Air dried	2.20 cd	2.92 cd	3.72 cd	5.65 cd
Freeze-dried (50%), Air dried	5.47 a	7.37 a	81.0 a	8.20 a

[a] Means within columns followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range tests.

The sample that was freeze-dried to 50% moisture, compressed, then air dried had the highest ratio and was the quickest to rehydrate. The final ratio was also higher than ideal indicating excessive rehydration. This is in agreement with Wisakowsky (1977) who found that freeze-dried carrot cubes compressed at 46% moisture rehydrated quicker and more completely than carrots compressed at lower moistures.

The lowest ratios were for the totally air dried sample. These carrots did not exhibit satisfactory rehydration after a 60-min soak.

Shear

The shear values ranged from 546 pounds for the totally air-dried carrots to 300 pounds for the carrots freeze-dried to 50% moisture, compressed, then air dried.

The reduction in texture in the sample freeze-dried to 50% moisture before compression and air drying was due to over-rehydration leading to a soft sample. Conversely, the high shear value for the totally air dried sample was due to lack of rehydration leading to an overly firm tissue.

Sensory evaluation

The sensory panel could differentiate between the totally air dried carrots and the other treatments. Little difference was noted between the other samples.

Scanning electron microscopy

A photomicrograph of air-dried carrot tissue is shown in Fig. 1A. Collapse of the cellular structure and tissue coagulation are apparent. This caused a barrier to rehydration. This condition also contributed to the toughness of the tissue which led to high shear values and low sensory panel scores for texture.

The effect of total freeze drying on the carrot tissue is visible in Fig. 1B. Definite cellular structure is apparent along with shrunken areas. These areas could act as barriers to rehydration and thus account for low rehydration ratios.

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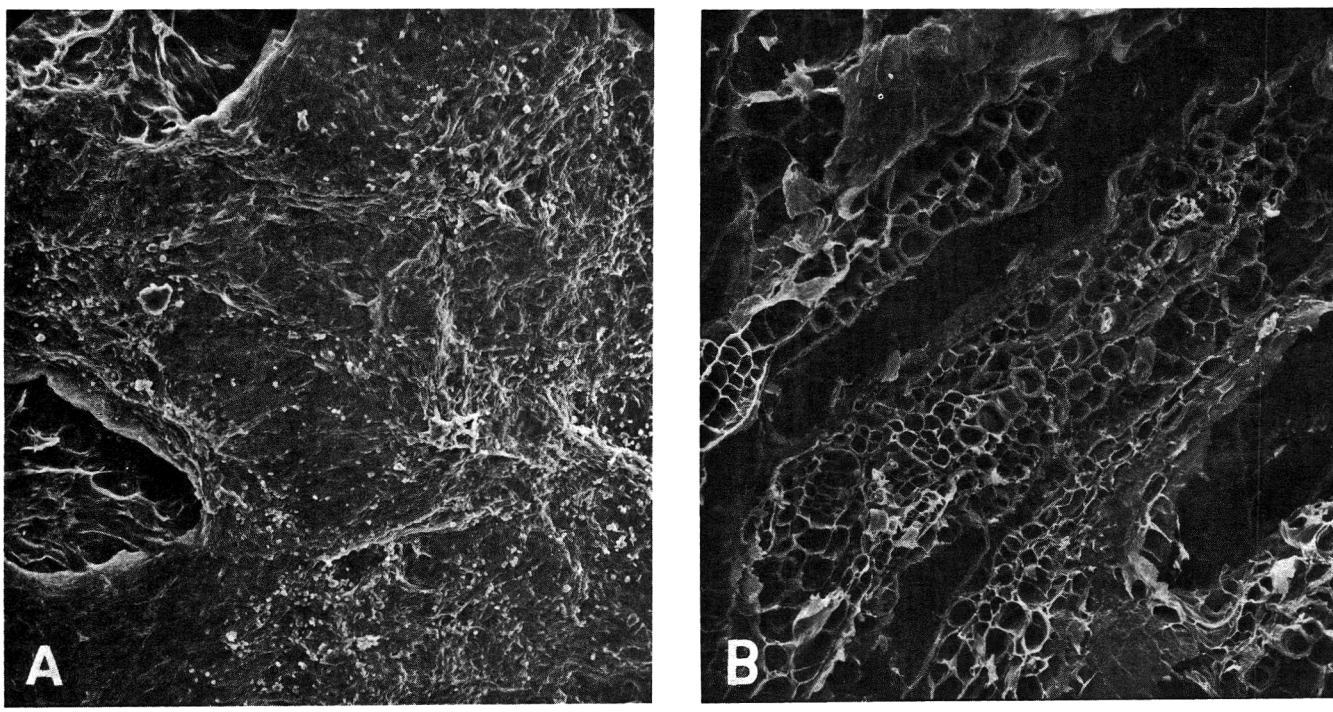

Fig. 1—Scanning electron photomicrograph of (A) totally air-dried and (B) freeze-dried carrot tissue.

Carrots that were freeze-dried to 50% moisture, compressed, then air dried are shown in Fig. 2. Large amounts of tissue disruption with large voids can be noticed. This is the cause of the observed rapid over-rehydration. The over-rehydration and tissue disruption led to the lower shear values. In general, as precompression moisture content increased, tissue damage increased. The least amount of damage took place in the samples at 10, 20 and 30% precompression moisture. The 20 and 30% samples did not exhibit extensive areas of compression. This is in agreement with Wisakowsky (1977) who found a precompression moisture of 46% to be optimum for rehydration of freeze-dried compressed carrot cubes.

Combining freeze-drying and air drying was demonstrated to give a quality dehydrated compressed carrot bar comparable to a completely freeze-dried carrot.

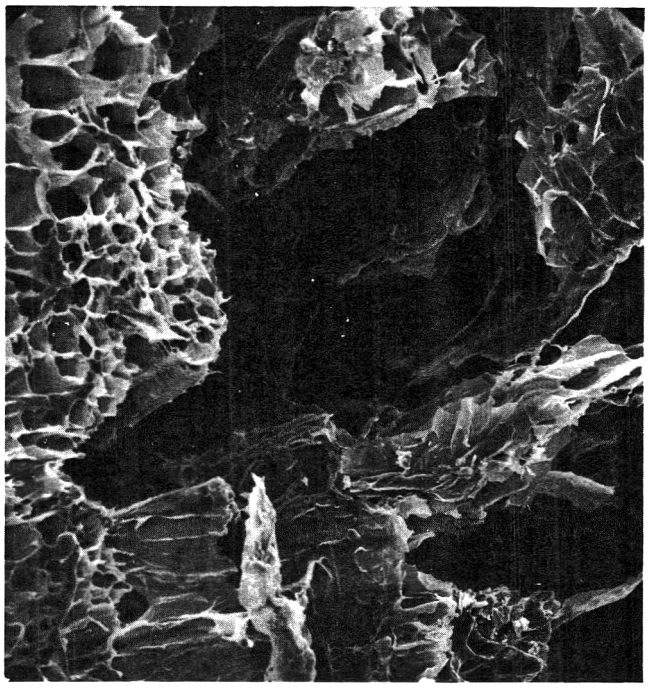

Fig. 2—Scanning electron photomicrograph of carrot tissue freeze-dried to 50% moisture, compressed, then air dried (ca 160X).

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Role of the Osmoticum in Bloater Formation of Pickling Cucumbers

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ABSTRACT

Changes in internal gas volume and pressure, and mass flow characteristics of fresh compared to brined cucumbers were measured to study the mechanism of bloater formation. Internal gas volume of fresh cucumbers decreased by about 50% and the resistance to mass flow of gases increased following storage in brine. Internal gas pressure increased by 81 mm Hg in cucumbers stored in brine and only 26 mm Hg in fresh cucumbers in brine following carbonation of the brine. Upon carbonation, cucumbers stored in both 2M NaCl and 3M ethylene glycol exhibited bloater damage accompanied by tissue dehydration, whereas fresh and water stored cucumbers did not bloat. Susceptibility of pickling cucumbers to bloater formation was proposed to be associated with a lowered internal tissue resistance to increased gas pressure.

INTRODUCTION

BLOATER FORMATION is a disorder of pickling cucumbers (*Cucumis sativus* L.) that occurs during brine fermentation. Bloated cucumbers are characterized by various forms of hollow regions in the internal tissue of the cucumbers (Etchells et al., 1974). Gas production, primarily CO_2, from the fermentation of brined cucumbers was implicated as the cause of bloater formation more than 40 years ago (Veldhuis and Etchells, 1939) and has since been associated with several microbial sources (Etchells and Bell, 1950; Etchells et al., 1945, 1968; Fleming et al., 1973a, b). Recent research has been directed toward understanding the mechanism of bloater formation (Fleming and Pharr, 1980).

Fleming et al. (1978) categorized susceptibility to bloater damage into three stages. Cucumbers exhibited little bloater damage if carbonated immediately after brining (stage I, low susceptibility), whereas bloater damage was severe if carbonation was started from 1 to 32 days after brining (stage II, high susceptibility). A reversion to low susceptibility (state III) occurred after 49 days of brining. Recently, a model for the mechanism of bloater formation was proposed by Fleming and Pharr (1980). They proposed that a liquid-clogged layer in the intercellular gas spaces of the epidermal and outer mesocarp tissue formed by brine entrance, acts as a differentially permeable barrier to the diffusion of N_2 and CO_2. A fresh fruit contains about 78% N_2 and only 6% CO_2 (Fleming and Pharr, 1980). Upon submergence of the cucumber into brine, microbial evolution of CO_2 causes a partial pressure gradient to develop, resulting in the diffusion of CO_2 from the exterior brine to the interior of the fruit. It was further postulated that there is a greater transport of CO_2 into the fruit than N_2 out of the fruit which causes the total internal gas pressure of the cucumber to exceed 1 atmosphere, resulting in bloater formation. The observation that cucumbers do not bloat upon artificial carbonation of the brine immediately following addition of brine was attributed to the presence of relatively continuous intercellular gas spaces in fresh fruit, allowing a more rapid exchange and equilibration of interior and exterior gases. However, there is no evidence or suggestion in the proposed mechanism, for the possible occurrence of mass flow of gases from cucumbers in brine upon increased internal gas pressure. In addition, the model fails to explain why the extent and severity of bloater damage may decrease with decreasing brine strength (Fleming et al., 1978).

This study was undertaken to gain a further understanding of physical factors associated with the susceptibility of brined cucumbers to bloater formation. Specific objectives were: (1) to develop a method and to measure mass flow of gases through fresh and brine- or water-stored cucumbers, (2) to measure changes in the internal gas pressure in fresh cucumbers, compared to brine- or water-stored cucumbers upon artificial carbonation of the brine, and (3) to test the hypothesis that a liquid-clogged layer of tissue develops during brine storage of cucumbers.

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MATERIALS & METHODS

Cucumbers

North Carolina size grade 3 pickling cucumbers (3.8–5.1 cm diameter), cv. Calypso, were obtained from a nearby grower on the day of harvest for most of the experiments and from hand-pollinations in a greenhouse for remaining experiments. Cucumbers used in experiments involving single fruit measurements were selected for uniform weight (±10% of the mean fruit weight). Bloater damage was evaluated according to Etchells et al. (1974), and bloater indices were calculated according to Fleming et al. (1977).

Gas exchange of cucumbers

Gas exchange of the internal atmospheres of fruit was accomplished by flowing N_2 through glass gas dispersion tubes at 300 ml/min for 1 hr around single fruit in 1500 ml containers before adding solution. Flow of N_2 was maintained at 50 ml/min following addition of solution to exclude O_2 from the system. For experiments involving samples of fruit, 1.7 kg of fruit were packed into 3.8 liter jars to give a 45:55 (w/v) pack-out ratio of fruit to brine. Each jar lid was equipped with a glass gas dispersion tube, graduated reservoir and a glass rod to support the reservoir as previously described by Fleming and Pharr (1980). Carbon dioxide was introduced through the gas dispersion tubes either immediately following addition of solution or following 48 hr of exposure to solution. Carbonation was initiated at a flow rate of 300 ml/min for the first 30 min to achieve saturation rapidly. The flow rate was adjusted to maintain 50 ml/min following the first 30 min.

Composition of solutions

Acidified, aqueous solutions were added to the containers or jars, while gas flow was maintained to exclude air from the system. The brining treatment used in all experiments was 10.6% (w/w) NaCl, 0.32% (v/v) glacial acetic acid, and 0.20% (w/w) sodium benzoate. NaCl was excluded from this mixture in certain experiments. The acidified, aqueous solutions containing sodium benzoate were used to suppress microbial growth during storage (Fleming and Pharr, 1980).

A 16.8% (v/v) ethylene glycol solution was used in one experiment to approximate the osmotic pressure of the 10.6% NaCl

solution. The concentration of ethylene glycol was interpolated from a table of osmosity values (Weast and Astle, 1979).

Expansion volume

Expansion volume of solution to the exterior of cucumbers was determined by the rise in solution level in the graduated reservoir and was expressed as a percentage of the volume of cucumbers (Fleming et al., 1973a).

Internal gas pressure

A cavity was made through the approximate center of the longitudinal axis of cucumbers with a 0.4 cm diameter cork borer. A no. 19 stainless steel, hypodermic needle was placed into the gas cavity, secured to the fruit surface and connected to a Hg manometer as described previously (Corey et al., 1982). Changes in pressure of the gas cavity were then monitored over a 10-hr period at 1-hr intervals. Cucumbers were cut longitudinally and examined for bloater damage following 10-hr carbonation. Fruit packed in glass jars were given the same gas exchange and solution treatments to provide internal controls. The internal controls verified whether a particular lot of fruit was susceptible to bloater damage for a given treatment. In addition, it was not possible to check the experimental set-up of bloater-susceptible fruit for gas leaks because of the possibility of inducing bloater formation by pressurizing the fruit. Thus, if a treatment rendered the cucumbers susceptible to bloating as verified by the internal controls, the results of internal gas pressure changes were used only for those fruit that displayed bloater damage.

Internal gas volume of tissue components

The internal gas volume of exocarp, mesocarp and seed regions from fresh fruit and fruit held for 48 hr in water or brine solutions was determined. A 25–35g sample of each tissue component obtained from a sample of three fruit was immersed in a 2.0M $MgSO_4$ solution adjusted to pH 2.5 with HCl as described by Jorge (1978). A 10-ml graduated pyrex tube was fitted to an inverted funnel with a rubber stopper all of which was placed in a sealed desiccator jar to trap the gases from the tissue. A vacuum pump was connected to the desiccator lid and the system was subjected to a 737 mm Hg (0.97) atmosphere) vacuum for 2 min. Gases exited the cucumber tissue, and were trapped and measured in the graduated tube upon return to atmospheric pressure.

Mass flow

The flow rate of pressurized air from the interior to the exterior of individual fruit was measured using the apparatus shown in Fig. 1. A gas cavity was bored in the fruit and then equipped with a hypodermic needle setup as previously described (Corey et al., 1982). A flow line was connected from the hypodermic needle in the fruit to a pressurized air line. Connections were made on the inflow line to go through a rubber stopper into a sealed desiccator jar. Individual fruit were pressurized internally at increasing increments of pressure as measured by a pressure gauge (Fisher Scientific Company, Raleigh, NC) connected to the inflow line. An outflow line was inserted through the rubber stopper and connected to a flow tube which was read for each static pressure setting. Flow rates were established for fresh fruit in air and fresh fruit submerged in water or brine by reference to a calibration chart for air flow. Fruit were also held for 48 hr in acidified, aqueous solutions with a continuous N_2-purge, following a N_2-exchange of the internal atmosphere and then tested for flow characteristics.

The flow rate for intact fruit was compared to that of fruit with ends removed for both fresh and brined (48 hr) cucumbers to determine if the skin is a major component of resistance to flow. Flow rates for fruit in this experiment were expressed on a per unit of surface area basis.

Since the mass flow apparatus (Fig. 1) was not sufficiently sensitive to measure flow rates less than about 12–15 cm^3/min, low flow rates were measured by gas entrapment. This was achieved by fitting a 10-ml, graduated pyrex tube filled with brine solution to an inverted funnel placed over the fruit in an open container. The fruit were pressurized internally as previously described, and the volume of gas collected in the graduated tube was read every 5 min for 25 min to establish a flow rate.

Percent change in weight of cucumbers

Individual cucumbers were labeled and weighed prior to packing in 3.8 liter jars. The fruit were reweighed following 48 hr exposure to water, brine or ethylene glycol solution, and the % change in weight calculated.

RESULTS

Internal gas pressure

The pressure of gases inside cucumbers stored for 48 hr in brine or water increased rapidly after carbonation was initiated (Fig. 2). After 4 hr carbonation, internal gas pressure increased by 81 mm Hg in fruit exposed to brine for 48 hr, and by 80 mm Hg in fruit exposed to water for the same length of time. In contrast, internal gas pressure increased by only 26 mm Hg in cucumbers carbonated immediately following addition of brine. All cucumbers brined for 48 hr prior to carbonation bloated. On a scale of slight, moderate, and advanced (Etchells et al., 1974), bloating was slight and was of the honeycomb, lens or balloon type. There was no bloater damage in any of the cucumbers carbonated immediately following addition of brine or after 48 hr exposure to water.

Internal gas volume of tissue components

The internal gas volume of both exocarp and seed regions from fresh fruit was lower than in mesocarp tissue (Fig. 3). Exposure of fresh cucumbers to brine or water for 48 hr

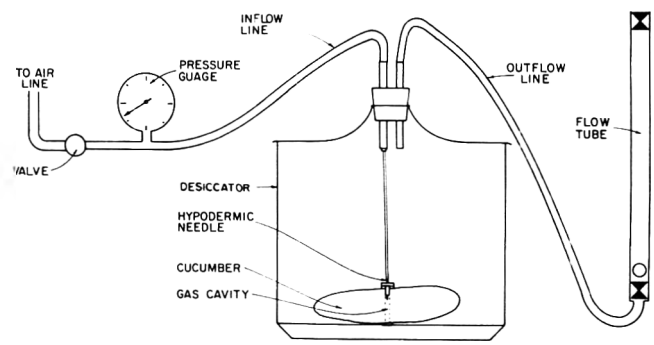

Fig. 1—Diagram of apparatus used for measuring mass flow of pressurized air through cucumber fruit.

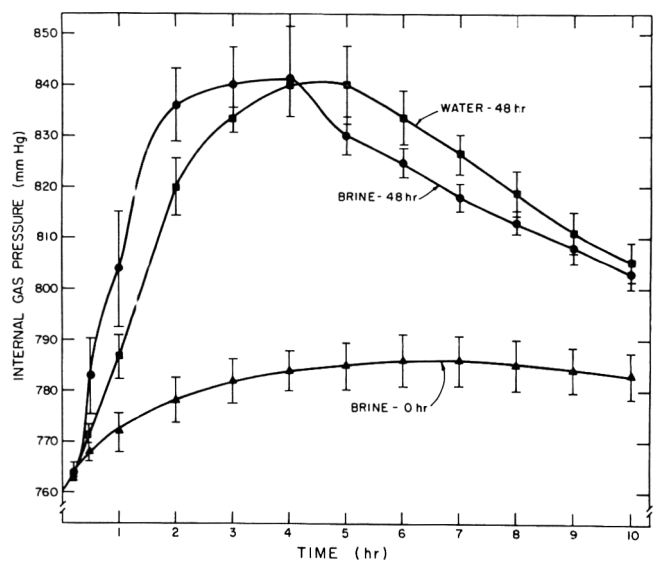

Fig. 2—Internal gas pressure changes in cucumbers held in carbonated brine or water. The cucumbers were N_2-exchanged before addition of liquid. Carbonation was begun either immediately (0 hr) or 48 hr after the addition of liquid. The liquid was continuously purged with N_2 before carbonation when carbonation was begun after 48 hr. Vertical bars represent one standard deviation.

resulted in a significant reduction in the internal gas volume of exocarp, mesocarp and seed region tissue (Fig. 3). Internal gas volume of both exocarp and mesocarp in cucumbers exposed to brine or water decreased by 50–60%. However, the internal gas volume of seed region tissue from fruit exposed to water was reduced by only 29% compared to 51% reduction in fruit exposed to brine.

Mass flow

Air under pressure readily flows through fresh cucumbers in air, indicating the presence of realtively continuous channels from the interior to the exterior of such fruit (Fig. 4). Upon submergence in brine or water, flow was not measurable (apparatus shown in Fig. 1) until an internal pressure of 138 mm Hg was reached in the cucumbers (Fig. 4). However, air bubbles flowing from the surface of the fruit were observed at pressures of 101 mm Hg for cucumbers submerged in water and 84 mm Hg for fruit in brine (Table 1). The flow rate through submerged, fresh fruit at a pressure of 227 mm Hg was only 31.9 $cm^3\ min^{-1}$ · fruit as compared to 97.5 $cm^3\ min^{-1}$ · fruit for fresh fruit in air.

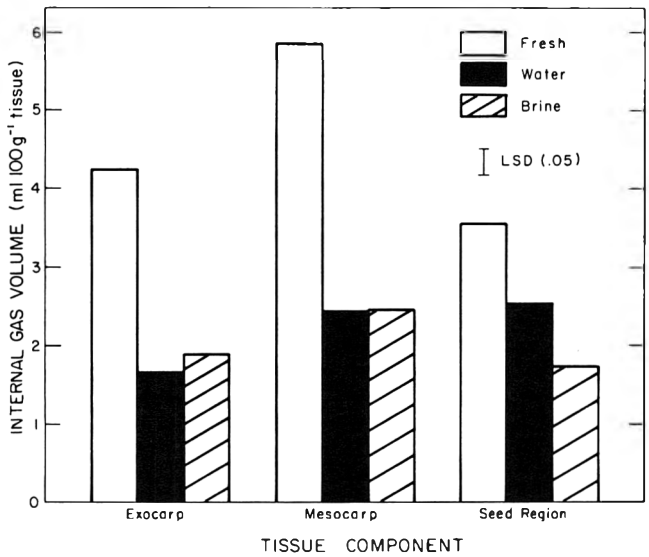

Fig. 3—Internal gas volume of exocarp, mesocarp and seed region tissue of fresh fruit, and of fruit held in brine or water solutions for 48 hr. The fruit were N_2-exchanged before liquid storage. Mean separation is indicated by vertical LSD bar, 5% level.

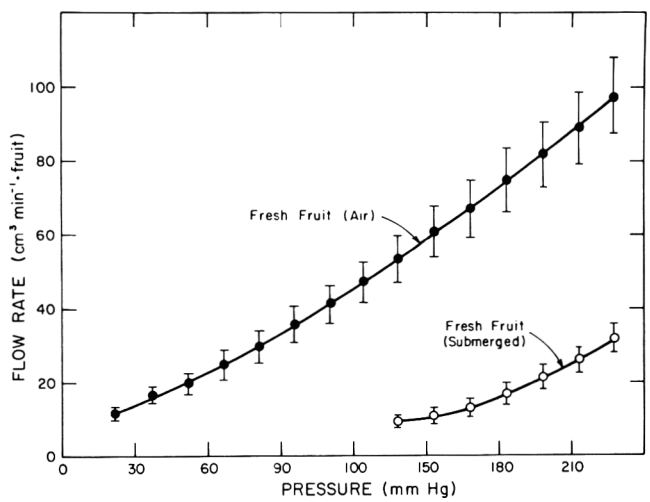

Fig. 4—Flow of pressurized air through fresh cucumbers in air or through cucumbers submerged in water or brine at various pressures. Each point represents the mean of 6–8 replications. Vertical bars represent one stardard deviation.

Gas flowed at a much greater rate in response to internal pressurization from fresh cucumbers with both ends removed than from intact fruit, as measured in air (Fig. 5). There was no gas collected from intact cucumbers pressurized at 70 mm Hg for 5 min following 48 hr brining. However, if both ends were removed from the cucumbers after the 48-hr brining, a flow rate of 4.1 ± 2.5 $cm^3\ min^{-1} \cdot cm^{-2}$ (4 replications) was measured in air at an internal pressure of 70 mm Hg (using the apparatus of Fig. 1).

A measurable flow did not occur through cucumbers exposed previously to water or brine for 48 hr even at pressures as high as 300 mm Hg. However, a slight flow was observed at lower pressures (Table 1) in the form of one to several streams of small air bubbles coming from the surface of each fruit. The pressure was increased to 300 mm Hg inside cucumbers exposed to water and maintained for 2 min. Fruit treated in this manner did not exhibit carpel separation, whereas carpel separation in fruit previously exposed to brine for 48 hr occurred at an average pressure of 202 mm Hg (Table 1). Since the internal gas pressure was increased rapidly and did not simulate closely the natural course of gas pressure development, carpel separation rather than bloater damage was used to describe damage to the

Table 1—Internal pressures for gas flow from cucumbers submerged in water or brine, and for carpel separation

Cover solution[a]	Time held in solution (hr)	Internal gas pressure for: Earliest observable flow (mm Hg)[b]	Carpel separation (mm Hg)
Without NaCl	0	101 ± 17[c]	–[d]
	48	186 ± 66	>288[e]
With NaCl	0	84 ± 7[c]	–[d]
	48	159 ± 20	202 ± 19[f]

[a] Cover solutions contained 0.32% (v/v) glacial acetic acid and 0.20% (w/v) sodium benzoate. NaCl, when added, was 10.6% (w/w).
[b] Values represent means of 4 replications ± 1 standard deviation.
[c] Measurements were made immediately after addition of solutions.
[d] Fruit were later held in solution for 48 hr following flow measurements and were therefore not tested for carpel separation.
[e] Pressure was increased from 186 mm Hg to 288 mm Hg within 30 s and maintained at 288 mm Hg for 2 min.
[f] Severe carpel separation occurred in all fruit.

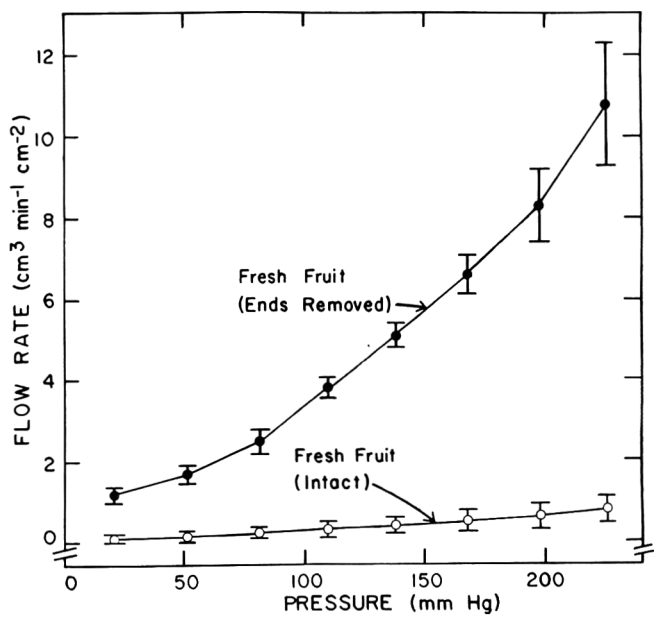

Fig. 5—Flow of pressurized air through intact fresh cucumbers and fresh cucumbers with both ends removed as measured in air. Each point represents the mean of 4 replications. Vertical bars represent one standard deviation.

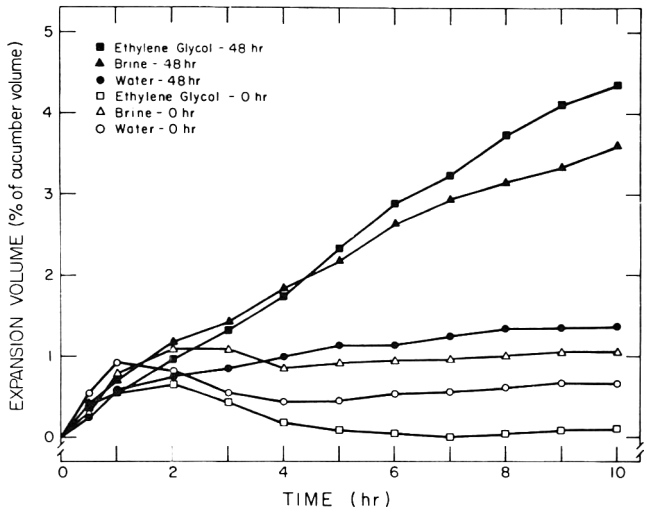

Fig. 6—Expansion volume changes in cucumbers held in carbonated solutions. The cucumbers were N_2-exchanged before addition of solutions. Carbonation was begun either immediately (0 hr) or 48 hr after addition of solution. The cover solutions were water, 16.8% (v/v) ethylene glycol or 10.6% (w/w) NaCl. Each point represents the mean of duplicate 3.8 liter jars, each containing 12–14 fruit.

internal tissue. Carpel separation in fresh cucumbers has been used as a measurement analogous to balloon bloating (Wehner and Saltveit, 1982).

Low flow rates determined by gas entrapment were measured to determine if mass transport of gases can occur through cucumbers in brine. At a pressure of 70 mm Hg, a flow rate of 8.7 ± 4.2 $\mu l\ min^{-1}$ · fruit (3 replications) was measured for fresh fruit immediately after submergence in brine. No gas was collected after 25 min at pressures of 70–100 mm Hg from cucumbers exposed to brine for 48 hr.

Effect of osmoticum on bloater formation

Changes in expansion volume after carbonation of N_2-exchanged cucumbers ranged from 0–1.1% in the three external solutions immediately following addition of the solutions (Fig. 6). Bloater damage was absent from these three treatments (Table 2). However, carbonation of fruit after 48 hr exposure to either osmoticum, brine or ethylene glycol, resulted in substantial expansion volume changes (Fig. 6). Bloater damage was significantly greater for cucumbers exposed to NaCl or ethylene glycol solutions than for fruit held only in acidified water for 48 hr prior to carbonation (Table 2). Cucumbers exposed to acidified water for 48 hr increased in weight by 1.65% of their initial weight as compared to losses of 6.15% and 7.43% for cucumbers held in brine and ethylene glycol, respectively (Table 3).

DISCUSSION

A major aspect of the model for the mechanism of bloater formation proposed by Fleming and Pharr (1980) is the formation of a continuous liquid-clogged region of tissue in the outer layer of brine-stored cucumbers. Internal gas pressure in excess of atmospheric pressure upon artificial carbonation of brined fruit was proposed to occur due to the greater inward transport of CO_2 compared to outward transport of N_2 in cucumbers possessing this hydrated layer, thereby causing bloater formation.

Evidence for the clogging of the intercellular passages following brine exposure was obtained in this study. A reduction in the internal gas volume of cucumbers exposed to 48 hr brining was measured throughout the fruit, including the interior seed region tissue (Fig. 3). Therefore blockage of intercellular avenues may occur not solely in the outer layer of tissue as postulated previously by Fleming and Pharr (1980). Further, since cucumbers exposed to brine become dehydrated (Table 3), the mode of clogging is probably not solely by intrusion of brine.

The clogging of internal gas spaces in brine-stored cucumbers may also be attributed to both an exosmosis of water and shriveling and collapse of the tissue (Fleming and Thompson, 1982) leading to the reduction in internal gas volume throughout the fruit (Fig. 3). In contrast, the reduction in internal gas volume of cucumbers exposed to water for 48 hr is attributed to the entrance of liquid water into the internal gas spaces, since these cucumbers increase in weight (Table 3).

Although there is a reduction in the internal gas volume of the interior tissue of brined cucumbers, the exocarp is the main component of resistance to the mass flow of gases through the fruit surface in both fresh fruit (Fig. 5) and brined fruit (see Results). Previous work of Fleming et al. (1973a) showed that cucumbers pierced to a depth of 1 inch with a bed of 20-guage needles did not bloat. In addition, bloater damage was also reduced in fruit that were peeled before brining.

The above observations along with mass flow measurements of brined cucumbers with ends removed suggest that mass flow may act to relieve pressure and subsequent bloating of brined cucumbers when resistance to gas transfer is artificially reduced. However, in intact cucumbers there is a reduction in mass flow of gases through brine- or water-exposed cucumbers as measured by an increase in the pressure required to provide observable flow from the cucumber surface compared to fresh fruit (Table 1). This provided additional evidence for the clogging of the intercellular avenues for gas exchange.

Evidence that fresh cucumbers possess relatively continuous intercellular spaces (allowing a more rapid transfer of gases than brine- or water- stored fruit) were obtained from the following observations: (1) lower internal gas

Table 2—Effects of cover solution and the time carbonation was initiated on susceptibility of cucumbers to bloater formation

Cover solution[a]	Time carbonation was initiated after addition of solution[b]: 0 hr — Expansion volume after 10 hr (%)	0 hr — Bloater index	48 h-[c] — Expansion volume after 10 hr (%)	48 h-[c] — Bloater index
Without NaCl	0.67	0	0.64	0.87
With NaCl	1.06	0	3.61	9.94
Ethylene glycol	0.12	0	4.35	18.75
LSD (0.05)	0.63	–	0.63	2.34

[a] Cover solutions also contain 0.32% (v/v) glacial acetic acid and 0.20% (w/v) sodium benzoate. NaCl, when added, was 10.6% (w/w). Ethylene glycol, when added, was 16.8% (w/w).
[b] Cucumbers were N_2-exchanged prior to addition of solution.
[c] Purged continuously with N_2 after addition of solution until carbonation was begun at 48 hr.

Table 3—Percent weight change of fresh cucumbers after 48 hr exposure to water, brine or ethylene glycol

External solution[a]	Change in weight from initial fresh weight (%)[b]
Without NaCl	+1.65 ± 0.95
With NaCl	–6.15 ± 1.47
Ethylene glycol	–7.43 ± 1.70
LSD (0.05)	1.01

[a] Cover solutions also contained 0.32% (v/v) glacial acetic acid and 0.20% (w/v) sodium benzoate. NaCl, when added, was 10.6% (w/w). Ethylene glycol, when added, was 16.8% (w/w).
[b] Values represent means of 16 cucumbers ± 1 standard deviation.

pressure in fresh cucumbers carbonated immediately following brine addition as compared to cucumbers carbonated following exposure to water or brine for 48 hr (Fig. 2), (2) higher internal gas volume of fresh cucumbers compared to brine- or water- stored cucumbers (Fig. 3), and (3) high flow rate of air through fresh cucumbers upon internal pressurization (Fig. 4). Those findings were consistent with the results and ideas of Fleming and Pharr (1980) that gases in fresh cucumbers can exchange rapidly with the ambient gaseous environment.

However, there is a substantial reduction in the mass flow of air through internally pressurized cucumbers immediately following submergence in either water or brine compared to the mass flow rate through the same cucumbers in air prior to submergence. This immediate reduction in the pressure-induced flow of gases through intact fresh cucumbers upon submergence may be explicable on the basis of opposing surface forces of the liquid at the interface of the liquid and openings on the surface of the fruit (Corey, 1982).

Nevertheless, there is the potential for mass flow of gases to occur through fresh cucumbers in brine. The lower internal gas pressure measured in fresh cucumbers carbonated immediately following brine addition compared to brine- or water- stored cucumbers (Fig. 2) may be due to mass flow of gases acting in the release of pressure. The fact that a measurable flow of air was obtained for fresh cucumbers in brine at a gas pressure of 70 mm Hg (i.e. 8.7 μl min^{-1} · fruit) indicated that internal gas pressures of that magnitude would probably not generally be reached in fresh cucumbers carbonated immediately following addition of brine.

The development of internal gas pressure is not a sufficient condition to account for the occurence of bloaters since water-stored cucumbers did not exhibit bloater damage. Further, the acquisition of susceptibility to bloater formation is due to an effect of the NaCl solution. The role of the NaCl in bringing about a bloater-susceptible condition in pickling cucumbers is apparently due to its effect as a strong osmoticum, causing the dehydration of the tissue (Table 3). This idea was supported by the expansion volume (Fig. 6 and Table 2), bloater index (Table 2) and weight loss (Table 3) resulting from 48 hr exposure to ethylene glycol solution having the same osmotic pressure as the NaCl solution. Apparently, there is a decreased tissue resistance to internal gas pressure in cucumbers exposed to the dehydrating effects of a hypertonic solution relative to the tissue resistance of fresh fruit or fruit exposed to a hypotonic solution such as water. Wehner and Saltveit (1982) found that internal air pressures of 0.68–2.0 atmospheres above atmospheric pressure were needed to cause carpel separation in several cultivars of fresh pickling cucumbers. Those measurements are in sharp contrast to the maximum internal gas pressure of about 0.1 atmosphere (80 mm Hg) above atmospheric pressure associated with bloater damage in brine stock as measured in this study. This further demonstrates the large difference in tissue resistance of fresh compared to brined cucumbers.

In summary, findings of this study suggest that the acquisition of susceptibility to bloater formation in pickling cucumbers is associated with the following mutually dependent conditions: (1) a change in the gas exchange properties of the cucumber such that an altered transport of gases occurs leading to the development of internal gas pressure, and (2) a decreased resistance of the tissue to internal gas pressure brought about by exposure to high solute concentration.

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Effect of Osmotic Agents and Concentration on Fruit Quality

H. R. BOLIN, C. C. HUXSOLL, R. JACKSON, and K. C. NG

ABSTRACT

Osmotic syrups can be reconcentrated and reused for osmotic water removal through at least five complete cycles without adversely affecting the fruit being concentrated, even though the properties of the osmotic medium do change. Syrup penetration rate into a fruit piece was faster with high fructose corn syrup (HFCS) than sucrose. Taste panel evaluation indicated that overall, sucrose was preferred as an osmotic medium over HFCS.

INTRODUCTION

WATER REMOVAL from piece-form foods is primarily done to lower water activity, so that microbial growth will be inhibited. However, with increased costs for energy and other resources, there is now a greater concern for the high costs of shipping, packaging, and storing products. Since most foods are composed largely of water, one way these costs could be reduced, would be by removing a portion of their water before packaging and distribution. This concept has been applied largely to food purees and liquids for many years. Concentration of piece-form foods must require a minimum use of energy and produce an acceptable product on rehydration.

One of the more energy efficient means of removing moisture from a food piece is by osmosis, since water does not have to go through a phase change. The technique of using the difference in osmotic potential between a product and the medium surrounding it has been used experimentally on various food items. Intermediate moisture products have been produced by immersing the fruit into a 70° brix sucrose solution to reduce their weight by fifty percent (Ponting et al., 1966; Moy et al., 1978). The products were further dried, using vacuum or hot air dehydration, to about 2% moisture. This procedure has also been investigated as an initial step in freeze dehydration (Hawkes and Flink, 1978). Others (Contreras and Smyrl, 1981), used a different osmotic medium such as corn syrup solids. However, most previous work was done using a small cube or thin piece of fruit in a large quantity of osmotic syrup, which provided good conditions for theoretical studies but could not be scaled up to a commercial production size operation. One limitation for large scale production using an osmotic concentration process is the quantity of diluted syrup left at the end of the process. For this procedure to become economically feasible, the syrup would need to be reconcentrated and reused. Use of multieffect evaporators for reconcentration of the syrups is the key factor in making this an energy efficient system for water removal, since only about 25% as much energy should be required by this procedure for water removal as compared to hot air dehydration.

No work has been done on the effects repeated reconcentration and recycling of syrup would have on its physical and chemical properties or on the properties of the product being concentrated. Also, for this procedure to become commercially feasible, additional information is needed on the effect the osmotic syrups have on the final product, regarding flavor and syrup penetration.

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MATERIALS & METHODS

THE FRUIT, *Pyrus malus*, *Prunus armeniaca* and *Prunus persica*, (Red delicious apples, Elberta peaches and Blenheim apricots), were all obtained fresh from a wholesale supplier. Sucrose and high fructose corn syrup - 90 (HFCS) were the osmotic mediums investigated, as well as a Sorbitol syrup for flavor evaluation. Malic acid was used to acidify the syrups where indicated. The osmotic procedure consisted of peeling, coring and slicing the apples into twelfths, or halving and pitting peaches and apricots. Six hundred grams of the fruit were put into a container holding 2400g of the osmotic medium. The container was placed in a 70°C water bath and the product was stirred at regular intervals. After 6 hr, the syrup was drained and reconcentrated using a laboratory vacuum concentrator operating at 60°C.

The 70° Brix reconcentrated syrup was then used to desiccate a fresh batch of fruit. The 4:1 syrup to fruit ratio was maintained, by adjusting the amount of fruit to fit the quantity of reconcentrated syrup available.

Color of the fruit and syrup were evaluated using a Hunter Color Difference Meter. One hundred grams of the syrup were poured into a 5.5 cm diameter cell, covered with a standard white tile and placed over a 5 cm diameter opening to be read. By using the white tile cover a greater difference in color reading was realized, since a greater percentage of the light transmitted through the syrup was reflected back through the sample and to the sensor. For fruit, the pieces were packed directly into a cup to a 3 cm depth. Syrup viscosity was determined with a Epprecht Rheomat 15 Viscosimeter. Water activity was determined with a Beckman Hygroline instrument, and moisture by the AOAC (1970) procedure.

Syrup cellular penetration was determined by the following procedure. Unpeeled, uncored 15 mm thick apple slices from the center area of the apple were immersed in the respective 70°C osmotic syrups. At the time specified, the slices were removed from the syrup, drained and excess suface liquids blotted off. Using a 15 mm diameter cork borer, a cylinder was cut from the apple slice and quickly frozen by immersion in liquid nitrogen. A freezing microtome operating at −25°C was used to cut 1 mm thick slices consecutively from the frozen cylinder, starting at the flat outside surface and progressing towards the center. The resultant pieces were put into 0.25 dram vials and sealed. Soluble solids were determined on each piece using an Abbe refractometer.

Sensory evaluation of the osmotically concentrated fruit was investigated to ascertain if any objectionable flavor changes occurred in the product from the concentration procedure. A twenty member consumer panel was used to evaluate the fruit pieces at two different times, with the testings being spaced at least four hours apart. The evaluation was done in partitioned booths equipped with subdued lighting. The panelists were instructed to evaluate the samples by two procedures, Hedonically and by ranking. In the Hedonic procedure a rating was used of one (dislike extremely) to nine (like extremely), and in ranking a one was given for the sample most preferred, down to a four for the one least preferred. The ranked results were analyzed statistically using the tables of Kahan et al. (1973). Three osmotic syrups were used in the taste panel evaluation, sucrose, HFCS, and sorbitol.

RESULTS & DISCUSSION

OSMOTIC SYRUPS reconcentrated through five complete cycles underwent definite physiochemical changes. One of these changes was the darkening of the syrups during

recycling (Fig. 1), where sucrose and high fructose corn syrups darkened almost linearly with the increased number of concentrations, as determined visually and by the decreased L value (0 = black and 100 = white). The two syrups darkened at a similiar rate when in contact with apples. However, darkening rate accelerated in HFCS when it was acidified either from adding acid directly to the syrup, or from acid being extracted during the osmotic concentration of a higher acid fruit, such as apricots. Acidification increased the slope of the darkening curve from 3.0 to 6.5, indicating more than a twofold rate increase.

The coloration of the osmotic syrup was also effected by the recycling. The red hue (+a) of the syrup increased slightly but the main initial pigmental shift was in the yellow (+b) area (Fig. 2). Acidification of the syrup increased "b" dramatically through the first two concentrations, followed by a decrease. This same effect was observed when apricots were desiccated with HFCS. Since apricots contain more acid than apples, a large quantity is leached into the syrup (Table 1A). This acid extraction into the syrup seems to be a major factor in initiating browning during the extraction and reconcentration because no color change occurred in syrups that were subjected to five heating cycles with no fruit.

As the syrups darkened there was also an increase in 5-hydroxymethyl furfural (5-HMF), which was determined by monitoring the development of the absorption peak at 284 mμ (Fukuchi et al., 1977). This compound is formed as one of the breakdown products of glucose, whose formation is catalized by the acids leached from the fruit and also heat.

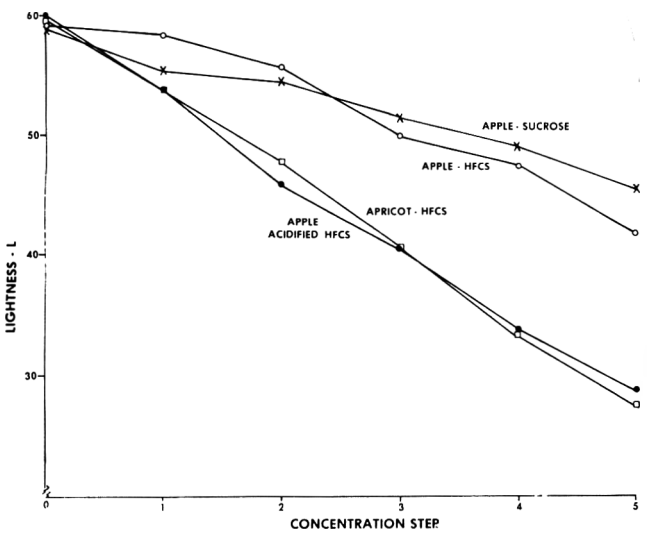

Fig. 1—Changes in lightness of osmotic syrups during repeated vacuum reconcentration and recycling.

Acids also can react as a catalyst in hydrolyzing sugar disaccharides to the monosaccharides. For instance, sucrose in the granular form or dissolved in water is chemically stable. However, if hydrogen ions are introduced, by acidifying the solution, hydrolysis can rapidly occur. During these osmotic drying – reconcentrating procedures, the fruit acids leached out of the apples into the syrup accelerated sucrose hydrolysis (Table 1B). The glucose increase in the syrup was because of sucrose hydrolysis and not from being leached out of the fruit since this glucose increase was followed by a similiar sucrose decrease. Also, from material balance studies no gain in syrup solids was noted, but in contrast, a 2–3% loss of solids occurred from migration into the fruit. The hydrolysis rate was linear with respect to recycle number for the first through fifth reconcentration and extraction indicating a first order reaction, which sucrose hydrolysis is known to be. With apricots this hydrolysis would be even more rapid because of their higher acid content; sucrose hydrolysis rate is acid dependent (Hoynak and Bollenback, 1966). There was also an increase with HFCS in reducing sugars and a decrease in total sugars during the osmotic dehydration-reconcentration sequence, but the change was small.

Viscosity of syrups relate to the amount of energy required for syrup movement, such as pumping, stirring,

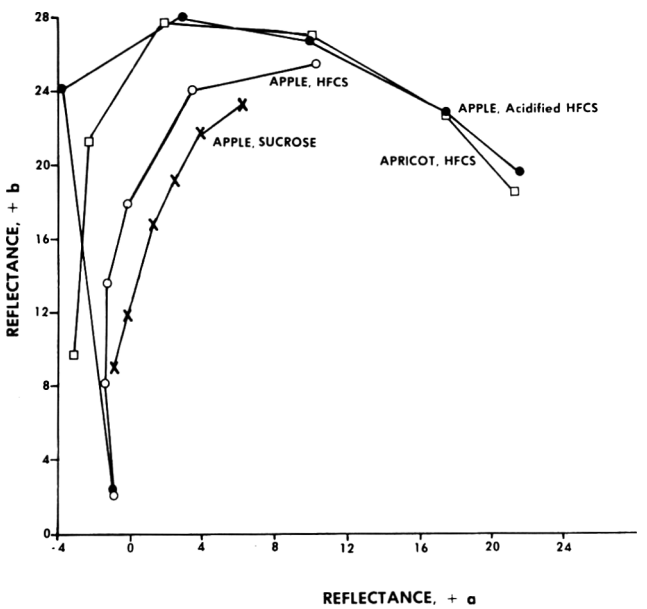

Fig. 2—Changes in color saturation of osmotic syrups during repeated vacuum reconcentration and recycling (lowest point is initial sample, second point is first recycled sample, third point is second recycled sample, etc.)

Table 1—Effect of syrup recycling on acidity and hydrolysis

Fruit	Syrup	Brix	Recycle Number 0	1	2	3	4	5	Slope[a]	Correlation coefficient
			A – Acidity (% as malic)							
Apple	HFCS	80	0.03	0.07	0.11	0.11	0.13	0.17	0.03	0.970
Apple	HFCS+Acid	80	1.03	1.05	1.18	1.18	1.11	1.09	0.01	0.417
Apple	Sucrose	70	0.03	0.05	0.07	0.07	0.12	0.13	0.02	0.980
Apricot	HFCS	80	0.03	0.13	0.19	0.19	0.43	0.48	0.09	0.990
			B – Sugars, analytical (%)							
Apple	Sucrose	70	66.6	54.2	54.0	48.7	37.7	33.7	–6.25	0.974
	Reducing[b] sugar	–	0	12.0	16.4	21.9	25.8	29.6	5.57	0.973

[a] Obtained from plotting viscosity, acidity, or sugars against recycle number
[b] From sucrose inversion.

Table 2—Taste panel evaluation of cut fruits dried to 50% weight reduction

		Hedonic					Ranking				
				Osmotic					Osmotic		
Fruit	(%) Acidity	Fresh	Air	Sucrose	HFCS	Sorbitol	Fresh	Air	Sucrose	HFCS	Sorbitol
Apple	0.3	7.5	5.9	5.8	5.1	5.8	1.2*	3.3	3.9**	3.3	3.4
Apricot	2.3	5.2	3.6	5.8	5.6	5.4	3.6**	3.9**	2.2*	2.5	2.8
Peach	0.5	6.9	5.7	6.2	6.0	6.4	2.5	2.9	3.0	3.6**	3.0

* Like most (P = 0.05)
** Like least (P = 0.05)

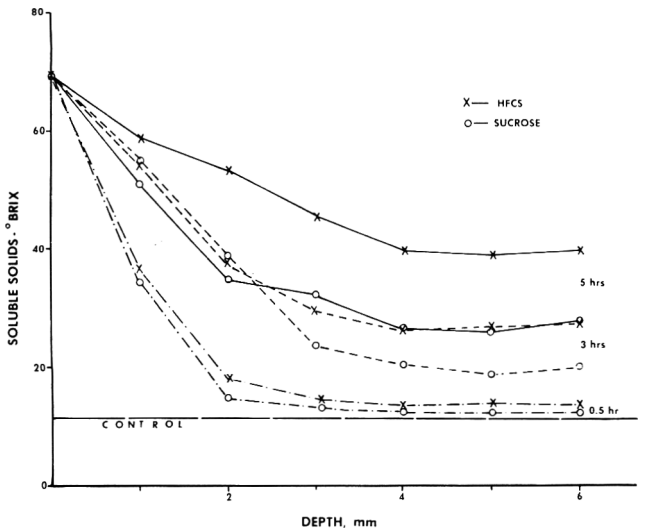

Fig. 3—Osmotic syrup penetration at 70°C into apple tissue during concentration.

etc. The commerical 80° brix HFCS is much more viscous than 70° sucrose, with the HFCS having a viscosity of 40_cp compared to 2.9_cp for sucrose. When 80° HFCS was diluted to 70° brix its viscosity dropped to 1.7_cp, which was less than 70° sucrose. There was no consistent change in syrup viscosity during the recycling operations.

Some syrup loss occurred during recycling steps, with the fruit accounting for approximatley 9–14% and another 10–15% being lost as coatings on the vessels. This latter quantity would be much less in a large scale commercial operation.

Even though the physiochemical properties of the syrups changed during repeated reconcentrations the fruit was not effected either visually or in flavor through these five recyclings as evaluated by a subjective informal consumer panel. However, if the syrup continued to darken during additional reconcentrations, eventually syrup or syrup constituants absorbed into the fruit may be detrimental to product quality. This syrup darkening rate would be less during actual commercial operations because of the percentage of new syrup that would be added each time to the system to compensate for that absorbed by the fruit piece.

The effect of HFCS, sucrose and also another possible osmotic medium, sorbitol, on fruit flavor was evaluated by a taste panel along with the fresh untreated and hot-air dehydrated fruit. In an overall comparison, apples and peaches were rated the highest in the fresh condition (Table 2). Apricots did not receive this same rating, probably because they contain a greater amount of acid which produces a more acceptable fruit brix/acid ratio because of the syrup adsorption. In the osmotic samples, sucrose was the osmotic medium of choice for apricots, with a significant number of panelists rating it highest Hedonically and by ranking. This was not the case with apples and peaches, where apples with sucrose as the osmotic medium were rated as liked the least. Sucrose seems to be more compatable at higher acid levels. With peaches, HFCS was ranked lowest, with the Hedonic rating being lower also. In general, when HFCS was rated low, comments indicated it was because of an excessive sweetnese imparted to the product. Sorbitol osmotic samples were neither liked or disliked significantly. In the overall general evaluation, sucrose was considered a better osmotic medium than HFCS.

Table 3—Component transfer in 70° brix sucrose and HFCS osmotic concentration at 70°C in apples

	Sucrose			HFCS		
Treatment time (hr)	Solids[a] gain (%)	Weight[b] loss (%)	Moisture[c] loss (%)	Solids[a] gain (%)	Weight[b] loss (%)	Moisture[c] loss (%)
0	0	0	0	0	0	0
0.5	7.3	16	27	8.5	14	26
1	8.8	24	37	8.8	22	36
3	7.0	40	54	10.7	37	55
5	8.2	45	61	12.3	44	65
7	8.8	48	65	13.6	47	70

[a] $\text{Solids gain} = \frac{\text{Total solids - Initial Solids}}{\text{Initial Gross Weight}} \times 100$

[b] $\text{Weight loss} = \frac{\text{Initial Gross Weight - Total Weight}}{\text{Initial Gross Weight}} \times 100$

[c] $\text{Moisture loss} = \frac{\text{Initial Moisture - Total Moisture}}{\text{Initial Moisture}} \times 100$

In an ideal osmotic situation there would be a semi-permeable membrane where the solvent molecules would permeate the membrane but not the solute molecules. In fruits, the cell wall membranes are living biological units which can stretch and expand under the influence of growth and the turgor pressure generated inside the cells. These cellular membranes, which are comprised mainly of parenchyma cells in the pom and drupes studied, freely allow the solvent molecules to pass through, but they also allow to a lesser degree passage of some of the solute molecules. This type of membrane is classified as a differentially permeable membrane, rather than a semi-permeable one. Also, some of the osmotic syrup may not activately migrate into the cells but may simply penetrate into the intercellular spaces. Two separate studies were initiated to determine the degree of solute (sugar molecule) penetration into the fruit; a histologic slicing procedure and a materials balance study.

In the study involving the histological slicing procedure with apples a 1–2 mm penetration was realized in the first 0.5 hr with both osmotic mediums (Fig. 3). For the first 3 hr of concentration, the migration rates into the outer areas were essentially the same for HFCS and sucrose. As the contact time increased, the HFCS began to absorb further into the tissue than the sucrose, with the differences becoming pronounced even at minimal depths. This was also indicated when the change in amount of solids in the apple piece were followed during osmotic concentration (Table 3). After being in the osmotic mediums for 3 hr the apples in HFCS had absorbed 50% more solids than those in the sucrose solution. After 5 hr extraction time, 70% more solids was absorbed by apples in HFCS than in sucrose, as

determined by both testing procedures. Sucrose, which is a disaccharide, would be expected to migrate slower than fructose which is a monosaccharide, whether the migration mechanism is explained by the conventional diffusion procedure, or the "bulk flow" theory of Ray (1960). Chandrasekaran and King (1972), in their study on the diffusion characteristics of sucrose and fructose, determined that fructose had a diffusion coefficient 32% higher than sucrose. Weight loss rate was similiar for both syrups, but moisture loss rate was slightly more rapid for HFCS. The final amount of water loss and solids gain with sucrose are similar to those observed by Islam and Flink (1982) in their work drying potato pieces in a 60% sucrose solution.

The water activity of a product affects its susceptiability to microbial proliferation. Some molds, in the presence of an adequate oxygen content and at optimum temperatures, can multiply at a a_w as low as around 0.7. However, most molds require more moisture. Yeast growth is not a problem until the product reaches an a_w of about 0.8 or 0.9. Reconcentration to the original brix did not have any effect on the water activity (a_w) of the syrups. However, the a_w of the 70° HFCS was different from the 70° sucrose with the former being 0.734 and the latter 0.832. With the more concentrated 80° HFCS a_w was 0.587, indicating a greater assurance against microbial spoilage.

The fruits that were concentrated with HFCS as the osmotic medium had a lower activity than those concentrated with sucrose, 0.904 compared to 0.951, and also a lower moisture content, 49% compared to 55%. The HFCS replaced more of the water in the cells because of its faster penetration rate. This also accounts, to a degree, for the taste panel finding that HFCS osmotically concentrated fruit was too sweet. Also, fructose produces a sweeter taste sensation than sucrose.

Of the osmotic syrups studied sucrose would seem to be the syrup of choice. It can be recycled a minimum of five times without affecting fruit quality even when no new syrup is added (in commercial operations about 10–15% new syrup would probably have to be added each time to compensate for loss). In addition, less sucrose is absorbed by the fruits per unit of time than with HFCS. Also, from the taste panel evaluation, the sucrose concentrated samples were rated highest overall. Even in the apple evaluation, when the fresh sample was omitted and only the concentrated products compared, the sucrose concentrated product was not statistically different than apple products osmotically dried with HFCS or sorbitol. Sorbitol is also indicated as a viable osmotic medium alternative.

To aid this dehydration procedure in becoming commercially adopted, one of the next possible steps could be to undertake an economic evaluation of the complete process to document its economic advantages. Another step would be to scale the process up, including the syrup recycling, to a pilot-plant size operation. This could be easily done in an existing plant that already contains tanks, a multiple-effect evaporator, and cold or frozen storage facilities. These studies could supply information that would make this process a commercial reality.

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Submerged Production of *Agaricus campestris* Mycelium in Peat Extracts

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ABSTRACT

The edible mushroom *Agaricus campestris* has been grown using peat extracts as the only substrate source. Shaker flask fermentations were conducted with *Sphagnum* peat extracts obtained by autoclaving peat mixed with 1.5% (v/v) H_2SO_4 for 2 hr at 121°C. Inoculum ratio, temperature, initial pH, fermentation time and agitation were tested in order to evaluate the effect of those factors on the mycelial growth, expressed as final dry mycelium concentration and biomass yield (g of dry mycelium produced per g of carbohydrates consumed). The best combination of operating variables, in the range of values investigated, are: 4% (v/v) inoculum ratio, 24°C, pH 6.0, 6 days and 150 rpm.

INTRODUCTION

SUBMERGED CULTURE PRODUCTION of edible fungi mycelium has potential as a food, food additive or a food supplement (Litchfield, 1968). Besides its value as a protein source, the mycelium of edible mushrooms are useful as a flavoring product for use in dehydrated foods, soups or sauces (Litchfield, 1967). Several substrates have been utilized in the submerged growth of different species of mushrooms (Worgan, 1968). Although a number of mushrooms give good yields in submerged culture, a satisfactory flavor and aroma have been found in only a few cases (Moustafa, 1960; Litchfield, 1967).

Peat, which consists primarily of organic residues rich in carbohydrates, minerals and other substances, is an important, frequently utilized component in the traditional production of mushrooms. Peat is employed as casing soil previous to the sphorophore growth in the production of *Agaricus campestris* mushroom fruit body (Smith and Berry, 1975). However, few attempts have been made to utilize peat extracts as a major substrate for the submerged growth of edible fungi (Martin 1982a). Previous reference in the literature to the submerged growth of *A. campestris* mycelium utilizing peat extracts as substrate has not been found.

This study is an initial step in the development of a pleasant flavored mycelium of the mushroom *A. campestris*. At present, it is the only species grown commercially in North America.

MATERIALS & METHODS

Peat extracts

Ground *Sphagnum* peat moss from Sundew Peat Bog, Newfoundland, Canada, was mixed with 1.5% (v/v) H_2SO_4 (33g peat/100g solution), autoclaved at 15 psig (121°C) for 2 hr and separated by pressing, followed by filtration through Whatman No. 1 filter paper.

Organism

Agaricus campestris NRRL 2334 (American Type Culture Collection), was adapted to growth in peat extracts by successive transfers (Martin, 1982b).

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Culture conditions

Adapted culture was aseptically inoculated to 100 mL sterile peat extracts in 250 mL shaker flasks and incubated in a Gyrotory water bath shaker (Model G76, New Brunswick Scientific Co., Inc.). The pH was adjusted (before inoculation) by addition of 15N NaOH. Inoculum ratio (4, 7, and 10% v/v), temperature (22, 24, 26, an 28°C), initial pH (5.5, 6.0, 6.5, and 7.0), fermentation time (4, 6, and 8 days) and agitation rates (100, 150, and 200 rpm) were tested.

Total carbohydrates (TCH)

TCH concentrations in peat extracts and in the fermented media were determined by the anthrone reagent method (Morris, 1948).

Mycelium dry weight

The culture medium, after fermentation, was filtered through oven dried (105°C to constant weight) Whatman No. 1 filter paper. The filter paper with the mycelium was oven dried (105°C) to constant weight.

RESULTS & DISCUSSIONS

THE EFFECT of the different variables analyzed on the biomass yield (g of dry mycelium produced per g of TCH consumed) can be seen in Table 1 and Fig. 1.

Inoculum ratio

The highest biomass yields were obtained at an inoculum concentration of 4% (v/v) (Table 1), the values decreasing, in general, with the inoculum concentration. This fact suggests that some inhibitory substance is produced in a previous stage of the cultivation of *A. campestris* from which the inoculum was taken, and transported with it; a larger inoculum implying a higher concentration of the inhibitory substance. Fermentations at higher rpm were conducted for the three inoculum ratios reported in this work, as is shown in Fig. 1 for the specific case of 4% (v/v). The fact that the biomass yield decreased in all cases at higher rpm, in addition to the relatively low mycelium concentrations obtained, excluded the possibility of dis-

Table 1—Effect of temperature, pH and inoculum ratio on the biomass yield of Agaricus campestris in peat extract medium at 150 rpm, 17.7 g/L initial TCH concentration; fermentation time: 6 days

Temp (°C)	Inoculum Ratio % (v/v)	Initial pH 5.5	6	6.5	7
22	4	0.31	0.58	0.30	0.20
	7	0.31	0.50	0.31	0.19
	10	0.29	0.45	0.27	0.18
24	4	0.65	0.73	0.62	0.58
	7	0.55	0.70	0.53	0.47
	10	0.51	0.59	0.53	0.48
26	4	0.33	0.55	0.28	0.19
	7	0.30	0.31	0.25	0.19
	10	0.30	0.26	0.26	0.17
28	4	0.10	0.17	0.17	0.10
	7	0.09	0.18	0.17	0.11
	10	0.09	0.21	0.16	0.10

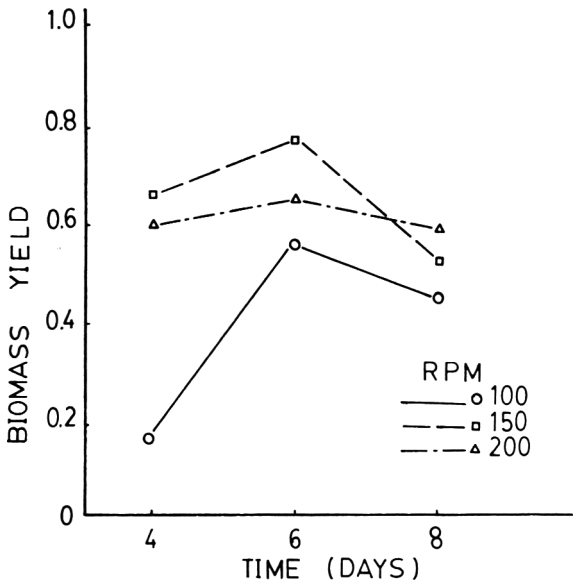

Fig. 1—Effect of fermentation time and agitation on the biomass yield of A. campestris in peat extracts at pH 6.0, 24°C, 4% (v/v) inoculum ratio. Initial TCH concentration: 25 g/L.

solved oxygen concentration being the limiting factor in the mycelium growth in the fermentations with larger inoculum ratios.

Growth temperature

The temperatures reported for the growth of *A. campestris* range between 15 and 35°C, with a preferred value of 25°C (Litchfield, 1967). In this research, the highest biomass yields were obtained at 24°C, and decreased markedly at higher temperatures (Table 1).

Initial pH

The pH range reported for *A. campestris* varies from 4–8, with the preferred value depending on the medium composition, e.g. 4.5 for a synthetic medium and between 5 and 7 for malt sprout extract (Litchfield, 1967). In this work the optimum initial pH value was found to be 6.0 (Table 1) for *A. campestris* growing in a peat extract medium.

Fermentation time

Using the best values previously observed for inoculum ratio, temperature and pH, fermentations were conducted to determine biomass yields at different agitation rates and fermentation times. Peat extract with 25 g/L of TCH concentration was utilized in these experiments.

Fig. 1 shows the biomass yields obtained in the new runs at three different rpm. The biomass yield generally increased from 4 to 6 days, and afterward it decreased. The mycelium concentration follows a similar pattern to the yield, and this suggests that after 6 days, autolysis of the mycelium occurs.

Agitation

Fig. 1 shows an optimum agitation of 150 rpm. It is supposed that a higher rpm implies a better oxygen transfer in the fermenting medium. The fact that the biomass yields are lower at 200 rpm (except the yield after 8 days of fermentation) could be attributed to a detrimental effect of increased shear stress on the mycelium, although good yields have been reported at higher rpm (Moustafa, 1960). The present work suggests that peat extracts can be a source of nutrients in the submerged production of *A. campestris* mushroom mycelium.

The best biomass yields obtained are comparable to those reported in fermentations with other substrate sources (Litchfield, 1967). However, the mycelium concentrations are low; the highest value, 2.96 g/L, corresponds to a biomass efficiency (g dry mycelium produced/g initial TCH concentration) of 0.12. At the end of the fermentations, a large fraction of the initial TCH concentration has not been consumed. This fact could result from two different causes:

(a) As it has been previously suggested (Quierzy et al., 1979), not all the carbohydrates present in peat are assimilable.

(b) The present work has been done with nonsupplemented peat extract medium. It is possible that some other nutrient, besides carbohydrates, becomes the growth limiting factor during the growth.

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Characterization of Exopolysaccharide Produced by *Corynebacterium* #98 in Cheese Whey Substrate

M. A. SHAMS and H. O. JAYNES

ABSTRACT

Corynebacterium #98 produced an orange pigmented gum when grown in a 1.5% whey medium containing added lactose, $MgSO_4$, and $FeSO_4$ at optimized conditions: pH 7.0, 30°C, agitation and aeration at 1090 ml/min. Gum rheology showed increasing viscosity with gum concentration. Viscosity varied inversely with temperature and NaCl concentration. Analysis indicated an ash content of 29.7% due mainly to potassium, sodium and phosphate. The structural components of the gum identified were rhamnose, xylose, mannose, glucose, galactose, and glucuronic and mannuronic acids in a weight ratio of 2:1:6:6:7:16:11.

INTRODUCTION

WHEY, a by-product of the cheese industry, is produced in large quantities and continues to be a disposal problem to both the dairy industry and city sewage systems. To lessen the magnitude of the problem, research on the effective utilization of whey has been encouraged. In past years several organisms, including species of *Hansenula, Alcaligenes, Arthrobacter,* and *Zooglea* have been screened for their ability to produce extracellular polysaccharides in whey substrates (Harrison, 1976; Rao, 1977; Stauffer and Zeeder, 1978). Laboratory investigations of these polysaccharides have shown them to possess promising properties when used in applications where plant gums are usually employed.

A coryneform bacterium, designated as number 98, which was isolated from sorghum molasses (Daeshel, 1979), was found to produce a slimy material in sorghum juice. Further investigation by Rodriguez (1980) showed that this microorganism produced a viscous, orange-pigmented exopolysaccharide in a modified whey substrate. The main purpose of this investigation was to optimize the production and recovery of the ghum produced by *Corynebacterium* #98 grown in a sweet whey substrate, and to characterize the gum's rheology and its composition.

MATERIALS & METHODS

Culture and polysaccharide synthesis

The culture used in this study was obtained from Dr. J.O. Mundt of the Microbiology Department of the University of Tennessee and was isolated in 1978. Rodriguez (1980) investigated this culture primarily for its polysaccharide production and designated it *Corynebacterium* #98. The culture was maintained on Brain Heart Infusion Agar (BHIA).

A 6% whey solution (w/v) was prepared by rehydrating dried whey with distilled water. After adjusting the pH to 5.0, the mixture was autoclaved for 15 minutes at 121°C which precipitated part of the whey protein to limit N in the medium. The precipitate was removed by filtration through two layers of Whatman No. 1 paper, and the volume was adjusted back to the original. The solution was then diluted fourfold with distilled water and 0.5% of a salt solution containing 0.2% $FeSO_4$ and 4% $MgSO_4$ was added. The culture medium was enriched with 2% lactose, raising the content of lactose to about 3%. The pH was then adjusted to 7.0 with 1N NaOH and the medium was autoclaved (121°C, 15 min).

A 50 ml aliquot of sterile Tryptic Soy Broth (TSB) was inoculated with two loops of culture #98 and incubated in a shaker-incubator at 30°C for 24 hr (shaker rate = 80 rpm). Five 50 ml aliquots of the whey substrate in 250 ml Erlenmeyer flasks were then inoculated with the 24 hr culture in TSB at a rate of 10% (v/v). The flasks were incubated in the same shaker-incubator with identical temperature and agitation. The incubation period usually lasted 7 days or until the whey substrate became viscous.

Fermentation for gum production was carried out in a Multigen fermentor (F-2000, New Brunswick Sci. Co., Addison, NJ). A 15% (v/v) inoculum was added to 1.5 liters of sterilized whey substrate. The three impellors in the fermentor were operated at 200 rpm and temperature was held at 30°C while air was sparged in at a rate of 1090 ml/min. Silicone antifoam was added at a level of 0.5 ml per 1000 ml of culture medium to supress foaming.

At the end of the fermentation, gum was precipitated in 1000 ml plastic centrifuge bottles which had a minimum adherence for the precipitated polymers. To 200 ml of the viscous liquid from the fermentor, 5 ml of 2M phosphate buffer at pH 7.6, 5 ml saturated KCl, and 800 ml of acetone were added. This solution was hand mixed for 30 sec then refrigerated overnight for precipitation. The precipitate was collected on Whatman No. 1 filter paper and homogenized into three volumes of hot distilled water in a Virtis homogenizer. The gum solution then was placed in glass petri dishes and dried in a Virtis freeze-drier (Gardiner, N-Y).

The freeze-dried gum was placed in a plastic bottle with a few marbles and put into a Twin Shell Dry Blender at 27 rpm for 30 minutes. The composite, powdered gum was kept refrigerated at 4°C for further analysis.

Rheological properties

The effect of gum concentration, salt (NaCl) concentration, pH and temperature on viscosity was studied. The levels of these factors were as follows: (1) gum concentration: 0.5, 1.5, 2.5% (w/v); (2) salt concentrations: 0.5, 1.0, 1.5% (w/v); (3) pH; 5, 6, 7; (4) temperature: 15, 25, 35, 45°C.

The aqueous solution of the gum for rheological determinations was prepared several hours prior to testing to permit the gum to rehydrate completely. Viscosity was measured with a Bookfield viscometer, model LVF. A jacketed, small sample adapter was attached, and measurements were taken with spindle SC4-18 at 60 rpm. Temperature of the circulating water was maintained with a Haake device, model E-52.

The experiment was conducted as a 3^3 factorial in randomized blocks of 9 combinations for a total of 27 experimental units per block. Each experiment was performed at four different temperatures which made a total of 108 observations. Data on viscosity were analyzed by analysis of variance (Sokal and Rohlf, 1969).

Composition of gum

Composition of the gum was determined using official methods (AOAC, 1975). Ash from the gum also was analyzed for calcium, potassium, magnesium, sodium, and iron by atomic absorption or flame emission. Phosphate (molybdovanado phosphate, ASTM, 1975), silica (molybdosilicate, AOAC, 1975), sulphate (gravimetric, APHA, 1975), and chloride ion (potentiometric, APHA, 1975) were also determined.

Two hydrolysis procedures (Adams, 1965) were used to assess the subunits in the polysaccharide. The procedure for α-D-hexoglycans involved refluxing 1.5-g samples of gum for 2.5 hr in 200 ml of water plus 20 ml HCl (sp gv 1.125). For β-D-hexoglycans, 1.5-g samples were mixed into 5 ml of 72% H_2SO_4 and held at 20°C for 45 min with stirring at 5 min intervals. Then the mixture was

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diluted with 140 ml water and autoclaved (121°C) for 1 hr. In both procedures, hydrolyzed mixtures were cooled, neutralized with 1N NaOH, and filtered through Whatman #4 paper. Then aliquots of 3 ml were placed in septum vials and freeze-dried. After drying, septa were placed on the vials, and the contents were derivatized with Tri-Sil "Z" (Pierce Chem. Co.) to permit analysis by gas-liquid chromatography (GC). Triplicate samples were hydrolyzed and analyzed.

The GC was equipped with 1.4m x 5 mm (o.d.) glass column packed with 1% SE 30 on Chromosorb Q (100-110 mesh). Oven temperature was maintained at 180°C while the inlet and flame ionization detector were set at 210°C. A Schimadzu E1A integrator was used to quantitate peaks, and sorbitol was used as an internal standard.

RESULTS & DISCUSSION

Production and yield

Polysaccharide production by the *Cornyebacterium* #98 in a whey substrate was found to be practical at optimum conditions. Gum production was terminated at 15 days with an average yield of 33 grams of gum from each batch of 1,500 ml of modified whey substrate.

Rheological properties

The gum was very soluble in hot water; however, it could be dissolved in cold water with high speed agitation for a short period of time. Limited observations that were made by varying rotation rate of the LVF viscometer indicated shear-thickening, or dilatent, properties for solutions of the gum (Glicksman, 1969). If true, this characteristic is very uncommon among microbial gums (Jeanes et al., 1961; Lilly et al., 1958).

The maximum mean viscosity observed was 29 cps at 15°C, 2.5% concentration. This viscosity was not as high as that evidenced by other microbial gums (Jeanes et al., 1961; Smiley, 1966); however, higher viscosity might be obtained at higher concentrations or in different environments.

Concentration of the gum gave a highly significant positive effect, with viscosity increasing as the gum concentration increased. The effect of pH was also significant in positively altering viscosity at the pH levels used as shown in Table 1. The use of a broader pH range could have resulted in greater variation.

Salt concentration had a significant, though rather small, inverse effect on viscosity of the aqueous gum solution. The effect of salt on the viscosity usually depends upon the the particular salt used, the concentration of gum, and salt concentration. Sodium chloride was used in this experiment, so it could be concluded that the gum might give different rheological reactions with other salts which could affect the viscosity in a different manner.

The effect of temperature upon viscosity also was inverse and highly significant. This characteristic is typical for many other microbial gums (Jeanes et al., 1961, 1965).

In determination of rheological properties of the gum, the linear and quadratic trends between each main effect also were considered since food rheology includes combined physical and chemical interactions. The interactions of the main effects were investigated and, as shown in Table 1, all two-way interactions were significantly different at a level of 0.01 except for pH*T and S*T. The significant two-way interactions were subjected to the Multiple Regression Range Test (Barr and Goodnight, 1979) and the trends are shown in Fig. 1, 2, 3, and 4.

Fig. 1 shows the effect of interaction between gum concentration and pH on viscosity. Regardless of the pH, viscosity increased as the gum concentration increased with a linear-quadratic trend. The effect of pH, though small, was significant ($P < 0.01$).

Fig. 2 shows the effect of the interaction between gum concentration and salt upon viscosity. It was observed that at low gum concentrations there was no change in viscosity as the salt level was increased. However, at higher gum concentrations (1.5 and 2.5%) there was a small, linear decrease in viscosity as salt level increased.

The interaction effect of pH and salt upon viscosity is illustrated in Fig. 3, which shows that viscosity decreased linearly with the addition of salt at pH 5. This trend was

Table 1—Analysis of variance of effects of gum concentration, salt concentration, pH, and temperature on viscosity of the gum

Source	DF	SS	MS	F
C (concentration)	2	4441.18	2220.59	7779.16**
pH	2	2.92	1.46	5.12**
C * pH	4	5.03	1.26	4.40**
S (salt)	2	11.92	5.96	20.88**
C * S	4	10.19	2.55	8.92**
pH * S	4	6.34	1.58	5.55**
T (temperature)	3	1107.71	369.24	1293.51**
C * T	6	1031.12	171.85	602.04**
pH * T	6	0.69	0.12	0.40^{NS}
S * T	6	1.57	0.26	0.92^{NS}
Residual error	68	19.41	0.28	

** Significant at the 0.01 level.
NS Not significant at the 0.05 level.

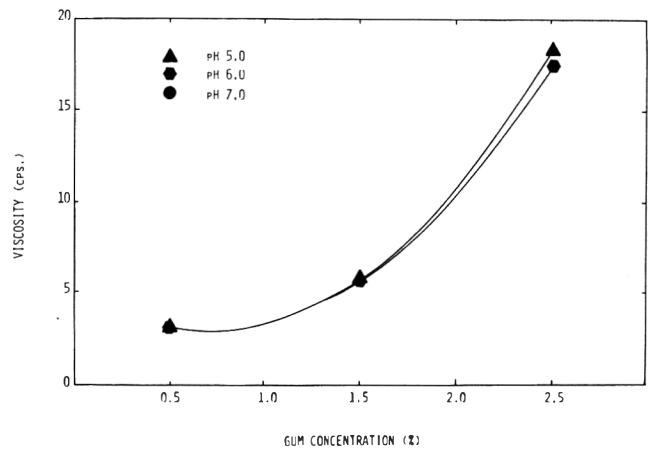

Fig. 1—Effect of concentration and pH on viscosity of gum solutions.

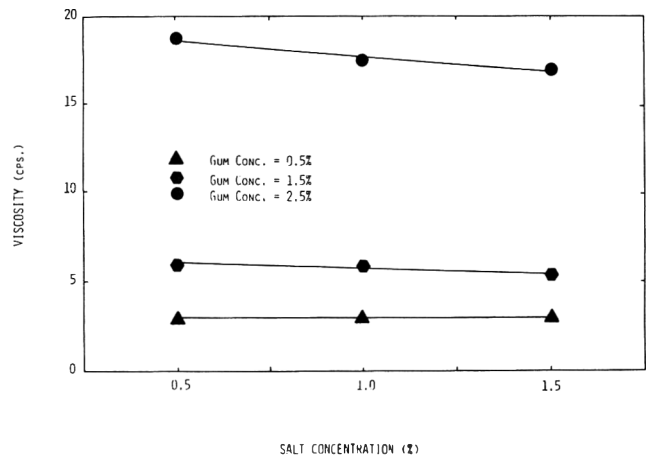

Fig. 2—Effect of concentration of gum and salt on viscosity of gum solutions.

quadratic at pH 6, while at pH 7 there was no effect on viscosity from the addition of salt.

Fig. 4 delineates the interactive effect of gum concentration and temperature upon viscosity. The trends were quadratic at all temperature levels, and viscosity increased with increasing gum concentration. Predictably, viscosity increased to a greater extend as temperature decreased.

Composition

Proximate analysis of the gum is listed in Table 2. The fat and protein present were attributed to cells remaining in the gum since the cells could not be separated from the gum (Brown and Lester, 1980). Mild sonification could separate part of the polymer from residual cells, but fragmentation of the polymer was inevitable (Rodriguez, 1980; Brown and Lester, 1980). Therefore, production of the gum, including the cells, could still be feasible since the *Corynebacterium* #98 did not survive after precipitation with acetone. If a nonpathogenic culture could be killed during the course of preparation, it could remain with the gum (Margaritis and Zajic, 1978; Rocks, 1971; Smiley, 1966).

The high ash content of the gum was investigated through different procedures which established the actual level of each mineral component. The level of each element and procedures used are shown in Table 3. The high concentration of potassium, 51.52%, indicated that the gum most likely was precipitated in the form of a potassium salt. Some residual potassium could have come from the saturated potassium chloride solution which was used in precipitating the gum. The phosphate concentration in the ash, 3.72%, is not unusual in microbial gums (Jeanes et al., 1961). This level of phosphate is believed to be related to the addition of phosphate buffer during the course of gum precipitation. The sodium content at a level of 5.71% was considered to be chiefly attributable to the whey ingredients and possibly to the addition of the antifoam agent.

The concentration of elements such as calcium, magnesium, iron, and silica in the ash were very low. No SO_4 was detected. It could be concluded that the levels of potassium, phosphate, sodium, and chloride ion encountered were not out of line with levels of these materials commonly found in foods.

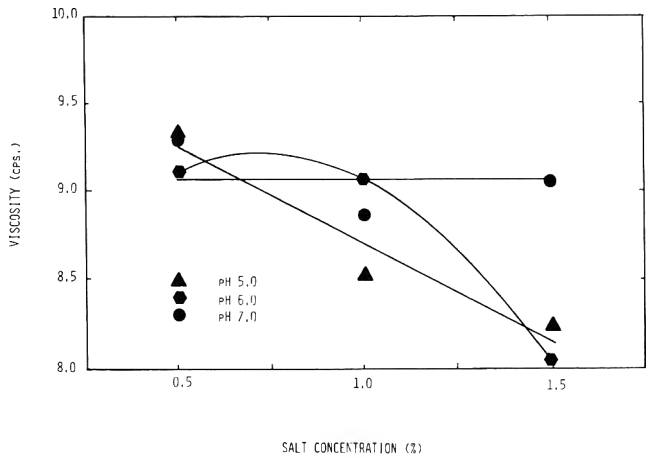

Fig. 3—Effect of concentration of salt and pH on viscosity of gum solutions.

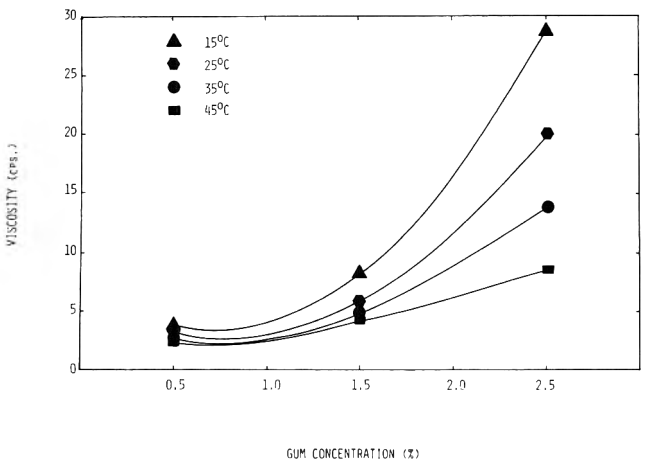

Fig. 4—Effect of concentration and temperature on viscosity of gum solutions.

Qualitative and quantitative subunit analysis of the gum

Two types of hydrolyses, for α- and β-linked D-hexoglycans, were performed to determine the kind of bonds existing in the gum's structure. Gum hydrolyzed by the β-linked D-hexoglycan procedure showed no peaks when derivatized samples were injected into the GC. This indicated no β-linkages between sugar components of the gum. Conversely, the derivatized samples resulting from α-linked d-hexoglycan hydrolysis produced seven peaks with good resolution in the GC, as shown in Fig. 5. To identify the peaks, different known sugars or sugar acids were derivatized and injected into the GC after injection of the gum sample. Corresponding retention times were used for identification of species. The constituents of the gum identified were rhamnose, xylose, mannose, galactose, glucose, glucoronic acid, and mannuronic acid. The relative amount of each residue in the gum samples were calculated using sorbitol as an internal standard.

The mean value for each component was calculated and the sugar with the lowest mean value, xylose, was taken as unity and the ratios of other components were extrapolated accordingly. On this basis, the polymer was composed of rhamnose, xylose, mannose, galactose, glucose, glucuronic acid, and mannuronic acid in the ratio of 2:1:6:6:7:16:11. However, such an assumption should be qualified. The

Table 2—Proximate composition of gum

Components	Percent[a,b]
Moisture	5.93
Carbohydrate	59.23
Ash	29.67
Protein	4.06
Fat	1.11

[a] Means of three observations.
[b] Values calculated on a freeze-dried basis.

Table 3—Mineral components in the ash of the coryneform gum with the method of determination

Component	Method of measurement	Percent in ash
Calcium (Ca)	Atomic absorption	0.80
Magnesium (Mg)	Atomic absorption	0.51
Potassium (K)	Atomic absorption	51.52
Iron (Fe)	Atomic absorption	0.02
Sodium (Na)	Flame emission	5.71
Phosphate ($PO_4^{=}$)	Molybdovanado phosphate	3.72
Silica (SiO_2)	Molybdosilicate	0.01
Sulfate (SO_4)	Gravimetric	0.00
Chloride ion (Cl^-)	Potentiometric	2.54

material contained residual cellular components which may well be the source of rhamnose and xylose. Their removal as polymer constituents would reduce the presumed complexity of the polymer to a level more often encountered in microbial gums. Also, in view of the observed viscosity levels, there is a distinct possibility of branching which would not be shown by the analytical procedures used.

CONCLUSION

IT WAS FOUND that lactose in a modified whey substrate could be utilized by *Corynebacterium* #98 to produce an orange-pigmented gum. Laboratory production indicated that commercial scale production of the gum might be feasible.

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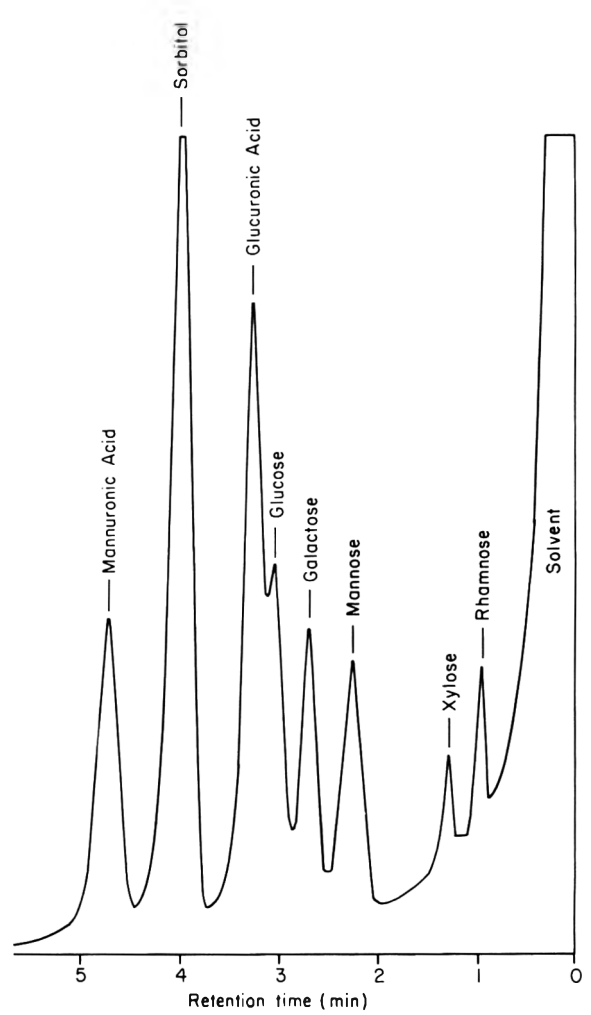

Fig. 5—Gas chromatogram of structural constituents of the gum.

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Influence of Selected Thermal Processing Conditions on Steam Consumption and on Mass Average Sterilizing Values

SANTI R. BHOWMIK and KAN-ICHI HAYAKAWA

ABSTRACT

The influence of can size, type of food (conductive or convective), retort temperatures, initial temperature of food and target sterilizing value on steam consumption was studied. A 2^5 factorial design of experiments was used. Steam consumption was measured by using steam flow meters. A mass average sterilizing value was computed for each process by using experimentally determined heat penetration parameters. Steam consumption was significantly high for processing larger cans compared to smaller cans both containing equal quantities of conduction heating food simulant. The can size had no significant influence on steam consumptions with the convection heating food simulant. Steam consumption and mass average sterilizing values were reduced significantly by employing a high retort temperature to obtain a high target F_p value. With a low target F_p value, the type of food simulant did not affect significantly mass average sterilizing value.

INTRODUCTION

RESEARCH in energy conservation in the thermal processing of canned foods has been stimulated by the recognized shortage of oil and rising cost of energy. Several researchers investigated energy utilization and conservation in thermal processing of canned foods. Rao and Katz (1976) developed computerized methods to estimate overall heat losses in food canning plants. Rao et al. (1976) studied thermal energy losses in vegetable canning plants. Singh (1978) conducted quantitative studies on thermal energy losses in canning plants with special attention to convection and radiation loss from a sterilizer body, evaporation from water surface, steam discharge through vents and bleeders, and sensible heat required for heat canned food. Lopez (1981) discussed energy usage for the thermal processing of canned foods in batch sterilizers. Griffith et al. (1979) reported substantial energy savings through the installation of an external heat exchanger to heat water in a continuous atmospheric, water cooker. Energy accounting in canning plants for several types of food products has also been done by Carroad et al. (1980), and by Singh et al. (1980).

The influence of several process-factors on steam consumption may be estimated from available scientific data. However, interactions among these factors could not be estimated from these data. Therefore, the present study was conducted to examine the interactions and main effects of five selected factors on the steam consumption. These factors are can size, food consistencies, retort temperatures, initial food temperatures, and target lethalities.

The processed canned food should retain the best possible quality for consumer acceptance. Therefore, it would be desirable to evaluate the influence of the above named factors on mass average sterilizing values, which are closely related to food quality. Hence this evaluation was also performed in the present investigation.

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MATERIALS & METHODS

FIG. 1 SHCWS a vertical still retort with all major components in our pilot plant used for this study. The system consists of: (A) Two steamflow meters installed serially. The first (Fisher and Porter Co., Warminster, PA, Model No. 10A 1152 A, full span = 5-87 kg per hour) recorded steam flow at the low range and the second (Model No. 10A 1152 A, full span = 80-455 kg/hr) recorded steam flow at the higher flow range. This combination insured precision in the measurement of steam flow (Performance precision = ± 1.0% of maximum flow rate). (B) Steam regulating valve, reverse acting, air activated, 1.27 cm valve (SER. No. 311988 of Honeywell Co., Morton Grove, IL). (C) By-pass valve. (D) Vertical still retort (Berlin Chapman, Serial No. 02136), height = 1015 mm, internal diameter = 765 mm, internal volume = 465 liters, outside wall insulated with a 39 mm thick layer of asbestos cement. (E) Steam distributor. (F) Condensate outlet valve. (G) Vent valve. This is a hand operated ball valve. A pointer mounted on the handle to easily reproduce valve opening during operation. (H) Vent pipe. (I) Bleeder. (J) Crate. (K) Several cans containing thermocouple junctions properly placed at different locations in each of them. The temperature inside the retort and at different locations inside the test cans were measured by 0.0762 mm Teflon insulated copper-constanan thermocouples (Omega Engineering, Conn.). The two temperature recorders used to collect the temperature data are a key-programable data recording instrument (Esterline Angus Instrument Corp., Indianapolis, Model No. PD-2064) and a strip chart recorder (Honeywell Electronik-16, Model No. 176303846, Serial No. D 4207773081, Type T, 10–177°C). (L) Cooling water inlet. (M) Temperature controller (Model No. 152P13P-83K1-11, range 10–177°C, Brown Instrument Co., Philadelphia, PA). (N) Small retort (internal diameter = 202 mm, height – 360 mm, internal volume = 11.5 liters) used to determine thermophysical properties of food simulants for all sizes of cans. (P_1) and (P_2) Pressure gages (Jas P. March, Chicago, IL, Borden Type, full span 6.9–690.0 kPa). (P_3) Pressure Transducer (Sensometrics Inc., Van Nuys, CA, Model No. SP 68E, Serial No. S/N J411, full span = 0–345 kPa. Accuracy = ± 0.5% full scale).

A 2^5 factorial design was used to examine experimentally the influence of the five selected processing factors which are cited in Introduction on steam consumption and on the food quality. The upper and lower levels of each factor employed for this factorial design were selected as follows. The lower F_p value of 3.0 min was chosen because of findings by Pflug and Odlaung (1978). According to these authors, a process using an F_p of 3.0 min with a z value of 10C° would be the minimum value for safe botulinum cook. The upper target of F_p value of 10 min was chosen after carefully examining published data (Desrosier, 1970) on the process lethalities. The initial food temperatures of 23 and 60°C were chosen based on published recommended heat processing conditions (Anonymous, 1976). The retort temperatures of 121 and 110°C were based on published data on heat processes for low acid foods. Material used as a food simulant was either an aqueous bentonite suspension (8% by weight) to simulate a conduction heating food product (Townsend et al., 1949) or distilled water to simulate a convection heating food product. The simulants were processed in 307x409 or 211x300 cans. The relative volumetric capacity ratio for the two cans is 2.5.

Each can was filled with a food simulant at 45°C to 93% of the net volume and was vacuum-sealed, 380 mm of Hg, using a semi-automatic can sealer (Rooney Machine Co., Bellingham, WA). The cans were placed in an environmental chamber at 3C° above the initial food temperature, overnight to ensure uniformity of sample temperatures. The 3C° difference was used to compensate the temperature drops of the cans, which was observed to occur when cans were transferred from the environmental chambers to the retort. Four hundred 211x300 cans or 155 307x409 cans were randomly filled in the retort crate per batch. The quantity of food simulant in each crate was same for both can sizes.

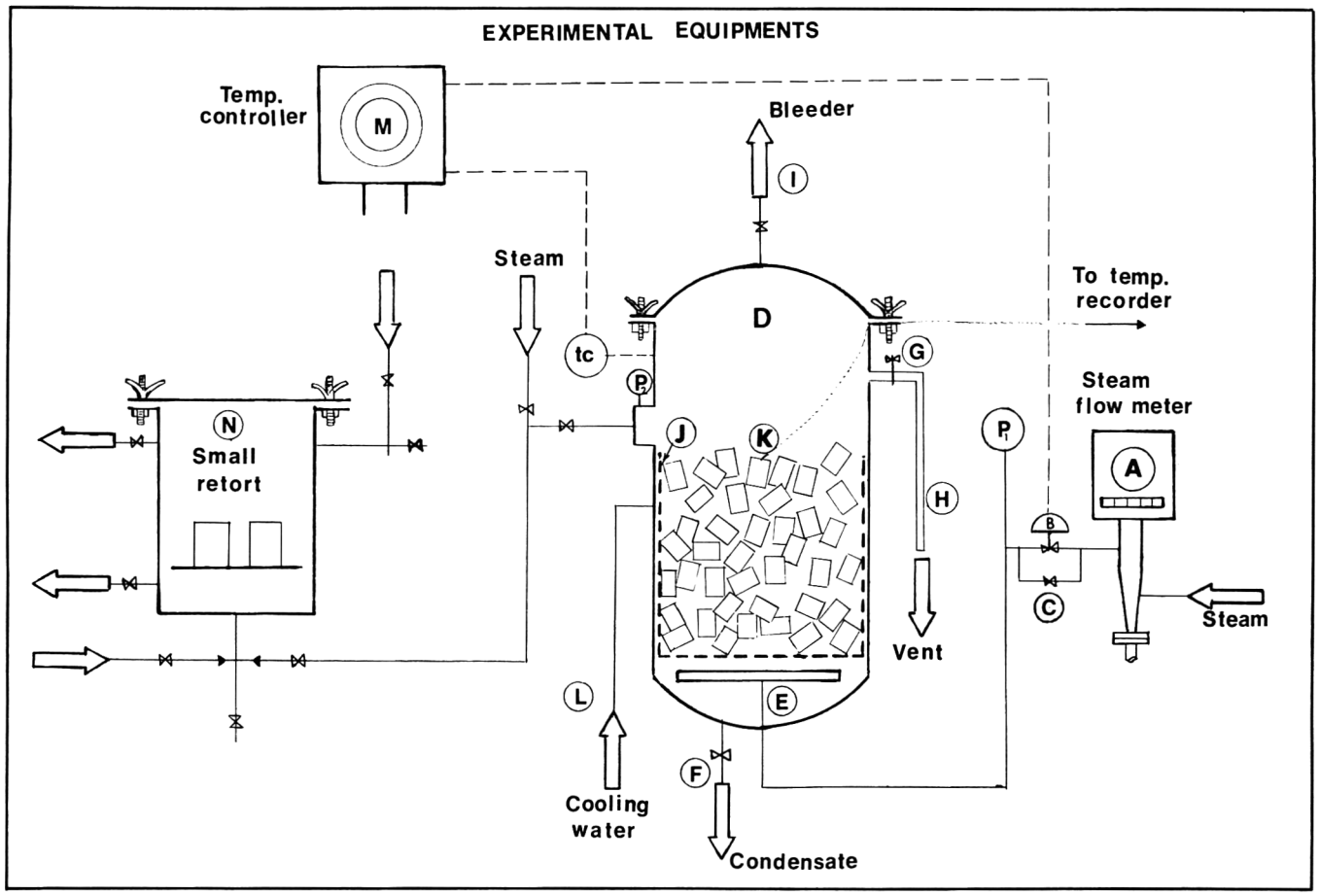

Fig. 1—Diagram of system used for experiment.

Table 1—Venting procedures used for processes with various retort temperatures and initial food temperatures

Retort temp. (°C)	Initial food temp. (°C)	Operation of the vent valve[a] Close ¼ opening at (min)	Close ½ opening at (min)	Close completely at (min)	Close steam by-pass valve at (min)	Net venting time (min)	Net come-up time (min)
121.0	23.0	4.0	4.5	5.0	4.0	5.0	6.5
121.0	60.0	3.0	3.5	4.0	3.5	4.0	6.0
110.0	23.0	4.0	4.5	5.0	4.0	5.0	5.5
110.0	60.0	3.0	3.5	4.0	3.5	4.0	5.0

[a] The vent valve is fully open at zero time, the moment steam was first introduced into the retort by opening a steam by-pass valve and setting retort temperature controller set-point.

Since over 35% of the steam requirement for a thermal process is consumed during venting (Singh, 1978), a reproducible venting procedure was developed for each combination of retort temperature and initial temperature of food simulants. Retort temperature and pressure were monitored during venting by a thermocouple and a pressure transducer respectively. Venting was completed when the pressure transducer indication was equal to the saturated steam pressure at the temperature indicated by the thermocouple. Table 1 shows the venting procedure developed. No significant variation in these procedures was observed between heat conduction and heat convection heating food simulants.

The come-up correction factors for the thermal processing of conduction heating simulant were determined by using a method developed by Uno and Hayakawa (1980). Since there was no published procedure available to determine come-up correction factor for heat-convection food, a Ball's correction factor (Ball and Olson, 1957) was applied to the cans containing distilled water.

The process times and mass-average sterilizing values were calculated from the heat penetration parameters of sample cans containing food simulants in a small retort whose temperature almost instantaneously reaches processing temperature at the moment of steam introduction (zero come-up time).

We assumed z = 10C° to determine proper heating schedules. A mass average sterilizing value was then estimated for each process schedule using z = 31C° since z values for the thermal degradation of several important nutrients are close to this assumed value (Lund, 1977). Hayakawa's (1977) computer program was used for all process estimations.

RESULTS & DISCUSSION

TABLE 2 shows f and j values determined from the heating and cooling curves of food simulants. Each value shown in this table represents the mean of six different experimentally determined values. Table 3 summarizes data on the heating times, mass-average sterilizing values, and steam consumption. Fig. 2 shows a typical data for a run showing the rate of steam consumption, retort temperature and time. The steam consumption curve in this figure differs from one reported in the literature (Lopez, 1981). This difference could be attributed to the manner of venting and the configurations of the retort. It was observed that the relative quantities of steam consumed for venting, for bringing the

Table 2—Values of f and j of food simulants[a]

Can size	Food simulant	f_h (min)	j_r	f_c (min)	j_c
307x409	Bentonite susp.	52.2 (3.9)	1.96 (2.9)	65.5 (2.7)	1.61 (4.5)
	Water	2.6 (6.1)	1.38 (4.2)	10.3 (5.1)	0.61 (3.2)
211x300	Bentonite susp.	29.8 (4.5)	1.97 (2.5)	39.5 (4.7)	1.61 (3.5)
	Water	1.8 (3.5)	2.49 (3.8)	7.5 (3.0)	0.86 (4.1)

[a] The tabulated values are averages of six experimental data. A value in each pair of parentheses is maximum deviation (%).

Table 3—Experimental design, calculated process time, steam consumption and mass average sterilizing values

Exp. no.	C_s	T_o (°C)	F_p (min)	T_a (°C)	F_t	t_b (min)	Mass avg. sterilizing value (min)	Steam consumed (kg)
1	S	23.0	3.0	110.0	L_d	43.0	18.0	39.0
2	L	23.0	3.0	110.0	L_d	43.0	18.0	38.0
3	S	60.0	3.0	110.0	L_d	42.0	18.0	37.0
4	L	60.0	3.0	110.0	L_d	42.0	18.0	36.0
5	S	23.0	10.0	110.0	L_d	133.0	57.0	63.0
6	L	23.0	10.0	110.0	L_d	134.0	57.0	62.0
7	S	60.0	10.0	110.0	L_d	133.0	57.0	59.0
8	L	60.0	10.0	110.0	L_d	133.0	57.0	58.0
9	S	23.0	3.0	121.0	L_d	8.0	5.0	32.0
10	L	23.0	3.0	121.0	L_d	8.0	5.0	31.0
11	S	60.0	3.0	121.0	L_d	7.0	5.0	30.0
12	L	60.0	3.0	121.0	L_d	7.0	5.0	29.0
13	S	23.0	10.0	121.0	L_d	15.0	12.0	38.0
14	L	23.0	10.0	121.0	L_d	15.0	12.0	36.0
15	S	60.0	10.0	121.0	L_d	14.0	12.0	32.0
16	L	60.0	10.0	121.0	L_d	14.0	12.0	31.0
17	S	23.0	3.0	110.0	S_d	89.0	30.0	54.0
18	L	23.0	3.0	110.0	S_d	125.0	39.0	62.0
19	S	60.0	3.0	110.0	S_d	82.0	29.0	44.0
20	L	60.0	3.0	110.0	S_d	113.0	38.0	60.0
21	S	23.0	10.0	110.0	S_d	180.0	110.0	79.0
22	L	23.0	10.0	110.0	S_d	218.0	79.0	99.0
23	S	60.0	10.0	110.0	S_d	173.0	103.0	76.0
24	L	60.0	10.0	110.0	S_d	205.0	78.0	95.0
25	S	23.0	3.0	121.0	S_d	46.0	26.0	49.0
26	L	23.0	3.0	121.0	S_d	74.0	39.0	64.0
27	S	60.0	3.0	121.0	S_d	40.0	25.0	43.0
28	L	60.0	3.0	121.0	S_d	63.0	38.0	54.0
29	S	23.0	10.0	121.0	S_d	58.0	37.0	51.0
30	L	23.0	10.0	121.0	S_d	91.0	55.0	70.0
31	S	60.0	10.0	121.0	S_d	52.0	36.0	47.0
32	L	60.0	10.0	121.0	S_d	80.0	53.0	66.0

retort to a process temperature after closing the vent valve, and for maintaining the retort temperature were approximately 40%, 10%, and 50% respectively. The rate of steam consumption was maximum during the later half of the venting period, dropped sharply after closing the vent valve, and was steady at a low rate after the come-up time.

The data in Table 4 show that the steam consumption for different runs were similar when the initial sample temperature, retort holding temperature, and type of food were similar. The quantity of steam used during the come-up time was moderately higher with a lower initial food temperature at the same retort temperature. Steam consumption rate was significantly lower during the holding period at the lower retort temperature. However, the total amount of steam used for a process at the lower retort temperature was higher than a process at the higher retort temperature due to a prolonged holding time.

To obtain quantitative information on the influence of the five selected factors on the steam consumption, the data were subjected to the analysis of variance by using a computer program (Barr et al., 1976). Table 4 shows abridged results of this analysis. The Table shows that all main factors influenced greatly the steam consumption. We were not able to analyze independently and separately each main factor since two interactions, $F_p \times T_a$ and $C_s \times F_t$, were significant at a 0.1% level and since one interaction, $F_p \times F_t$, was significant at a 5% level.

We examined the effect of the two highly significant interactions on the steam consumption by plotting average steam consumption against the target F_p values for different heating temperatures, Fig. 3, and by plotting the steam consumption against different can sizes for different type of food simulators, Fig. 4. The vertical bar on each plotted point represents a 95% confidence interval. From Fig. 3, we observe clearly that the steam consumption was significantly reduced by lowering the F_p value when the retort temperature was 110°C. However, this reduction was not significant when the retort temperature was 121°C. Fig. 4 clearly shows that the smaller cans required significantly less steam than the larger cans when their contents were of a conduction heating type. However, there was no significant difference in the steam consumption when the canned content was of a convection heating type.

The mass average sterilizing values were also subjected to the analysis of variance, Table 5. We found highly significant three main effects, one highly significant interaction and two significant interactions. The influence of two selected interactions, $F_p \times T_a$ and $F_p \times F_t$, on the mass average sterilizing values was examined graphically, Fig. 5 and 6. Fig. 5 shows that the smaller target F_p value resulted in a significantly smaller mass average sterilizing value compared to that of the larger F_p value when the retort temperature was 110°C. However, the target F_p values did not influence significantly when the retort temperature was 121°C. Furthermore, the retort temperatures did not affect significantly the mass average sterilizing values at the F_p values of 3 min. Fig. 6 shows that there was a less distinctive interaction between F_p and F_t compared with the one shown in Fig. 5. The heat conduction simulator produced slightly greater reduction in the mass average sterilizing value, when the F_p value was reduced from 10 (min) to 3, compared to the reduction observed with the heat convection simulator.

As shown with Table 5, the can sizes and initial temperatures did not influence significantly the mass average sterilizing values. However, we observed complicated influence of these two factors from Table 3. With the convection heating simulant, there was no observable influence of can sizes and of initial temperatures on the mass average sterilizing. This could be due to a uniform temperature distribution in the sample throughout a process and to an extremely high rate of heat penetration in the sample. We observe different relationships with the conduction heating simulant. The data show that the smaller the can sizes, the smaller the mass average sterilizing values for all runs except runs 21 through 24, with which the relationship was reversed. Reasons for this reversal were examined below.

By examining a computational procedure and basic definition of the mass average sterilizing value, we found that the mass average sterilizing value became greater when a heating time became longer or when the temperature distribution in the food became more uniform throughout a

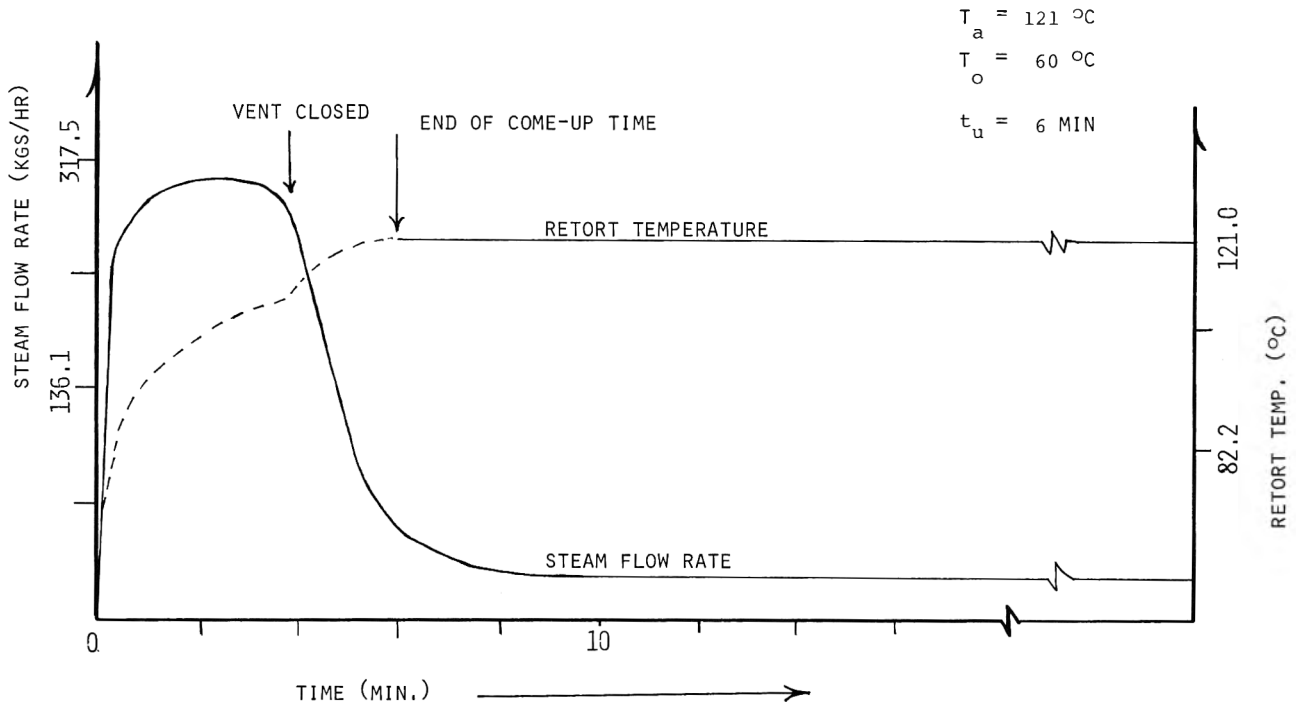

Fig. 2—Typical relationship between steam consumption rate and retort temperature for run 28.

Table 4—Analysis of variance on steam consumption

Source of variation	F-ratio[a]	Significance[b]
Can size (C_s)	33.68	***
Food type (F_t)	348.89	***
Initial food temp (T_o)	19.30	***
Retort temp (T_a)	163.96	***
F_p-value (F_p)	174.97	***
$C_s \times F_p$	1.92	—
$C_s \times T_o$	0.01	—
$T_o \times F_p$	0.09	—
$C_s \times T_a$	0.07	—
$F_p \times T_a$	101.59	***
$C_s \times F_t$	59.02	***
$T_o \times F_t$	0.46	—
$F_p \times F_t$	7.30	*
$T_a \times F_t$	0.24	—
$T_o \times T_a$	0.07	—

[a] All triple or higher interactions were pooled together to estimate an error variance for computing F-ratio. The value of this variance and its degree of freedom are 56.61 and 16, respectively.
[b] Single, double and triple asterisks respectively signify 5(%), 1(%) and 0.1(%) levels of significance. Sources without any asterisk did not significantly influence the amount of steam consumption.

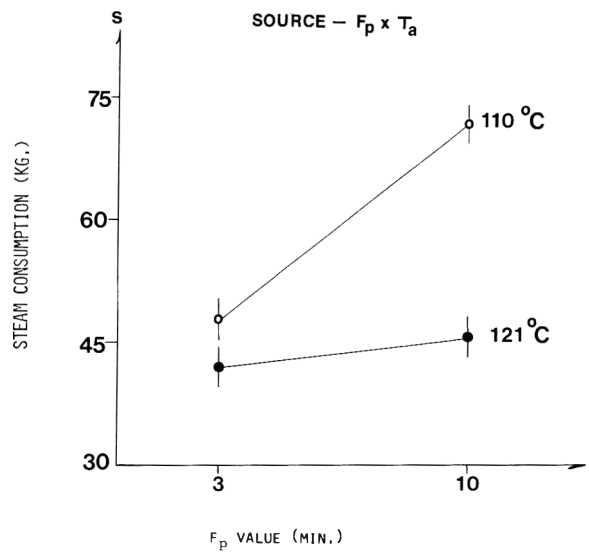

Fig. 3—Mean steam consumption for two-way classification according to retort temperatures and F_p values.

thermal process, provided that all other processing conditions were kept unchanged.

The exceptional runs, runs 21 through 24, were for the larger target F_p value and for the lower holding temperature of the retort. Therefore, they required longer heating times, t_b, than those of other runs. The average ratio of t_b values for the larger cans and for the smaller cans is equal to 1.2 for the exceptional runs while it is equal to 1.4 for runs 17 through 32. Therefore, the ratio of the exceptional runs was smaller than that of the other run. Since the smaller cans have the smaller f values, Table 2, the temperature distribution in these cans are more uniform compared to those in the larger cans throughout the thermal processes. Because of the smaller t_b ratio for the exceptional runs, the influence of the temperature uniformity of the mass average sterilizing value was most likely more pronounced compared with the influence of the processing time on the same value. This likely caused the reversed relationship stated above.

The food simulators used for our study were either thermally conductive or convective although most commercially canned foods exhibit both modes of heat transfer. The selection of these simulants was to provide two extremes of heat transfer mechanism within which a broad range of food products can be categorically identified.

Although the smaller cans significantly reduced the steam consumption when the food simulator was of conduction heating type, overall energy requirements should be carefully examined since smaller cans require more tin plates for their fabrication, and require more handling unit mass of food processed in comparison with the larger cans.

CONCLUSION

STEAM CONSUMPTION during heat processing was highly affected by all five factors tested. Mass average sterilizing

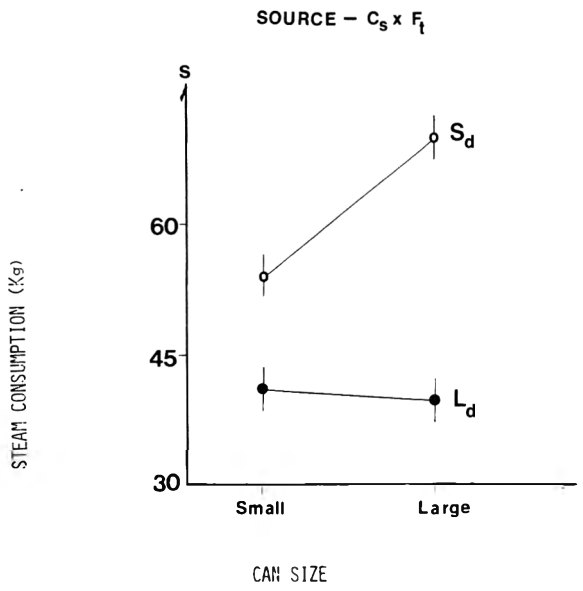

Fig. 4—Mean steam consumption for two-way classification according to can size and food types.

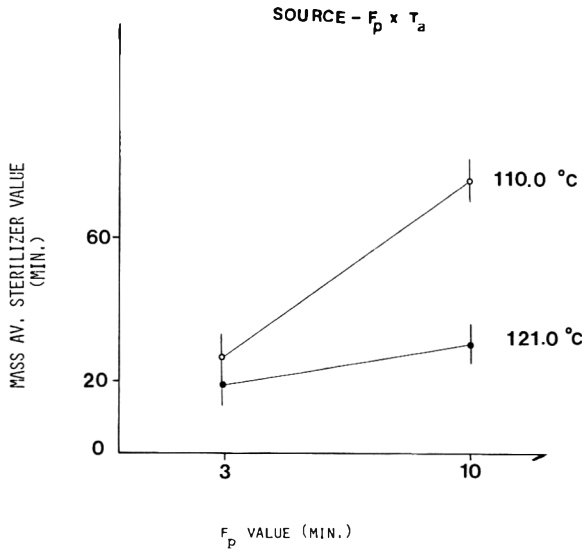

Fig. 5—Mean mass average sterilizing value for two-way classification according to retort temperatures and F_p values.

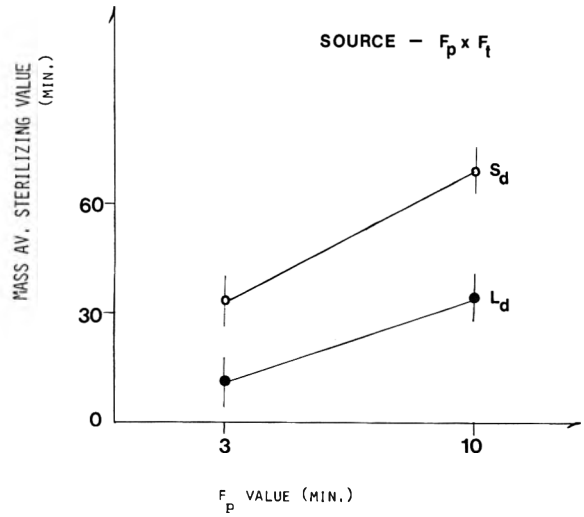

Fig. 6—Mean mass average sterilizing value for two-way classification according to food types and F_p values.

Table 5—Analysis of variance on mass average sterilizing value

Source of variation	F-ratio[a]	Significance[b]
Can size (C_s)	0.29	–
Food type (F_t)	101.23	***
Initial food temp (T_o)	0.10	–
Retort temp (T_a)	92.39	***
F_p-value (F_p)	110.46	***
C_s x F_p	2.33	–
C_s x T_o	0.20	–
T_o x F_p	0.05	–
C_s x T_a	5.04	*
F_p x T_a	49.09	***
C_s x F_t	0.21	–
T_o x F_t	0.05	–
F_p x F_t	5.05	*
T_a x F_t	0.52	–
T_o x T_a	0.02	–

[a] All triple or higher interactions were pooled together to estimate an error variance for computing F-ratio. The value of this variance and its degree of freedom are 61.96 and 16, respectively.
[b] Single and triple asterisks respectively signify 5(%) anc 0.1(%) levels of significance. Sources without any asterisk did not significantly influence the amount of steam consumption.

values were influenced by retort temperature, types of food simulants and target F_p values. However, in both cases, there were three significant interactions. The following observations were made based on careful examination of these interactions:

Steam consumption was considerably higher in the case of large cans compared to small cans. Can size had no significant influence on steam consumption when processing heat convection food simulants. Steam consumption and mass average sterilizing values were low for processes with a high retort temperature and with the high F_p value. The type of food did not affect mass average sterilizing value considerably when the low F_p value was used.

NOMENCLATURES

C_s	Can size
F_p	Sterilizing value (min)
F_t	Food type
f	Slope index of heating or cooling curve of sample food simulator (min)
j	Intercept coefficient of heating or cooling curve of the sample food simulant
L	Large, 307x409, can
L_d	Liquid food simulator, water
M_m	Mean of mass average sterilizing value (min)
M_s	Mean of steam consumption (Kg)
S	Small, 211x300, can
S_d	Solid food simulator, 8% bentonite suspension
T_a	Retort temperature (°C)
T_c	Temperature at the thermal center of a can (°C)
T_o	Initial temperature of food simulant (°C)
t_b	Calculated process time (min)

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—Continued on page 225

Development of High-Protein Low-Cost Nigerian Foods

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ABSTRACT

Nigerian foods, akara, chin-chin, and puff-puff, were supplemented with full-fat cottonseed, defatted cottonseed, peanut, sesame or soybean flours. The foods were evaluated using a nine-point Hedonic scale by African and non-African panels. Foods were evaluated for appearance, texture, absence of greasiness, palatability and overall acceptability. The African panel rated all food products for all characteristics significantly (P=0.001) higher than the non-African panel. Protein quality evaluations indicated that full-fat cottonseed, defatted cottonseed and defatted peanut flours could be used to increase the quantity and quality of protein in traditional Nigerian foods.

INTRODUCTION

AKARA is the most common food product in Africa containing cowpeas (Wilson, 1971). Deep fat fried balls prepared from akara provide a tasty fresh product available at specific times to the consumer (Dovlo et al., 1976). The size of akara balls determined whether they are to be eaten as an appetizer or as a main dish (Hachten, 1970). The nutritional value of cowpeas is associated with a high protein value, ranging from 20–25%. Puff-puff, soft, golden brown balls of wheat flour batter fried in deep fat, is a major snack food in most African countries. Chin-Chin is crisp, slightly sweet golden brown knots of pastry (Wilson, 1971). These deep fat-fried cakes are made in different shapes and styles (Hachten, 1970). Chin-chin is often flavored with vanilla extract, nutmeg, caraway seed, orange or lemon rind. The objectives of this study were to develop high-protein, low-cost Nigerian foods: chin-chin, puff-puff, and akara using combinations of various high protein flours; and to evaluate their protein quality.

MATERIALS & METHODS

THE RECIPES for chin-chin and puff-puff were adapted from Cookery Book for the Tropics (Vincent, 1970) and for akara from Kitchen Safari (Hachten, 1970). Each experimental product was prepared by the same method as the control, except for the substitution of either full-fat or defatted cottonseed flour, soybean flour, peanut flour or sesame seed flour (Table 1). Preliminary taste panels indicated the substitutions most acceptable in the chin-chin and puff-puff recipes was 30% of the all purpose wheat flour and in the akara recipe 25% of the cowpeas. The recipes were standardized by metric weight measurements, temperatures for cooking (191°C), and fried to an even golden brown color.

The taste panel consisted of 23 Africans familiar with the three foods and 24 untrained non-Africans. A nine-point hedonic scale, in which an increase in quality paralleled an increase in numerical value, was used in evaluating the product. The highest rating to be assigned was a score of 9 (excellent) and the lowest a score of 1 (unacceptable). A randomized complete block experimental design was used. Analysis of variance, covariance and Newman-Keuls analysis were used to evaluate the significance (P=0.05) of the differences between the food products.

Proximate analyses (AOAC, 1975) were obtained for all flours (Table 1). The chemical score was calculated by using the protein and amino acid content of the prepared foods and the amino acid requirements for adults as recommended by the Food and Agricultural Organization (FAO, 1970, 1973). The composition of the total solids of each food product prior to deep fat frying was calculated (Table 2). Following the frying, each food was dried at 65°C in an oven for not less than 24 hr, ground, and stored at −15°C until mixed in a diet for assay. Samples of each dried food were taken for proximate analyses (Table 2). These data were used to calculate the composition of the total solids of each product after frying.

Protein (N X 6.25) efficiency ratio (PER) was determined (AOAC, 1975) for each food variation (Table 3). Casein control diets contained 8, 18.5, 25 or 32.7% fat. Ten male Holtzman (Holtzman Co., Madison, Wisc.) rats, 21–24 days of age weighing 55–60g were assigned in such a manner as to balance the total weight of each group. Water and diets were provided ad libitum.

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RESULTS & DISCUSSION

Organoleptic

The taste panel data for akara, puff-puff and chin-chin are presented in Table 3. The African panel rated all the food products significantly (P=0.001) higher for all characteristics than the non-African panel. The higher scores by the African panel may be attributed to the foods being culturally and traditionally acceptable. The akara products in which soybean or sesame flour replaced part of the cowpeas were rated more desirable more frequently by both panels than were the original cowpea akara or the akara in which either cottonseed flour or peanut flour were used.

The African panel preferred the chin-chin with no substitution for all characteristics except appearance. The non-

Table 1—Proximate analysis (percent) of various flours

Flours	Moisture	Protein	Fat	Fiber	Ash	Nitrogen free extract
All purpose wheat	10.40	10.69	1.00	0.20	0.46	77.25
Whole kernel cottonseed (Full-fat)[a]	6.20	39.13	35.45	1.48	4.30	13.44
Defatted cottonseed[a]	9.00	55.41	3.24	0.80	7.43	24.12
Defatted groundnut (Peanut)[a]	5.80	60.24	0.60	2.80	4.65	25.91
Soybean (Full-fat)[a]	8.80	38.88	21.40	3.40	5.08	22.44
Cow peas[a]	8.90	21.88	1.20	2.70	3.80	61.52
Sesame seed[b]	4.00	60.00	1.00	5.00	6.00	

[a] Analyses performed at the Food Protein Research & Development Center, Texas A&M Univ. of Pope Testing Laboratories, Inc.
[b] Source: Sesame Products, Inc. Paris, TX 1977.

Table 2—Percentage protein and fat of total solids of the food before and after frying

Products[a]	Before		After	
	Protein	Fat	Protein	Fat
Akara				
CP	27.5	6.0	20.9	24.9
FC	30.8	14.0	21.7	36.9
DC	34.7	6.8	24.0	32.0
G	35.5	6.2	25.7	29.5
Chin-Chin				
APW	11.1	21.0	9.8	32.0
FC	16.7	27.5	13.6	40.4
DC	20.8	21.3	16.4	30.3
G	20.8	20.6	17.8	29.7
Puff-Puff				
APW	17.3	7.5	14.0	30.6
FC	22.4	13.7	17.0	41.9
DC	25.4	7.9	18.7	30.6
G	26.2	7.4	19.1	33.8

[a] FC, full-fat cottonseed; DC, defatted cottonseed; G, defatted peanut

Table 3—Taste panel scores[a] assigned by African and non-African panels to akara, chin-chin and puff-puff

	AKARA					
	CP[b]	S	FC	DC	G	SB
Appearance						
African	8.33	8.34[c]	8.26[d]	8.29	8.27	8.33
Non-African	7.09[c]	7.14[c]	7.31[c]	6.81[d]	7.02[c]	7.26[c]
Texture						
African	8.54[c]	8.32[d]	8.42	8.45	8.38	8.50[c]
Non-African	6.86	6.97	6.78[d]	6.88	6.97	7.05[c]
Absence of greasiness						
African	8.35	8.35	8.39	8.46[c]	8.30[d]	8.51[c]
Non-African	6.71[d]	7.00[c]	6.88	6.81	6.81	7.31[c]
Palatability						
African	8.43[c]	8.36	8.28[d]	8.38	8.39	8.46[c]
Non-African	7.00[c]	6.93	6.79[d]	6.86	7.00[c]	6.90
Overall acceptability						
African	8.27	8.38[c]	8.20[d]	8.27	8.26	8.32
Non-African	6.81	7.03[c]	6.90	6.64[c]	6.95	7.22[c]

	CHIN-CHIN					
	APW	S	FC	DC	G	SB
Appearance						
African	8.28[c]	7.44[d]	7.82[d]	8.63[c]	7.90[d]	8.11[c]
Non-African	7.29[c]	5.34[d]	6.77[c]	6.73[d]	6.77[c]	6.94[c]
Texture						
African	8.23[c]	7.56[d]	7.88[c]	7.70[d]	7.82[c]	7.85[c]
Non-African	6.74[c]	5.31[d]	6.05[c]	5.61[c]	5.47[d]	5.84[c]
Absence of greasiness						
African	8.01[d]	7.41[d]	7.56[d]	7.51[d]	7.72[c]	7.91[c]
Non-African	6.87[c]	5.71[d]	6.20[c]	5.89[c]	5.80[c]	5.78[c]
Palatability						
African	8.28[c]	7.73[d]	7.73[d]	7.79[c]	7.72[d]	7.93[c]
Non-African	6.61[c]	4.86[d]	5.60[c]	5.27[d]	5.21[d]	5.49[d]
Overall acceptability						
African	8.33[c]	6.67[d]	7.90[d]	7.91[d]	7.74[d]	8.20[c]
Non-African	6.19[c]	4.69[d]	5.39[c]	5.27[d]	5.13[d]	5.38[c]

	PUFF-PUFF					
	APW	S	FC	DC	G	SB
Appearance						
African	8.19[c]	8.01[c]	7.97[d]	8.23[c]	7.96[d]	7.96[d]
Non-African	6.50[c]	5.55	5.41[d]	6.48[c]	5.77[d]	5.48[d]

(Continued in next column)

Table 3—Continued

	PUFF-PUFF					
	APW	S	FC	DC	G	SB
Texture						
African	8.15[c]	8.25[c]	8.07[d]	8.24[c]	7.93[d]	8.10[c]
Non-Africar	6.54[c]	5.92[d]	6.05	6.27[c]	5.52[d]	5.30[d]
Absence of greasiness						
African	8.00[d]	8.00[d]	8.08[d]	8.15[c]	8.03[d]	8.07[d]
Non-Africar	5.10[d]	4.79[d]	4.94[d]	5.05[c]	5.05[c]	4.70[d]
Palatability						
African	8.05[c]	8.10[c]	7.88	8.22[c]	7.82[c]	8.04[c]
Non-Africar	5.57[c]	5.81[c]	5.65[c]	5.41[d]	5.27[c]	5.29[d]
Overall acceptability						
African	8.18[c]	8.16[c]	7.91[d]	8.23[c]	8.03[c]	8.14[c]
Non-Africar	5.64[c]	5.44[c]	5.34	5.19[d]	5.34	5.33[d]

[a] Based on a 9-point scale with 9 = Excellent, 1 = Unacceptable.
[b] CP, Cowpeas; APW, all purpose wheat; S, sesame seed; FC, full-fat cottonseed flour; DC, defatted cottonseed flour; G, peanut flour, SB, soybean flour.
[c,d] When mean scores within a given panel differ, c was significantly (P = 0.05) higher than d.

Table 4—Chemical scores for food products using FAO pattern as reference[a]

	Chemical scores	
	Methionine + cystine	Tryptophan
Akara		
CP	13*	24
FC	15*	30
DC	16*	35
G	16*	34
Chin-Chin		
APW	10*	10*
FC	13*	22
DC	15*	28
G	15*	26
Puff-Puff		
APW	19	17*
FC	22*	29
DC	23*	35
G	23*	33

[a] FAO, 1970; FAO, 1973
* Limiting amino acid

African panel preferred chin-chin without any substitutions for all characteristics.

The soybean flour was the most desirable substitution for the all purpose flour.

The African panel preferred the puff-puff containing the defatted cottonseed. The non-African panel preferred the puff-puff containing the all purpose flour for all characteristics except the absence of greasiness.

Amino acid profile

The substitution of the seed flours for cowpeas or wheat flour increased the percentage protein (Table 2) in all food products. The substitution of the seed flours increased the chemical scores of the limiting amino acids (Table 4) methionine and cystine for all foods.

Protein efficiency ratio

The percentage of protein and fat in the total solids of food products was calculated on the basis of the recipes prior to deep fat frying (Table 2). Following the frying of the products proximate analyses were obtained and the protein and fat in the total solid were calculated. There

was a large increase in the fat content of all the products as a result of the deep fat frying. In all the fried food products the percentage of fat exceeded the percentage of protein (Table 2).

The AOAC (1975) procedure for the determination of protein quality states that the minimum level of fat shall be 8% of the diet fed to rats. Protein quality evaluation of foods having greater fat content than the prescribed level of 8% has not met with great success. Hurt et al. (1975) investigated the protein quality of a beef-vegetable product and a casein control diet at 8, 16 and 24% fat levels. Food consumption, weight gain and efficiency of food utilization for the rats fed the casein control diet and the beef-vegetable product were significantly decreased by increasing the lipid content in the diets. The animals consumed less of the diet as the level of fat was increased. This trend was noted for the rats fed both the casein and the test diet. However, when the caloric content of the diets was considered, it was apparent that growth was less efficient for the rats fed the diets with higher fat content. Staub (1978) has suggested that the PER method can be used to evaluate both simple and complex food products; but studying high moisture and high fat diets stretch the PER assay to its limit.

The effect of the high fat content in the foods was tested by feeding the control casein diet containing 8, 18.5, 25 and 32.7% fat and the PER values are shown in Table 5. The inclusion of fat in the diet above the 8% set by the AOAC lineraly decreased the PER value of casein. The unadjusted values (Table 5) were adjusted for casein equivalent to 2.50 and for the effect of the level of fat in the diet by a computer program to arrive at the adjusted values (Table 5).

The addition of the seed flours to akara did not significantly increase the protein quality of the product containing the seed protein cowpea. Although akara was high in fat content after frying the high level of protein in akara resulted in PER values above 2.00. In both the chin-chin and puff-puff products the fat to protein ratio was considerably higher than for akara (Table 2). The high fat content in the diet reduced the food intake and the weight gained by the rats. Thus, the PER values were much lower than would be expected. Puff-puff was not a good source of protein and supplementation with seed flours made it an even poorer source. The addition of the seed flours to chin-chin improved the protein quality with defatted cottonseed producing the best improvement. Supplementation of chin-chin, therefore, would be advantageous for the Nigerian people.

Table 5—Percentage of fat in the diet and PER for akara, chin-chin and puff-puff

	Fat	PER	
		Unadjusted	Adjusted
Akara[a]			
CP	12.0	1.82	2.16
FC	17.0	1.96	2.34
DC	13.0	2.10	2.09
G	12.0	1.68	2.00
Casein	8.0	3.22	2.50
Chin-chin			
APW	32.7	1.25	1.24[d]
FC	18.5	1.69	1.66[c]
DC	29.7	1.69	2.08[b]
G	16.7	1.42	1.74[c]
Casein	8.0	3.11	2.50
Puff-puff			
APW	21.8	0.63	0.57[b]
FC	24.6	−0.12	−0.13[c]
DC	16.4	−0.06	−0.07[c]
G	17.7	−0.27	−0.32[c]
Casein	8.0	3.22	2.50
Casein	18.5	3.07	
Casein	18.5	2.98	
Casein	25.0	2.77	
Casein	32.7	2.46	

[a] FC, fat-full cottonseed; DC, defatted cottonseed; G, defatted peanut.
[b] Is significantly (p=0.05) higher than c; [c] is significantly (P=0.05) higher than d (Newman-Keuls multiple range test).

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Heat and Mass Transfer During the Warm Water Blanching of Potatoes

A. N. CALIFANO and A. CALVELO

ABSTRACT

A model of heat and mass transfer with simultaneous chemical reaction is proposed for analyzing the influence of operating variables on the reducing sugar content at the surface of blanched potatoes. This content is partially responsible for the color of the finished product. The involved parameters (potato thermal conductivity, heat transfer coefficient of the system and kinetic constants for overall reaction of reducing sugar generation) were evaluated in separate experiments. The apparent diffusion coefficient of reducing sugars in potatoes was the only parameter obtained from blanching experiments. Temperature and concentration profiles and effect of blanching temperature on surface reducing sugar content are analyzed using the developed model. The possible underestimation of the diffusion coefficient when simultaneous starch hydrolysis is not taken into account is also considered.

INTRODUCTION

THE BLANCHING of potatoes prior to frying has several advantages, e.g., improved texture of the final product, reduction of fat absorption and better color of fried products by decreasing the reducing sugar content on the surface.

It is widely accepted that sugar concentration, especially reducing sugars, modify the final coloration in French fries, due to caramelization or a Maillard reaction (Smith, 1975; Shallenberger et al., 1959; Schwimmer et al., 1957; Hoover and Xander, 1963; Rodgers and Binsted, 1972; Birch, 1977). Thus, several authors have reported that a darker final product corresponds to higher content of reducing sugars (Burton, 1962, 1968, 1969; Wisler, 1968; Gould, 1969; Burtea and Mirea, 1977).

The reducing sugar content of potatoes is naturally low, but on storing the tubers at temperatures below 4°C, to prevent sprouting or dehydration, a substantial increase of the reducing sugars takes place. Therefore, the tubers must be reconditioned prior to frying, either by holding at 20°C for several weeks or by water blanching.

In the French frying process the blanching operation consists in placing the potato strips on conveyor belts that carry them through a hot water bath. The usual range of water temperature is from 60–80°C and residence times vary between 5–20 min (Weaver et al., 1975; Duckworth, 1979). During the process, the sugar concentration decreases, resulting in a lighter and more uniform color in the product surface.

Another possibility is to use two blanchers in series. The first blancher to decrease the sugar concentration, while the second one, containing a diluted sugar solution, may be used as an aid in adjusting the surface sugar concentration to a desired level. (Smith, 1975).

In order to design the operating conditions of the process, in both cases, it is necessary to know the reducing sugar transfer rate to or from the blanchers and its dependence on temperature.

In a previous work performed on the changes of sugar content in potatoes during blanching, Califano and Calvelo (1979) found evidence that besides the mass transfer to the bath there exists an internal generation of reducing sugars, probably due to an enzymatic hydrolysis of starch. A similar effect has been reported by Ikemiya an Deobald (1966) and by Sistrunk (1977) in sweet potatoes. Consequently, both mechanisms must be taken into account when predicting the rate of change in the surface concentration of reducing sugars.

Moreover, as blanching times are of the same order as those required for heating the potato pieces, the described mechanisms work simultaneously with an unsteady state heat transfer from the bath to the product.

The present paper proposes a mathematical model for heat and mass transfer with a simultaneous chemical reaction to simulate the adjusting operation of the reducing sugar content in potato by means of blanching at moderate temperatures.

The involved physical parameters (thermal conductivity, heat and mass transfer coefficients, diffusion coefficient, etc.) as well as the chemical parameters (rate constant and activation energy for the generation of reducing sugars) are obtained by means of complementary experiments. In this way, it is possible to analyze the operating conditions and the relative importance of the different mechanisms.

MATHEMATICAL MODEL

Equations

By considering a potato sphere of R radius initially at temperature T_o, in contact with a stirred fluid at temperature T_f, and assuming that the heat of reaction is negligible, the heat transfer balance results: (Bird et al., 1964)

$$\frac{\partial T}{\partial t} = \alpha \left(\frac{2}{r}\frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial r^2}\right) \quad (1)$$

where $\alpha = k/\rho C_p$ is the potato thermal diffusivity, supposed to be constant.

The corresponding initial and boundary conditions are:

$$t \leq 0; \quad T = T_o; \quad 0 \leq r \leq R \quad (2)$$

$$t > 0; \quad r = R; \quad h(T_f - T) = k\frac{\partial T}{\partial r} \quad (3)$$

$$t > 0; \quad r = 0; \quad \frac{\partial T}{\partial r} = 0 \quad (4)$$

Where h is the heat transfer coefficient at the sphere-fluid interface and k the potato thermal conductivity.

By considering a potato sphere with a C_o initial reducing sugar concentration (expressed as moles of glucose per m^3 of potato) and the water-bath with a C_f concentration (expressed as moles of glucose per m^3 of fluid), the mass transfer equation and the corresponding initial and boundary conditions will be: (Bird et al., 1964)

$$\frac{\partial C}{\partial t} = \frac{1}{r^2}\left(\frac{\partial}{\partial r} r^2 D_G \frac{\partial C}{\partial r}\right) + k' \quad (5)$$

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$$t \leq 0; \quad C = C_o; \quad 0 \leq r \leq R \tag{6}$$

$$t > 0; \quad k_L(C_f - C) = \epsilon D_G \frac{\partial C}{\partial r}; \quad r = R \tag{7}$$

$$t > 0; \quad \frac{\partial C}{\partial r} = 0; \quad r = 0 \tag{8}$$

where D_G is the apparent diffusion coefficient of reducing sugars in potato, depending on temperature according to the Stokes-Einstein equation: $D_G = KT/\mu_w$ (Skelland, 1974). In this equation μ_w is the water viscosity at temperature T and K is a constant which depends on the system. For aqueous glucose solutions K = 1.98 x 10^{-15} kg m/s^2 K (Califano, 1981). •

In Eq. (7) k_L is the mass transfer coefficient at the potato-fluid interface and ϵ is the volumetric fraction of liquid in the potato, which transforms the concentration per unit volume of liquid into concentration per unit volume of potato according to $C' = C/\epsilon$. (Thus, $C_f = C_f'/\epsilon$ when using the proposed model).

The reaction rate term in Eq. (5) was considered as a zero order reaction with a temperature dependence given by:

$$k' = A \exp [-E_a/R_gT] \tag{9}$$

The zero order kinetic assumption was based on a previous work (Califano and Calvelo, 1979) in which the rate of glucose production was found to be independent of its concentration. This kinetic behavior may be extended to the production of reducing sugars on the assumption that fructose might not play an inhibiting role on the reaction.

Moreover, for proposing Eq. (9) a constant concentration of hydrolytic enzymes and an excess of starch were considered.

Solution method

Eq. (1) to (4) were written in terms of finite differences applying the Crank-Nicholson method (Jenson and Jeffreys, 1963). The Thomas algorithm (Von Rosenberg, 1971) was used for solving the tridiagonal matrix formed each time.

Eq. (5) to (9) were also solved by the Crank-Nicholson method, supplying each time the corresponding temperature profile obtained from the solution of Eq. (1) to (4).

Once the concentration profile was obtained as a function of time by means of an IBM Series 1 Computer, average concentration was calculated according to:

$$\bar{C} = \frac{3}{R^3} \int_0^R C(r)\, r^2\, dr \tag{10}$$

The integral was solved numerically by means of a Simpson's algorithm.

MATERIALS & METHODS

Heat transfer with simultaneous generation of reducing sugars

In order to obtain information on kinetic constants it was necessary to separate reducing sugar generation from mass transfer to the blanching bath. Thus, experiments on heating of potato spheres were carried out, and the results obtained evaluated by considering $k_L = 0$ in the mathematical model proposed above.

Kennebec potatoes (harvest 1980) were previously placed in a cold-storage room at 4°C, simulating industrial conditions, thus obtaining a product with a high initial content of reducing sugars. Samples, avoiding the internal medulla, were extracted from peeled tubers.

Air from a blower was circulated through a heater with electronically controlled resistances and introduced into a 0.4m/side cubic container with four fans placed at the corners of an imaginary tetrahedron. The potato sphere was placed at the center of the container. This design was developed in order to obtain a uniform temperature over the surface of the sphere through a constant local heat transfer coefficient. Each potato sphere of 2.7 cm. diameter was heated for a certain time and the center and surface temperatures were recorded. The air temperature was also recorded. The sphere was covered with a thin protective film ("Parafilm") to avoid dehydration.

Temperatures were measured by means of copper-constantan thermocouples connected to a multichannel potentiometric recorder.

In each case, the initial concentration of reducing sugars was measured on a control sphere, extracted from the same area as the treated one, and the final concentration was determined on the heated sphere. Samples were subjected to an extraction with hot ethanol (80%); reducing sugars were determined by Somogyi's method (1945) and expressed as glucose. The specific weight of each tuber was determined by using a picnometer.

The heat transfer coefficient, h, of the system was determined by replacing the potato sphere with an acrylic one, (3.04 cm. diameter), and known thermal conductivity (k_a = 0.207 W/mK), covered with the same protective film. Thermocouples were placed in the acrylic sphere at different depths and on the surface. Then, the coefficient, h, was obtained by comparing the recorded experimental temperatures with those predicted by an unsteady state heat transfer model. The value between 60° and 68°C was h = 55 W/m^{2}K.

Heat and mass transfer with simultaneous generation of reducing sugars

The apparent diffusion coefficient of reducing sugars in potatoes was determined by blanching potato spheres, and comparing results with those coming from the corresponding model.

Potato spheres of 2.25 cm diameter were blanched in a container filled with distilled water at a controlled temperature. Stirring was strong enough to secure uniformity of heat transfer coefficient. Temperatures at the center of the sphere and in the blanching bath were recorded and initial and final concentrations of reducing sugars were measured. Duplicates of experiments were performed in order to determine initial and final water content.

The heat transfer coefficient for the blancher was determined by replacing the potato sphere with an acrylic one of 1.84 cm diameter, with thermocouples placed at several positions. Temperatures were compared with those predicted by an unsteady state heat transfer model. The value was h = 350 W/m^{2}K.

RESULTS & DISCUSSION

Heat transfer with simultaneous generation of reducing sugars

Table 1 shows the change in the reducing sugar content during the heating of potatoes without blanching.

Initial concentrations of the reducing sugars (C_o) range from 61–129 x 10^{-3} g-mole/kg potato and the air temperature (T_f) between 59.6° and 68.2°C. The concentrations are average values of duplicate determinations on each extract; the error of the averages was ± 0.5 x 10^{-3} g-mole/kg potato with a confidence limit of 0.95. An increment of about 20% in the content of potato reducing sugars due to the heating process was observed. The average specific weight of potatoes was 1070 kg/m^3. *–Continued on next page*

Table 1–Heat transfer with simultaneous reducing sugar generation

T_o(°C)	T_f(°C)	Time (s)	Sugar conc x 10^3 (expressed as glucose) (g-mole/kg potato) Initial	Final	ρ (kg/m^3)
22.8	61.9	1806	100.6	112.4	1069
20.3	61.6	1809	128.9	142.7	1077
17.8	59.6	1812	71.2	85.0	1069
19.0	60.0	1812	61.0	73.7	1073
23.0	60.7	1233	80.0	87.4	1062
22.8	60.7	1211	83.7	91.1	1074
18.3	60.7	2448	91.6	108.0	1065
20.3	62.1	2421	93.9	114.1	1064
15.6	66.4	1206	88.6	97.0	1076
20.2	68.2	2406	62.0	88.1	1066
20.3	66.2	2416	86.8	111.8	1069
21.8	61.7	1805	81.1	96.7	1073
21.3	63.7	1807	90.5	105.7	1075
21.5	67.8	1203	95.2	103.3	1062
21.8	59.6	2411	103.3	119.2	1071
22.8	60.7	2410	87.6	101.6	1070

The heat transfer with simultaneous chemical reaction model proposed (considering $k_L = 0$), was compared with the experimental final average concentration.

The thermal properties supplied to the model were k = 0.549 W/m K (Rao et al., 1975); C_p = 3579 J/kgK (Misener and Shove, 1976) and h = 55 W/m^{2}K. A satisfactory correlation was obtained between the experimental thermal histories and those calculated by using the model.

Different pairs of values of the pre-exponential factor (A) and the activation energy (E_a) in the kinetic equation for reducing sugar generation were fed into the model, calculating the variance (s^2) and the sum of residuals (S.R.) in each case (Box et al., 1978) as:

$$s^2 = \sum_{i=1}^{N} (C_{exp} - C_{calc})^2/(N-2) \qquad (11)$$

$$S.R. = \sum_{i=1}^{N} (C_{exp} - C_{calc}) \qquad (12)$$

In a first approximation those pairs that minimized the variance were selected, but as changes are small near the minimum, the pair in which the sum of residues was the closest to zero, was finally chosen. The values obtained were A = 33.7 g-mole/kg potatoes and $E_a = 4.184 \times 10^4$ J/g-mole.

Fig. 1 shows the experimental final concentrations as a function of the values predicted by the model. As it may be observed, there exists a good agreement between both sets of data. The scattering between the experimental concentrations and those predicted by the model with the adopted parameters was s = 1.89 x 10^{-3} g-mole/kg potato.

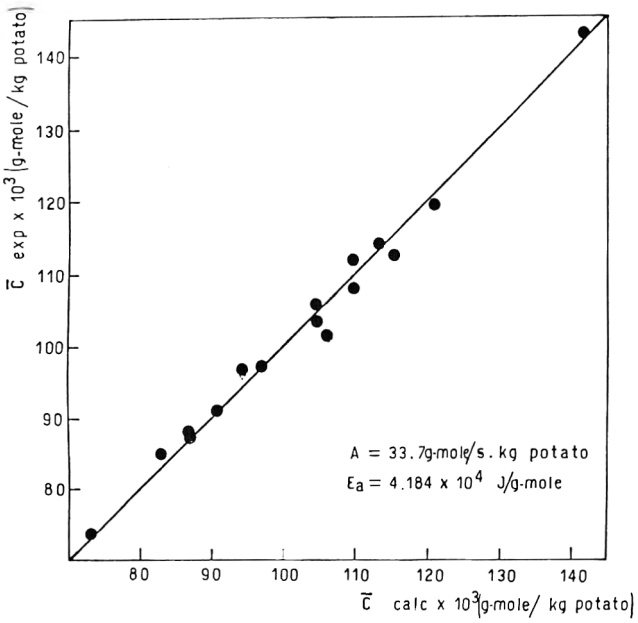

Fig. 1—Experimental final concentrations in terms of predicted values for heating of potato spheres.

The described experiments were not performed to elucidate the involved mechanisms in the production of reducing sugars during heating. Instead, the objective was to obtain adequate parameters for modelling the blanching operation. However, the activation energy obtained is comparable with values reported in the literature for different enzymes capable of causing a reducing sugar increase in similar biological systems (Table 2).

Heat and mass transfer with simultaneous generation of reducing sugars

Results on changes of the reducing sugar content during potato blanching experiments are presented in Table 3.

The blanching temperature T_f ranged from 59.4–70.4°C, while the initial concentrations of reducing sugars were between 79 and 119.6 x 10^{-3} g-mole/kg potato.

The average initial and final water contents of the samples were 79.8 and 80.0% respectively, showing no significant water transfer during the process. This fact justifies the use of Eq (2) in the model, where the convective term has been neglected since $N_B = 0$ and $x_a \ll 1$.

Concentrations are average values of duplicates measured on each extract. The error of the average was ± 0.6 x 10^{-6} g-mole/kg potato ($P \leqslant 95\%$).

Final concentrations reported in Table 3 were compared with those predicted by the proposed model when feeding the computer program the experimental blanching conditions and the corresponding heat and mass transfer properties. Several values of the apparent diffusion coefficient of glucose in potato were supplied to the program for each run and the coefficient which minimized the variance and the sum of residues was chosen.

The heat transfer parameters fed to the program were those already reported. A satisfactory agreement between experimental thermal histories and those predicted by the model was obtained.

The mass transfer coefficient k_L was evaluated from the heat and mass transfer analogy as:

$$k_L = \frac{h}{C_T\, C_{P_w}} \left(\frac{Pr}{Sc}\right)^{2/3}_{w} \qquad (13)$$

where $Sc = \mu/\rho D$ and $Pr = C_p\mu/k$. The fluid properties were evaluated at the bath temperature; C_T is the total molar concentration of water. Values of k_L ranged from 3.6–4.1 x 10^{-6} m/s depending on the blanching temperature.

The apparent diffusion coefficient obtained changed with temperature according to the Stokes-Einstein equation as proposed. For 60°C the correlated value was $D_G = 11.3 \times 10^{-10}$ m^2/s.

Fig. 2 shows the experimental final concentrations as a function of those computed by using the model, the kinetic constants previously evaluated and the apparent diffusion coefficient obtained. The scattering between the experimental concentrations and those calculated by the model is = 1.9 x 10^{-3}g-mole/kg potato. It may be concluded that the model simulates adequately the blanching experiments.

As the apparent diffusion coefficient was obtained by comparing average concentrations and taking into account

Table 2—Activation energies reported in the literature for enzymes capable of causing a reducing sugar increase in several biological systems

Authors	Origin	Enzyme	E_a (J/g-mole)	Temperature range (°C)
Ikemiya and Deobald (1966)	Sweet potato (Raw juice)	α-amylase	3.09 x 10^4	30–70°C
	Sweet potato (Raw juice)	α-amylase	3.03 x 10^4	30–70°C
	Sweet potato (Extract)	α-amylase	2.88 x 10^4	30–70°C
Poulsen et al. (1980)	—	Glucoamylase	1.3 x 10^4	46–65°C

Table 3—Heat and mass transfer with simultaneous reducing sugar generation

T_o(°C)	T_f(°C)	Time (s)	Sugar conc x 10^3 (expressed as glucose) (g-mole/kg potato) Initial	Final	Water content (%) Initial	Final	ρ (kg/m^3)
24.4	60.0	601	104.4	87.8	80.6	79.4	1112
25.1	60.6	600	92.0	77.8	80.6	81.6	1077
23.6	59.7	300	109.3	98.4	80.3	77.3	1096
25.0	60.3	301	110.5	98.3	83.0	85.6	1079
25.8	60.2	901	90.6	78.6	81.4	76.5	1074
27.8	59.4	600	87.9	77.8	83.4	83.4	1079
26.3	60.3	900	101.3	84.5	79.2	80.7	1076
25.4	60.2	1200	102.8	79.9	77.6	80.5	1074
27.1	60.1	1200	96.3	77.8	77.9	78.0	1092
28.1	65.5	307	112.9	102.6	79.6	82.4	1095
24.6	65.6	300	80.4	70.9	78.5	80.4	1091
26.9	64.5	480	111.1	95.0	79.3	78.1	1079
26.2	65.4	190	115.0	102.7	81.8	80.5	1093
18.4	66.1	480	79.0	70.3	77.4	79.0	1103
28.5	70.2	181	97.3	83.4	78.1	82.7	1110
26.0	70.2	180	102.7	95.9	80.5	79.5	1103
26.0	70.4	180	84.4	76.7	78.7	78.9	1097
28.8	60.7	600	118.6	98.4	78.2	78.0	1104
25.6	69.1	60	105.2	97.8	81.7	81.0	1101
24.5	69.3	62	102.6	100.5	78.1	78.8	1103
26.2	69.3	120	119.6	113.9	79.4	79.7	1075
25.3	69.3	120	100.4	94.7	79.7	78.1	1064

that the contribution of diffusion at low temperatures is small, the correlated value must be considered more reliable in the temperature range between 60° and 70°C. (The heating period, besides, is short).

By considering the potato as consisting of a solid matrix (starch, cellulose, pectic substances and some proteins) and a water solution, and assuming that such a matrix has little interaction with the solute, the apparent diffusion coefficient, D_G, used in this work is related to the molecular coefficient, D_{AB}, according to the equation $D_G = D_{AB}/\Omega$, where the tortuosity factor, Ω is defined as the ratio between the distance covered by the solute and the straight line in the direction of the concentration gradient (Califano, 1981). The tortuosity factor obtained was $\Omega = 1.26$.

Stahl and Loncin (1979) reported an effective diffusion coefficient of 6.1 x 10^{-10}m^2/s for cyclohexanol in potato tissue, for a water content of 86%, at 20°C. The corresponding diffusivity in water was 8.7 x 10^{-10}m^2/s at the same temperature. Taking into account that the effective diffusion coefficient used by these authors is related to the molecular difusivity as $D_{ef} = D_{AB}\,\epsilon/\Omega$, the tortuosity value for this case is $\Omega = 1.23$ (the volumetric fraction of solution respecting the solid matrix ϵ has been considered equal to the water content in a wet basis; $\epsilon = 0.86$). This tortuosity factor value shows very good agreement with that obtained from the reported blanching experiments.

Predicted temperature and concentration profiles

Fig. 3 presents temperature and concentration profiles as predicted by the developed model at different blanching times. A sharp decrease of the concentration near the potato surface is observed due to the diffusion of the reducing sugars to the blancher. At the same time, in the central region, the reducing sugar concentration was increased 10% with respect to the initial value as a consequence of the enzymatic hydrolysis.

A slight maximum is also shown in Fig. 3 due to the simultaneous effect of sugar generation in the potato tissue

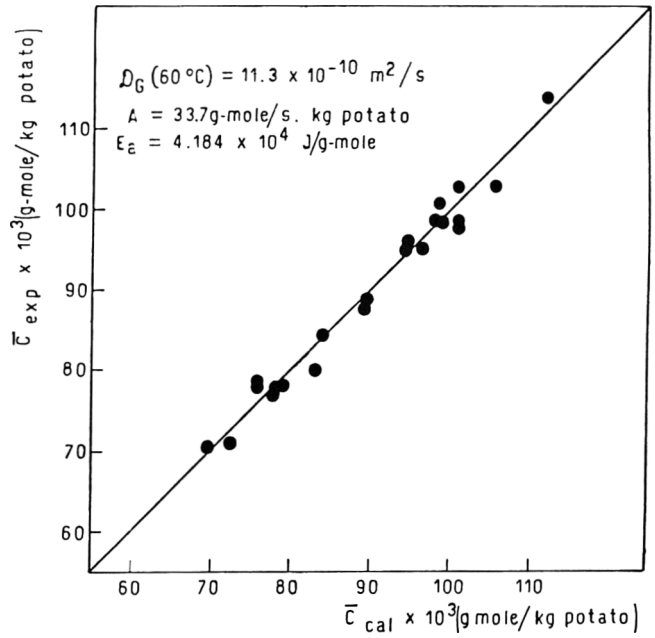

Fig. 2—Experimental final concentrations in terms of predicted values for blanching of potato spheres.

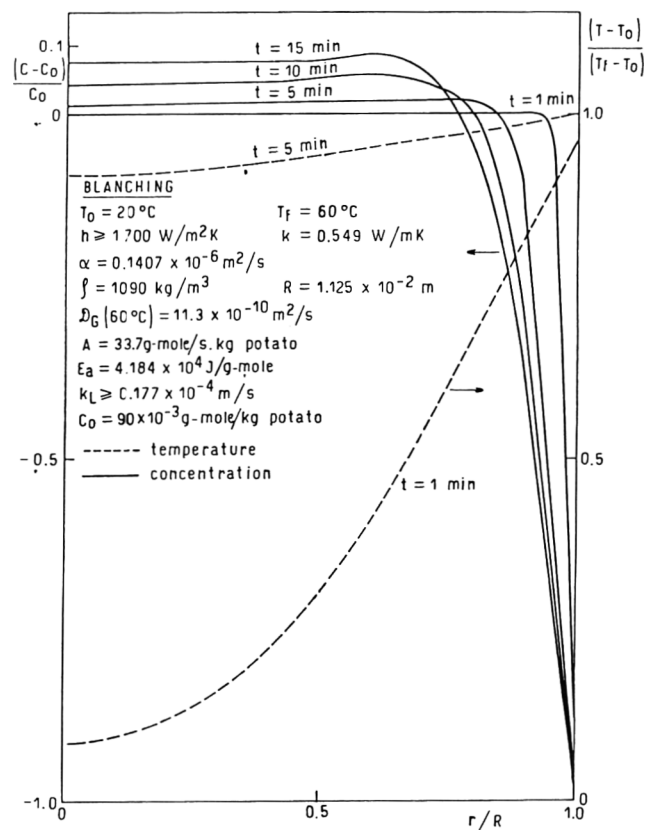

Fig. 3—Temperature and concentration profiles as predicted by the developed model at different blanching times.

and the corresponding sugar transfer to the bath.

Moreover, as heat transfer in this system is faster than mass transport, (very low values of Lewis number) an isothermal transfer of reducing sugars during most of the processing time is achieved.

Effect of changes in the blanching temperature

It must be considered that when raising the bath temperature the following properties increase: (a) mass transfer

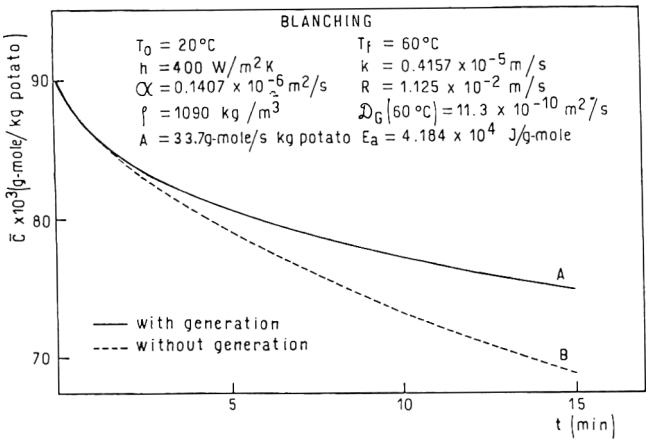

Fig. 4—Average concentration of a potato sphere in terms of blanching time as computed with and without simultaneous generation of reducing sugars.

coefficient; (b) diffusion coefficient; (c) kinetic constants of the sugar generation process. However, such increments do not affect the surface concentration of reducing sugars in the same way. Thus, the effect (a) tends to decrease the concentration when temperature rises while effects (b) and (c) tend to increase it.

As a consequence, the effect of temperature on the surface concentration is not significant (For a sphere of 2.25 cm diameter a temperature change from 60° to 75°C produces a reduction of less than 2% in the surface concentration for the same time).

A model of diffusion with simultaneous chemical reaction as compared to a pure diffusion model

As has been mentioned, there may be a reducing sugar increase of about 20% when considering generation without transfer to the fluid. This increase is reduced to 10% for usual processing times (less than 15 min).

This increment seems of little significance when compared to the initial sugar content but it becomes important with respect to the amount of reducing sugars transferred to the blanching bath. (Values up to 30% can be reached for usual operating conditions).

Fig. 4 presents the average concentration in the potato sphere calculated by using the mass transfer model with simultaneous generation of sugars (curve A) and the concentration computed applying a model where the simultaneous generation of sugars is not taken into account (diffusion model, curve B).

The diffusion model prediction favors the use of shorter periods of time in order to achieve the same concentration drop.

Similarly, it is possible to determine how the value of the diffusion coefficient obtained by the described experiments could be falsified when a diffusion model without simultaneous generation of sugars is used.

By applying the optimization method already described and the developed model without chemical reaction, the diffusion coefficient D_G at 60°C that minimized the variance and the sum of residues between experimental data and computed values was determined. The result led to $D_G = 4.95 \times 10^{10} m^2/s$ at 60°C which, compared to the value obtained when considering sugar generation, involves an underestimation of about 56%.

CONCLUSIONS

(1) A mechanism of reducing sugar generation was detected in warm water potato blanching, which takes place simultaneously with the transfer of solutes to the blanching bath.

(2) The kinetic constants of the sugar generation process were determined by assuming a zero order reaction.

The activation energy obtained was of about 4×10^4 J/g-mole in the range of 60–70°C. This value may be compared with those reported by other authors for the enzymatic generation of reducing sugars in similar systems.

(3) Experimental results on the loss of reducing sugars under laboratory conditions which simulate blanching were interpreted in terms of a heat and mass transfer model with simultaneous chemical reaction.

(4) The experimental data were adjusted by an apparent diffusion coefficient of reducing sugars in potato which depended on temperature. The value obtained was in accordance with the data reported by other authors in similar systems. The tortuosity factor agreed with the value calculated from data reported by Stahl and Loncin (1979) on potato diffusivity, accepting that potatoes consist of an insoluble matrix (starch, cellulose, pectic substances, etc.) and an aqueous phase (equal to the water content of the potato) through which the diffusion process occurs.

(5) The blanching temperature had a small effect on the reducing sugar content, due to its influence on the simultaneous mechanisms of generation and mass transfer to the blanching bath.

(6) Although, for blanching times and temperatures analyzed, the increase of reducing sugars was about 10% of the initial potato content, the increment became important when compared with the amount of sugars transferred to the bath (the ratio reached values of about 30%).

Not considering the reducing sugar generation effect leads to an underestimation in the blanching time necessary to obtain a certain reduction of sugars. It also leads to errors of about 56% when determining the apparent diffusion coefficient from blanching experiments.

(7) The described phenomenon of reducing sugar generation becomes less important at temperatures higher than 75°C because of the simultaneous destruction of the intervening enzymes. Above that temperature the starch gelatinization process is also significant.

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NOMENCLATURE

- A = pre-exponential factor (g-mole/s.kg potato)
- C = reducing sugars concentration in the potato (g-mole/m^3 potato)
- C' = reducing sugars concentration in the potato (g-mole/m^3 fluid)
- C_o = initial concentration (g-mole/m^3 potato)
- $\bar{C}$ = average concentration (g-mole/m^3 potato)
- C'_f = fluid concentration (g-mole/m^3 fluid)
- C_f = fluid concentration (g-mole/m^3 potato)
- C_{calc} = concentration predicted by the model
- C_{exp} = experimental concentration
- C_T = total molar concentration
- C_p = specific heat (J/kg K)
- C_{p_w} = specific heat of water (J/kg K)
- D_{AB} = molecular diffusion coefficient (m^2/s)
- D_G = apparent diffusion coefficient of reducing sugars in the potato (m^2/s)
- D_{ef} = effective diffusion coefficient (m^2/s)
- E_a = activation energy (J/g-mole)
- h = heat transfer coefficient (W/m^2 K)
- k = thermal conductivity (W/m K)
- k_a = acrylic thermal conductivity
- k' = apparent overall kinetic constant
- k_L = mass transfer coefficient based on concentration (m/s)
- K = constant, defined as $K = \mu_w D_G/T$ (kg m/s^2 K)
- Le = Lewis number = D_G/α
- N = number of determinations
- N_B = diffusion flux of component B with respect to fixed coordinates (g-mole/m^2 s)
- Pr = Prandtl number = $C_p\mu/k$
- r = radius (m)
- R = radius of the sphere (m)
- R_g = ideal gas constant (J/K g-mole)
- s^2 = variance
- S.R. = sum of residuals
- Sc = Schmidt number = $\mu/\rho D$
- t = time (s)
- T = absolute temperature (K)
- T_o = initial temperature (K)
- T_f = fluid temperature (K)
- x_A = molar fraction of the specie A

Greek Letters

- α = thermal diffusivity (m^2/s)
- ϵ = volumetric fraction of liquid in potato with respect to the whole potato.
- Ω = tortuosity factor
- μ = viscosity (kg/m s)
- μ_w = water viscosity (kg/m s)
- ρ = density (kg/m^3)

THERMAL PROCESSING CONDITIONS & STEAM CONSUMPTION. . . From page 216

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Effect of Delayed Germination by Heat-Damaged Spores on Estimates of Heat Resistance of *Clostridium botulinum* Types E and F

R. K. LYNT, D. A. KAUTTER and H. M. SOLOMON

ABSTRACT

The effect of delayed germination by heat-damaged spores on estimates of heat resistance of *Clostridium botulinum* types E and F was studied by analysis of data from thermal death time tube experiments. Type E strains were studied in crabmeat and nonproteolytic and proteolytic type F strains in phosphate buffer and crabmeat. The date each culture became positive was recorded and tabulated by week. The decimal reduction times (D) and z values for each strain were calculated at several different time intervals and compared. Although both D and z values increased during the incubation period, D values as much as fourfold, only proteolytic strains showed substantial increases in z values.

INTRODUCTION

DELAYED GERMINATION of heat-damaged spores was recognized many years ago as a problem in heat-resistance studies (Bigelow and Esty, 1920; Dickson et al., 1925). The heat resistance of PA3679 and *Clostridium botulinum* was compared in cultures for surviving spores which were incubated for at least 3 months before they were considered sterile (Townsend et al., 1938). A study of the effect of incubation temperature on the apparent heat resistance of spores of *C. botulinum* types A and B showed that differences caused by media were often eliminated by prolonged incubation (Williams and Reed, 1942).

In studies of the heat resistance of type E and both proteolytic and nonproteolytic type F, the nonproteolytic strains were found to have low heat resistance, whereas the proteolytic strains, like those of types A and B, were found to have high heat resistance (Lynt et al., 1977, 1979, 1981). Delayed germination of heat-damage spores required an incubation time of 10 wk in cultures for surviving spores in type E experiments and 6 months in all type F experiments. Obviously, data on the heat resistance of food spoilage bacteria, including *C. botulinum*, would be more readily obtainable if incubation times could be shortened. When solid media have been used for recovery, incubation times generally were much shorter (Odlaug and Pflug, 1977; Odlaug et al., 1978; Roberts et al., 1965; Stumbo et al., 1950). After exposure of *C. perfringens* spores to heat, recovery of the stress-damaged spores peaked in a few days. Although recovery declined when the spores were incubated in an agar medium, some spores were recovered throughout the 90-day incubation period in a broth medium (Futter and Richardson, 1972).

This study evaluated the effect of delayed germination of estimates of heat resistance by re-examining the data from previous experiments and using survivor data at various times during incubation of broth cultures.

MATERIALS & METHODS

Strains and spore stocks

The Alaksa, Beluga and G21-5 strains of type E *C. botulinum* and the nonproteolytic 190, 202 and 610 as well as the proteolytic 4YRC, PC and Langeland strains of type F were studied. Spore stocks were grown in 500-ml Wheaton bottles containing 350 ml of typticase-peptone-glucose-yeast extract (TPGY) broth. When the cultures were fully sporulated, the spores were harvested, washed three times in distilled water and concentrated by centrifugation. The spores were finally resuspended in a small amount of distilled water for seeding 150 ml of buffer or 454g of crabmeat to give a final count of 10^4–10^6 spores per ml or g. Each experiment was planned to cover a range of time intervals so that a gradation occurred between the last time surviving spores were observed in all tubes to the time beyond which no spores survived in any tube.

Preparation of buffer

Buffer was prepared from 0.067M solutions of Na_2HPO_4 and KH_2PO_4, which were combined with constant stirring and monitoring until a pH of 7.0 was reached. The buffer was dispensed in 150-ml quantities and sterilized by autoclaving at 121°C for 15 min. Buffer was refrigerated until used, at which time 1.0 ml of the spore stock was added to each 150 ml needed for the experiment. After being sealed and stored in an ice bath (or under refrigeration for proteolytic strains) for the duration of each experiment, at least three representative tubes of the seeded buffer were used to determine the actual spore count in that experiment. Spores were counted by the 3-tube most probable number (MPN) method, with TPGY as the culture medium. Vegetative cells were not considered to be present since the method of preparation of the spore stock makes this highly unlikely.

Preparation of crabmeat

Crabmeat was purchased in 1-lb (454g) cartons as needed from a local dealer as "fresh-picked crabmeat" and sterilized in 454-g portions by autoclaving 40 min at 121°C in flat, shallow pans. After being autoclaved, the crabmeat was refrigerated overnight; the following morning it was aseptically transferred to a sterile Waring Blendor in which the spore stock was uniformly blended into the meat to form a smooth paste. After being tubed and stored in an ice bath for the duration of the experiment, at least three representative tubes, each containing 1g of crabmeat, were used to determine the spore count by the 3-tube MPN method.

Experimental procedure

The thermal death time (TDT) experiments were conducted by the TDT tube technique at 160, 165, 170, 175, 180 and 185°F for the type E and the nonproteolytic type F strains and at 210, 215, 220, 225 and 230°F for the proteolytic type F strains. Four replicate experiments were performed for each set of conditions. The results were cumulated to give approximately 40 tubes per time interval for each temperature with each strain for calculations. For each experiment, 100 replicate 10 x 75 mm heat-resistant glass TDT tubes were filled with either 1.1 ml of seeded buffer from a Cornwall pipet, or 1 g of the seeded crabmeat from a specially adapted grease gun (Stumbo et al., 1945). The tubes were immediately flame-sealed and stored in an ice bath or refrigerated until needed. They were bundled into groups of 3 or 4, attached to a sinker to ensure complete immersion in the bath, and labeled with an identifying tag. The 100 tubes were dropped, in groups of 10, at each time interval into a 50-gallon (378.6-liter) water or silicone bath controlled at the desired temperature. Precision of the bath temperature was indicated by a straight line recording produced by the monitoring copper-constantan thermocouples on a potentiometer-recorder chart. Thermocouples used for this purpose had been previously checked against a calibrated thermometer. Accuracy of the thermocouples was checked periodically. All tubes were removed simultaneously

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at the end of the experiment and returned immediately to the ice bath or chilled in running tap water.

Tubes of the nonproteolytic strains were held in the ice bath until they were opened for culturing. Proteolytic type F strains were held at room temperature. Either 1 ml of seeded buffer or the entire contents of seeded crabmeat from each tube was cultured in a separate tube of TPGY broth, which had been boiled 10–15 min to exhaust dissolved oxygen just before inoculation. Each culture contained a Durham tube. All type E and nonproteolytic type F cultures were incubated at 26°C for 2 wk (or less if they showed growth). Proteolytic type F cultures were incubated at room temperature only. All tubes without growth in 2 wk were sealed with a layer of vaspar to maintain anaerobiosis and further incubated at room temperature for at least 6 months. TPGY broth is an anaerobic medium with enough thioglycolate to maintain anaerobiosis for at least 2 wk but not for extended incubation. If no growth was observed in 6 months, the tubes were considered to be sterile. Cultures from tubes having the longest heat exposure with growth in all 10 and those with growth at longer exposure times were tested for botulinum toxin by intraperitoneal injection of 0.5 ml of undiluted culture fluid into mice. Only toxic cultures were counted. The possibility of contamination from the handling involved in filling, sealing, opening and culturing the TDT tubes required proof that the culture was from spores surviving the heat treatment. Nontoxic cultures were rarely found.

Calculations

Correction of the time of exposure for thermal lag and lethality during lag was made by the graphic method (Anellis et al., 1954). The heat penetration curve for each temperature of the bath was plotted on inverted 3-cycle semilogarithmic paper as the average number of °F below bath temperature for at least five determinations. Temperatures were charted at 11-sec intervals by a potentiometer-recorder from a copper-constantan thermocouple located as close as possible to the geometric center of 1.1 ml of buffer or 1g of crabmeat in a TDT tube. The time to reach bath temperature, thus determined, and corresponding temperatures from the penetration curve and the uncorrected TDT curve were the basis for the corrections. Decimal reduction times (D) were calculated from the corrected data by Schmidt's probability method (1957). This method uses a modification of the general formula, D = U/log A-log B, in which D = LD_{50} (in min)/log A + 0.16, where log A is the log of the initial count per tube and log B, the log of the number of survivors per tube at LD_{50} (0.69), is −0.16. The Karber method of calculating the LD_{50} from the number of positive and negative cultures at each time interval was used. Because of skips and tailing in the data, use of the LD_{50} seemed more appropriate than methods using absolute end points. D values were calculated for each temperature for each strain.

The data obtained from these experiments were subjected to statistical analysis. The standard error of each 10-wk LD_{50} for type E and each 26-wk LD_{50} for type F was determined and the upper and lower 95% confidence limits were calculated. The mean logarithm of all initial spore counts of each strain was used to calculate D values at all temperatures for that strain. The D values thus calculated from the cumulated data of replicate experiments at each temperature were plotted and the heat-resistance curve was drawn as a straight line by visual best fit on semilogarithmic paper, with time on the logarithmic (vertical) axis and temperature along the arithmetic (horizontal) axis on the assumption that the destruction of spores by heat is logarithmic. For each of these curves the temperature increment in F° for one log cycle (z) was determined.

Delayed germination

The date when growth first appeared in each positive culture was recorded. Positive cultures were tabulated by week. Any culture requiring more than 1 wk to show evidence of growth was counted as delayed. D values were calculated at 1 wk, 2 wk and 4 wk in addition to the final incubation time for all strains and at 13 and 20 wk for the type F strains.

RESULTS & DISCUSSION

NORMAL SPORES of these 9 strains of *C. botulinum* types E and F grow out very quickly in TPGY broth. Spore counts of the stock suspensions made by the MPN method in this medium did not change after prolonged incubation. Therefore, for purposes of this study, any culture requiring more than 1 wk to grow out was considered delayed.

Tables 1, 2 and 3 show the percent of positive cultures exhibiting delayed germination in heat-resistance experiments with these organisms. Type E strains Alaska, Beluga and G21-5 showed the lowest percentages of delayed germination. The nonproteolytic strains of type F, 190, 202 and 610 showed much higher rates of delayed germination, but with neither the type E strains nor these did the extent of delay correlate with the temperature of exposure. Among the nonproteolytic type F strains delayed germination was high with strains 610 and 190, which were also the most and least heat-resistant strains, respectively, at these temperatures. Delayed germination was most extensive in experiments with the 3 proteolytic strains of type F, PC, Langeland and 4YRC, and among these strains it correlated with their heat resistance. But, like the nonproteolytic strains, they showed less delayed germination in crabmeat than in buffer.

Regardless of the percentage of cultures showing delayed germination, these cultures cannot be ignored in measuring

Table 1 — Percent of positive cultures for surviving spores showing delayed germination in heat -resistance experiments on 3 strains of Clostridum botulinum type E in crabmeat

Temp (°F)	Strain		
	Alaska (%)	Beluga (%)	G21-5 (%)
165	11	10	0
170	6	5	7
175	14	5	10
180	16	25	8
185		18	
Overall percentage at all temps	12	12	5

Table 2 — Percent of positive cultures showing delayed germination in heat-resistance experiments on 3 nonproteolytic strains of Clostridium botulinum type F

Temp (°F)	Strain			
	190	610	202	
	in buffer (%)	in buffer (%)	in buffer (%)	in crabmeat (%)
160	34		29	
165	77		25	
170	49	50	51	23
175	53	45	37	23
180	60	96	31	39
185		37		25
Overal percentage at all temps	55	58	33	26

Table 3 — Percent of positive cultures for surviving spores showing delayed germination in heat-resistance experiments on 3 proteolytic strains of Clostridium botulinum type F

Temp (°F)	Strain			
	PC	Langeland	4YRC	
	in buffer (%)	in buffer (%)	in buffer (%)	in crabmeat (%)
210	94	38	46	
215	97	94	71	72
220	95	66	62	74
225	73	50	47	55
230	80	68	70	53
Overal percentage at all temps	88	56	62	53

Table 4 — Effect of delayed germination on D and z values of Clostridium botulinum type E in crabmeat, calculated from survival data at intervals during incubation

Temp (°F)	D value (min) Alaska strain 1[a]	2	4	10
165	8.10	8.69	9.96	10.05 (± 2.15)[b]
170	2.66	2.66	2.82	2.82 (± 0.70)
175	1.33	1.47	1.47	1.49 (± 0.44)
180	0.55	0.65	0.71	0.71 (± 0.19)
185				
z value (F°)	10.5		11.0	11.0
	Beluga strain 1	2	4	10
165	10.77	11.22	11.22	11.22 (± 3.46)
170	2.80	2.88	2.91	2.94 (± 2.14)
175	1.18	1.19	1.19	1.19 (± 0.75)
180	0.37	0.53	0.84	0.84 (± 0.29)
185	0.15	0.15	0.33	0.33 (± 0.13)
z value (F°)	10.3		11.7	11.7
	G21-5 strain 1	2	4	10
165	6.15	6.15	6.15	6.15 (± 1.06)
170	1.53	1.62	1.70	1.70 (± 0.72)
175	0.66	0.70	0.71	1.09 (± 0.52)
180	0.45	0.49	0.49	0.51 (± 0.30)
z value (F°)	11.0		11.3	12.8

[a] Weeks of incubation.
[b] 95% confidence limits in parentheses.

Table 5 — Effect of delayed germination on D and z values of nonproteolytic strains of Clostridium botulinum type F in phosphate buffer, calculated from survival data at intervals during incubation

Temp (°F)	D value (min) Strain 190 1[a]	2	4	13	20	26
160	26.13	29.87	29.93	30.43	30.88	31.88 (± 16.60)[b]
165	6.66	7.29	7.51	8.49	8.80	9.07 (± 3.50)
170	0.59	1.00	1.17	1.53	1.60	1.66 (± 1.22)
175	0.27	0.43	0.44	0.83	0.91	1.03 (± 0.55)
180	0.13	0.14	0.16	0.22	0.24	0.25 (± 0.20)
185						
z value (F°)	7.5		8.0	9.3	9.5	9.7
Temp (°F)	Strain 202 1	2	4	13	20	26
160	36.87	39.60	39.74	41.84	42.41	42.41 (± 17.40)
165	9.72	11.49	11.78	12.21	12.68	12.68 (± 6.87)
170	2.63	2.90	3.12	3.94	4.29	4.29 (± 2.32)
175	0.60	0.70	0.77	0.89	0.91	0.93 (± 0.44)
180	0.20	0.23	0.23	0.31	0.33	0.33 (± 0.25)
185						
z value (F°)	8.7		8.8	9.4	9.5	9.5
Temp (°F)	Strain 610 1	2	4	13	20	26
160						
165						
170	3.62	4.75	5.03	6.06	6.47	6.64 (± 2.68)
175	1.28	1.53	1.63	2.05	2.10	2.12 (± 1.89)
180	0.33	0.46	0.57	0.78	0.78	0.84 (± 0.68)
185	0.17	0.25	0.26	0.32	0.36	0.37 (± 0.38)
z value (F°)	10.3		11.1	11.2	11.3	11.3

[a] Weeks of incubation.
[b] 95% confidence limits in parentheses.

heat resistance. The influence of delayed germination on the calculation of D values is shown in the remainder of the tables. Type E was studied in crabmeat only (Table 4). With the lowest percentages of delayed germination, type E nevertheless showed a general rise in D values during the incubation period until all surviving spores were accounted for. In some instances there was no increase, but in others, D values almost doubled during the incubation period because of delayed germination. The z values for type E remained virtually unchanged throughout the incubation period.

Among the nonproteolytic type F strains, D values reflected the greater percentage of cultures showing delayed germination. In the nonproteolytic type F strains in 0.067M phosphate buffer (Table 5), delayed germination accounted for up to 2-, 3- and 4-fold increases in D values over the 6-month incubation. Crabmeat seemed to offer protection to the spores of strain 202, giving higher D values than in phosphate buffer (Table 6), but the overall effect of delayed germination appeared to be the same. The greatest effect, however, at 180°F in crabmeat and 170°F in phosphate buffer, corresponded to the temperatures with the highest rate of delayed germination in each substrate. The z values for type F showed a slight increase.

Proteolytic strains of type F (Tables 7 and 8), showing the highest percentages of delayed germination, also showed 2-, 3- and 4-fold increases in D values over the 6-month incubation, but crabmeat afforded them no protection. The D values were approximately the same as in phosphate buffer. However, the z values of the corresponding heat-resistance curves increased over the 6-month incubation period in both phosphate buffer and crabmeat. The z value of strain 62A in phosphate buffer, based on a 3- to 6-wk incubation period in an agar recovery medium, was reported as 16.3 (Stumbo et al., 1950), which, although somewhat lower, is still very close to our results for the PC and Langeland strains at 4 wk in a broth recovery medium.

A least squares plot of the heat-resistance curves, which sometimes gives a better logarithmic fit to the D values, was tried for comparison of z values with those determined by visual best fit. The differences were small and not statistically significant, but for nonproteolytic stains were affected in the same manner as those determined by visual best fit. For the proteolytic strains, z values calculated by least squares reached the peak earlier, but extrapolated D_{250} values were approximately the same as those determined by visual best fit.

Occasionally, germination of a culture of either proteolytic or nonproteolytic type F was delayed until the last week of the 6-month incubation period; thus, further incubation might have yielded additional positive cultures. At the same time, however, with little or no difference between 20- and 26-wk D values, longer incubation for greater precision in estimates of D values seemed unwarranted. On the other hand, D values calculated at the end of a few days or

Table 6 — Effect of delayed germination on D and z values of Clostridium botulinum nonproteolytic type F strain 202 in crabmeat, calculated from survival data at intervals during incubation

Temp (°F)	D value (min) 1[a]	2	4	13	20	26
170	7.59	8.52	8.77	9.28	9.32	9.50 (± 4.64)[b]
175	2.85	3.14	3.33	3.61	3.63	3.64 (± 1.85)
180	0.68	1.03	1.17	1.18	1.18	1.20 (± 0.73)
185	0.41	0.47	0.49	0.53	0.53	0.53 (± 0.25)
z value (F°)	10.6		11.0	11.0	11.5	11.5

[a] Weeks of incubation.
[b] 95% confidence limits in parentheses.

1 wk may be underestimated by ½ to ¾ of that based on a 6-month incubation period. Translated into processing time, such an underestimate could have the effect of reducing a calculated 12D process to an actual 3D process, eroding the margin of safety and giving a false sense of security.

There is no indication that the usual heat processes for low-acid canned foods are not adequate to destroy proteolytic type F strains; on the contrary, if the curves based on 6 months of incubation are extrapolated, the D_{250} would be from 0.14–0.23, or about the same as the proteolytic types A and B, which is the basis for safe processes. However, similar extrapolation at 1 wk gives an entirely different picture. With no delayed germination the D_{250} would be only 0.02–0.05 min, very low values in terms of heat processing. One might therefore conclude that type F is no problem, when actually it poses the same problem to heat-processing safety as types A and B (proteolytic). Elsewhere, heat-resistance studies with types A and B showed z values in food substrates to be <18, the z value by which F_0 is defined, and that such deviations may result in an underesimate of F_0 (Perkins et al., 1975). This also appears to be true if delayed germination is not accounted for by adequate incubation times.

The nonproteolytic strains would be readily destroyed by safe heat processes for low-acid canned foods, whether delayed germination is taken into account or not; nevertheless, where a low heat process or "pasteurization" is used, it is just as important that it be adequate to destroy spores of the nonproteolytic strains as for canning processes to destroy spores of the proteolytic strains. An underestimate of heat resistance could be equally disastrous. Unless adequate consideration is given to delayed germination of heat-damaged spores, the heat resistance of *C. botulinum* may be underestimated by both the D and z values.

Table 7 — Effect of delayed germination on D and z values of proteolytic strains of Clostridium botulinum type F in phosphate buffer, calculated from survival data at intervals during incubation

Temp	D value (min)					
	PC strain					
(°F)	1[a]	2	4	13	20	26
210	13.15	16.40	18.05	19.76	22.68	23.22 (± 5.23)[b]
215	5.20	8.45	9.70	11.78	11.96	12.02 (± 5.74)
220	2.72	3.45	4.11	5.44	6.24	6.33 (± 2.86)
225	0.76	1.69	2.59	3.24	3.33	3.33 (± 2.27)
230	0.93	1.10	1.30	1.76	1.82	1.82 (± 1.28)
z value (F°)	14.6		17.2	18.0	18.1	18.1
Temp	Langeland strain					
(°F)	1	2	4	13	20	26
210	12.46	16.12	17.50	19.18	19.28	19.38 (± 5.23)
215	5.18	5.95	7.48	8.73	8.76	8.76 (± 2.39)
220	2.37	3.04	3.80	4.64	4.72	4.74 (± 2.12)
225	1.33	1.66	2.17	2.60	2.60	2.60 (± 0.90)
230	0.72	1.35	1.59	1.78	1.79	1.79 (± 1.16)
z value (F°)	14.2		17.0	19.0	19.1	19.1
Temp	4 YRC strain					
(°F)	1	2	4	13	20	26
210	6.89	8.25	9.70	11.84	12.07	12.19 (± 4.16)
215	3.35	4.06	4.79	5.30	5.35	5.35 (± 2.42)
220	1.16	1.66	2.37	3.35	3.46	3.55 (± 2.04)
225	0.93	1.48	1.73	2.07	2.07	2.09 (± 1.00)
230	0.54	0.88	1.19	1.45	1.45	1.45 (± 0.70)
z value (F°)	17.5		21.8	23.2	23.7	25.3

[a] Weeks of incubation.
[b] 95% confidence limits in parentheses.

Table 8 — Effect of delayed germination on D and z values of proteolytic Clostridium botulinum type F strain 4YRC in crabmeat, calculated from survival data at intervals during incubation

Temp	D value (min)					
(°F)	1[a]	2	4	13	20	26
215	3.30	4.50	4.93	5.05	5.07	5.07 (± 2.80)[b]
220	2.19	3.36	3.65	4.02	4.02	4.02 (± 2.41)
225	1.32	1.71	2.06	2.14	2.14	2.14 (± 1.08)
230	0.42	0.99	1.21	1.35	1.35	1.35 (± 1.06)
z value (F°)	14.5		20.5	23.0	23.0	23.0

[a] Weeks of incubation.
[b] 95% confidence limits in parentheses.

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Application of Two-Level Fractional Factorial Designs in Development of a Soybean Whipped Topping

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ABSTRACT

The feasibility of developing a whipped topping formula based on Illinois Soybean Beverage was investigated. Two-level fractional factorial designs were shown to be efficient for statistical analysis of a large number of ingredients. Foam stability was the major consideration in selecting ingredients for a formula. Rapid cooling of the homogenized mix was the processing factor controlling overrun while stability depended on composition of the mix. A hydrophilic-hydrophobic emulsifier blend was most effective. The model system thus derived contained 3% soybean solids, 30% fat, 7% sucrose, 1% emulsifier, and 59% water; it gave 270% overrun, complete stability over 2 hr and stiffness similar to commercial products.

INTRODUCTION

WHIPPED TOPPING is one of the most successful fabricated nondairy products and imitates cream whip. It is usually based on sodium caseinate, vegetable fat, carbohydrates and emulsifiers (Glicksman, 1975). Additives such as stabilizers, corn syrup solids and salts are also widely incorporated into various formulas (Knightly, 1968). Since 1979, the price of sodium caseinate has increased sharply with a possible supply shortage due to inflation and the international dairy market. Investigation has been intensified to find caseinate substitutes that provide the needed functionality at a reasonable price.

Nelson et al. (1975, 1977) utilized the entire soybean cotyledon to produce a beverage. The product had a bland taste, excellent suspension stability and smooth mouthfeel. Their aqueous soybean emulsion was named Illinois Soybean Beverage (ISB). Starting from the ISB, numerous dairy analogs were formulated (Wei et al., 1975), including flavored soy beverage, yogurt, ice cream and diet margarine. They concluded the ISB had great potential as a basis for other dairy analogs. Research on these products is a complex process due to the large number of variables involved.

Factorial designs are useful in handling complicated food formulation problems (Thalheimer and Rusch, 1970). Compared to the one-variable-at-a-time method, these designs require fewer tests per variable studied and allow a number of independent variables to be changed simultaneously in a systematic fashion. In the early stages of experimental studies, two levels of each variable are most often used. With a few very simple calculations (no computer needed), the results can indicate major trends among the variables and so determine a promising direction for further experimentation.

The number of runs required by a full factorial design increases geometrically with the number of variables. It is generally the case that for more than five variables, the full factorial need not be performed. This is so since higher order interactions (three-factor, four-factor, etc.) are likely to be of negligible magnitude and therefore need not be estimated (Box and Hunter, 1961). Further, as the number of variables increases, the likelihood of all variables being important decreases, i.e., only a few variables are truly significant. These conditions contribute to redundancy in full factorial designs, i.e., many of the main effects and interactions which can be estimated are found to be insignificant. Fractional factorial designs exploit this redundancy and enable the experimenter to consider a large number of variables with a small fraction of the full factorial requirements for tests. Fractional factorial designs are of great value at the early stage of an investigation, when it is frequently good practice to use a preliminary experimental effort to look at a large number of variables superficially rather than a small number thoroughly (Box et al., 1978). However, in such a two-level design, the variables to be tested and their high and low levels should be carefully chosen so that important effects will not be overlooked.

The objective of this research was to investigate the feasibility of using ISB as the sole protein source in a whipped topping formulation. The project was divided into three stages. In the first stage, two-level fractional factorial designs were applied to study a large number of the ingredients commonly used in sodium caseinate whipped topping to obtain a preliminary formula. Processing conditions were evaluated with this formula in the second stage. Lastly, a model system with the characteristics of a whipped topping and the least number of essential ingredients (Pour-El, 1976) was developed and compared with commercial products.

MATERIALS & METHODS

Ingredients

ISB was prepared from Williams variety soybeans according to Luttrell et al. (1981). The base was frozen at −23°C and thawed at room temperature overnight before use. To improve the freeze-thaw stability, 6.8% sucrose was added to the ISB during the heating stage before homogenization (Yeh et al., 1982). The ISB contained 9.3% soybean solids including 4.7% protein and 2.3% lipid. The remaining 2.3% was assumed to be cell wall material.

Based on a literature search (Knightly, 1968; Kolar et al., 1979; Anonymous, 1977a, b; Andreasen, 1973), the following ingredients were chosen for evaluation as ingredients with ISB:

Vegetable Fat. Hydrol 92 (partially hydrogenated coconut oil, Durkee Industrial Food Group, SCM Co.).

Emulsifiers. Dur-Em 114 (Mono- and diglycerides, Durkee Industrial Food Group, SCM Co.); Span 60 (sorbitan monostearate, ICI Americas Inc.); Tween 60 (polyoxyethylene (20) sorbitan monostearate, ICI Americas Inc.); Lactoden F-15 (lactylated monoglyceride, Grinsted Chemical Co.).

Carbohydrates. Sucrose; Star-Dri 42R (Corn Syrup Solids, 42 D.E., A.E. Staley Manf. Co.).

Stabilizers. Methocel F 50 (hydroxypropyl methyl cellulose, The Dow Chemical Co.); Kelgin MV (sodium alginate, Kelco Division of Merck and Co., Inc.); Avicel RC-581 (Microcrystalline cellulose, FMC Co.).

Salt. Sodium chloride.

Preparation of whipped topping

Each ingredient in the test formula was added to either the aqueous or lipid phase, according to its solubility or recommenda-

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tion of its supplier. The two phases were heated separately and merged at 87.8°C. The liquid mixture was homogenized, cooled, and stored at 1.1°C overnight before whipping.

Five hundred ml of the liquid mixture was whipped on a 3-speed Hobart Kitchen Aid mixer at the highest speed in a constant temperature room at 23.9°C. The mixing bowl and wire whip attachment were prechilled on ice before use. Whipping was completed in 3 min.

Evaluation of whipped topping

General evaluation (Sanders, 1979) of the whipped topping can yield a considerable amount of information. The foam was evaluated at the first stage for the following characteristics: homogeneity of air cell distribution, stability, stiffness and stand-up property. Organoleptically, the whipped topping was tasted for its richness, sweetness, gumminess and greasy after-taste.

Overrun (Min and Thomas, 1977) was determined by the following formula:

$$\% \text{ overrun} = [(W_1 - W_2)/W_2] \times 100$$

where W_1 = weight of a given volume of liquid and W_2 = weight of the same volume after whipping.

Stability against syneresis (Griffin et al., 1970) was measured as the drainage volume (ml) collected from 100g of whipped topping after 2 hr standing at 23.9°C. The sample was placed in a 5-inch, long stem funnel with perforated porcelain disk at the apex. The drips were collected in a 100 ml graduate cylinder.

The soybean model system and commercial products were further compared for stiffness and mouthfeel. Stiffness was measured with a Brookfield LVF Synchro-Lectric Viscometer (Brookfield Engineering Laboratories, Stoughton, MA), which was mounted on a Helipath stand (Sanders, 1979). The T-E unit spindle rotating at 60 rpm was descended slowly through the foam; stiffness was reported in Brookfield Units (B.U.).

A multiple comparisons test (Larm[illegible], 1977) was performed to evaluate mouthfeel of the whipped toppings within 30 min after preparation. A commercial, frozen pre-whipped topping, labeled R, was presented with the samples as reference. Four samples including another reference were coded with 3-digit random numbers for the panel. Twenty-three untrained panelists were asked to compare the mouthfeel of each coded sample with R and to give information about the direction and magnitude of the preference. Seven panelists did not give the same rating to R and coded R so their evaluations were discarded. In case of the remaining 16 panelists, numerical scores from a hedonic scale of 1 through 9 were assigned to their rating with "no difference" given 5, "extremely better than R" given 9 and "extremely worse than R" given one. An analysis of variance was conducted on these scores.

Experimental procedure and data analysis

To demonstrate the feasibility of formulating a soybean whipped topping, three sets of 2^{7-4} fractional factorial designs were applied in the preliminary study to examine a wide variety of ingredients (Box et al., 1978). Table 1 gives the ingredient variables, their levels of use, the structure of the experimental designs and the results of the two measurable responses: overrun and stability.

Two concentration levels were used to evaluate each ingredient variable. The high level for each ingredient was selected according to literature values for sodium caseinate whipped topping (Andreasen, 1973; Anonymous, 1977b; Kolar et al., 1979, Knightly, 1968). In case of the basic components including soybean solids, vegetable fat and emulsifier, the low levels were assigned at 50% or less than the high levels. Other ingredients, including stabilizers, corn syrup solids and sodium chloride, were tested on the present-absent basis for their essentially to the formula. Therefore, their low levels were assigned as zero. Kelgin MV was combined with Methocel F 50 as one variable and served as a secondary stabilizer. Sucrose was added to each sample at a fixed level (7%) for its contribution to sweetness. Each formula was balanced to 100% with tap water. The merged phases were homogenized at a pressure of 3500 psi/500 psi, bottled, and cooled in an ice water bath.

The basic assumption of the experimental designs employed herein is that the interactions higher than two factors can be considered as insignificant (Box and Hunter, 1961). The first set of experiments was designed to estimate the average main effects of ingredient variables. Since Set I was a 2^{7-4} resolution III fractional factorial, these main effects are confounded with groups of three 2-factor interactions. In order to separate the average main effects from their confounding interactions, a second 2^{7-4} resolution III fractional factorial design was performed (set II in Table 1) and its results are added to Set I to create a 2^{7-3} resolution IV design. For this combined design, average main effects are now confounded with 3-factor interactions which are assumed to be negligible. Hence, all average main effect estimates are obtained. Using the same general principle, a third 2^{7-4} fractional factorial design was constructed (set III in Table 1) such that when combined with Set I, it separates the 2-factor interactions involving soybean solids from the average main effects and other 2-factor interactions. This was done since the major interest in this research was soybean solids. The sequence of the experiments was randomized within each set.

From the experimental design and results, the average main effects and 2-factor interactions for each response were calculated. Table 2 shows the linear combinations of effects (excluding higher order effects) which can be estimated from each of the three 2^{7-4} fractional factorial designs. The estimates of effects for both overrun and stability are given in Table 2. When the results of Set I and Set II are combined, "clear" estimates of the average main effects of all variables are obtained. When Set I and Set III are combined, "clear" estimates of the average main effect of variable I (soybean solids) and all its 2-factor interactions are obtained. If Set II and Set III are combined, some groups of two 2-factor interactions are also separated out.

When experiments are not replicated and hence no direct estimate of experimental error is available, the estimated effects can be plotted on normal probability paper to investigate their statistical significance. If the level changes of the variables have no real effect

Table 1—Preliminary study: variable levels of ingredients with experimental results from three 2^{7-4} fractional factorial designs

Ingredient	Variable number	Level, wt%	
		+	−
Soybean solids (ISB)	1	5	1.67
Hydrol 92	2	20	10
Star-Dri 42R	3	5	0
Dur-Em 114	4	0.5	0.17
Methocel F 50/Kelgin MV	5	0.5/0.1	0/0
Avicel RC-581	6	0.25	0
Sodium chloride	7	0.1	0
Sucrose (kept constant at 7%)			
Water (balance to 100%)			

Set-run	Variable							Responses	
	1	2	3	4	5	6	7	Overrun %	Stability ml
I-1	−	−	−	+	+	+	−	62	10
2	+	−	−	−	−	+	+	15	96
3	−	+	−	−	+	−	+	50	4
4	+	+	−	+	−	−	−	51	2
5	−	−	+	+	−	−	+	116	41
6	+	−	+	−	+	−	−	39	52
7	−	+	+	−	−	+	−	22	19
8	+	+	+	+	+	+	+	13	1
II-1	+	+	+	−	−	−	+	89	45
2	−	+	+	+	+	−	−	81	2
3	+	−	+	+	−	+	−	89	1
4	−	−	+	−	+	+	+	173	35
5	+	+	−	−	+	+	−	86	0
6	−	+	−	+	−	+	+	58	4
7	+	−	−	+	+	−	+	93	0
8	−	−	−	−	−	−	−	241	85
III-1	+	−	−	+	+	+	−	105	0
2	−	−	−	−	−	+	+	200	93
3	+	+	−	−	+	−	+	173	0
4	−	+	−	+	−	−	−	158	2
5	+	−	+	+	−	−	+	101	23
6	−	−	+	−	+	−	−	176	65
7	+	+	+	−	−	+	−	57	19
8	−	+	+	+	+	+	+	71	0

on the product performance, their estimated effects arise solely due to chance causes and hence should be normally distributed with a mean of zero. On normal probability paper, such effects would fall on a straight line centered about zero and be used to estimate constant variance α^2. Those effects, which failed to fall on the straight line, indicate the presence of significant effects on the product performance, i.e., they arise from a normal distribution with nonzero mean. This approach was used to evaluate the estimated effects for both overrun and stability.

When significant interaction effects are present, it is difficult to interpret their influence on the response by simply examining the magnitude and sign of the estimate. To more clearly interpret this interaction effects, the experimental designs were collapsed (averaged) into the two dimensions of various two-factor interactions. In this way, the manner in which the change levels of one variable influence the effect of the other variable can be clearly seen (Montgomery, 1976). A preliminary soybean whipped topping formula was derived from the results of the factorial analysis and general evaluation of each product.

Two important processing conditions, homogenization pressure and cooling method, were studied with the preliminary formula. One-variable-at-a-time method was used. Two homogenization pressures, 3500 psi/500 psi and 2000 psi/500 psi, were tested and the pressure which gave better product performance was used to study cooling method. Three cooling methods were available: (1) the sample was bottled after homogenization and then cooled in an ice water bath; (2) the sample was discharged from the homogenizer onto the wall of a bucket which was chilled on ice; and (3) the sample was passed directly from the homogenizer through a plate heat exchanger with ice water as cooling medium.

Composition of the preliminary formula was further examined so that only the least number of essential ingredients would be selected for the model system. Two other emulsifier systems, Lactodan F-15 and Span 60/Tween 60 (60:40) were also tested individually and compared with Dur-Em 114 at the same concentration.

RESULTS & DISCUSSION

NORMAL PLOTS of the estimated effects from the preliminary study (Tables 1 and 2) for overrun and stability are given in Figs. 1 and 2, respectively. Also shown in Figs. 1 and 2 are graphs which indicate the magnitude and direction of those average main and interaction effects found to be significant.

Table 2–Estimated effects and their abbreviated confounding pattern from the experimental designs and results in Table 1

	Estimated effects		Confounding pattern
	Overrun	Stability	
Set I			
1_1 =	–33	19	1 + 24 + 35 + 67
1_2 =	–24	–43	2 + 14 + 36 + 57
1_3 =	3	0	3 + 15 + 26 + 47
1_4 =	29	–29	4 + 12 + 56 + 37
1_5 =	–10	–23	5 + 13 + 46 + 27
1_6 =	–36	7	6 + 23 + 45 + 17
1_7 =	5	15	7 + 34 + 25 + 16
Set II			
$1'_1$ =	–49	–20	1 – 24 – 35 – 67
$1'_2$ =	–71	–18	2 – 14 – 36 – 57
$1'_3$ =	–12	–2	3 – 15 – 26 – 47
$1'_4$ =	–67	–40	4 – 12 – 56 – 37
$1'_5$ =	–11	–25	5 – 13 – 46 – 27
$1'_6$ =	–25	–23	6 – 23 – 45 – 17
$1'_7$ =	–21	–1	7 – 34 – 25 – 16
Set III			
$1''_1$ =	–42	–30	1 – 24 – 35 – 67
$1''_2$ =	–31	–40	2 – 14 + 36 + 57
$1''_3$ =	–58	3	3 – 15 + 26 + 47
$1''_4$ =	–43	–38	4 – 12 + 56 + 37
$1''_5$ =	2	–18	5 – 13 + 46 + 27
$1''_6$ =	–44	6	6 + 23 + 45 – 17
$1''_7$ =	12	8	7 + 34 + 25 – 16
1_0 =	97	25	average

The analysis of overrun results (Fig. 1) indicated that variables 1 (soybean solids), 2 (Hydrol 92) and 6 (Avicel RC-581) had significant average main effects on the aeration; the interaction effects of 12 and 15 were also significant. Soybean solids was the most significant variable because it showed a significant average main effect (variable 1) and participated in two significant two-factor interactions (12 and 15). High overrun occurred at low soybean solids. Kolar et al. (1979) reported that, in case of most whipped topping formulations made with soy isolates, the soybean solids were used at a lower level than sodium caseinate because of the high viscosity and excellent emulsifying properties of soy protein. However, if a high level of soybean solids in the formula is desirable, the presence of stabilizers, Methocel F 50/Kelgin MV, would provide complementary functional properties and improve overrun (Fig. 1). There was a big interaction between soybean solids and vegetable fat. In other words, the main effect of vegetable fat depended on the level of soybean solids. For high soybean levels, the overrun value was not markedly affected by the level of Hydrol 92 and was generally low. Increasing the level of Hydrol 92 only slightly improved overrun. However, the effect of Hydrol 92 was very large at low soybean levels. A low level of Hydrol 92 gave very high overrun. When Hydrol 92 level was increased, overrun dropped off dramatically. No strong interaction between Hydrol 92 and Avicel RC-581 was seen; they both have similar effects on overrun, i.e., the higher their concentration, the lower the overrun.

From stability analysis (Fig. 2), variables 2 (Hydrol 92), 4 (Dur-Em 114) and 5 (Methocel F 50/Kelgin MV) showed significant average main effects; the interaction effect combination of 23 + 45 was also significant. Since variables 2, 4, and 5 showed significant average main effects on stability, it was assumed that the two-factor interactions involving these variables were likely to be the dominant one in the combination. From the summary of the significant effects, emulsifier appeared to be the critical variable. Stable whipped toppings could be formulated with a high level of Dur-Em 114 and high levels of at least one of the following: Hydrol 92 and Methocel F 50/Kelgin MV.

Samples were evenly distributed over the range of study in the factorial design. Therefore, general evaluation of the foam characteristics as well as the objective test provided valuable information in determining ingredient selection. Six formulas were selected because they gave stable and homogeneous foams with good stand-up property. Samples I-8, II-2 and III-8 produced overly stiff foam structures while samples I-3, II-6 and III-7 gave desirable stiffness. The formulas were compared side-by-side and some obvious trends were observed. Five of the six formulas selected contained a high level of fat; this showed that a high fat level was desirable. Richert (1979) reported that typical food foams were either quite high in fat or very low. Compositions between the two extremes were difficult to whip and the foams were usually unstable. The high level of fat was generally selected to imitate the richness of cream whip. As indicated from stability analysis in Fig. 2, these six stable samples did contain high levels of emulsifier. Whipped toppings from those formulas, containing both Star-Dri 42R and Methocel F 50/Kelgin MV, were very stiff and gummy. Products with desirable stiffness came from the formulas containing no corn syrup solids and only one of the stabilizer variables, Avicel RC-581 or Methocel F 50/Kelgin MV.

A preliminary formula was derived from the factorial study. Stability was the major concern in the selection of ingredients and level of use. High levels of emulsifier and

vegetable fat were adopted for this reason. High levels of Avicel RC-581 were selected to provide desirable stiffness. The concentration of soybean solids was slightly higher than the low level in Table 1 to improve the overrun. Table 3 gives the preliminary formula and product performance under various processing conditions.

Table 3 shows that, although the samples homogenized at 2000 psi/500 psi gave slightly better overrun and stability than those processed at 3500 psi/500 psi, the effect of homogenization was not significant. This was probably due to the fact that both 2000 psi and 3500 psi are considered high pressures (Knightly, 1968).

The cooling method comparison indicated that the effect of fast cooling was very significant in improving overrun. Rapid cooling of an oil-in-water emulsion to below the melting point of its lipid phase would fix the shape of the fat globules and minimize their coalescence. A certain degree of fat globule agglomeration was inevitable and desirable (Knightly, 1968). In the study of ice cream emulsions, Thomas (1981) reported that whippability and structural properties of the emulsions depended on the rate of fat clumping. If large fat clumps were present initially, or were formed too early, desirable overrun would be difficult to obtain. The same phenomenon would apply to whipped topping emulsion. Within the present range of study, stability of the products was largely dependent on the composition of the formula and was not significantly improved by the processing conditions. The stability improved slightly as the overrun improved markedly (Table 3). This was possibly due to the substantially increased volume. The experimental conditions for the preparation of liquid whipped topping mixture were therefore fixed as follows: the warm mixture of the two phases should be homogenized at the pressure of 2000 psi/500 psi and cooled rapidly with an ice-water circulated plate heat exchanger.

According to the manufacturer (Avicel Application Bulletin, No RC-29, FMC. Co., Philadelphia, PA), the functional property of Avicel RC-581 can be replaced by an increase in fat content. Therefore, the soybean whipped topping model system was derived by the elimination of the Avicel and increasing levels of vegetable fat from 20% to 30%. Soybean solids content was adjusted from 2% to 3% due to their interaction effect with vegetable fat for higher overrun (Fig. 1). Concentration of emulsifier was raised to 1% to assure sufficient emulsifier for good stability (Fig. 2).

Table 4 gives the model formula and product performance of various emulsifier systems. No drip was observed from any of the samples. This was probably due to the high dose of emulsifier. The Span 60/Tween 60 combination

Table 3—Effect of homogenization pressure and cooling method: preliminary formula, processing conditions and results of overrun and stability

Ingredient	wt %
Soybean solids	2
Hydrol 92	20
Sucrose	7
Dur-Em 114	0.5
Avicel RC-581	0.25
Water	70.25

Processing conditions		Responses	
Homo. pressure psi/psi	Cooling method	Overrun %	Stability ml
3500/500	Ice bath	85	4.0
2000/500	Ice bath	98	3.5
2000/500	Chilled bucket	200	3.0
2000/500	Plate heat exchanger	215	0.5

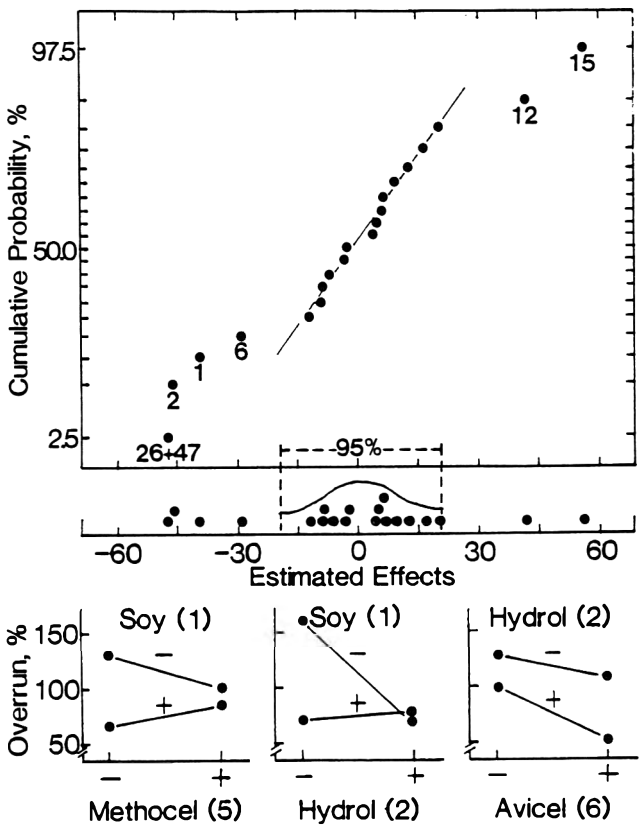

Fig. 1—Overrun: Normal plot of estimated effects from preliminary study (Tables 1 and 2) with summary of significant average main effects and two-factor interactions.

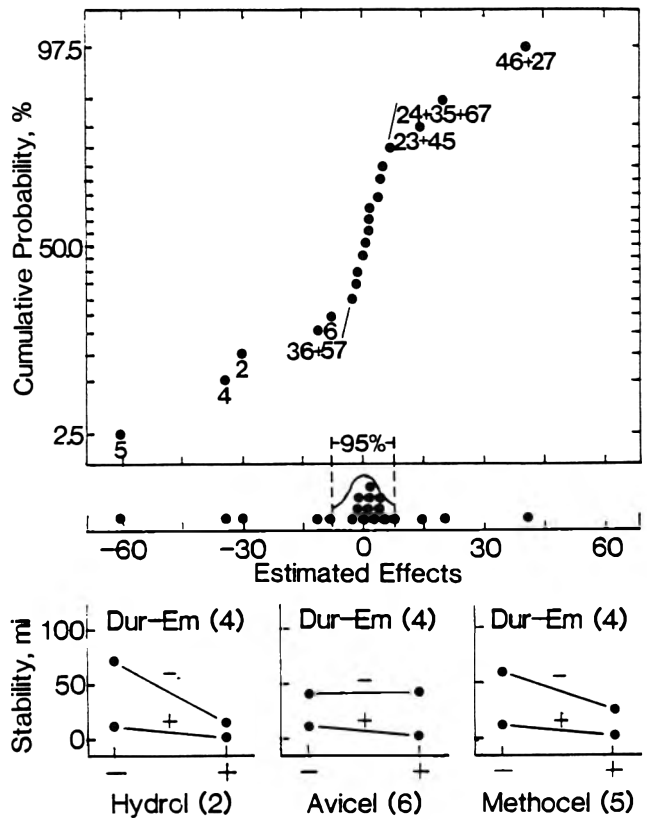

Fig. 2—Stability: Normal plot of estimated effects from preliminary study (Tables 1 and 2) with summary of significant average main effects and two-factor interactions.

gave significantly higher overrun and obvious better stand-up property than either Dur-Em 114 or Lactodan F-15. It is generally recognized (Rosen, 1978) that a mixture of emulsifier, commonly a hydrophobic-hydrophilic mixture, is better than an individual emulsifier. The results also agreed with Min and Thomas (1977) that a blend of water-soluble and fat-soluble emulsifiers was more effective than either type alone in obtaining better overrun and stiffness of a sodium caseinate whipped topping.

Andreasen (1973) reported that the development of nondairy whipped toppings has gone further than mere provision of substitutes for whipping cream. A number of these formulated products have gained their own market. The soybean whipped topping containing Span 60/Tween 60 was compared with three commercial products: Whipping cream and two nondairy whipped toppings. The results of their performance as shown in Table 5 indicated that the soybean product was very similar to these nondairy toppings and showed better overrun and stability than cream whip. Stability should be the major consideration in the formulation to meet a specific product requirement. Analysis of variance, (Table 6) of the multiple comparisons test indicated that there was no significant preference of mouthfeel among these samples. The soybean whipped topping was bland in taste and its texture was as acceptable as the commercial products.

Table 4—Effect of emulsifier: model formula, composition of emulsifier and results of overrun, stability and stiffness

Ingredient	wt %
Soybean solids	3
Hydrol 92	30
Sucrose	7
Emulsifier	1
Water	59

Emulsifer	Responses		
1% Wet Basis	Overrun (%)	Stability (ml)	Stiffness
Dur-Em 114	240	0	Good
Lactodan F-15	229	0	Fair
Span 60/Tween 60 (60:40)	274	0	Excellent

Table 5—Performance of whipped topping made from soybean model system containing Span 60/Tween 60 as compared with performance of whipping cream and two nondairy toppings

Product	Performance			
	Overrun (%)	Stability (ml)	Stiffness (Brookfield Units)	Mouthfeel mean score (9 is best)
Soybean Whipped Topping	274	0	93	4.6
Powdered Whipped Topping	254	0.5	>100	4.4
Frozen Pre-Whipped Topping	257	0	>100	5.0
Whipping Cream	183	14	93	4.8

Table 6—Analysis of variance of multiple comparisons sensory evaluation scores in Table 5

Source of variance	df	Sum of squares	Mean squares	F
Samples	3	3.42	1.14	0.40
Judges	15	74.11	4.94	1.71
Error	45	129.83	2.87	
Total	63	207.36		

CONCLUSION

A product development task should be divided into two stages: variable screening and optimization. This report covers the first stage. Two-level fractional factorial designs were shown to be efficient for systematic study of a large number of ingredient variables; normal plots clearly showed those with significant effects on overrun and foam stability. Foam stability was the major consideration in developing a preliminary formula. Rapid cooling of the homogenized whipped topping mixture was the processing factor controlling overrun. A soybean whipped topping model system was derived based on the general trends obtained for the statistical analysis and literature data. Three emulsifier types were evaluated with the model system for the comparison of their effectiveness; a blend of hydrophilic and hydrophobic emulsifiers gave the best overrun and stiffness. The soybean model system was compared with whipped cream and two commercial caseinate whipped toppings for their overrun, stability, stiffness and mouthfeel. It was concluded that Illinois Soybean Beverage can be used as the sole protein source to replace caseinate in a whipped topping. The second stage, a response surface analysis of the model system, will be given in a subsequent paper to demonstrate in detail the interrelationship among soybean solids and the other essential ingredients at optimal product performance.

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Effect of Heat Processing on Hemagglutinin Activity in Red Kidney Beans

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ABSTRACT

The effect of temperature in destroying the hemagglutinin (lectin) activity in red kidney beans has been determined. Heating presoaked beans at 100°C for 15 min or at 80°C for 2 hr, or pressure cooking (15 psi) for 45 min without presoaking, decreased the hemagglutinin activity to below detectable levels. At 65°C, no significant decrease was observed even after 12 hr heat treatment. Commercially canned beans have lectin levels similar to beans pressure cooked for 30 min.

INTRODUCTION

AMONG THE MANY antinutrients present in plant foods are a group of heat-labile proteins or glycoproteins known as lectins or hemagglutinins. They are found in many commonly eaten foods (Nachbar and Oppenheim, 1980) but are probably most concentrated in legumes. One of the richest sources is the red kidney beans (*Phaseolus vulgaris*).

Lectins are toxic when ingested in large quantities. Diets containing raw bean meal have been shown to be responsible for decreased growth and death in rats (Jaffe, 1980). In addition, cases of toxicity have been reported in humans who had eaten raw or partially cooked red kidney beans (Noah et al., 1980).

Most toxic or antinutritional effects of legumes can be partially or wholly eliminated by heating (Liener, 1962) and this has been clearly shown to be true of lectins in soybeans (Liener and Hill, 1953) and common beans (*P. vulgaris*) (Honavar et al., 1962) but the effect of temperature and the time required to eliminate hemagglutinin activity have not been systematically studied.

Conventional household cooking methods usually involve high temperatures, either in pressure cooking or in boiling or simmering. These methods could effectively destroy hemaglutinin activity in the legume in a short time (Stein, 1976). However, lower temperature methods of cooking such as now generated by slow cookers, are often employed in the preparation of beans for human consumption and the question has arisen as to whether this treatment is adequate to destroy the hemagglutinin activity of the lectins. A recent study of Bender (1981) in fact showed an increase in activity after heat treatment at 80°C for 45 min.

This study was undertaken to determine the conditions of time and temperature required to destroy the hemagglutinin activity in red kidney beans. We consider this important because of the growing number of reports of gastrointestinal symptoms after eating raw, soaked or partially cooked red kidney beans (Noah et al., 1980) and because of the popular move towards eating raw and less processed foods.

MATERIALS & METHODS

Sample preparation

Two brands of raw red kidney beans and five brands of canned red kidney beans were obtained from the local supermarket. Raw beans from all packages of the same brand were mixed well before allocation to the different treatment groups.

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Fifty gram portions of each brand of raw beans were treated as follows: (1) soaked in 190 ml water for 10½ hr at room temperature, drained, weighed and freeze-dried; (2) soaked in 190 ml water for 10½ hr, drained, redispersed in 330 ml water and heated at various temperatures (100°, 80° or 65°C) and times (5 min–12 hr); the beans were drained, weighed and freeze-dried; (3) similar to (2) except that the beans were not presoaked and were heated in 1750 ml water in a pressure cooker (15 psi, 121°C) for various lengths of time (5–60 min).

Prior to addition of the beans, water was preheated to the required temperature (either 65° or 80° using a water bath or 100°C using a hot plate) or was brought to boiling temperature in the case of pressure cooking. Water and beans returned to the desired temperature within 2–3 min after addition of the beans, and this was designated as zero time. The temperature of the beans, in all cases, differ only slightly (≤ 2°C) from that of the cooking water. All containers were covered to prevent loss of moisture during heat treatment.

Canned beans were drained well and then freeze dried before analysis.

Lectin analysis

Freeze-dried samples were ground in an analytical mill and analyzed for lectin activity according to the method described by Simpson et al. (1978). Samples were extracted with 0.9% sodium chloride solution using a Sorval omnimixer for 2 min. Starting with 25 μl of the extract, serial twofold dilutions were made with saline on Cooke microtiter V plates. Trypsinized rabbit red blood cell suspension (25 μl) prepared according to Lis and Sharon (1972), was then added. The plates were covered with polyethylene film to prevent moisture evaporation and shaken on a platform shaker for 10 min. After 2½ hr, agglutination patterns were observed with the aid of a microscope. Hemagglutinin activity was expressed as the reciprocal of the highest dilution giving positive agglutination. Concanavalin A was used as the standard in all determinations.

All raw and freeze-dried samples were analyzed for moisture content (AOAC, 1980) to allow calculation of the hemagglutinin activity on a moisture-free basis. The results of all hemagglutinin and moisture analyses are the means of two or more analyses per brand. Duplicate lectin analyses were in agreement with only a few cases where the difference was equivalent to one dilution.

RESULTS & DISCUSSION

HEMAGGLUTININ ACTIVITIES of raw kidney bean brands A and B were 140×10^4 and 270×10^4 HU/g sample, respectively. Fig. 1 shows the mean hemagglutinating activity of the two brands. Soaking the beans did not reduce the hemagglutinin activity. The apparent increase is equivalent to one serial dilution and is insignificant.

Presoaking followed by heating at 100°C for 15 min decreased the lectin activity to below detectable levels. However, heating at this temperature for 1 hr was necessary to cook the beans to the point where they could be considered edible i.e. could be flattened easily with a fork, a common household test to ensure that beans in general are properly cooked.

At 80°C, lectin activity decreased to below detectable levels in 2 hr. At this point, the beans were still firm under fork pressure and did not soften appreciably until the end of the 10 hr cooking period.

Lectin concentration did not decrease significantly under 65°C treatment even after 12 hr of cooking. At 12 hr, beans were still quite firm under the fork pressure and thus not considered fit for consumption.

–Continued on next page

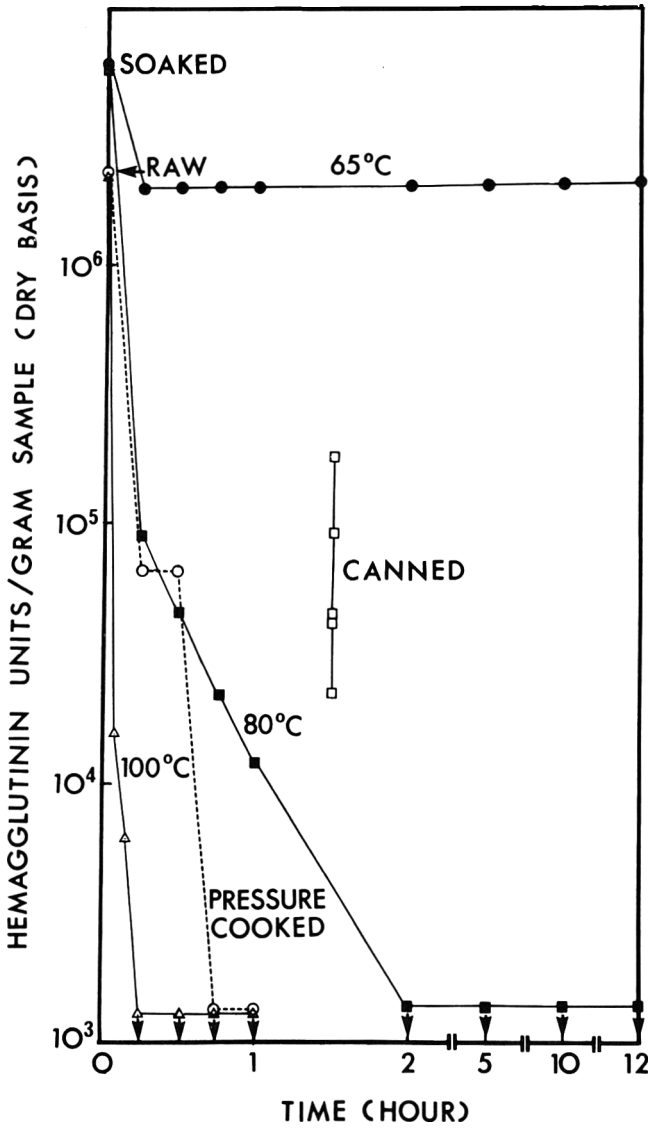

Fig. 1—Hemagglutinin activity in red kidney beans processed at different temperatures and times.

Pressure cooking without presoaking the beans decreased the lectin concentration to below detectable levels in 45 min. However, 60 min pressure cooking was required to soften the beans sufficiently to be edible. Commercially canned red kidney beans frequently eaten without further heating varied in lectin content but the levels were close to those of beans pressure cooked for about 30 min.

In this study, to estimate the variability between trypsinized rabbit cells prepared on different days, a lectin standard (concanavalin A) was routinely tested with the sample extracts. Results showed similar agglutination patterns with erythroctyes used on different days suggesting little variability in our preparations.

One source of error in the described method of lectin analysis is the identification of the visual agglutination end point. Because of the serial twofold dilution involved, an error equivalent to one dilution in the estimation of lectin activity are usual. Therefore a difference in activity equivalent to one dilution was not considered significant.

No significant lectin changes were observed after soaking the beans. Previously reported losses (Noah et al., 1980) could be related to leaching due to longer soaking time and larger volume of soaking water. An increase in lectin activity during the first 45 min heating at 80°C reported by others (Bender, 1981) was also not observed in this study.

It appears that cooking beans to the point where they might be considered edible is more than sufficient to destroy virtually all of the hemagglutinating activity of lectins. In all of the laboratory cooked samples, the point of no detection of hemagglutinating activity was reached before the beans were considered edible. High lectin activity was found in kidney beans heated in water at 65°C for up to 12 hr but at no point were they considered cooked adequately for human consumption, being a firm rubbery texture and generally unpalatable. It is possible, however, that such beans may be used in "raw" vegetable salads and in this form may have been responsible for the gastrointestinal symptoms reported (Noah et al., 1980).

Relatively high lectin activity levels found in edible commercially canned red kidney beans may be due to smaller volumes of processing water thus less leaching of lectins. There have been no reports of toxicity from canned kidney beans.

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Sunflower Butter: Nutritional Evaluation and Consumer Acceptance

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ABSTRACT

The nutritional and sensory quality and physical characteristics of commercially and experimentally processed sunflower butters were evaluated. The analyses included: proximate analyses, calories, available lysine, in vitro protein digestibility, C- and DC-PER, phytic acid, a 9-point hedonic test, Gardner color, and spreadability determinations. Sunflower butter was found to have a good overall nutritional value with a protein quality approximately equal to that of peanuts. Roasting conditions had a significant impact on nutritional and sensory quality, color and spreadability of sunflower butter. Taste panelists generally rated sunflower butter lower than peanut butter.

INTRODUCTION

THE USE OF OILSEEDS in human foods has become increasingly important. Sunflower seeds in particular have unique sensory, nutritional and functional properties which could expand the range of their use in foods (Sosulski, 1979). For example, sunflower seeds have been used as a nut substitute in confectionery and bakery formulations and have been found to be very acceptable (Lorenz, 1978).

Recently, sunflower butter and spreads have appeared on supermarket shelves in several Midwestern and New England states and have become available for institutional markets throughout the USA (Hannigan, 1981; Lynch, 1981). According to Lynch (1981), the incentive behind the increased availability of sunflower butter was the 1980 peanut crop shortfall that caused peanut butter prices to rise and decreased product availability. Although sunflower butter can be used like peanut butter, it must be stressed that sunflower butter does not taste or look like peanut butter. The chlorogenic acid present in sunflower products may cause a greenish-gray color and distinct bitter flavor. Sunflower butter was shown to be lower in protein, fat and calories, and higher in calcium, phosphorus, iron and many B-vitamins than peanut butter (Falk and Holm, 1981).

With the expanding interest in food nutritional quality and the importance of consumer acceptance in the introduction of new products, sunflower butter needs extensive evaluation. Sunflower butter could potentially constitute the single largest end product prepared from confectionery sunflower seeds. In this study, the nutritional, sensory quality and physical characteristics of commercially available sunflower butters and sunflower butters prepared in the laboratory under various roasting conditions were evaluated.

MATERIALS & METHODS

Samples and treatments

The sunflower butters (200g sample size) used included commercially available sunflower butter samples purchased from a supermarket (Sigco Sun Products) and a health food store (Erewhon, Inc.), and experimentally prepared sunflower butters which simulated home preparation techniques. The experimental butters were prepared from sunflower seeds supplied by a local company. Seeds were divided into three treatment groups: raw (untreated), conventionally roasted and microwave roasted. Seeds were roasted in a force air oven at 165°C for 20 min (Falk and Holm, 1981). Microwave roasted seeds were prepared in a Genius II Panasonic microwave oven for 4 min per 1.5 cups at the high power select setting. Peanut butter samples consisted of three name brands and one health food brand (old fashion style) for use in calorie and sensory analyses.

A 200-g sample from each treatment was processed into sunflower butter by grinding in a food processor for 10 min. Myvatex (stabilizer) – 1.5%, dextrose – 5.5% and salt – 2.0% were added to all the laboratory samples prior to grinding in the food processor for another 10 min (Falk and Holm, 1981).

Nutritional evaluation

All analyses were done in triplicate. Proximate analyses of moisture, fat, ash and protein (6.25 X N) were determined according to standard methods (AOAC, 1980). Calories were determined by Parr bomb calorimetry. DNP-lysine (available lysine) was separated and quantified by high pressure liquid chromatography using a UV detector set at 436 nm (Carpenter, 1960; Peterson and Warthesen, 1979). In vitro protein digestibility was determined by two methods: an enzymatic method (Hsu et al., 1977) and a discriminant method (Jewell et al., 1980). Protein hydrolysis was done in 6N HCl under vacuum at 105°C for 24 hr using norleucine as an internal standard (Tkachuk and Irvine, 1969). All amino acids except tryptophan were determined on a Beckman amino acid analyzer (Spackman et al., 1958). Tryptophan was determined by using a modification of the Hopkins-Cole method (Vollmer, 1972). Chemical scores were determined by the FAO (1973), and Osborne and Voogt (1978) method. The C-PER and DC-PER values were calculated according to the methods of Satterlee et al. (1979) and Jewell et al. (1980). Phytic acid was assayed by the method of Harland and Oberleas (1977). Dietary fiber was determined by the enzyme-modified neutral detergent fiber (ENDF), (Robertson and Van Soest, 1977).

Sensory evaluation

Sensory quality attributes including appearance, aroma, texture, flavor and aftertaste were measured by 66 untrained consumer panelists using a 9-point hedonic rating scale (9 – like extremely to 1 – dislike extremely) (Amerine et al., 1965; Johnston, 1979). The panel members consisted of faculty, staff and students. The test was performed in partitioned booths with fluroescent lighting. Samples were randomly coded and served individually with water and unsalted crackers distributed between samples. Sample size consisted of one Tbl of each butter served in individual containers.

Physical characteristics

Sample color was determined on a Gardner Tristimulus IX-23 Colorimeter using the L, a, b scale compared to the white standard (XL-23-246-D). Spreadability was assayed on a Stevens-LFRA Texture Analyzer at 22°C with a 60° cone plunger, a 20 mm penetration distance, and a speed of 0.5 mm/sec.

Statistics

All data were statistically analyzed by analysis of variance ($P \leq 0.05$) and Duncan's multiple-range test (Steel and Torrie, 1980).

RESULTS & DISCUSSION

Proximate analyses

The proximate analyses of the sunflower butter samples

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are shown in Table 1. Moisture levels for the commercial samples ranged from 0.5–1.3% and the experimental sample values ranged from 1.5–1.8%. The health food store brand contained significantly lower moisture. The oil content of the commercial samples ranged from 46.0–49.0% and that of the experimental samples ranged from 43.0–47.0%. Generally, sunflower butter contains slightly lower oil content than peanut butter (McWatters and Young, 1978). The protein content of the commercial samples ranged from 22.0–25.0% and experimental sample values ranged from 22.0–24.0%. The health food store brand had significantly higher protein content than the supermarket brand. This can be attributed to the added sugar, salt and stabilizers found in the supermarket brand. Sunflower butter contains the same protein level as peanut butter (McWatters and Young, 1978). The ash content of the commercial samples ranged from 3.4–3.9% and the experimental sample values ranged from 3.7–4.4%. According to Falk and Holm (1981), the high level of minerals in sunflower butter can be accounted for by calcium, phosphorus, iron, sodium and potassium. Finally, the ENDF levels for commercial samples ranged from 2.6–4.6% and the experimental sample values ranged from 5.7–5.9%. The health food store and experimental samples had significantly higher dietary fiber levels than the supermarket sample. As expected, there were only slight differences between the proximate analyses of the experimental sunflower butter samples.

Table 1—Proximate analyses of sunflower butter[a]

	Moisture (%)	Oil (%)	Protein (%)	Ash (%)	ENDF (%)
Supermarket brand	1.3a	49.0a	22.0a	3.9a	2.6a
Health food store brand	0.5b	46.0ab	25.0b	3.4b	4.6b
Experimental					
Raw	1.5ac	46.0ab	23.0a	4.2c	5.9c
Conventionally roasted	1.8c	47.0ab	24.0b	4.4d	5.8c
Microwave roasted	1.7c	43.0b	22.0a	3.7e	5.7c

[a] Values in an entire column followed by a common letter are not significantly different at $P \leq 0.05$ (a through e). The letters in each column are presented for ease of comparison within the categories of brands and experimental butters.

Table 2—Amino acid content of sunflower butter[a]

Amino acids	Supermarket brand	Health food store brand	Experimental[b] R	CR	MR
Lysine	5.0	4.1	5.2	3.8	4.4
Methionine	2.8	2.3	2.3	2.7	2.2
Cystine	1.4	1.3	1.3	1.4	1.3
Threonine	3.5	4.5	4.2	4.5	4.7
Isoleucine	5.2	4.6	5.3	5.2	4.8
Leucine	9.8	8.7	8.9	9.0	8.3
Valine	6.0	5.5	6.7	6.1	5.6
Phenylalanine	8.5	7.8	8.5	8.1	6.8
Tyrosine	3.7	3.8	4.3	3.7	3.9
Tryptophan	1.6	1.4	1.5	1.5	1.2
Aspartic acid	11.2	11.5	10.5	12.2	11.6
Proline	7.2	6.2	5.1	7.9	7.6
Serine	5.0	5.7	5.0	5.8	5.4
Glutamic acid	22.8	20.2	20.8	25.1	19.7
Glycine	7.5	6.3	4.5	6.8	6.3
Alanine	5.5	6.0	5.4	6.7	5.8
Histidine	6.9	4.9	5.3	4.8	7.1
Arginine	8.9	12.1	11.5	10.4	12.7
Ammonia	4.5	3.8	3.2	3.7	3.6

[a] g/100g protein.
[b] R=Raw, CR=Conventionally roasted, and MR=Microwave roasted.

Nutritional quality

The amino acid and protein quality data on sunflower butter samples are shown in Tables 2 and 3. The chemical scores of the amino acid profile for the commercial samples ranged from 65–75 and for the experimental samples from 57–77. According to Osborne and Voogt (1978), the maximal chemical score (whole egg) is 100. The most limiting amino acid for all the sunflower butters was lysine. Of the experimental samples, raw sunflower butter had a better score than the roasted samples with the conventionally roasted sample having the lowest value. The in vitro protein digestibilities for the commerical samples ranged from 81–83% and those of the experimental samples from 80–81%. According to Jewell et al. (1980), the sunflower protein digestibility was approximately equal to the digestibilities of soy and cottonseed protein. Since a human protein quality (PQ) predictive model has not been finalized, both C-PER and DC-PER were evaluated in this study (Jewel et al., 1980). The ANRC casein values for C-PER and DC-PER were 2.7 and 2.6, respectively, compared to 2.5 for the in vivo PER method. The C-PER's for the commercial samples ranged from 2.3–2.5 and the experimental samples ranged from 2.2–2.5. The DC-PER's for the commercial samples were 1.8 and the values for the experimental samples ranged from 1.4–2.1. Of the experimental samples the conventionally and microwave roasted samples had lower in vitro PER values than the raw samples. According to Jewell et al. (1980) peanut flour has a C-PER and DC-PER of 2.1 and 1.6, respectively. By comparison, sunflower and peanut protein have approximately the same protein quality.

The nutritional values of the sunflower butter samples are shown in Table 3. Calories for the commercial samples ranged from 7.2–7.3 Kcal/g and the values for the experimental samples ranged from 6.9–7.0 Kcal/g. The calorie content of the sunflower butter was approximately equivalent to the peanut butter samples evaluated which ranged from 6.8–7.1 Kcal/g. Available lysine for the commercial samples ranged from 4.8–7.3 mg/g on a defatted, dry weight basis and the values for the experimental samples ranged from 5.3–6.2 mg/g. The phytic acid level of the commercial samples ranged from 1.4–1.6% on a defatted, dry weight basis and the values for the experimental samples

Table 3—Nutritional quality of sunflower butter[a]

	Calories (Kcal/g)	Protein digestibility (%)	C-PER	DC-PER	Available lysine[b] (mg/g)	Phytic acid[b] (%)
Supermarket brands	7.2a	83.0a	2.5a	1.8a	4.8a	1.6a
Health food store brand	7.3b	81.0ab	2.3b	1.8a	7.3b	1.6a
Experimental						
Raw	7.0c	80.0b	2.5c	21.b	5.3c	1.8b
Conventionally roasted	6.9c	81.0ab	2.2b	1.4c	6.2d	1.8b
Microwave roasted	7.0c	81.0ab	2.2b	1.8a	5.9d	1.8b

[a] Values in an entire column followed by a common letter are not significantly different at $P \leq 0.05$ (a through d). The letters in each column are presented for ease of comparison within the categories of brands and experimental butters.
[b] Values on a defatted, dry weight basis.

Table 4—Sensory analysis of sunflower butter and peanut butter[a,b]

	Appearance	Aroma	Texture	Flavor	Aftertaste
Sunflower butter					
Supermarket brand	5.1a	5.3a	5.4a	6.0a	5.1a
Health food store brand	2.8b	4.0b	2.7b	3.4b	2.6b
Experimental					
Raw	2.8b	4.3b	2.7b	3.7b	2.6b
Conventionally roasted	3.5c	4.6b	3.9c	5.0c	2.9b
Microwave roasted	2.6b	4.3b	3.1b	5.0c	2.4b
Peanut butter					
National brands	7.1–7.8d	7.3–7.5c	7.5d	6.9–7.7d	6.8–7.1c
Old fashion style	6.2e	6.5d	5.9a	5.0c	5.8a

[a] Values in an entire column followed by a common letter are not significantly different at $P \leq 0.05$ (a through e). The letters in each column are presented for ease of comparison within the categories of sunflower brands, experimental butters, and peanut butter.
[b] Hedonic scale: 9=like extremely; 1=dislike extremely.

were all 1.8%. Cheryan (1980) showed defatted peanut meal to have 1.7% phytic acid which is approximately equivalent to that of defatted sunflower butter. Although Falk and Holm (1981) noted that sunflower butter has high levels of calcium and iron, the bioavailability of these minerals needs to be evaluated due to the phytic acid level present.

Sensory quality

The sensory analyses data for the sunflower and peanut butter samples are shown in Table 4. The supermarket sunflower butter sample was rated significantly higher in all sensory attributes than either the health food store or the experimental samples. The conventionally roasted sample was rated higher in all attributes except for that of flavor than either the raw or microwave roasted samples. The flavor rating for both conventionally and microwave roasted samples were the same. At best, sunflower butter's sensory qualities were rated in the categories (a) neither like nor dislike or (b) like slightly. In general, the commercial sunflower butters were rated lower in all attributes than their corresponding peanut butter samples. However, the supermarket sunflower butter sample was rated approximately equivalent in most sensory attributes to the old fashion style of peanut butter sample. Enhancement of all sunflower butter sensory characteristics will be required before a significant impact on consumer acceptance can occur.

Physical characteristics

The color and spreadability values of sunflower butter samples are shown in Table 5. The color values for the commercial samples were (a) L values reflecting the degree of lightness, ranged from 37.7–41.9, (b) a values reflecting the degree of redness, ranged from 2.9–7.3, and (c) b values reflecting the degree of yellowness, ranged from 15.3–17.3. Generally, the sunflower butters evaluated were darker and less red and yellow than the peanut butter samples evaluated by McWatters and Young (1978). Of the experimental samples the conventionally roasted sunflower butter was darker than the raw or microwave roasted samples. This probably helped to mask the slight gray-greenish color inherent in sunflower products due to chlorogenic acid (Robertson, 1975). The spreadability scores for the commercial sunflower butters ranged from 128–386g and the experimental samples ranged from 303–508g. The health food brand had lower spreadability than the supermarket brand. The poor spreadability of the health food brand was probably due to the absence of stabilizers, allowing oil separation. The experimental sunflower butters showed an inverse relationship between spreadability and the degree of roasting (raw samples had the highest spreadability while the conventionally roasted sample had the lowest spreadability). Both roasted sunflower butters had better spreadability than the raw sample, which was too firm. The load/penetration values from the Stevens LFRA Texture Analyzer have been shown to correlate favorably with taste panel spreadability scores (Marrs et al., 1980).

Table 5—Color and spreadability values of sunflower butter[a]

	Color			Spreadability
	L	a	b	Load (g)
Supermarket brand	37.7a	7.3a	17.3a	386a
Health food store brand	41.9b	2.9b	15.3b	128b
Experimental				
Raw	46.8c	1.6c	13.9c	508c
Conventionally roasted	43.9d	2.9b	15.8b	303d
Microwave roasted	46.9c	3.3b	13.1c	432a

[a] Values in an entire column followed by a common letter are not significantly different at $P \leq 0.05$ (a through d). The letters in each column are presented for ease of comparison within the categories of brands and experimental butters.

CONCLUSIONS

THE DATA PRESENTED in this study show that sunflower butter has good nutritional value and moderate to low sensory scores. The proximate analyses of the sunflower butter samples showed it to have slightly less oil content and equivalent protein level when compared to literature peanut butter values. The nutritional analyses of sunflower butter showed the C-PER values to range from 2.2–2.5 and the DC-PER values to range from 1.4–2.1 with the raw sunflower butter having slightly higher protein quality than the other samples. Sunflower butter and peanut butter had approximately the same protein quality, calorie content and phytic acid level. Taste panelists rated sunflower butter lower than peanut butter in all sensory attributes evaluated. Of the commercial samples, the supermarket sunflower butter sample was rated approximately equivalent in sensory quality to the old fashion style of peanut butter. In the experimental sunflower butters the conventionally roasted samples had higher sensory scores, darker color and better spreadability than the raw samples. Additional studies are needed to help improve the sensory attributes of sunflower butter, thereby improving consumer acceptance of this unique product.

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—Continued on page 242

Analysis of Direct Contact Paper and Paperboard Food Packaging for N-nitrosomorpholine and Morpholine

JOSEPH H. HOTCHKISS and ALEX J. VECCHIO

ABSTRACT

Analytical methods were developed to estimate the nitrosatable morpholine and apparent N-nitrosomorpholine content of paper and paperboard direct contact food packaging. Examination of 34 different paper or paperboard food packages indicated 9 to be contaminated with detectable levels of apparent N-nitrosomorpholine. All 12 packages analyzed for nitrosatable morpholine were positive with an average level of 380 μg/kg. Migration of apparent N-nitrosomorpholine and morpholine to food was demonstrated when paper or paperboard and dry food were incubated in a closed container for 3 days at 100°C.

INTRODUCTION

SMALL AMOUNTS of volatile N-nitrosamines (NAs) have been detected in a number of commercial products including pesticides (Kearney, 1980), cosmetics (Fan et al., 1977), rubber nursing nipples (Havery and Fazio, 1982), tobacco products (Hoffmann et al., 1979) and foods (Scanlan, 1975). These findings have raised concerns because of the ability of most NAs to produce tumors in laboratory animals (Odashima, 1980).

Research into the NA contamination of foods has, in part, focused on the sources of NA precursors as well as the occurrence and formation of NAs during processing and cooking. For example, research has shown that the amino acid proline is a precursor to N-nitrosopyrrolidine in fried bacon (Bharucha et al., 1979) and that plant alkaloids can serve as precursors to N-nitrosodimethylamine in malted barley (Mangino et al., 1982). Singer and Lijinsky (1976) have surveyed foods for nitrosatable secondary amines and in addition to other amines, found morpholine in nearly all samples at levels of 300–9000 μg/kg. As pointed out by these authors, morpholine is not thought to be a naturally occurring amine, hence its occurrence in food is most likely due to contamination. Rounbehler and Fine (1981) have reported the occurrence of morpholine in bacon.

N-nitrosomorpholine (NMOR) has been reported to occur sporadically in foods. Fazio and Havery (1981) found soy protein isolates contained NMOR and Gray et al. (1981) identified NMOR in cured heated chicken frankfurters. In these reports the authors have speculated that the use of morpholine as a corrosion inhibitor in boiler feed waters has led to the occurrence of NMOR in the food. Fajen et al. (1979) have found NMOR in high concentrations in certain industrial environments where morpholine or morpholine derivatives are used. Our purpose in this investigation was to determine if direct contact food packaging materials contained morpholine and/or NMOR as contaminants and if these contaminants could potentially migrate to foods.

MATERIALS & METHODS

All packaging materials were obtained through retail stores or directly from manufacturers. Dichloromethane (DCM) was redistilled in glass. Nitrosamine standards were obtained commercially (Aldrich or Sigma) and used without further purification. Water was distilled and not deionized. All other chemicals were analytical reagent grade or better. Blank analyses were run on each new container of all reagents including DCM and water.

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NMOR in paper and paperboard

Five gram samples were ground dry on high speed (Waring Blender Model 7011) for 30 sec. After addition of 120 ml $1NH_2SO_4$ containing 10 mg/ml $NH_4SO_3NH_2$ the sample was reblended 15 sec on low speed. One ml internal standard containing N-nitrosodipropylamine (NDPA) at 250 ng/ml and 50 ml DCM were added and again blended 15 sec on low speed. The blender contents were filtered under vacuum through #1 Whatman filter paper (9 cm) covered by 0.5 cm Celite 545. The solid was reblended after addition of DCM and water (50 ml each) and filtered as above. Filtrates were combined and the aqueous portion extracted in a separatory funnel. After removal of the DCM, 20g NaCl were added and the aqueous layer further extracted with DCM (2 X 25 ml). All DCM extracts were combined, washed with 25 ml 1N NaOH (backwashed with DCM 1 X 15 ml), and dried by passing through 20g anhydrous Na_2SO_4 held in a fritted glass bottomed funnel. The DCM was concentrated to ca 4 ml in a Kuderna-Danish apparatus (60°C) and further concentrated to 1 ml under N_2 stream at ambient. Eight μl aliquots were injected into the GC-TEA (see below).

The recovery of the internal standard (NDPA) averaged 84% and when negative paperboards were spiked at 10 μg/kg with NMOR recovery averaged 81%. Some analyses were carried out with the addition of 57 μg 2,6,-dimethylmorpholine. N-nitroso-2,6,-dimethylmorpholine was not detected indicating that nitrosation did not occur during sample workup. Limit of detection of NMOR was approximately 3 μg/kg. The NMOR values reported here are not corrected for recovery.

We have not confirmed the identity of the NMOR found by mass spectrometry and, hence, the values reported should be considered as "apparent" in keeping with international recommendations (IARC, 1978). We did, however, photolyze all positive samples (Doerr and Fiddler, 1977) and in all cases the NMOR and NDPA peaks disappeared.

Nitrosatable morpholine in paper and paperboard

Five gram samples were ground, filtered and reground as above except the DCM and internal standard were not added. In place of H_2SO_4, 0.1N HCl which did not contain $NH_4SO_3NH_2$ was used. The combined filtrates were washed with DCM (1 X 25 ml) which was discarded. The filtrate, 20 ml glacial acetic acid, 2.0g $NaNO_2$ and NDPA (internal standard) were stirred at room temperature for 2 hr. The nitrosation was quenched with 6.6g $NH_4SO_3NH_2$. The NMOR was extracted with DCM (3 X 50 ml), the DCM washed with 20 ml 1N NaOH (backwashed 1 X 15 ml DCM) dried and concentrated as above. Eight μl aliquots were analyzed by GC-TEA (see below). The recovery of morpholine added to paperboard at 400 μg/kg and analyzed as NMOR using this procedure averaged 95%.

The morpholine content of flour which had been incubated with paperboards containing morpholine was estimated by steam distilling 20g of flour (Goodhead and Gough, 1975) and subjecting the distillate to nitrosation as given above. Subtraction for the morpholine content of the flour prior to incubation was necessary. Recovery of added morpholine was 90%.

NMOR in foods

Twenty-five gram samples of food were vacuum distilled from mineral oil as described previously (Hotchkiss et al., 1980) to

estimate the NMOR content of foods before and after incubation with paperboards containing NMOR.

Detection & Quantitation

NAs were detected and quantitated against external standards by a Gas Chromatograph-Thermal Energy Analyzer (GC-TEA). Conditions were as follows: Column, 10 ft X 0.125 in. o.d. SS packed with 10% Carbowax 20M + 2% KOH on 100/200 Chromosorb WHP; Temperatures: injector 190°C, column 160°C isothermal, interface 200°C, pyrolyzer 525°C, trap −150°C; Flow rates, column 25 cc/min; TEA pressure 2.3 mm Hg.

RESULTS & DISCUSSION

TABLE 1 lists the types of food containers analyzed and the resulting NMOR levels found in each. Nine of 34 contained detectable levels of NMOR at concentrations ranging from <3 to 33 μg/kg. The average concentration of detectable samples was 12 μg/kg. With three exceptions these levels are at or below the levels for other volatile NAs which might be found in foods such as fried bacon (Gray, 1981) and as such may not represent a significant direct source of NMOR in foods, assuming these values to be representative. One sample contained 2.9 μg/kg N-nitrosodimethylamine (NDMA).

We investigated the possibility that the NMOR was associated with the outer virgin layer of paper which is applied to improve printability and not with the inner food contact surface. A paperboard sample which had given a positive NMOR response when analyzed was carefully separated down the center into inner and outer layers. Subsequent analysis of the inner layer indicated that it contained NMOR at levels similar to the paperboard when analyzed whole. We conclude that apparent NMOR is associated with the inner paperboard layer and not just the outer printed layer.

Paper and paperboards which were both positive and negative for NMOR were analyzed for nitrosatable morpholine (Table 2). All the samples tested contained morpholine which could be nitrosated to yield NMOR. The morpholine contents ranged from 98 to 842 μg/kg ($\bar{X}$ = 380 μg/kg). Those materials which contained NMOR, on the average, contained more morpholine. The major exception to this generalization was sample L (Table 2). However, four different samples containing this same brand of product were analyzed and three out of four contained NMOR.

A majority of the packages analyzed had contained food prior to analysis. We did, however, also analyze several unused packages and in all cases the paper and paperboard packaging contained morpholine and in some cases NMOR. We also analyzed the food contained in certain packages for NMOR. We carefully removed with a small spatula 20g of flour most closely (0.5 cm) in contact with the outer wall of the package. That flour nearest the bag wall contained 1.1 μg/kg NMOR. The paper itself contained 33.0 μg/kg NMOR. We have assumed that migration occurs from high to low concentration and that the relatively high apparent NMOR content of the paper was responsible for detectable levels in the flour. Morpholine was detected in flour from a similar package at 18 μg/kg.

The fact that food packaging may contain contaminants is only significant if those contaminants migrate to the food. In addition to the analyses of foods described above we demonstrated migration of NMOR from paperboards and paper which were positive for NMOR to foods which were negative. These data indicate that when flour or ground pasta is incubated with small pieces of paperboard in a closed container at 100°C migration can occur (Table 3). The amount of NMOR found after incubation was less than that originally in the paperboard, hence, it is unlikely that heating caused formation of NMOR during incubation. Similar results could be demonstrated for morpholine. These severe conditions do not, however, conclusively indicate that migration occurs under normal conditions of storage. Only after long term incubations at room temperature would such conclusions be possible. Further work is underway in our laboratory to clarify this point.

Table 1—N-nitrosomorpholine content of direct contact food paper and paperboard packaging

Package Type	Number Tested	NMOR[a]
Round paperboard (oats)	1	ND[b]
Coated carton (milk)	4	ND
Paperboard (cereal)	1	ND
Paperboard (dinner)	3	2ND, 6.1
Paperboard (frozen)	2	ND
Paperboard (rice)	1	ND
Ovenable board (tray)	1	tr[c]
Paperboard (pasta)	11	7ND, tr, 15.1, 8.9, 11.7
Composite can (frozen juice)	2	ND, 2.9[d]
Paper sack (sugar)	1	ND
Paper sack (flour)	4	ND, 13.1, 33.0, 4.3
Round paperboard (salt)	1	ND
Paperboard (corn meal)	1	ND
Paperboard (starch)	1	ND

[a] μg/kg (uncorrected for recovery)
[b] not detected
[c] trace (< 3 μg/kg)
[d] N-nitrosodimethylamine

Table 2—Nitrosatable morpholine content of paper and paperboard used for the packaging of food

Sample	NMOR	Morpholine[a]
A	15.1	426
B	tr[b]	223
C	3.6	560
D	8.9	347
E	13.1	812
F	tr	238
$\bar{X}$		434
G	ND[c]	98
H	ND	132
I	ND	329
J	ND	445
K	ND	113
L	ND	842
$\bar{X}$		327

[a] μg/kg (uncorrected for recovery)
[b] trace (< 3 μg/kg)
[c] not detected

Table 3—Migration of apparent N-nitrosomorpholine from paperboard to flour or ground pasta after heating to 100°C[a]

	Experiment			
Container	1	2	3	4
Before	152[b]	304	62	119
After	136	273	ND	ND
Test food				
Before	ND[c]	ND	ND	ND
After	23	40	31	53

[a] 3.6—26g of paperboard or paper and 20—26g of flour or ground pasta were incubated in a closed container at 100°C for 72 hr.
[b] total ng
[c] not detected

The source ot the morpholine and apparent NMOR is not known. However, as has been pointed out by several authors, morpholine is used as a corrosion inhibitor in boiler feed water and large amounts of steam and water are used in paper and paperboard manufacture. A recent Environmental Protection Agency report dealt with the use of amines in boiler feed water (EPA, 1980). In 1978 an estimated 1,800–2,200 metric tons of morpholine were used as neutralizing amines in boiler feed water. This morpholine conceivably could be transferred to products which contact large amounts of steam such as paper and paperboard. The source of the NMOR is less clear. NMOR could be formed in the steam or subsequently in the paperboard. It is also possible, that NMOR is a contaminant in the morpholine. Commercially available amines have been shown to be contaminated with up to 53 mg/kg of the corresponding N-nitroso derivative (Spiegelhalder et al., 1978).

Our data suggests that paper and paperboard packaging materials may indirectly contribute trace amounts of morpholine and in some cases apparent NMOR to the diet. Morpholine nitrosation is facile and the possibility exists that dietary morpholine could be nitrosated *in vivo* (Mirvish, 1975). We believe alternatives to morpholine which do not form stable N-nitroso derivatives should be investigated as replacements for secondary amines in boiler feed waters.

Hoffmann et al. (1982) have independently made similar conclusions concerning NMDR and morpholine in packaging materials.

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SUNFLOWER BUTTER. . . From page 239

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Enterotoxin C_2 Production by *S. aureus* in Entree Salads

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ABSTRACT

Staphylococcus aureus strain 361 was inoculated into salads containing chicken, macaroni, potato, or artificially flavored soy product. Egg was added to some. The pH averaged 6.2 in the control made with water dressing rather than vinegar, 4.7 with home-style cooked salad dressing, and 4.9 with commercial salad dressing. Following incubation at 37°C for 8 or 24 hr, pH, total counts, and enterotoxin C_2 were determined. Enterotoxin was detected at 8 hr in all macaroni and potato dressing types. At 8 hr, enterotoxin was not detected in most soy product or chicken salads. By 24 hr the lowest levels were in the soy product salads but all products contained toxin. When the inoculum level was reduced below the 10^5 CFU per g used in the main study, enterotoxin production was delayed but occurred at 8 hr if the inoculum level was 10^3.

INTRODUCTION

FOODS which have a preparation step after cooking may become contaminated with microorganisms which can then multiply even under somewhat unfavorable conditions. Thus, it is not surprising to find that staphylococcal food poisoning remains one of the leading causes of foodborne illness (Center for Disease Control, 1978) and that the largest number of cases reported was due to consumption of different types of salads. Within the 1969–1979 period, Center for Disease Control data show that chicken salad was responsible for 16 outbreaks (1935 cases); egg salad, 8 outbreaks (848 cases); potato and macaroni salads, 27 outbreaks (1887 cases); and salad dressing, 2 outbreaks (11 cases).

Although the optimal pH for growth of enterotoxigenic *Staphylococcus aureus* is near 7, growth will occur at pH extremes depending on other factors such as type of food, type of acid, concentration of ingredients such as salt, sugar, and spices, and the buffering capacity of some of the ingredients such as egg. Entree salads are normally refrigerated so that proliferation of microorganisms is prevented, but at higher temperatures the acidity may be insufficient to prevent growth and production of enterotoxin. Longree et al. (1959) found that both turkey salad and potato salad made with egg supported growth of *S. aureus*. Numbers after 20 hr of incubation at 30°C increased to 10^8 from the initial inoculum level of 10^3. Scheusner et al. (1973), using *S. aureus*, strain 243 (producing enterotoxin B), was able to detect enterotoxin after 154 hr incubation in laboratory medium at 37°C at pH 5.15 but not below pH 5.0.

This study was initiated to study the potential for support of staphylococcal enterotoxin production of both plant and animal protein sources at the pH of entree salads. Hard-cooked egg was included in some of the salads, since it is a common protein ingredient in potato and macaroni salads. *S. aureus*, strain 361, which produces enterotoxin C_2 (SEC_2) was used.

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MATERIALS & METHODS

Preparation of ingredients

Potato. Red potatoes were peeled, eyed, cut into 1 or 2 cm cubes, and cooked in boiling water for 10–15 min, then drained.

Macaroni. Bulk generic-brand macaroni was put into boiling water and simmered for 15 min, then drained.

Soyameat. Canned, sliced soyameat, artificial chicken-flavored, (Worthington Foods, Division of Miles Laboratories, Inc., Worthington, OH) was drained and cut into 2 cm pieces aseptically.

Chicken. Canned chunk chicken containing both light and dark meat (Swanson, Campbell Soup Co., Camden, NJ), was used as purchased.

Egg. Eggs were hard-cooked for 15 min, transferred aseptically into sterile ice water, shelled and chopped using aseptic techniques.

Vinegar dressing. Cooked dressing contained 28g sugar, 1.2g salt, 1.0g dry mustard, 3.0g corn starch, 2g dry onion flakes, 20 ml white vinegar, and 40 ml distilled water. The ingredients were cooked over direct heat with constant stirring until thickened, then covered with foil. The pH was 3.4.

Water dressing. Cooked clear water dressing contained the same ingredients as the vinegar dressing with the exception of the substitution of an equal amount of water for the vinegar. The pH was 4.9.

Commercially prepared cooked dressing. Miracle Whip (Kraft, Inc., Chicago, IL) salad dressing was purchased. The pH was 3.4. All ingredients were brought to room temperature (21°C) before use.

Salads were prepared with and without egg with the exception of chicken salad which was prepared only without egg. The high-mayonnaise level used was that of Longree et al. (1959). The composition of the salads with egg was 75g potato, macaroni, or soyameat; 5g egg; and 20g of dressing. In salads without egg, the amount of potato, macaroni, soyameat, or chicken was increased to 80g. Controls without dressing consisted either of 95g of the other ingredients in total and 5g of egg or 100g without egg. The ingredients, such as potato, macaroni, soyameat, and egg, were weighed aseptically into sterile half-pint jars. No effort was made to control the relative proportion of yolk and white when chopped egg was added to the salads. However, in a subsequent experiment either chopped egg white or egg yolk was added to soyameat salad prepared with commercial dressing in order to determine if differences due to egg fraction were detectable after 24 hr incubation at 37°C.

The inoculum was prepared by centrifuging (9400 x *g*, 10 min, 4°C) 5 ml of an 18–24 hr culture of *Staphylococcus aureus*, strain 361 which produces SEC_2, decanting the culture fluid, and resuspending the cells in peptone water (0.1% Bacto-peptone). Each jar was inoculated with 10 ml of an appropriate dilution of the suspended cells to give a level of 10^5 organisms per g of salad. Macaroni salad prepared with commercial dressing and egg was also inoculated with low numbers of *S. aureus* ranging from 10–1000 CFU per g of salad. The uninoculated control was salad to which 10 ml of sterile peptone water were added. The liquid was distributed over the surface and then the mixture shaken.

Within 15 min, 20g of salad dressing were added to each jar with the exception of those done without dressing. The jars were covered, shaken to mix the ingredients, and incubated at 37°C for 8 and 24 hr. A different jar was used for each sampling time.

At 8 and 24 hr, 90 ml of sterile distilled water were added to each jar and the contents were blended at high speed for 2–3 min. The pH was determined before the sample was further diluted with 50 ml of distilled water. CFU were determined by plating appropriate dilutions of the blended sample on plate count agar (Difco).

Samples were centrifuged for 10 min at 32,800 x *g* at 4°C and the supernatant was collected. The precipitate was reextracted with 50 ml of distilled water and recentrifuged. The combined supernatants were placed in dialysis bags to be concentrated using Aqua-

cide II-A (Calbiochem, San Diego, CA). The concentrated sample (approximately 15 ml) was centrifuged for 10 min at 32,800 x *g* at 4°C and the supernatant assayed for SEC_2 using the microslide gel double diffusion technique as adapted by Fung et al. (1976). Some of the samples were also extracted and assayed for enterotoxin by the method of Bennett and McClure (1980).

The minimum level of SEC_2 which could be detected in these salads by microslide gel diffusion assay was about five times greater following the direct concentration method than after chloroform extraction and chromatographic separation. The detection limit in the direct method was 5 μg/100g food; in the purified > 1 μg/100g in the foods tested.

Three replications were included for each product except that for comparisons of level of inoculum, two were done.

RESULTS & DISCUSSION

MAYONNAISE and salad dressings retard spoilage and growth of pathogens due to their high concentration of acetic acid. Data compiled by Smittle (1977) indicated that those commercial dressings with a pH of 4.1 or less are lethal to staphylococci. Although both the commercial and laboratory-prepared vinegar dressing used in our experiments had a pH value of 3.4, the addition of salad ingredients increased the pH to 4.0 and above resulting in ten to 100-fold increases in staphylococci after 8 hr incubation at 37°C (Table 1). However, as expected, the CFU values were highest in entrees without vinegar: those prepared with water-based salad dressing and controls containing no dressing.

Generally, if the initial pH (pH_i) was high (>5.8), the pH decreased by 8 hr and continued to decrease for the duration of the experiment (Table 1). If the pH_i was low (3 to 5.6), pH had increased by 8 hr and either remained at that level or showed only a slight decrease by 24 hr.

As expected, enterotoxin levels were highest for products with water-based dressing and controls containing no dressing (Table 1). However, SEC_2 was detectable in all macaroni and potato salads. Size of the potato pieces made little difference with the possible exception of SEC_2 levels after 24 hr incubation in salads made with commercial dressing.

Addition of hard-cooked egg increased the initial pH, especially in products prepared with water-based dressing, and in some cases, also increased the enterotoxin levels. Longree et al. (1959) found little difference in final counts in comparing high and low levels of added egg. Soyameat salad containing either chopped egg yolk or chopped egg white showed little difference in pH after 24 hr incubation but CFU was approximately 10 times greater (60×10^6 vs 30×10^5) in the salad containing the egg white. SEC_2 was detectable in the salad with the egg white but not in the salad with egg yolk.

Harbrecht and Bergdoll (1980) found strain differences in their work with *S. aureus*-contaminated hard-boiled eggs. SEA and SEC_1 were produced in much smaller amounts than SEB which may explain partly why the addition of boiled egg to salads did not have a greater effect on the production of SEC_2.

Of the three vegetable protein salads, soyameat salads provided the least favorable environment for *S. aureus*. Growth in soy salads after 8 hr incubation lagged behind growth in other salads. By 24 hr, the differences were not noticeable. The low 8 hr CFU was reflected in the barely detectable level of SEC_2 produced. After 24 hr, despite the

Table 1—Entree salads with four dressing treatments, inoculated with 10^5 CFU per g of S. aureus strain 361 and incubated at 37°C for 8 and 24 hr

			8 hr			24 hr		
Entree	Salad dressing	Initial pH	pH	CFUx10^a/g	SEC_2 μg/100g	pH	CFUx10^a/g	SEC_2 μg/100g
Macaroni Salad (no egg)	None	6.2	5.9	60^7	10	5.3	22^8	11
	H_2O	5.9	5.7	33^7	10	4.9	18^7	16
	Vinegar	4.2	4.5	45^5	6	4.5	89^6	8
	Commercial	4.3	4.6	15^7	4	4.5	58^7	12
Macaroni Salad (with egg)	None	6.6	6.1	85^7	17	5.7	92^8	18
	H_2O	6.7	5.8	72^7	15	4.9	65^7	18
	Vinegar	4.7	4.6	51^5	6	4.6	45^6	9
	Commercial	4.9	4.7	12^7	12	4.6	27^7	19
Potato Salad (small pieces no egg)	None	6.2	5.5	14^8	15	5.0	18^8	14
	H_2O	5.8	5.6	35^7	15	5.0	91^7	16
	Vinegar	4.0	4.7	15^6	4	4.6	13^6	8
	Commercial	4.1	4.7	55^6	8	4.7	31^7	9
Potato Salad (large pieces no egg)	None	6.4	5.6	43^7	16	5.0	20^8	13
	H_2O	5.8	5.7	29^7	16	5.0	58^7	17
	Vinegar	4.0	4.7	12^6	5	4.7	10^6	12
	Commercial	4.1	4.7	32^6	5	4.7	73^6	4
Potato Salad (large pieces with egg)	None	6.5	5.6	16^8	15	5.0	36^8	14
	H_2O	6.8	5.8	77^7	15	4.9	12^8	15
	Vinegar	4.9	4.8	30^5	3	4.8	23^6	13
	Commercial	4.9	4.8	69^6	3	4.7	35^7	14
Soyameat Salad (no egg)	None	6.2	5.7	92^6	4	5.5	13^8	17
	H_2O	5.6	5.7	24^6	3	5.3	34^7	3
	Vinegar	4.8	4.8	17^5	2	4.7	49^6	3
	Commercial	4.8	4.8	98^5	2	4.8	17^6	<1
Soyameat Salad (with egg)	None	6.2	5.5	27^7	1	5.5	16^8	14
	H_2O	6.1	5.5	19^7	3	5.2	72^7	4
	Vinegar	5.1	4.8	84^5	3	4.9	77^6	7
	Commercial	4.9	4.9	21^6	2	4.9	19^7	5
Chicken Salad (no egg)	None	5.6	6.3	30^7	1	6.1	19^8	14
	H_2O	6.4	6.2	29^7	1	5.7	15^8	3
	Vinegar	5.6	5.6	71^6	<1	5.4	24^7	16
	Commercial	5.6	5.7	14^7	<1	5.5	38^7	16

[a] = Exponent 5-8

Table 2—Comparison of three levels of inoculum with S. aureus 361 for macaroni salad with egg incubated at 37°C for 8 and 24 hr

		8 hr			24 hr		
Salad dressing	CFU/g	pH	CFUx10^a/g	SEC_2μg/100g	pH	CFUx10^a/g	SEC_2μg/100g
None	0	6.4	nd	nd	5.6	nd	nd
	10	6.3	96^4	nd	5.7	56^6	nd
	100	6.4	82^5	nd	5.5	39^7	11
	1000	6.1	20^7	2.6, 3.5	5.2	18^8	>11, 20
Commercial	0	4.8	nd	nd	4.6	nd	nd
	10	4.5	36^3	nd	4.6	94^5	nd
	100	4.7	20^4	nd	4.6	62^6	nd
	1000	4.6	19^6	nd, 0.2	4.6	15^7	0.2, 0.2

[a] = Exponent 3-8
[b] not detectable (nd)

high CFU, the amount of SEC_2 produced was very low with the exception of the controls and one replication of soy salad made with commercial dressing. Addition of egg had little effect.

Chicken salads, despite the relatively high pH, showed little change in pH at 8 hr and SEC_2 was barely detectable. However, by 24 hr, with still little change in pH, the CFU and enterotoxin levels had increased. McKinley et al. (1974) observed a much depressed growth of *S. aureus* in chicken salad than on chicken.

The major series of salads was done with an inoculum level of 10^5 CFU per g. To investigate spoilage potential at lower levels of contamination, macaroni salad with egg was inoculated with 10^1, 10^2, or 10^3 CFU of *S. aureus* per g. Although multiplication was greater in the controls without salad dressing, increases of at least 10^3 occurred within 8 hr at all levels of inoculum (Table 2). Enterotoxin was detected at 8 and 24 hr only if the inoculum was 10^3 or greater. The initial inoculum level is important since larger inocula are needed to initiate growth and enterotoxin production in an unfavorable environment than otherwise. Genigeorgis et al. (1971) observed growth and enterotoxin C production in experimental broths at pH 4.00 only if the inoculum was 10^8 cells per ml.

The microbial environment would be expected to vary greatly within the salads. Air adsorbed to surfaces (Woodburn and Morita, 1978) may exclude contact with the dressings, thus giving a more favorable environment. The buffering effect of the proteins will be greater also at the particle surface. The heterogeneity of mixtures contributes to problems in predicting growth effects (Christiansen and King, 1971).

Although salad dressings themselves do not support the growth of staphylococci, when they are mixed with low-acid ingredient in entree salads, including those without meat or poultry but with potato, soy, or macaroni, *S. aureus* was able to multiply and produce enterotoxin at 37°C. The time required was longer if the level of inoculum was low. Meat salads have been shown to have retarded multiplication of *S. aureus* but to still support growth (Lewis et al., 1953, and Doyle et al., 1982). The present findings of the production of enterotoxin C_2 if multiplication of *S. aureus* has occurred emphasize the hazard not only with meat and poultry salads but also with potato, soy products, and macaroni. Thus, all entree type salads must be refrigerated if not to be consumed within 2–3 hr.

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Effect of Processing Parameters on Trypsin Inhibitor and Lectin Contents of Tortillas from Whole Raw Corn-Soybean Mixtures

F. R. DEL VALLE, M. L. PICO, J. L. CAMACHO, and H. BOURGES

ABSTRACT

Trypsin inhibitor and lectins in raw corn-soybean blends (92/8 and 84/16 w/w, dry basis) cooked in limewater decreased with cooking time and increasing lime concentration, with total inactivation occurring, in all cases, after 30 min. These factors, determined in raw corn-soybean (92/8 and 84/16 w/w, dry basis) tortillas decreased with increasing hot plate contact time; total inactivation of both occurred only with 92/8 tortillas after 60 sec. Antinutritional factor inactivation rates were considerably higher for hot plate cooking than for limewater boiling. Soaking cooked blends in liquor after turning off the heat and allowing to cool, followed by grinding into a dough had little effect on antinutritional factors, while washing cooked blends with water resulted in some reduction of both factors. Corn-soybean tortillas prepared with normal heat treatment had no residual factors, while those prepared with minimum heat treatment had insignificantly low residual trypsin inhibitor and no lectins.

INTRODUCTION

IN PREVIOUS WORK Del Valle and Perez-Villaseñor (1974) found that it was possible to enrich tortillas with soy proteins by applying the traditional tortilla-making process, normally utilized with whole raw corn, to mixtures of whole raw corn and soybeans. Del Valle et al. (1976) further found that the same procedure of cooking whole raw corn-soybean mixtures in place of whole raw corn alone, could also be used for enrichment of industrially produced tortilla flour. In both cases, it was found that this procedure appreciably increased protein quantity and quality of tortillas and tortilla flour, with degree of enrichment dependent upon soy level in the mixture. Subsequent work by other investigators (Bressani et al., 1974; Franz, 1975; Green et al., 1976 and 1977; Bressani et al., 1979) has verified these results.

Since soybeans are known to contain antinutritional factors, most important of which are trypsin inhibitor and lectins (hemagglutinins), it was thought that it would be desirable to determine effect of different processing parameters on inactivation of these factors when preparing tortillas by the method described. Previous related work by Bressani et al. (1979) showed that trypsin inhibitor content of 85/15 corn-soybean blends decreased with cooking time in limewater, with total inactivation occurring after 30 min. It was also found that trypsin inhibitor inactivation was apparently unaffected by lime concentration. These authors, however, did not investigate all processing parameters involved in tortilla preparation, and particularly did not consider lectins which, like trypsin inhibitor, are known to be antinutritional factors. Moreover, some tortilla making processes are known to involve a low degree of heat treatment, and such aspects were also not considered.

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Del Valle and Perez-Villaseñor (1974) gave a complete description of the traditional process used for making tortillas. Briefly, the process involved cooking corn in limewater, turning off the heat after cooking and letting the cooked corn steep in the cooking liquor, decanting the liquor, washing the corn several times with fresh water and grinding the corn, usually in a stone mill into a dough or "masa." Thin pancakes prepared from the dough, either by hand or pressing in a tortilla-making machine, were then cooked on a hot plate. Normally, one side of the pancake was cooked for a short time, then turned over and the other side cooked for approximately the same time. The pancake was finally turned over again and the first side cooked once more, for the same length of time.

Wide variations in processing parameters were found when this process is applied in practice. Thus, limewater concentration, cooking time in boiling water, soaking time after cooking, number of washings of cooked corn with fresh water and hot plate cooking time can all vary within fairly wide limits. In corn-soybean tortillas, all of these factors, obviously, could have an effect on antinutritional factor content of the final product.

In this work, a thorough study of the effect of principal tortilla-mkaing parameters and variations of tortilla-making processes on trypsin inhibitor and lectin contents of corn-soybean blends and tortillas was carried out.

MATERIALS & METHODS

THE EXPERIMENTAL WORK was divided into three parts. In the first part, effect of limewater cooking parameters was investigated; in the second part, effect of hot plate cooking per se was studied; finally, in the third part, effect of two tortilla-making processes on trypsin inhibitor and lectin content was investigated: one with normal and one with minimum degree of heat treatment. It might be noted that most tortilla-making processes encountered in practice fall within the two procedures of the third part. This part of the study was also used to determine effects of soaking after cooking, and washing and grinding after soaking, on trypsin inhibitor and lectin contents of cooked corn-soybean blends.

Effect of limewater cooking

In the limewater cooking study, whole raw corn-soybean mixtures with 8% and 16% soya (w/w, dry basis) were cooked in boiling limewater containing 0%, 2% and 4% calcium hydroxide in the manner described by Del Valle and Perez-Villaseñor (1974). Samples were withdrawn after cooking for 0, 15, 30, 45, and 60 min, thoroughly washed with tap water and immediately ground in a laboratory mill. Trypsin inhibitor and lectin contents of the ground samples were determined utilizing the methods of Kakade et al. (1974) and Jaffé et al. (1974). In the case of the 8% soybean blends, pH of samples cooked for 30 and 60 min was also measured after washing and grinding: the cooked samples were mashed into a dough with a pestle inside a mortar, and pH meter electrodes inserted into the dough for pH measurement.

Effects of hot plate cooking

In the hot plate cooking study, the effect of hot plate treatment per se was determined. Raw tortilla pancakes were prepared by mixing raw soybean flour, made by grinding whole raw soybeans in a laboratory mill, with commercial tortilla flour at 8% and 16% levels (w/w, dry basis); adding water to form a dough (120 ml of water per 100g mixed flour); weighing 30-g portions of dough, and

pressing these to a standard size (15 cm diameter) in a hand tortilla-making machine. The pancakes were then cooked on a hot plate, alternating sides, allowing each side to contact the hot plate for 15 seconds. Trypsin inhibitor and lectin contents were determined in tortillas, by the methods previously described, after total contact times of 0, 15, 30, 45, and 60 sec, and reported on a moisture-free basis. Hot plate temperature was controlled at 200°C.

Effect of tortilla-making processes

In the third part of the study, the process with normal heat treatment was investigated as follows. Whole, raw corn-soybean mixtures, with 8% and 16% soybeans (w/w, dry basis), were cooked in limewater containing 2% and 4% calcium hydroxide, as previously described, for 30 min. After cooking, the heat was turned off and the mixtures were allowed to soak in the cooking liquor for 10 hr. The liquor was decanted, the cooked mixtures were washed three times with fresh tap water and ground into a dough using a stone mill. Thirty-gram dough balls were weighed, pressed into standard size (15 cm diameter) tortillas using a hand tortilla machine and cooked on a hot plate, controlled at 200°C, allowing a 15-sec contact time for each side. Samples for trypsin inhibitor and lectin determinations were withdrawn after cooking and soaking, after washing and grinding, and after cooking on the hot plate for total contact times of 0, 15, 30, 45 and 60 sec. The process with minimum heat treatment was similar to that for normal heat treatment, except that the corn-soybean mixtures were not cooked in limewater; instead, the limewater was heated to boiling, the heat was turned off and the mixtures were immediately added and allowed to soak in the limewater for 10 hr. As in the former case, mixtures with 8% and 16% soybeans (w/w, dry basis), treated in limewater containing 2% and 4% calcium hydroxide, were studied. Samples for trypsin inhibitor and lectin content were withdrawn at the same points as before, i.e., after soaking in limewater, after washing and grinding, and after total hot plate contact times of 0, 15, 30, 45 and 60 sec. Trypsin inhibitor and lectins were determined by methods previously described, and reported on a moisture-free basis.

Soybeans utilized were of the *Tropicana* variety. Proximate analysis (AOAC, 1970) and trypsin inhibitor content (Kakade et al., 1975) of both raw materials, corn and soybeans, were determined and are reported on a moisture-free basis.

Table 1—Results of proximate analyses and trypsin inhibitor determinations in whole raw corn and soybeans

COMPONENT	CORN	SOYBEANS
Protein	8.5%	35.5%
Moisture	11.5	7.1
Ash	1.7	5.1
Lipids	5.2	15.6
Crude fiber	2.2	7.5
Carbohydrates	71.0	29.2
Trypsin inhibitor (TIU/MG[a])	1.9	46.3

[a] TIU/MG = Trypsin inhibitor units per milligram sample, dry basis

Table 2—Effect of limewater cooking parameters on trypsin inhibitor and lectin contents of corn-soybean blends

Percent soya in blend	Percent lime in limewater	Cooking time, minutes	Trypsin inhibitor, TIU/MG[a]	Lectins[b] Type of Blood Rabbit	Human
8	0	0	4.6[c]	4	1
		15	0.8	2	1
		30	0	0	0
	2	15	0.6	1	0
		30	0	0	0
	4	15	0	1	0
		30	0	0	0
16	0	0	7.2[d]	6	2
		15	2.5	2	0
		30	0	0	0
	2	15	1.9	2	0
		30	0	0	0
	4	15	0	1	0
		30	0	0	0

[a] TIU/MG = Trypsin inhibitor per milligram sample, dry basis
[b] Maximum dilution of 1:10 corn-soybean blend dispersion (dry basis) in 1% NaCl solution which still agglutinates red blood cells
[c] Calculated value from raw material trypsin inhibitor contents = 5.4 TIU/MG
[d] Calculated value from raw material trypsin inhibitor contents = 9.0 TIU/MG

RESULTS & DISCUSSION

TABLE 1 REPORTS data on proximate analyses and trypsin inhibitor contents of raw corn and soybeans. Tropicana variety soybeans were found to have relatively low protein and fat contents when compared with other varieties, whose corresponding values may be as high as 46% and 26%, respectively, with average values of approximately 40% and 20% (Smith and Circle, 1978). Trypsin inhibitor content of these soybeans, on the other hand, was considered normal (Rackis, 1974). It is interesting to note that raw corn was found to possess some trypsin inhibitor activity, although this was, of course, very low.

Limewater cooking study

Table 2 contains data on the limewater cooking study. Trypsin inhibitor content of uncooked corn-soybean mixtures was directly proportional to soy content, and closely paralleled values calculated considering inhibitor content of raw materials and mixture soybean level. Both trypsin inhibitor and lectin contents decreased with increasing cooking time, verifying Bressani et al.'s (1979) results as far as trypsin inhibitor is concerned. Also, in all cases, trypsin inhibitor and lectin levels were reduced to zero after cooking for 30 min, irrespective of mixture soy level or lime concentration, again verifying Bressani et al.'s (1979) results in the case of trypsin inhibitor. Since processes utilized for manufacture of tortillas or tortilla flour, both domestic and industrial, normally employ limewater cooking times of the order of 30 min, these results indicate that tortillas utilizing these processes would probably be free from both trypsin inhibitor and lectins.

An important finding was that increasing lime concentration accelerated trypsin inhibitor inactivation; this was consistently observed in mixtures containing both levels of soybeans and for the three lime concentrations studied. These results are in disagreement with those of Bressani et al. (1979), who found that lime dose had no effect on heat inactivation of trypsin inhibitor when cooking corn-soybean mixtures in limewater. One possible explanation for this discrepancy might be that in this study, fairly high lime concentrations were utilized (up to 4%), while Bressani et al. (1979) employed a maximum concentration of only 2%. It is interesting to note, however, that in this study a definite effect of lime concentration was observed even at the 2% level. Another, although less plausible, explanation could lie in the different procedures employed for determining effect of lime dose on trypsin inhibitor. Bressani et al. (1979) measured inhibitor in soybeans manually separated from corn in limewater cooked mixtures, while in the present study inhibitor content was measured in the total mixtures themselves. Presence or absence of corn when determining inhibitor content could have affected results, although this possibility was not checked in the present work. If this were the case, however, no explanation for the phenomenon is apparent at this time.

A possible explanation for the effect of lime concentration on heat inactivation of antinutritional factors observed in this work could be the known fact that proteins are denatured by pH extremes as much as by heat. Both trypsin inhibitor and lectins are proteins, and an increased lime concentration led to increased blend pH (Table 3), with a consequent increase in denaturation or inactivation of these factors.

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The experimental data (Table 2) suggest that increasing lime concentration also accelerated heat inactivation of lectins, although the results are not so clear as those obtained with trypsin inhibitor. An interesting observation is that lectin agglutinating activity to human blood was much lower, and more rapidly destroyed, than to rabbit blood.

Hot plate cooking study

Data obtained in the hot plate cooking study are reported in Table 4. Again, both trypsin inhibitor and lectin contents decreased with increasing cooking time. In this study, however, trypsin inhibitor was reduced to zero only in the case of the 8% soybean blend, and after cooking for 60 sec. Lectin agglutinating activity to human blood was reduced to zero only after heating for 30 sec in both cases (8% and 16% soybeans), but agglutinating activity to rabbit blood was never totally destroyed.

These results are interesting for a number of reasons. It is apparent that hot plate heat treatment was much more effective in destroying antinutritional factors than boiling in limewater. Comparing data in Tables 2 and 4, it may be seen that for both the 8% and 16% soybean blends, boiling in limewater for 15 min was approximately equivalent to heating in the hot plate for 45 sec, since trypsin inhibitor and lectins were reduced to roughly the same levels. This is probably because: (1) heat treatment in the hot plate occurred at a much higher temperature than in boiling limewater (200°C vs 100°C), and it is known that heat inactivation of antinutritional factors increases strongly with temperature (Del Valle, 1981); and (2) raw tortillas cooked on the hot plate had a much higher initial moisture content than raw whole corn-soybean blends boiled in limewater; it is also known that antinurtitional factor heat inactivation rate increases with moisture content (Del Valle, 1981).

The data in Table 4 indicate that hot plate cooking alone would be insufficient to totally inactivate antinutritional factors contained in corn-soybean tortillas, since most tortilla-making processes employ hot plate contact times of the order of 45 sec. This situation, however, would not normally be encountered in practice since, due to previous heat treatment in boiling limewater, in no case would antinutritional factor inactivation depend solely upon hot plate heat treatment. Actually, hot plate heat treatment should be viewed as a safety factor since, as Table 4 shows, a total hot plate contact time of 45 sec would further lower residual trypsin inhibitor and lectins, remaining after cooking in limewater, by at least 75% and 50%, respectively.

Tortillas containing high proportions of soybeans obviously possessed higher levels of antinutritional factors (Table 4); as a result, total inactivation times for these factors would necessarily be higher than those required for tortillas containing lower soybean proportions. Also, as in the case of limewater cooking study (Table 2), human blood agglutinating activity of the lectins was lower, and was destroyed more rapidly, than that of rabbit blood.

Two tortilla-making processes

Data obtained in the tortilla-making processes applying normal and minimum heat treatment are reported in Tables 5 and 6, respectively. As might have been expected, tortillas prepared with normal heat treatment were totally free from trypsin inhibitor and lectins. In the case of blends containing 8% soybeans, boiling in limewater was sufficient to destroy all trypsin inhibitor, all hemagglutinating activity to human blood, and most activity to rabbit blood. With 16% soybean blends, some trypsin inhibitor remained after cooking in limewater which was, however, removed by washing. Table 5 also shows that rabbit blood lectins remaining after cooking were similarly eliminated by washing. These observations are interesting because they show that some trypsin

Table 3—pH of washed and ground 92.8 corn-soybean blends after cooking in limewater for different times

Lime conc in limewater	pH of blend after cooking in limewater for 30 Min	60 Min
0%	6.5	6.5
2%	9.6	9.7
4%	10.0	10.1

Table 4—Effect of hot plate cooking on trypsin inhibitor and lectin contents of corn-soybean tortillas

Percent soya in blend	Cooking time sec.[a]	Trypsin inhibitor, TIU/MG[b]	Lectins[c] Type of Blood Rabbit	Human
8	0	4.6	4	1
	15	2.7	3	0
	30	1.6	2	0
	45	0.9	2	0
	60	0	2	0
16	0	7.2	6	2
	15	5.2	4	1
	30	2.9	3	0
	45	2.0	3	0
	60	1.0	3	0

[a] Total cook ng time, alternating sides, 15 seconds each side
[b] TIU/MG = Trypsin inhibitor units per milligram sample, dry basis
[c] Maximum dilution of 1:10 corn-soybean blend dispersion (dry basis) in 1% NaCl solution which still agglutinates red blood cells

inhibitor and lectins (although not in very large amounts) are probably leached out into the liquor during cooking, and are subsequently removed by washing. It might be noted that reductions in trypsin inhibitor and lectins after washing and grinding, as reported in Tables 5 and 6, have been attributed wholly to washing because it was found that dough temperature after grinding was too low (35°C) to assign any significant antinutritional factor reduction to grinding.

The process with minimum heat treatment (Table 6) showed totally different results. The procedure of heating limewater to boiling, turning off the heat, adding whole raw corn-soybean mixtures and letting them soak for 10 hr produced relatively little reduction of both antinutritional factors. This treatment – i.e., soaking for different periods of time in limewater initially at boiling temperature – is similar to that which would be encountered in processes with preliminary limewater cooking. It may be concluded that soaking in cooking liquor, per se, would probably be of limited effectiveness in reducing antinutritional factor activity in normal tortilla-making processes due, most probably, to the low heat applied, since liquor temperature begins to drop after the heat is turned off. It is conceivable that in some processes liquor temperature could drop at a slower rate than in normal ones, so that an important future experiment would be to measure rate of temperature drop of soaking liquor and correlate it with effectiveness in inactivation of antinutritional factors.

As in the process with normal heat treatment, washing and grinding produced a small reduction of both trypsin inhibitor and lectins remaining after soaking. With the exception of tortillas containing 8% soybeans, however, trypsin inhibitor remaining after washing and grinding could not be totally eliminated by hot plate cooking. Fortunately, the residual activity of this factor in fully cooked tortillas was too low to be of any significance (Liener, 1979).

Table 6 clearly shows that, for equal soybean levels, blends cooked in higher limewater concentration exhibited faster hot plate inactivation rates for trypsin inhibitor than

Table 5—Effect of process with normal heat treatment on tryps in inhibitor and lectin contents of corn-soybean tortillas

Percent soya in blend	Percent lime in limewater[a]		Trypsin inhibitor, TIU/MG[b]	Lectins[c] Type of Blood Rabbit	Human
8	2	Raw blend	4.6	4	1
		After cooking and soaking[a]	0	1	0
		After washing and grinding	0	0	0
		After cooking on hot plate[d]			
		15 Sec	0	0	0
		30 Sec	0	0	0
		45 Sec	0	0	0
		60 Sec	0	0	0
	4	Raw blend	4.6	4	1
		After cooking and soaking[a]	0	0	0
		After washing and grinding	0	0	0
		After cooking on hot plate[d]			
		15 Sec	0	0	0
		30 Sec	0	0	0
		45 Sec	0	0	0
		60 Sec	0	0	0
16	2	Raw blend	7.2	6	2
		After cooking and soaking[a]	0.9	1	0
		After washing and grinding	0	1	0
		After cooking on hot plate[d]			
		15 Sec	0	0	0
		30 Sec	0	0	0
		45 Sec	0	0	0
		60 Sec	0	0	0
	4	Raw blend	7.2	6	2
		After cooking and soaking[a]	0.8	1	0
		After washing and grinding	0	0	0
		After cooking on hot plate[d]			
		15 Sec	0	0	0
		30 Sec	0	0	0
		45 Sec	0	0	0
		60 Sec	0	0	0

[a] Blend was cooked for 30 min in boiling limewater, heat was turned off and blend was soaked in liquor for 10 hr
[b] TIU/MG = Trypsin inhibitor units per milligram sample, dry basis
[c] Maximum dilution of 1:10 corn-soybean blend dispersion (dry basis) in 1% NaCl solution which still agglutinates red blood cells
[d] Total cooking time, alternating sides, 15 sec each side

Table 6—Effect of process with minimum heat treatment on trypsin inhibitor and lectin contents of corn-soybean tortillas

Percent soya in blend	Percent lime in limewater[a]		Trypsin inhibitor, TIU/MG[b]	Lectins[c] Type of Blood Rabbit	Human
8	2	Raw blend	4.6	4	1
		After soaking[a]	3.1	3	1
		After washing and grinding	2.4	2	0
		After cooking on hot plate[d]			
		15 Sec	2.1	1	0
		30 Sec	1.9	0	0
		45 Sec	1.2	0	0
		60 Sec	0.8	0	0
	4	Raw blend	4.6	4	1
		After soaking[a]	3.0	3	1
		After washing and grinding	2.5	2	0
		After cooking on hot plate[d]			
		15 Sec	1.4	1	0
		30 Sec	0.9	0	0
		45 Sec	0	0	0
		60 Sec	0	0	0
16	2	Raw blend	7.2	6	2
		After soaking[a]	6.4	4	2
		After washing and grinding	6.1	2	1
		After cooking on hot plate[d]			
		15 Sec	4.6	1	0
		30 Sec	3.1	0	0
		45 Sec	2.7	0	0
		60 Sec	2.2	0	0
	4	Raw blend	7.2	6	2
		After soaking[a]	6.9	2	0
		After washing and grinding	6.7	1	0
		After cooking on hot plate[d]			
		15 Sec	4.4	0	0
		30 Sec	2.6	0	0
		45 Sec	1.9	0	0
		60 Sec	1.3	0	0

[a] Limewater was heated to boiling, heat was turned off, blend was added and soaked for 10 hr
[b] TIU/MG = Trypsin inhibitor units per milligram sample, dry basis
[c] Maximum dilution of 1:10 corn-soybean blend dispersion (dry basis) in 1% NaCl solution which still agglutinates red blood cells
[d] Total cooking time, alternating sides, 15 sec each side

those cooked in lower concentrations. This effect is the same as that observed in the limewater cooking experiments, which was previously explained as being due to additional protein denaturation by higher pH's. This was the only case in which effect of lime dose on hot plate heat treatment could be studied, since tortillas prepared directly from cooked corn-soybean mixtures were employed; in the hot plate cooking experiments previously discussed (Table 4), tortillas were prepared from industrial tortilla and raw soybean flours.

SUMMARY & CONCLUSIONS

EFFECTS of processing parameters on trypsin inhibitor and lectin contents of corn-soybean tortillas prepared by lime cooking of whole raw corn-soybean mixtures may be summarized as follows.

(1) Cooking time in boiling limewater and on the hot plate produces the largest reduction of both trypsin inhibitor and lectins. Due to higher temperature and higher initial blend moisture content involved, hot plate cooking exhibits higher inactivation rate than limewater boiling. Total reduction of antinutritional factors is higher when boiling in limewater, however, due to considerably longer processing time employed as compared with hot plate cooking.

(2) Trypsin inhibitor and lectin inactivation rates on boiling in limewater appear to increase with increasing lime concentration due, probably, to additional protein denaturation by higher pH's.

(3) Soaking in cooking liquor after boiling in limewater and turning off the heat results in relatively little reduction of trypsin inhibitor and lectins, because temperature begins to drop after the heat has been turned off.

(4) Some trypsin inhibitor and lectins appear to be leached out into the liquor during cooking and removed by subsequent washing. This effect, however, produces relatively little reduction of both antinutritional factors.

(5) Grinding of cooked corn-soybean mixtures into a dough involves relatively low temperatures and produces very low temperature rise; consequently, this process prob-

—Continued on page 252

Effect of Thermal Processing and Agitation in 100% Steam on the Seal Strength of Retortable Pouches

R. A. ROOP, J. S. MARKS, and P. E. NELSON

ABSTRACT

Retortable pouch seals were tested before, during and after rotary and still processing in 100% steam. Evaluation included tensile, compression, and static load testing. As expected, thermal processing had an effect on the pouch material, but rotary agitation did not affect seal strength.

INTRODUCTION

ALL FOOD CONTAINERS intended to preserve products at ambient temperatures must be sealed properly. Though one function of the seal is to keep product contained, the most important function is to prevent microbiological recontamination.

Retort pouches must have high quality seals for these same reasons. The flexible nature of these packages presents problems not associated with rigid containers. Pouches undergo flexing, bending, and stretching during processing which causes special concern over seal integrity.

Care must be taken to prevent the internal pressure of retort pouches from greatly exceeding the external pressure. Pouches cannot withstand large internal pressure due to their flexible nature and seal strength. Though racking restricts expansion beyond a maximum, it does not prevent bursting. To avoid this problem, pouches have been processed using hot water with overriding air pressure or a steam-air mixture (Lampi, 1977; Pflug et al., 1963; Pflug, 1964). Processing with 100% steam allows the internal pressure to exceed the steam pressure due to the partial pressure of trapped air (Davis et al., 1960; Pflug et al., 1963). Steam-air and water with overriding pressure have been recommended by many researchers (Pflug et al., 1963; Rubinate, 1964; Lampi, 1977; Whitaker, 1971). This does not, however, mean that 100% steam cannot be used. Rubinate (1964) recommended that pouches processed in 100% steam at 121°C have no more than 10 ml of residual air. Whitaker (1971) calculated pressure differentials for 0.5 lb of product and 5 ml of trapped air in a pouch that had an expansion volume of 35 ml. He heated them to 126.7°C and concluded that overriding air was not necessary under those conditions, except during cooldown. Davis et al., (1960) measured internal pressures of pouches during processing using a differential manometer. They concluded that the maximum pressure differences occurred during cooldown.

Steam is the most efficient heating medium currently available under normal production conditions. Pure steam has less heating variation than either of the other media (Lampi, 1977). Pflug (1964) stated, "In comparing the temperature distribution study one outstanding conclusion can be made, heating in 100% steam is in a class by itself as far as uniformity and rapidity of retort come-up; the other heating media. . . are all less efficient than 100% steam because a longer come-up time is required and wider ranges in temperature exist during the come-up time."

The purpose of this study was to test the seal strength of retort pouches and to determine the effects of heat and agitation on seal strength during processing in 100% steam.

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MATERIALS & METHODS

Tensile strength

Twelve retort pouches (130 mm x 178 mm) made from laminated polyester/aluminum foil/polypropylene (Reynolds Metals, foil thickness = 0.00035 mil) were filled with 180 ml of H_2O and sealed with a vacuum sealing machine (Swiss Vac Model 500, Transvac Maschinen Ag, Kriens/Lupen, Switzerland) at 325 mm Hg vacuum to minimize residual air. Four pouches were given a rotary cook (reel speed = 6.5 rpm) and 4 were still cooked, horizontally, for 25 min at 121°C in a FMC Steritort with 100% steam. These pouches were confined during the process in a cylindrical rack described by Roop and Nelson (1982). The remaining four pouches received no treatment. After heating, the pouches were emptied and cut into strips 2 cm wide and 7 cm long. The seal was centrally located in each of these strips. Each strip was labeled as either the manufacturer's bottom seal, or Swiss Vac top seal.

Pouch Strips were randomly selected and pulled apart with an Instron Universal Testing Machine. The strips were aligned between the clamps and the tests were conducted at a crosshead speed of 2.54 cm/min. Tensile strength was recorded as the maximum force recorded before strip failure. Results were analyzed statistically by Analysis of Variance (ANOVA).

Compression testing

Compression load testing was performed on 44 pouches, of the same material, measuring 130 mm x 178 mm. These pouches were filled with 180 ml H_2O and sealed at 325 mm Hg vacuum to minimize residual air. Eleven of these pouches were confined in the cylindrical rack and processed for 25 min in 100% steam at 121°C using a FMC Steritort at a reel speed of 6.5 rpm. Eleven pouches were rotated at 6.5 rpm for 25 min at room temperature and 11 pouches were cooked horizontally in the rack without agitation at 121°C for 25 min. The remaining 11 pouches received no treatment. After treatment, pouches were tested for compression strength with the Instron.

A compression burst cell was constructed from "Plexiglas" (Fig. 1). It consisted of a 134 mm x 176 mm ram which could be lowered into a "Plexiglas" box with very close tolerances. The box rested upon a load cell which measured the force applied during each test. The pouches were placed horizontally in the box one at a time and the ram was lowered at 0.13 cm/minute until bursting occurred. The maximum force before bursting was recorded. Results were analyzed statistically by ANOVA.

Static load testing

In a third experiment the tensile strength of seals during processing at 121°C was examined. The procedure involved a slight modification of the work by Pflug and Long (1966). One hundred strips, 1 cm wide, were cut from unheated pouches of the same material, so the seal was centrally located. Each end of a strip was rolled onto a 3.2 mm diameter wooden rod cut to 13 mm length. One end was attached to a stationary rack and a weight was hung on the opposite end. Weights of 200, 300, 400, 500, and 600 grams were used. Strips with weights were autoclaved for 30 min at 121°C. The strips were examined after heating and classified as failure or nonfailure.

Table 1 —Tensile strength (kg) of pouch seals after processing

	Treatments[a]											
Seal[b]	A1	A2	A3	A4	S1	S2	S3	S4	N1	N2	N3	N4
MS	3.97	3.91	3.79	3.94	3.85	4.04	4.07	3.78	3.51	3.71	3.63	3.46
MB	3.15	3.83	3.79	3.82	3.89	3.60	3.82	3.37	3.52	3.65	3.39	3.59
ST	2.77	3.32	3.63	3.23	3.21	3.03	3.50	2.78	2.72	3.98	3.67	3.00

[a] A-Agitated at 121°C; S-Still processed at 121°C; N-No treatment
[b] MS-Manufacturer's side seal; MB-Manufacturer's bottom seal; ST-Swiss Vac top seal

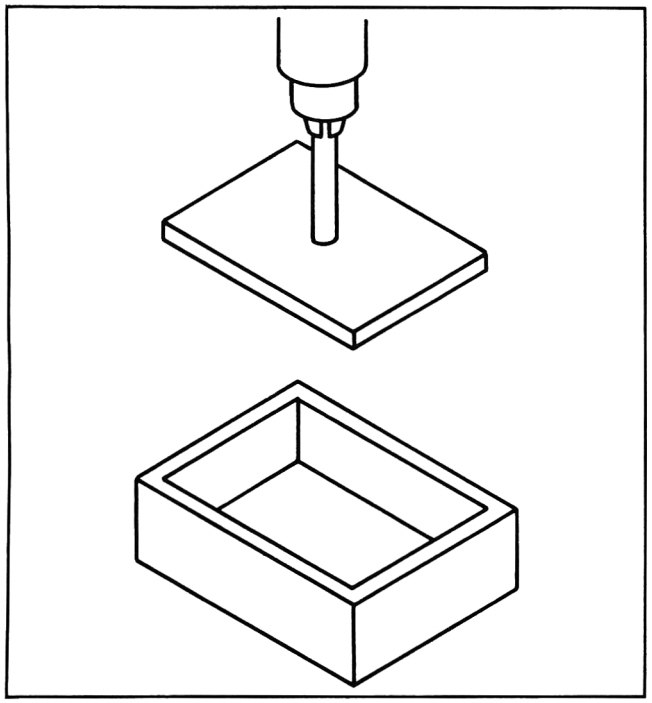

Fig. 1—Cell used to contain pouches during compression testing.

RESULTS & DICUSSION

TABLE 1 lists the tensile strengths of seals after various treatments. The Swiss Vac top seal and manufacturer's bottom seal were compared because they were symmetrically equivalent during processing. The Swiss Vac seals were weaker than the manufacturer's seals when evaluated by ANOVA ($F_{1,18}$ = 7.13, p-value = 0.0102). This difference was expected because the seals were formed under different conditions on different machines. None of the tensile strengths was affected by agitation when compared within seal types ($F_{2,18}$ = 0.037, p-value = 0.964). Because all pouches were from the same source, there was no effect due to material differences. During the rotary process, the side seals received most abuse because the motion was side over side, not end over end. When all of the manufacturer's side seals processed under varying conditions were compared, a large difference in strengths was detected ($F_{2,11}$ = 11.94, p-value = 0.0029). Upon further examination of the data using the Student-Newman-Keuls' (SNK) range test, no difference was found between manufacturer's side seals processed by either rotary or still cooks. The manufacturer's side seals of the unheated samples, however, showed significantly lower tensile strength than those of the other two samples at α = 0.05 level of confidence. The heat processed pouches were less flexible than the untreated pouches. Heating evidently altered the physical characteristics of the pouch polymers resulting in greater tensile strength. In each case, the pouch material failed before the manufacturer's seal. The seals, therefore, were stronger than the pouch material.

Compression test results are listed in Table 2. Large differences were found between compression strengths of

Table 2—Compression burst force of pouches after processing

Process type	Temperature 23.8°C				121°C			
	kg				kg			
Rotary process	449	499	506	492	370	265	381	399
	487	503	490	465	306	340	240	422
	474	422	494		315	449	442	
Still process	431	463	522	533	404	363	374	472
	458	463	522	535	385	381	385	451
	481	415	458		490	415	317	

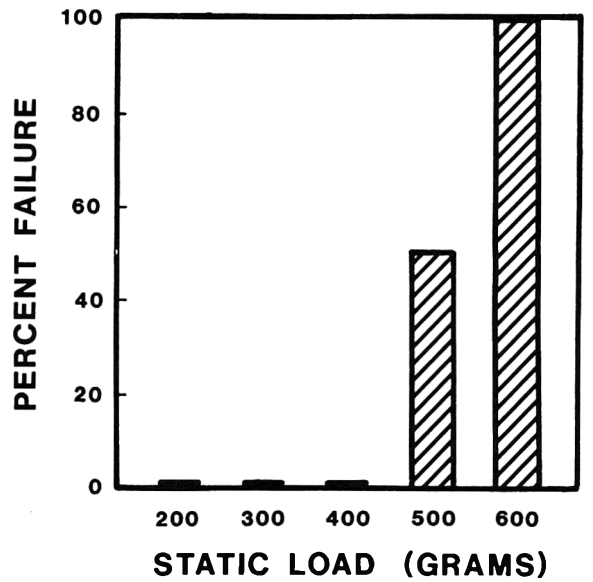

Fig. 2—Percent failure of retort pouch seal strips experiencing various static loads at 121°C for 30 min.

heated and unheated pouches ($F_{1,40}$ = 44.22, p-value = 0.001). The unheated samples were stronger than the heated samples. The opposite was true of the tensile strengths. This conflict apparently resulted from the difference between the two test procedures. Tensile strength testing applied force to the strip perpendicular to the seal only. Compression testing applied force in all directions. The compression strength, therefore, was equal only to the strength of the weakest point of the entire pouch. The reduced strength after heating does not mean that the pouches were unacceptable. The weakest pouch withstood a force of 265 kg. Strength of pouches subjected to agitation showed no significant difference from those that were not agitated during sterilization ($F_{1,40}$ = 2.4, p-value = 0.132).

Results of the static stensile strength test at 121°C are illustrated graphically in Fig. 2. The strength at 121°C was found to be at least 400g per cm of seal width. Pre-process strength was regained upon cooling as verified by the previous post-process tensile strength evaluations. The combined results are shown in Fig. 3. These experiments support the literature (Beverly, 1979; Corning, 1979) that care must be exercised during processing to prevent pouch failure.

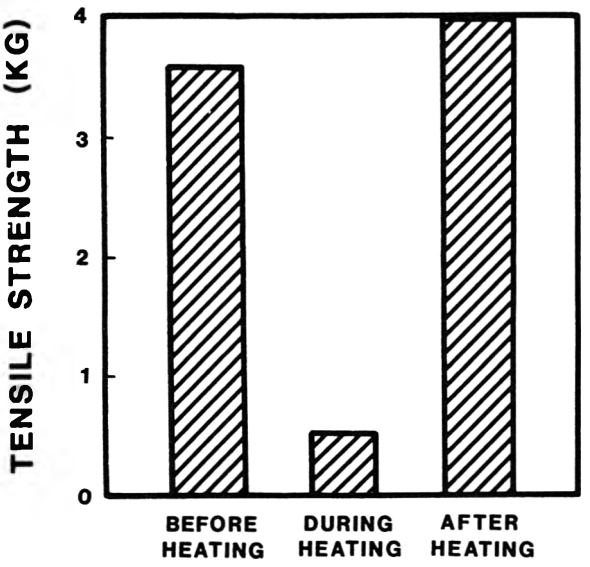

Fig. 3—Comparison of retort pouch seal strip tensile strength with respect to heat exposure.

CONCLUSIONS

THE SEAL STRENGTH of retort pouches containing water was not affected by agitation and processing in 100% steam. Failures during testing were due to the material strength strength being lower than the seal strength. This indicated that seals were sufficiently strong to withstand extreme stress situations. Further research is suggested using actual food products.

Thermal processing increased the tensile strength yet decreased the compression strength. This conflict of data demonstrates the necessity of using several tests when drawing conclusions on seal strength or pouch integrity.

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TORTILLAS FROM CORN-SOYBEAN MIXTURES. . . From page 249

ably results in negligible change in trypsin inhibitor and lectins.

(6) In all experiments performed, lectin agglutinating activity to human blood was appreciably lower and more easily destroyed by heat treatment or increasing lime concentration, than to rabbit blood.

(7) Tortillas prepared by limewater cooking of whole raw corn-soybean mixtures by the traditional method would probably be free from trypsin inhibitor and lectins if normal heat treatment, resulting from employment of usual processing conditions (i.e., boiling in limewater for 30 min and cooking 45 sec on a hot plate, alternating sides, 15 sec each side) were applied. In this case most antinutritional factor activity would be destroyed by limewater cooking, with additional heat treatment applied on the hot plate serving as a safety factor. If, on the other hand, a heat treatment lower than normal were applied, tortillas with residual trypsin inhibitor, but no lectins, could result. Trypsin inhibitor level, however, would probably be too low to be of any significance.

It can be concluded that, because of the reduction of the concentration of trypsin inhibitor and lectins, corn-soybean tortillas prepared by the method described in this paper would probably be suitable for human consumption.

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Effects of Heat and Hydrogenation on Cyclopropenoid Fatty Acid Composition of Baobab (*Adansonia Suarezensis*) Seed Oil

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ABSTRACT

The seeds of the baobab *Adansonia suarenzensis* (Bombacaceae) contain 46.2% oil used for cooking by the malagasy population. This oil gives a positive Halphen test showing the presence of cyclopropenoic fatty acids (CPEFA). Composition analysis of derivatized fatty acid methyl esters was done by gas-liquid chromatography (GLC). The *A. suarenzensis* seed oil contains mainly palmitic (46%) stearic (3.5%) oleic (21%) and linolenic (12%) acids. Effect of heat on CPEFA content of boabab oil was studied at 133°C and 180°C. Major decomposition of CPEFA at 180°C shows that deodorisation would be the main step in the oil refining process. Hydrogenation of the baobab oil using an industrial hydrogenating catalyst results in CPEFA content decreasing rapidly.

INTRODUCTION

BAOBAB SEED OIL is largely consumed as edible oil by the Malagasy population and many hundred tons of seeds are processed yearly in local oil plants for this purpose. The baobab trees belong to the genus *Adansonia* (Bombacaceae family) and are widespread in Madagascar (Perrier de la Bâthie and Hochreutiner, 1955). The seeds of one of them, *A. suarenzensis*, an endemic Malagasy baobab, are largely used for cooking after some refining. There has been no previous work on the fatty acid composition of the oil of these seeds.

Cyclopropenoic fatty acids (CPEFA) such as malvalic (8,9-methylene-heptadec-8-enoic) and sterculic (9,10-methylene-octadec-9-enoic) acids are commonly found in higher plant families specially in Malvaceae, Sterculiaceae and Bombacaceae. These results were reviewed by Carter and Frampton (1964) and Phelps et al. (1965). The presence of CPEFA was not reported in *Adansonia* seeds by Thomas and Boiry (1913), Cmelik (1963) and Bahieldin and Moniemi (1979), although it was said in earlier investigation (Carter and Frampton, 1964) that these oils gave a positive Halphen test (Halphen, 1897) which is characteristic of the occurence of CPEFA in oils (Bianchini et al., 1981). Since these CPEFA are responsible for some physiological disorders in fowl (Phelps et al., 1965) and should have some cocarcinogenic properties (Sinnhuber et al., 1968; Lee et al., 1971) it would be unwise to consume such oils. Procedures for reducing or removing cyclopropenoids from oils have been developed by Nordby et al. (1962) and Berry (1980a, b). Crude cottonseed oil, which contains CPEFA, heated at 150°C for 4 hr or 200°C for 1 hr did not give a positive Halphen reaction (Evans et al. 1960). Hydrogenation studies (Wilson et al., 1961) of oils containing both sterculic and malvalic acids showed that prolonged hydrogenation in ethanol produced cyclopropanoic fatty acids (CPAFA) such as dihydrosterculic, dihydromalvalic and branched-chain products.

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Our work was done in three parts. In the first or identification phase, the occurrence of CPEFA and CPAFA in *A. suarenzensis* seed oil was studied with different methods which permit the assay of CPEFA. In the second and the third part, effects of temperature and hydrogenation upon the CPEFA stability of baobab oil were determined.

MATERIALS & METHODS

Materials

The seeds of *A. suarenzensis* were collected in Antsiranana (Madagascar) area in 1979. Standard specimens of malvalic, sterculic and dihydrosterculic acids were obtained from kapok seed oils (Bianchini et al., 1981). Fatty acid methyl ester reference standards were obtained through Sigma Chemical Company. Hydrogenating catalyst was obtained through Königswarter (G.F.R.). All other reagents used were of analytical grade.

Extraction of oil and analyses

The oven-dried seeds were ground into a fine powder and extracted with petroleum ether (bp 40–60°C) in a Soxhlet apparatus. The solvent was removed using a rotary evaporator under reduced pressure. The *A. suarenzensis* seeds contained 46.2% oil. The Halphen color test, proton magnetic resonance (PMR), and hydrobromic titration of CPEFA were performed according to the method described previously (Bianchini et al., 1981).

Preparation of fatty acid methyl esters and silver nitrate derivatives

Methyl esters of the oil fatty acids were prepared by transmethylation using sodium methoxide in methanol as described by Luddy et al. (1960). The methyl esters containing CPEFA were either used for direct gas-liquid chromatography or reacted with methanol saturated with silver nitrate according to the method described by Schneider et al. (1968), to obtain stable CPEFA ester derivatives. The normal fatty acid esters and the CPEFA ester derivatives were recovered from the reaction mixture in the usual manner.

Gas-liquid chromatography

An Intersmat IG 12 DFL gas chromatograph equipped with a flame ionization detector and a glass injector was used for the analysis. Two glass capillary columns were employed: a 45m long 0.35 mm i.d. coated with Carbowax 20M and a 20m long, 0.40 mm i.d. coated with BDS. Temperatures used were 190°C for the Carbowax 20M column and 150°C for the BDS column: 200°C for the detector and 220°C for the inlet. The flow-rate of hydrogen used as carrier gas was 5 ml/min with a split ratio of 5/100.

Gas chromatograph peaks were identified by comparison with pure methyl esters through their relative retention times under identical conditions. Kapok seed oil was used as reference standard to identify CPEFA and CPAFA methyl esters. The area of each peak was obtained on a Spectra-physics minigrator linked directly to the gas chromatograph.

Effect of heat

The effect of heat on CPEFA of *A. suarezensis* was studied in the following manner: 50 mg of fatty acid methyl esters were put under nitrogen in glass-sealed tubes. The temperature of the tubes was raised to 133 ± 1°C or 180 ± 1°C in a drying oven. The composition of fatty acid methyl esters was controlled at regular intervals of time by GLC using the Carbowax 20M column at 190°C.

–Continued on next page

Hydrogenation

In a glass autoclave (50 mm x 10 mm i.d.) 2g of fatty acid methyl esters of baobab seed oil and 16 mg of hydrogenating catalyst (75% of saturated triglycerides and 25% of Nickel) were brought to 155 ± 1°C, under 0.3–1.0 bar of hydrogen pressure. Aliquot parts were removed at regular time intervals and evaluated by GLC using the Carbowax 20M column at 190°C.

RESULTS & DISCUSSION

Occurrence of cyclopropene fatty acids (CPEFA)

The oil and the fatty acid methyl esters prepared by sodium methoxide transesterification gave a positive Halphen color test, thereby indicating the presence of CPEFA. The oil showed the typical PMR signal at 9.2τ for the cyclopropene moiety (Pawlowski et al., 1972). The methyl esters of the oil had a characteristic infrared (IR) band at 1010 cm^{-1} and a weak band at 1852 cm^{-1} for cyclopropene moiety. There was no indication in the spectrum of an hydroxyl or terminal acetylenic group. The ultraviolet (UV) spectra (228–315 nm) indicated no conjugation in the oil. GLC analysis of the methyl esters containing CPEFA treated with methanol saturated with silver nitrate, showed the presence of malvalic and sterculic ester derivatives in addition to normal fatty esters by a comparison of the equivalent chain length values (Bianchini et al., 1981). Methyl esters prepared from the oil had the composition, determined, on glass capillary columns, shown in Table 1. The saturated fatty acids formed 50% of the total fatty acid composition of the *A. suarenzensis* sample investigated, with the predominance of palmitic (46%) and stearic (3.5%) acids. The mono- and polyunsaturated fatty acid contents were 23 and 13% respectively, with oleic (21%) and linoleic (12%) acids predominating. CPEFA amounted to 11% and consisted of malvalic (7%) and sterculic (4%) acids. CPAFA were also present and dihydrosterculic acid (19:CA) came up to 1.8% of the total fatty acid profile.

Table 1—Fatty acid composition of the baobab (A. suarenzensis) seed oil after reaction of their methyl esters with $AgNO_3$ in methanol, as determined by gas-liquid chromatography.

Fatty acid	ECL[a]	Area % by GLC
Saturated		
C 14 : 0	14.00	0.1
C 15 : 0	15.00	0.1
C 16 : 0	16.00	45.6
C 17 : 0	17.00	0.3
C 18 : 0	18.00	3.5
C 20 : 0	20.00	0.5
C 22 : 0	22.00	0.1
Monounsaturated		
C 16 : 1 ω 7	16.29	0.3
C 17 : 1	17.21	0.7
C 18 : 1 ω 9	18.21	21.2
C 18 : 1 ω 7	18.27	1.2
C 20 : 1 ω 9	20.13	tr
Polyunsaturated		
C 17 : 2	17.66	0.3
C 18 : 2 ω 6	18.66	12.5
C 18 : 3 ω 3	19.29	0.4
Cyclic		
C 18 : CE	b	7.0
C 19 : CE	c	4.3
C 19 : CA	19.23	1.8

[a] Equivalent chain lengths determined on wall-coated open tubular glass capillary column at 190°C (Carbowax 20M).
[b] Four peaks at ECL 20.37, 20.42, 21.70 and 21.82.
[c] Four peaks at ECL 21.34, 21.39, 22.70 and 22.80.

Direct GLC analysis of methyl esters, without derivatization with silver nitrate showed on chromatograms two new peaks which were attributed to methyl malvalate (18:CE) and methyl sterculate (19:CE) according to Bianchini et al. (1981). The total CPEFA content were found to be in good agreement with hydrobromic titration (11.7%), PMR titration (12.2%) and GLC analysis 12% (Bianchini et al. (1981). As essentially the same results were obtained on the two columns only those for the carbowax column were given in this work.

The presence of CPEFA and CPAFA was not reported in *Adansonia* seeds (Cmelik, 1963; Bahieldin and Moniemi, 1979). Since CPEFA have some toxicological effects upon experimental animals (Scarpelli, 1974; Ferguson et al., 1976) the consumption of this oil may be a health hazard for the population.

Effect of heat on CPEFA of baobab oil

Conventional cooking methods such as boiling in water and frying in cooking oil may modify the fatty acid composition, since it is known that cyclopropene ring is quite unstable (Carter and Frampton, 1964). The isolated cottonseed oil and sterculic acid, are reported not to respond to the Halphen test and exhibited no biological activity when subjected to temperatures above 200°C (Evans et al., 1960; Norby et al., 1962). Berry (1980a) did not observe the disappearance of CPEFA when the seeds and leaves of *Gnetum gnemon* were cooked in boiling water. Therefore, we investigated the effect of temperature on CPEFA content in baobab oil at 133°C and 180°C. The results, obtained at different time intervals, are given in Table 2. The decomposition of CPEFA (malvalic, 18:CE + sterculic, 19:CE) was relatively great, at 180°C since the malvalic + sterculic content, which was 10.5%, decreased to 3.3% (Table 2). At 133°C the decomposition is less and 8.9% of CPEFA was recovered after 24 hr heating. The composition of dihydrosterculic acid (19:CA) is relatively constant either at 133°C or at 180°C. CPAFA are not responsible for physiological disorder in animals. During these trials unknown products were not formed (besides polymerisation of CPEFA) and the composition of all normal fatty acids increased slightly because of the disappearance of CPEFA.

This process seems to be a convenient method for the elimination of CPEFA in baobab seed oil for the preparation of edible oil. The deodorization step will be the one that causes greatest loss of CPEFA content during the oil refining. Such results were observed in the case of cottonseed oil processing, by Harris et al. (1964).

CPEFA hydrogenation of baobab seed oil

Shenstone and Vickery (1959) showed that hydrogenation destroyed the biological activity of malvalic and sterculic acids. Prolonged hydrogenation studies of oils containing CPEFA (Wilson et al. 1961) showed that dihydroderivatives and branched-chain products, but none having straight chains, was produced. The fatty acid composition was followed by GLC at regular time intervals. These fatty acid profiles are given in Table 3. CPEFA content (10.5%) steadily decreases during hydrogenation to 1.8% after 160 min reaction time. Dihydrosterculic acid gradually increases from 1.6% to 3.1% after 2 hr reaction time and then decreased to 1.5%. This fact can be explained because one mole of hydrogen is fixed in the double bond of sterculic acid to give dihydrosterculic acid. This compound attracts another hydrogen molecule to give, theoretically, a mixture of two branched acids (9- and 10-methyl-octadecanoic) and nonadecanoic acid (Hofman and Lucas, 1950). We have

Table 2—Effect of heat on the fatty acid composition of the baobab (A. suarenzensis) seed oil for various time intervals

Time (hr)	Fatty acids (per cent by wt)												
	0		1		2		4		8		16	24	
Temperatures (°C)	133	180	133	180	133	180	133	180	133	180	180	133	180
Saturated													
Total	48.1	48.1	49.0	48.2	49.1	49.6	48.7	51.0	57.4	52.3	52.3	48.4	52.5
Monounsaturated													
Total	23.5	23.5	24.5	24.2	24.6	24.1	25.3	24.1	25.0	24.8	25.0	24.4	25.5
Polyunsaturated													
Total	16.0	16.0	16.1	16.5	15.6	15.7	15.9	16.0	12.1	16.1	16.5	16.3	17.0
Cyclic													
C 18 : CE	5.2	5.2	4.6	4.5	4.4	4.4	4.5	3.7	2.0	2.6	1.8	4.6	1.6
C 19 : CE	5.3	5.3	4.3	4.8	4.1	4.4	4.0	3.6	1.8	2.5	1.8	4.3	1.7
C 19 : CA	1.6	1.6	1.4	1.7	1.8	1.5	1.5	1.4	1.6	1.5	2,6	1.7	1.7
Total	12.1	12.1	10.3	11.0	10.3	10.3	10.0	8.7	5.4	6.6	6.2	10.6	5.0
Unknown[a]													
18.52	0.1	0.1	0.1	tr[b]	tr	tr	0.1	tr	0.1	tr	tr	0.1	tr
19:15	0.1	0.1	tr	tr	0.1	0.1	tr	tr	tr	tr	tr	0.1	tr
19:77	0.1	0.1	tr	0.1	0.2	0.1	tr	0.1	tr	0.1	tr	0.1	tr
Total	0.3	0.3	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.1	tr	0.3	tr

[a] Equivalent chain lengths determined on Carbowax 20M glass capillary column.
[b] Less than 0.1%.

Table 3—Changes in fatty acid content of the baobab (A. suarenzensis) seed oil during hydrogenation at 155° C with nickel (pressure: 0.3—1.0 bar)

Time (min.)	Fatty acids (per cent by wt)					
	0	20	40	60	120	160
Saturated						
Total	48.1	47.6	47.9	49.2	49.4	50.5
Monounsaturated						
Total	23.5	24.8	25.5	27.9	30.7	36.5
Polyunsaturated						
Total	16.0	16.6	16.0	13.7	10.4	4.7
Cyclic						
C 18 : CE	5.2	4.0	2.8	2.0	1.8	1.5
C 19 : CE	5.3	4.2	3.1	1.4	0.4	0.3
C 19 : CA	1.6	1.9	2.3	2.5	3.8	1.5
Total	12.1	10.1	8.2	5.9	5.3	3.3
Unknown[a]						
16.84	–	–	0.2	0.1	0.2	0.2
16.94	–	–	tr	0.2	0.2	0.2
17.49	–	–	0.9	1.1	1.5	1.6
17.86	–	0.3	0.2	0.3	0.6	0.7
18.52	0.1	tr	tr	–	1.6	1.8
18.60	–	0.4	0.9	1.3	0.1	0.3
18.81	–	0.3	–	0.3	0.2	0.3
Total	0.1	1.0	2.1	3.3	4.4	5.1

[a] Equivalent chain lengths determined on Carbowax 20M glass capillary column at 190° C.

confirmed the results of Wilson et al. (1961) in observing the formation of the straight chain fatty acid which would appear with an Equivalent Chain Length (ECL) value of 19.00. During hydrogenolysis polyunsaturated fatty acids (mainly linoleic) are almost eliminated since the concentration drops from 15.7% to 4.7% for linoleic and from 0.2% to trace for linolenic acids. Monounsaturated fatty acids are mainly represented by oleic acid and its content rises from 21% to 33% (Table 3). This increase essentially comes from the partial hydrogenolysis of linoleic acid. The saturated fatty acid content increases slightly (48% to 50%) during hydrogenation. Unknown compounds are mainly branched chain fatty acids formed during the cyclopropenic ring opening of malvalic acid at ECL value 17.49 (1.6%) and sterculic acid at ECL value 18.52 (1.8%) (Table 3).

During hydrogenolysis of the baobab oil, the content of CPEFA regularly decreases, in the same way as polyunsaturated fatty acids. To produce an edible oil, hydrogenation appears to be an interesting process for the elimination of CPEFA without increasing too much the saturated fatty acid content.

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—Continued on page 259

Effects of Calcium Addition on Stability and Sensory Properties of Soy Beverage

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ABSTRACT

Pasteurized or thermally processed soy beverages (6% soy solids) were fortified to a comparable level of cow's milk with 25 mM (or 30 mM) calcium using mixtures of calcium citrate and tricalcium phosphate. These fortified pasteurized products had acceptable sensory properties. Addition of these calcium salts did not adversely affect protein stability of the beverage. Calcium citrate addition caused a decrease in beverage pH and viscosity. Thermally processed (still retort and agitort) canned beverages containing calcium salts were stable for 6 months when stored at 1°C or at room temperature.

INTRODUCTION

SOY BEVERAGE, or soymilk as it is sometimes called, has been consumed in the Orient for hundreds of years. Recent efforts involving marketing this product in the United States as either a novel beverage or a nonlactose containing milk substitute have met with limited success.

It would be highly desirable for a milk substitute such as soymilk to contain an amount of calcium (Ca) equivalent to cow's milk (25 mM). Soybeans contain approximately 0.20% calcium and thus a beverage consisting of 6% soy solids would contain only 3 mM calcium. Fortification efforts have largely been unsuccessful due to the tendency for calcium to coagulate the protein and cause gelation of the soy beverage during storage.

The objective of this investigation was to determine if mixtures of calcium citrate and tricalcium phosphate [$Ca_3(PO_4)_2$] could be used in the fortification of a stable and acceptable pasteurized and/or sterilized soy beverage. Both of these salts contain biologically available calcium (Stearns and Jeans, 1934-1935; Steggerda and Mitchell, 1946). The major criteria for judging the success of fortification were based on sensory evaluation of beverage flavor and measurement of stability, pH and viscosity during storage.

MATERIALS & METHODS

SOY BEVERAGE was prepared as described by Nelson et al. (1975, 1976, 1977) and Kuntz et al. (1978). Dehulled soybeans (Bonus, 1975) were blanched, ground, diluted with water, homogenized, and mixed with sugar and water. The beverage was divided into aliquots after the sugar and salt were dissolved. Weighed samples were individually reheated to 85°C and calcium salts were added during the reheating step just prior to the second homogenization.

Mixtures of food grade calcium citrate and tricalcium phosphate were added to soy beverage. Pasteurized and sterilized soy beverage products containing these calcium salts were produced from the same batch of soybeans. These products were evaluated for their stability, pH, sensory and viscometric properties.

Sterile canned products were prepared as described by Lutrell et al. (1981) and McCune (1980) using a still retort and agitort. The cans in the still retort were processed for 42 min at 121°C; those in the agitort, for 13 min at 121°C. The thermally processed cans were stored vertically at room temperature or 1°C for later sampling.

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Half-gallon containers of pasteurized soy beverage were stored under quiescent conditions at 1°C for 10 days. Then, approximately 20 ml were removed via pipette from the upper part of the container. A sample was taken from the bottom of the container in the same manner. Duplicate analysis of each of the three samples were made for protein and calcium using the methods of AOAC (1975).

Viscosity of soy beverage was measured using a Model RV-3, rotational HAAKE Rotoviscometer (HAAKE, Inc., Saddlebrook, NJ) equipped with the NV sensor system and a No. 50 measuring head. Samples for viscosity measurements were taken from the middle of containers after shaking. Shear rates of 172, 345, 690, 1380 sec^{-1} corresponding to 32, 64, 128, and 256 rpm were used to measure shear stress. Each soy beverage was measured at least three times at 25°C. Viscosity was determined by the equation: viscosity = shear stress/shear rate. The consistency coefficient (b) and flow behavior index (n) were calculated as described by Forster and Ferrier (1979).

Six to ten staff members of the Dept. of Food Science participated in sensory evaluation of the soy beverages. The panelists had previous experience testing soy beverage, but were otherwise untrained. Samples were warmed to room temperature coded with three digit numbers and presented randomly to panelists.

Triangle tests were conducted during the initial screening process. Panelists were presented with two sets, each containing three samples of soy beverage. One set contained two identical control samples of soy beverage with added calcium.

Further sensory evaluation involved rating soy beverage on a nine point quality judgment scale. Panelists rated each of four samples: a control plus three samples containing combinations of calcium salts for flavor and chalkiness. Flavor is the overall impression of aroma and taste. Chalkiness refers to the sensation of particulate matter in the mouth. The data was subjected to analysis of variance testing (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Sensory evaluation of pasteurized and sterilized products

In triangle tests involving addition of calcium citrate to freshly prepared pasteurized soy beverage, panelists were only able to correctly identify the odd sample 21 out of 52 times (not significant $p < 0.05$). Thus the addition of calcium citrate to the fresh beverage was not detectable by the panelists.

In quality judgement rankings of various fortified and nonfortified fresh products, soy beverage samples containing 30 mM calcium (calcium citrate:tricalcium phosphate = 1:1) had significantly ($P < 0.05$) higher flavor ratings than samples without calcium.

Another quality test, involved ranking calcium citrate and tricalcium phosphate containing soy beverage. Panelists repeatedly commented that they could not detect differences. It was concluded that addition of calcium citrate, tricalcium phosphate, or mixtures of both did not adversely affect the flavor or chalkiness of freshly pasteurized or sterilized soy beverage.

Evaluation of stored pasteurized beverages

Soy beverage products were stored at 1°C for 10 days, following which, protein, calcium content, viscosity and sensory properties were measured (Table 1). The protein concentration in the top and bottom of the container were similar indicating that calcium citrate and tricalcium phos-

phate did not adversely affect beverage stability. Approximately 10 mM calcium from the calcium citrate remained in the top of the beverage. Almost none of the calcium from the tricalcium phosphate remained in solution. This is not unexpected since tricalcium phosphate is virtually insoluble in cold water while calcium citrate is slightly soluble. Sensory panels were unable to distinguish differences in flavor or chalkiness between beverages. The viscometric properties (at shear rate of 690 sec^{-1}, only) of these stored pasteurized soy beverages are depicted in Table 1. There was no significant difference between flow behavior indexes or consistency coefficients of the products tested at each of the four shear rates. However, products containing calcium citrate had lower viscosity than those without calcium citrate. The pH of the stored products was: control, 7.55; both calcium citrate containing products, 7.35; and the 25 mM calcium as $Ca_3(PO_4)_2$ product was 7.63.

Evaluation of stored sterile beverages

Two storage studies were conducted with sterile soy beverage products. Soy beverages containing either 30 mM added calcium (Ca citrate) or no added calcium (control) were thermally sterilized using either a still retort or agitort. The viscosity of the samples was measured after 0, 1, 3, 4, and 6 months storage at 1°C and room temperature. The four products (soy beverage with and without added calcium citrate processed by still retort or agitort) generally decreased in viscosity over the storage period. Table 2 shows data at 0 and 6 months storage for soy products at a shear rate of 690 (sec^{-1}). The initial viscosity values were higher for the products processed via agitort than the retort. Storage temperature did not affect the viscometric properties of the beverage. Calcium addition did not affect the still retort products. However, when compared with the agitort control beverage, the agitort plus calcium sample had a marked decrease in viscosity during storage. The flow behavior index indicated that all four of the products were pseudoplastic.

A separate 6 month storage study was conducted (data not shown) using four thermally processed (by still retort) products: no added calcium (control), 25 mM added calcium [20 mM as Ca citrate plus 5 mM calcium as $Ca_3(PO_4)_2$], 25 mM calcium (as Ca citrate), and 25 mM calcium [as $Ca_3(PO_4)_2$]. The addition of 25 mM calcium as calcium citrate or tricalcium phosphate to soy beverage had little effect on the b and n values. As in the first study, viscosity decreased slightly during storage. Storage temperatures (1°C or room temperature) did not affect viscosity characteristics. The viscosity of the beverage containing a mixture of calcium citrate and tricalcium phosphate was lower than either the control beverage or the products made with only calcium citrate or tricalcium phosphate. After a total storage of 18 months, these products were observed to be free from any major defects.

The pH of the sterilized soy beverages was measured during both storage studies. Thermal processing caused a small reduction in pH as compared with the pasteurized products. McCune (1980) reported a similar trend. There was little additional change in pH over the 6 month storage period.

Taste panelists found no significant difference in flavor or chalkiness between the control sample (no added calcium) and samples containing calcium citrate (25 mM Ca)

—Continued on page 263

Table 1—Stability, viscosity properties and sensory evaluation of pasteurized soy beverages after 10 days storage at 1°C

	Stability[a]		Viscosity properties[b]			Sensory tests[c]	
	Protein (dry) g/100g	Calcium (dry) g/100g	Centipoises	b	n	Flavor	Chalkiness
Control			5.7	0.12	0.89	7.7	7.4
Top	28.1	0.13					
Bottom	30.0	0.16					
Calcium Citrate (15 mM Ca) + $Ca_3(PO_4)_2$ (10 mM Ca)			4.9	0.14	0.86	7.4	7.0
Top	29.4	0.53					
Bottom	28.4	1.40					
Calcium Citrate (20 mM Ca) + $Ca_3(PO_4)_2$ (5 mM Ca)			4.9	0.13	0.85	7.5	7.2
Top	29.1	0.52					
Bottom	29.0	1.20					
$Ca_3(PO_4)_2$ (25 mM Ca)			5.7	0.14	0.87	7.6	7.2
Top	28.8	0.22					
Bottom	29.1	1.30					

[a] Average of two samples
[b] Viscosity, expressed in centipoise, was measured at a shear rate of 690 (sec^{-1}); b = consistency coefficient; n = flow behavior index.
[c] Quality judgment rating (1—9): 1 = poor flavor or very chalky; 5 = acceptable in flavor and chalkiness; 9 = excellent flavor or least chalky. Ten panelists evaluated 4 samples in 5 sessions. There were no significant differences between beverages.

Table 2—Viscosity properties of sterile (agitort and still retort) soy beverages stored at room temperature or 1°C for 6 months[a]

	No Ca Addition						30 mM Ca as Ca Citrate					
	Agitort			Still Retort			Agitort			Still Retort		
Storage Time	Centipoises	b	n	Centipoises	b	n	Centipoises	b	n	Centipoises	b	n
Initial	10.0	0.49	0.76	7.4	0.29	0.80	8.8	0.49	0.74	7.1	0.24	0.82
Six Months												
Room temp	10.0	0.30	0.76	6.4	0.09	0.87	7.7	0.15	0.84	6.5	0.11	0.85
1°C	10.6	0.32	0.76	6.5	0.09	0.88	6.2	0.08	0.88	6.7	0.11	0.86

[a] Viscosity, expressed in centipoise, was measured at a shear rate of 690 (sec^{-1}); b = consistency coefficient; N = flow behavior index.

A Research Note

Effect of Initial Acidity of Plain Yogurt on Acidity Changes during Refrigerated Storage

JOSEPH P. SALJI and ANWAR A. ISMAIL

ABSTRACT

The relationship between initial acidity of plain yogurt and the changes in this acidity during refrigerated storage has been investigated. Yogurt samples with initial pH 4.5 ± 0.1, pH 4.2 ± 0.1 and pH 3.9 ± 0.1 were prepared under laboratory conditions and stored at 4°C and 7°C for 3 wk. pH and titratable acidity were monitored weekly. Results indicated that samples with the lowest initial acidity (high pH) showed the highest increase in acidity during refrigerated storage (pH 4.59–4.15). Samples with high initial acidity (low pH) remained fairly stable (pH 3.82–3.77). Acidity changes during refrigerated storage were more pronounced at 7°C compared to 4°C (pH 4.59–4.27 at 4°C; pH 4.59–4.15 at 7°C). Changes in acidity were maximal for the first week and minimal thereafter.

INTRODUCTION

ACIDITY is one of the major indices for consumers' acceptability of plain yogurt since acid and flavor development go hand in hand in this fermented product of bacterial symbiosis. Although tartness constitutes part of the natural and desirable flavor to yogurt eaters, high acidity in the product is seldom appealing to consumers.

Wide variation in acidity ranging between pH 4.53–3.27 has been reported for commercial yogurt (Duitschaever et al., 1972; Kroger and Weaver, 1973; Davis and McLachlan, 1974; O'Neil et al., 1979). Information is lacking, however, concerning the relationship between initial acidity of plain yogurt and the change of acidity during refrigerated storage. This type of information can be useful to yogurt manufacturers in estimating the acidity of their product at point of sale if the initial acidity of samples and time and temperature of storage are known.

The objective of this experiment was to study the effect of three levels (commercially representative) of initial acidity of plain yogurt on acidity changes during 3wk of refrigerated storage.

MATERIALS & METHODS

IN THIS EXPERIMENT, the composition of milk used in the preparation of yogurt, the type of yogurt culture and the processing and storage conditions were maintained as close as possible to the commercial conditions of plain yogurt as it prevails in Riyadh, the Capital of Saudi Arabia. It is to be noted, however, that earlier studies (Salji et al., 1982) indicated that the local commercial plain yogurt was predominantly made from HTST-pasteurized milk and no differences were observed in acidity changes in yogurt made from vat-pasteurized milk in comparison with yogurt made from HTST milk. Vat pasteurization of milk was used in this experiment simply for its convenience.

Reconstituted milk was prepared from commercial whole milk powder under laboratory conditions and according to the manufacturers' instructions to yield whole milk of 3.6% fat and 13.0% total solids. The milk was heat-treated at 87°C–30 min, cooled down to 43°C and inoculated with a 3% yogurt culture (Hansen CH-1). Incubation was carried for different periods of time to yield yogurt samples with three different initial acidities, namely, pH 4.5 ± 0.1, pH 4.2 ± 0.1 and pH 3.9 ± 0.1. Replicates of samples with the same initial acidity were refrigerated at 4°C and 7°C for 3 wk. pH and titratable acidity were monitored weekly. The pH of the product was measured on a digital pH meter (Orion, Model 701A) and titratable acidity was carried out on a 9-g sample diluted with 9 ml of Co_2-free water according to standard methods (AOAC, 1980).

RESULTS & DISCUSSION

CHANGES in pH and titratable acidity of plain yogurt are shown in Fig. 1 and 2, respectively. Acidity changes were found to be maximal during the first week of storage and minimal thereafter. Samples with low initial acidity (high pH) showed relatively the highest increase in acidity. As shown in Fig. 1, after the first week of storage at 4°C, decreases in pH of 0.32 (4.59–4.27), 0.06 (4.18–4.12) and 0.01 (3.82–3.81) were obtained for samples with initial acidities of pH 4.59, 4.18, and 3.82, respectively.

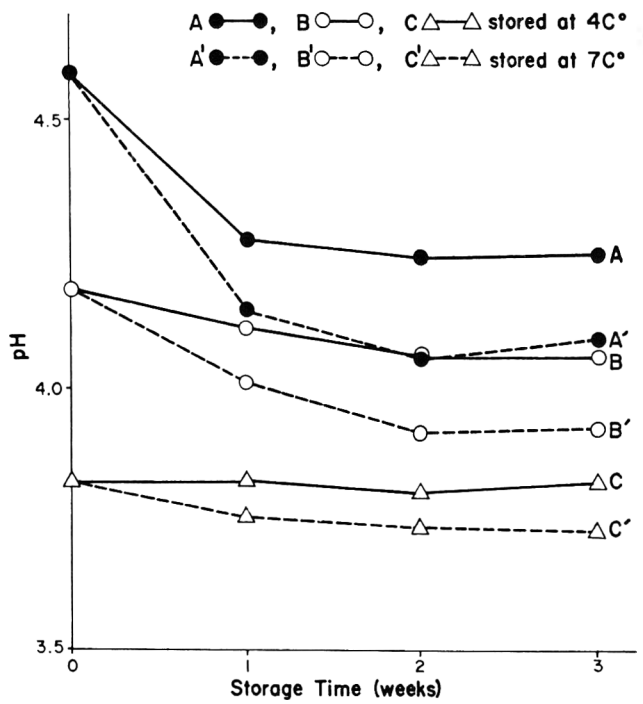

Fig. 1—Changes in pH of plain yogurt during 3-wk of refrigerated storage.

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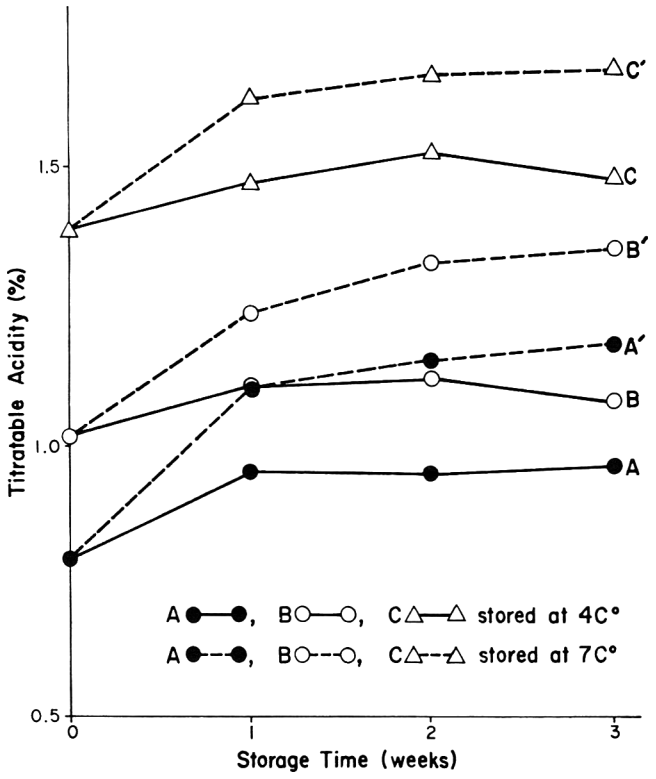

Fig. 2—Changes in titratable acidity of yogurt during 3-wk of refrigerated storage.

The change was more pronounced at 7°C storage as shown in the decrease in pH of 0.44, 0.17 and 0.05 for initial acidities of pH 4.59, 4.18 and 3.82, respectively.

Changes in titratable acidity, as shown in Fig. 2, showed an increase of 0.17% (0.79–0.96), 0.09% (1.01–1.10) and 0.10% (1.38–1.48) for samples stored at 4°C and with initial titratable acidities of 0.79%, 1.01%, and 1.38%, respectively. The change in titratable acidity at 7°C storage was 0.21% (0.79–1.10), 0.22% (1.01–1.23) and 0.24% (1.38–1.62) for the same samples, respectively. Changes in titratable acidity, as the data indicated, did not seem to be much affected by the initial acidity of the samples as did the changes in pH. pH measurement, therefore, can be considered a more sensitive tool than titratable acidity in monitoring acidity changes during refrigerated storage of yogurt.

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HEAT AND HYDROGENATION OF BAOBAB OIL... From page 255

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A Research Note

In Vitro Interactions Between Dietary Fiber and ^{14}C-Vitamin D or ^{14}C-Vitamin E

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ABSTRACT

Free ^{14}C-vitamin D_3 decreased between 86.8–92.9% when incubated at 37°C with various fibers in the presence of 0.84% saline. The addition of bile as sodium taurocholate reversed the decrease in free vitamin D_3 and was related directly to the concentration of the bile salt. Free ^{14}C-vitamin E was also decreased in saline solutions with the fiber sources of hard red spring (HRS), soft white winter (SWW) wheat brans or alphacel cellulose (AC). In turn the decrease in free ^{14}C-tocopherol was reversed by the addition of bile. Citrus pectin (CP) had no influence on the ^{14}C-vitamin E.

INTRODUCTION

THE ROLE OF DIETARY FIBER in nutrition and good health is currently a topic of extensive research. Among the many claims made in the support of an increase in dietary fiber consumption are included reports of lower fasting levels of triglycerides and cholesterol, and alterations in bile salt metabolism and fecal steroid concentration (Heaton and Pomare, 1974; DeGroot et al., 1963; Pomare and Heaton, 1973). However, within the possible useful facet, are several adverse side effects resulting in changes in the bioavailability of essential nutrients.

The purpose of the present study was to investigate the effect of several levels of dietary fiber sources and bile as sodium taurocholate on free vitamin D and vitamin E.

EXPERIMENTAL

Chemical sources

Vitamin E, vitamin D_3, methylbenzethonium hydroxide (hyamine hydroxide) and sodium taurocholate were obtained from the Sigma Chemical Company (St. Louis, MO). Samples of certified hard red spring (HRS) and soft white winter (SWW) wheat brans (30 mesh) were obtained from the American Association of Cereal Chemists. Citrus pectin (CP) and alphacel cellulose (AC) were obtained from ICN Nutritional Biochemicals (Cleveland, OH). [3,4-^{14}C]-dl-α-tocopherol (35.8 μCi/μmole) was obtained from LaRoche Inc. (Basel, Switzerland). [4-^{14}C] Vitamin D_3 (25 mCI/mmole) was purchased from Amersham/Searle (Arlington Heights, IL).

In vitro method

DL-α-[3,4-^{14}C]-tocopherol or [4-^{14}C] vitamin D_3 was mixed with 1 mM of the respective unlabelled carrier compound in 100% ethanolic solution. All incubations contained 5 ml of either a solution of 0.84% saline or 0.84% saline containing bile as sodium taurocholate (pH = 5.1–5.2), with a known amount of the fiber being tested. The procedure was as described previously (Omaye et al., 1982). The amount of vitamin remaining with dietary fiber was calculated as the difference between the amount of labeled vitamin added and the amount recovered in the supernatant and compared to sample (blank) incubated without fiber. Loss of ^{14}C-vitamin in blanks was not larger than 24%. Blanks were arbitrarily fixed to 100%.

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Data were analyzed statistically by Student's t-test or by analysis of variance. Values are expressed as per cent of initial added radioactivity.

RESULTS & DISCUSSION

IN SPITE OF the possible benefits derived from certain fiber consumption i.e., hypocholesterolemia (Lin et al., 1957; Leveille and Sauberlich, 1966), the subsequent binding of bile (Story and Kritchevsky, 1975), could result in less micellar formation necessary for essential lipids and/or fat-soluble vitamin absorption. Both vitamin D and vitamin E became less available (soluble vitamin remaining after centrifugation and is not trapped or bound to the fiber) in saline solutions containing HRS, SWW and AC (Table 1 and 2). Analysis of variance over all fiber substances demonstrated that there was a significant increase in radioactivity with increasing fiber concentrations (Table 1) for vitamin D samples: HRS (in sodium taurocholate), $P < 0.001$; and CP (in sodium taurocholate), $P < 0.001$; and CP (in sodium taurocholate), $P < 0.05$. Analysis of fibers in saline or AC and SWW in sodium taurocholate was not significant (NS) for vitamin D samples. Analysis of variance over all fiber substances illustrated that there was a significant change in radioactivity with increasing fiber concentration (Table 2) for vitamin E samples: HRS, and SWW (in saline), $P < 0.005$ and HRS and SWW (in sodium taurocholate), $P < 0.025$. Analysis of variance for CP or AC was not significant (NS) for vitamin E samples. Vitamin D, but not vitamin E, was less available in saline solutions of pectin.

Results from recent work imply that dietary fiber influences the bioavailability of fat-soluble vitamins (de-Lumen et al., 1982). Also we have found that the plasma levels of vitamin E and vitamin A are lower in rats fed 20%

Table 1—Vitamin D remaining in supernatant of centrifuged fiber samples[a]

Fiber substance (mg)	Vitamin D content, % Saline	Vitamin D content, % Sodium taurocholate
Hard Red Spring Wheat Bran (40)	11.3 ± 1.3	39.9 ± 5.6
Hard Red Spring Wheat Bran (100)	9.3 ± 3.1	52.8 ± 2.6
Hard Red Spring Wheat Bran (200)	7.1 ± 2.8	102.9 ± 3.2[b]
Soft White Winter Wheat Bran (40)	10.3 ± 2.9	55.7 ± 4.2
Soft White Winter Wheat Bran (100)	10.7 ± 3.3	62.8 ± 3.1
Soft White Winter Wheat Bran (200)	13.2 ± 0.9	75.9 ± 17.1[b]
Citrus Pectin (20)	7.6 ± 1.3	33.3 ± 5.7
Citrus Pectin (40)	9.1 ± 2.8	61.5 ± 13.7[b]
Citrus Pectin (60)	10.7 ± 6.8	103.7 ± 21.1[b]
Alphacel Cellulose (20)	9.7 ± 1.4	71.7 ± 7.7
Alphacel Cellulose (60)	9.9 ± 3.1	86.9 ± 16.0[b]
Alphacel Cellulose (120)	9.8 ± 1.4	92.1 ± 3.2[b]
No Fiber	100.0 ± 10.3	100.0 ± 16.5[c]

[a] Each observation is the mean ± S.E.M. of three incubations. Each substance was incubated with 5 ml of 0.84% NaCl or 0.84% NaCl containing 100 μmole, 240 μmole, or 480 μmole of sodium taurocholate for the three respective fiber concentrations.
[b] Significantly different ($P < 0.05$) compared to fiber substance in saline incubation.
[c] Containing 480 μmole of sodium taurocholate.

Table 2—Vitamin E remaining in supernatant of centrifuged fiber samples[a]

Fiber substance (mg)	Vitamin E content, %	
	Saline	Sodium taurocholate
Hard Red Spring Wheat Bran (40)	13.1 ± 3.8	58.1 ± 4.5[b]
Hard Red Spring Wheat Bran (100)	14.4 ± 1.1	78.4 ± 2.0[b]
Hard Red Spring Wheat Bran (200)	34.2 ± 1.4	77.1 ± 2.4[b]
Soft White Winter Wheat Bran (40)	17.9 ± 4.5	74.9 ± 0.7[b]
Soft White Winter Wheat Bran (100)	40.9 ± 0.1	73.9 ± 0.1[b]
Soft White Winter Wheat Bran (200)	41.8 ± 3.5	82.4 ± 1.0[b]
Citrus Pectin (20)	104.8 ± 2.2	96.1 ± 1.0
Citrus Pectin (40)	104.9 ± 3.5	104.5 ± 3.0
Citrus Pectin (60)	96.2 ± 5.3	96.9 ± 2.5
Alphacel Cellulose (20)	54.1 ± 0.7	96.2 ± 1.5[b]
Alphacel Cellulose (60)	46.8 ± 1.4	93.7 ± 0.1[b]
Alphacel Cellulose (120)	43.9 ± 0.7	97.6 ± 0.2[b]
No Fiber	100.0 ± 0.9	100.0 ± 2.2[c]

[a] Each observation is the mean ± S.E.M. of three incubations. Each substance was incubated with 5 ml of 0.84% NaCl or 0.84% NaCl containing 100 μmole, 240 μmole or 480 μmole of sodium taurocholate for the three respective fiber concentrations.
[b] Significantly different ($P < 0.05$) compared to fiber substance in saline incubation.
[c] Containing 480 μmole of sodium taurocholate.

wheat bran diets compared to 5% (unpublished results). Including bile as sodium taurocholate in the incubation mixture had a marked influence on increasing the availability of both fat-soluble vitamins (Table 1 and 2). In the bile of mammals, bile acids are present as glycine and taurine conjugates. The proportion of glycine to taurine conjugates varies with species. In the rat the bile acids are conjugated predominantly with taurine (Hargreaves, 1968) and since our intial in vivo work will eventually be in the rat model, we use sodium taurocholate as an appropriate example of a bile salt. Other constituents of bile were not included, so as not to complicate the systems.

Based on the properties of sodium taurocholate as well as other components of bile, there are at least two possible explanations for why fat-soluble vitamins are more free in solutions containing bile. First, bile has a detergent action on the particles, decreasing the surface tension of the particles and allows with agitation, to break the fat globules into minute sizes. Second, and even more important than that of the emulsifying function, bile has a hydrotropic function. Alternatively, pH effects or changes in nonspecific particle adhesion may account for the effects of added bile on vitamin availability. Ions of bile salts become physically absorbed to the lipids, and the electrical charges of these ions then presumably increase the solubility of the lipids. Without the presence of bile salts in the intestinal tract, up to 40% of the lipids can be lost into the stools (Eastwood, 1973). Obviously these relationships between bile and lipids are also relevant to bile and fat-soluble vitamins. The underlying mechanisms of action for the loss in availability of fat-soluble vitamins after incubating with various fibers can only be speculative at this time, but are under study.

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A Research Note

Effect of Fermentation Time on the Inositol Phosphates of Bread

N. R. NAYINI and P. MARKAKIS

ABSTRACT

Bread was made by an AACC procedure using whole wheat flour and 70%-extraction wheat flour. Five different fermentation times were applied. Six inositol phosphates and inorganic phosphate were separated, identified and quantified in the bread. It was found that phytate decreased and the inorganic phosphate increased, with the largest decrease in phytate occurring during first 30 min of fermentation. The content in intermediate inositol phosphates also changed and it was only after considering their phosphorus content that an overall phosphorus balance could be achieved.

INTRODUCTION

MYO-INOSITOL 1,2,3,4,5,6-HEXAKIS (dihydrogen phosphate), also known as phytic acid (PA), is nutritionally important because it may bind multivalent minerals (e.g. Zn, Fe) and render them unavailable for absorption in the intestinal tract. Phytate salts constitute 1–2% of the dry weight of cereals and oilseeds. Wheat contains an average of 1.1% and wheat bran about 5% of PA (Lolas et al., 1976). The enzyme phytase (E.C. 3.1.3.8) dephosphorylates PA on successive steps terminating with the formation of inositol and phosphoric acid. Courtois and Joseph (1948) isolated several intermediates of the enzymic hydrolysis of PA. Phytase is present in seeds in which its activity increases manyfold during germination (Chang, 1967; Lolas and Markakis, 1977). Phytase is also present in bacteria, yeasts and fungi. Significant decrease in PA content was observed during the tempeh fermentation of soybeans (Sudarmadji and Markakis, 1977) and the oncom fermentation of peanuts (Fardiaz and Markakis, 1981). The disappearance of phytate and the concurrent appearance of inorganic phosphate in yeast-raised bread was observed by several investigators (Pringle and Moran, 1942; deLange et al., 1961; Harland and Harland, 1980; Tangkongchitr et al., 1981), but no attention was paid to the intermediate inositol phosphates. In this work an effort was made to follow the fate not only of the phytate and inorganic phosphorus, but also that of the five intermediate inositol phosphates as affected by the fermentation of the wheat dough.

MATERIALS & METHODS

WHOLE WHEAT FLOUR and 70%-extraction wheat flour were obtained from a local market. The dough formulation was: 100g flour; 3g yeast; 1.5g salt; 5.0g sucrose; and 100 ml water. The bread was prepared according to the AACC, 10-10 method (1962) but with fermentation periods of 0, 30, 60, 90 and 120 min.

The loaves were air-dried, ground and stored in a desiccator until they were analyzed. The inositol phosphates were extracted from 1g of ground sample with 10 ml of 3% trichloracetic acid. The suspension was shaken mechanically for 45 min and subsequently centrifuged for 15 min at 12,000 x *g*. One ml of the supernatant was chromatographed on a Dowex-1 x 8 (200-400 mesh, Cl^- form) column, 1.1 x 10.5 cm. Linear gradient elution was performed with 600 ml of 0.0–1.0N HCl, 2 ml/min. Five ml fractions were collected, dried with air-jets and subjected to total phosphorus (P) determination using Allen's method (1940). On the basis of P analysis the fractions of a subsequent, identical run were grouped into peaks, combined, air-dried and hydrolyzed with 6N HCl at 110°C in sealed ampoules for 48 hr. The hydrolysate was subjected to inositol determination according to Agranoff et al. (1958) and to P assay (Allen, 1940). The molar ratio, inositol:P, allowed the identification of the inositol phosphate corresponding to each peak.

RESULTS & DISCUSSION

THE CHROMATOGRAPHIC SEPARATION of the six inositol phosphates, i.e. monophosphate (IP), diphosphate (IP_2), triphosphate (IP_3), tetraphosphate (IP_4), pentaphosphate (IP_5), and hexaphosphate (IP_6), plus phosphoric acid (P_i), is illustrated in Fig. 1. A more quantitative account of these fractions in the two types of bread prepared after fermentation of varying duration is given in Table 1.

Fig. 1 indicates that the separation of inositol phosphates and P_i is satisfactory. It is noteworthy that bread made of either flour (whole wheat and 70%-extraction) contained all six types of inositol phosphate before being subjected to the panary fermentation. In a previous communication (Ferrel, 1978), only phytic acid was reported as present in wheat flour. Table 1 shows that IP_6 is the dominant inositol phosphate in both of the unfermented breads analyzed, although whole wheat bread (WWB) contains more than twice the amount of IP_6 present in 70%-extraction bread (70B). The remaining inositol phosphates collectively contain almost as much P as IP_6 in WWB, and 1.5 times as much in 70B.

Fermentation reduced the phytate content with the fastest decrease occurring during the first 30 min. Breads prepared after 120 min fermentation sustained a 72–77% loss of IP_6. In the literature, the figures pertaining to phytate destruction in breadmaking vary greatly: from 100% for white bread (Pringle and Moran, 1942), to 40–50% for whole wheat bread (deLange et al., 1961), to 13% for village flat breads made in Iran (Reinhold, 1972).

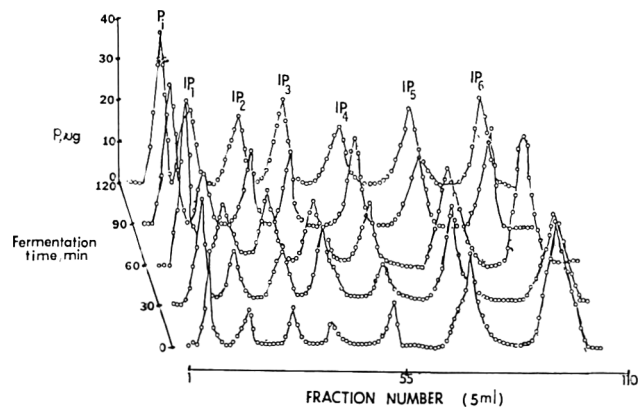

Fig. 1–Elution pattern of inositol phosphates on Dowex 1 x 8 (Cl^-) column.

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Table 1—Wheat flour bread contents in inositol phosphates as a function of fermentation time (mg of P present in each of the fractions/100g of bread, dry basis)

Fermentation time, min	Type of bread[b]	P_i[a]	IP_1	IP_2	IP_3	IP_4	IP_5	IP_6	Total phosphorus
0	WWB	45	36	37	52	98	191	398	857
	WB	40	62	29	94	33	63	192	513
30	WWB	95	68	116	127	89	157	206	858
	WB	57	32	102	75	39	92	116	514
60	WWB	110	88	104	100	112	175	175	862
	WB	64	43	71	98	51	83	101	511
90	WWB	131	79	122	160	90	139	133	854
	WB	77	50	43	71	92	114	73	520
120	WWB	157	112	125	112	128	127	92	860
	WB	110	56	48	108	40	101	54	517

[a] P_i=inorganic phosphate; IP_1 to IP_6=inositol phosphates containing 1 to 6 phosphates per inositol residue.
[b] WWB=whole wheat bread; WB=70%-extraction wheat bread.

As the phytate content decreased the inorganic phosphate content increased. But not even after 120 min of fermentation can the loss of IP_6 be accounted for by the P_i rise. Only when the P content of the intermediate inositol phosphates is taken into consideration can an acceptable P balance be achieved. Tangkongchitr et al. (1981) could account for almost all of the phytate P loss by the increase in inorganic P, but this occurred after 8 hr of fermentation by which time perhaps all of the intermediate inositol phosphates were dephosphorylated. In our work, the intermediate inositol phosphates content in bread fluctuated with fermentation time, which is probably due to several dephosphorylation reactions occurring simultaneously.

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CALCIUM ADDITION TO SOY BEVERAGE. . . From page 257

or $Ca_3(PO_4)_2$ (25 mM Ca) in stored, retorted samples (not shown). However, the product containing a mixture of calcium citrate (20 mM Ca) and $Ca_3(PO_4)_2$ (5 mM Ca) was rated significantly lower in flavor and was chalkier than the control sample.

McCune (1980), using the same processing parameters for soy beverage, reported the following consistency coefficients and flow behavior index values following 3-day storage: pasteurized beverage (0.11 and 0.91), agitort (0.12 and 0.91), and still retort (0.17 and 0.89). He reported that thermal processing increased the viscosity of soy beverage. The type of thermal processing (agitort vs still retort) had no effect on the beverage's viscosity. In the present study soy beverage processed via agitort was more viscous than one produced by still retort. Also, flow behavior index values show that the beverage was more pseudoplastic than that described by McCune.

In this study, the addition of calcium salts to soy beverage improved its nutritional profile while not adversely affecting acceptability. Future research in this area should involve using calcium salts in combination with other nutrients to further improve the nutritional and sensory properties of the beverage.

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A Research Note

A High Performance Liquid Chromatographic Method for Chlorogenic Acid Determination in Sunflower Seeds

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ABSTRACT

A new high performance liquid chromatographic (HPLC) method for chlorogenic acid determination in sunflower seeds is described. This method offers a relatively rapid and highly selective way to separate chlorogenic acid from caffeic acid and other polyphenolic compounds in sunflower seeds. The HPLC method results are compared with those of the spectrophotometric method and no significant difference is shown between methods using confectionery seeds but significant differences are shown with the oilseed varieties.

INTRODUCTION

SUNFLOWER SEEDS are a good source of protein, but the presence of polyphenolics constituents restricts their use in the food industry (Assogna et al., 1978). Caffeic and chlorogenic acids are the major polyphenolic compounds found in sunflower seeds (Sabir et al., 1974; Sosulski, 1979). At alkaline pH levels, caffeic acid produces a slight pink discoloration and chlorogenic acid causes a dark green discoloration of food products and may also decrease the degree of protein digestibility (Cater et al., 1972; Robertson, 1975; Dryden and Satterlee, 1978).

A rapid, highly selective, high performance liquid chromatographic (HPLC) technique for separating and quantifying both chlorogenic and caffeic acid would be useful primarily in the development of new sunflower varieties with lower chlorogenic acid content and to monitor chlorogenic acid levels during processing of sunflower meal into high protein derivatives for food applications. Previous HPLC techniques for chlorogenic acid determination have been reported. One approach involved electrochemical detection which is not commonly available in most laboratories (Felice et al., 1976). Another method utilized a phosphate buffer system in the mobile phase, which requires extra pump cleaning steps (Walter et al., 1979).

MATERIALS & METHODS

Samples

Sunflower seeds from a confectionery variety, an oilseed variety (Hybrid 894) and a purple oilseed genotype (Neagra de Cluj) were obtained from the North Dakota State Univ., Dept. of Agronomy. All seeds were hand separated into their hulls and kernels. Samples were dried for 2 hr at 75°C. Hulls were then ground with a Wiley Mill to pass a 40-mesh sieve and the kernels were masticated with a tissue grinder. Subsequent analyses were done in duplicate.

Extraction

Approximately 50 mg of each sample were accurately weighed into a 125-ml erlenmeyer flask. Seventy ml of 70% methanol was added, and the sample was shaken for 2 hr (Fleming and Sosulski, 1977). Each sample was filtered through a Whatman #4 filter paper and quantitatively transfered into a 100 ml volumetric flask.

Determination

Chlorogenic acid and caffeic acid levels were determined by both spectrophotometric and HPLC methods. The spectrophotometric method used a Perkin-Elmer double beam spectrophotometer at 324 nm and 313 nm (Cater et al., 1972; Walter et al., 1979). The HPLC method used a Waters Associates system including a U6K injector, both a 6000A and a M45 solvent delivery system, a Model 440 absorbance detector set at 313 nm and a Data Module. The separation conditions include a μ Bondapak C_{18} column (3.9 nm ID x 30 cm); a mobile phase consisting of 35% methanol (Solvent A) and 65% of 1% acetic acid (Solvent B) at a flow rate of 1.5 ml/min, attenuation of 0.5, chart speed of 1.0 cm/min, and injection of 10-25 μl. After each sample set, the column was washed with 25 ml of tetrahydrofuran (THF).

Standards of chlorogenic acid and caffeic acid were obtained from Sigma Chemical Company. Sample peaks were spiked with appropriate standards as a verification step, and standard curves were prepared at 2, 5 and 10 μg/ml.

Calculation

$$\% \text{ chlorogenic acid} = \frac{\mu\text{g/ml} \times 100 \text{ ml}}{1000\ \mu\text{g/ml} \times \text{mg sample (dry wt)}} \times 100$$

Statistics

All data were statistically analyzed by analysis of variance and Duncan's multiple-range test (Steel and Torrie, 1980).

RESULTS & DISCUSSION

THE CHROMATOGRAM of the HPLC method is shown in Fig. 1. The retention times are 3.8 min for chlorogenic acid and 4.8 min for caffeic acid. In all samples, caffeic acid was found only in small amounts. This may be due to the conversion of trans caffeic acid to its cis-isomer and the subsequent formation of esculetine (Milic et al., 1968).

The content of chlorogenic acid in the sunflower seed samples is reported in Table 1. The hull and kernel chlorogenic acid levels determined in this study compared favorably with those reported by Dorrell (1976). Comparing methods showed variable results depending on the type of sunflower seed examined. The confectionery seed showed no significant difference between the use of either 313 nm or 324 nm on the spectrophotometric method, or between the spectrophotometric and HPLC methods. The oilseed kernel and hull showed no significant difference between

Table 1—The % chlorogenic acid in sunflower seeds[a]

	% Chlorogenic acid		
	Spectrophotometric		HPLC
Sunflower seed	324 nm	313 nm	313 nm
Confectionery			
Hull	0.18a	0.18a	0.15a
Kernel	1.15a	1.15a	1.09a
Oilseed			
Hull	0.49a	0.50a	0.19b
Kernel	1.60a	1.55a	0.89b
Purple genotype			
Hull	1.43a	1.83b	0.70c
Kernel	1.90a	1.84a	1.25b

[a] Values in a row followed by a common letter are not significantly different at $P \leq 0.5$ (a through c).

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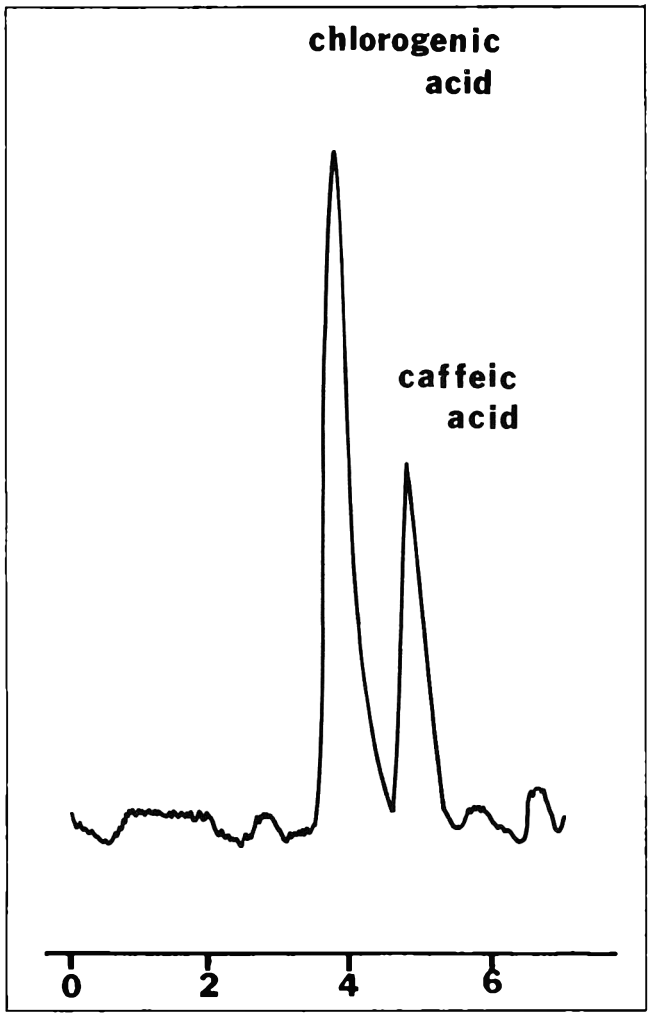

Fig. 1—Chromatogram of chlorogenic and caffeic acid standards.

the two wavelengths used with the spectrophotometric methods, but there was a significant difference between the spectrophotometric and HPLC methods. This indicated that extracted compounds other than chlorogenic acid may give an overestimation if only the spectrophotometric method is used. With the purple genotype seed, the hull portion showed a significant difference in the spectrophotometric method between 313 nm and 324 nm; the HPLC results differed significantly from the spectrophotometric results. The anthocyanin pigments found in the hull may account for this discrepancy (Vaccarie et al., 1982). The major pigment is a glycone peonidin which has two distinct absorption peaks in the UV range with a gradual absorbance increase below 350 nm. This may account for the higher apparent chlorogenic acid content detected at 313 nm as compared to 324 nm. The kernel showed no significant difference between the spectrophotometric method at 313 nm or 324 nm, but a significant difference was detected between the spectrophotometric and HPLC methods. Consequently, an HPLC method of separating and quantifying chlorogenic acid would be the preferred method for use in breeding programs and evaluating processing techniques to lower chlorogenic levels in oil sunflower seeds and their products. However, this method would be less critical when evaluating confectionery sunflower seed.

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A Research Note

Snack Crackers Containing Whole-Grain Triticale Flour: Crispness, Taste, and Acceptability

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ABSTRACT

Snack crackers containing 25, 40, 55, and 70% whole-grain triticale flour were prepared. The crackers did not differ in breaking strength or crispness as measured with the Instron Universal Testing Machine. Sensory panelists used the technique of magnitude estimation coupled with word concepts to evaluate the crispness, taste, and overall acceptability of the crackers. Crackers containing 25 and 40% triticale flour tasted better and were more acceptable than were those containing 55 and 70% triticale flour. Panelists did not find differences in crispness. Protein contents of the crackers ranged from 11.78 to 12.97%. It was demonstrated that triticale flour can be used to produce acceptable, increased-protein snack crackers.

INTRODUCTION

TRITICALE, a hybrid of wheat and rye, has higher protein and lysine contents than does wheat (Lorenz, 1974; Lorenz et al., 1974; Anon., 1975a; Hulse, 1974). Because triticale offers a potentially high protein efficiency ratio (Kissell and Lorenz, 1976), it can be used in a variety of baked products to improve the nutritional value of products for populations that subsist largely on bread and cereal products. Its mild rye flavor has been shown to be preferred over the flavor of whole-wheat products (Lorenz et al., 1972; Anon., 1975b). Triticale has been used successfully in breads, noodle products, cookies, cakes, doughnuts, muffins, extruded breakfast cereals, and pancake and waffle mixes (Tsen, 1974; Lorenz and Welsh, 1974).

Research on the use of triticale in crackers has not been reported. In general, little research has been published on crackers in spite of the fact that crackers and cookies rank second in popularity among bakery foods (Tarone and Matthews, 1982). Katz and Labuza (1981) recently reported a study on the effect of water activity on subjective and objective crispness of several snack food products, including saltines.

A limited preliminary study had shown that 25 and 50% whole-grain triticale crackers were equivalent in flavor desirability and tenderness to all-wheat crackers. A trend toward increased desirability of the triticale crackers also was noted in our preliminary work. Therefore, the objective of this study was to compare the performance of a wider range of levels of triticale flour in snack crackers and determine if an acceptable increased-protein snack cracker could be made. Because the all-wheat crackers and 25% triticale crackers were found to be similar, the 25% cracker was considered to be the control in the present study. Crispness, taste, overall acceptability, and protein content of the crackers were evaluated.

MATERIALS & METHODS

CRACKERS were prepared according to modifications of a formula published by Casella (1966). Whole-grain triticale flour was substituted for all-purpose wheat flour at the 25, 40, 55, and 70% levels.

Flour (90g total), baking soda (0.85g), salt (3.0g), and cultured buttermilk powder (0.57g) were sifted into a mixing bowl and 100 strokes with a pastry cutter were used to cut in the vegetable shortening (28g). Water (40 ml) and eggs (27g) were added and mixed with a KitchenAid mixer with a cake paddle at speed 4 for 1 min. The dough was kneaded for 1 min and then was rolled out with 0.12-cm thick metal strips used as guides. Crackers, 9 cm long and 2.5 cm wide, were cut for objective measurements and crackers half as long were prepared for sensory evaluation. The crackers were placed on baking sheets and pricked with a meat tenderizer block that was 3.4 × 2.3 cm and had 48 pins.

The dough was baked for 3 min in each of three ovens of varying top and/or bottom temperatures to simulate the procedure for baking crackers as described by Matz (1968). The crackers were allowed to cool for approximately 15 min and stored in plastic bags for later evaluation.

Crackers were subjected to a snap test with the 3.18-mm thick blade of a Bailey shortometer-like attachment to an Instron Universal Testing Machine equipped with a 50-kg load cell. Crosshead and chart speeds were 50 and 100 mm/min, respectively. The maximum force required to break the crackers (breaking strength) and the initial slope of the force-deformation curve (crispness) were determined.

The crackers were evaluated by a panel of 18 female Food Science and Nutrition students ranging in age from 20 to 35. For testing panelists were seated at individual booths equipped with red lights. Crackers were presented on a white plate and the order of testing was randomized. Water was provided for rinsing between samples.

The panelists were trained to use magnitude estimation coupled with word concepts as described by Moskowitz (1977). Crispness, taste, and overall acceptability were evaluated. Samples that were perceived to be crisp were assigned positive values while those that were not perceived to be crisp were assigned a value of zero. The judges assigned positive values to those crackers that tasted good and to those that they liked, zero to crackers that were neither good nor poor in taste or that they neither liked nor disliked, and negative values to those crackers that were poor in taste or that they disliked. Judges also used magnitude estimation to assign scores to the concept scales for crispness, taste, and overall acceptability (Footnote. Table 1). All sensory evaluation data were normalized as described by Katz and Labuza (1981).

The experiment was replicated on five days. Order of preparation was randomized for each replication. Statistical differences among treatments were evaluated with analyses of variance. When appropriate, the Student-Newman-Keuls test (Sokal and Rohlf, 1969) was used for mean separation.

Protein contents of the whole-grain triticale flour and crackers containing the four levels of triticale flour were determined according to the Kjeldahl method (AOAC, 1975).

RESULTS & DISCUSSION

RESULTS from the objective tests for texture are shown in Table 2. The crackers did not differ with respect to breaking strength or crispness. The latter suggests that levels of triticale flour above 25% may not offer additional interference in the gluten formation of the wheat flour to result in increased crispness. Triticale flour is deficient in quality and quantity of gluten. As suggested by Matz (1968) this may be desirable for making crackers because weak flours lead to little oven spring and can produce tender, friable crackers. It also is possible that the level of water was very low in the crackers so that all crackers were crisp regardless of the level of triticale flour.

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Table 1—Crispness, taste, and overall acceptability scores[a,b] of snack crackers containing four levels of triticale

Characteristic	Triticale, %			
	25	40	55	70
Crispness[c]	1.002a	0.884a	0.967a	0.985a
Taste[c]	0.842a	0.798a	0.638b	0.572b
Acceptability[c]	1.049a	1.073a	0.815b	0.800b

[a] Means of 5 replications of 18 judges.
[b] Means followed by like letters do not differ ($P > 0.05$).
[c] Concept scores (Medians of 5 replications): Extremely crisp = 1.81; very crisp = 1.36; moderately crisp = 0.90; slightly crisp = 0.45; not crisp = 0.00. Very good taste = 1.41; good taste = 0.71; neither good nor poor taste = 0.00; poor taste = —0.71; very poor taste = —1.41. Like extremely = 1.81; like very much = 1.36; like moderately = 0.90; like slightly = 0.45; neither like nor dislike = 0.00; dislike slightly = —0.45; dislike moderately = —0.90; dislike very much = —1.36; dislike extremely = —1.81.

Table 2—Breaking strength, crispness, and protein contents of triticale crackers

	Triticale, %			
	25	40	55	70
Breaking strength,[a] kg	0.88	0.86	0.82	0.80
Crispness,[a] kg/mm	1.27	1.23	1.35	1.27
Protein,[b] %	11.78	12.09	12.36	12.97

[a] Means of five replications.
[b] N X 6.25; Means of three determinations.

Sensory scores for crispness, taste, and overall acceptability are shown in Table 1. As with the objective measurement for crispness, triticale crackers did not differ with respect to crispness. The verbal concepts of the sensory scores showed that the judges rated all crackers as moderately crisp. The 25 and 40% crackers were given higher scores for taste and acceptability than were the 55 and 70% crackers. Conceptually the 25 and 40% crackers were rated as "good-very good" in taste whereas the crackers with higher percentages of triticale were rated lower than the concept of good taste. All crackers were rated positively with respect to taste.

All crackers were rated as acceptable. The 25 and 40% crackers were rated between "like moderately" and "like very much" whereas the higher triticale crackers were rated close to "like moderately".

Protein content of the triticale flour used in this study was found to be 15.4% (as-is basis). Triticale flour protein has been reported to range from 9.2–18.8%, with wheat flour ranging from 7.8–14.0% (Lebsock, 1974). Protein contents for the crackers containing triticale flour are shown in Table 2. These levels are higher than reported protein values of 9.2% (Tarone and Matthews, 1982) and 9.90% (USDA, 1977) for saltines and a reported value of 11.1% for whole-wheat crackers (Tarone and Matthews, 1982). Thus the triticale crackers contain favorable amounts of protein, which also should be of good quality. The higher protein content also may be attributed partially to the inclusion of egg in the cracker formula. The potential for production of an acceptable whole-grain triticale snack cracker that is higher in protein than commonly used crackers has been demonstrated.

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A Research Note

Effect of Cooking on Cholesterol Content of Patties Containing Different Amounts of Beef, Textured Soy Protein and Fat

KI SOON RHEE and GARY C. SMITH

ABSTRACT

Ground beef patties were prepared to contain 0, 10, 20 or 30% rehydrated textured soy protein (TSP) and 8, 16 or 27% fat. Patties were cooked from the frozen state on a rack in a preheated oven at 177°C to an endpoint internal temperature of 75°C. The cholesterol content of raw ground beef patties decreased as the amount of TSP increased and also decreased as the fat content decreased from 27 to 16 or 8%. At initial fat levels of 8 and 16%, there were no significant differences in cholesterol retention (per-patty basis) during cooking among patties with initial TSP (rehydrated) levels of 0, 10, 20 and 30%; at an initial fat level of 27%, however, patties containing TSP (10, 20 or 30%) retained substantially higher ($P < 0.05$) amounts of cholesterol when cooked than did those without TSP.

INTRODUCTION

TEXTURED SOY PROTEIN (TSP) products are widely used as ground meat extenders, and cooking properties and sensory quality of ground beef patties containing TSP have been studied extensively. However, there exist no studies reporting the effects of incorporating TSP in ground beef on cholesterol content. This study was undertaken to determine the effect of cooking on cholesterol content of beef patties extended with TSP and the interaction between TSP and fat levels on cholesterol content of cooked patties.

MATERIALS & METHODS

A 3 x 4 FACTORIAL DESIGN (12 experimental groups) was used. Ground beef patties were formulated to contain three different levels of fat and 0, 10, 20 or 30% rehydrated TSP. Rehydrated TSP was prepared by mixing one part of TSP (from Central Soya) with two parts of tap water.

Lean beef from the rounds of U.S. Good grade carcasses and subcutaneous fat from the same carcasses were ground separately through a 9.52 mm plate and sub-sampled for rapid fat analysis by the modified Babcock method (Salwin et al., 1955). Appropriate quantities of ground lean, ground fat, and rehydrated TSP (if needed) were hand-mixed, ground through a 9.52 mm plate, hand-mixed again, and ground once more through a 4.76 mm plate. The final ground beef mixes, which were prepared to contain three different levels of fat, had mean fat contents of 8.4, 16.2, and 27.0%, respectively, when fat contents were determined on the extracts prepared by the procedure of Folch et al. (1957).

Patties (115g, 9.2 cm diameter, 1.7 cm thickness) were formed mechanically and stored at −20°C until analyzed or cooked. Three patties from each experimental group were cooked from the frozen state on a rack in a preheated oven at 177°C to an endpoint internal temperature of 75°C and removed from the oven immediately. Two raw patties or three cooked patties per experimental group were frozen in liquid nitrogen and thoroughly powdered in a Waring Blendor, and then analyzed in duplicate (from the lipid extraction step) for cholesterol content as described previously (Rhee et al., 1982). The cholesterol assay procedure consisted of extraction of total lipids, saponification of the extracted lipids and determination of cholesterol in the nonsaponifiable fraction by a colorimetric method (Searcy and Bergquist, 1960).

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Data were subjected to analysis of variance and Duncan's multiple range test (Duncan, 1955) for mean separation.

RESULTS & DISCUSSION

Raw patties

Cholesterol content of raw ground beef patties ranged from 63.3 mg/100g (72.8 mg/patty) with 0% rehydrated TSP and 27% fat to 40.7 mg/100g (46.8 mg/patty) with 30% rehydrated TSP and 8% fat (Table 1). No significant interaction ($P > 0.10$) was found between TSP level and fat level in analysis of variance and, therefore, means across the three fat levels within each TSP level and also means across the four TSP levels within each fat level were used for mean separation to determine the effects of TSP and fat levels, as shown in Table 1.

Cholesterol content decreased as the amount of TSP increased because of the absence of cholesterol in TSP; the decrease in cholesterol content was almost proportional to the increase in the amount of TSP. Raw patties containing 8 or 16% fat had significantly less cholesterol than did patties containing 27% fat (Table 1). There was no significant difference in cholesterol content between patties with 8% fat and those with 16% fat.

Cooked patties

The amount of cholesterol in a cooked patty would be influenced by total weight losses during cooking, distribution of the weight loss between volatile (evaporation) loss and drip loss, and the composition of drippings. All of these cooking loss factors might be influenced by initial TSP and fat contents and possibly by the interaction of TSP and fat contents. Mean total cooking losses decreased ($P < 0.05$) as the amount of TSP increased (data not shown in tabular form).

Since the initial evaluation by analysis of variance of the cholesterol data for cooked patties revealed that the inter-

Table 1 – Cholesterol content of raw beef patties[a]

Fat (%)	Rehydrated textured soy protein (%) 0	10	20	30	Means for effect of fat
	mg Cholesterol/100g				
8	60.6	55.6	46.8	40.7	50.9b
16	62.9	55.3	48.4	45.3	53.0b
27	63.3	57.6	53.7	48.4	55.8a
Means for effect of rehydrated TSP	62.3a	56.2b	49.6c	44.8d	
	mg Cholesterol/patty				
8	69.7	63.9	53.8	46.8	58.6b
16	72.3	63.6	55.7	52.1	60.9b
27	72.8	66.2	61.8	55.7	64.1a
Means for effect of rehydrated TSP	71.6a	64.6b	57.1c	51.5d	

[a] Means in a column or row which are not followed by the same letter are significantly different ($P < 0.05$).
Cholesterol was determined in duplicate (from the lipid extraction step) on a pooled sample of two raw patties per treatment.

Table 2 – Cholesterol content of cooked beef patties[a]

Fat (%)	Rehydrated textured soy protein (%) 0	10	20	30
	mg Cholesterol/100g			
8	91.3 a	69.4 b	66.4 b	54.5 c
16	86.3 a	73.3 b	62.5 c	56.4 c
27	74.8 a	69.4 b	62.5 c	65.2 c
	mg Cholesterol/cooked patty			
8	66.8 a	59.0 b	55.4 b	46.8c
16	64.5 a	62.0 a	55.1 b	52.7b
27	58.1 a,b	57.8 a,b	54.7 b	59.4a
	Cholesterol content in a patty as % of that in all-beef (no TSP) patty at the same % of fat			
8	100a	88.4b	82.9b	70.0c
16	100a	96.1a	85.5b	81.7b
27	100a	99.5a	94.1b	102.2a
	Retention (%) of cholesterol during cooking on a per-patty basis			
8	95.8a	92.3a	103.0a	99.9a
16	89.2a	97.4a	99.0a	101.2a
27	79.8c	87.4b	88.5b	106.7a

[a] Means in the same row within a data set followed by a common letter are not significantly different ($P>0.05$).
Means in the same column within a data set accompanied with a common sideline are not significantly different ($P>0.05$).
Cholesterol was determined in duplicate (from the lipid extraction step) on a pooled sample of three cooked patties per treatment.

action between TSP level and fat level was highly significant ($P < 0.001$), the effect of TSP level was evaluated at each fat level and the effect of fat level (only for mg cholesterol/100g and mg cholesterol/patty) was evaluated at each TSP level (Table 2).

At initial fat levels of 8 and 16%, the amount of cholesterol in 100g of cooked sample or in an entire cooked patty tended to decrease as the amount of TSP increased, but the decreases in cholesterol content were not proportional to the increase in the amount of TSP as was the case in the initial uncooked patties. At an initial fat level of 27%, however, the amount of cholesterol per cooked patty did not decrease with an increase in the amount of TSP.

No consistent trend was observed as to the effect of the initial fat level on the cholesterol content per cooked patty. However, among patties containing no TSP, those with an initial fat level of 27% had less cholesterol per patty when cooked than did those with an initial fat level of 8%; the patties with 27% fat initially might have lost a substantial amount of fat (with accompanying loss of cholesterol) as drippings during cooking.

The percentage retention (per-patty basis) of cholesterol during cooking was computed to determine the effect of TSP level on cholesterol retention. The addition of different amounts of TSP to patties did not result in significant differences in cholesterol retention at initial fat levels of 8 and 16%; there were no significant differences in cholesterol retention among patties with 0, 10, 20 or 30% rehydrated TSP. However, at an initial fat level of 27%, patties containing TSP retained substantially higher amounts of cholesterol, with a complete retention shown for patties with an initial TSP content of 30%. At the high fat level, i.e., 27%, the binding effect of TSP on the fat present in patties became obvious; fat retention (per-patty basis) in cooked patties increased as the amount of TSP increased at an initial fat level of 27% with no obvious or consistent fat-binding effect observed at initial fat levels of 8 and 16% (data not presented in tabular form).

CONCLUSIONS

1. The cholesterol content (per-gram or per-patty basis) of raw ground beef patties can be decreased by adding TSP and also by decreasing the fat content to 16% or lower.
2. The cholesterol content (per-gram or per-patty basis) of cooked patties can also be decreased by incorporating TSP in raw patties if the fat content of raw patties is 16% or lower.
3. If the fat content of raw patties is high (27%), patties containing TSP retain more cholesterol on a per-patty basis when cooked.

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A Research Note

Changes in Phytase Activity and Phytate During Germination of Two Fababean Cultivars

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ABSTRACT

Two fababean cultivars were germinated over a 10-day period and the levels of phytate and activity of phytase monitored. In both cultivars there was a marked increase in phytase activity which was accompanied by a concomitant decrease in phytate. The enzyme level reached a maximum 6 days following germination and was much higher in Ackerperle compared to Diana. Nevertheless a marked reduction in phytate levels was evident at the end of 10 days which accounted for a decrease of 71.2% and 77.3% in Ackerperle and Diana, respectively.

INTRODUCTION

PHYTATE is widely distributed in nature and represents the major storage form of phosphorus in mature grains and legumes. The ability of phytate to complex and reduce the availability of zinc in diets containing plant proteins is a problem of general concern to nutritionists (Atwal et al., 1980; Davies and Nightingale, 1975; Forbes and Parker, 1977). The hydrolysis of phytate is catalyzed by the enzyme phytase (myoinositol hexaphosphate phosphohydrolase E.C. 3.1 3.8) to inositol and free orthophosphate. This enzyme has been reported in soybeans, corn seeds, lettuce seeds, dwarf beans, mung beans, triticale and wheat (Chang, 1967; Gibbins and Norris, 1963; Mandal et al., 1972; Mager et al., 1980; Sartirana and Bianchetti, 1967; Singh and Sedeh, 1979). The presence of relatively high levels of phytate in fababeans (Latta and Eskin, 1980; Griffith and Thomas, 1981) prompted an investigation of phytase in several cultivars of fababeans currently licenced in Western Canada.

MATERIALS & METHODS

Germination

Seed samples of fababeans (*Vicia faba* L. minor var. Diana and Ackerperle) were provided by Dr. Laurie Evans, Dept. of Plant Science, Univ. of Manitoba. The dry seeds were germinated at the Regional Laboratories (Food Production and Inspection Branch, Agriculture Canada, Winnipeg, Manitoba) over a 10-day period in accordance with the International Rules for Seed Testing. The growth medium was composed of 5 parts sand:1 part water measured on a volume basis. The samples were then placed in a chamber maintained at a constant temperature (20°C), humidity (70%) and under continuous fluorescent light (80 ft-c).

Enzyme extraction

Crude phytase extracts were obtained from the seeds following the procedure described by Lolas and Markarkis (1977). Finely ground lyophilized fababeans were extracted with a 10:1 ratio of 2% $CaCl_2$ to beans (50 ml $CaCl_2$:5g beans) and mechanically studied at room temperature for 30 min. The crude mixture was centrifuged at 20,000 x *g* for 30 min at 3°C and the supernatant filtered through a Whatman #1 filter paper. The crude phytase extract was treated with ammonium sulfate and the fraction obtained between 35–80% collected. This fraction was found to contain all the phytase activity.

Enzyme assay

Phytase activity was monitored using the procedure of Lolas and Markarkis (1977). The reaction mixture contained 0.2 mL of 0.6M acetate buffer, pH 5.3:0.15 mL of 2mM sodium phytate (Sigma Chemical Co., St. Louis, MO) previously adjusted to pH 5.3 with 1 N HCl:0.02 mL enzyme solution and 0.83 mL distilled water. The total volume was 1.2 mL with final concentrations of buffer and phytate being 0.1M and 0.25 mM, respectively. The mixture was incubated at 50 ± 1°C for 30 min in a water bath. The reaction was terminated by the addition of 1 mL of cold 1.54M trichloroacetic acid. The mixture was chilled in an ice bath for 15 min and then centrifuged at 30,000 x *g* for 10 min. The supernatants were filtered through Whatman #1 filter paper and a 1 mL aliquot removed for inorganic phosphorus determination according to the method of Chen et al. (1956). The protein content in the supernatant was measured by the procedure of Lowry et al. (1951). Phytase activity was determined as μgPi/mg protein/30

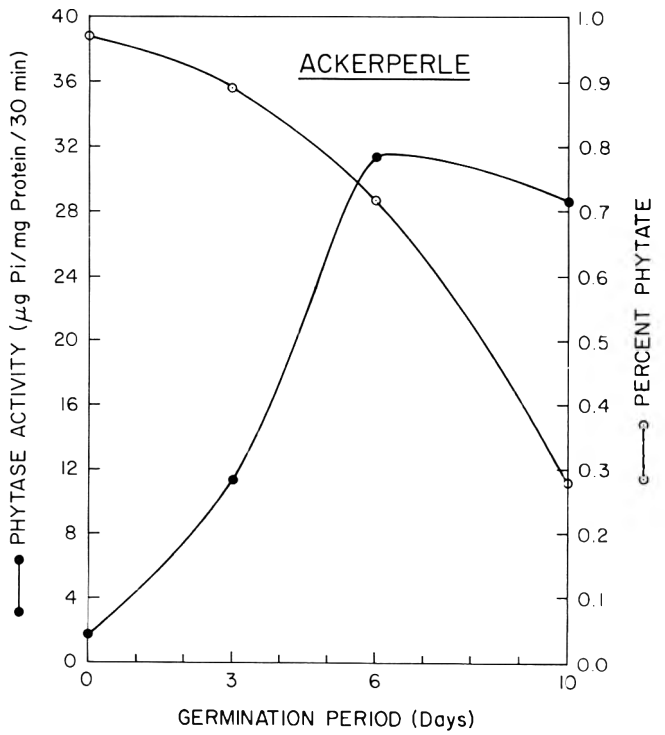

Fig. 1—Changes in phytase activity and percent phytate during germination of Vicia Faba L. minor var. Ackerperle.

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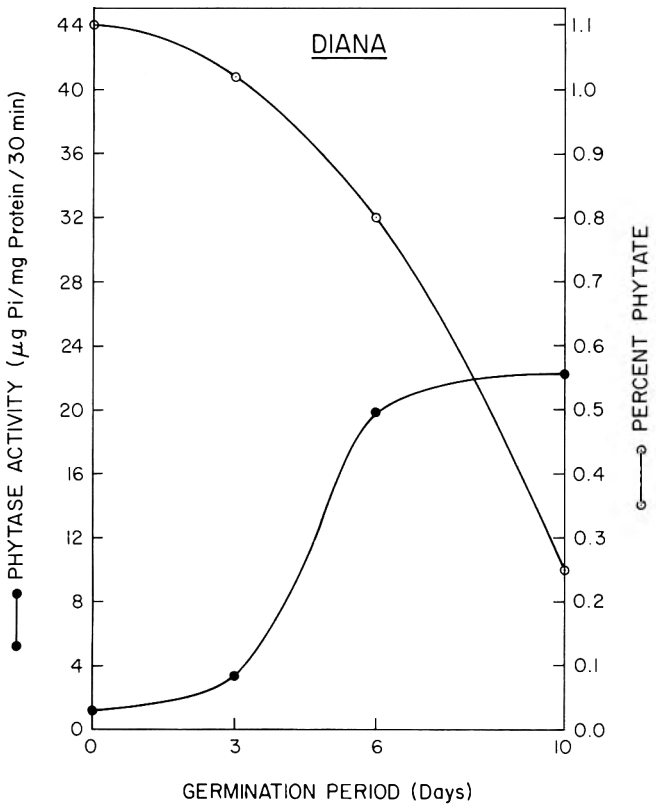

Fig. 2—Changes in phytase activity and percent phytate during germination of Vicia Faba L. minor var. Diana.

min. and values corrected against a control with boiled enzyme.

Phytate levels

Phytate was extracted and measured following the procedure described by Latta and Eskin (1980).

RESULTS & DISCUSSION

THE RESULTS shown in Fig. 1 and 2 indicate a rapid rise in phytase activity for both varieties with a concomitant reduction in phytate. The level of phytase was somewhat lower in Diana which also reflected a slightly higher level of phytate of 1.10% compared to 0.97% in Ackerperle. The higher level of phytase during germination of Ackerperle reached a maximum following 6 days germination and then levelled off. An increase in phytase activity was also observed in Diana but reached only two-thirds of the activity of Ackerperle and then levelled off after 6 days. In both cases the level of phytate decreased gradually during the first 3 days of germination with a rapid decline between days 6 and 9. The overall reduction in phytate at the end of the 10-day germination period accounted for 71.2% and 77.3% for Ackerperle and Diana, respectively. It is clear that simple germination of fababeans substantially reduce the level of phytate due to the utilization of phosphorus during the increase in metabolic activity. This reduction of phytate should result in an improvement in the nutritional quality of the bean.

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A Research Note

Vitamin C Retention of Potato Fries Blanched in Water

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ABSTRACT

Vitamin C retention was determined microfluorometrically for French fries heated in water. Vitamin C retention for 1.3 cm (½ in.) water blanched French fries ranged from 83.2–54.1%. The French fry blanch times were 5, 10 and 15 min at 66°C, 77°C and 88°C. The apparent Ea was 4.0 Kcal/mole.

INTRODUCTION

POTATOES, *Solanum tuberosum*, continue to be an important vitamin C source, particularly for individuals who do not regularly consume other fresh or frozen vegetables and fruits (American Medical Assoc., 1974). Potato products contain from 3–21 mg of vitamin C per 100g depending upon initial concentrations of vitamin C in the raw potatoes and the method of processing (Watt and Merrill, 1963; Augustin et al., 1979). The initial levels of vitamin C in potatoes are dependent on several factors including production, potato cultivar, harvest and storage conditions, as well as length of storage (Augustin et al., 1978; Vitek and Mihelle, 1978; Shekar et al., 1978).

Thermal processes such as blanching, soaking and frying will decrease the vitamin C content of potato products (Bring et al., 1963; Pelletier et al., 1977; Augustine et al., 1978). Fried shoestring potatoes had total vitamin C retention of 75.4–84.6% (Bring, 1966). Whole peeled potatoes heated in boiling water for 25–30 min had vitamin C retentions of 66–77%, while vitamin C retentions of 66 to 80% were reported for fried potatoes (Pelletier et al., 1977).

Rognerud (1972) reported significant decreases in ascorbic acid retention during heating of vegetables in water due to leaching. The leaching losses in some cases two to three times greater than losses due to thermal degradation.

METHODS & MATERIALS

Product

Russet Burbank potatoes, *Solanum tuberosum*, were stored at 7°C for 5 months. Each potato was peeled and cut into an even number of strips. Half of the strips from each potato were assigned to the control group and half were assigned to the processed group. Sufficient potatoes to provide eight French fries per sample were used. The French fry dimensions were 1.3 cm x 1.3 cm x 9.6 cm (0.5 in x 0.5 in x 3.0 in), a commercial 0.5 in. French fry. Half of the strips from each potato were allotted to a control group and immediately weighed and frozen in liquid nitrogen as the control. The remaining potato strips were immediately heated in water, weighed and frozen in liquid nitrogen. All potato strips, after liquid nitrogen freezing, were stored at −20°C and analyzed within 24 hr for vitamin C.

Temperature measurement

Iron-constantin thermocouples (Omega Engineering No. GG-J-36, 36 guage) with fiberglass insulation were prepared by electric welding. Epoxy glue was added to stiffen the thermocouple ends and facilitate insertion into the potato strip. The thermocouples were examined under a low power microscope to insure relatively uniform size and spherical shape. None of the epoxy glue was allowed on the metal tip of the thermocouple. The thermocouples were checked in ice water and boiling water to insure they were functioning correctly. To ease insertion of the thermocouple, a small hole 2.0–2.5 cm long was formed with a needle through the end of the potato strip. The thermocouples were inserted approximately 3.0–3.5 cm into the center of 2–3 potato strips for each experiment. Due to the insertion of the thermocouple lengthwise through the ends, rather than the sides of the potato strips, and the small diameter of the wire, any heat transfer affecting the thermocouple tip by conduction was minimized. The thermocouples were attached to a recording potentiometer (Esterline Angus Model D-2020). Temperatures were recorded at one minute intervals during blanching.

Water blanching

The water-blanched potato strips were heated in a noncirculating water bath (Precision Scientific Co., No. 6606). Blanch times of 5, 10 and 15 min and blanch water temperatures of 66, 77 and 88°C used in this experiment are in the range used by potato processors in the Pacific Northwest.

Vitamin C concentration was determined fluorometrically with a filter fluorometer (G. K. Turner No. 111), set at a 1x sensitivity, with a narrow pass primary filter (G. K. Turner No. 110-811; 7-60, 360 nm wavelength peak) and a sharp cut secondary filter (G. K. Turner No. 110-816; 2A, cut-off wavelength at 415 nm). The microfluorometric procedure (AOAC, 1975) was used to determine vitamin C concentration. The heated potato strip extraction solutions were centrifuged at 12,000 x *g* for 15 min after extraction rather than filtered, since filtration was impossible due to the gelatinization of the starch. Ascorbic acid and dehydroascorbic acid have equivalent antiscorbutic activity (Johnson, 1979). The combination of the two forms will be referred to as vitamin C.

For moisture analysis, frozen French fries were chopped in a pre-cooled Waring Blendor for 1 min and 3–7g of the potato samples were weighed into a pre-weighed aluminum drying pan. The samples were pre-dried for 1 hr at 102°C and dried to a constant weight at 70–75°C under a vacuum of 69 cm Hg.

The percent fat was assumed to be the same as that reported for boiled, baked and fresh potatoes in Watt and Merrill (1963), 0.1%.

RESULTS & DISCUSSION

VITAMIN C RETENTIONS at the various times and temperatures during water blanching indicated highly significant ($P < 0.01$) differences. Blanch time and blanch water temperatures were determined statistically to be the best variables for estimating vitamin C retention. The model of blanch time and blanch water temperature accounts for 97.5% of the variation in the blanching data. The apparent Ea for vitamin C was 4.0 Kcal/mole. This vitamin C Ea is about 1/10 the value of the Ea determined in a system where leaching was not a factor (Lathrop and Leung, 1980). The apparent Ea in this experiment arises from two sources of vitamin C loss, leaching and heat degradation. Under commercial processing conditions there will be differences in fry size, specific gravity of the potatoes, solids concentration in the blanch water, product flow rate and potato variety that will affect vitamin C content. Because of these parameters this model system is not directly comparable to commercial processing conditions.

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Table 1 – Vitamin C in water blanched French fries (mg/100g)

Temp (°C)	Control	5 min	Control	10 min	Control	15 min
65.6	10.66 ± 0.12[a]	8.88 ± 0.14	10.06 ± 0.26	7.61 ± 0.32	11.70 ± 0.96	8.06 ± 0.42
76.7	12.19 ± 1.51	9.66 ± 1.01	12.42 ± 1.17	8.70 ± 0.40	12.44 ± 0.06	7.66 ± 0.28
87.8	11.60 ± 0.09	8.88 ± 0.14	11.30 ± 0.63	7.29 ± 0.78	11.45 ± 0.70	6.19 ± 0.32

[a] $\bar{x} \pm s_{\bar{x}}$

Vitamin C retention

The vitamin C concentrations of the thermally processed potato fries (Table 1) were divided by the vitamin C concentrations of the control potato fries (Table 1) to give vitamin C retentions in per cent. The ranges in vitamin C retention for water blanched potato fries were 54.1–83.2%. The mean percent retention for process time and temperature combinations are presented in Table 2. Pelletier et al. (1977) reported vitamin C retentions in pared potatoes heated in boiling water of 66 ± 6% to 77 ± 7%. The vitamin C retentions observed here are in that range.

The retention values for vitamin C in water blanched potatoes reported by Augustin et al. (1979) are significantly greater than the retention values reported in this study. A possible reason for this discrepancy is the fact that the two groups used somewhat different blanching procedures. The blanching procedures used in our investigation involved a small noncirculating water bath and water free of any solids at the onset of the blanch treatment; whereas the blanching procedure used by Augustin et al. (1979) involved a commercial processing operation. In the latter case, the blanch water apparently contained a significant amount of solids, which in turn, meant smaller concentration gradients than in the laboratory system. Therefore, differences in results can be explained on the basis of differences in leaching losses between the two systems.

An air blanching experiment to separate leaching losses from thermal degradation losses was conducted. In addition, experiments to separate heating and leaching losses during frying were conducted. Vitamin C retention did not consistently decrease with increases in heating time and temperatures in these experiments as expected. Because of these inconsistencies, it was determined that evaluation of vitamin retention wasn't meaningful. Browning reaction products fluoresce and have excitation and emission maxima at 350 and 430 nm, respectively (Adhikar and Tappel, 1973). The quinoxaline formed from dehydroascorbic acid and o-phenylenediamine also has excitation and emission maxima at 350 and 430 nm (Deutsch and Weeks, 1965). These browning reaction products may interfere with the fluorometric analysis of vitamin C. Deutsch and Weeks (1965) evaluated several compounds for interference but did not include Maillard browning reaction products.

Table 2 – Vitamin C retention (%) in water blanched potato strips

Water temp (°C)	Blanch Time		
	5 min	10 min	15 min
66	83.2 ± 1.7[a]	75.7 ± 1.6	68.9 ± 3.1
77	79.3 ± 2.6	70.1 ± 3.8	61.6 ± 2.1
88	76.6 ± 0.2	64.5 ± 3.7	54.1 ± 2.0

[a] $\bar{x} \pm s_{\bar{x}}$

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A Research Note

Cellulose, Hemicellulose and Lignin Content of Raw and Cooked Processed Vegetables

JOSEFA HERRANZ, CONCEPCION VIDAL-VALVERDE and ENRIQUE ROJAS-HIDALGO

ABSTRACT

The NDF, ADF, cellulose, hemicellulose and lignin contents of five frozen vegetables (raw and boiled) and five canned vegetables (two of them fried) have been studied. In general, it was observed that boiling resulted in an increase in the NDF, ADF and cellulose content. There was also a slight increase in hemicellulose values. The lignin contents of most vegetables did not change upon boiling. When the cooking process was frying, a drastic decrease of NDF, ADF, cellulose, and lignin contents occurred, while the amount of hemicellulose changed slightly in the vegetables analyzed.

INTRODUCTION

A CLOSE RELATIONSHIP has been established between several illnesses and low dietary fiber intake. Digestive tract diseases such as colon cancer (MacLennan et al., 1978), hiatus hernia (Burkitt, 1981) and intestinal diverticulosis (Gear et al., 1979); circulatory diseases such as varicose veins (Burkitt, 1976) and haemorrhoids (Stuart, 1978); and certain metabolic disturbances such as diabetes mellitus (Jenkins et al., 1976; Potter et al., 1981) are among those related to the dietary fiber intake. There is also an effect on lipid metabolism, since an increased dietary fiber intake leads to fecal elimination of a higher level of cholesterol and bile salts (Huang et al., 1978; Jenkins, 1980; Kritchevsky, 1980; Vahouny et al., 1981). A knowledge of the contents of the dietary fiber and its components in foods, is required to be able to prepare diets which could help correct or prevent these aliments, since each of the fiber components plays a different physiological role (Huang et al., 1978; Zivelrsmit, 1979).

Previously, we determined the dietary fiber composition of raw and cooked vegetables (Herranz et al., 1981) and fruits (Vidal-Valverde et al., 1982). In order to supplement these results, we describe here the data obtained for frequently consumed raw and cooked processed vegetables.

MATERIALS & METHODS

THE VEGETABLES ANALYZED, labeled "select" or "first" quality, were obtained from a local supermarket. About 1 kg of each canned vegetable was drained and weighed. A portion of some of them was cooked. A sample of about 1 kg of frozen vegetables was selected and a portion was defrosted and weighed, while another portion was subjected to the culinary process, drained and weighed. Vegetables were homogenized in a Waring Blendor.

The dry matter content was calculated from weight loss after heating in a vacuum oven at 35°C to constant weight.

The Van Soest method (1963a, b) and that of Van Soest and Wine (1967, 1968) for the determination of the neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin, were used. In the case of starch-rich vegetables such as peas and broad beans, the modification described by McQueen and Nicholson (1979) was employed. For fried vegetables, we followed the method of Van Soest and Robertson (1980). Each analysis was replicated three or four times on the same sample.

The effect of cooking on the dietary fiber components of the edible portion is shown on a dry basis. The mean values obtained for frozen or canned vegetables, and those corresponding to the same vegetable subjected to a culinary process have been submitted to student's "t" test.

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RESULTS & DISCUSSION

THE NDF, ADF, cellulose, hemicellulose, lignin and water contents of five frozen vegetables (raw and boiled) and five canned vegetables (two of them fried) were determined (Table 1).

The frozen vegetables, upon boiling, showed an increase ($P \leq 0.01$) in NDF, with the exception of artichokes, which decreased ($P \leq 0.001$). The ADF and cellulose contents also increased, although, in the case of spinach the values were not significant. Hemicellulose values also increased with cooking, but this increase was significant ($P \leq 0.01$) only in spinach, while artichokes show a significant decrease ($P \leq 0.001$). The lignin contents present only a slight variations as a result of cooking.

Canned vegetables are normally consumed without cooking. Only peas and tomatoes were subjected to a frying process. We observed that the NDF, ADF, cellulose and lignin content decreased drastically ($P \leq 0.02$) during this heating process, while the hemicellulose contents showed no significant statistical change.

In frozen and canned vegetables, the effect of cooking on the dietary fiber components and water contents is quite similar to that reported for fresh vegetables (Herranz et al., 1981).

Information in the literature regarding the dietary fiber components of processed vegetables is limited. Southgate (1978) and Southgate et al. (1976) gave values for frozen and canned peas, and Holloway et al. (1977) for canned peas.

From our work we conclude that processed vegetables, like fresh vegetables, are important sources of dietary fiber components.

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Table 1—Content in some components of dietary fiber in processed vegetables (means ± SD)

VEGETABLES	Edible matter proportion of wt purchased	g/100g Fresh matter edible portion						g/100g Dry matter edible portion				
		NDF	ADF	Cellulose	Hemi-cellulose	Lignin	Water	NDF	ADF	Cellulose	Hemi-cellulose	Lignin
ARTICHOKES												
Canned	1.00	1.39±0.04	1.11±0.03	0.94±0.01	0.28±0.05	0.17±0.03	87.7	11.34±0.33	9.05±0.24	7.67±0.08	2.28±0.41	1.39±0.24
Frozen												
raw	1.00	2.16±0.08	1.16±0.03	1.01±0.02	1.00±0.09	0.15±0.01	86.4	15.88±0.59	8.53±0.22	7.43±0.15	7.35±0.66	1.10±0.07
boiled (35 min)	1.03	1.47±0.02	1.12±0.02	0.95±0.06	0.35±0.03	0.16±0.04	89.5	14.00±0.19	10.67±0.19	9.05±0.57	3.33±0.29	1.52±0.38
ASPARAGUS												
Canned	1.00	0.94±0.01	0.69±0.00	0.62±0.01	0.25±0.01	0.07±0.01	93.2	13.82±0.15	10.15±0.00	9.12±0.15	3.68±0.15	1.03±0.15
BROAD BEANS												
Frozen												
raw	1.00	4.59±0.23	3.96±0.08	3.46±0.12	0.63±0.24	0.50±0.04	74.8	18.21±0.91	15.71±0.32	13.73±0.47	2.50±0.95	1.98±0.15
boiled (35 min)	1.10	4.55±0.09	3.77±0.28	3.36±0.26	0.78±0.29	0.38±0.04	80.4	23.21±0.46	19.23±1.43	17.14±1.33	3.98±1.48	1.94±0.20
GREEN BEANS												
Canned	1.00	1.33±0.01	1.29±0.02	1.09±0.04	0.04±0.02	0.21±0.02	92.1	15.92±0.13	16.41±0.25	13.80±0.51	0.51±0.25	2.66±0.25
Frozen												
raw	1.00	1.28±0.01	1.23±0.04	1.08±0.02	0.05±0.04	0.15±0.06	91.0	14.22±0.11	13.67±0.44	12.00±0.22	0.55±0.44	1.66±0.66
boiled (40 min)	1.02	1.25±0.02	1.18±0.03	1.03±0.05	0.07±0.04	0.16±0.06	93.4	18.93±0.30	17.88±0.45	15.60±0.75	1.06±0.60	2.42±0.90
PEAS												
Canned												
Canned	1.00	3.20±0.17	3.27±0.16	3.13±0.07	traces	0.24±0.03	82.0	17.87±0.95	18.17±0.89	17.48±0.39	traces	1.34±0.17
Canned and fried (5 min)	1.01	3.56±0.12	3.31±0.04	3.18±0.13	0.25±0.13	0.14±0.10	51.8	7.39±0.25	6.87±0.08	6.60±0.27	0.52±0.27	0.29±0.21
Frozen												
Frozen	1.00	3.15±0.10	2.93±0.20	2.62±0.12	0.22±0.22	0.31±0.10	77.2	13.81±0.43	12.85±0.88	11.49±0.52	0.96±0.96	1.35±0.43
boiled (35 min)	0.97	3.81±0.16	3.27±0.10	2.88±0.07	0.54±0.19	0.39±0.05	82.9	22.28±0.94	19.12±0.58	16.84±0.40	3.15±1.11	2.28±0.29
SPINACH												
Frozen												
raw	1.00	3.34±0.22	1.52±0.03	1.21±0.01	1.82±0.22	0.31±0.05	88.5	29.04±1.91	13.22±0.26	10.52±0.09	15.83±1.91	2.69±0.43
boiled (12 min)	1.13	3.66±0.17	1.28±0.05	1.00±0.05	2.38±0.18	0.28±0.04	91.0	40.67±1.89	14.22±0.56	11.11±0.56	26.44±2.00	3.11±0.44
TOMATO												
Canned												
Canned	1.00	0.76±0.00	0.66±0.02	0.54±0.01	0.10±0.02	0.12±0.01	92.9	10.70±0.00	9.30±0.28	7.65±0.14	1.42±0.28	1.70±0.14
Canned and fried (20 min)	0.67	1.15±0.06	0.87±0.01	0.69±0.01	0.28±0.06	0.18±0.02	77.0	5.00±0.26	3.78±0.04	3.00±0.04	1.22±0.26	0.78±0.09

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A Research Note

Size Distribution of High Weight Species in Pectin Fractions from Idared Apples

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ABSTRACT

The apparent MW of two apple pectin fractions were investigated using gel permeation chromatography. Both fractions behaved similarly on Sephadex G–200, Sepharose 6B, and Bio Gel 50m. Seventy percent of galacturonic acid residues eluted in a continuous distribution in the range 12.5 million to 1 million Daltons. Ten percent lay above this range, twenty percent below. These high weight species (possibly aggregates) do not appear to be artifacts of the extraction and handling procedures used, and may reflect an intrinsic property of some pectic substances.

INTRODUCTION

MOLECULAR WEIGHTS of apple pectin have been reported to be in the range 60,000–200,000 Daltons (Knee, 1978; Doesburg, 1957; Joslyn and Deuel, 1963). However, exclusion from Sephadex G-200 (Barrett and Northcote, 1965) and electron microscopic observations on stained material (Hanke and Northcote, 1975) have suggested that aggregation of pectin molecules to form higher weight species may take place. In the current work solutions/suspensions of two pectin fractions from stored Idared apples were observed to be turbid and the size distribution of the species present was investigated.

MATERIALS

PLANT MATERIAL, extraction, handling and analytical procedures used have previously been described (O'Beirne et al., 1982). Gel permeation chromatography of pectin fractions was on Sephadex G-200, Sepharose 6B (Pharmacia Inc.) and Bio Gel 50m (Bio Rad Laboratories). The exclusion limits for linear polysaccharides in these gels were respectively 200,000, 1 million and 12.5 million (estimated) Daltons. LKB 2137 columns, inside diameter 2.6 cm, were used. Samples containing approximately 500 μg/ml galacturonic acid residues were applied at 1–2% of the gel bed volume in 0.01M phosphate, 0.001M Na_2EDTA, pH 6.9. Void volume was determined using exclusion/part exclusion of Dextran 2000 as recommended by Pharmacia Inc. Fractions of 7.5 ml were collected.

Viscosity was measured at 30°C in an Ostwald viscometer. Intrinsic viscosity (η) was obtained by extrapolating the specific viscosity/concentration values obtained to zero galacturonic acid concentration.

RESULTS & DISCUSSION

Two pectin fractions, water soluble pectin and chelator (EDTA) soluble pectin, both extracted nondegradatively from stored Idared apples (O'Beirne et al., 1982) were studied. The presence of high MW species in these fractions was suggested by the fact that solutions/suspensions in phosphate buffer were visibly turbid, and that the level of galacturonic acid residues could be reduced by millipore (0.2μ) filtration (e.g., with water-soluble pectin the level was reduced from 418 to 314 μg/ml). Both fractions were excluded by Sephadex G-200 as Barrett and Northcote (1965) had observed for their apple pectin fraction from Bramley's Seedling. This was found at 5.5, 6, and 11 months storage and when phosphate buffers of different concentration (1.0M, 0.01M) were used for elutions, and indicated species of MW > 200,000 Daltons. Pectic substances of the fractions entered Sephadex G-200 after transelimination (4 hr at 100°C, neutral pH), a treatment which cleaves esterified portions of the rhamnogalacturonan mainchain.

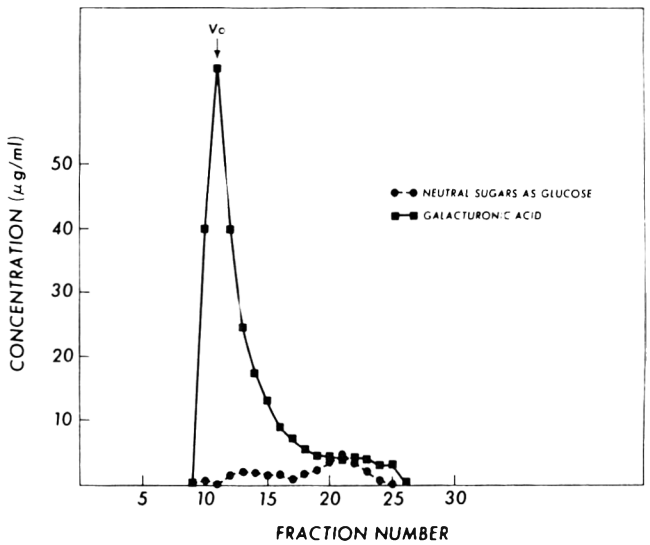

Fig. 1—Elution pattern of water-soluble pectin on Sepharose 6B (Idared apples stored 11 months).

The elution patterns of both water soluble pectin and chelator soluble pectin were similar to one another with either Sepharose 6B or Bio Gel 50m. Eighty percent of galacturonic acid residues were excluded by Sepharose 6B, indicating that 80% of the species present had apparent MWs > 1 million Daltons (Fig. 1). Only 10% of galacturonic acid residues were excluded by Bio Gel 50m (Fig. 2a, b) indicating that this excluded fraction was > ca. 12.5 million Daltons. In order to minimize contamination with cytoplasmic protein, pectic substances were also extracted with 2% TCA (trichloroacetic acid; Knee, 1978), and eluted from Bio Gel 50m. Both unconcentrated and concentrated (precipitation with 80% acetone and re-dissolution) TCA extracts gave elution patterns similar to those in Fig. 2.

Little neutral sugar eluted with the galacturonic acid residues of chelator soluble pectin (Fig. 2b). With water-soluble pectin, however, a broad neutral sugar 'peak' was seen with both Sepharose 6B and Bio Gel 50m (Fig. 1, 2a). Its K_{av} on Sepharose 6B corresponds to MW of ca. 150,000 Daltons. This fraction appears to be the neutral sugar-rich fraction of water-soluble pectin which does not bind to cation exchange resins (Knee, 1970; O'Beirne et al., 1982). Spot tests (Dorée, 1947) on freeze-dried

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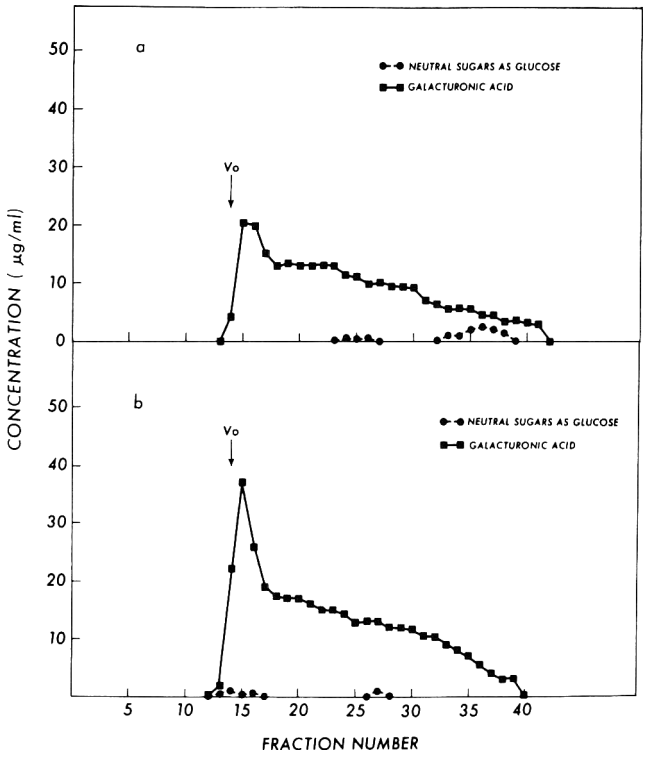

Fig. 2—Elution patterns on Bio Gel 50m of (a) water soluble pectin from Idared apples stored 6 months, and (b) chelator soluble pectin from Idared apples stored 11 months.

samples of water-soluble pectin and chelator soluble pectin indicated that cellulose was absent.

Gel permeation chromatography data indicate that both fractions occur as high weight species, 70% lying in a continuous distribution between 1 million and ca. 12.5 million Daltons. Pectic substances examined here appeared similar to those used by other workers in their molecular weight determinations. For example, the intrinsic viscosity (η) of the 2% TCA extract was 6.4 dL/g, almost identical to that of Knee (1978). If the true MW of apple pectic substances is in the range 60,000–200,000, it is likely that the high weight species observed here represent aggregates of lower MW polymers. These high weight species do not appear to be artifacts of extraction or concentration since several extraction procedures were used, and both dilute and concentrated 'solutions' produced similar elution patterns. In addition, Barrett and Northcote (1965) have reported exclusion from Sephadex G-200 of an apple pectin fraction extracted by different means.

Since there were extremely low levels of neutral sugars throughout the elution sequence for chelator soluble pectin (Fig. 2b) these do not appear to be required for interchain associations of the magnitude indicated here. Instead, associations of the rhamnogalacturonan mainchains (Rees, 1969) may be involved. Whatever the mechanism, the elution patterns were unaffected by the considerable drop in degree of esterification between 5.5 and 11 months storage (O'Beirne et al., 1982). Pectin and cellulose have been observed together in cell wall fragments (Grant et al., 1969) but no cellulose was present in these fractions.

Considering both the consistency of the size distribution of these species and its randomness, it is possible that they may reflect an intrinsic property of apple pectin. Whether some apple pectin occurs as high weight aggregates *in vivo* is unknown, but loss of neutral sugar sidechains during senescence (Bartley, 1976) and the low a_w in the cell wall would favor such an occurrence.

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A Research Note

GLC-MS Analysis of Volatile Constituents in Rabbiteye Blueberries

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ABSTRACT

Steam volatile oils were obtained from Rabbiteye blueberries (*Vaccinium ashei* Reade, cv. Tiftblue) in amounts of ca. 25 ppm of the berry and analyzed by capillary gas-liquid chromatography-mass spectrometry. Major components identified were ethyl acetate, limonene, hexanol, cis-2-hexenol, heptanol, cinerolone, β-ionone, terpinene-4-ol, 2-undecanone, α-terpineol, 1-carveol, nerol and eugenol. Of the 42 compounds identified, 29 have not been previously reported as constituents of blueberry volatiles.

INTRODUCTION

THREE TYPES OF BLUEBERRIES are grown commercially in the United States. The lowbush dwarf type (*Vaccinium angustifolium* Ait.) is native to the northeastern United States and is grown mainly in Maine. The highbush blueberry (*V. corymbosum* L.) introduced into the eastern United States in 1920 (Draper, 1979), is now grown extensively in North Carolina, New Jersey and southern Michigan and constitutes a majority of the annual harvest in the United States (45,000 metric tons in 1980). The Rabbiteye blueberry (*V. ashei* Reade) is native to the southeastern United States and has been improved through breeding for adaptability to commercial production. This cultivar has received recent attention as a cash crop to developing rural areas.

The flavor components of blueberries have received little attention. In 1970, Hall et al. reported that fruits of lowbush blueberries contain acetaldehyde, methyl acetate, ethanol and ethylene. Parliment and Kolor (1975) identified 21 compounds in highbush blueberry including low molecular weight esters, alcohols, aldehydes, acyclic terpenes and cyclic terpenes. These authors made a minor attempt to relate three of the compounds identified to blueberry aroma. Volatiles of another member of the genus *Vaccinium* were studied by Von Sydow and Anjou (1969) and Von Sydow et al. (1970) who investigated the volatiles from bilberry fruit (*V. myrtillas*) and identified a large number of compounds. They concluded that trans-2-hexenal, ethyl 3-methylbutyrate and ethyl-2-methylbutyrate were largely responsible for the bilberry aroma. The volatiles of Rabbiteye blueberries, however, have not been reported.

The purpose of this investigation was to identify the volatile compounds of the Rabbiteye blueberry, Tiftblue, and in subsequent studies establish the relative importance of these compounds to blueberry aroma for quality control in processes that are being developed.

MATERIALS & METHODS

SAMPLES OF BLUEBERRIES were obtained at the firm ripe stage of maturity from the University of Georgia Horticultural Farm, Athens, GA. Two hundred gram samples of the berries were selected and placed in a Waring Blendor with 200 ml of distilled water; the container was sealed and the berries were macerated for 3 min at medium speed. The resulting slurry was placed in a 3-L round-bottom flask with an additional 400 ml of distilled water; the flask was connected to a Likens and Nickerson steam distillation, continuous pentane extraction apparatus (Likens and Nickerson, 1964) and the pentane and blueberry macerate were boiled for 8 hr. After cooling, the flask containing the 120 ml of pentane was removed, cooled to ca 40°C, maintained at this temperature, and then concentrated to ca 0.5 ml with a gentle stream of high purity nitrogen. The extract was further concentrated to about 100 μl by allowing it to stand at room temperature.

GLC-MS analyses

Analyses were performed with a Perkin-Elmer Model 900 gas-liquid chromatograph on samples ranging from 0.2 to 1.0 μL in volume. The chromatograph was connected by means of an effluent splitter to a DuPont 21-490B mass spectrometer equipped with differential pumping on the analyzer section. Separations were made on a 91.4m x 0.0762cm stainless steel, open-tubular column coated with GE-SF 96(50) plus 1% Igepal CO 880 and with a 50m x 0.05cm glass column coated with Superox 4 (Alltech Associates). Glc conditions were: carrier gas inlet pressures, 0.6 kg/cm^2; injector and manifold temperature at 250°C; and columns held at 60°C for 6 min then programmed to 210°C at 3°/min.

Mass spectrometer conditions were: ion source temperature, 175°C; scan rate, 10 sec per decade; ionizing voltage, 70 ev; and ion source pressure, 2×10^{-5} Torr. Compounds were identified by comparison of their mass spectra and GLC retention times (RT) with those of known standards. Compounds were considered to be positively identified when their mass spectra and GLC RT agreed with those of a known standard. Compounds identified solely on the basis of comparison of their mass spectra with standards in the literature are designated "tentatively identified."

Table 1—Volatile compounds of Rabbiteye Bluberry identified by GLC-MS

acetaldehyde	thymol
acetone	cineralone[a]
ethanol*	benzyl alcohol
ethyl acetate*	piperonal
2-pentanone	2-ethylhexanol*
1-pentene-3-ol*	linalool*
toluene	n-butylphenylether[a]
2-furfural	2-undecanone
hexanal*	geraniol*
γ-terpinene	nerol*
2 isomeric C_4 alkylbenzenes	2-decyne[a]
hexanol	β-ionone
limonene*	terpinene-4-ol[a]
trans-2-hexenal*	2-trisdecanone
5 methylfurfural	cis-caran-3-ol[a]
cis-2-hexenol*	α-cedrene[a]
heptanol*	dimethyl suberate
p-cymene	geranyl formate[a]
α-terpineol*	eugenol
carveol[a]	linalool acetate
sabinol[a]	ethyl myristate

* Previously identified in highbush blueberry (Vaccinium corymbosum).
[a] Identified solely on the basis of a comparison of their mass spectra with standards in the literature.

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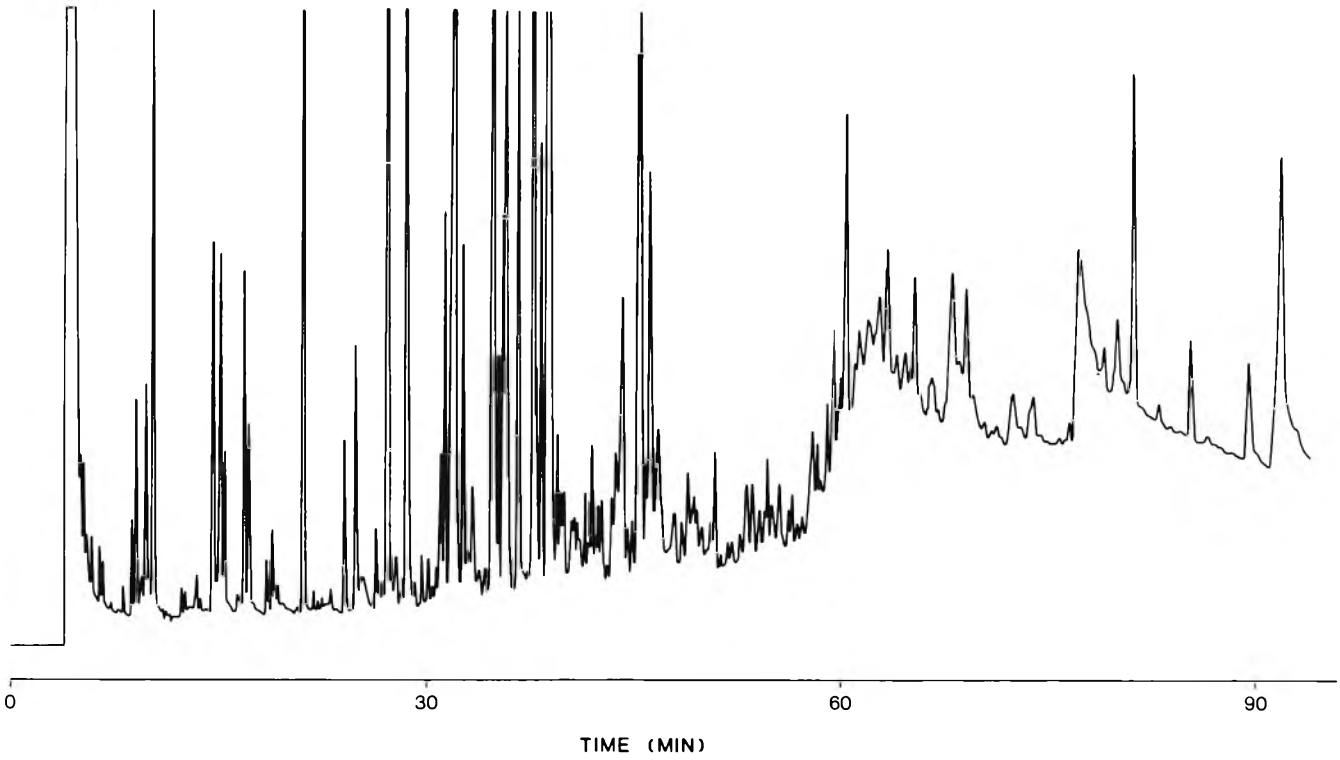

Fig. 1—Chromatogram of a pentane concentrate of Rabbiteye blueberries with a glass capillary column (50 m x 0.05 cm) coated with Superox 4.

RESULTS & DISCUSSION

WHEN REMOVED from the extraction apparatus, the pentane extract possessed a typical blueberry aroma which was not lost with subsequent concentration. This concentrate was stable for extended periods of several weeks when closed and stored under refrigerated conditions as evidenced by GLC analyses and comparison of subsequent chromatograms.

Identifications of the neutral components of the extracts are given in Table 1. Of the 42 compounds identified, 29 have not been previously reported in either lowbush or highbush blueberries. Also, the same compliment of C-6 compounds were found in Rabbiteye blueberries as was previously reported in highbush blueberries by Parliment and Kolor (1975). Eighteen terpenes were present of which ten were identified—five being previously identified by the preceding authors in highbush blueberries. Newly identified terpenes and terpene esters are γ-terpinene, p-cymene, carveol, sabinol, thymol, cineralone, β-ionone, terpinene-4-ol, cis-caran-3-ol, α-cedrene, geranyl formate and linalool acetate. This class of compounds appears to constitute the preponderance of the aroma that is characteristic of blueberries. Other classes of compounds present in considerable quantities in the extracts were ketones, alcohols, and esters. Furfural and 5-methylfurfural were also identified but were probably artifacts formed during the extraction process from the dehydration of pentoses and 6-desoxyaldohexoses in the acid macerate (pH 3.25). Ethyl myristate was present in the extract and has previously been identified as a natural constituent in molasses residue (Furia and Bellanca, 1971). The presence of toluene, two isomeric C_4 alkylbenzenes and p-cymene is not unexpected since aromatic hydrocarbons are widely distributed in plant materials (Gerarde and Gerarde, 1961; von Sydow and Anjou, 1969).

Fig. 1 is a typical GLC chromatogram of the Rabbiteye blueberry extracts. The elution pattern appears to follow both increasing molecular weight and polarity of the compounds. A further study is needed to relate the identified compounds to aroma threshold levels and evaluate the effects of processes on these volatiles.

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Reference to a company or product name does not imply approval or recommendation by the United States government.

A Research Note

Whole Body, Liver and Plasma Cholesterol Levels in Rats Fed *Thermophilus, Bulgaricus* and *Acidophilus* Milks

S. R. PULUSANI and D. R. RAO

ABSTRACT

In five dietary treatments 70 rats (146g) were fed: (1) chow + water, (2) chow + skim milk, (3) chow + skim milk fermented by *Streptococcus thermophilus*, (4) chow + skim milk fermented by *Lactobacillus bulgaricus*, and (5) chow + skim milk fermented by *Lactobacillus acidophilus*. After 4 wk of feeding, the mean levels of cholesterol in plasma (mg/dl) and whole body (mg/g dry matter) for treatments 1 through 5 were: 61.3, 54.7, 56.0, 57.1, and 58.1, and 3.68, 3.58, 3.27, 3.13, and 3.00, respectively ($P > 0.05$). Whole body lipids, liver lipids, liver cholesterol and liver weights were not significantly different. Results indicate that various fermented milks do not significantly alter the distribution of cholesterol among various body pools.

INTRODUCTION

IN 1974, Mann and Spoerry serendipitously discovered that consumption of large quantities of fermented milk by the Maasai tribesmen in Africa actually lowered their serum cholesterol level and counteracted the hypercholesterolemic action of Tween 20. Later, it was demonstrated that unfermented milk is also hypocholesterolemic both in man (Mann, 1977b; Howard and Marks, 1977) and animals (Malinow and McLaughlin, 1975; Boguslawski and Wrobel, 1974; Bernstein et al., 1977; Ahmed et al., 1978; Nair and Mann, 1977; Kritchevsky et al., 1979). Mann (1977a, b) claimed that hypocholesterolemic activity is more in fermented milk than in whole milk. Commercial plain yogurt used in these studies (Mann, 1977a, b) is prepared by using a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. We have recently demonstrated that metabolites produced in milk fermented by *Streptococcus thermophilus* lower plasma cholesterol levels in vivo and inhibit cholesterogenesis in the liver in vitro in rats (Rao et al., 1981). However, we observed that there was a nonsignificant but sizable increase in liver cholesterol levels in rats fed extracts from *Thermophilus* milk. This prompted us to examine whether fermented milk lowers the plasma cholesterol level by mere redistribution of cholesterol among various body pools. The purpose of this investigation was to study the effectof feeding skim milk fermented by *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* on cholesterol distribution in various body pools in rats.

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MATERIALS & METHODS

IN A COMPLETELY RANDOMIZED experiment, a total of 70 white Wistar male rats of 146g average initial weight were assigned to five dietary treatments in two replications. The treatments were: (1) chow + water, (2) chow + skim milk, (3) chow + skim milk fermented by *Streptococcus thermophilus*, (4) chow + skim milk fermented by *Lactobacillus bulgaricus*, and (5) chow + skim milk fermented by *Lactobacillus acidophilus*. The skim milk was fermented as described previously (Pulusani et al., 1979), except the length of incubation was 24 hr. Fermented milk was prepared fresh everyday. Rats in groups 2 through 5 received no water. The rats were housed individually (22°C ± 2 and 10 hr light and 14 hr dark cycle), and received their assigned diets for 4 wk ad libitum. Weight gains and consumption of chow and milks were recorded. At the end of 28 days, after an overnight fast, the rats were lightly anesthetized by ether for blood sample (about 10 ml) collections from abdominal aorta. After sacrifice by exsanguination, livers were weighed and portions rinsed in 0.15M KCl and frozen for total cholesterol and lipid analysis. Whole bodies (minus the liver and blood collected) were also frozen for total lipid and cholesterol analysis. Blood plasma was frozen until analyzed for total cholesterol. Before analysis, frozen whole bodies were dried in an oven at 105°C. Dried whole bodies were then ground in a Wiley Mill to a particle size of about 2 mm.

Total lipids were extracted by the method of Folch et al. (1957). Total cholesterols in plasma were determined as described by Baginski and Zak (1970). Total cholesterols in liver and whole body were determined as digitonin precipitable sterols in the unsaponifiable fraction of the extracted fat (Baginski and Zak, 1970). All statistical analyses were conducted as per Steele and Torrie (1960).

RESULTS & DICUSSION

THE AVERAGE WEIGHT GAINS (g/28 days) or rats for treatments 1, 2, 3, 4 and 5 were: 113.1 133.1, 116.0, 117.4, and 128.0, respectively, with no significant differences ($P > 0.05$). The fluid intake ranged from 40–50 ml/day and no signs of diarrhea were evident in skim milk group. When expressed on metabolic body weight basis the consumption of fermented milk by rats in this experiment (40 ml/0.29 kg) compares well with the consumption of sour milk by Maasai tribesmen (40 ml/0.24 kg) assuming that these tribesmen consume up to 4 liters/day (Mann and

Table 1—Weight gain feed consumption and feed efficiency of rats fed various fermented milks

PARAMETER	Diet				
	Chow + Water	Chow + Skim milk	Chow + *Thermophilus* milk	Chow + *Bulgaricus* milk	Chow + *Acidophilus* mil
Weight gain (g/rat/28 days)	113[a]	133[a]	116[a]	117[a]	128[a]
dry matter consumption (g/rat/28 days)	516[c]	566[ab]	559[b]	582[ab]	599[a]
Feed/gain	4.56	4.25	4.81	4.97	4.67

[a,b,c] Means bearing same superscript in the same row are not significantly different at 5% level.

Table 2—Cholesterol distribution, lipid levels and liver weights of rats fed various fermented milks

Parameter	Diet				
	Chow + Water	Chow + Skim milk	Chow + *Thermophilus* milk	Chow + *Bulgaricus* milk	Chow + *Acidophilus* milk
Whole body cholesterol[b] (mg/g dry matter)	3.68[a]	3.58[a]	3.27[a]	3.13[a]	3.00[a]
Liver cholesterol[b] (mg/100 g wet weight)	347[a]	349[a]	301[a]	306[a]	352[a]
Plasma cholesterol[c] (mg/dl)	61.3[a]	54.7[a]	56.0[a]	57.1[a]	58.1[a]
Liver lipids[c] (mg/g)	46.9[a]	43.2[a]	47.2[a]	59.2[a]	48.3[a]
Whole body lipids[c] (% of dry matter)	21.6[a]	17.6[a]	18.7[a]	20.1[a]	19.1[a]
Liver weight[c] (g)	8.2[a]	8.6[a]	8.4[a]	8.5[a]	8.6[a]

[a] No significant differences among treatments for any parameter.
[b] n = 6.
[c] n = 14.

Spoerry, 1974). The corresponding total dry matter consumptions for these groups were: 516.0, 566.4, 559.3, 582.2 and 599.0, respectively (Table 1). Rats fed *Acidophilus* milk consumed significantly more dry matter than the rats fed the other diets (except *Bulgaricus* milk group), and rats fed chow + water consumed significantly less dry matter than the rats fed chow + milk or fermented milks ($P < 0.05$).

The cholesterol distribution pattern among various body pools is shown in Table 2. The cholesterol content in the body (minus the cholesterols in blood collected and the liver) ranged from 3.00–3.68 mg/g of dry matter for various treatments with no significant ($P > 0.05$) differences. If anything, there was less cholesterol in the whole bodies of rats fed various fermented milks. Similarly, plasma and liver cholesterols were not affected by the various dietary treatments. Liver lipids, whole body lipids and liver weights were also not affected by the dietary treatments. To our knowledge, no published information is available to compare our results. Thus, from our investigation, it appears that cholesterol distribution among various body pools is not affected by milk fermented by various lactic cultures. Therefore, previously observed hypocholesteremic effect of fermented milk (Mann, 1977b; Hepner et al., 1979; Rao et al., 1981) may be due to: (1) an increased excretion of cholesterol or its metabolites, and (2) inhibiton of cholesterogenesis by metabolites produced by lactic cultures (Rao et al., 1981). In the former mechanism it is possible that lactic cultures metabolize the cholesterol secreted in the bile to products not absorbable and thus increase the rate of excretion of cholesterol. Sterol balance studies are needed to substantiate such mechanism.

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A Research Note

A Study on the Growth Potential of *Staphylococcus aureus* in *Boletus edulis*, A Wild Edible Mushroom, Prompted by a Food Poisoning Outbreak

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ABSTRACT

A dish of *Boletus edulis*, a wild edible mushroom, in vinegar caused staphylococcal food poisoning in 13 of 35 diners in a restaurant. Enterotoxicosis was confirmed by detection of toxins A and D in the dish. *Staphylococcus aureus* growth potential in *B. edulis* was studied by inoculating fresh and frozen and thawed bolete with *S. aureus* strains VTTE 530, 757, 793 and 805 and storing for 3 days at 15 and/or 21°C. Essentially no staphylococcal growth was observed in frozen and thawed mushrooms contaminated with strains 530, 793 or 805 and stored at 15 or 21°C. In fresh *B. edulis* the same strains showed slight growth at 21°C. Frozen and thawed bolete inoculated with strains 530 and 757 (isolated from mushroom soup, nonenterotoxigenic) supported staphylococcal growth in 2 days at 21°C from a level of 4.8×10^4 and 5.4×10^3 to 2.0×10^6 and 7.0×10^7 cfu/g, respectively. Enterotoxin was not detected in these samples.

INTRODUCTION

OF 35 EMPLOYEES of a firm lunching at a restaurant 13 became ill. Within 1-4 hr after lunch symptoms of nausea, severe and frequent vomiting, fatigue, diarrhea and in some cases headache and abdominal cramps appeared. Routine microbiological tests of different buffet dishes gave no indication of the causative agent. On the basis of interviews with the affected and unaffected persons, suspicions centered on *Boletus edulis* (a highly valued wild mushroom) in vinegar. This was taste tested by a volunteer member of the municipal laboratory staff. Typical staphylococcal food poisoning symptoms, as described above, followed 3.5 hr after cosumption of the mushroom.

A sample of these mushrooms was sent to the Food Research Laboratory on the basis of poisoning symptoms for analysis for staphylococcal enterotoxins, although no living staphylococci had been observed in the sample. The mushrooms were identified as a nonpoisonous species of the genus *Boletus* and the sample mixture was found to contain high levels of enterotoxin. As to the best knowledge of the authors no earlier reports of staphylococcal food poisoning originating in fresh, unheated and unprocessed mushrooms exist, the growth potential of *Staphylococcus aureus* was investigated together with the enterotoxin formation capability in mushrooms experimentally contaminated at the Laboratory.

MATERIALS & METHODS

Enterotoxin detection

A sample of *B. edulis* in vinegar (7g of mushroom pieces and 54g of vinegar, pH 4.0) was analyzed for enterotoxins A, B, C, D and E by the Niskanen and Lindroth (1977) method. Control toxins and antisera were purchased from Serva Feinbiochemica, Heidelberg (A, D) or supplied by Prof. M.S. Bergdoll, Food Research Institute, Univ. of Wisconsin, Madison (B, C, E).

The applicability and enterotoxin recovery efficiency of the method were tested by adding 0, 1, 3 or 10 μg of purified enterotoxin A into a mushroom-vinegar sample containing 50g of *B. edulis*, 50 ml of distilled water and 0.3 ml of glacial acetic acid, final pH 3.5.

Mushroom identification from the food poisoning sample

In order to verify that the mushroom pieces in the sample actually belonged to nonpoisonous species of *Boletus*, mushroom pieces were examined microscopically for spores and other characteristic features (Leclair and Essette, 1970).

Growth experiments with *S. aureus* strains

S. aureus strains VTTE 530, 757, 793 and 805, previously isolated from foods or feed, were cultivated in brain heart infusion broth (Difco) for 18-20 hr at 37°C with agitation (120 rpm). Bacterial suspensions were diluted 4000-fold with tap water yielding a final count of 5×10^5 to 1×10^6 cfu/ml on Baird-Parker agar. A member of the laboratory staff immersed his hands in staphylococcal water and air dried them for a few minutes. Fresh or frozen and thawed *B. edulis* (thawing overnight at 4°C; used as such or after rinsing with a small amount of distilled water), 300g in weight, were sliced with contaminated hands yielding a staphylococcal inoculum of 1.1×10^3 to 4.8×10^4 cfu/g of mushroom. Mushrooms were put into a plastic box and stored at 15 and/or 21°C for 3 days. During storage 11g subsamples were taken at 24-hr intervals and analyzed for aerobic plate count (plate count agar, Difco; 30°C 72 hr) and staphylococci (Baird-Parker agar, Difco; 37°C 48 hr). Coagulase production of colonies showing typical staphylococcal appearance was tested on coagulase agar (Difco) plates containing 7% coagulase plasma EDTA (Difco). Enterotoxin analyses were carried out for 50g samples of mushrooms contaminated with *S. aureus* strains VTTE 530 and 757 and stored for 3 days at 21°C. The enterotoxin formation capability of the strains was tested by the method of Donnelly et al. (1967).

RESULTS & DISCUSSION

SEROLOGICAL ENTEROTOXIN DETECTION revealed 0.01 μg toxin A and 0.001 μg toxin D per g of the mushroom-vinegar sample. Control analysis with a laboratory-made bolete-vinegar mixture with no added toxin produced a clear negative result. All tests with added toxin showed the enterotoxin recovery efficiency of the method to be around 5%. This value is equal to the recovery efficiency of the methods of Barber and Deibel (1972), Niskanen and Lindroth (1976) and Reiser et al. (1974) as reported by Niskanen and Lindroth (1976) as well as to the recovery efficiency of the method currently being used by the FDA (Bergdoll and Bennett, 1976; ICMSF, 1978) and calculated from the data of Bennett and McClure (1980). Thus the detected toxin values would correspond to actual concentrations of 0.2 μg of enterotoxin per g of mushroom-vinegar sample. Toxin concentration per g of mushroom has obviously been lower since it is probable that enterotoxins, as water-soluble compounds, have partly been extracted from the mushrooms by vinegar, which comprised most of the analyzed sample.

A microscopic analysis of the mushroom pieces from the food poisoning sample revealed fusiform spores typical of

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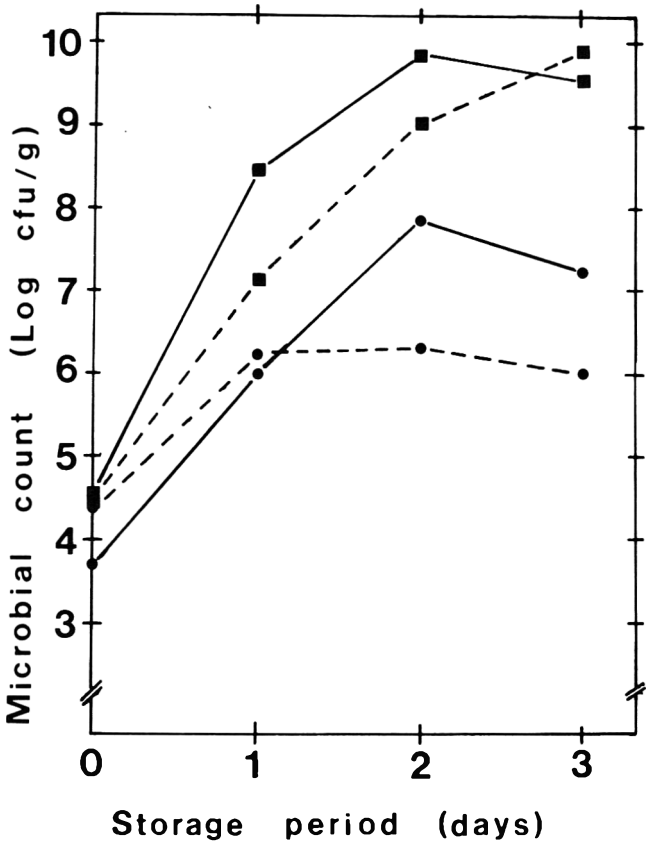

Fig. 1—Microbial counts in frozen and thawed B. edulis, *rinsed with water, inoculated with* S. aureus *strains VTTE 530 and 757 and stored at 21°C. Symbols: ■ aerobic plate count, ● coagulase positive staphylococci, – – – strain 530, ___ strain 757.*

the species of *Boletaceae* family. The structure of the flesh on cap and stipe pieces was also characteristic of a *Boletus* species. The fusoides spores place the mushrooms in the genus *Boletus*. Neither were fruiting body pieces that had turned bluish nor red pieces of tubes and upper stipe, all characteristic of poisonous species of the genus *Boletus* (Rumack and Salzman, 1978), detected. Poisonous *Boletus* species have, moreover, not been found in the region where these mushrooms have been picked.

On the basis of the history of the mushroom dish in question, although no precise time-temperature values were available, contamination by *S. aureus* most probably occurred at some point between mushroom growth and cleaning, reaching a staphylococcal level producing enterotoxins before the hot vinegar mixture was added. For this reason research sought to analyze the growth potential of *S. aureus* in *B. edulis*. *B. edulis* used in laboratory experiments had an initial staphylococcal count of less than 100 and an initial aerobic plate count of 400 to 2.1×10^4/g. No significant growth of staphylococci nor increase in aerobic plate count during storage was observed in frozen and thawed bolete contaminated with *S. aureus* strains 530, 793 and 805 and stored at 15 or 21°C. Fresh mushrooms were stored at room temperature (21°C), after which a markedly higher aerobic plate count level was recorded. Staphylococcal content, on the other hand, increased only slightly (strains 530, 793 and 805).

Both the growth of staphylococci (strains 530 and 757) and increase in aerobic plate count was observed in frozen and thawed mushrooms, that had been rinsed with distilled water. This was done to remove possible antimicrobial substance(s) liberated from mushroom cells ruptured during the freezing-thawing process before staphylococcal contamination (Fig. 1). Maximum values were recorded for coagulase positive staphylococci after two days of incubation at 21°C. These values were 2.0×10^6 for strain 530 (isolated from smoked horse meat) and 7.0×10^7 cfu/g for strain 757 (isolated from mushroom soup causing food poisoning). Staphylococci levels then slightly declined, apparently due to the influence of competing microbial flora. Enterotoxin was not detected in these samples. Toxin production tests revealed that strain 530 produced enterotoxin A but that the strain 757 had become nonenterotoxigenic during the laboratory storage phase.

The results indicated that food poisoning was caused by staphylococcal enterotoxin found in the "mushroom in vinegar" dish and that certain *S. aureus* strains can reach a level of 10^6 cfu/g in *B. edulis*, thereby exceeding the value generally regarded as minimal for detectable entrotoxin production. However, the probability that abundant *S. aureus* growth would appear in fresh, unprocessed *B. edulis* is slight.

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A Research Note

A Scheme for the Rapid Preparation of Protein Hydrolyzates for Amino Acid Analysis

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ABSTRACT

An accelerated procedure for preparing acid hydrolyzates of food and feed proteins prior to amino acid analysis has been developed. This scheme features the simultaneous removal of oxygen from 12 samples and from hydrolysis reagent; accelerated hydrolysis of continuously stirred samples at 145°C for 1.25 hr; and semi-automated filtration of the pH-adjusted, diluted hydrolyzates. Using this scheme, one technician can prepare 12 samples for analysis in approximately ½ day. Hydrolyzates of casein and durum wheat flour were prepared by this method by the traditional approach, and by an intermediate method; then analyzed by automated ion exchange chromatography. Resulting profiles were generally comparable for the different schemes.

INTRODUCTION

ADVANCES in the automated chromatographic analysis of amino acids have made the preparation of protein hydrolyzates the rate-limiting step in determining amino acid profiles of proteins. Unlike the analysis itself, sample preparation is still usually done by manual operations on one sample at a time. The work reported here is aimed at expediting the preparation of acid hydrolyzates in which the common protein amino acids other than cystine and tryptophan can be determined.

The most widely accepted method for acid hydrolysis is that of Moore and Stein (1963), which features deaeration of 6N HCl-sample slurries by freezing, evacuation and thawing in glass tubes; sealing the tubes by torch; hydrolyzing at 110°C for 18–72 hr; evaporating the acid; making to volume; and filtering prior to analysis.

The skill and time required for sealing hydrolysis mixtures in glass tubes have been reduced by the use of stopcock equipped flasks (Kurtzman et al., 1965), screw cap test tubes (Roach and Gehrke, 1970; Savoy et al., 1975), and specially designed hydrolysis tubes (Conkerton, 1973; Anon., 1980). Removal of air in conjunction with these devices is simpler than freezing and thawing under vacuum, as it usually involves a combination of partial evacuation and nitrogen purging.

An accelerated hydrolysis scheme which gave comparable results to the 110°C, 24 hr method, was reported by Roach and Gehrke (1970). It features hydrolysis at 145°C for periods of time from 1–8 hr.

Hubbard and Finney (1976) reported that removal of HCL following hydrolysis was expedited by the use of an apparatus for evaporating 10 samples simultaneously. Spitz (1973) demonstrated that removal of the HCl could be replaced by pH adjustment.

A device which enables 12 samples in screw cap tubes to be simultaneously prepared for hydrolysis in about 1 hr (Phillips, 1981a) and a method for automated, unattended filtration of diluted, pH adjusted hydrolyzates (Phillips, 1981b) have recently been reported. This paper describes the integration of these devices into a scheme which allows one person to produce 12 ready-for-analysis hydrolyzate samples in about ½ day and the application of this scheme to two food protein materials.

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MATERIALS & METHODS

SAMPLES OF ANRC CASEIN and durum wheat flour were hydrolyzed by each of three schemes. Scheme A was similar to the traditional method of Moore and Stein (1963). Samples of casein (50–100 mg) and durum wheat flour (150–300 mg) were weighed into acid cleaned 18 mm diameter glass test tubes. The tubes were constricted in a flame and 10 ml of 6N HCl (a 1:2 dilution of reagent grade 12N HCl) were added. Contents of the tubes were frozen in a dry ice-isopropanol slurry and were deaerated by three cycles of thawing and refreezing under vacuum. Following a final freezing of the sample, the tubes were evacuated and sealed in a torch flame. Samples were hydrolyzed for 24 hr at 110°C in a forced air oven. Following hydrolysis, the tube contents were quantitatively transferred to beakers, and the pH adjusted to 2.20 using sodium hydroxide. Hydrolyzates were made to volume in 50 ml volumetric flasks with citrate buffer (pH 2.2). Diluted, pH-adjusted hydrolyzates were filtered through 47 mm diameter, 0.22 μm pore Millipore filters (Millipore Corp., Bedford, MA) prior to amino acid analysis.

In hydrolysis Scheme B, samples of casein (50–100 mg) and durum wheat flour (150–300 mg) were weighed into acid-cleaned 25 x 120 mm test tubes (KIMEX) equipped with Teflon-lined screw caps. Air was removed from the samples and from hydrolysis reagent using the 12 place deaerator as previously described (Phillips, 1981a). The sample tubes and 6N HCl – 0.5% phenol were introduced into the apparatus and were deaerated by three cycles of alternate evacuation (10–15 min) and N_2 purging (5 min). Ten ml of acid were measured into each tube and the caps threaded on under a blanket of nitrogen. The tubes were placed in a force draft oven at 110°C for 24 hr after which the hydrolyzates were pH adjusted, diluted, and filtered as a Scheme A.

In the integrated hydrolysis Scheme C, samples of casein (approximately 10 mg) and durum wheat flour (approximately 100 mg) were weighed into screw cap test tubes and 0.5 ml of norleucine internal standard (0.25 μmoles/ml) were added. Deaeration and acid transfer were carried out as in Scheme B. Hydrolysis was performed for 1.25 hrs in a Fisher Hi-Temp oil bath fitted with a circular, oscilating, 12-place test tube rack. Glycerol maintained at 145 ± 3°C was used as the heating medium. The contents were pH adjusted as in Schemes A and B. Volume was adjusted to approximately 30 ml as the presence of internal standard eliminates the need for exact control of volumes. Aliquots of each hydrolyzates were placed in sample cups and filtered on the autofilter as described by Phillips (1981b).

Hydrolyzate samples produced by the above methods were analyzed on a Durrum D-500 automatic amino acid analyzer as previously described (Phillips, 1981b).

RESULTS & DISCUSSION

AMINO ACID RECOVERIES resulting from the application of the three hydrolysis schemes to casein and wheat flour are given in Tables 1 and 2, respectively. Kwolek and Cavins (1971) analyzed the variation inherent in amino acid analysis data from the literature. They found the mean relative standard deviation, considering all sources of variation, to range from 6–14% depending on the individual amino acid. From this they concluded that duplicate values must vary from each other by 17–40% (depending on the individual amino acid) to be significantly different. Applying the same criteria to means of 4 values, the expected variation would be 12–28%. For most amino acids, the variation

Table 1—Results of amino acid analysis of casein following acid hydrolysis under various conditions (g amino acid/16g nitrogen)

Amino acid	Hydrolysis procedure A	B	C
Asp	7.54 ± 0.50	7.32 ± 0.15	7.76 ± 0.29
Thr	4.58 ± 0.30	4.09 ± 0.12	4.28 ± 0.11
Ser	5.93 ± 0.30	5.28 ± 0.22	5.78 ± 0.29
Glu	22.41 ± 2.15	22.88 ± 0.70	23.63 ± 0.58
Pro	11.21 ± 0.90	10.89 ± 0.30	11.10 ± 0.49
Gly	1.94 ± 0.10	1.90 ± 0.03	1.93 ± 0.05
Ala	3.24 ± 0.30	3.10 ± 0.05	3.06 ± 0.08
Val	6.84 ± 0.50	6.86 ± 0.12	6.34 ± 0.13
Met	1.10 ± 0.22	1.93 ± 0.06	2.98 ± 0.10
Ile	5.51 ± 0.50	5.55 ± 0.05	5.11 ± 0.05
Leu	9.85 ± 0.70	9.64 ± 0.15	9.52 ± 0.10
Tyr	6.01 ± 0.60	6.17 ± 0.24	5.58 ± 0.13
Phe	5.29 ± 0.50	5.21 ± 0.18	4.78 ± 0.16
His	3.26 ± 0.28	2.97 ± 0.05	2.87 ± 0.03
Lys	8.52 ± 0.27	8.21 ± 0.12	8.21 ± 0.10
Arg	3.97 ± 0.26	3.77 ± 0.10	3.67 ± 0.14
Recovery[a]	91.3 ± 7.0	91.2 ± 9.6	91.7 ± 2.1
Number of replicates	4	4	4

[a] $\text{Recovery} = \frac{\text{g amino acid + } NH_3 \text{ Nitrogen}}{\text{g sample Nitrogen}} \times 100$

Table 2—Results of amino acid analysis of durum wheat flour following acid hydrolysis under various conditions (g amino acid/16g nitrogen)

Amino acid	Hydrolysis procedure A	B	C
Asp	5.15	4.78 ± 0.24	4.94 ± 0.07
Thr	2.72	2.86 ± 0.36	2.76 ± 0.05
Ser	2.47	3.75 ± 0.70	4.99 ± 0.14
Glu	37.54	35.71 ± 2.00	35.51 ± 0.34
Pro	12.15	11.53 ± 0.88	11.90 ± 0.37
Gly	3.77	3.46 ± 0.25	3.48 ± 0.06
Ala	3.40	3.09 ± 0.26	3.16 ± 0.03
Val	4.78	4.37 ± 0.33	3.96 ± 0.03
Met	0.00	0.94 ± 0.13	1.41 ± 0.13
Ile	4.75	4.16 ± 0.21	3.78 ± 0.02
Leu	8.11	7.39 ± 0.30	7.31 ± 0.05
Tyr	2.30	2.40 ± 0.30	3.16 ± 0.05
Phe	5.04	4.73 ± 0.50	4.74 ± 0.06
His	3.86	2.09 ± 0.22	2.32 ± 0.01
Lys	2.45	2.28 ± 0.05	2.33 ± 0.03
Arg	3.06	3.93 ± 0.31	4.26 ± 0.13
Recovery[a]	92.3	79.2 ± 5.4	81.1 ± 0.8
Number of replicates	1	4	4

[a] $\text{Recovery} = \frac{\text{g amino acid + } NH_3 \text{ Nitrogen}}{\text{g sample Nitrogen}} \times 100$

between values obtained by the different schemes in the present study were <10% and more often ≤5%. There were exceptions, however. Most notably Scheme C gave much higher values for methionine than Scheme A, while Scheme B gave intermediate values. Other amino acids which might be expected to be affected by hydrolysis procedure are serine, threonine and tyrosine due to lability and valine and isoleucine due to their slow release. In casein; threonine, serine and tyrosine are higher (but not significantly so) for Scheme A; while in wheat flour, recovery of serine and tyrosine (but not threonine) was considerably higher for Scheme C than for Schemes B and A. For serine there was also a large difference between Schemes A and B. These observations are in agreement with reported effects due to antioxidants (Blackburn, 1978) and to hydrolysis temperature and time (Roach and Gherke, 1970). Scheme A recovery of histidine was much higher for wheat flour but only slightly higher for casein than Scheme B or C recoveries. Recoveries of valine and isoleucine would be expected to be affected by hydrolysis temperature and time but not by the presence of oxygen or antioxidants. In casein, the differences between recoveries of these amino acids were too small to be considered significant, while in wheat flour they probably are significant. Scheme C does not eliminate the problems of destruction of some amino acids and slow release of others. Studies in this laboratory, supported by the findings of Roach and Gherke (1970), have shown that 1.25 hr hydrolysis time was optimal for the recoveries of methionine, serine, threonine and tyrosine but produced lower values for valine and isoleucine than did longer times. For more accurate results it is necessary to hydrolyze for times of up to 6 hr and to extrapolate the results (Phillips, unpublished data; Roach and Gherke, 1970).

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A Research Note

Influence of Temperature on Germination and Growth of Spores of Emetic and Diarrheal Strains of *Bacillus cereus* in a Broth Medium and in Rice

K. M. JOHNSON, C. L. NELSON, and F. F. BUSTA

ABSTRACT

Germination and growth of *Bacillus cereus* spores from emetic and diarrheal strains were measured in Trypticase soy broth (TSB) and in autoclaved rice/beef extract from 5°–55°C. Growth for some strains occurred from 15°–50°C, and little difference was noted between responses of diarrheal and emetic types or between media, except a higher maximum population was achieved in rice. Germination was more extensive in rice than in TSB at <15°C and was generally more extensive for diarrheal strains in either medium.

INTRODUCTION

Bacillus cereus is known to cause two distinct food-borne illness syndromes. One is characterized by emetic symptoms which most frequently result from the consumption of contaminated rice. The other syndrome is characterized by diarrheal symptoms and a wide variety of foods have been implicated (Gilbert, 1979). Previous work in our laboratory suggested that there was little difference between the heat resistance of diarrheal and emetic strains; however, diarrheal strains were capable of more extensive germination than emetic strains in Trypticase soy broth (TSB) at 30°C in 2 hr (Johnson et al., 1982). Since rice is associated with the emetic syndrome, one questions if the rice might stimulate the growth of emetic strains. Therefore, the objective of this work was to characterize growth and germination of four strains of *B. cereus* spores over a range of temperatures in TSB and in a rice medium.

MATERIALS & METHODS

SPORE SUSPENSIONS of diarrheal strains F4433/73 and B4ac, and emetic strains F4810/72 and F4165/75 were prepared using fortified nutrient agar and stored as previously described (Johnson et al., 1982). To reduce variation among trials, the four individual strains were evaluated simultaneously.

Germination and growth was studied in duplicate trials at 5°, 15°, 25°, 30°, 35°, 45°, and 55°C in rice. A 3.5-g sample of package grade, long grained white rice of the Starbonnet variety (Riviana Foods) was heated with 7 ml of 1.0% beef extract (Difco) in 25 x 150 mm screw-capped tubes for 15 min at 121°C. The rice/beef extract mixture, equilibrated at the appropriate temperature, was inoculated with 1 ml of heat-shocked (70°C for 15 min) spores to achieve a population of approximately 10^5 spores/g. For each trial, a tube was removed at each of ten specified sampling times over a 24-hr period. A 150 mm stainless steel letter opener was used to dislodge the rice, which was subsequently transferred to a Whirlpak bag with 90 ml of 0.1% peptone. The mixture was blended by stomaching for 15–30 sec and the toal *B. cereus* population was determined by plating appropriate dilutions on mannitol egg yolk polymyxin agar (MYP, Mossel et al., 1967). The heat resistant population was determined by plating a heat-shocked (70°C for 15 min) aliquot. Plates were incubated at 30°C for 17–24 hr. The percentage of germinated spores was defined as:

$$\% \text{ germinated} = \left[1 - \frac{\text{heat resistant population}}{\text{total population}}\right] \times 100$$

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Germination and growth of the four strains was studied in TSB from 10–55°C in 5°C intervals, excluding 20°C. Strain B4ac was also used for one trial at 5°C. One to three replicate trials were performed for each temperature. Individual 100 ml portions of TSB, equilibrated at the appropriate temperature, were inoculated with heat-activated spores to achieve an initial population of approximately 10^5 spores/ml. The broth was agitated vigorously prior to each sampling. For each trial, 3 ml samples were removed at each of ten specified sampling times over a 24-hr period. One milliliter was diluted and plated on MYP and the remaining 2 ml were heat shocked prior to plating. Plates were incubated as described for rice samples.

RESULTS & DISCUSSION

DATA or geometrically averaged generation times, maximum populations, and percentage of germinated spores in rice and in TSB are presented in Fig. 1. Growth of one or

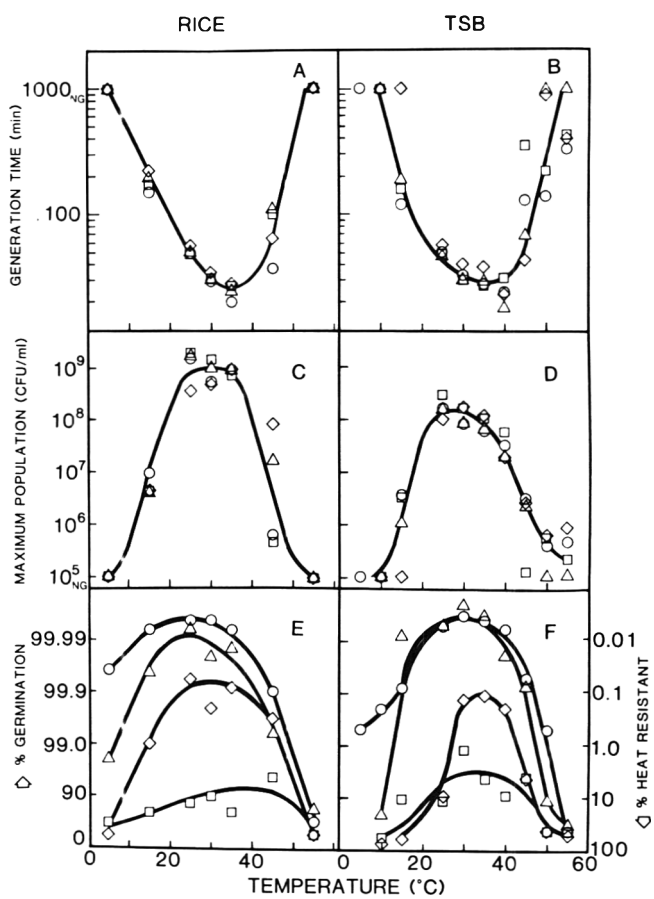

Fig. 1—Generation time (A and B), maximum population (C and D), and percentage of germinated spores (E and F) of Bacillus cereus spores in rice made with 1% beef extract (1:2) and in Trypticase soy broth at various constant temperatures. Emetic strains F4165/75 (◇) and F4810/72 (□); diarrheal strains B4ac (○) and F4433/73 (△). N.G. indicates "no growth" and points were arbitrarily plotted at 1000 min for generation time and 10^5 for maximum population.

more strains occurred between 15° and 50°C. Generation times for all strains in both media were similar from 25°–40°C, and the fastest growth occurred between 35° and 40°C with a generation time of 18–27 min (Fig. 1, A and B). Parry and Gilbert (1980) previously reported little difference between the growth rate of several strains of *B. cereus* in boiled rice at 22°C. At 45°C, strains B4ac and F4810/72 in rice demonstrated an initial decline in the population prior to the subsequent increase in numbers (Fig. 2). Rappaport and Goepfert (1978) previously reported injury of vegetative cells of *B. cereus* attributed to incubation at 47°C. The generation times calculated at 45°C in our work may, in fact, be a measure of the rate of repair, of growth, or of both at 45°C. The same phenomenon was observed in TSB with incubation greater than or equal to 45°C for all strains except F4165/75.

Although the lag time (data not presented) and generation time were generally the same in rice and TSB for a given strain, a higher maximum population was achieved in rice (Fig. 1, C and D). The highest populations were observed between 25° and 35°C. Emetic strain F4165/75 was capable of extensive growth at 45°C in rice, whereas growth for other strains was slower or declined as previously described (Fig. 2). It should be noted that strain F4165/75 was the most heat resistant of the four strains (Johnson et al., 1982).

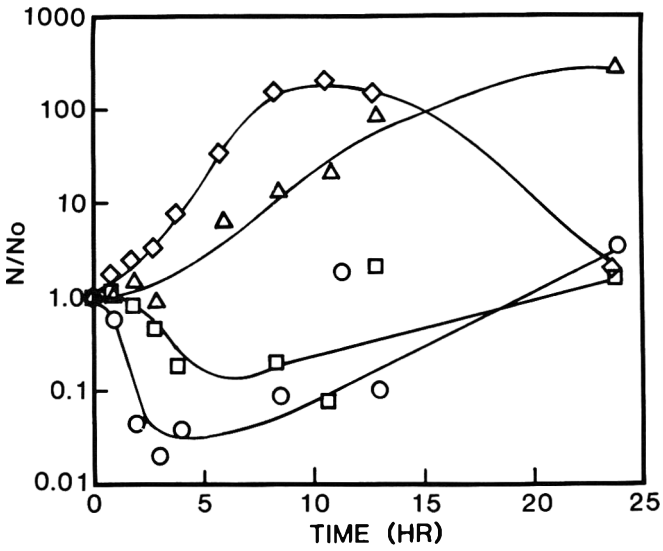

Fig. 2—Growth of Bacillus cereus from heat shocked (70°C for 15 min) spores in rice made with 1% beef extract (1:2) at 45°C. Emetic strains F4165/75 (◇) and F4810/72 (□); diarrheal strains B4ac (○) and F4433/73 (△).

Differences between the extent of germination in rice and in TSB was dependent upon strain (Fig. 1, E and F). Germination in rice at 5°C was more extensive than that in TSB at 10°C for all strains tested. Strain F4165/75 was capable of >99% germination from 15–45°C in rice, and from 30–40°C in TSB. Diarrheal strain F4433/73 had greater germination in TSB except at low temperature. This may have also been true for emetic strain F4810/72; however, the low level response of this strain, in comparison to the others, produced variability which makes detection of differences difficult. Little difference was noted between germination in rice and TSB for diarrheal strain B4ac, except at low temperatures. Overall, germination of emetic strains was less than that of diarrheal strains in either medium at ≤40°C.

These data provide useful information on the basic growth and germination characteristics of *B. cereus*. While little difference in growth rate was observed among the four strains, a higher maximum population was observed in rice due to a delay of the onset of stationary phase. Rice did not appear to favor the growth of emetic strains over the diarrheal strains. Although differences were observed between the germination of the individual strains in the two media, the differences were not related to strain type. Germination was greater for one emetic strain in rice and for the other in TSB. However, rice did improve germination for all strains at low temperature. This, in addition to the higher maximum populations produced in rice, illustrates the potential risk in translation of laboratory media data to phenomena which occur in food.

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A Research Note

Nutritional Quality of Raw, Precooked and Canned Albacore Tuna (*Thunnus alalunga*)

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ABSTRACT

The nutritional quality of raw, precooked and canned tuna was assessed by determining the total amino acids, in-vitro protein digestibility, Computed Protein Efficiency Ratio (C-PER), Tetrahymena Relative Protein Value (RPV), and fluorodinitrobenzene reactive lysine, together with selected vitamin and mineral assays. Amino acid composition, in-vitro protein digestibility, C-PER and Tetrahymena RPV remained almost the same for the raw, precooked and canned tuna. The percent retention of FDNB-reactive lysine was 91% for the precooked tuna and 80–85% for canned tuna. Thiamin retention for the canned tuna was about 5%, while niacin and riboflavin ranged from 71–73% and 49–50% respectively. The values for Cu, Fe, K, and Ca were significantly lower in canned tuna.

INTRODUCTION

THE VALUE of fish for human nutrition is due to its relatively high protein content and the good digestibility and high biological value of fish proteins. The amount of the B-vitamins, minerals and trace elements in fish is also very important nutritionally (Geiger and Borgstrom, 1962).

Published data on the effects of processing, especially heat processing, on the nutritional quality of fish are meager (Lopez and Fellers, 1948; Rice and Beak, 1953). Conventional canning of tuna often involves a long precooking period followed by heat sterilization of the fish meat in vacuum-sealed cans in the still-retort. This study was undertaken to determine changes in nutritional quality of tuna which occur at various stages of the canning process. Because of the number of analyses done on raw, precooked and canned samples, we have limited our study to two fish. We recognize the limitations of this decision, but wished to emphasize changes in particular nutrients as a result of processing, rather than doing a broad compositional survey.

MATERIALS & METHODS

TWO ALBACORE TUNA (*Thunnus alalunga*), frozen whole for three months since capture, were obtained commercially in Dec., 1981, and thawed in a tank of water for five hr. The fish weighed approximately 10 and 14 kg. Samples from the thawed, raw fish were obtained from six 1-in. cores per fish using a cork borer (Carlson et al., 1960). The fish were then dressed, washed, and precooked in a steam chest at atmospheric pressure (average temperature 100°C) for 3 hr. For comparison, samples from the raw and precooked fish were freeze-dried, then pulverized to produce a uniform sample.

After precooking the fish were placed in a 2°C cold room overnight. The fish were cleaned and the meat "standard" packed in 303 × 406 cans. Three pieces of solid meat were placed in each can with sufficient fragments to give a net weight of 350 gm. Ten gm of salt was then placed on top of the meat, and distilled, deionized water was next added, leaving a headspace of about 10 mm.

Five cans from each fish were treated in a steam retort at two processing temperatures, 115°C and 121°C, for 120 and 95 min respectively (Lerke, 1981). The cans were allowed to cool, stored at room temperature, and opened a week after processing. The contents were drained through a number 8 mesh screen. The drained meat was lyophilized, homogenized to a fine powder and stored at −20°C for subsequent analysis.

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Analytical techniques

Samples were analyzed for crude protein (AOAC, 1975), N × 6.25. The amino acid composition was determined using an automated amino acid analyzer equipped with a Durrum chromatography column and an Autolab integrator. The fish samples were hydrolyzed for 24 hr at 110°C with 6N HCl under vacuum. Tryptophan was determined by an alkaline hydrolysis method (Hugli and Moore, 1972), and the sulfur-containing amino acids were analyzed by using a performic acid pretreatment of samples followed by acid hydrolysis with 6N HCl (Moore, 1963).

The in-vitro protein digestibilities of the samples and Animal Nutrition Research Council (ANRC) sodium caseinate were determined by the method of Satterlee et al. (1979) using a multienzyme mixture of trypsin, chymotrypsin, peptidase and a bacterial protease. The Computed Protein Efficiency Ratio (C-PER) values for the fish samples were computed using a C-PER procedure program (Satterlee et al., 1977, 1979, 1982). Tetrahymena Relative Protein Values (RPVs) were assessed by the method of Baker et al. (1978). A stock culture of *Tetrahymena thermophila* WH_{14} (no. 30008) was purchased from American Type Culture Collection, Rockville, MD.

Chemical estimates of reactive lysine in the samples were made by the 1-fluoro-2, 4-dinitrobenzene (FDNB) extractive method of Carpenter (1960). Thiamin, niacin and riboflavin contents were determined by AOAC procedures (1975). Samples for atomic absorption spectrophotometric analysis of minerals were prepared by wet digestion using 5 ml of concentrated nitric acid and 2 ml of 70% perchloric acid per g of lyophilized sample for 1.5 hr at 150°C and at 210°C for another 1.25 hr.

Table 1—Amino acids (g/16g N) in albacore tuna

Amino acid	Raw[a]	Precooked[a]	Canned[b] 115°C	Canned[b] 121°C
Essential				
His	6.59	5.58	5.07	5.07
Ile	4.49	4.43	4.42	4.47
Leu	8.24	8.13	8.30	8.33
Lys	8.97	8.70	8.65	8.67
Met	3.04	3.06	2.66	2.72
Cys	1.62	1.55	1.45	1.47
Phe	3.62	3.59	3.68	3.81
Thr	4.57	4.48	4.66	4.69
Trp	1.13	1.07	1.15	1.19
Val	5.31	5.15	5.20	5.33
Nonessential				
Ala	5.73	5.68	5.67	5.72
Arg	5.60	5.51	5.63	5.64
Asp	9.36	9.19	9.28	9.28
Glu	12.83	12.62	12.52	12.57
Gly	4.39	4.40	4.64	4.56
Pro	4.13	3.94	3.94	3.94
Ser	3.45	3.67	3.77	3.82
Tyr	3.70	3.61	3.78	3.79

[a] Mean of two analyses
[b] Mean of six analyses

Table 2—Vitamin and mineral contents of raw, precooked and canned tuna[a]. Na values in canned samples reflect added NaCl

	Raw	Precooked	Canned 115°C	Canned 121°C
Vitamins[b]				
Thiamin (μg/100g)	189 ± 36d	97 ± 14e	9 ± 0f	11 ± 3f
Riboflavin (μg/100)	305 ± 16d	215 ± 13e	150 ± 28f	152 ± 24f
Niacin (mg/100g)	28 ± 1d	20 ± 0e	20 ± 1e	19 ± 1e
Minerals[c]				
Na (g/100g)	0.27 ± 0.03d	0.21 ± 0.05d	2.31 ± 0.03e	2.39 ± 0.14e
K (g/100g)	0.91 ± 0.03d	0.73 ± 0.03e	0.63 ± 0.00f	0.63 ± 0.03f
Ca (mg/100g)	2.94 ± 1.47d	1.20 ± 0.85e	0.80 ± 0.29e	0.80 ± 0.46e
Fe (mg/100g)	8.76 ± 0.40d	7.43 ± 2.09d	5.47 ± 1.05e	5.59 ± 2.39e
Cu (mg/100g)	2.54 ± 1.42d	0.35 ± 0.21e	1.03 ± 0.26d,e	0.63 ± 0.03e

[a] Values reported are on a dry weight basis. Means followed by the same letter are not significantly different ($P < 0.05$) from each other.
[b] Each mean ± standard deviation represents four determinations for the raw and precooked samples and six determinations for the canned samples.
[c] Mean ± standard deviation (N = 6).

RESULTS & DISCUSSION

THE AMINO ACID CONTENT remained essentially the same after heat processing except for histidine and the sulfur amino acids (Table 1). In-vitro protein digestibilities of the precooked and canned samples decreased slightly. Precooking resulted in an 0.8% loss in digestibility. Heat processing at 115°C and 121°C resulted in 2.2 and 1.8% overall losses, respectively. The losses are statistically significant ($P < 0.05$) when compared to the digestability of raw fish. The small decrease in digestability could mean that the availabilites of several amino acids were involved rather than only the reactive amino acids such as lysine and the sulphur containing amino acids. This could be due to the formation of inter- and intramolecular bonds which are resistant to digestive enzymes (Hurrell et al., 1976).

Precooking and retorting of the canned tuna did not affect protein quality of the product as determined by the C-PER and Tetrahymena RPV procedures. The C-PER value for all the samples were 2.6, showing excellent agreement between the Tetrahymena RPV and the C-PER values. Satterlee et al. (1977, 1979) have previously reported excellent correlation between the rat Protein Efficiency Ratio (PER) and the C-PER assays after doing extensive assessment of protein quality of a variety of food proteins. There are also reports that show a high correlation exists between the rat (PER) and the Tetrahymena assay (Satterlee et al., 1977; Baker et al., 1978). Therefore, the C-PER procedure seems to be a practical method for evaluating protein quality.

Reactive lysine was moderately decreased during precooking and a further decrease resulted after heat treatment in the still retort. The percent retention of lysine in the precooked tuna was 91% and 80–85% for canned tuna. The observed loss of lysine has little practical importance, as the content of lysine in the raw product is high to start with.

Thiamin, being a heat-labile vitamin, was significantly lower in both the precooked and the canned samples (Table 2). There was about a five percent retention of the thiamin content in the canned samples as compared to the raw samples. This loss may be due not only to heat destruction but also to leaching of the vitamin into the discarded liquid portion of the cans. This was not assessed, however. Niacin and riboflavin, although not usually considered heat-labile vitamins, were also significantly reduced in the precooked and canned samples. The retention of niacin in the canned samples was 71–73%, while the retention of riboflavin was 49–50%. Again, leaching may account for all or part of the differences. In canned mackerel, the retention of thiamin, niacin and riboflavin have been reported to be 30%, 71% and 64% respectively. These values were obtained from analysis of both the solid and liquid portions (Watt and Merrill, 1975).

Results for the mineral content of the samples showed that there was a general trend whereby the precooked and canned samples have a lower level of each of the minerals assayed. Copper and calcium particularly were poorly retained in the precooked and canned samples. As with the vitamins, leaching of the minerals into the liquid portion could be responsible for the low retention in the meat.

These results suggest that while there were losses in some vitamins and minerals during processing of tuna, the protein nutritive values were not drastically altered.

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A Research Note

A Headspace Gas Chromatographic Method for the Rapid Analysis of Ethanol in Canned Salmon

THOMAS A. HOLLINGWORTH, JR. and HAROLD R. THROM

ABSTRACT

A rapid, simple method using a headspace sampling technique and gas chromatographic detection has been developed for the analysis of ethanol in canned salmon. This method was used to analyze canned salmon samples of four species of Pacific salmon (sockeye, pink, coho, and chum) for ethanol content. The highly significant correlation between ethanol content and sensory classification of decomposition in canned salmon found in previous work using different analytical methodology was confirmed in this study ($r = 0.92$, $p < 0.001$, Pearson's correlation; $r = 0.95$, $p = 0.001$, Spearman's correlation). This relationship has potential practical application for the confirmation of an initial sensory classification of the product.

INTRODUCTION

RECENTLY, we reported (Hollingworth and Throm, 1982) that ethanol content in canned salmon is highly correlated to the sensory classification of decomposition of the product. It was therefore suggested that this relationship would be useful to confirm the initial sensory classification of canned salmon.

However, the gas chromatographic method used for analysis presented major problems with respect to turnaround time and column contamination. Therefore, if the correlation between ethanol content and sensory classification were to prove useful, an improved method of analysis would have to be found.

We now report the development of a simple, rapid headspace method for the quantitative analysis of ethanol in canned salmon aqueous phase.

MATERIALS & METHODS

Apparatus

Gas chromatograph. Hewlett-Packard Model 5880A, equipped with a flame ionization detector (Hewlett-Packard, Avondale, PA).

Syringes. Hamilton gas tight, No. 1005-LTN (5.0 ml capacity) and No. 1010-LTN (10.0 ml capacity) (the Anspec Company, Inc., Ann Arbor, MI).

Headspace vials. Glass vials, screw cap, Kimble Cat. No. 60910-L, 23 mm x 85 mm, 6 dram (approx. 22 ml) capacity (Ace Glass, Inc., Vineland, NJ) fitted with a perforated screw cap (Cat. No. 95053) with a Teflon-faced liner (Cat. No. 9522), both screw cap and liner from Alltech Associates, Los Altos, CA.

Continuously adjustable digital microliter pipette. Gilson Pipetman Model P-20, 1-20 µl range (Cat. No. P-20), equipped with Rainin Certified Disposable Microliter Pipette Tips (Cat. No. RC-20), both from West Coast Scientific, Inc., Berkeley, CA.

Reagents

Absolute ethyl alcohol (U.S.P., U.S. Industrial Chemicals Co., New York, NY).

Sodium chloride crystal (Baker Analyzed Reagent, J.T. Baker Chemical Co., Phillipsburg, NJ).

tert-Butyl alcohol (Baker Analyzed Reagent, J.T. Baker Chemical Co., Phillipsburg, NJ).

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Canned salmon samples

Canned salmon samples of four species of Pacific salmon, sockeye (*Oncorhynchus nerka*), pink (*Oncorhynchus gorbuscha*), coho (*Oncorhynchus kisutch*) and chum (*Oncorhynchus keta*) were collected from a variety of sources which included commercially packed cans from various processors and locations, salmon that contained naturally occurring decomposition which was canned and retorted under FDA supervision, and from salmon that was allowed to decompose in Alaskan canneries under as near normal conditions as possible.

Canned salmon aqueous phase

The can was opened and the liquid phase was drained into a 250 ml beaker while pressing the lid against the contents. The salmon remaining in the can was retained for sensory analysis. The liquid phase was transferred to a 250 ml separator and the oil and aqueous phases allowed to separate. The aqueous phase was drained into a stoppered cylinder and stored until analysis. If the sample was not analyzed immediately, it was stored either in the refrigerator for up to 2 days or in the freezer for longer periods of time.

Sensory classification of sample

Immediately after removal of the liquid phase, the salmon remaining in the can was examined by a sensory panel of 3 to 4 qualified examiners and assigned to one of the three sensory classes of decomposition that are utilized by the Food and Drug Administration (Hollingworth and Throm, 1982). The analyst who performed the ethanol analysis was not informed of the sensory panel results prior to the analysis.

Preparation of headspace standards and samples

Five standard solutions containing ethanol in distilled water of the following concentrations: 11.0 ppm, 21.9 ppm, 43.8 ppm, 76.6 ppm and 109.5 ppm were prepared. Exactly 5.0 ml of each of the standard solutions was transferred into a glass headspace vial using a 5 ml pipet. Then 16.1 µl of a 1858.6 ppm standard solution of tert-butyl alcohol in distilled water was added to each using a Gilson Pipetman to give a tert-butyl alcohol (the internal standard) concentration of 6.0 ppm. After gentle mixing for a few seconds, 3.0g NaCl was added. Each vial was sealed with the perforated screw cap, swirled vigorously for 2 min, and let stand for at least 5 min before 5 ml of headspace was injected into the gas chromatograph. A calibration curve was constructed from analysis of the 5 standards. The same general procedure was used for the preparation of samples.

Gas chromatographic analysis

A 6 ft x 4 mm i.d. glass column packed with Porapak QS (100-120 mesh) (Waters Associates, Inc., Milford, MA) was used for analysis. Operating conditions were as follows: (a) temperatures – injector, 200°C; detector, 250°C; column, 166°C; (b) gas flow rates – carrier gas (nitrogen), 50 ml/min; hydrogen, 43 ml/min; air, 421 ml/min.

Prior to analysis, 5 ml of air was injected to ensure that neither the gas tight syringe nor air were contaminated. For analysis, 5 ml of headspace was withdrawn from the vial into the syringe in a single, slow, continuous action; then injected. If for any reason a second injection was required, a new standard was prepared. Between injections, the syringe was "pumped" a minimum of 10 to 15 times to avoid carry over contamination. Peak areas of ethanol (R.T. = 2.6 to 2.7 min) and the internal standard, tert-butyl alcohol (R.T. = 6.9 to 7.1 min), were used for quantitation. Reproducibility as

Table 1—The relationship between ethanol concentration (ppm) and sensory classification in various species of canned salmon

Sockeye[a]			Pink[b]			Coho[c]			Chum[d]		
Class 1	Class 2	Class 3	Class 1	Class 2	Class 3	Class 1	Class 2	Class 3	Class 1	Class 2	Class 3
0	78	170	1	8	225	TR	28	48	0	33	200
0		400	2	49	240	1	29		TR	52	
TR[e]			2	63	275	1	30		2	230	
TR			2			2	35		7	300	
TR			4			7			7		
TR			5			7			13		
8			5			14			14		
11			14			22					
11			22								

[a] Total number of canned sockeye salmon samples = 12
[b] Total number of canned pink salmon samples = 15
[c] Total number of canned coho salmon samples = 13
[d] Total number of canned chum salmon samples = 12
[e] TR = Trace (less than 1 ppm ethanol)

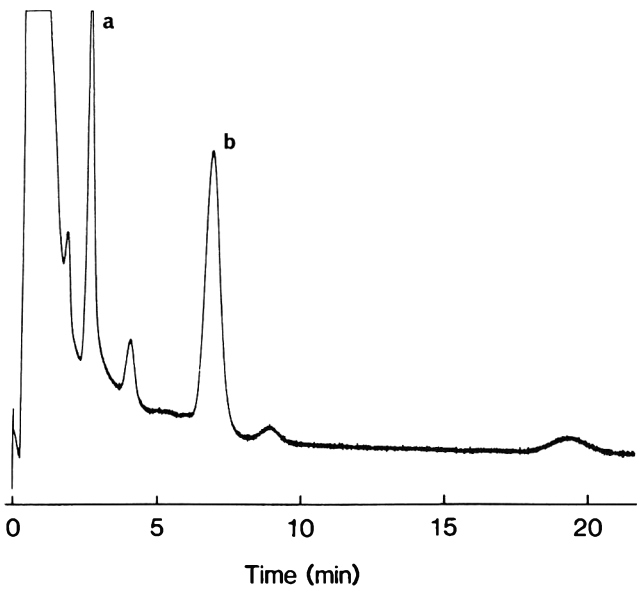

Fig. 1—Chromatogram from the injection of 5 ml headspace from a sample of canned salmon aqueous phase containing 25 ppm ethanol and 6.0 ppm tert-butyl alcohol (internal standard): a = ethanol; b = tert-butyl alcohol.

determined from recoveries and multiple sample injection was excellent (less than 10% variance). Samples were analyzed using the same techniques and quantitated from the calibration curve. However, when analysis of a sample indicated an ethanol concentration higher than that of the most concentrated standard, the original aqueous phase was accurately diluted with distilled water to give a value falling within the calibration limits.

The analysis turnaround time was approximately 21 min due to a late eluting peak at approximately 20 min.

Recoveries of ethanol from spiked (25.1 ppm and 75.2 ppm) canned salmon aqueous phase were 106% and 101%, respectively (average of two determinations).

RESULTS & DISCUSSION

AS INDICATED in the introduction, major problems were encountered with the gas chromatographic method used in the previous work (Hollingworth and Throm, 1982).

Since the method involved the direct injection of the filtered canned salmon aqueous phase onto a gas chromatographic column, the column tended to become contaminated during the course of a day's analyses. To overcome this problem, routine purging and cleaning with multiple injections of the ethanol standard solutions at the beginning and end of each day were required, thus greatly decreasing usable analysis time. A second problem associated with the method was the time (more than 45 min) required for the analysis of each sample due to late eluting peaks.

In view of these facts, a method was sought that would not require extensive initial cleanup. Machata (1972) reported that the concentration of ethanol in blood could be effectively analyzed by gas chromatographic headspace analysis. It thus appeared that this approach should be adaptable to the analysis of ethanol from canned salmon. Upon investigation, a method was developed which has less than half the turnaround time required by the previous method and which completely eliminates the contamination problem. These results are illustrated in Fig. 1.

The results of the analysis of the canned salmon samples are shown in Table 1. A total of 52 samples were analyzed and the degree of statistical significance and correlation between ethanol concentration and sensory classification of decomposition were determined by both the Pearson's correlation test and the nonparametric Spearman's correlation test. The results of this statistical analysis were as follows: $r = 0.9163$, $p < 0.001$, Pearson's correlation; $r = 0.9517$, $p = 0.001$, Spearman's correlation. These results confirm and extend those found in our previous work using different analytical methodology (Hollingworth and Throm, 1982).

In the previous study, tentative ranges of ethanol concentration were selected for confirmation of an initial sensory classification. The tentative ranges suggested were: sensory class 1, 0–24 ppm ethanol; sensory class 2, 25–74 ppm ethanol; and sensory class 3, 75 ppm ethanol and above. The excellent correlation found in the present study strongly suggests that these guidelines will need little if any adjustment in future work.

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A Research Note

A Simple Method for Evaluating Textural Changes of Frozen Fish Minces

DIETRICH KNORR and JOE M. REGENSTEIN

ABSTRACT

A simple and objective method for the evaluation of fracture force of frozen fish minces is presented. Fracture force determinations during frozen storage of red hake (*Urophycis chuss*) minces indicated a relative coefficient of variation of 4.7% and a significant correlation between fracture force data of frozen samples and force of compression data of thawed samples. No significant changes of fracture force of frozen samples have been observed between 2 and 45 days of storage, but fracture force increased five times between 45 and 90 days of frozen storage.

INTRODUCTION

QUALITY DETERIORATION during storage of frozen fish products has long been noted (Love, 1956; Matsumoto, 1980; Mills, 1975; Shenouda, 1980). It is also well recognized that the storage stability of minced fish is less than that of the whole fish muscle (Laird et al., 1981; Sikorski et al., 1976) and that the quality changes occurring during frozen storage of fish are of great commercial importance (Mills, 1975).

Shenouda (1980) recently reviewed theories on protein denaturation during frozen storage and reported that undesirable changes in fish texture (extra firmness, toughness, springiness, sponginess, stringiness, dryness, rubbery texture, lack of succulence, loss of water-holding properties, or loss of juiciness) are recognized as being due to protein changes during frozen storage, particularly the myofibrillar proteins. The textural changes of fish muscle and fish minces during frozen storage have been assessed both subjectively (Gill et al., 1979; Laird et al., 1981; Love, 1956, Sorensen 1976) and objectively (Buttkus and Tarr, 1962; Dassow et al., 1962; Dunajsky, 1979; Gill et al., 1979; Love, 1958; Sorensen, 1976). All these methods require thawing of the frozen samples prior to testing which lengthens the total procedure. Furthermore, since deterioration of frozen fish is usually accelerated by increasing the temperature, thawing of frozen fish samples contributes an additional variable which affects the results of texture evaluation.

The present study aimed, for the above mentioned reasons, to develop a fast, simple and objective method for the evaluation of textural changes of fish mince during frozen storage.

MATERIALS & METHODS

RED HAKE (*Urophycis chuss*) was obtained as whole, ungutted fish from Gloucester, MA, kept on ice for approximately 10 hr, filleted, and then forced through 1 mm orifices (Meat grinder model N-50, Hobart Manufacturing Co., Troy, OH). The minced samples were frozen in aluminum trays (60 x 25 x 6) at −26°C for 30 hr. The frozen blocks were then band-sawed into 25 x 50 x 10 mm and 25 x 25 x 25 mm pieces. Four samples each were heat sealed in polyethylene bags, kept for up to 30 min at −26°C, and finally stored at −18°C. Texture measurements (TM table model, CT 200 lb load cell, Instron Corp, Canton, MA) were carried out at a crosshead speed of 25.4 mm · min^{-1} and a chart speed of 254 mm · min^{-1}.

The 25 x 25 x 25 mm samples (covered with aluminum foil to prevent drying) were thawed at 40°C for 3 hr and then compressed to 50% of their original height. The maximum force of compression was determined. During compression the samples were placed on two layers of Whatman No. 1 filter paper (diameter 9 cm) to determine the water loss during compression. The frozen samples (25 x 50 x 10 mm) were placed on an especially designed test cell (Fig. 1) and tested at room temperature within 30 sec after removal from frozen storage. The fracture force and deflection of frozen samples till fracture were recorded. All measurements were made with four to eight replications. Statistical analyses were carried out using a statistical package of Plato (Control Data Corp., St. Paul, MN).

RESULTS & DISCUSSION

DATA ON STORAGE TIME dependent changes of force of compression of thawed (25 x 25 x 25 mm) samples as well as data on the changes of fracture force and deflection of

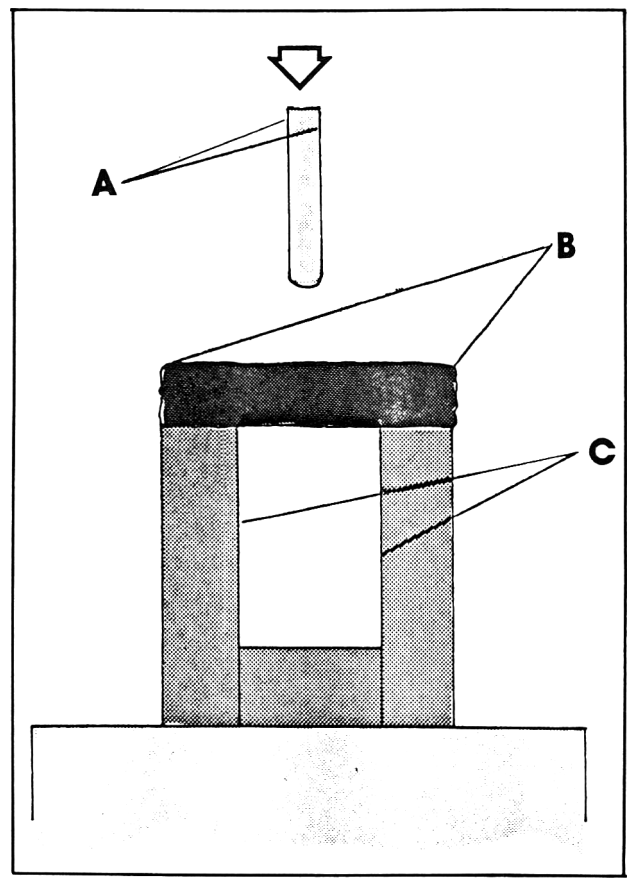

Fig. 1—Test cell for fracture force determination of frozen fish samples: (A) Bar (6 x 25 x 100 mm) attached to the crosshead of the Instron Universal Testing Machine; (B) Fish mince sample (25 x 50 x 10 mm); (C) Supporting parallel bars (inner distance 25 mm, outer distance 50 mm).

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Table 1—Force of compression (50%) of thawed minced red hake samples vs storage time, and fracture force and deflection of frozen samples vs storage time

Storage time (days)	Thawed samples force of compression (N)	Frozen samples fracture force (N)	Deflection mm
2	7.54 ± 0.62 (n=4)[a]	79.2 ± 9.1 (n=7)[a]	0.93 ± 0.32 (n=7)[a]
5	6.82 ± 2.51 (n=4)[a]	76.7 ± 6.2 (n=4)[a]	0.70 ± 0.26 (n=4)[a]
10	8.42 ± 0.86 (n=4)[a]	80.7 ± 7.5 (n=4)[a]	0.95 ± 0.27 (n=4)[a]
15	33.90 ± 2.58 (n=4)[b]	74.6 ± 13.3 (n=4)[a]	0.70 ± 0.22 (n=4)[a]
30	–	88.7 ± 18.7 (n=4)[a]	0.88 ± 0.46 (n=4)[a]
45	–	61.9 ± 17.7 (n=8)[a]	1.13 ± 0.10 (n=8)[b]
60	–	220.5 ± 24.3 (n=4)[b]	0.80 ± 0.34 (n=4)[a]
90	–	312.6 ± 63.6 (n=4)[c]	1.08 ± 0.42 (n=4)[a]

[abc]Means with different letters within a column are significantly different ($P < 0.01$).

frozen samples (25 x 50 x 10 mm) of minced red hake are given in Table 1. These results indicate no significant changes of force of compression of thawed samples between 2 and 10 days of frozen storage but a significant ($P < 0.01$) increase of force of compression between the 10th and 15th day of frozen storage. The loss of water during compression from 100% to 50% of initial height during this period ranged from 1.2–2.9g H_2O/sample of 14 ± 2g.

Insignificant ($P < 0.01$) changes of fracture force of frozen samples (25 x 50 x 10 mm) were observed between 2 and 45 days of storage. A regression analysis of fracture force vs storage time (2 to 45 days) resulted in the following equation: Y (fracture force) = 81.64 – 0.33 X (storage time), s.e. = 14.37. Fracture force increased significantly after 45 days of storage (Table 1).

No consistent trends were found in the changes of deflection of frozen samples (until fracture) vs storage time. The correlations ($R = -0.29$) between fracture force and deflection were insignificant ($P < 0.01$).

The development of dryness and toughness of fish during frozen storage has been reported indicating changes in the myofibrillar proteins (Sikorsky et al., 1976; Laird et al., 1981). Results of raw texture panel evaluations of minced cod and haddock by Laird et al. (1981) indicate a deterioration of textural properties of thawed samples within 36 days of frozen storage at –15°C. Hiltz et al. (1976) reported that in minced flesh of silver hake (*Merluccius bilivearis*), the rate of deterioration during frozen storage was twice as fast as in fillets. These authors also indicated a faster deterioration of red hake than silver hake, cod or haddock. Laird et al. (1981) note that whether or not these rapid changes in the textural properties of minced fish are due to the same mechanism which produce toughening in unminced fillets has yet to be established.

The most interesting effect of storage time in our study was the delay of textural changes in the frozen samples in comparison to the thawed samples, indicating a possible additional effect of thawing on textural changes of frozen minced hake.

However, although a significant correlation has been found between force of compression data of thawed samples and means of fracture force of frozen samples (storage time 2–15 days), whether or not compression data and fracture data of minced fish are related has yet to be established on a more general basis.

In summary the results of this study indicate the following:

(1) A method to determine the textural changes of frozen minced fish has been developed providing data with a relative coefficient of variation of 4.7% and a significant correlation between fracture force data of frozen samples and force of compression data of thawed samples.

(2) Major advantages of the method presented are savings of time for routine quality control tests of frozen minced fish due to absence of the usually required thawing procedures of 3 to 6 hours. Furthermore the additional influence of the thawing procedure on the texture properties of minced fish is eliminated when testing the frozen samples.

(3) While no significant changes of the fracture force of frozen samples have been observed between 2 and 45 days of storage time, fracture force increased 3.5 times and 5.0 times between 45 and 60 days and 45 and 90 days respectively.

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A Research Note

Effects of Postmortem Storage and Temperature on Muscle Protein Degradation: Analysis by SDS Gel Electrophoresis

PETER J. BECHTEL and FREDERICK C. PARRISH, JR.

ABSTRACT

The proteolytic breakdown of the major contractile proteins of bovine longissimus muscle was examined during postmortem storage by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Samples of muscle stored at 4°C for 14 days exhibited little proteolysis of the major contractile proteins; however, samples stored at 37°C for 1 day showed significant degradation of myosin heavy chains and almost complete proteolysis of this protein by day 14. Major degradation products of the myosin heavy chains included a series of polypeptides having molecular weights between 145,000 and 125,000. These experiments demonstrate that substantial degradation of the myosin heavy chain and other muscle proteins can occur during the storage of meat, and this phenomenon was highly temperature dependent.

INTRODUCTION

ONE MECHANISM POSTULATED to contribute to meat tenderization, as well as to other meat properties, is the enzymatic proteolysis of myofibrillar proteins. A number of proteolytic enzymes that utilize myofibrillar proteins as substrates have been purified from skeletal muscle (Pennington, 1977). These include the calcium-activated proteases (Huston and Krebs, 1968; Dayton et al., 1976), alkaline proteinases (Noguchi and Kandatsu, 1971), cathepsins D (Okitani et al., 1972; Schwartz and Bird, 1977), B (Schwartz and Bird, 1977) and L (Okitani et al., 1980), and serine protease (Yasgawa et al., 1978).

A number of postmortem studies using myofibrils or protein fractions isolated from skeletal muscle have been performed in which proteolytic breakdown of the contractile proteins was monitored by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) (Hay et al., 1973; Samejima and Wolfe, 1976; Penny, 1974; Olson et al., 1977; Cheng and Parrish, 1978; Dutson et al., 1978; Robbins et al., 1979; Penny and Ferguson-Pryce, 1979). Although these studies have shown that some lower molecular weight polypeptides are degraded during postmortem storage, major alterations in the content of actin and myosin heavy chain polypeptides were not observed. A recent report by Ikeuchi et al. (1980) reported some degradation of rabbit skeletal muscle myosin and troponin was observed during storage for up to 12 hr at 37°C.

The purpose of this study was to examine the proteolytic breakdown of the major bovine contractile proteins during postmortem storage of meat.

MATERIALS & METHODS

MUSCLE SAMPLES were obtained from the M. longissimus of 454–500-kg; 12–18-month-old beef animals. Muscle samples obtained within 1 hr after exsanguination were cut approximately 2.5 cm thick and 6.3 cm in length, wrapped in paper toweling, soaked in 0.1M NaN_3, then wrapped in plastic wrap and stored at 4°, 23° and 37°C. After storage for the designated time, cores were removed from the muscle samples and weighed. Then, 0.5g of the core sample was homogenized (Kontes glass homogenizer) in 14.5 ml of the SDS sample buffer (2% SDS, 10 mM sodium phosphate, pH 7.0). These diluted samples were held in a boiling water bath for 5 min and then stored at −70°C.

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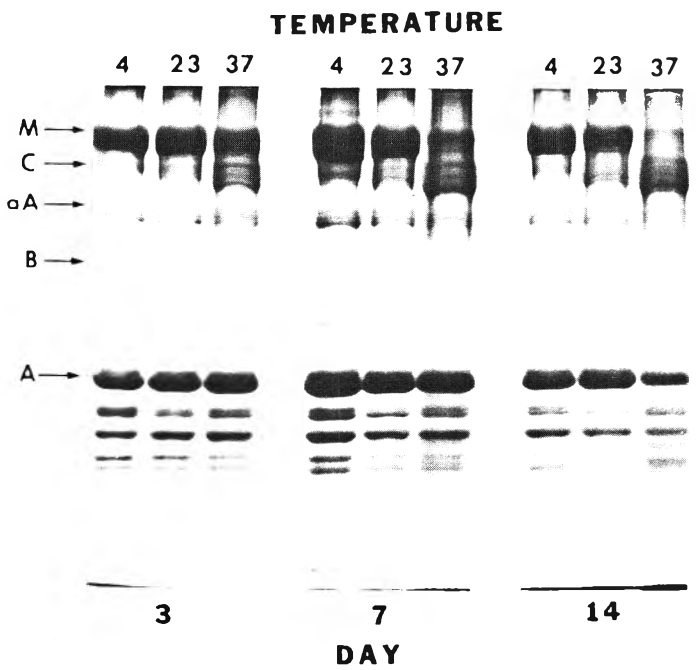

Fig. 1—Effect of temperature and storage time on the electrophoretic patterns of muscle proteins. Bovine skeletal muscle was removed at death and split into aliquots and stored at 4°, 23° and 37°C. Samples taken on days 3, 7 and 14 were homogenized and subjected to electrophoresis on an 8% polyacrylamide gel with a 5% polyacrylamide stacking gel in the presence of SDS. Samples were prepared in an identical manner and 5 μl applied to each lane. Protein standards include (M) myosin, (C) C-protein, (aA) α-actinin, (B) bovine serum albumin, and (A) actin.

Other samples of M. longissimus were exicsed shortly after death, cooled to 4°C and ground through a 0.25 in plate. Samples of 10g were weighed into beakers and 20 ml of phosphate-buffered saline (pH 7.0) and 3 ml of 0.1M NaN_3 were added to each sample. Treatments included the addition of (1) 3.0 ml of 0.1M $CaCl_2$, (2) 0.3 ml of 1M $MgCl_2$, or (3) 1.5 ml of 0.2M EDTA. Bacterial growth was inhibited by adding 300 μl of a solution containing penicillin (10,000 units/ml) and streptomycin (10,000 units mcg/ml) and 200 μl of amphotericin B (250 mcg/ml). These samples were then stored at 4°, 23° and 37°C. After various storage periods, 0.5g of samples (ground meat plus phosphate-buffered saline) were removed and diluted with 4.5 ml of SDS sample buffer, held in a boiling water bath for 5 min and then stored at −70°C. Samples for electrophoresis were prepared as previously described (Callaway and Bechtel, 1981). After examination of samples there was no evidence of bacterial proteolytic activity in any of these experiments.

RESULTS & DISCUSSION

SAMPLES of whole muscle were stored at 4°, 23° and 37°C and aliquots were taken on days 3, 7 and 14. As shown in Fig. 1, SDS electrophoresis of samples stored at 4°C for 3, 7 or 14 days showed little proteolytic degradation; however, samples stored at 23°C showed an increased accumulation of proteolytic breakdown products, but the most profound and significant degradation was observed from samples stored at 37°C. The accumulation of protein degradation product at 37°C was observed after 3 days and the content of degradation products increased after 7 and 14 days of postmortem storage. The molecular weights of the most prominent breakdown products observed after 14 days' storage at 37°C ranged from 125,000–145,000 Daltons and appeared to coincide with the loss of myosin heavy chain. The other major contractile protein, actin, was not degraded to the same extent as the myosin heavy chain during storage at 37°C (Fig. 1). The results from other experiments (not shown) indicated that the protein degradation observed in Fig. 1 was occurring uniformly at both the surface and internal regions of the meat samples during storage.

The effect of metal ions on the degradation of the contractile proteins was examined by using ground muscle and the samples were stored at 4° or 37°C for 1 or 7 days. The polyacrylamide gel in Fig. 2 showed that after 7 days at 4°C little degradation of the major skeletal muscle proteins occurred; however, after 7 days at 37°C, degradation of myosin heavy chain was observed in all treatments. In samples to which calcium (10 mM) was added, myosin heavy chain was degraded to a number of lower molecular weight polypeptides, many having molecular weights between 145,000 and 125,000 Daltons. Similar qualitative results were obtained in the samples containing magnesium ions, but in this experiment, it is evident that less myosin heavy chain was degraded. Samples stored at 37°C to which EDTA (10 mM) was added gave a polypeptide pattern that was qualitatively different from either the calcium or magnesium treatments. Two of these major polypeptide breakdown products had molecular weights of 110,000 Daltons and 90,000 Daltons (Fig. 2). The pH of all the different treatments was measured and determined to be 6.2–5.8.

Degradation of the major contractile proteins can best be shown by examining gel lanes from different experiments. Degradation of myosin heavy chain is readily observed on all lanes in which whole muscle or ground muscle samples were incubated at 37°C for 3 or more days. In these experiments, it is difficult to determine the status of C-protein and M-line protein because several of the myosin heavy chain degradation products have similar polypeptide molecular weights when determined by SDS gel electrophoresis. The Z-line protein, α-actinin, is present although in diminished quantities after prolonged incubations at 37°C, and the other major contractile protein actin is degraded to only a limited extent even after prolonged

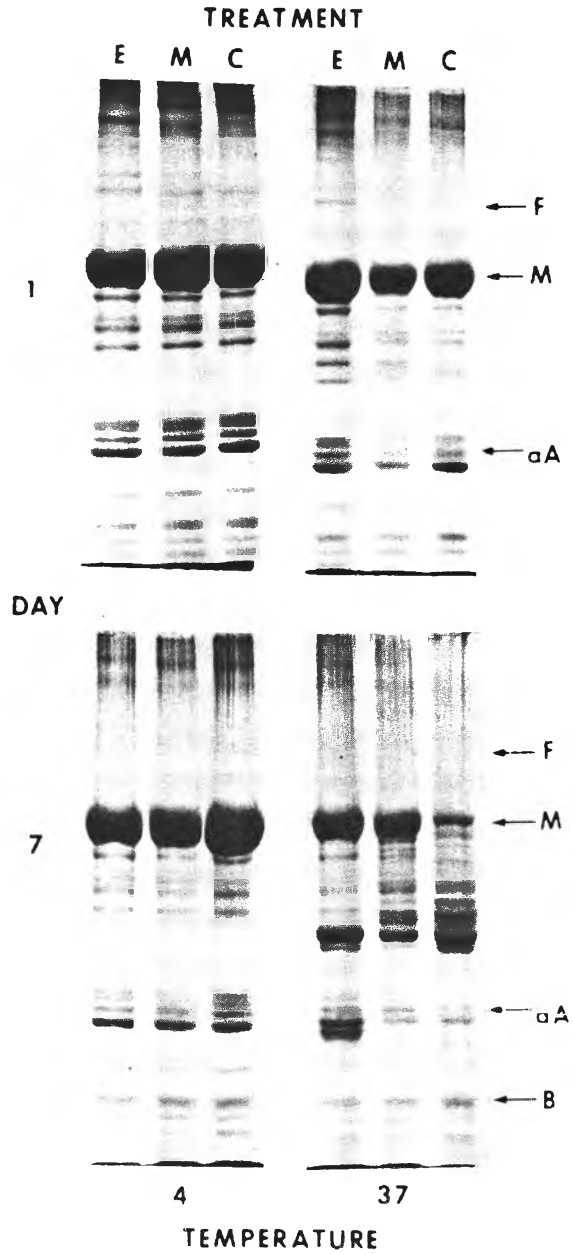

Fig. 2–Effect of storage time, temperature and divalent cation on the electrophoretic pattern of muscle proteins. Bovine skeletal muscle was removed at death, ground and aliquots mixed with two volumes of phosphate-buffered saline plus either 8.3 mM EDTA (E), 9.0 mM magnesium chloride (M) or 8.7 mM calcium chloride (C). Samples were removed on days 1 (upper) and 7 (lower), prepared for electrophoresis and 10 μl applied to each lane of a 5% polyacrylamide gel with 3% stacking gel. Protein standards include (F) filamin, (M) myosin, (aA) α-actinin, and (B) bovine albumin.

storage at 37°C (Fig. 1). An interesting observation was that the most stable contractile proteins are associated with the thin filaments of muscle while the thick filament proteins seem more labile at 37°C. Even after 14 days at 37°C, major segments of the myosin heavy chains are present as 125,000–145,000 Daltons molecular weight polypeptides and have not been degraded to smaller peptides and amino acids.

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A Research Note

Effect of Heat and Alkali on Lysinoalanine Formation in Fish Muscle

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ABSTRACT

Alkali-treated fish products such as lutefisk and sodium tripolyphosphate-treated fillets were analyzed for lysinoalanine (LAL). No detectable amounts of LAL were found in these products both of which had pH values between 8.0 and 8.5 even after they had been heated to ordinary cooking temperatures (162°C) for up to 30 min. There was no measurable quantity of LAL found in samples at pH 10 heated for 60 min at 90°C; however, measurable quantities of LAL were found in similarly heated samples at pH 12 and 13. Thus, LAL formation is not an apparent problem in the ordinary processing of fish.

INTRODUCTION

CHANGES that are induced in proteins as a result of processing are of concern to nutritionists because they can lower the quality and nutritive value of foods. The presence of lysinoalanine (LAL) in several processed and cooked foods was demonstrated by Sternberg et al. (1975). LAL is formed when dehydroalanine, derived from cystine-cysteine or serine, is cross-linked with lysine (Bohak, 1964; Friedman, 1977). The formation of LAL is accompanied by a decrease in nutritional value of the food, and LAL itself has been shown to cause pathological changes in rat kidneys (Gould and MacGregor, 1977; Woodward and Short, 1973) although not in other species (DeGroot et al., 1976).

Alkali and alkaline salts are often used in dried, canned, and frozen fish products. Lutefisk, a traditional Scandanavian fish product, is treated with strong alkali in its preparation (McClane, 1977; Davidson, 1979). Sodium pyrophosphate is used to prevent the formation of struvite in canned fish, and mixtures of sodium tripolyphosphate and sodium hexametaphosphate are sometimes used to control the drip in frozen fish fillets.

It has been shown that the susceptibility of a protein to respond to heat and alkali to form LAL depends on the configuration and the amino acid composition of the protein molecule (Bohak, 1964; Asquith et al., 1969; Friedman, 1977; Haraguchi et al., 1980; Annan and Manson, 1980); therefore individual proteins have different maximum conditions for LAL formation (Fujimaki et al., 1980; Hasegawa et al., 1981).

The purpose of this study was to determine: (1) the LAL content in commmercially prepared lutefisk; (2) the formation of LAL in frozen fillets treated with tripolyphosphate; and (3) the effects of heat and pH on the formation of LAL in fish muscle protein.

MATERIALS & METHODS

Fish

Fresh Puget Sound Pacific whiting (*Merluccius productus*) and cod (*Gadus macrocephalus*) were obtained from local processors.

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Amino acid analysis

Amino acid analyses were done on a Beckman 118 CL Amino Acid Analyzer. Weighed aliquots of the samples were hydrolyzed in 6 N HCl in a Kontes heating block at 110°C for 24 hr according to the method of Spackman et al. (1958). Results are expressed as grams amino acid/100 grams protein. In these determinations, cystine was not oxidized to cysteic acid for quantitative measurement.

Treatments

Lutefisk, which had been obtained from a local market processed and ready for cooking, was cut into small pieces, randomly mixed, and divided into three groups. One of the groups served as a control while the other two were wrapped in gauze and boiled in water for 10 and 30 min, respectively. All samples were freeze-dried.

Fillets of Pacific whiting were dipped for 30 sec in 7.5% sodium tripolyphosphate (TPP) and 2% salt (NaCl) according to the method of Spinelli and Wieg (1968). Part of each fillet was frozen as an uncooked control and the remainder was baked in aluminum-covered dishes for 20 min at 162°C. After cooling, all samples were freeze-dried.

Samples of freeze-dried Pacific whiting and cod were ground, weighed, and suspended in 5 ml H_2O. The pH was adjusted with NaOH before bringing the final concentration to 1 mg of fish muscle/mL. The tubes were covered and heated in a constant 90°C water bath for the desired length of time. After heating, the tubes were rapidly cooled in ice and aliquots were taken for hydrolysis. Table 1 lists the conditions studied, the range of pH values, temperatures, and length of time used to determine the formation of LAL in fish muscle.

RESULTS & DISCUSSION

NO LYSINOALANINE (LAL) was detected in the lutefisk boiled for 10 or 30 min nor was LAL found in the sodium tripolyphosphate-treated Pacific whiting fillets. The similar conditions in both of these studies were alkaline treatment at cold temperatures. Lutefisk, which originated in the Scandinavian countries, is kept cold during the alkali treatment and the alkali is washed out before cooking (Davidson, 1979). The final pH is between 8.0 and 8.5 The Pacific whiting fillets were also treated under chilled conditions and the 30-sec exposure resulted in a surface pH of 8.0–8.5.

In order to better define the conditions under which LAL would form in fish muscle protein, samples of freeze-dried Pacific whiting and cod were solubilized and the pH adjusted over the range shown in Table 1. The effect of heating and alkali pH treatments is illustrated in Fig. 1. Lysinoalanine was detected only after heating the fish muscle suspension for 60 min at 90°C at a pH of 12 or greater. Extending the time of heating markedly increased the formation of LAL with an accompanying decrease in lysine.

Table 1—Conditions tested to determine the formation of LAL in fish muscle

Temp (C)	Time	pH
0°	60 min	7,9,13
	180 min	7,13
90°	60 min	6,7,8,9,10,12,13
	180 min	7,9,13

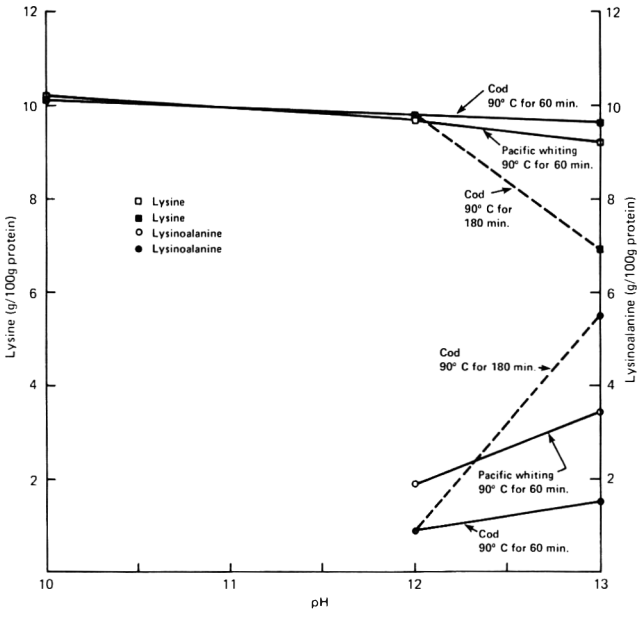

Fig. 1—Effect of the duration of heat and alkali pH on lysinoalanine formation in fish muscle.

The results indicate that neither heat nor high pH alone influenced the formation of LAL nor changed the amino acid composition. The sample at pH 7, even though it was heated at 90°C for 180 min, had no significant change in amino acid values nor was LAL formed. Also, the pH 13 sample held at 0°C had no abnormalities in the amino acid pattern. Heating the sample at pH 13 for 180 min at 90°C resulted in a marked deviation in amino acid values. Accompanying the formation of LAL was a 51% decrease in threonine and serine, 40% in arginine, 25% in lysine, and 28% in histidine. Similar results of the effects of heat and pH on these amino acids in fish protein concentrate have been reported by Fujimaki et al. (1980).

Thus, using the formation of LAL as a criteria for protein quality, it appears that fish processing which involves alkaline treatments can be practiced with no change in nutritional quality as long as the fish is not subjected to long periods of high temperature in the alkaline state.

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A Research Note

Free Amino Acids of Raw and Cooked Ground Beef and Pork

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ABSTRACT

Ground beef and ground pork were cooked for 15 min on an electric griddle heated to a surface temperature of 121°C. Cooking reduced the total free amino acid (FAA) content from 25.23 to 17.60 mg/25g fresh weight in pork and from 32.77 to 30.09 mg/25g fresh weight in beef. Sixteen FAA were detected in both beef and pork; most individual FAA decreased during heating. Residues remaining after extraction of FAA from raw pork, when rehydrated (70% moisture) and heated (121°C, 15 min), contained only trace amounts of FAA. Free amino acids apparently were not produced under the cooking conditions employed in this study.

INTRODUCTION

FREE AMINO ACIDS (FAA) are important precursors of flavor compounds of meats and other foods (Macy et al., 1964). During cooking, FAA react with sugars and possibly lipid oxidation products to produce heterocyclic compounds that contribute to the flavor and aroma profiles of cooked foods (Shibamoto, 1980).

Recent interest in the FAA content of foods and of the reactions that occur between these and other food constituents during cooking stems from the isolation of mutagenic heterocyclic amines from cooked meat (Kasai et al., 1981; Spingarn et al., 1980). Mutagens have also been detected in smoke condensate from pyrolized proteins and in tars from pyrolized amino acids, particularly tryptophan, serine, and glutamic acid (Matsushima and Sugimura, 1981). The formation of mutagens during the cooking of meat is both time and temperature dependent (Pariza et al., 1979) and is influenced by method of cooking (Nader et al., 1981). Nader et al. (1981) did not detect mutagens in beef cooked by microwave irradiation.

To our knowledge, the precurors of the basic mutagens formed during the heating of meat at normal cooking temperatures have not been conclusively identified. However, the formation of mutagens has been demonstrated in sugar-ammonia model systems (Spingarn and Garvie, 1979) and it has been suggested that mutagens may be produced by browning reactions between sugars and free amino acids or other amines during cooking (Spingarn et al., 1981). It has also been reported that the fat content of meat has an influence on the formation of mutagens (Spingarn et al., 1981).

The levels of FAA in cooked meat may be governed not only by initial levels in raw meat but also by formation of FAA through protein hydrolysis during cooking, by heat degradation of FAA, or by a combination of these processes. The purpose of this study was to determine the effects of cooking (frying) on the FAA content of ground pork and beef. Free amino acids were extracted from raw and cooked samples and from raw pork residue that had been extracted for FAA and subsequently rehydrated to 70% moisture and heated to 121°C.

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MATERIALS & METHODS

FRESH GROUND BEEF (22% fat) was obtained from a local market. Ground pork was prepared in the laboratory from lean Boston Butt, pork fat, and salt. The ingredients were mixed in a Hobart mixer and passed through a Toledo chopper (9.52 mm plate). The final pork mixture contained 40% fat and 2% salt.

Samples of ground beef and pork, both raw and cooked, were extracted with a 6:2:2 v/v (52.4%:23.3%:24.3% w/w) methanol-chloroform-water solvent system. Methanol-chloroform-water solvent systems have been used to extract both FAA (Bieleski and Turner, 1966; Young et al., 1974) and lipids (Bligh and Dyer, 1959) from biological materials. Reference to the methanol-chloroform-water phase diagram published by Bligh and Dyer (1959) indicated that a solvent to sample ratio (v/w) of 4 would produce a monophasic extract with samples containing less than 60% moisture and a biphasic extract with samples containing more than 60% moisture.

Raw samples (25g) were slurried with 75 ml of 6:2:2 solvent in a Sorvall Omni Mixer, transferred to a 150-ml corex centrifuge bottle with 25 ml additional solvent, mixed, and centrifuged at 16,300 x *g* for 15 min. The extracts were decanted into a separatory funnel and separated into two phases by adding 10 ml chloroform followed by 10 ml water and mixing. After phase separation the lower chloroform layer containing extracted lipid was discarded and the aqueous methanol layer was retained for FAA analysis. The sample residues were extracted four additional times with methanol chloroform-water and the aqueous methanol extracts retained for FAA analysis.

Samples of cooked beef and pork, were prepared by heating 25g (raw weight) portions for 15 min on an electric griddle preheated to a surface temperature of 121°C. The samples were stirred occasionally to insure uniform heating and attained a final internal temperature of about 90°C. The cooked samples were transferred quantitatively to an Omni Mixer and extracted in the same manner as raw samples.

Aqueous extracts were monitored for FAA by thin layer chromatography (TLC) on cellulose plates (Avicel, Analtech, Newark, DE) to test for completeness of FAA extraction. TLC plates were developed in a 65:25:10 butanol-acetic acid-water solvent system and amino acids detected by spraying with ninhydrin (0.25% in butanol) and heating for 5 min at 100°C.

After removal of methanol with a rotary evaporator, the aqueous extracts were freeze-dried. The freeze-dried residues were dissolved in a measured volume of pH 2.2 sodium citrate buffer, filtered through a Millipore membrane filter (0.2μm) and analyzed for amino acids on a Durrum D-500 amino acid analyzer (Phillips, 1981).

The residue from uncooked pork remaining after extraction of FAA was weighed and sufficient distilled water added to give a final water content of 70%. The raw protein residue was then heated at 121°C for 15 min on a griddle and analyzed for FAA content as described for raw and cooked samples.

RESULTS & DISCUSSION

THE THIN-LAYER CHROMATOGRAMS of the aqueous extracts indicated that most of the FAA was removed in the first two extractions; FAA were not detected by TLC in the 4th and 5th extracts. Quantitative analyses of freeze-dried residues from all extracts revealed that over 98% of total extractable FAA were removed during the first three extractions while less than 0.5% were removed during the 5th extraction (Table 1). Total FAA data for all samples – both raw and cooked – are shown in Table 2. The levels of total FAA in both beef and pork were decreased by cooking, although some individual FAA showed increases

Table 1—Extraction of free amino acids from raw ground pork[a]

Amino acid	Extraction number 1	2	3	4	5
Alanine	3.4468	0.5464	0.2423	0.0778	0.0104
Arginine	0.4945	0.0713	ND[b]	ND	ND
Aspartic Acid	0.6319	0.0861	0.0414	0.015	0.0020
Cysteine	ND	ND	ND	ND	ND
Glutamic Acid	4.3676	0.5886	0.2671	0.0972	0.0208
Glycine	2.3793	0.2852	0.1319	0.0465	0.0128
Histidine	0.1484	0.0570	0.0298	0.0098	0.0019
Leucine	1.3523	0.2084	0.0990	0.0288	0.0029
Lysine	0.9999	0.1447	0.0581	0.0241	0.0066
Methionine	0.2345	0.0375	0.0975	ND	0.0008
Isoleucine	0.3537	0.0676	0.0337	0.0095	0.0009
Phenylalanine	0.7390	0.1315	0.0654	0.0220	ND
Proline	1.1566	0.1439	0.0780	0.0236	0.0052
Serine	2.9517	0.4269	0.1191	0.0682	0.0135
Threonine	0.6977	0.1042	0.0522	0.0173	ND
Tryptophan	ND	ND	ND	ND	ND
Tyrosine	0.1074	T[c]	T	ND	ND
Valine	0.2552	0.1056	ND	ND	ND
Totals	20.3165	3.0049	1.3955	0.4398	0.0778

[a] Values based on 25g raw weight
[b] ND = not detected
[c] T = trace

Table 2—Total free amino acid values of raw and cooked ground beef and ground pork[a]

Amino acids	Raw pork	Cooked pork	Raw beef	Cooked beef
Alanine	4.3237	3.3586	7.445	6.1618
Arginine	0.5658	0.4078	0.3623	0.2606
Aspartic Acid	0.7764	0.5641	0.9204	0.855
Cysteine	ND	ND	ND	ND
Glutamic Acid	5.3413	3.6619	5.7823	4.9199
Glycine	2.8557	1.7890	1.9960	1.6281
Histidine	0.2469	0.2233	0.6464	0.7888
Leucine	1.6914	1.1481	2.2809	2.1129
Lysine	1.2334	0.9219	1.3237	1.2470
Methionine	0.3703	0.2309	0.8088	0.6818
Isoleucine	0.4654	0.3996	1.1599	1.0574
Phenylalanine	0.9579	0.6253	1.3296	1.3664
Proline	1.4073	0.9129	0.8865	0.8181
Serine	3.6594	2.0452	4.5350	6.1701
Threonine	0.8714	0.6555	1.4050	0.3028
Tryptophan	ND[b]	ND	ND	ND
Tyrosine	0.1074	0.2036	T[c]	ND
Valine	0.3608	0.4554	1.8875	1.7183
Totals	25.2345	17.6031	32.7693	30.0850

[a] Values based on 25 g raw weight
[b] ND = not detected
[c] T = trace

(valine in pork; histidine, phenylalanine, and serine in beef). Only traces of FAA (alanine, aspartic acid, glutamic acid, glycine, leucine, lysine, proline, serine) were detected in the extracted raw pork residue that was rehydrated and heated to 121°C and these together amounted to 0.08 mg/25g fresh sample.

Free amino acids are undoubtedly released by cathepsins and other proteolytic enzymes following slaughter and the actual levels observed in meat would be expected to be governed by time and temperature of aging and storage. Niewiarowicz (1956) studied the formation of FAA in beef during aging at 4°C for 18 days and although all common amino acids except tryptophan were observed at day one, the levels of FAA increased with time of aging. Spinelli-Gugger et al. (1980) observed much higher levels of FAA in fried "country cured" bacon than in raw untreated pork bellies.

The values reported here for FAA in raw and cooked pork and beef are lower than those published by Baldwin et al. (1976) when adjusted to the dry, fat-free basis used by those authors. Free amino acids decreased during roasting, but high levels of FAA were found in drippings (Baldwin et al., 1976). However, drip losses were not reported and direct comparisons with the data reported here are not possible.

Macy et al. (1964) reported that heat caused substantial losses of sugars and free amino acids in meat extracts. Our data indicate partial loss of most FAA during cooking of both ground beef and pork. Although we did not analyze the extracts for compounds other than FAA, the solvent system used in this study would be expected to also extract simple sugars and other low molecular weight compounds that would likely be involved in browning reactions. Only traces of FAA were detected in the residue that was wetted to 70% moisture and heated to 121°C for 15 min, although considerable mutagenic activity was produced by this treatment (Penet, 1982). These observations are interpreted to indicate that FAA are not generated under the mild cooking conditions employed in this study and further that FAA are not a necessary requirement for the formation of mutagens during cooking.

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A Research Note

Thiobarbituric Acid Values and Glutathione Perioxidase Activity in Meat from Chickens Fed a Selenium-Supplemented Diet

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ABSTRACT

Selenium glutathione peroxidase (SeGSHpx) was assayed in breast and leg muscle from chickens fed a basal (0.09 ppm Se) and a Se-supplemented (0.31 ppm Se) diet. The same muscles were ground and made into 50g patties and stored 4 days at 4°C. TBA values were determined on the stored patties. SeGSHpx activity was higher ($p < 0.05$) in muscles from the Se-supplemented chickens than in muscles from the non-Se supplemented birds. The higher SeGSHpx activity was accompanied by lower ($p < 0.05$) TBA numbers in the stored chicken patties.

INTRODUCTION

POLYUNSATURATED FATTY ACIDS undergo oxidation in the presence of oxygen and catalysts. This reaction can occur during processing and storage of raw chicken meat, resulting in an off-flavor in the cooked product. In the living animal there are metabolic functions that serve to retard this oxidation or to prevent accumulation of lipid oxidation products. The selenium-dependent glutathione peroxidase (SeGSHpx) is a part of one such system (Ganther et al., 1976). If the SeGSHpx system is viable in post-rigor muscle, then it may be able to retard oxidative flavor changes during storgae of chicken meat.

Omaye and Tappel (1974) found the amount of selenium (Se) fed to chickens to be related semi-logarithmically to the amount of measured GSH-Px activity in chicken muscle. Combs and Regenstein (1980) obtained 2-thiobarbituric acid (TBA) values (as an index of lipid oxidation) on freezer-stored muscle from chickens fed various dietary supplements, including Se. When compared to their respective controls, TBA values were lower (indicating less oxidation) in meat from some of the dietary treatments which contained Se supplements. Plasma SeGSHpx activity was measured and was found to be greatest for the Se-supplemented birds. No values for muscle SeGSHpx were reported.

In the present study muscles from chickens fed two amounts of Se were assayed for SeGSHpx activity. TBA values were obtained on the same muscles after a 4 day storage period. The objective was to determine whether an increase in muscle SeGSHpx activity in response to a dietary Se supplementation would result in lower TBA values in the stored chicken meat.

MATERIALS & METHODS

DAY OLD male Hubbard broiler chickens were placed randomly into two groups. One group was fed the basal diet (Table 1). The second group was fed the basal diet supplemented with 0.25 ppm Se as sodium selenite (Na_2SeO_3). A Se analysis (Whetter and Ullrey, 1978) of the diets showed the basal diet to contain 0.09 ppm Se, and the supplemented diet, 0.31 ppm Se. No supplemental vitamin E or synthetic antioxidant was added to the diet. The chickens were housed in a battery brooder with wire floors.

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At 39 days of age the chickens were killed and skin and feathers removed. The breast and legs (including thighs) were removed and placed in separate sterilized, polyethylene bags (Whirl-Pak, Markson Science, Inc.). Each bag was wrapped in aluminum foil and stored at −18°C. Twenty-four hours prior to each analysis day the carcass parts from two chickens were placed in a refrigerator (4°C) to thaw. At the time of analysis the muscle tissue was removed from the bone and trimmed of all visible connective tissue and gross blood vessels. The breast and leg muscles were minced separately in a food processor (La Machine, Moulinex Products, Inc.), and made with a home-style pattie maker into 50g uniformly shaped patties. The patties were stored for 4 days at 4°C. SeGSHpx activity was assayed from the meat immediately after mincing. TBA analyses were done at this time and after four days of pattie storage. Maximum freezer storage time was 60 days; minimum storage time, five days. There was no apparent increase in initial TBA values of meat removed from the freezer after 5 days over the initial TBA values of meat removed after 60 days.

Enzyme activity was measured using the glutathione reduction coupled assay procedure of Paglia and Valentine (1967), with the modification described by DeVore and Greene (1982). Lipid oxidation was assessed by the 2-thiobarbituric acid method of Tarladgis et al. (1960). Results are expressed as mg TBA reacting substances/kg tissue. In this method malonaldehyde, as 1,1,3,3-tetraethoxypropane, serves as the standard.

The means reported in Table 2 are a pool of two studies. Diet (Table 1) and length of time on the diet were identical for both experiments. The values from each experiment were tested by Student's t test and were not significantly different. The two studies were considered to be testing the same population and are therefore reported as one experiment.

The Statisitcal Analysis System (Barr et al., 1976) procedures were used in the evaluation of data by analysis of variance. When treatment effects (F test) were significant, pairwise comparison among means was made using Fisher's test of least significant difference.

Table 1—Composition of basal diet

Ingredient		%
Corn		53.5
Soybean meal		37.5
Poultry fat		5.0
$CaCO_3$		1.37
Dicalcium phosphate		1.63
Salt		0.50
DL-Methionine		0.20
Vitamin mix[a]		0.25
Trace mineral mix[b]		0.05
Calculated analysis:		
Vitamin E	14.5 IU/kg diet[c]	
Total fat	7.43% of diet[d]	
Polyunsaturated fatty acid	2.37% of diet[d]	

[a] Vitamin mix provides (per kg/diet): vitatmin A, 11,000 IU; vitamin D_3, 1,100 ICU; riboflavin, 4.4 mg; Ca pantothenate, 12.0 mg; nicotinic acid, 44.0 mg; choline Cl, 220.0 mg; vitamin B_6, 2.2 mg; menadione (as MSBC), 1.1 mg; thiamine, 2.2 mg; folic acid, 0.55 mg; D-biotin, 0.11 mg; vitamin B_{12}, 6.6 mcg.
[b] Trace mineral mix provides (mg/kg of diet: Mn 60; Zn, 50; Fe, 30; Cu, 5; I, 1.05.
[c] National Research Council (1977)
[d] Edwards (1964)

Table 2—Effect of a dietary Se supplement on SeGSHpx activity in freshly minced chicken breast and leg muscles and TBA values for minced and stored (4 days, 4°C) patties from the same muscles[a]

	SeGSHpx[b]		TBA[cd]	
	Basal diet	+ 0.25 ppm Se	Basal diet	+ 0.25 ppm Se
Breast	1.9 ± 0.38[e]	4.0 ± 1.1[f]	1.0 ± .47[h]	0.4 ± 0.12[i]
Legs	2.4 ± 1.3[e]	9.7 ± 1.7[g]	1.0 ± .44[h]	0.3 ± 0.12[i]

[a] Mean ± standard deviation for nine muscles per treatment
[b] Values are expressed as nmole NADPH oxid/min/mg protein
[c] mg TBA reacting substances/kg tissue
[d] Initial TBA for basal — 0.4 ± 0.23; for diet + 0.25 ppm Se = 0.3 ± 0.066.
[e,f,g] Means with like superscripts are not significantly different ($p < 0.05$) between rows and columns for SeGSHpx activity.
[h,i] Means with like superscripts are not significantly different between rows and columns for TBA numbers.

RESULTS & DISCUSSION

VALUES for SeGSHpx activity of the freshly minced meat compared with TBA values for the stored patties are presented in Table 2. The SeGSHpx activity was higher, and TBA values lower ($p < 0.05$) in muscles from chickens fed the Se supplemented diet than in muscles from chickens fed the basal diet. Thus, in muscles from the Se supplement-fed chickens, the expected increase in SeGSHpx activity did occur and was accompanied by lower TBA values in the same muscles after the 4 day storage peroid.

The SeGSHpx activity was within the range observed by Omaye and Tappel (1974) for chicken muscle. These workers did not report the type of skeletal muscle tested. In the present study enzyme activity was higher ($p < 0.05$) in the leg than in the breast muscle of the Se supplemented birds (Table 2). A similar trend was indicated for muscles from chickens fed the basal diet, but these differences were not significant. This difference in SeGSHpx activity between leg and breast muscles was not reflected in TBA numbers for the two muscles from either diet treatment variable.

In the coupled assay procedure the substrate hydrogen peroxide is considered to measure activity of the selenium dependent glutathione perioxidase only, while cumene-hydroperoxide measures both selenium dependent and non-selenium activities (Lawrence and Burk, 1976). The substrate for determining the activity reported in Table 2 was hydrogen peroxide. All samples were assayed using cumene-hydroperoxide as well. When cumene-hydroperoxide was used as substrate, the level of activity was the same as that obtained with hydrogen peroxide (values not reported). This would indicate that chicken muscle contains only the SeGSHpx. Similar findings were reported for beef muscle SeGSHpx (DeVore & Greene, 1982) and rat muscle (Lawrence and Burk, 1978).

Comparison of the data from the present study with other data obtained from the TBA method of Tarladgis et al (1960) shows the TBA values for the Se supplemented treatment groups to be similar to those obtained on meat treated with phenolic antioxidants (Greene et al., 1971). The TBA values for the meat from chickens fed the basal diet are within the range of those reported by Igene et al (1979) for stored chicken carcasses or parts. They are lower than those of Chen and Waimaleongora-ek (1981) for ground chicken meat. Differences in endogenous Se and antioxidant factors such as vitamin E as well as differences in handling the meat during sample preparation may account for this.

According to this study, higher SeGSHpx activity was found in muscle from chickens fed a Se supplemented diet compared to muscles from chickens that did not receive a Se supplement. Samples which exhibited the higher SeGSHpx activity also had lower TBA values after 4 days of refrigerator storage. It is suggested from these findings that further study of the activity of the SeGSHpx system in post-rigor chicken muscle would be warranted.

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Effect of Addition of Chloride Salts on Rancidity of Ground Pork Inoculated with a *Moraxella* or a *Lactobacillus* Species

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ABSTRACT

Two experiments were conducted to determine rancidity development in raw-refrigerated ground pork treated with different types and levels of chloride salts. In the first experiment, raw ground pork was inoculated with either a *Moraxella* or a *Lactobacillus* species. In the second experiment, samples were not inoculated. In both experiments, samples were treated with either NaCl, KCl, or $MgCl_2$ at ionic strength of either 0.73 or 0.37 and stored at 5°C. Regardless of inoculation, in both experiments addition of any chloride salt increased ($P < 0.05$) TBA values over those values for controls (no added salt). Addition of NaCl resulted in the highest ($P < 0.05$) TBA values among inoculated samples, but these differences were not apparent for noninoculated samples.

INTRODUCTION

SODIUM CHLORIDE (salt), in relatively high brine concentrations of 4–8%, is added to processed meats for its effects on sensory, functional and preservation properties. As much as 40–60% of daily salt intake may come from commercially processed foods (IFT, 1980). However, increasing concern with the association between dietary sodium intake and hypertension has promoted voluntary efforts by food processors to reduce the amount of sodium in processed foods. Many studies have been conducted with processed meat products on complete or partial replacement of sodium chloride with other chloride salts relative to the effects that such reductions or replacement may have on sensory, functional and processing properties (Terrell and Olson, 1981).

In addition to these properties, shelf-life stability of processed meats made with reduced levels of NaCl or with other chloride salts replacing NaCl, needs investigation. Sodium chloride promotes the development of rancidity (lipid peroxidation) in both refrigerated and frozen meat products (Ockerman and Crespo, 1981; Zipser et al., 1964; Watts and Peng, 1947; Wiesman and Ziemba, 1946). Studies on the effect of KCl and $MgCl_2$ on the development of rancidity have been conducted only on frozen meat products (Zipser et al., 1964; Watts and Peng, 1947). Watts and Peng (1947) found no accelerating effect of KCl (1.94%) on rancidity in frozen raw ground pork. In the study by Zipser et al. (1964), KCl at a concentration of 5.1% (equimolar to 4% NaCl) did not accelerate the development of rancidity in frozen cooked ground pork but provided some protection when compared to the control samples with no added salt.

This study was a companion study to that of Terrell et al. (1983), but that study only concerned microbial growth and did not address rancidity development. The objectives of the present study were to determine effects of NaCl, KCl and $MgCl_2$, at two different ionic strengths, on rancidity development in refrigerated raw ground pork that was either inoculated or not inoculated with two different species of microorganisms.

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MATERIALS & METHODS

IN THE FIRST EXPERIMENT, ground pork samples (100g for each trial involving one bacterial species) prepared from six pork shoulder-cuts, aseptically removed from Hampshire x Yorkshire crossbred barrows, were inoculated with either a *Moraxella* or a *Lactobacillus* species (Terrell et al., 1983); three shoulder-cuts (from three different animals) were used for each experiment involving a single bacterial species. Preparation of the inocula, method of inoculation and bacterial count data are presented in the companion paper (Terrell et al., 1983). To each inoculated sample, either no chloride salt (control) or one of the following types and ionic strengths of chloride salt was added: 0.73 or 0.37 NaCl; 0.73 or 0.37 KCl; and 0.73 or 0.37 $MgCl_2$. Addition of these chloride salt was based on raw meat weight to achieve ionic strengths equivalent to 2.5 or 1.25% NaCl. Samples inoculated with the *Moraxella* species were stored 12 days while those inoculated with the *Lactobacillus* species were stored 10 days at 5°C in Whirl-Pak bags.

In the second experiment, two of the same shoulder-cuts from the same animals that were used for inoculation with the *Lactobacillus* species in the first experiment, were used. These ground pork samples (60g/treatment) were not inoculated, but were frozen and stored (–20°C) for 2 wk before they were thawed, treated with chloride salts and then stored at 5°C for either 5 or 10 days.

The extent of rancidity development was determined by the 2-thiobarbituric acid (TBA) test of Tarladgis et al. (1960), as modified by Rhee (1978). In this modification, 5 ml of a 0.5% solution of propyl gallate and EDTA, for each 10g sample, were added during the blending process. Data were analyzed by analysis of variance and the Duncan (1955) multiple range test.

Table 1 – TBA values of refrigerated, inoculated ground pork treated with different chloride salts

Chloride salt	Ionic strength[a]	Percent salt	TBA no. (mg malonaldehyde/kg)[b] of pork inoculated with: Moraxella[c]	Lactobacillus[d]
NaCl	0.73	2.50	14.4[e]	12.0[e]
	0.37	1.25	10.2[f]	9.5[f]
KCl	0.73	3.19	10.7[f]	7.3[g,h]
	0.37	1.60	8.9[f,g]	6.0[h]
$MgCl_2$	0.73	1.36	6.6[g,h]	7.7[g]
	0.37	0.67	4.9[h]	5.8[h]
Control	0	0	0.6[i]	1.5[i]

[a] Based on a mean moisture content of 58.6% (range: 57.7 — 59.0%) in ground pork samples used; $Y = \frac{1}{2} \Sigma Mz^2$, where Y = ionic strength, M = molarity, and z = charge of the ion.
[b] Mean value of data on ground pork samples from three animals.
[c] Samples stored for 12 days at 5°C, not vacuum packaged.
[d] Samples stored for 10 days at 5°C, not vacuum packaged.
[e,f,g,h,i] Means within a column which are not followed by a common superscript letter are significantly different ($P < 0.05$).

Table 2 — TBA values of refrigerated, noninoculated ground pork treated with different chloride salts[a]

Chloride salt	Ionic strength	Percent salt	TBA no. (mg malonaldehyde/kg)[b] 5 days	10 days
NaCl	0.73	2.50	6.0[c]	7.7[c]
	0.37	1.25	5.0[c,d]	6.9[c]
KCl	0.73	3.19	5.0[c,d]	7.6[c]
	0.37	1.60	3.7[d]	6.5[c]
$MgCl_2$	0.73	1.36	4.9[c,d]	6.5[c]
	0.37	0.67	4.7[c,d]	6.1[c]
Control	0	0	0.9[e]	2.2[d]

[a] Stored at 5°C, not vacuum packaged.
[b] Mean value of data on ground pork samples from two animals.
[c,d,e] Means within a column which are not followed by a common superscript letter are significantly different ($P < 0.05$).

RESULTS & DISCUSSION

TBA VALUES for inoculated samples are shown in Table 1. Within a chloride salt treatment when *Moraxella* was inoculum, ionic strength (0.73 vs. 0.37) affected TBA values for the NaCl treatment ($P < 0.05$) but did not affect those values for either the KCl or $MgCl_2$ treatment ($P > 0.05$); TBA values were higher for NaCl at an ionic strength of 0.73 than at 0.37. Among the three chloride salts, NaCl, at 0.73 ionic strength, produced higher TBA values than either ionic strength of KCl or $MgCl_2$. Samples made with $MgCl_2$ at an ionic strength of 0.37 had lower TBA values than those made with KCl. When *Lactobacillus* was inoculum, samples made with NaCl had the highest TBA values irrespective of ionic strength. There was no difference ($P > 0.05$) in TBA values between the two ionic strengths of KCl in *Lactobacillus*-inoculated samples; within the NaCl or $MgCl_2$ treatment, ionic strengths of 0.37 resulted in lower TBA values than ionic strengths of 0.73. Regardless of the species of bacteria used for inoculation, all chloride salt treatments produced higher TBA values than controls. Although microbial counts were not determined in this study, the companion study (Terrell et al., 1983) showed that mean *Moraxella* counts (log_{10}/g) of ground pork samples at day 0 and after 12 days of refrigerated storage were 5.13 and 5.06, respectively and mean *Lactobacillus* counts at 0 and 10 days were 5.81 and 8.37, respectively.

TBA values for noninoculated samples, during 5 and 10 days of storage, are shown in Table 2. Within a chloride salt treatment, ionic strength did not affect ($P > 0.05$) TBA values. However, addition of any chloride salt resulted in higher TBA values than those for controls.

Although ionic strength did not affect ($P > 0.05$) TBA values when samples were not inoculated, or when *Moraxella*-inoculated samples were treated with KCl or $MgCl_2$, or when *Lactobacillus*-inoculated samples were treated with KCl, there was a numerical trend in all experiments for samples with the higher ionic strength of each chloride salt to have higher TBA values than those of samples with the lower ionic strength.

TBA values of inoculated samples containing NaCl were considerably higher than those of noninoculated samples with NaCl. The same was true for samples containing KCl that were inoculated with the *Moraxella* species. These differences in TBA number could be related to microbial activity since it is well known that some microorganisms cause development of oxidative rancidity (Jensen, 1954). A synergistic effect of these species on rancidity development could exist in samples containing chloride salts, particularly with NaCl and to a lesser extent with KCl and $MgCl_2$.

On the other hand, inoculated and noninoculated ground pork samples were of different animals and/or were handled slightly different, and this perhaps could have been responsible for some of the differences in TBA values.

The significance of the present study is in demonstrating that addition of any of the three chloride salts, i.e., NaCl, KCl and $MgCl_2$, accelerates rancidity development in raw-refrigerated ground pork, regardless of inoculation of samples with microorganisms. In previous studies (Watts and Peng, 1947; Zipser et al., 1964), rancidity values of KCl-treated ground pork samples were either not different from, or were lower than, those of controls. However, there were differences between the present study and the latter two studies in regard to the state of meat (raw vs. cooked), the method for measuring rancidity, KCl concentration, or storage temperature. Additional studies are needed to elucidate mechanisms associated with differences among various types and/or levels of chloride salts and their effects on rancidity development, during refrigerated and frozen storage, in both raw and cooked meat products. Also needed are studies determining whether certain microorganisms have synergistic effect on rancidity development in meat products containing chloride salts.

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A Research Note

A Modified Sample Preparation Method for Measuring Meat Tenderness by the Kramer Shear Press

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ABSTRACT

Tenderness of steaks and diced meat was evaluated by sensory panel, Warner-Bratzler shear press and modified Kramer shear press which employed coarse grinding of meat through a food chopper and shearing duplicate 20g samples of ground meat by the Kramer shear device. Highly significant ($P < 0.01$) correlation coefficients of −0.88 and −0.80 were obtained for the steaks and diced meat, respectively, between sensory score and modified Kramer shear value. A correlation value of 0.90 between W-B shear values and modified Kramer shear values was also highly significant. The results strongly indicate that the modified sample preparation method is a simple, reliable and versatile method which is less influenced by experimental variables and is applicable to almost any kind of meat products.

INTRODUCTION

THE WARNER-BRATZLER (W-B) shear device, as first described by Bratzler (1932), has been most widely used for measuring tenderness. Even though this device would serve as a reliable measure of meat tenderness, experimental variables such as uniformity of sample size, direction of muscle fibers, presence and absence of connective and fatty tissues and anatomical location must be carefully controlled in order to insure reliable results (Bratzler, 1949). Besides, this method has been primarily applied to steaks or roasts from which uniform core samples of 1.25–2.5 cm in diameter can be drilled. On the other hand, the Kramer shear press, originally developed by Kramer et al. (1951) has been less frequently applied to meat products. Even though the correlation with sensory panels have been of about the same magnitude as for the W-B shear or slightly higher (Pearson, 1963), the conventional method requires meat slices of uniform thickness, same surface area and same muscle fiber direction. Such requirements in obtaining proper samples are often difficult to achieve and limit the versatility of these methods to selected meat products such as steaks or roasts.

The present study describes a modification of Kramer shear press method which eliminates the need for careful sample preparation and improves its versatility of application to almost any kind of meat products.

MATERIALS & METHODS

Study 1

Two rib steaks of 2.54 cm in thickness were cut out from the 12th rib area of the carcasses of 12 well-fed steers. The carcasses were graded from high Good to average Choice. Each steak was inserted with a metal meat thermometer, broiled to an internal temperature of 71°C and cooled down to room temperature. For the W-B shear test, three cores of 1.9 cm in diameter were removed from medial, dorsal and lateral positions of each steak using a cork borer mounted on a power drill. The core samples were sheared by a W-B device mounted on an Instron at a crosshead speed of 20 cm/min.

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For the modified Kramer shear test, the sheared (cut in half) meat samples from the W-B test were combined with additional chunks of meat removed from the remaining steak and approximately two-thirds of the pooled meat chunks were coarsely ground through a hand food chopper (chopper #2, Universal Chopper Division, Union MFG Co., Conn.). The coarse cutter was used for grinding, and the size of the front openings through which meat was extruded was 1.5 x 0.7 cm. After gentle mixing, duplicate 20g samples were weighed, placed evenly on the bottom of the test cell (6.7 x 6.7 cm) and sheared by the Kramer shear device mounted on an Instron at a crosshead speed of 20 cm/min. The shear value measured from the peak force was expressed as kg force per 20g sample. This modified method consists of simply grinding meat coarsely and subjecting 20g samples to shearing, instead of carefully preparing meat slices, uniform in thickness and surface area, as required in the conventional Kramer shear method.

The remaining one-third of the meat chunks was used for the sensory evaluation of tenderness by 6 trained panel members on a 9-point hedonic scale; 1 = extremely tough, 2 = very tough, 3 = tough, 4 = slightly tough, 5 = satisfactory, 6 = slightly tender, 7 = moderately tender, 8 = very tender and 9 = extremely tender.

Study 2

Two rib steaks of 2.54 cm in thickness were cut out from the 12th rib area of the carcasses of 12 grass-fed steers. The carcasses were graded from low Standard to average Good. The sample preparation, shear test and sensory evaluation were identical to study 1.

Study 3

As an example of application to other kinds of meat products to which the W-B shear or the conventional Kramer shear method is difficult to apply, chucks and rounds of steers and cows were boned, boneless meat chunks stuffed into a nylon bag of 17 cm in diameter, cooked in boiling water to an internal temperature of 87°C, cooled in cold water and finally diced into 2 x 2 x 2 cm cubes. A 100g portion of the diced meat was coarsely ground, and duplicate 20g samples were sheared by the Kramer shear device as described in study 1. Another portion of diced meat was served for the sensory evaluation of tenderness as described in study 1.

For all the studies, simple correlation coefficients were calculated among sensory scores, W-B shear values and modified Kramer shear values. Regression equations were also drawn from the pooled data of study 1 and 2 and from those of study 3 to show the relationship between the sensory score and the shear value determined by the modified Kramer method.

RESULTS & DISCUSSION

TABLE 1 SUMMARIZES RESULTS from the three studies of the relations among sensory tenderness, W-B shear and modified Kramer shear values. Data from 48 rib steaks of different grades, ranging from low Standard to average Choice grade, revealed that the modified Kramer shear can be a useful tool for measuring tenderness of beef steaks. Correlation coefficients between sensory score and modified Kramer shear values were highly significant ($P < 0.01$) with an average value of −0.88. They were higher than those between sensory score and standard W-B shear. Correlation values obtained between the modified Kramer and the W-B shear were also highly significant ($P < 0.01$) with an average value of 0.90, indicating that the modified Kramer shear described in this study can successfully substitute for the standard method of W-B shear. Bailey et al. (1962) sheared

Table 1—Mean values and correlation coefficients among sensory tenderness, W-B shear value and modified Kramer shear value

Study	Kinds of meat	n	Mean values ± S.E.			Correlation coefficient		
			Sensory[a] score	W-B, Shear, kg/core	Modified Kramer shear, kg/20g	Sensory vs. W-B shear	Sensory vs. modified Kramer	W-B shear vs. modified Kramer
1	Rib steak (high Good~ ave. Choice)	24	5.1 ± 0.2	7.3 ± 0.2	119.8 ± 4.7	−0.76**	−0.87**	0.92**
2	Rib steak (low Standard~ ave. Good)	24	4.4 ± 0.3	9.0 ± 0.5	137.2 ± 5.6	−0.85**	−0.89**	0.88**
3	Diced meat	96	4.6 ± 0.2	–	127.2 ± 4.2	–	−0.80**	–

[a] 1 = extremely tough, 2 = very tough, 3 = tough, 4 = slightly tough, 5 = satisfactory, 6 = slightly tender, 7 = moderately tender, 8 = very tender, 9 = extremely tender.
**$P < 0.01$.

a 2 5/8 inch square section cut out from steak using the Kramer shear device and reported the overall correlation value of −0.74 between sensory score and Kramer shear. The modified sample preparation employed in this study gave a higher correlation value of −0.88.

For the 96 samples of diced meat in study 3, the correlation value of −0.80 was highly significant ($P < 0.01$) indicating that the modified method can be successfully applied for tenderness measurement of other irregular shaped meat products such as canned meat, diced meat and sliced meat.

Table 2 shows two regression equations, one derived from steaks and the other from diced meat, which describe the relationship between the sensory score and the modified Kramer shear value. The two equations were very similar; equation 1 giving a slightly higher shear value than equation 2 for the same sensory score. An average shear value of 117 kg per 20g meat was the borderline value for the satisfactory tenderness.

The modified shear method has several advantages over the standard W-B shear or the conventional Kramer shear; (1) it does not require careful sample preparation in terms of uniformity of sample diameter or thickness, muscle fiber direction, anatomical location and presence of connective tissue strands; (2) the shear data are more representative of the whole piece of meat studied because the whole piece is ground and well mixed before weighing; (3) coefficient of variation (<5.0%) between replicate measurements from the same muscle or products is much smaller than that (10~40%) of the other two methods; (4) it is a highly versatile method applicable to almost any kind of meat cut or product.

Two experimental variables can affect the test results. One is the content of moisture which is in turn largely affected by the degree of cooking. However, this variable can affect any method of tenderness measurement and is not unique to this method. The other variable is the size of the meat particles after grinding. Since smaller particles tend to give lower shear values at the same sample weight and tend to fall through the bottom slits of the shear cell, it is recommended that the size of the extrusion opening should be approximately 1.0 cm^2 or slightly larger. As long as the same kind of grinder and plate size is used, the size of the meat particles can be closely controlled.

Table 2—Regression equations describing the relationship between the sensory score and the modified Kramer shear value

Sensory score	Shear values, kg/20g meat	
	Study 1 and 2	Study 3 (diced meat)
	Y = −18.49X + 211.93	Y = −17.42X + 203.09
1 = extremely tough	193	185
2 = very tough	175	167
3 = tough	156	150
4 = slightly tough	138	132
5 = satisfactory	119	115
6 = slightly tender	101	98
7 = moderately tender	83	80
8 = very tender	64	63
9 = extremely tender	46	45

In conclusion, the proposed modified method of sample preparation for the Kramer shear press is a simple, reliable and versatile one and is less influenced by experimental variables.

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A Research Note

HPLC Determination of Erythorbate in Cured Meats

KEN LEE and S. MARDER

ABSTRACT

Erythorbate was measured in nitrite-cured meats using ion-paired HPLC. With tetrabutylammonium formate as the ion pair, good separations were obtained with a linear response up to 300 ppm of erythorbate. Recoveries were complete and agreed with values obtained by titration with 2,6-dichloroindophenol. The method is specific and free from interferences from other reducing substances in cured meats. Erythorbate and its isomer, ascorbate, are not distinguished under these conditions.

INTRODUCTION

HPLC METHODS for the determination of ascorbic acid in foods are sometimes desirable over the conventional titration with 2,6-dichloroindophenol, (AOAC, 1980) especially with turbid or pigmented foods. Augustin et al. (1981) reported titration was difficult and overestimated the ascorbic acid content of deep-fat fried products, and thus developed a HPLC reverse-phase ion paired method based on the work of Sood et al. (1976). Use of reverse phase ion-pairing is advantageous as many polar interferences would elute in the dead volume and hydrophobic compounds would be in low concentration in the aqueous extract. It is desirable to measure the erythorbic acid (D-isoascorbic acid) present in nitrite-cured meats, commonly added as a curing-aid. The method of Augustin et al. (1981) was not appropriate for the analysis of erythorbic acid in cured meats. We experienced some precipitation, presumably of phosphate, upon injection. Other workers have also experienced this problem (Watada, 1981). This note describes a procedure for analysis of ascorbic acid in cured meats and other foods. The method is free from a precipitation problem but retains the advantages of the reverse phase ion-pairing HPLC analysis.

MATERIALS & METHODS

Apparatus

A Model 6000A pump, U6K injector, 30 cm reverse-phase C18 column (all Waters Assoc.), Model III 254 nm detector (Gilson) and Model SP 4100 integrator (Spectra-Physics) was used.

Mobile phase

5.0 x 10^{-3}M tetrabutylammonium hydroxide (Eastman Chem) in water was adjusted to pH 4.77 by dropwise addition of 20% formic acid and degassed by millipore filtration or sonication. About 15 min of mobile phase at 1.5 ml/min were allowed for equilibrium with the column before analysis. Mobile phase was followed and preceeded by 50:50 water:methanol until a stable baseline was established.

Standard and sample preparation

50.0g of cured meat plus 50.0 ml of 6.0% HPO_3 (metaphosphoric acid was prepared daily and stored at 4°C) were blended for 1 min. The solution was filtered through glass wool until 10–15 ml were collected, and further purified by passing through a Waters Sep-Pac C18 cartridge just prior to injection. A 20 microliter injection of cured meat extract initially containing 200 ppm erythorbate would typically give a response of one-half the linear range. Standards were prepared by spiking samples with known amounts of erythorbate to yield final concentrations in the range of 0 to 300 ppm.

Chromatography

Mobile phase contained 0.005M tetrabutyl ammonium formate (pH 4.77) flowing at 2.0 ml/min. 20 ml to 100 ml injections of samples or standards diluted in mobile phase were injected onto a pre-equilibrated reverse phase column. Standards were run at the beginning and end of each analysis and peak areas were converted to milligrams erythorbate by electronic integration.

DISCUSSION

ERYTHORBATE was successfully measured in cured meats and other foods using ion-paired HPLC. This method retained the minimal sample preparation and accurate recoveries of the method of Augustin et al. (1981) which employed tridecylammonium-formate as the pairing agent for the analysis of ascorbic acid in potatoes. However, use of the tridecylamine pairing ion resulted in precipitation and column clogging when used for analysis of erythorbate and ascorbate in cured meats. A smaller ion-pairing molecule, tetrabutylammonium-formate, was used in this work. The ion-pair did not precipitate and exhibited good separa-

–Continued on page 308

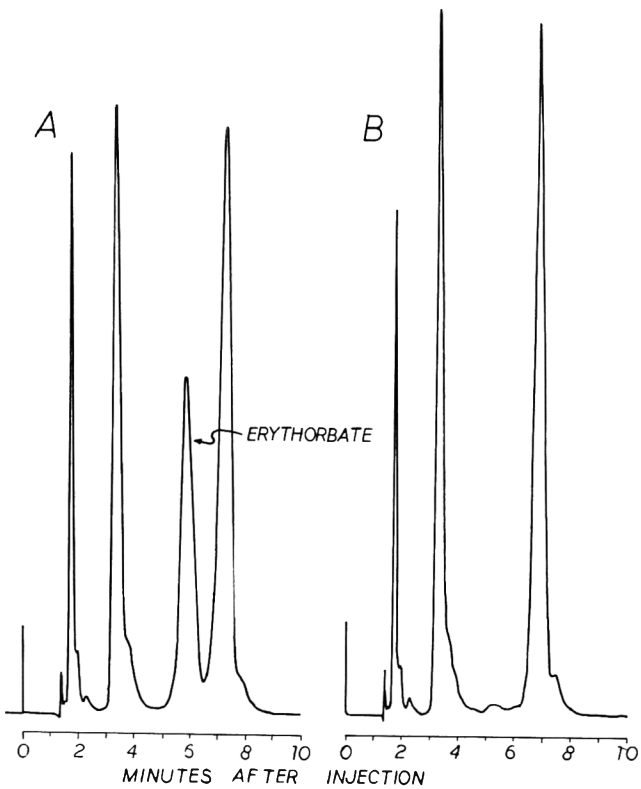

Fig. 1–Cured meat extracts containing added erythorbate (A) or with no erythorbate (B).

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A Research Note

Comparison of Nutrients in Raw, Commercially Breaded and Hand-Breaded Shrimp

I. H. AHAMAD, R. M. RAO, J. A. LIUZZO and M. A. KHAN

ABSTRACT

Three different brands of commercially breaded shrimp were analyzed for breading composition and nutrients (protein, carbohydrates, fat, B-vitamins, calcium and iron) for comparison with hand-breaded laboratory samples. All analyses were on a 100g basis. The percent breading, carbohydrates and calories were higher in the commercial samples than in the hand-breaded shrimp. However, the hand-breaded shrimp exhibited slightly higher values of niacin, thiamin and riboflavin when compared to the commercial samples. An average of 41.8 mg of calcium and 0.97 mg of iron were found in commercial samples while the hand-breaded samples contained 32.4 mg and 1.1 mg. respectively, for these two minerals.

INTRODUCTION

FROZEN RAW BREADED SHRIMP is the food prepared by coating one of the optional forms of shrimp with safe and suitable batter and breading ingredients (Food & Drug Administration, 1973). Either fresh or frozen shrimp can be used for breading, but the usual practice is to use fresh shrimp as these are generally of a higher quality than frozen ones. The breading process involves several steps consisting of sample preparation, coating with batter followed by breading, frying and packaging for frozen storage.

Standards of Identity (Food & Drug Administration, 1973) specify that breaded shrimp, that are produced and marketed in the United States, should contain not less than 50% shrimp material. Gagnon and Fellers (1958) conducted a survey of retail bought breaded shrimp from 27 cities throughout the United States and found variations in the percentage breading between 26.2 and 72.5% and in total nitrogen content between 1.18 and 3.16%. A negative correlation was found between percentage breading and total nitrogen content.

The nutrient composition of breaded shrimp is determined by its component ingredients i.e., batter, breading and shrimp. The percentage breading which influences the nutritional composition of the finished product, may itself be affected by factors such as the quality of raw shrimp used for breading, methods of handling and breading, and ambient temperature and humidity during processing and storage of the product.

The present study was undertaken to analyze the breading and nutrient content of commercially breaded shrimp purchased in local markets and to compare them with ideally hand-breaded laboratory samples.

MATERIALS & METHODS

Procurement of sample

Arrangements were made with three retail outlets in Baton Rouge, LA to procure three commercially breaded shrimp samples which were processed and packaged in three different geographical areas of the United States, i.e., the Gulf Coast, the Atlantic Coast and the Great Lakes Region. At the time of purchase, these samples were frozen and packaged in a moisture proof paperboard container. The frozen breaded shrimp were arranged in layers and packaged. The shrimp used were of 20 count range. Altogether, three purchases of the same sample were made at 2-wk intervals and the samples were put in frozen storage.

Good quality fresh white shrimp (*Penaeus setiferus*) of 20 count size were bought at a seafood market in Baton Rouge, for the laboratory prepared breaded shrimp samples. The raw shrimp were stored in crushed ice until they were further processed.

Breading materials were purchased from commercial outlets in Louisiana.

Preparation of hand-breaded laboratory samples

A portion of the fresh shrimp was shelled (leaving the terminal segment of the shell intact), deveined and split down the center. They were then washed, flattened on absorbent paper towels and individually weighed in order to determine the amount of breading to be used for obtaining a ratio of 50% breading in the finished product.

The procedure for preparing batter and laboratory breaded shrimp closely simulated the commercial procedures. The breaded shrimp were arranged in layers in moisture proof paperboard containers and were stored in the freezer at −15°C.

Before analysis, half of the samples of raw, hand-breaded and commercially breaded shrimp was removed from the freezer and thawed. They were cut in smaller pieces and each 1.5 lb-cut sample was individually blended with 10 ml aliquots of water until a homogeneous blended mixture was obtained. The homogeneous blended samples were kept in screw-top sample bottles which were covered with aluminum foil to keep out light, and labelled. They were then kept in frozen storage at −15°C for 5 wk and used for nutrient analysis.

Methods of analysis

Moisture. Moisture content of the samples was determined by the Weight Difference method. The shrimp were placed in individual 6 cm diameter aluminum cups and dried in an oven at 100°C for 24 hr to determine the loss in weight.

Breading. Six shrimp from each box of a particular brand were used and the average breading content determined. Any ice glaze which had accumulated on the frozen shrimp was removed and weighed. Then the shrimp were washed in running water until all the breading material had been removed and blotted on paper towels and then weighed. The percentage breading was determined by difference in weights.

Calories. Gross calories (heats of combustion) were determined by using an adiabatic bomb calorimeter and the gross calories were calculated from the formula,

$$\text{Kcal} = \frac{\text{Water equivalent} \times \text{temp rise}}{\text{net sample weight}} -$$

(acid + wire correction factor) × 0.5555

Carbohydrate. Carbohydrate was determined by difference from moisture, protein, fat and ash. the remainder after subtracting these nutrients was assumed to be total carbohydrate.

Protein, thiamin, riboflavin, niacin, calcium, iron, fat and ash. These nutrients were analyzed according to the methods recommended by AOAC (1975) in specification 39.032, 43.038, 43.044, 14.014, 2.096, 18.039 and 18.02, respectively. For fat analysis, the Goldfish apparatus was used for extraction.

—Continued on next page

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RESULTS & DISCUSSION

A SUMMARY of breading levels, calories, protein, carbohydrates, fat, thiamin, riboflavin, niacin, calcium and iron are present in Table 1.

It was found that while values of some of the nutrients in the different samples were relatively consistent, values for other nutrients exhibited a greater range of variability among the samples.

The nutrient values that fluctuated least include fat, niacin, iron and calories. The fat content averaged 0.73% for the laboratory breaded shrimp and the three commercial samples, while niacin in these samples ranged from 1.7 mg to 2.1 mg, with an average of 1.9 mg/100g sample. Iron values were from 0.92 mg to 1.05 mg per 100g sample. Protein, carbohydrate and calcium exhibited some variability among samples but the small differences are to be attributed to the geographical variations of the samples. The nutrient showing the widest range of values among the sample was thiamin. The hand-breaded laboratory sample contained 24.5 mg of thiamin per 100g of sample whereas the values for the commercial samples were 18.3, 11.6 and 12.9 mg/100g for A, B and C samples, respectively. These values are rather low compared to the reported values of 26.5 mg/100g (Watt and Merrill, 1963). One of the reasons for the low values of thiamin found in commercially breaded shrimp samples may be due to the presence of the enzyme thiaminase in the raw shrimp. According to Lubitz et al. (1943), the *Penaeus genus* of shrimp is especially rich in thiaminase, the presence of which induces an abnormally low thiamin value in the muscle tissue of shrimp after death.

The results of this investigation indicate that there can be substantial differences in the nutritional values among commercially breaded shrimp compared to the ideally hand-breaded laboratory samples. Breading ingredients, methods of breading, shrimp species used for breading, and handling and processing raw shrimp prior to breading are some of the influencing factors which affect the nutritional composition of breaded shrimp.

Table 1—Summary of nutrient levels in raw, commercially breaded and hand breaded shrimp

Nutrients	per 100 gram samples[a]				
	R	A	B	C	X
Breading (%)		47.30	50.50	56.70	50.00
Calories (Kcal)	87.10	139.20	141.40	147.80	140.60
Protein (g)	16.80	15.40	12.00	11.70	15.60
Carbohydrate (g)	2.70	17.00	21.20	23.10	19.20
Fat (g)	0.78	0.85	0.62	0.69	0.75
Thiamin (mg)	29.60	18.30	11.60	12.90	24.50
Riboflavin (mg)	45.00	12.30	31.70	17.70	21.10
Niacin (mg)	3.60	1.80	1.70	1.90	2.10
Calcium (mg)	69.60	47.10	36.60	41.80	32.40
Iron (mg)	1.40	0.93	0.92	1.06	1.05

[a] R is the raw shrimp used in the hand-breaded laboratory sample; A, B, C are commercial samples; X is the hand-breaded laboratory sample.

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HPLC OF ERYTHORBATE IN CURED MEAT. . . From page 306

tion of the erythorbate from other components of the meat extract.

Erythorbate eluted at 5.7 min when chromatographed at 2 ml/min and pH 4.77 as shown in Fig. 1. Optimization of the retention time was possible through adjustment of the pH of the mobile phase. A higher pH caused longer retention. Commercially available samples of bologna contained 220 ± 7.3 ppm erythorbate by HPLC analysis, compared to 238 ± 12.7 ppm by titration with 2,6-dichloroindophenol. The HPLC method was more precise and measured slightly less erythorbate than the titrimetric assay. The precision of the titration was impaired due to the vague end point in the opaque white meat extract. It is also possible that the indophenol responded to other reducing substances in the meats.

Peak identity was verified by chromatography of spiked samples of cured meat which were produced without any added erythorbate. The mean recovery of added erythorbate spikes was 98 ± 1.2%. The additive-free cured meat is also shown in Fig. 1. Both erythorbate and ascorbate were used for spiking, and did not differ in chromatographic behavior. Differentiation of these isomers is possible under different chromatographic conditions (Bui-Nguyen, 1980), and may be desirable as erythorbate has about one-twentieth the biological activity of ascorbate. This procedure was also successfully used for the analysis of tomato juice, tomato soup, and orange juice (not shown).

The ion-pairing HPLC procedure as described was accurate and precise in measurement of erythorbate in nitrite-cured meats. It was also appropriate for turbid or colored foods in which the colorimetric titration was difficult.

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A Research Note

Influence of Storage Times After Cooking on Warner-Bratzler Shear Values of Beef Roasts

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ABSTRACT

Fifty longissimus and 49 biceps femoris roasts were cut from five Choice-grade steer carcasses and used to evaluate differences in Warner-Bratzler shear values at different times after cooking. Five longissimus roasts and four or five biceps femoris roasts were cut from both sides of each carcass. Roasts were cooked to an internal temperature of 71°C then wrapped in aluminum foil and chilled (4°C) for 2, 24, 48, 72 or 168 hr prior to shear analysis. No differences in shear values by carcass side or by length of chill after cooking existed for biceps femoris roasts. Longissimus roasts were more tender when taken from the left side and when chilled for 2 hr vs longer chill periods. Roasts chilled for 24, 48, 72 or 168 hr had similar shear values. Cores taken from the medial portion of the longissimus roasts had lower shear values than cores taken from the central or lateral portions. Longissimus roasts were always more tender than roasts from the biceps femoris muscle and they were less variable in tenderness.

INTRODUCTION

THE WARNER-BRATZLER SHEAR apparatus is widely used in the objective evaluation of meat tenderness (Cover et al., 1962c; Amerine et al., 1965; Sharrah et al., 1965; Szczesniak and Torgeson, 1965; Culler et al., 1978; Francis et al., 1981). Bratzler (1954) theorized that differences in time between cooking and shearing of cores might influence shear values as he expected warm cores to have lower shear values than cold cores. The AMSA (1978) suggested that cooling time prior to coring of cooked meat be at least 2 hr or to an internal sample temperature of 20°C. Carlin and Harrison (1978) failed to mention chill time before coring in their suggestions for meat evaluation. Researchers have allowed cooked meat to equilibrate to room temperature before shearing (Rodgers et al., 1963; Culler et al., 1978; Gokalp et al., 1978; Cross et al., 1979; Francis et al., 1981); others (Boccard et al., 1979) have sheared hot cores, while Craddock et al. 1974), Misock et al. (1976) and Field et al. (1978) have chilled cooked meat overnight at 2–4°C prior to shearing. Bratzler (1954) reported more uniform cores could be made from chilled, cooked meat with shear values on these cores more accurately reflecting differences in tenderness of meat.

Published shear values from similar roasts cooked to the same degree of doneness and allowed to chill for different lengths of time after cooking are lacking. The objective of the present study was to evaluate the influence of length of storage after cooking on tenderness. Influences of carcass side, core location and anatomical location on Warner-Bratzler shear values were also reported.

MATERIALS & METHODS

FIVE CHOICE-GRADE cross bred steers fed on a finishing diet were slaughtered in the University of Wyoming abattoir after an overnight stand without feed but with access to water. Carcasses which ranged in weight from 299–362 kg were chilled and aged (4°C) for 7 days prior to cutting. Longissimus muscles from the 13th rib and from the 1–5 lumbar region from both sides of the carcass were cut into adjacent roasts approximately 5 cm thick. The medial portion of the biceps femoris muscle was removed and cut into five roasts approximately 5 cm thick. One biceps femoris muscle from the left side of one animal was lost. Therefore, 50 longissimus roasts and 49 biceps femoris roasts were used in this study. Adjacent longissimus roasts from each muscle on each side were numbered 1 through 5 beginning with the roasts from the 13th rib. Biceps femoris roasts from each muscle were also numbered 1 through 5 but four of these roasts were obtained by cutting two roasts from the center portion, and then dividing each of these roasts in half by cutting parallel to the muscle fibers. The portion of the biceps femoris muscle adjacent to the vastus lateralis muscle was number 1; the portion of the first cut adjacent to the semitendinosus muscle was numbered 2; the portion of the second cut adjacent to the vastus lateralis muscle was numbered 3; the portion of the second cut adjacent to the semitendinosus muscle was numbered 4; and the small tapered roast posterior to roasts 3 and 4 was numbered 5. Roasts were stored 2 months at −25°C before they were thawed at 4°C and roasted in the convection oven to an internal temperature of 71°C. One roast from each anatomical location of each muscle from both sides of the carcass was stored 2, 24, 48, 72, or 168 hr after roasting. The roasts stored 2 hr were held at 20°C while those stored 24, 48, 72, or 168 hr were held at 4°C. After the specified chill period, three 2.54 cm diameter cores were removed, parallel to the muscle fibers, from the medial (core 1), central (core 2) and the lateral (core 3) portions of each longissimus roast and each core was sheared three times. Two or three 2.54 cm diameter cores were removed from each biceps femoris roast and a total of nine shear values were recorded.

Statistical analysis

Least-squares analysis of variance of the data was used for longissimus roasts to obtain means by storage time, carcass side, anatomical location and core location. The same variables were included for both muscles except core location which was variable for the biceps femoris roasts. Interactions between storage time, anatomical location and carcass side were also included in the models tested. Differences between means were tested using the Duncan's Multiple Range test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

WARNER-BRATZLER SHEAR VALUES for the biceps femoris muscle, which is higher in connective tissue, were always higher than for longissimus roasts, supporting the work of Ramsbottom and Strandine (1948), Cover et al. (1957, 1962a) and Batcher and Dawson (1960) (Table 1). Snowden and Wiedemann (1978) suggested that amount and nature of connective tissue not converted to gelatin in cooking may be of importance in determining the influence of collagen on toughness; Cover et al. (1957, 1962b) indicated that biceps femoris muscle contains more and tougher connective tissue than longissimus muscle. Cores from longissimus roasts which were sheared 2 hr after cooking had significantly lower ($P < 0.05$) resistance to shearing than did cores taken 24, 48, 72, or 168 hr after roasts were cooked (Table 1). Thoroughly chilled cores would be expected to require more force to shear than either raw (Segars et al., 1974) or freshly cooked roasts. There were no differences among cores taken from roasts which were stored at 4°C for 24 hr to 168 hr prior to shearing for either muscle.

Longissimus roasts from the left side of the carcass were

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Table 1—Least-squares means for Warner-Bratzler shear values of beef by chill time, carcass side, core location and anatomical location

Variable	Longissimus muscle		Biceps femoris muscle	
	Number	Mean	Number	Mean
Chill time after roasting, hr[a]				
2	10	7.69[e]	10	9.42
24	10	8.44[f]	9	9.94
48	10	8.16[f]	10	10.53
72	10	8.45[f]	10	10.00
168	10	8.36[f]	10	10.04
Standard error		0.18		0.49[g]
Carcass side[b]				
Right	50	8.39[e]	50	10.30
Left	50	8.05[f]	49	9.67
Standard error		0.11		0.33[g]
Core location[c]				
1 (medial)	100	7.35[e]		
2 (central)	100	8.39[f]		
3 (lateral)	100	8.91[f]		
Standard error		0.08		
Anatomical location[d]				
1	20	8.03	20	10.30
2	20	8.26	20	10.96
3	20	8.45	20	10.05
4	20	8.46	20	9.89
5	20	7.90	19	8.64
Standard error		0.18		0.56[g]

[a] Time after cooking that 2.54 cm diameter cores were removed and sheared.
[b] Side of carcass from which the roasts originated.
[c] Location for cores from the longissimus muscle: 1 = medial; 2 = central; 3 = lateral position. Core location for the biceps femoris muscle would not be meaningful since some roasts were from medial portions of the muscle and others were from lateral portions.
[d] Anatomical location for adjacent longissimus and biceps femoris roasts (See Materials & Methods).
[e,f] Values with different superscripts within columns are significantly different ($P<0.05$).
[g] Standard error of the means is the largest value when unequal numbers were present in a treatment group.

more tender ($P < 0.05$) than roasts from the right side while biceps femoris roasts were not different between carcass sides (Table 1). Weir (1953) suggests that longissimus muscle from the right side of hogs had lower shear values than roasts from the left side of animals although no explanation for the difference was given. The left side of the beef carcass contains more kidney fat than does the right side. As a result, meat may have chilled at a slower rate, resulting in less cold shortening in the 13th rib to 5th lumbar portion in the longissimus muscle from the left side when compared to the same anatomical location on the right side.

A number of researchers (Hiner and Hankins, 1950; Paul and Bratzler, 1955a, b; Batcher and Dawson, 1960) reported that the anterior portion of the longissimus is more tender than the posterior portion, while Ramsbottom et al. (1945) reported that the anterior end of the longissimus is tougher than the rest of the muscle. However, no differences in tenderness due to anatomical location of roasts from longissimus muscle existed (Table 1).

Segars et al. (1974) suggested that greater variation in mechanical properties of muscle tissue was across the muscle (lateral to medial) rather than along the length of the muscle (origin to insertion) and the position in which a core is taken within a muscle cross section may have an effect on shear value (Sharrah et al., 1965), with those cores closest to the inner edge of the biceps femoris muscle being lower in shear value (Sharrah et al., 1965). Differences in core locations within biceps femoris muscle were not analyzed in our study since two cores were removed from smaller roasts and three cores were removed from roasts large enough to allow for removal of uniform cores. Some cross sections of the biceps femoris muscle were also cut into two different roasts. Conflicting reports in the literature make it difficult to assess tenderness within the longissimus muscle. Shear values for cores taken at positions closest to the fat edge of the longissimus muscle have been lower than those closest to the vertebrae (Cover et al., 1962c). Alsmeyer et al. (1965) indicated that the medial portion, Tuma et al. (1962) suggested the central portion and Howard and Judge (1968) reported the lateral portion of the longissimus muscle to be most tender. In our study, cores taken in the medial portion of longissimus muscle were significantly more tender ($P < 0.05$) than cores from the central or lateral positions regardless of the length of chill time before shearing (Table 1). Carlin and Harrison (1978) recommended that cores be taken from medial and lateral positions of longissimus muscle in order to minimize differences within muscle. No significant interaction existed between location, carcass side or storage time after roasting for either muscle and no differences in variability among shear times existed (Table 2).

Table 2—Means, standard deviations and coefficients of variation for roasts by sheartime

Sheartime[a]	Muscle							
	N	Longissimus			N	Biceps femoris		
		$\bar{X}$(kg)	SD[b](kg)	CV[c]		$\bar{X}$(kg)	SD[b](kg)	CV[c]
2 hr	10	6.6	1.0	14.3	10	9.2	1.5	16.5
24 hr	10	7.4	1.3	17.2	9	9.8	1.3	13.6
48 hr	10	7.4	1.4	18.6	10	10.3	2.1	20.8
72 hr	10	7.7	0.9	11.4	10	9.4	2.2	23.0
168 hr	10	7.7	0.7	8.7	10	10.1	2.3	22.9

[a] Sheartime is the storage time after cooking in which roasts were sheared.
[b] SD = standard deviation of the means.
[c] CV = coefficient of variation for each of the means.

Overall, position of the core and time between cooking and shearing may well affect shear values but cores removed between 24 and 168 hr after roasting had similar values. Based on data presented here meat should be chilled at least 24 hr after cooking in order to give the most consistent shear values.

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A Research Note

Chillpack Studies on Farm-Raised Channel Catfish

R. J. REED, G. R. AMMERMAN, and T. C. CHEN

ABSTRACT

The shelf-life of commercially processed pond raised catfish held in two different storage environments was assessed in 1981 by sensory and microbiological examinations. An experienced panel rated samples held in a chillpack environment (−2.2°C) significantly higher than samples stored in ice for 7 days for odor and flavor, and overall mean after cooking. There was no significant difference between raw samples held 7 days in ice and 19-day-old chillpack samples. Total plate counts increased more rapidly in ice pack samples than chillpack samples.

INTRODUCTION

IN 1969, 3.2 million pounds live weight of farm-raised catfish were processed (Ayers, 1980). Over 56 million pounds live weight of channel catfish were processed in 1981 (Anonymous, 1981). The most common form of catfish marketed was fresh dressed product packed in direct contact with ice and approximately 60% of all the fish sold was packed in this manner (Ayers, 1980).

The handling and distribution of ice-packed fish has some major disadvantages. The ice used accounts for approximately 30% of the weight which is shipped and contributes substantially to transportation costs. The shelf-life of ice-packed fish has been reported to be 7 days (Beuchat, 1973). 11 days (Lovell and Ammerman, 1974), and 12 days (Heaton et al., 1972). This study was conducted to determine the effectiveness of storing fresh catfish without ice at a temperature below that of ice (−2.2°C) – sometimes referred to as the chillpack.

MATERIALS & METHODS

A RANDOMIZED complete block experimental design with four replications was used to compare the effect of cold storage environments on the shelf-life of farm raised channel catfish (*Ichtalarus punctatus*). This research was conducted in 1981.

The fish were harvested at 9:00 a.m., transported live to the processing plant, and held in concrete tanks alive until 11:00 a.m. They were shocked using 110 volts of electricity, deheaded by use of a band-saw, the body cavity opened with a knife, and the eviscera removed by use of vacuum. The fish were skinned using a Townsend Membrane skinner, packed in ice within 20 min of slaughter, and transported by automobile to the laboratory. Total time from pond to being placed under experimental conditions was 6 hr and time from deheading was 4 hr. The experimental units were 5 – 7 oz. whole fish.

At the laboratory the fish were divided into three groups and prepared for storage. One lot was IQF frozen and placed in individual 1.5 mil polyethylene bags and sealed at ambient pressure. This lot was held at from 0 to −23°C. The second lot was held packed in ice in a cardboard shipping case lined with a 1.5 mil polyethylene bag. The third lot was placed in 10-S styrofoam trays and covered with saran wrap.

These tray-packed fish were held in a freezer equipped with a White-Rogers No. 7818 thermostat and maintained at a temperature of −2.2°C, ±1.7°C to simulate a chillpack. The chillpack involves holding the product at its freezing point throughout the channels of trade and offering it for sale as a fresh product.

A sensory test utilizing a 9-point rating scale for panelists to evaluate attributes was employed. A score of 9 corresponded with "liked extremely" and 1 to "disliked extremely." The 10 member untrained sensory panel consisted of employees and students from the Food Science Institute who had previous experience on sensory panels evaluating a variety of other products including fish. The sensory panel sessions were conducted in an open area in the Food Processing laboratory with even lighting provided by F40CW florescent tubes.

Fish from each storage environment were prepared for evaluation by wrapping the samples in aluminum foil and baking for 45 minutes in an electric oven preheated to 176.7°C (350°F). The fish were cooled for 10 minutes, coded with a three digit random number and presented to the panel members with a glass of water. Each panelist was presented a whole cooked fish from the ice pack and chillpack and frozen treatments. One raw sample from the same two treatments was presented for evaluation by the panel members.

Odor, flavor, and texture scores were assigned on the 9-point scale for the cooked samples. Raw samples were evaluated for odor, color, and appearance. Cooked and raw chillpack samples were evaluated for sensory attributes on days 7, 11, 15, and 19. Since the ice packed fish exhibited signs of spoilage by day 15, cooked samples of fish which had been frozen were served to the panelists in place of the ice packed fish after day 15.

Fish samples were examined on days 0, 4, 7, 11, 15, and 19 to determine microbiological counts. Day 0 refers to the day after the fish were slaughtered. Total plate counts were determined using a swab technique as described by Mountney (1976). Bacto Nutrient Broth (BBL) was utilized in making serial dilutions. A pour plate technique was employed utilizing Standard Methods Agar (BBL). Psychrophilic counts were obtained by incubating plates at 20°C for 3 days. Additional platings using Peptone Iron Agar (Difco) were employed on two replications as a differential media for detection of hydrogen sulfide producing organisms.

RESULTS & DISCUSSION

TABLE 1 ILLUSTRATES the results of the sensory evaluations of the raw fish. The ice packed fish changed significantly ($P < 0.01$) in all attributes between day 11 and day 15 and again between day 15 and day 19, and reached their spoilage point between day 11 and day 15. Spoilage was indicated by bad odor and sliminess. This corresponds to results reported by Heaton et al. (1972) that channel cat-

Table 1—Mean sensory panel scores of raw channel catfish evaluated on day 7, 11, 15 and 19 and held in chillpack and ice-pack storage*

	Factor					
	Odor		Color		Appearance	
Day	Ice	Chill	Ice	Chill	Ice	Chill
7	7.98^{a}	8.03^{a}	7.09^{ab}	6.69^{b}	7.52^{ab}	6.71^{cd}
11	7.67^{a}	8.08^{a}	7.21^{ab}	7.79^{a}	7.09^{bc}	7.86^{a}
15	5.80^{b}	7.97^{a}	6.04^{c}	7.24^{ab}	6.30^{d}	7.30^{abc}
19	2.88^{c}	8.17^{a}	3.83^{d}	7.49^{ab}	3.95^{e}	7.54^{ab}

^{a,b,c,d,e}Means in the same attribute not sharing a letter in common differ significantly ($P < 0.01$) by the Student Newman-Keuls Test.

* 9 = liked extremely; 1 = disliked extremely

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Table 2—Mean sensory panel scores of cooked channel catfish for day 7 ice pack, day 19 chillpack, and frozen control fish*

Treatment	Odor	Flavor	Texture
19 day chill	7.88[a]	7.71[a]	7.56[a]
7 day ice	7.59[b]	7.23[b]	7.28[a]
Frozen	7.94[a]	7.62[a]	7.66[a]

[a,b]Means in the same column not sharing a letter in common differ significantly ($P < 0.05$) by the Student Newman-Keuls Test.
* 9 = liked extremely; 1 = disliked extremely

fish could be held in crushed ice 12 days and retain acceptable quality while fish held in plastic bags at 0.56°C retained their acceptability for 8 days. Ammerman et al. (1973) reported that the first significant difference in iced channel catfish occurred on the 10th day from slaughter when compared to a frozen control by use of a triangular difference test.

There was no change in the odor of the raw chillpacked fish through 19 days of the test. There was no significant ($P = 0.01$) change in the color or appearance of the chillpacked fish from day 11 through 19.

No significant ($P > 0.05$) change occurred in any sensory characteristic of the cooked, chillpacked fish throughout 19 days of storage. Data in Table 2 compare the mean panel scores of cooked chillpacked fish on day 19, ice-packed fish on day 7, and the frozen control fish. The 7 day ice-pack fish had a significantly ($P < 0.05$) lower rating in odor and flavor than the chillpack or frozen fish. There was no significant difference in texture scores ($P > 0.05$).

Data show that bacterial counts increased more rapidly on fish held in the ice-pack environment than on fish held in the chillpack environment. Psychrophilic and psychrotrophic counts had reached log 7.0/cm^2 in the ice-packed fish samples by day 15. The initial mean psychrotropic count for storage treatments was log 3.9/cm^2. This was higher than the initial mean psychrophilic count of log 2.5/cm^2. However, the number of psychrophiles increased at a faster rate than did the number of psychrotrophs.

As a result of this study, it was concluded that a chillpack environment (−2.2°C) would provide a means by which the shelf-life of fresh pond raised channel catfish could be extended.

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STORAGE TIME AFTER COOKING VS W-B SHEAR. . . From page 310

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A Research Note

Yields, Proximate Composition and Mineral Content of Finfish and Shellfish

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ABSTRACT

Yields of edible flesh, proximate composition, and mineral content were determined in two samplings of six finfish and four shellfish from the Chesapeake Bay region. Although there was considerable variation, most species have high protein (above 20%), low fat (less than 5%) and are relatively good sources of macrominerals, including calcium, phosphorus, magnesium and potassium. The ratio of sodium to potassium ranged from 1:4 to 1:15. These species, particularly the shellfish, are excellent sources of the trace elements, including copper, iron, zinc and manganese. Minerals are very high in certain shellfish.

INTRODUCTION

PER CAPITA CONSUMPTION of both finfish and shellfish has increased during recent years (Lane, 1981). The level of consumption was 13.3 pounds in 1979 with shellfish representing approximately 25% of the total per capita intake (Exler and Weihrauch, 1977). Many predict that seafood production will double by 1990 (Sprague and Arnold, 1972) and will become an increasingly larger source of nutrients for the U.S. population. However, there is only scant information available about the composition, particularly the essential mineral content, of major seafood species. An examination of nutrient composition tables, such as USDA Handbook No. 8 (Watt and Merrill, 1963) and USDA Handbook No. 456 (Adams, 1975) demonstrated that data have not been reported for several species and for several important minerals.

This study represents an analyses of the major seafood species marketed in the Chesapeake Bay region of the eastern U.S. Data were generated in a manner designed to provide useful information for consumers and the industry.

MATERIALS & METHODS

SAMPLES of the ten seafood species were obtained from markets in eastern Virginia on two different occasions, spring and early summer 1980, and during late summer and fall 1981. The following species were obtained: bluefish (*Pomatomus saltatrix*), croaker (*Micropogon undulatus*), flounder (*Pseudopleuronectes americanus*), sea bass (*Centropristes striatus*), gray sea trout (*Cynoscion nobilis*), spot (*Leiostomus xanthurus*), clams (*Mercenaria mercenaria*), crab (*Callinectes sapidus*), scallop (*Placopectens magellanicus*), and oyster (*Crassostrea virginica*). Each sample, consisting of several fish, was delivered to the laboratory whole and uncleaned packed in ice within 2 days of the catch.

Yield

The percent of edible portion was determined by weighing the whole fish or shellfish. Finfish were filleted by cutting at the pectoral fin behind the head and gill, turning the knife, and cutting away the flesh, including skin and scales, from the backbone from the head to the tail. The skin was then sliced away from the flesh, leaving the edible portion of the fish. The process was repeated for the other side of the fish. The edible flesh of each was weighed and the yield was calculated as a percentage of the total weight. When possible, the yield of edible raw flesh was determined for the shellfish. One-half of the fillets or flesh from the shellfish were broiled to the same degree of doneness, i.e., until flaky and lightly-browned or between 5–8 min per side. Actual broiling time varied with size of the fillet. After cooling, the cooked samples were weighed again and the yield of edible, cooked product was calculated. Crabs were boiled and the cooked flesh was removed.

Raw and cooked samples were ground in a blender, the blades of which were coated with teflon to prevent metal contamination, and a homogenous mixture was obtained. Extreme care was exercised to separate bones from the flesh portion.

Proximate analyses

Moisture, total ash, and nitrogen was determined in raw and cooked samples using the AOAC methods (AOAC, 1970). Protein was calculated using the factor of 6.25 x nitrogen (Pomeranz and Meloan, 1978). Total fat was extracted from dried samples with petroleum ether using the Soxhlet procedure.

Mineral analyses

Raw and cooked samples were ashed using a nitric acid-perchloric acid digestion procedure. The ash was taken up in dilute HCl and appropriate dilutions were made. Calcium, magnesium, sodium, potassium, iron, copper, zinc and manganese were determined using a Perkin-Elmer 503 atomic absorption spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT). Phosphorus was analyzed by the colorimetric method of Chen et al. (1956). Internal standards were utilized to determine recoveries and to assess the reliability and duplication of analyses.

RESULTS & DISCUSSION

YIELDS of edible flesh for finfish and shellfish (Table 1) range from about 10% for clams to 40% for flounder. Percentages of cooked edible flesh ranged from 6% for clams to 31.8% for bluefish. The cooked flesh, expressed as a percentage of raw flesh, was in a much narrower range. The percentages of yield were considerably lower for the shellfish. Yields from these samples are in general agreement with information on flounder (Brooke et al., 1962; Ousterhoust, 1960; Wangler, 1960) and on spot and gray sea trout (Ousterhout, 1960). Cooking methods, such as baking and grilling result in lower edible amounts because of the water loss when compared to steaming or poaching. The broiling in this study would tend to cause significant water loss.

Proximate composition data (Table 1) indicate that both finfish and shellfish are good sources of protein and are relatively low in fat. The percentages of protein (N x 6.25) range from around 9% in clams and oysters to about 21% in flounder. Increased percentages in the the cooked product suggest loss of moisture, but represent a more appropriate set of data for estimating actual composition as consumed. The percentage of ash or total mineral matter is around 1.1–1.5%, except for crab.

Moisture ranged from about 70% to about 82% in the raw products, but was reduced in the cooked process. The proximate analyses of these species compare closely to other reports except for the percentage of fat.

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Minerals

The content of several inorganic elements, essential to the human, are summarized in Table 2. The concentrations were higher in the cooked than in the raw samples, a reflection of water lost during the cooking process.

Except for sodium and potassium, values (Table 2) were in the general range reported by others (Sidwell et al., 1973; Gordon and Roberts, 1977). The sodium levels are in the general range reported by others, including Sidwell et al. (1973) and Thurston (1958), but the potassium concentrations are somewhat higher. Previous investigators have suggested ratios of sodium:potassium in the range 1:4 to 1:5. Data from these products suggest a much wider ratio, from 1:4 to about 1:15.

The trace or micronutrients have received some attention in previous investigators, but comparisons are difficult between other reports and the data generated from this work. Copper, iron, zinc and manganese are reported (Table 2) as mcg/100g edible flesh. Concentrations of copper range from 24.2 in sea bass to 76.7 mcg/100g in bluefish, with the exception of oysters which have a very high concentration of copper, 60.2 mg/100g. In cooked finfish, iron ranges from 123.4–583.4 mcg/100g, zinc ranges from 404.6–1024.0 mcg/100g, and manganese ranges from 12.5–38.3 mcg/100g. Shellfish have very much higher concentrations for iron, zinc and manganese. Oysters are especially rich in these micronutrients. Concentrations are expressed in mg/100g (Table 2) in several products for these trace minerals.

Composition information presented herein, representing two samplings of major species marketed in the Chesapeake Bay area, confirm that seafood can be characterized as low to moderate fat, high protein, and relatively rich sources of several important inorganic nutrients. Shellfish are rich sources of the microminerals.

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–Continued on page 316

Table 1–Yields of edible flesh and proximate composition of raw and cooked flesh from skinned finfish and shellfish[a]

Species	Raw edible flesh as % of total wt.	Cooked flesh as % of total wt.	Moisture (%)		Protein (%)		Fat (%)		Ash (%)	
			Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Finfish										
Bluefish	37.5	31.8	70.16	62.74	19.56	24.40	2.00	1.98	1.12	1.35
Croaker	22.2	15.2	79.37	68.90	18.13	27.10	1.90	2.78	1.08	1.68
Flounder	40.0	31.5	77.00	70.96	21.23	26.05	1.19	2.06	1.23	1.48
Sea Bass	26.9	21.1	79.80	74.17	18.46	26.14	2.08	1.56	1.09	1.42
Sea Trout (Gray)	35.1	24.7	76.98	71.38	18.62	24.16	3.41	4.41	1.13	1.40
Spot	32.8	25.5	70.23	56.47	19.70	27.14	10.24	14.86	1.20	1.74
Shellfish										
Blue Crab	–	24.0	78.83	74.89	16.42	21.18	0.77	1.21	2.06	2.42
Clams	10.1	6.0	81.68	69.00	9.70	15.44	1.18	1.61	1.52	2.15
Oysters	11.8	7.2	82.20	70.96	8.59	12.22	2.36	3.89	1.40	1.94
Scallops	–[b]	–[b]	77.91	68.70	15.08	22.34	1.00	1.59	1.22	1.44

[a] Each value represents the mean of two samplings; proximate analyses represent the mean of duplicate analyses from two different samplings.
[b] Flesh was obtained from different crabs for raw and cooked samples. Scallops were received in shucked form, thus yields were not obtained.

Table 2–Mineral content of raw and cooked edible flesh from skinned finfish and shellfish[a]

	Calcium (mg/100 g)		Phosphorus (mg/100 g)		Magnesium (mg/100 g)		Sodium (mg/100 g)		Potassium (mg/100 g)		Copper (mcg/100 g)		Iron (mcg/100 g)		Zinc (mcg/100 g)		Manganese (mcg/100 g)	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Finfish																		
Bluefish	6.8	9.6	243.8	274.9	42.3	50.5	31.8	29.7	327.3	431.0	76.6[b]	96.30	451.60	583.40	763.2	1024.0	22.05	12.50
Croaker	15.0	25.6	209.6	261.6	39.8	54.2	55.5	88.2	345.0	461.5	51.10	81.68	372.45	416.36	371.5	580.3	31.75	25.10
Flounder	13.2	18.4	220.2	289.4	47.0	58.4	32.9	35.9	595.4	694.5	28.2[c]	25.87	102.85	123.45	393.8	404.6	14.95	38.32
Sea Bass	10.4	22.7	193.7	246.3	41.4	47.2	55.6	49.4	355.7	471.0	24.25	57.95	136.25	154.85	341.2	510.8	12.70	13.20
Sea Trout (Gray)	14.3	9.6	217.2	235.0	50.0	51.8	40.9	45.4	553.7	631.1	47.7[c]	47.21	225.65	291.50	471.0	550.0	18.55	30.25
Spot	14.3	22.8	186.0	277.8	41.8	58.5	28.6	43.1	496.1	672.7	66.7[c]	91.15	319.15	539.90	369.8	639.0	22.30	32.85
Shellfish																		
Blue Crab	34.4	59.2	276.9	245.6	10.05	7.90	62.9	57.2	660.0	478.8	–	1061.30	–	674.17	–	54.24[b]	–	201.85
Clams	32.8	47.1	144.7	225.0	9.24	10.62	55.8	57.4	314.1	306.6	39.7[c]	58.58	2.46[b]	6.77[b]	2.50[b]	3.63[b]	16.87[b]	27.07[b]
Oysters	61.0	55.4	165.0	211.8	54.4	66.2	111.8	151.7	229.0	325.4	60.2[b]	79.30[b]	7.65[b]	8.23[b]	82.46[b]	99.29[b]	386.65	468.10
Scallops	9.2	12.4	201.6	267.3	56.0	69.0	87.3	113.2	412.2	519.7	37.4[c]	50.98	107.10	219.85	1218.7	1540.1	29.15	33.0

[a] Each value represents the mean of duplicate analyses from two different samplings.
[b] Expressed as mg/100g.

A Research Note

Effect of Stewing on Purine Content of Broiler Tissues

LOUIS L. YOUNG

ABSTRACT

The purine content of raw and stewed broiler tissues was evaluated. Stewed breast had slightly more adenine and stewed thigh had slightly more guanine than their raw counterparts. The analyses indicated that all of the purines were considerably higher in cooked skin than in raw skin. This obsrevation seems to be the result of extraction of other nitrogenous components (possibly collagen) from the skin. Hypoxanthine was the only purine which was studied that was extracted from the tissues in large amounts.

INTRODUCTION

FOODS which elevate serum uric acid are usually removed from the diets of hyperuricemic patients in order to control episodes of gout. Many of the foods which have this effect contain high levels of nucleic acids (Smith, 1971). Some reports indicate that this restriction may not always be warranted. Clifford et al. (1976) found that the effectiveness of nucleic acids in elevating serum uric acid levels in human volunteers varied with the component purines in the nucleic acid. Oral hypoxanthine, adenosine-5'-monophosphate (AMP), guanosine-5'-monophosphate (GMP) and inosine-5'-monophosphate (IMP) produced a greater hyperuricemic effect on subjects with frank gout than on either normouricemic or hyperuricemic subjects. Of the purine compounds normally found in foods, only adenine and hypoxanthine significantly elevated serum uric acid. This effect was especially pronounced in gout patients.

Data are needed concerning the purine content of foods in order to make dietary recommendations for gout sufferers and to have a technical basis for regulating the composition of our food supply. Very few such studies have been published. Clifford and Story (1976) evaluated the adenine (ade), guanine (gua), hypoxanthine (hyp) and xanthine (xan) content of a variety of raw foods. They concluded that the absolute and relative amount of each of the purines varied and that foods which were high in total purines were not necessarily high in those conducive to hyperuricemia.

Murphy et al. (1979) cited unpublished data by Bauermann (1978) and Weir and Clifford (1978) to conclude that mechanically deboned poultry presents no health hazard at any level in terms of its purine content.

Young (1980) evaluated the purine content of several poultry tissues. He reported that the most prominent purine in raw muscle tissues was hypoxanthine but that the hypoxanthine content of skin was low. He found that raw liver contained high levels of total purine but these were composed almost totally of adenine and guanine. Mechanically deboned chicken and chicken meat had somewhat lower levels of all the purines due to the high fat content of these products.

In the only report that I am aware of concerning the purine content of cooked foods, Young (1982) found that adenine and guanine were higher in roasted than raw broiler meats. He reported that hypoxanthine was extracted in the roasting process and there was a potential buildup of this purine in the cooking juices. This finding led to the present research. Chicken is often cooked by stewing. It was postulated that the hot water might alter the purine content of broiler tissues. The objective of this research was to evaluate the effect of stewing on the purine content of broiler tissues.

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METHODS & MATERIALS

Sampling and cooking

Twenty-four broilers were obtained from a local commercial processor. They were packed in ice and transported to the laboratory where they were split through the keel and along the dorsal midline. One-half of each bird was placed in a plastic bag and stored at 5°C. The other half was immersed in boiling water until it reached an internal temperature of 80°C. Internal temperature was monitored with a thermister inserted into the thickest part of the breast. After cooking, each half-bird was placed in a plastic bag and stored at 5°C. The next day the skin, breast and thigh tissues of each half carcass were removed, placed in individual glass sample jars, frozen and held at −20°C for purine analysis.

Purine analysis

The purines were analyzed using a previously described method (Young 1980, 1982). This method was modification of that used by Clifford and Story (1976). Samples were hand-chopped to a uniform particle size of about 2.5 x 2.5 mm and mixed. Nitrogen in the samples was evaluated by the macro-Kjeldahl procedure (AOAC, 1980). The samples were then freeze-dried, extracted with diethyl ether and then the dry, fat-free residue was ground with a Wiley mill. Nitrogen was evaluated in the resultant powder by the macro-Kjeldahl procedure (AOAC, 1980). The powders were digested in 1.0M $HClO_4$ for 1 hr at 100°C. The hydrolysates were centrifuged for 30 min at 80,000g and purine compounds in the supernatants were separated on HPLC using a cation exchange column and isocratic elution with 0.05M phosphate buffer, pH 2.0. Purine standards were purchased from Sigma Chemical Company. The purine content of each standard was evaluated spectrophotometrically using UV absorption data published by Sober (1970). All analyses were done singly since previous work has shown good repeatability on duplicate analyses (Young, 1980). The level of xanthine was not evaluated in these experiments because (1) previous work has shown that this purine is present only in very limited amounts in broiler tissues (Young, 1980), (2) xanthine has no effect in raising serum uric acid levels (Clifford et al. 1976), and (3) xanthine cannot always be separated from nonpurine compounds using this HPLC procedure. The HPLC data were converted to mg purine/100 g tissue using the NN analyses data and assuming that the total N in the samples was unaffected by analytical procedures.

Statistical analysis

The purine content of each sample was expressed on a wet basis and also as mg purine per 100 mg N. Least-square means were calculated for content of adenine, guanine and hypoxanthine in raw and stewed breast, thigh and skin. The value for raw and stewed tissues were compared using Student's t-test (Steel and Torrie, 1960). Differences were considered significantly different if $P > |t| \leq 0.05$ under H_0 raw = stewed.

RESULTS & DISCUSSION

STEWING INCREASED the adenine and guanine in breast

and thigh very slightly (Table 1). The only statistically significant differences were in the cases of adenine in breast and guanine in thigh. There was no significant difference in the hypoxanthine content of these tissues. On a nitrogen basis (Table 2), the adenine and guanine content of two stewed muscle tissues were remarkably similar to their raw counterparts. Hypoxanthine on a N basis was significantly reduced when breast and thigh were stewed. These results are very similar to those previously reported by Young (1982) for roasted broiler tissues. It appears that the hot water used in the cooking process extracted more of the hypoxanthine than of the other purines.

All purines in the cooked skin appeared to be higher than in the raw counterparts (Table 1) whether expressed on a wet or N basis. These observations can be rationalized by assuming that some of the non-purine nitrogenous material in skin was extracted in the cooking process. This behavior would be expected of tissues such as skin which contains large proportions of collagen since this protein is soluble in hot water (West and Todd, 1961). However, from a practical point of view the issue may be moot since the purines which exacerbate hyperuricemia (adenine and hypoxanthine) are lower in raw and stewed skin than in stewed breast or thigh tissues (Table 1). Moreover, skin represents less than 20% of the weight of water-cooked broiler parts (Hudspeth et al., 1974).

It appears that the effect of water cooking on the purine content of broiler tissues is similar to that of roasting: adenine and guanine increase somewhat due to removal of fat and moisture and part of the hypoxanthine is extracted by the hot water. This information may be of benefit to physicians, dietitians and others interested in recommending diets for gout sufferers.

Table 1—Purine content of raw and stewed broiler tissues (wet basis)

Tissue	Purine (mg/100g tissue)					
	Adenine		Guanine		Hypoxanthine	
	Raw	Stewed	Raw	Stewed	Raw	Stewed
Breast	21.11^{b}	24.61^{c}	26.49^{bc}	29.86^{c}	130.83^{d}	129.41^{d}
Thigh	19.91^{b}	22.57^{bc}	24.58^{b}	28.51^{c}	99.03^{c}	95.20^{c}
Skin	13.04^{a}	21.06^{b}	18.80^{a}	27.92^{bc}	27.10^{a}	44.80^{b}

^{a,b,c,d}Values within each purine group (raw and stewed) having different superscripts differ significantly, $P \leq 0.05$.

Table 2—Purine content of raw and stewed broiler tissues (nitrogen basis)

Tissue	Purine (mg/100 mg N)					
	Adenine		Guanine		Hypoxanthine	
	Raw	Stewed	Raw	Stewed	Raw	Stewed
Breast	0.61^{a}	0.58^{a}	0.76^{a}	0.71^{a}	3.75^{e}	3.05^{c}
Thigh	0.69^{a}	0.69^{a}	0.86^{b}	0.87^{b}	3.45^{d}	2.91^{c}
Skin	0.93^{b}	1.17^{c}	1.36^{c}	1.55^{d}	1.93^{a}	2.49^{b}

^{a,b,c,d,e}Values within each purine group (raw and stewed) having different superscripts differ significantly, $P \geq 0.05$.

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COMPOSITION OF FINFISH AND SHELLFISH. . . From page 314

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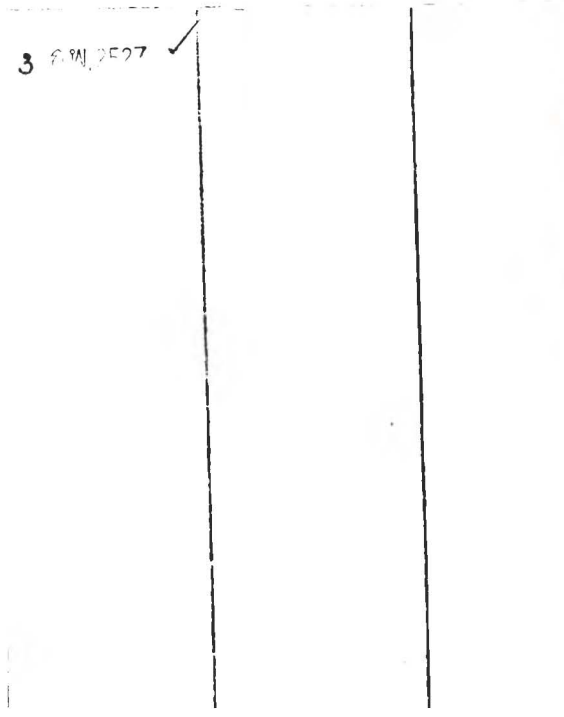

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