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PUBLICATION	WHERE TO FIND IT	DEADLINES
Call for Symposia Proposals and Guidelines for Symposia Organizers	*Food Technology,* January 1983, pp. 6 and 120–126	Receipt of preliminary proposals: May 15, 1983. Notice of acceptance or rejection of preliminary proposals: July 15. Receipt of final proposals: October 1. Notice of acceptance or rejection of final proposals: November 15, 1983
Call for Volunteered Papers and Official Abstract Forms	*Food Technology,* September 1983, pp. 89–96, and October 1983, pp. 163–172	Receipt of abstracts: December 1, 1983. Notice of acceptance or rejection of papers: February 1, 1984
Guidelines for Poster Presentations	*Food Technology,* September 1983, p. 88	Same as Call for Volunteered Papers
Rules Governing IFT's Graduate Paper Competitions	*Food Technology,* September 1983, pp. 86–87	Same as Call for Volunteered Papers
Call for Undergraduate Research Paper Competition Papers	*Food Technology,* September 1983, p. 87	Notify URPC chairman: February 15, 1984. Receipt of abstracts: March 15, 1984
Call for Films for Film Theater	*Food Technology,* September 1983, p. 86	Receipt of films: December 30, 1983
Call for Nominations for Achievement Awards and Fellows	*Food Technology,* September 1983, pp. 82–83.	Receipt of nominations: Industrial Achievement Award, December 1, 1983 International Award, December 1, 1983 Appert Award, December 15, 1983 Cruess Award, December 15, 1983 Babcock-Hart Award, January 1, 1984 Prescott Award, January 1, 1984 Fellows, February 1, 1984
Call for Nominations for New Phi Tau Sigma Award for Service to the Profession of Food Technology	*Food Technology,* September 1983, p. 84, and October 1983, p. 40.	Receipt of nominations: January 1, 1984
Call for Nominations for New General Foods Chair in Food Science	*Food Technology,* September 1983, p. 85, and October 1983, p. 42.	Receipt of nominations: December 30, 1983
Preliminary Program and Registration Forms for 1984 IFT Annual Meeting	Mailed to all IFT members on March 1, 1984. Program also published in *Food Technology,* May 1984	Receipt of advance registration forms: May 15, 1984
IFT–IUFoST Basic Symposium, "Chemical Changes During Food Processing," to be held in Anaheim, Calif., on June 9–10, 1984	Program, abstracts, and registration forms: *Food Technology,* March 1984	Receipt of advance registration forms: May 15, 1984
IFT Short Course, "Product Development & Marketing—Bridging the Gap," to be held in Anaheim, Calif., on June 13–15, 1984	Program and registration forms: *Food Technology,* March 1984	Receipt of advance registration forms: May 15, 1984
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Official Listing of Exhibitors and Booth Numbers	*Food Technology,* May 1984	—
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IFT 84 Food Expo Directory of Exhibits	Distributed to all registrants at Annual Meeting	—
Resume Forms and Position Description Forms for Employment Bureau	*Food Technology,* March 1984	Receipt of forms: May 1, 1984

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ERRATUM NOTICE

• *J. Food Sci.* 48(3): 703-707 (1983). Effect of water activity and moisture content on the stability of beet powder pigments – E. Cohen and I. Saguy. Fig. 2, page 704, is a duplicate of Fig. 6. The correct Fig. 2 is reprinted herewith, Please correct accordingly.

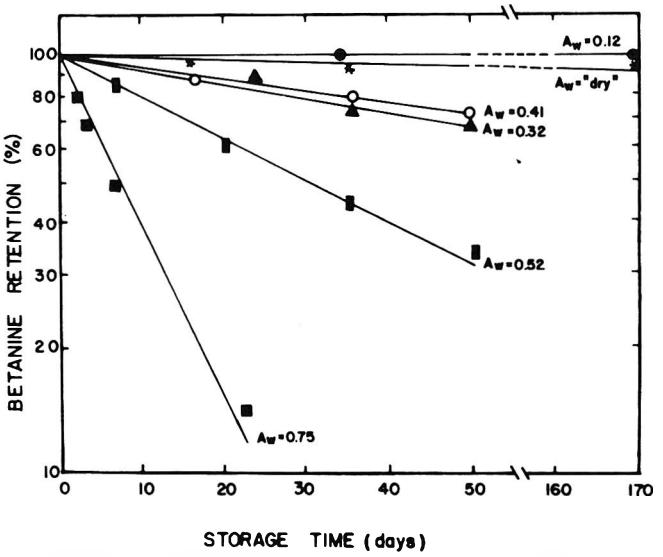

Fig. 2—Effect of a_w on betanine retention during storage at 35°C.

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Effect of pH and of Succinylation on the Water Retention Properties of Coagulated, Frozen and Thawed Egg Albumen

PATRICIA WONG GOSSETT and ROBERT C. BAKER

ABSTRACT

Egg albumen was modified to improve water retention properties of the coagulated, frozen and thawed product. Albumen was found to contribute more to expressible moisture (%EM) of coagulated whole egg mixture than yolk. For frozen and nonfrozen treatments, albumen (pH > 9.5) reduced %EM in both the coagulated albumen and whole egg mixtures made with pH-adjusted albumen. Succinylated albumen (≥0.2% g anhydride/g albumen) reduced %EM for frozen and nonfrozen treatments. Albumen at pH 10.5 and 11.0 required less time to attain an equivalent cook value than at pH 7.0, 8.0, 9.0, and 10.0.

INTRODUCTION

IT IS OF COMMERCIAL INTEREST to improve the functionality of egg proteins with respect to their water retention properties under various processes of heating and freezing. The success of egg products such as omelets, egg burgers, or quiches that have been pre-cooked, frozen, and thawed prior to serving, has been limited by their poor water retention properties and undesirable textural qualities, the most notable being toughness.

O'Brien et al. (1982) found that adding xantham gum (0.1% w/w), applying moist heat during cooking, or freezing with liquid nitrogen or carbon dioxide minimized moisture loss and shear force values of pre-cooked, frozen whole-egg omelets. In addition, sodium tripolyphosphate (0.5% w/w) minimized the expressible moisture of the omelets. Feiser and Cotterill (1982) found that the volume of the serum pressed from cooked, frozen, thawed, reheated scrambled eggs decreased as the pH of the liquid whole egg increased from 6.0 to 7.0. The albumen appears to be basic to the problem. Davis et al. (1952) and Cotterill (1977) claimed that freezing of cooked egg albumen caused the gel to become rubbery and water to separate from the thawed matrix. Freezing of cooked yolk, however, did not cause major defects (Cotterill, 1977).

In this study, protein modification was employed to alter the functionality of the proteins. By raising the pH of the protein environment, the added negative charges might cause increased repulsion among the peptide chains, enabling the protein matrix to entrap more water (Hamm, 1975). By succinylating the proteins, the water retention properties of the gel might be improved: succinylation of the proteins would result in amino groups binding to succinate anions with two carboxyl groups causing a net gain in negative charge (Means and Feeney, 1971). The increased repulsion between the carboxyl groups may decrease protein-protein interactons but increase protein-water interactions (Kinsella and Shetty, 1979).

The goals of this study were to determine whether pH or succinylation of the raw albumen had any effect on the expressible moisture (%EM) of the coagulated albumen before and after freezing. In addition, preliminary studies were done to determine (1) which component (albumen, yolk, or both) was responsible for the observed increase in moisture; and (2) how the length of storage affected the %EM.

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MATERIALS & METHODS

Eggs

All eggs used in this study were of the Babcock strain of White Leghorn hens from the Cornell Univ. Poultry Farm. Eggs varied in age from 1–14 days and were stored in a cooler with an average temperature of 3°C as shell eggs.

Sample preparation

Liquid eggs were prepared by breaking shell eggs, hand-separating into albumen and yolk, and blending the albumen in one liter quantities in a Waring Blendor at 14400 rpm for 3–5 sec. If less than one liter quantities were blended, an Erlenmeyer flask was inserted into the blender jar to cover the top of the sample to minimize incorporation of air during blending. The pH of raw albumen was adjusted with 1N HCl or 1N NaOH and readjusted to the appropriate pH after 6 hr of equilibration at 7°C. A Fisher Accumet (Model 230/A) pH/ion meter equipped with a standard combination electrode was used. The raw succinylated samples were prepared by slow addition of 0.1 – 0.7g succinic anhydride (Sigma Chemical Co., St. Louis, MO) in 0.1g increments per 100g albumen with the pH kept at 8.5 with 1N NaOH. The succinylated samples were stirred continuously for 30 min after equilibration of pH to 8.5. All treated albumen samples were diluted with distilled, deionized water to give equal protein concentration of 9.1% (w/w) in each treatment. The samples were stored in one liter containers with snap-on lids at 3°C until use.

Determination of extent of succinylation

The ninhydrin assay (Franzen and Kinsella, 1976; Moore, 1968) was used to estimate the extent of succinylation. Ninhydrin solution (Sigma Chemical Co., St. Louis, MO) (0.25 ml, 2% ninhydrin and 0.3% hydrindantin in 75% dimethyl sulfoxide and 25% 4M lithium acetate buffer, pH 5.2) was added to a 1% protein solution (0.25 ml, prepared from lyophilized succinylated samples.) The mixture was heated at 100°C for 5 min and cooled. Distilled water (10 ml) was added and the absorbance at 580 nm was measured against a water/ninhydrin solution blank. The difference in absorbances between the unmodified and modified proteins indicated the change in free amino groups available for binding with ninhydrin.

Coagulation

Coagulation of the samples was done by either one of two methods.

Oven method. 50g samples at room temperature were placed in 150 ml beakers and coagulated in a conventional oven (General Electric) at 177°C for 20–22 min. The positions of the beakers in the oven were randomized.

Water bath method. 75g samples were coagulated in a jacketed cylinder (3.9 cm, inner diameter) made of high grade aluminum alloy at Cornell Univ. The jacketed cylinder was connected to a circulating water bath with a flow rate of two liters/min. Internal temperatures were measured with a thermocouple thermometer (Cole-Parmer, Digi-Sense, Model 8520–50, time constant = 5 sec.)

Freezing and thawing

After oven-coagulation, each sample was removed from its container, vertically cut into halves, and one half was analyzed imme-

diately for %EM while the other half was placed in a 4 x 8 x 2 in. polyethylene bag and frozen in a −25°C freezer for 24 hr. The frozen samples were placed in a 7°C cooler for approximately 12 hr and then removed to room temperature about 4 hr prior to analysis for %EM.

Expressible moisture

The technique of Jaurequi et al. (1981) was modified to measure the expressible moisture of precooked samples. A 1-g coagulated sample was placed on a pre-weighed cup of three pieces of Whatman #3 (5.5 cm) and one piece of Whatman #50 (7.0 cm) filter paper with the #50 on top. The assembly was centrifuged in a 50 ml polycarbonate tube at 2°C in a centrifuge (Sorvall RC-2B) at 8000 rpm (7710 x G) for 15 min with the SS-34 rotor. The wet filter paper was weighed on a Mettler H20T balance after removal of the sample. The total moisture of each sample was determined by drying a 1.5-g sample in a Boekel 2000 watt drying oven (105°C) for at least 18 hr. The percent expressible moisture per unit weight was calculated as follows:

$$\%\ EM = \frac{\text{Wt of moisture lost/g sample} \times 100}{\text{Wt of total moisture/g Sample}} = \frac{(w_w) \times 100}{(w_s)\ (W_w/W_S)}$$

Where: w_w = wt of moisture on filter paper; w_s = wt of sample for centrifugation; W_w = wt of total moisture = $W_S - W_{S(dried)}$; W_S = wt of Sample before drying for total moisture; $W_{S(dried)}$ = wt of dried sample.

For some frozen treatments, drip was present in the plastic bag in which the sample had been frozen. This drip was filtered through Whatman #54 filter paper and measured as ml drip/g albumen. This figure was incorporated into the %EM calculation:

$$\%EM = \frac{[w_s\ (\text{ml drip/g}) + w_w] \times 100}{[w_s\ (\text{ml drip/g}) + w_s]\ \dfrac{[W_w + W_S\ (\text{ml drip/g})]}{[W_S + W_S\ (\text{ml drip/g})]}}$$

For all expressible moisture experiments, three samples per treatment were made and the entire study was repeated. Thus each point in Fig. 1, 2, and 3 is an average of six observations.

Cook value

The time required for the pH-adjusted or succinylated samples to receive an equivalent heat treatment was determined. Samples were coagulated using a water bath at 80°C, while the temperature of the center of the albumen sample was recorded every 0.5 min. Cook values were determined by the following equation:

$$\text{Cook value} = C = \int_0^t 10^{\left(\frac{T(t)-100}{Z_c}\right)} dt$$

where: t = time (min); T = Temperature (°C). Z_c was defined as the heating temperature rise in °C needed for a 10-fold increase in reaction rate for the coagulation process, and was found to be 41.7°C for albumen (Gossett, 1983). A cook value of 1 min is defined as any heat treatment equivalent to 1 min of heating at 100°C. To determine the time required for each treatment to attain a cook value of 5 min, cook values were calculated from internal albumen temperature versus time data, and plots of cook values versus time were integrated by weighing the areas under the curve up to 5 min. Determinations were done in duplicate.

Effect of albumen versus yolk on %EM

Various mixtures of whole egg containing 40, 50, 60, 70, and 80% albumen by weight were prepared, oven-coagulated, frozen, thawed, and measured for %EM.

Effect of time frozen versus %EM of albumen

Control albumen (pH 9.0) samples were oven-coagulated and frozen for 0, 1, 3, 5, 8, and 12 days. The %EM was measured at 0 days and at 1, 3, 5, 8, and 12 days after appropriate freezing and thawing.

Effect of pH on %EM

Raw albumen samples (pH 4.0 – 11.0 at 0.5 intervals) were prepared, oven-coagulated, frozen, thawed, and analyzed for %EM as described.

pH-Adjusted albumen added to yolk

Raw albumen samples adjusted to pH 9.0, 9.5, 10.0 and 10.5 were added to yolk in a ratio of 64% treated albumen to 36% yolk and magnetically stirred until mixed. The whole egg mixtures were oven-coagulated, frozen, thawed, and analyzed for %EM as described.

Effect of succinylation on %EM

Raw albumen samples succinylated at levels of 0 – 0.7% (g anhydride/g albumen) at 0.1% intervals were prepared, oven-coagulated, frozen, thawed, and analyzed for %EM.

Statistical analysis

A two-factor analysis of variance for a split-plot design was computed for all studies except the study on the time frozen versus %EM for which an analysis of variance for a completely random design was computed. If a significant F value was noted after computation of an analysis of variance, a protected least significant difference (LSD) test at the 5% level of significance was performed to detect any significant differences (Snedecor and Cochran, 1976).

RESULTS & DISCUSSION

Effect of albumen versus yolk on %EM

The %EM of coagulated whole egg mixtures of varying albumen content are shown in Fig. 1. For both frozen and nonfrozen treatments, the %EM increased significantly as the percentage of albumen in the mixture increased indicating that the albumen was the component mostly responsible for %EM. This observation might be expected because albumen is 89% water and yolk is only 47.5% water (Osuga and Feeney, 1977) and a whole egg mixture of 40, 50, 60, 70 or 80% albumen corresponded to 64, 68, 72, 76 or 80% water, respectively. On the basis of this experiment, it was decided to focus on the albumen rather than the whole egg.

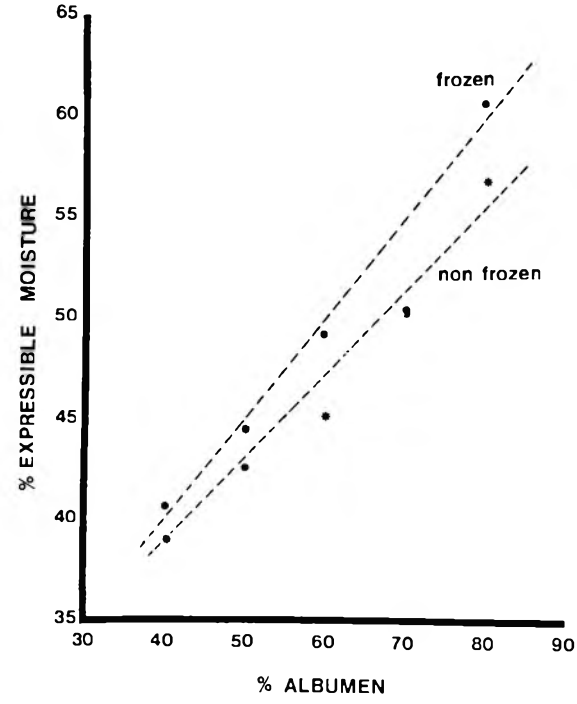

Fig. 1—%EM of coagulated whole egg mixtures versus percentage of egg albumen in the whole egg mixture.

Effect of time frozen versus %EM of albumen

The %EM for coagulated, frozen and thawed albumen samples that had been frozen for 1, 3, 5, 8, or 12 days were significantly higher than the %EM for the non-frozen samples (Table 1). The results indicated that a frozen treatment of at least one day should be adequate to show differences between frozen and non-frozen treatments and that additional storage did not increase %EM.

Effect of pH on %EM

The effect of pH on the raw albumen (pH 4.0 – 11.0) on the %EM of the coagulated, frozen and thawed albumen was determined (Fig. 2). In general, as the pH of the raw albumen increased above pH 9.5, the %EM decreased for both frozen and nonfrozen treatments. For the nonfrozen treatment, pH levels of 10.5 and 11.0 exhibited significantly ($P < 0.05$) lower %EM than the pH 9.0 control, and for the frozen treatment, pH 10.5 and 11.0 differed significantly ($P < 0.05$) from the frozen control at pH 9.0.

The times required to reach a cook value of 5 min for pH 7.0, 8.0, 9.0, and 10.0 albumen were significantly higher than that required of the pH 10.5 and 11.0 samples (Table 2). %EM measurements were then repeated for pH 9.0, 10.0, 10.5 and 11.0 albumen, but this time samples were individually coagulated using an 80°C water bath and cooled with 5°C water until an average cook value of about 5.75 min was attained for each sample. This was done in order to account for differences in heat transfer among the samples which was not done previously. Coagulated albumen at pH 10.0, 10.5, and 11.0 were significantly ($P < 0.05$) lower in %EM than the control albumen at pH 9.0 for both frozen and nonfrozen treatments (Table 3).

Albumen at higher pH values retained water better after coagulation and after freezing which seems to agree with the hypothesis that increased negative charge on the proteins may have caused repulsion among the side chain groups along the protein, and the expanded proteins may have then allowed increased hydration or increased entrapped water.

Fig. 2 also shows that the control albumen (pH 9.0) exhibited approximately a 5% difference in %EM between the nonfrozen and frozen treatments. Apparently this 5% difference is detectable by the consumer in precooked, frozen and thawed egg products.

Table 1–%EM of coagulated albumen (pH 9.0) versus time frozen[a]

Days frozen	%EM
0	67.6 ± 2.3[b]
1	74.7 ± 2.6[c]
3	75.1 ± 3.2[c]
5	74.3 ± 1.5[c]
8	73.4 ± 1.4[c]
12	76.9 ± 2.1[c]

[a] 3 samples/treatment, 2 replicates; For Day 0: 15 samples/treatments, 2 replicates
[bc] Means with different superscripts are significantly different at $P \leq 0.05$.

Table 2–Time (min) to reach an equal cook value for pH-adjusted or succinylated albumen with 80°C water bath[a]

Treatment	Time (min) to reach a cook value of 5 min:
pH 7.0	25.5 ± 0.1[b]
8.0	24.8 ± 0.8[bc]
9.0	25.3 ± 0.7[bc]
10.0	23.4 ± 0.2[cd]
10.5	22.2 ± 1.8[de]
11.0	20.2 ± 0.3[e]
Succ. 0.0% w/w	24.8 ± 0.5[b]
0.1	26.2 ± 0.8[b]
0.2	25.4 ± 0.0[b]
0.4	24.3 ± 0.4[b]

[a] 2 replicates/treatment; $Z_c = 41.7$°C; temperatures measured at the center of the albumen.
[bcde] Means in the same column with different superscripts are significantly different at $P \leq 0.05$.

pH-adjusted albumen added to yolk

Raw albumen samples at pH 9.0, 9.5, 10.0, and 10.5 were tested in a whole egg system as described. The %EM for the pH 10.0 and 10.5 treatments were significantly lower from the pH 9.0 treatment for both nonfrozen and frozen treatments (Table 4). Also significant ($P < 0.05$) were the differences between nonfrozen and frozen treatments at each pH level tested. Thus pH-adjusted albumen at higher pH levels could function to decrease %EM in a whole egg system, which agrees with the findings of Feiser and Cotterill (1982) and O'Brien (1981).

Effect of succinylation of %EM

Succinylation modifies proteins to possibly retain more water. The %EM of albumen succinylated at different levels were measured for nonfrozen and frozen treatments. The %EM decreased with increasing levels of succinylation up to 0.4% (g anhydride/g albumen), with treatments at 0.2% or greater being significantly lower in %EM than the control (0%) for both non-frozen and frozen treatments (Fig. 3). The extent of succinylation for the 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7% (w/w) levels was estimated as 1, 11, 21, 32, 41, 56, and 70%, respectively. Ball et al. (1979) estimated that succinylated albumen at the 0.1, 0.2, 0.3, 0.4, and 0.5% (w/w) levels blocked free amino groups by 10, 24, 40, 48, and 60%, respectively.

No significant differences among the succinylated samples were found in the time required to reach a cook value of 5 min (Table 2). Thus it was not necessary to repeat %EM measurements to account for differences in heat transfer.

It appeared that as proteins were succinylated up to a certain level, such as to 0.4% (g anhydride/g albumen),

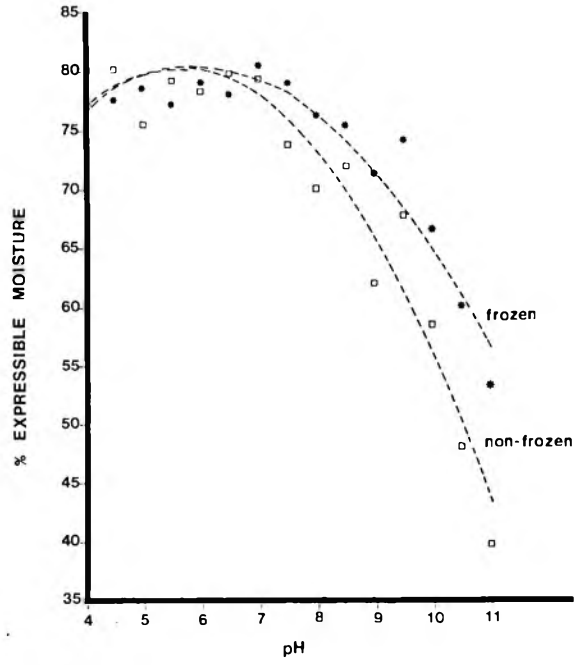

Fig. 2–%EM of coagulated albumen versus pH of raw albumen for frozen and nonfrozen treatments.

Table 3—%EM of coagulated albumen versus pH of raw albumen for frozen and nonfrozen treatments[a]

Treatment	Non-Frozen	Frozen
pH 9.0	75.0 ± 5.0[b]	78.9 ± 0.1[b]
10.0	56.4 ± 2.2[c]	67.5 ± 0.5[c]
10.5	46.3 ± 3.9[d]	63.2 ± 0.5[d]
11.0	41.1 ± 2.6[d]	53.8 ± 0.7[e]

[a] Cook value = 5.75 min, Z_c=41.7°C, 3 samples/treatment, 2 replicates.
[bcde] Means in the same column with different superscripts are significantly different at P≤0.05.

Table 4—%EM of coagulated whole egg mixtures versus pH of albumen in mixture[a]

pH			
Albumen	Whole egg mixture	Nonfrozen	Frozen
9.0	7.36	49.4 ± 0.5[d]	57.1 ± 1.3[b]
9.5	7.77	47.6 ± 0.2[de]	55.7 ± 1.6[bc]
10.0	8.34	40.6 ± 0.4[ef]	49.8 ± 2.0[c]
10.5	9.05	37.8 ± 2.8[f]	49.5 ± 2.8[c]

[a] 3 samples/treatment, 2 replicates.
[bcdef] Means with different superscripts are significantly different at P≤0.05.

the succinyl groups and their negative charges contributed to the general expansion of the protein which then might have allowed greater retention of water. Succinylation of other proteins showed similar trends: succinylated casein at approximately 40% substitution increased water adsorption capacity (Schwenke et al., 1981); heat-coagulated whey protein that has been succinylated exhibited high water absorption properties (Thompson and Reyes, 1980).

CONCLUSIONS

ALBUMEN was found to be the component contributing most to the %EM of precooked, frozen and thawed whole egg mixtures. A significant increase in %EM was observed in albumen samples frozen 1, 3, 5, 8 or 12 days when compared to the nonfrozen control. Raw albumen adjusted to pH 9.5 or greater exhibited significantly ($P < 0.05$) decreased %EM in the coagulated albumen and whole egg gels for frozen and nonfrozen treatments. Raw albumen adjusted to pH 10.5 and 11.0 required less time to attain the same cook value than the pH 9.0 control.

Raw albumen succinylated up to 0.4% (g anhydride/g albumen) exhibited decreased %EM with increasing levels of succinylation. Further levels of succinylation exhibited no further decrease in %EM of the coagulated gel. Raw albumen succinylated up to 0.4% (w/w) did not differ significantly in the times required to attain the same cook value.

It is hoped that the results from this study will contribute to the understanding of modified proteins and their behavior in food systems with respect to water retention properties. The safety, sensory, and nutritional aspects of these modified proteins should be determined before using them in food systems.

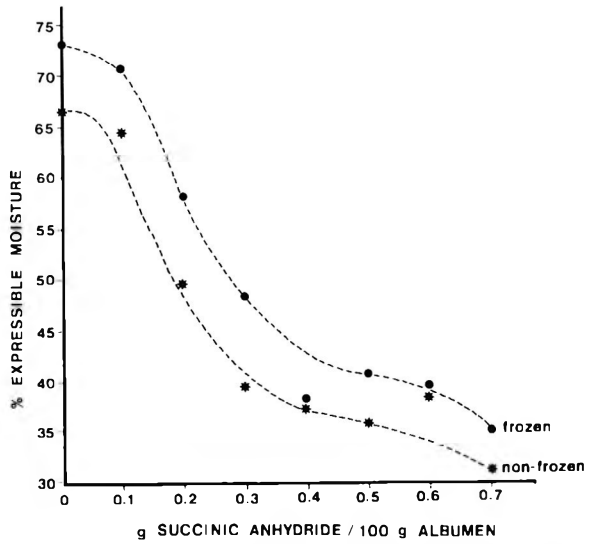

Fig. 3—%EM of coagulated albumen versus level of succinylation for frozen and nonfrozen treatments.

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Selected Rheological Properties of pH-Adjusted or Succinylated Egg Albumen

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ABSTRACT

Apparent viscosities of pH-adjusted or succinylated raw egg albumen were measured at 10, 20, and 30°C using a Haake rotary viscometer. Viscosities decreased with increasing temperature. Rheological power law model parameters indicated that as pH approached 5–6 and 11, albumen became more Newtonian. Control albumen (pH 9.0) and succinylated albumen were pseudoplastic. Intrinsic viscosities of pH 9.0, 10.0, and 11.0 albumens were 0.083, 0.092, and 0.102 dl/g, respectively, and that of succinylated albumens (0, 0.1, 0.2, and 0.4% w/w) were 0.052, 0.147, 0.149, and 0.184 dl/g, respectively. Apparent viscosities of coagulated albumen, measured by capillary extrusion, increased with pH but did not change with succinylation. Yield forces and rigidity moduli of these gels increased with pH but not with succinylation level.

INTRODUCTION

THE SUCCESSFUL DEVELOPMENT of new egg products such as precooked, frozen, and thawed omelets, quiches, or egg burgers have been limited by problems resulting from moisture loss during various heating and freezing processes. Gossett and Baker (1983) found that by adjusting the pH to alkaline values (pH 9.0–11.0) or by succinylating raw egg albumen, the expressible moisture of these egg proteins after coagulation and freezing is reduced. Before using these modified proteins in food products, it is necessary to characterize the rheological behavior of the raw and coagulated albumen for further insight into end-product characteristics.

Although the viscosities of egg albumen and pH-adjusted albumen have been studied (Tung et al., 1981; Pitsilis et al., 1975) the viscosities of high pH albumen or of succinylated albumen have not. The viscosity of the fluid albumen provides information about the flow properties of the bulk fluid but for understanding about the macromolecular changes of albumen occurring at high pH or with succinylation, the intrinsic viscosity should be measured. Also, knowledge of the behavior of coagulated gels is essential for understanding end-product textural properties. Dunkerley and Hayes (1980) and Egelansdal (1980) have both measured the gel strength of egg proteins as a function of pH but not at the very high pH levels. Hickson et al., (1982) have studied rheological properties of heat-coagulated egg albumen. To date, the rheological behavior of succinylated albumen has not been published although Ball and Winn (1979) have studied acylated gels.

The goals of this study were to (1) measure the apparent viscosity of pH-adjusted or succinylated albumen and quantify the rheological behavior by fitting the data to the power law model; (2) measure the intrinsic viscosity of pH-adjusted or succinylated albumen; and (3) measure the apparent viscosity, yield force, and modulus of rigidity of the coagulated pH-adjusted or succinylated albumen gels.

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MATERIALS & METHODS

Eggs and sample preparation

The source of eggs, sample preparation, and estimation of extent of succinylation were as described in Gossett and Baker (1983).

Raw albumen

Apparent viscosity. Raw albumen was adjusted from pH 4.0 through 11.0 at unit intervals or succinylated at levels of 0, 0.1, 0.2, 0.3, and 0.4% (g anhydride/g albumen) (Gossett and Baker, 1983). A Haake Rotating Viscometer equipped with a NV narrow-gapped concentric cylinder spindle and cup was used to measure shear stress. Sample size was 12 ml. Temperature was controlled with a circulating water bath, and maintained at ±0.5°C. Shear stresses were measured within 1 min after equilibration of the scale reading at shear rates from 38.8–3140 sec^{-1} at 10, 20, and 30°C for each pH level or succinylated treatment. Two readings per treatment were made, and the entire experiment was repeated twice.

Calculation of viscosity parameters were as follows:

$$\eta = K \times U \times S$$

where η = apparent viscosity, cp; K = calibration constant, 0.059 (See next paragraph); U = speed factor; rpm = 583.2/U; S = scale reading (normal scale).

$$\tau = A \times S$$

where τ = shear stress, dynes/cm^2; A = shear stress factor = (K x B)/100.

$$\gamma = B/U$$

where B = rate of shear factor = 3140; γ = shear rate, sec^{-1}.

The NV rotor assembly was calibrated with a glycerol solution (65% w/w) having a viscosity of 25.3 cp at 10°C (Hodgman, 1959). After measuring the shear stresses of the solution at various shear rates at 10°C, the calibration constant K was calculated as follows: K = 25.3/(U x S).

Intrinsic viscosity. Albumen was prepared by adjusting the pH to 9.0 or by succinylating at levels of 0, 0.1, 0.2, and 0.4% (g anhydride/g albumen). Frozen samples (–25°C, 24 hr) of these pH-adjusted or succinylated albumen were freeze-dried for 48 hr in a Stokes freeze dryer (plate temperature, 10°C).

Solutions for the pH-adjusted samples were made by dissolving freeze-dried pH 9.0 albumen in pH 9.0, 10.0, or 11.0. 0.025 $NaHCO_3$ buffer + 150 mM KCl to give concentrations of 0.5, 1.0, 2.0, and 4.0 g albumen/dl. Solutions of succinylated samples were made by diluting in 0.05M tris(hydroxymethyl)ammomethane buffer (pH 8.5) to give concentrations of 0.5, 1.0, 2.0, and 4.0g albumen/dl. Dilutions were prepared in tared 25 ml volumetric flasks, which were also weighed containing the diluted samples. The weights of the diluted samples were used to calculate the density of the solutions.

The intrinsic visosity measurements were made using a Cannon-Fenske Routine Viscometer (K311), size 50, with calibration constant C = 0.00396 at 20°C. The capillary viscometer was kept at 20.0 ± 0.5°C by a circulating water bath. Since the temperature of the sample as well as the bath was critical in this measurement, the temperature of the 10 ml sample inside the viscometer was also measured prior to flow by inserting an unmounted, bare thermocouple (Cole-Parmer 8520-90, time constant = 3 sec) and reading the temperature of the sample. The time of flow was recorded by a Lemania stopwatch (0.1 sec).

For each concentration at each pH or level of succinylation, three flow times were measured. The entire experiment was repeated twice.

Coagulated albumen

Apparent viscosity. An Instron Universal Testing Machine (Model TM) equipped with a capillary extrusion apparatus (Fig. 1) was used to measure the apparent viscosity, the yield force, and the modulus of rigidity of the coagulated albumen. The capillary extrusion apparatus was made of high grade aluminum alloy at Cornell University.

Samples (75g) were coagulated in one of the jacketed cylinders of the capillary extrusion apparatus. The cylinder was connected to a circulating water bath (80°C, two liters/min), and the internal albumen temperature was measured every 30 sec with a thermocouple (Cole-Parmer, Digi-Sense, Model 8520-50, time constant = 5 sec). Samples were coagulated with 80°C water and then cooled with 5°C water to an average cook value of 5.75 min. One cook value is the time equivalent to heating at 100°C for 1 min (Gossett, 1983). Albumen at pH 7.0, 8.0, 9.0, 10.0, 10.5, and 11.0 and succinylated albumen at the 0, 0.1, 0.2, and 0.4% (g anhydride/g albumen) levels were coagulated in this manner. The coagulated samples were removed from the cylinder with a spatula and cut into cylinders approximately 2.5 cm in height, which were put into the capillary cell and into the orifice cell. The average temperature of the samples prior to extrusion was maintained at 18 ± 1°C.

Samples were extruded as follows: The piston, suspended from the CCT load cell of the Instron Universal Testing Machine, was manually lowered into the capillary cell until the end of the piston was approximately 1.5 cm above the sample. Then the piston was lowered at a crosshead speed of 0.508 cm/min (0.2 in/min). Extrusion was completed when a constant force value was obtained. The average temperature of the extruded samples was 20°C'

The same procedure was performed for the extrusion of the sample through the orifice cell. Subtraction of the force required to extrude the albumen through the orifice from that required to extrude the albumen through the capillary cell allowed for correction of the pressure drop due to end effects. A typical force-distance curve is shown in Fig. 2, where F_y is the yield force required prior to extrusion; F_c and F_o are the forces required to extrude the gel through the capillary and orifice cells, respectively; and L_y is the distance the gel is deformed at the yield point.

Three samples were extruded at each pH level or succinylation level for both capillary and orifice attachments.

The apparent viscosity was calculated in poises according to Mayfield et al. (1978):

$$\eta_{app} = \frac{\tau}{\gamma} = \frac{\frac{[(F_c - F_o)/A]\,R}{2L}}{4Q/\pi R^3}$$

where F_c = force to extrude through capillary; F_o = force to extrude through orifice; A = 11.95 cm^2; R = 0.15 cm; L = 3.3 cm; Q = 0.101 cm^3/sec.

Yield force. The yield force was obtained from the force-distance curves of the capillary extrusion. The yield force, F_y, shown in Fig. 2 just as extrusion began, was taken to be the force in dynes required to initiate flow.

Apparent modulus of rigidity. The apparent rigidity modulus was obtained from the force-distance curves from the capillary extrusion studies. The distance, L_v, that the gel compressed in the capillary cell before extrusion occurred was noted (Fig. 2). Calculation of the modulus was as follows:

$$G = \frac{\sigma}{\epsilon} = \frac{\text{stress}}{\text{strain}} = \frac{dF/A}{dL/L} \text{ (Van Wazer et al., 1963)}$$

where $\sigma = F_y/A = F_y/\pi R^2$; $\epsilon = L_y/L$.

$$G = \frac{F_y/\pi R^2}{Ly/L}$$

where R = 1.95 cm = radius of gel; L = 2.5 cm = height of gel; F_y = yield force; L_y = length deformed before extrusion.

Statistical analysis

For each treatment, a one-way analysis of variance was computed for a completely random design. When a significant F ratio was obtained as a result of the analysis of variance, a protected least significant difference (LSD) test was performed at the 5% level of significance (Snedecor and Cochran, 1976).

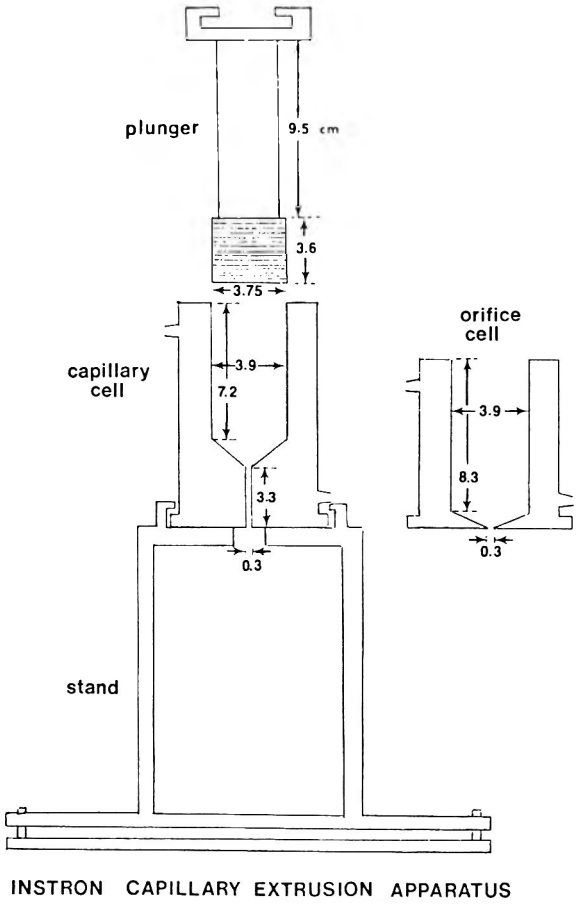

Fig. 1—Capillary extrusion apparatus for measuring apparent viscosity, yield force, and modulus of rigidity of coagulated gels.

RESULTS & DISCUSSION

Raw albumen

Apparent viscosity. The shear stresses for pH-adjusted and succinylated albumen were calculated for various shear rates at 10, 20, and 30°C, and the corresponding apparent viscosity was calculated for each sample.

The albumen appeared to be pseudoplastic, except for the pH 5.0–6.0 and 11.0 treatments which appeared to be Newtonian (Fig. 3). In general, the apparent viscosities of pH 4.0–8.0, 10.0 and 11.0 samples at comparable shear rates appeared to be less than that of pH 9.0 albumen. Succinylated albumen at 20°C appeared to be pseudoplastic and, at comparable shear rates, the apparent viscosity decreased as the level of succinylation increased (Fig. 4). The apparent viscosities of all treatments tended to decrease with increasing temperatures.

To quantify the non-Newtonian behavior of the pH-adjusted and succinylated albumen, the data were fitted to a power law model. For each treatment-temperature combination, the shear stress was plotted against shear rate to determine if a yield stress existed. For both pH and succinylation treatments, no apparent yield stresses were apparent; that is, the plots of shear stress versus shear rate gave straight lines through or near the origin. Linear regression of plots of ln (shear stress) versus ln (shear rate) yielded the values k from the intercept and n from the slope (average r^2 = 0.99). The n and k values are shown in Table 1 for 10, 20, and 30°C.

The n value for the control albumen (pH 9.0) was less than 1.0 (e.g. 0.83 at 20°C), implying pseudoplasticity. At or about pH 6.0 and 11.0, the n value approached 1.0 at all three temperatures and Newtonian behavior was indicated. The k values decreased at about pH 6.0 and 11.0, implying less viscosity at these pH levels.

Pitsalis et al. (1975) found that at pH 5.0–6.0, the viscosity of egg albumen decreased to about 3 cp at 36.7 sec^{-1} shear rate at 18°C, increased to over 6 cp at pH 9.0, and then decreased to less than 5 cp at pH 10.0. Our results agreed with those observations. Perhaps at pH 5.0–6.0, lower viscosities were observed because the isoelectric points of ovalbumin, ovotransferrin, ovomucin, and ovoinhibitor were approached and some of the protein precipitated out of solution, leaving a less viscous medium that approached Newtonian-like behavior. At pH 11.0 the isoelectric point of lysozyme was approached and perhaps the lysozyme-ovomucin complex was dissociated at these pH levels, leaving the albumen less viscous and more Newtonian-like.

The lysozyme-ovomucin complex contributes to the viscous nature of albumen and at higher pH levels, the complex is dissociated, causing thinning of the albumen (Osuga and Feeney, 1977). At pH 11.0, the proteins might be partially denatured, resulting in a loss of solubility and a decrease in viscosity.

The n value increased slightly with the level of succinylation while the k value decreased slightly, implying that albumen became less viscous and more Newtonian-like with succinylation (Table 1). However, the standard deviations of k and n for the succinylated samples were large enough to prevent a claim of any differences among the succinylated samples.

From the preceding viscosity measurements, changes in flow characteristics of the bulk fluid are obtained. However to gain insight on the macromolecular level, the intrinsic viscosity should be measured.

Intrinsic viscosity. The kinematic viscosities of pH 9.0, 10.0, and 11.0 albumen were measured because at these pH values: (1) there were differences in the amount of expressed moisture from the coagulated albumen; (2) the proteins might expand or unfold under these conditions;

Table 1—Power law model constants k and n for various levels of pH and succinylation at 10, 20, and 30°C[a]

Treatment		10°C		20°C		30°C	
		k	n	k	n	k	n
pH	4.0	0.110	0.94	0.072	0.85	0.062	1.00
	5.0	0.057	0.88	0.020	1.11	0.053	0.96
	6.0	0.052	1.05	0.016	1.04	0.082	1.06
	7.0	0.024	1.05	0.060	0.97	0.095	0.91
	8.0	0.130	0.88	0.161	0.84	0.067	0.91
	9.0	0.115	0.87	0.152	0.83	0.082	0.90
	10.0	0.164	0.82	0.177	0.88	0.064	0.95
	11.0	0.030	1.08	0.043	1.04	0.010	1.13
Succ.	0% w/w	0.633	0.72	0.281	0.79	0.103	0.87
	0.1	0.166	0.89	0.171	0.84	0.076	0.93
	0.2	0.204	0.87	0.327	0.77	0.042	1.04
	0.3	0.151	0.89	0.114	0.90	0.046	0.98
	0.4	0.156	0.89	0.089	0.93	0.067	0.94

[a] Triplicate determinations; average standard deviation for k_{pH} = 0.047; for n_{pH} = 0.13; for $k_{succ.}$ = 0.141; for $n_{succ.}$ = 0.12.

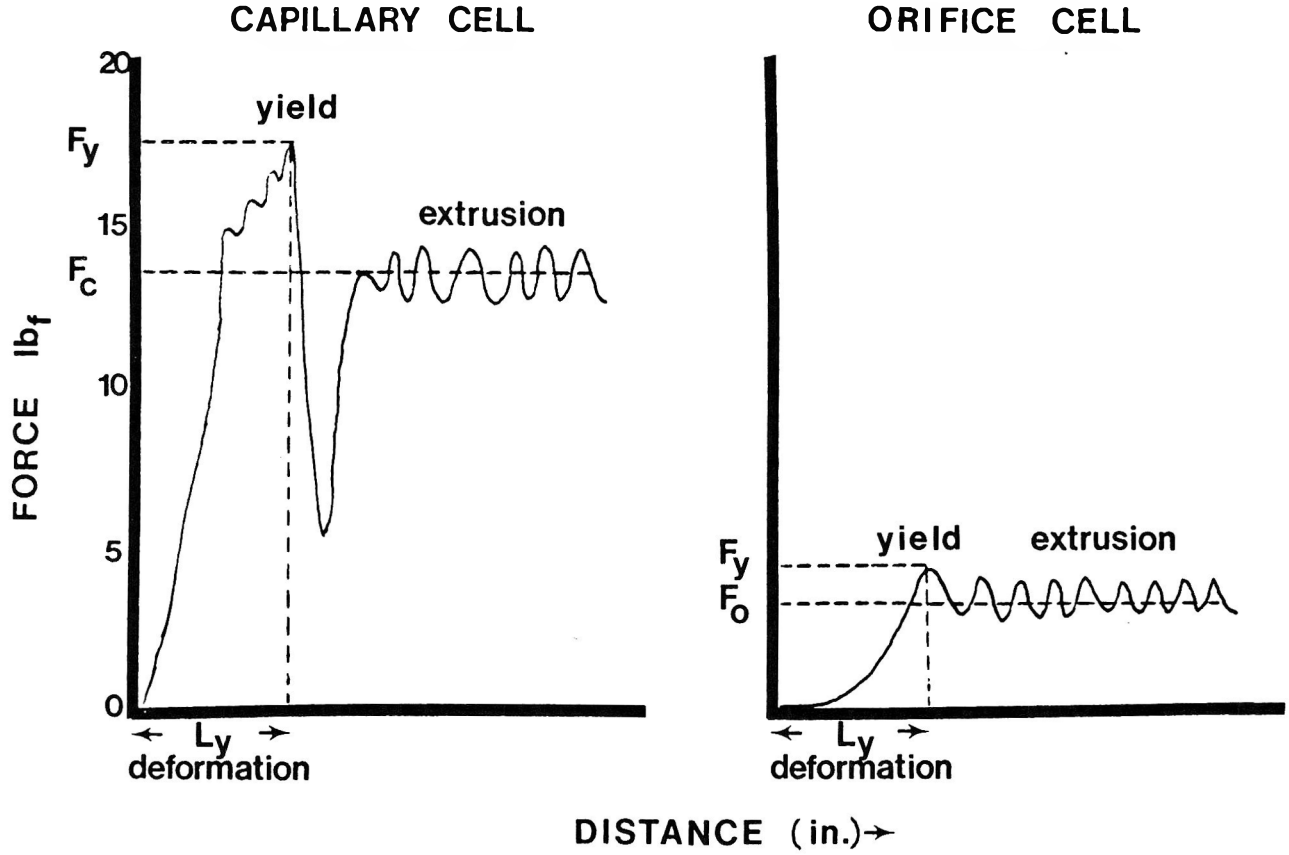

Fig. 2—Typical curves from capillary extrusion experiments: F_y = yield force required prior to extrusion; F_c = force required to extrude gel through capillary cell; F_o = force required to extrude gel through orifice cell; L_y = length gel is deformed at yield point.

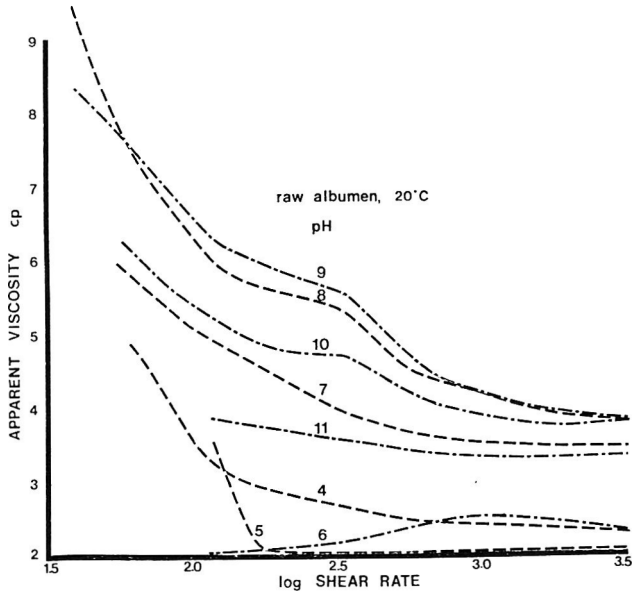

Fig. 3—Apparent viscosity versus log shear rate for raw pH-adjusted albumen at 20° C.

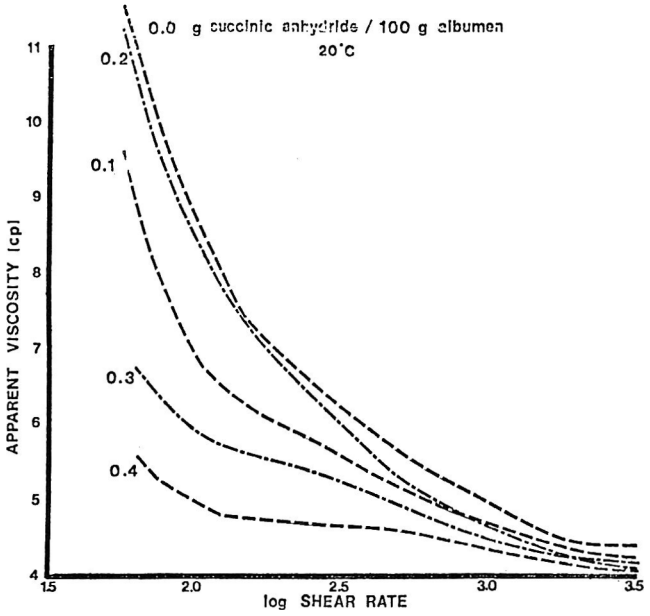

Fig. 4—Apparent viscosity versus log shear rate for raw succinylated albumen at 20° C.

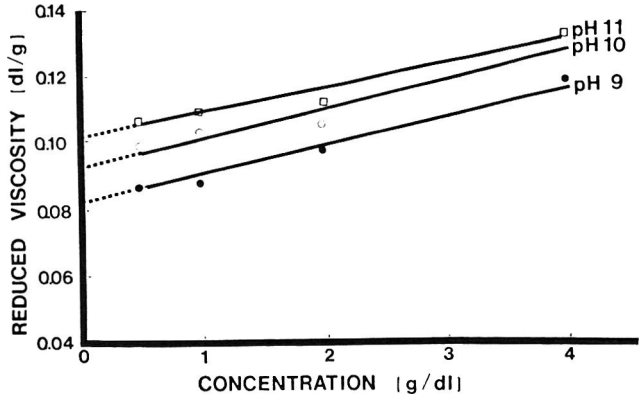

Fig. 5—Reduced viscosity versus concentration plots for pH-adjusted albumen. Intercept = intrinsic viscosity; pH 9.0, 10.0, and 11.0 0.025M $NaHCO_3$ buffer + 150 mM KCl.

Table 2—Regression equations for reduced viscosity versus concentration plots for pH-adjusted and succinylated albumen[a]

Treatment	Regression Equation	Correlation	Intrinsic viscosity (dl/g)
pH 9.0	y = 0.0087x + 0.0827	0.96	0.083
10.0	y = 0.0087x + 0.0923	0.97	0.092
11.0	y = 0.0070x + 0.1015	0.98	0.102
Succ. 0%	y = 0.0053x + 0.0515	0.98	0.052
0.1	y = 0.0097x + 0.1465	0.99	0.147
0.2	y = 0.0141x + 0.1487	0.99	0.149
0.4	y = 0.0069x + 0.1840	0.98	0.184

[a] 3 replicates/treatment

and (3) the proteins in dilute solution did not precipitate out. The kinematic viscosity multiplied by the density of the dilute solution gave the viscosity of the solution, which was used to calculate the reduced viscosity. From plots of the reduced viscosity versus the concentration of the solutions, the intrinsic viscosity was noted at the intercepts after linear regression of the data (Tanford, 1961; Fig. 5 and Table 2).

The kinematic viscosities of succinylated samples (0, 0.1, 0.2, and 0.4% g anhydride/g albumen) were measured and used to calculate reduced and intrinsic viscosities (Fig. 6 and Table 2). Concentrations of the solutions were calculated on the basis of g/dl where the weight of the succinylated albumen included the weight of the succinic anhydride added.

The intrinsic viscosity for pH 9.0 albumen was less than that for pH 10.0 which was less than that for pH 11.0 (Fig. 5). The data agreed with the hypothesis that increased net negative charges on the proteins might cause increased repulsion among the protein side groups and cause a general increase in volume or change in shape. From intrinsic viscosity values alone, it was not possible to tell whether the increase was due solely to increased hydrodynamic volume of the protein molecules or to increased axial ratio of the molecules from a change in shape (Scheraga, 1961).

The intrinsic viscosity of the succinylated samples increased compared to the control nonsuccinylated sample, and they also increased as the level of succinylation increased (Fig. 6). These increases might be due to changes in the net negative charge associated with the protein side groups causing a general expansion or a change in shape (e.g. elongation) from repulsive forces. Another explanation would be the incorporation of succinyl groups.

Coagulated albumen

Apparent viscosity. For pH-adjusted albumen, the apparent viscosities increased with increasing pH, with the pH 10.5 and 11.0 gels being significantly higher ($P < 0.05$) than the pH 9.0 control gel (Table 3). Succinylation had little effect on the apparent viscosities of the gels.

Yield force. For pH-adjusted albumen, the yield force increased with pH, with the pH 10.5 and 11.0 treatments being significantly higher ($P < 0.05$) than the pH 9.0 control gel (Table 3). The yield force required to initiate flow of the succinylated gels did not change with increasing levels of succinylation.

Apparent rigidity modulus. The apparent rigidity modulus was taken to be a measure of deformation characteristics of the gels rather than stress-relaxation qualities. For pH-adjusted albumen, the modulus of rigidity generally

Table 3—Apparent viscosity, yield force, and apparent rigidity modulus (20°C) for pH-adjusted or succinylated albumen gels[a]

Treatment	Apparent viscosity (P)	Yield force (dynes x 10^6)	Apparent rigidity modulus (dynes/cm^2 x 10^6)
pH 7.0	116.7 ± 38.9[b]	5.1 ± 1.1[b]	3.4 ± 2.5[b]
8.0	127.8 ± 38.9[bc]	6.3 ± 1.5[b]	4.9 ± 1.7[b]
9.0	155.5 ± 58.8[bc]	7.5 ± 0.1[b]	3.6 ± 1.2[b]
10.0	277.8 ± 29.4[cd]	12.2 ± 2.4[bc]	15.0 ± 5.5[cd]
10.5	329.6 ± 121.9[de]	19.7 ± 9.3[c]	21.1 ± 3.6[d]
11.0	476.3 ± 152.9[e]	18.5 ± 8.4[c]	7.9 ± 7.2[bc]
Succ. 0%	146.3 ± 51.6[b]	6.5 ± 2.7[b]	2.6 ± 0.4[b]
0.1	98.1 ± 32.6[b]	6.6 ± 1.8[b]	3.1 ± 1.5[b]
0.2	102.8 ± 57.4[b]	4.7 ± 1.8[b]	2.0 ± 0.3[b]
0.4	164.8 ± 14.0[b]	7.3 ± 1.6[b]	1.8 ± 0.5[b]

[a] Coagulated with 80°C water; triplicate determinations
[bcde] Means in the same column within a treatment with different superscripts are significantly at $P \leq 0.05$.

increased with pH; pH 10.0 and 10.5 treatments were significantly higher ($P < 0.05$) than the pH 9.0 control treatment (Table 3). The rigidity modulus did not change with succinylation.

The results indicated that high pH gels were more viscous, required a higher yield force, and were more rigid than pH 9.0 control gels. On the other hand, the succinylated gels did not appear to be different from the control gel in viscosity, yield force, or rigidity.

SUMMARY

THE APPARENT VISCOSITIES of raw pH-adjusted albumen and succinylated albumen decreased with increasing shear rates, implying pseudoplasticity. For pH 5.0–6.0 and 11.0 treatments, however, the albumen exhibited Newtonian behavior. The apparent viscosities generally decreased with increasing temperatures.

The intrinsic viscosities of pH 9.0, 10.0, and 11.0 albumen were 0.083, 0.092, and 0.102 dl/g, respectively, while that of succinylated (pH 8.5) albumen at the 0, 0.1, 0.2, and 0.4% (g anhydride/g albumen) levels were 0.052, 0.147, 0.149, and 0.184 dl/g, respectively. The increased intrinsic viscosities with increasing pH or succinylation implied that the proteins may have increased in volume or changed shape.

For coagulated pH-adjusted gels, the apparent viscosity, the yield force, and the apparent rigidity modulus increased for the higher pH treatments. These same rheological quantities did not appear to change with level of succinylation.

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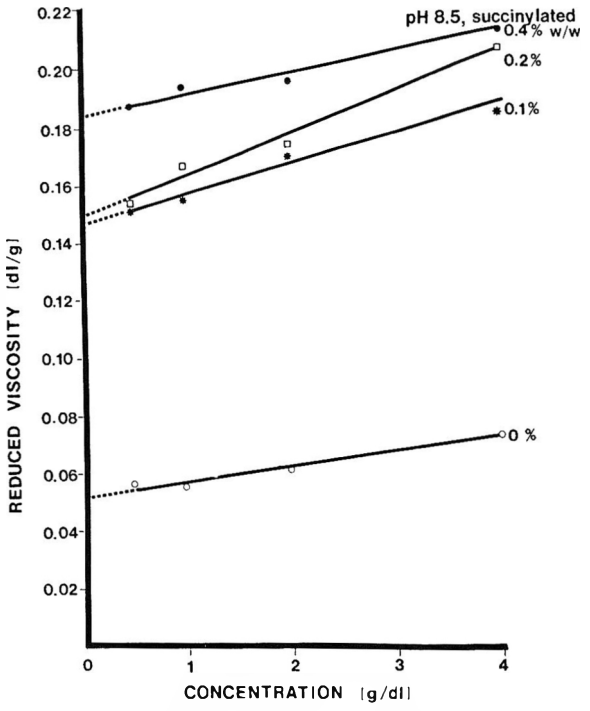

Fig. 6—Reduced viscosity versus concentration plots for succinylated albumen. Intercept = intrinsic viscosity; 0.5M tris(hydroxymethyl)aminomethane buffer, pH 8.5.

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A New Method to Quantitate the Coagulation Process

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ABSTRACT

A nondestructive method to quantitate the coagulation process has been developed and used to obtain kinetic data on the heat-induced gelation of egg albumen. It involves continuous monitoring of the force exerted by the gel as coagulation takes place and requires only one sample to observe the total process. From the information on the maximum force exerted by the gel, rate constants, Z values, and activation energies were calculated. This approach was applied to evaluate other coagulation processes such as the enzymatic coagulation of milk and the gelation of gelatin by cooling.

INTRODUCTION

COAGULATION of food proteins by heat, enzymes, or other agents involves complex phenomena. Many of the methods that observe coagulation entail measuring individual samples after discrete time intervals of treatment, such as heating, until coagulation or gelation is completed. A common method is to measure gel strength at various heating times (Hickson et al., 1982; Shimada and Matsushita, 1980a, b, 1981; Beveridge et al., 1980). Others have observed disappearance or appearance of electrophoretic bands of proteins at different heating times and temperatures (Matsuda et al., 1981; Shimada and Matsushita, 1980a). Turbidity, optical density, or absorbance measurements at various wavelengths of the supernatant of centrifuged samples of protein solutions after various heat treatments have also been reported by several workers (Shimada and Matsushita, 1980a, b, 1981; Sato and Nakamura, 1977; Nakamura et al., 1978). Hegg and Lofqvist (1977) have measured the change in optical rotation of ovalbumin after various heating periods. Still another method is to measure the protein concentration or the weight of the precipitate after centrifugation of protein solutions heated to different times (Nakamura et al., 1978; Hegg et al., 1979). Schweid and Toledo (1981) have observed gelation of meat batters by measuring the pressure induced by the meat batter against the pistons of a "dynamic tester" attached to an Instron Machine as a function of temperature and time of heating. Viscosity measurements of heated samples have also been made (Sato and Nakamura, 1977). However, no method involving the continuous measurement of heat-induced gelation has thus far been reported in the literature.

The goals of this study were (1) to develop a method for continuous measurement of heat-induced gelation; (2) to calculate rate constants, activation energies, and a Z_c value for the heat-induced gelation of egg albumen; and (3) to calculate rate constants for the enzymatic coagulation of milk and the gelation of gelatin on cooling.

MATERIALS & METHODS

Sample preparation

Albumen. Albumen samples (pH 9.0) were prepared as described by Gossett and Baker (1983).

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Milk. Homogenized skim milk was used. The enzyme was a 1/40 dilution of Hansen's Cheese rennet. These were stored at 3°C until use. At time of use, 0.5 ml of rennet was added to 24.5 ml of milk and measured immediately.

Gelatin. Gelatin (Knox) was dissolved in distilled water at 100°C to give a concentration of 14.52 g/liter (equivalent to one package/two cups water), and samples were used immediately.

Description of apparatus

A Cahn Scanning Electrobalance (Model 2000, recording) was used from which a wire probe (nickel-chromium, wire cable cord, bent at one end to form a hook, 0.508 mm diameter, 232 mg, 135 mm long) was suspended (Fig. 1). The electronic unit of the electrobalance was connected to a linear recorder (Unicam AR 25). Unless otherwise stated, the electrobalance was used on the 100 mg force range, and the chart speed was 5 min/cm. Samples (25 ml) were contained in a jacketed cylinder (3.9 cm, inner diameter) which was positioned under the electrobalance so that the end of the probe was centered in the sample. A plexiglass cap with a 3 mm hole in the center and a slit along the raidus was placed over the top of the cylinder, and the probe hung through the center of this cap. The cap minimized background noise. The cylinder was connected to an 11 liter circulating water bath having a flow rate of approximate 1 liter/min unless stated otherwise.

Procedure

Prior to each run, the probe was cleaned by flaming and then carefully hung on the triangular hanger of the electrobalance with a pair of tweezers. Samples (25 ml) were placed into the jacketed cylinder which was positioned under the electrobalance by adjusting a jack stand on which the cylinder stood. The water bath, maintained at the appropriate temperature, was connected to the cylinder. To initiate a run, the water bath and the recorder were turned on simultaneously. The samples were allowed to coagulate or gel over a period of 30 min or until maximum force was observed. A graph of force versus time was obtained for each of four runs.

In separate runs, the internal temperature at the center of triplicate samples was measured with a thermocouple thermometer (Cole-Parmer, Model 8520-50, time constant = 5 sec) as a function of time as coagulation or gelation occurred. As a result of good reproducibility of the data, measurements were obtained for triplicate runs rather than in quadruplicate.

Heat-induced gelation of albumen

Albumen was coagulated at circulating water bath temperatures of 60, 65, 70, 75, 80, 85, 90, and 95°C.

Rate constants. Analysis of the data assumed that heat coagulation of egg albumen followed first order kinetics (Chick and Martin, 1911, 1912). Assuming the following reaction:

$$\text{Raw (R)} \rightarrow \text{Coagulated (C)}$$

Then

$$\frac{-d[R]}{dt} = k\,[R]$$

In terms of C, where R_o = initial raw concentration of albumen:

$$\frac{d[C]}{dt} = -k[R_o - C].$$

Putting C and R in terms of Force (f), the f at C = f, and f at R_o = 0:

$$\frac{d[f]}{dt} = -k\,(0 - f) \quad \text{or} \quad \frac{df}{f} = k\,dt.$$

Integrating between f_o at time = 0 and f at time = t:

$$\int_{f_o}^{f} \frac{df}{f} = k \int_{0}^{t} dt$$

$$(\ln f - \ln f_o) = kt$$

$$\ln f = \ln f_o + kt$$

A plot of ln f versus t should yield a straight line of slope k.

The natural log of the force values at 0.5 min intervals were plotted against the time of coagulation for force values from t_o = start to t = time where maximum force was observed. Examination of the ln (force) versus time curves showed a broken line characteristic of a two-step process. Therefore regression of the resulting plots into two straight lines was attempted. The slopes of these lines were taken as rate constants k_1 and k_2. Each rate constant reported was an average of four determinations. An analysis of variance for a completely random design was computed for k_1 and for k_2.

In addition to the rate constants k_1 and k_2, the time at which the two lines corresponding to k_1 and k_2 intersected was calculated by simultaneously solving the two equations. The time for each treatment to reach maximum force was recorded as well as the value of the maximum force.

Activation energies. The natural log of k_1 was plotted against the inverse temperature ($°K^{-1}$) for 65, 70, 75, 80, 85, 90, and 95°C. The slope of this line yielded $-E_{a1}/R$, where R = 1.987 cal/°K mole and E_{a1} = activation energy in cal/mole for the first step of the coagulation process. The same exercise was performed with k_2 from which E_{a2} for the second step was found.

Z_c value. A Z_c value is defined as the necessary rise in heating temperature required for a 10-fold increase in the reaction rate for chemical and sensory changes (Dagerskod, 1977). This is analogous to the Z value used in thermal processing which is the temperature change required to change the thermal death time by a factor of 10 (Lund, 1975).

The heating time in minutes required to attain a force of 5, 10, or 15 mg was taken as an indicator of reaction rate at each temperature. The ln (heating time) was linearly regressed against heating temperature and from this equation, Z_c was calculated for each force.

Enzymatic coagulation of milk

A 24.5 ml sample of milk was added to the jacketed cylinder at 20°C, followed by 0.5 ml of rennet. The circulating water (30°C, 2 liters/min) and the recorder (chart speed 10 min/cm, full scale 10 mg force) were immediately started. From the force-time curves obtained rate constants were calculated as described earlier.

Gelation of gelatin by cooling

A 25 ml aliquot of the gelatin solution (30°C) was placed in the jacketed cylinder. The water bath (0°C, 2 liters/min) was turned on at the same time as the recorder (chart speed 5 min/cm and full scale 10 mg force.) Force-time curves were obtained and rate constants were calculated as described.

Statistical analysis

Statistical treatment was computed by an analysis of variance. When a significant F ratio was obtained, a protected least significant difference (LSD) test was performed at the 5% level of significance (Snedecor and Cochran, 1976).

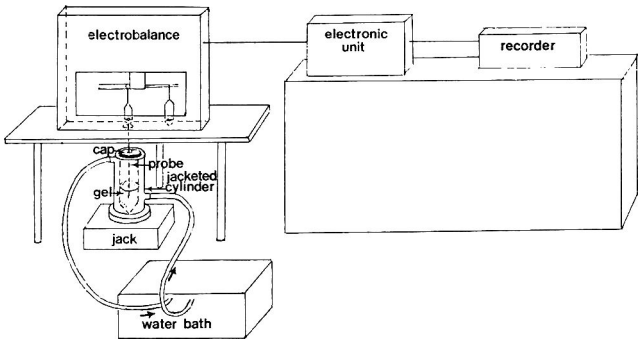

Fig. 1—Diagram of the set-up for the new method of quantitating coagulation.

RESULTS & DISCUSSION

Heat-induced gelation of albumen

Typical electrobalance force versus time curves for albumen heated at different temperatures from 60–95°C indicated the force (mg) increased until a maximum force value was attained (Table 1). As the temperature of heating increased, the maximum force exerted by the albumen increased. In general, it took less time to reach maximum force as the temperature of heating increased. The corresponding internal temperature of the albumen at peak force increased with increasing heating temperature.

The increase in force with time was initially assumed to be associated with the force exerted by the gel on the sensing probe as gelation took place and was thought to be correlated with gel strength. However, the electrobalance force for treatments coagulated with 65–95°C heating water decreased after a maximum force was attained which was thought to be due to expansion of the gel once the gel was coagulated. This expansion probably raised the sensing probe and decreased the observed force. Thus, the force may be an expression of gelation as well as thermal expansion. Changes in geometric configuration of the gelling assembly need to be explored to differentiate between the contribution of each of the two processes. Although the true source of this force is not yet known, it is the author's opinion that in the initial stages of this measurement, gelation is the predominant process.

Plots of internal temperature versus time data (Fig. 2) indicated that albumen treated at 60, 65, and 70°C exhibited a final internal temperature of less than 56°C, which is lower than the coagulation temperature reported for pH 9.0 albumen (Gossett, 1983).

Rate constants and activation energies

First and second order kinetic analyses provided poor fit to the data. First order kinetics and the existence of two reactions during the heat coagulation of the albumen, as exemplified by the broken line curve (Fig. 3), were used to analyze the data. The ln (force) versus time plots were regressed to yield two straight lines with slopes k_1 and k_2 ($r^2 \geq 0.92$).

In addition to the rate constants k_1 and k_2 (Table 2), the time at which the first step "stopped" and the second step "started" was estimated by solving simultaneously the two straight line equations for time. The internal temperatures corresponding to these times were obtained from temperature-time data. Values for k_1 increased with increasing heating temperatures as well as k_2 for 90° and 95°C heating temperatures.

The classic picture of heat coagulation of proteins involves a two-step sequential process in which the first step

Table 1—Maximum electrobalance force, time required to attain peak force, and internal albumen temperature at time of peak force for egg albumen (pH 9.0) at various temperatures of heating

Water bath temp	Maximum force (mg)[a]	Time (min)[a]	Internal albumen temp (°C)[b]
60°C	3.3 ± 0.9	15.3 ± 1.3	51.3 ± 2.5
65	6.4 ± 1.8	15.5 ± 1.9	51.7 ± 1.5
70	11.3 ± 1.5	13.3 ± 2.7	55.3 ± 0.6
75	13.9 ± 2.0	15.4 ± 4.5	58.3 ± 2.1
80	14.1 ± 1.6	12.3 ± 2.7	61.7 ± 1.2
85	26.9 ± 6.6	12.4 ± 1.8	63.0 ± 1.0
90	79.9 ± 15.0	13.6 ± 1.5	68.7 ± 0.6
95	> 100	11.1 ± 2.5	70.7 ± 3.1

[a] Quadruplicate determinations
[b] Triplicate determinations; temperature at time of peak force measured at center of sample.

Table 2—First order rate constants for heat coagulation of egg albumen (pH 9.0) at various temperatures of heating

Water bath temp	k_1 (min^{-1})[a]	k_2 (min^{-1})[a]	Time (min)[ac]	Int. albumen temp[b]
60°C	0.329 ± 0.100[d]	0.037 ± 0.008[d]	4.83 ± 1.36	48.3 ± 5.0°C
65	0.377 ± 0.150[d]	0.036 ± 0.006[d]	5.44 ± 1.88	53.7 ± 0.6
70	0.421 ± 0.143[de]	0.046 ± 0.012[d]	4.42 ± 1.11	51.7 ± 4.9
75	0.477 ± 0.179[def]	0.051 ± 0.011[d]	4.54 ± 1.51	52.0 ± 4.4
80	0.617 ± 0.083[ef]	0.064 ± 0.026[d]	3.01 ± 0.49	53.3 ± 3.8
85	0.662 ± 0.041[f]	0.067 ± 0.021[d]	3.09 ± 0.14	52.0 ± 5.2
90	0.690 ± 0.277[f]	0.157 ± 0.010[e]	2.04 ± 0.33	47.3 ± 7.1
95	1.223 ± 0.128[g]	0.231 ± 0.089[f]	1.59 ± 0.45	50.7 ± 4.0
Avg. R^2 for regression:	0.92	0.94		

[a] Quadruplicate determinations
[b] Triplicate determinations; temperature at time as described in c:
[c] Time at which first step "ends" and second step "starts."
[defg] Means in the same column with different superscripts are significantly different at $P \leq 0.05$.

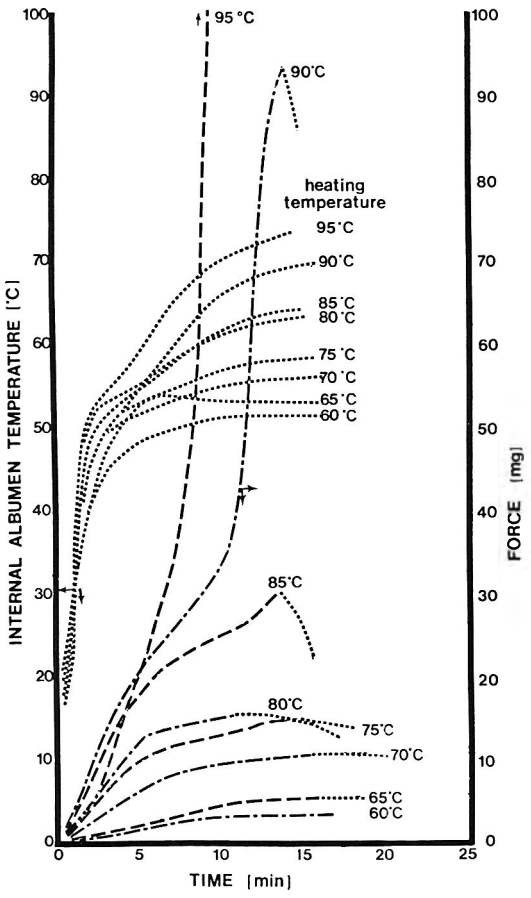

Fig. 2—Typical plots of electrobalance force versus time of heating of egg albumen (pH 9.0) at various temperatures of heating (dashed lines) with corresponding internal albumen temperatures versus time of heating (dotted lines) at different temperatures of heating. Plots shown terminate at or shortly after maximum force values.

is the denaturation of the proteins and the second step the aggregation of the unfolded proteins (Ferry, 1948). In this study, it was assumed that the two processes did not overlap and k_1 was interpreted to be the rate constant for the denaturation process, while k_2 the rate constant for the aggregation process. For albumen heated at various temperatures, the rates of denaturation (k_1) and of aggregation (k_2) increased with increasing heating temperature. The time at which the first step "ended" and the second step "started" was found to decrease with increasing heating temperature suggesting that at higher temperatures, denaturation occurred faster and the onset of aggregation was expedited.

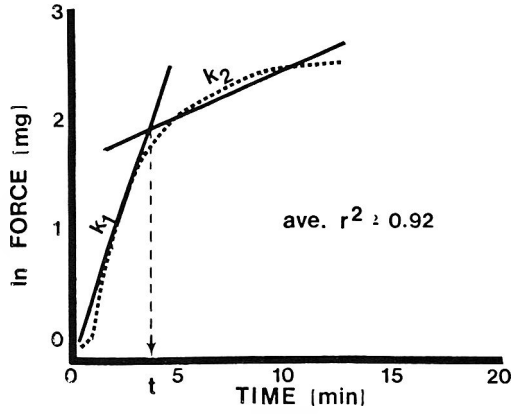

Fig. 3—Plot of ln (force) versus time showing rate constants k_1 and k_2 and t, the time where the first step "stops" and the second step "starts."

The Arrhenius equation was used to calculate the activation energies which describe this temperature dependency of rate constants (Lund, 1975):

$$k = s\, e^{(-E_a/RT)}$$

where k = rate constant (min^{-1}), s = frequency factor (min^{-1}), E_a = activation energy (cal/mole), R = gas constant (1.987 cal/°K mole), and T = absolute temperature (°K).

The ln k versus 1/T plots using heating temperatures of 65–95°C yielded E_{a1} to be 8.7 kcal/mole and E_{a2} to be 14.4 kcal/mole (Fig. 4). Dwek and Navon (1972) estimated the activation energy for denaturation of egg albumen between temperatures of 65–95°C as approximately 24 kcal/mole by comparing fluidities and hardness of the albumen at various temperatures. This value may represent the activation energy for the entire denaturation and aggregation processes since no distinction between the two was made.

Z_c value. To quantitate the coagulation process, a 10-fold change in heating time versus temperature for a constant force value was used to calculate Z_c values. At heating temperatures from 65–95°C, a force of at least 5 mg was exerted by the gels; from 70–95°C, a force of at least 10 mg was exhibited; and from 75–95°C, a force of at least 15 mg was shown (Fig. 2). The times required to heat the gels to attain 5, 10, or 15 mg force at various temperatures of heating were noted (Table 3).

A plot of ln (heating time) needed to attain 5 mg force versus the heating temperature, and linear regression of the data, yielded Equation 1 (Table 3). The equation was solved for heating temperature when ln (heating time) = 0 and = 2.303 and the difference in heating temperatures

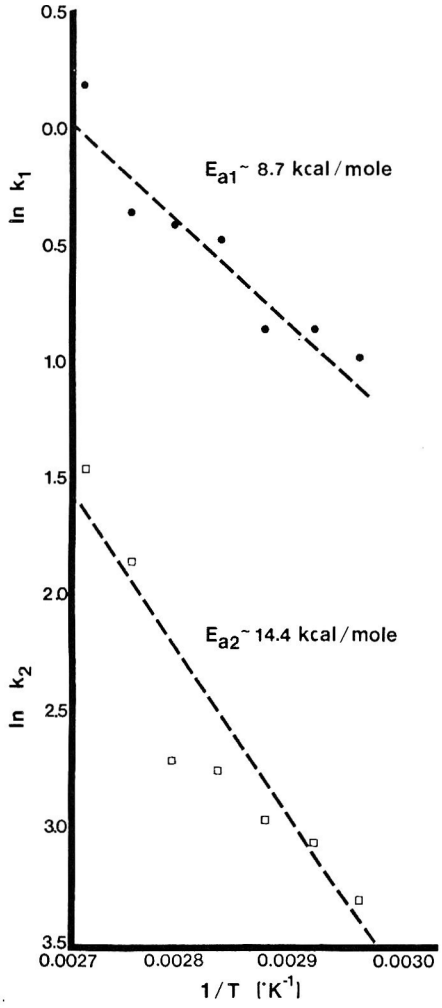

Fig. 4—Plots using the Arrhenius relation: 1n k versus 1/T; slope = $-E_a/R$. Each point is an average of four determinations. Equations: 1n k_1 = 11.9 − 4391 (1/T), r = 0.94; 1n k_2 = 18.0 − 7272 (1/T), r = 0.93.

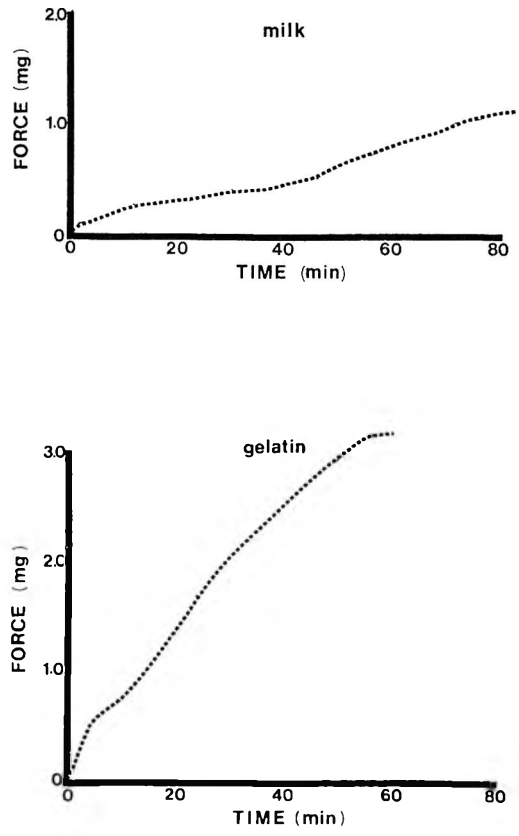

Fig. 5—(a) Typical plot of electrobalance force versus time of coagulation for enzyme-coagulated milk, 30°C; (b) Typical plot of electrobalance force versus time of gelation for gelatin, cooled from 30 to 1°C. Both plots terminate at maximum force values.

was 40.3°C for a 10-fold increase in heating time. Similarly, differences in heating temperature of 43.6°C and of 41.1°C were found for a 10-fold increase in heating time using Equations 2 and 3 (Table 3) for 10 and 15 mg, respectively. An overall average of 41.7 ± 1.7°C was set equal to Z_c.

Literature values for Z_c values for various reactions vary. Leonard et al. (1964) used a value of 33.2°C for Z_c for chemical reactions resulting in changes in quality factors such as destruction of thiamin. Dagerskod (1977) quoted values for Z_c ranging from 17°C for changes in texture of cooked potatoes to 24°C for changes in tenderness of cooked meat to 35°C for changes in fresh appearance of sterilized peas. Lund (1975) listed Z values for changes in texture and overall cook quality for beets at 19°C; peas, 32°C; corn, 37°C; and broccoli, 44°C. Chick and Martin (1911) estimated that "agglutination" process of egg proteins was accelerated 2 to 2.5 times/10°C rise in temperature. Thus the value of 41.7°C for Z_c obtained in these studies was comparable to literature values.

Table 3—Heating times required to attain various electrobalance force values at various heating temperatures

Heating temp	Heating time (min)[a] to attain: 5 mg	10 mg	15 mg
65°C	10.63 ± 5.64	—	—
70	3.62 ± 0.93	9.77 ± 2.90	—
75	3.93 ± 1.79	8.05 ± 4.25	15.00[b]
80	2.70 ± 0.54	6.54 ± 1.84	10.00[b]
85	1.58 ± 0.81	2.97 ± 0.50	4.46 ± 1.07
90	1.89 ± 0.34	2.93 ± 1.29	4.82 ± 1.92
95	1.55 ± 0.62	3.30 ± 1.91	5.31 ± 2.03

Regression equations for ln (heating time) vs heating temperature

Eq (1) 5 mg: ln (heating time = 5.64 − 0.0571 (heating temperature) correlation = 0.906
Δ heating temperature = 40.3°C

Eq (2) 10 mg: ln (heating time) = 5.96 − 0.0528 (heating temperature) correlation = 0.909
Δ heating temperature = 43.6°C

Eq (3) 15 mg: ln (heating time) = 6.72 − 0.0561 (heating temperature) correlation = 0.836
Δ heating temperature = 41.1°C

Z_c = average Δ heating temperature = 41.7 ± 1.7°C

[a] Quadruplicate determinations
[b] One observation available

Milk and gelatin

It was of interest to see if this new method of measuring heat-induced coagulation could be adapted to measure the coagulation or gelation of systems that (1) were enzymatically coagulated, such as milk at 30°C, and (2) were gelled, such as gelatin, by cooling from 30 to 0°C.

During coagulation of milk, increases in force were observed for about 80 min after which slight decreases in force were seen (Fig. 5a). The force readings were less than 2 mg, which was much lower than those exhibited by albumen gels. Initial sample temperature was 20 ± 1°C, reaching 27°C after 4 min. Plots of 1n (force) versus time gave straight lines and one rate constant, k_{milk}, was cal-

culated for each determination, making k_{milk} = 0.029 ± 0.007 min^{-1} (average r^2 = 0.95). Ernstrom and Wong (1974) stated that the reaction velocity of enzyme-catalyzed milk clotting was proportional to the enzyme concentration. If this is true, then treatment of the data using first order kinetics is reasonable.

The gelatin gels exerted increasing force values up to about 3.5 mg for at least 50 min after which slight decreases in force were observed (Fig. 5b). Initial internal sample temperature was 30 ± 1°C, reaching 1°C after 14–15 min. The modulus of rigidity for gelatin gels was found by Ferry (1948) to be proportional to the square of the concentration of gelatin, implying second order kinetics. However, a plot of reciprocal force versus time did not yield a linear plot. Plots of ln force versus time did yield straight lines (average r^2 = 0.94), making $k_{gelatin}$ = 0.035 ± 0.004 min^{-1}. It is reasonable to assume that the force measured on the electrobalance was not related to the rigidity modulus and therefore gelation as measured by the electrobalance may indicate a different kinetic mechanism.

Although this method was used to measure coagulation or gelation processes, conditions must be optimized for each system before meaningful data can be extracted. Since these milk and gelatin gels were much "weaker" than albumen gels, a more sensitive probe would be desirable. In addition, the thermal expansion characteristics of different types of gels need to be investigated and their role in coagulation or gelation measurements established.

SUMMARY

A NEW, nondestructive technique was developed to quantitate coagulation which offered the advantage of measuring the entire coagulation process with one sample, rather than with many samples measured at different time intervals throughout the process. The method involved using a microbalance transducer system which continuously monitored changes in force exerted by the gel as gelation took place. The sensing device was a straight wire suspended from the electrobalance. Kinetic data obtained from the gelation of egg albumen allowed calculation of (a) rate constants, which increased with increasing heating temperatures; (b) activation energies, E_{a1} = 8.7 kcal/mole and E_{a2} = 14.4 kcal/mole; and (c) a Z_c value of 41.7°C. In addition, it was found that the maximum force exerted by the gels increased as the temperature of heating increased. This method appeared to be adaptable to systems that are enzymatically coagulation at a constant temperature (milk) and that are gelled by cooling (gelatin).

Future research using this method should be directed towards finding the physical meaning and source of the measured force. Besides gelation or coagulation, it is possible that thermal expansion or surface tension properties are responsible for this force.

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Cotyledon Structure of Resting Peanut (*Arachis Hypogaea* L. cv. Florunner) Seed Before and After Hydraulic Pressing

W. E. SCHADEL, W. M. WALTER JR., and CLYDE T. YOUNG

ABSTRACT

The cotyledon structure of resting peanut (*Arachis hypogaea* L. cv. Florunner) seed before and after hydraulic pressing was investigated with light and scanning electron microscopy. Observations were made of the appearance of cell walls and the major subcellular components: spherosomes (oil reserve bodies), aleurone grains and starch grains. Major findings include previously unreported cell wall damage and surface fissures that may be important to processors who express the oil from peanuts.

INTRODUCTION

VARIOUS PROCESSING CONDITIONS which utilize hydraulic pressing have been developed to obtain partially defatted peanuts (Vix et al., 1967; Pominski and Spadaro, 1980). This processing involves three mechanical operations: (1) pressing, (2) reconstitution, and (3) drying and roasting. Raw (with skins) or blanched peanuts are hydraulically pressed to remove the oil, which removes up to 80% of the oil in the peanuts (Vix et al., 1967), thereby reducing the calorie content while maintaining the protein value.

In order to achieve the maximum efficiency of the peanut oil pressing, it is important to understand the cotyledon structure of the resting peanut (*Arachis hypogaea* L.) seed. Peanut seed anatomy and cytology have been investigated by Woodroof and Leahy (1940), Yarbrough (1949), Bagley et al. (1963), Jacks et al. (1967), and Vaughan (1970). The changes induced by the hydraulic pressing of peanut seed have been studied by Woodroof and Leahy (1940), Neucere and Hensarling (1963), and Yatsu (1981). The present study provides additional information of the cotyledon structure of the resting peanut seed before and after hydraulic pressing.

MATERIALS & METHODS

JUMBO, cv. Florunner, peanut cotyledons were hydraulically pressed at 1800–2000 psi in a 600-ton Albright Nell cage press. Oil content was reduced from 49.9 (w/w) to 32.6% (w/w) by this process. Tissue blocks (2 mm^3) of unpressed (native) and pressed cotyledons were fixed, embedded, sectioned, and stained for light microscopy using the methods of Yatsu (1981). Starch and aleurone grains (protein bodies) were stained differentially with 1% acid fuchsin specific for protein (Feder and O'Brien, 1968). Identically fixed tissue for scanning electron microscopy was dehydrated in a graded series of aqueous ethanol (10, 25, 50, 75, 95, and 100% ethanol), followed by a graded series of ethanol-amyl acetate (10, 25, 50, 75, 95, and 100% amyl acetate). Carbon dioxide was used as the transitional fluid in a Ladd Critical Point Dryer. The tissue was then gold-coated in a Polaron E 5000 Diode Sputtering System. Samples were observed and photographed at 20 KeV with an ETEC Autoscan microscope.

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RESULTS & DISCUSSION

Unpressed peanut

The embryo of the raw, unpressed peanut consists of two cotyledons and a small radicle and plumule known as germ or "heart." Earlier workers (Woodroof and Leahy, 1940) reported that the blanching process removed the hearts as well as the skins (6–7.5% of the weight). However, in many cases today, the spin blanching process does not split the nuts and, thus, the hearts are retained (Pominski and Spadaro, 1980). The primary concern of processors of partially defatted peanuts is the tissue of the peanut seed cotyledons which constitutes about 96% of the seed weight.

The peanut seed cotyledon contains three kinds of tissue: (1) epidermis, (2) vascular, and (3) parenchyma. The epidermis consists of a layer of cells which covers the surface of the cotyledon. This epidermal layer is united by a thick cutin which is most abundant on the rounded, outer surface. The epidermal cells of the rounded, outer surface are more or less rectangular in outline. Woodroof and Leahy (1940) reported that stomata are scattered irregularly over the epidermal surface of the cotyledons of Spanish peanuts, and Yarbrough (1949) reported "somewhat simplified stomates occurring on the abaxial surface only" of Virginia Bunch cotyledons. The numerous stomata (Fig. 1) of Jumbo, cv. Florunner, peanuts are present only on the flat, inner (adaxial) surface of the resting seed cotyledon.

The vascular system of the peanut seed extends through each cotyledon of the embryo. Woodroof and Leahy (1940) stated that one series of six to eight bundles follows the curvature of the outer surface and another series of four to six centrally located. Our observations confirm that the vascular system makes up only a small part of the embryo.

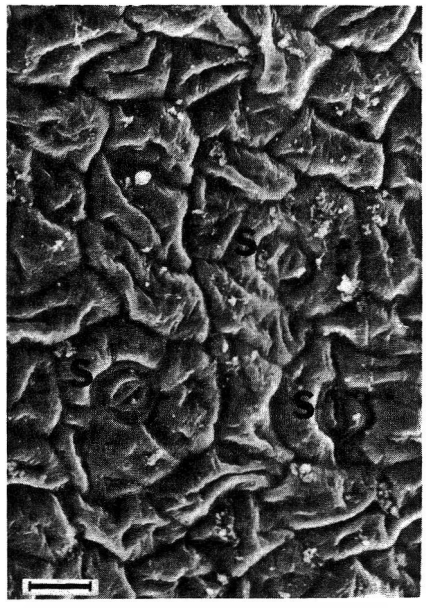

Fig. 1—Scanning electron photomicrograph of the epidermal cells with stomata (S) on the falt, inner surface of the unpressed cotyledon. Marker: 20μ.

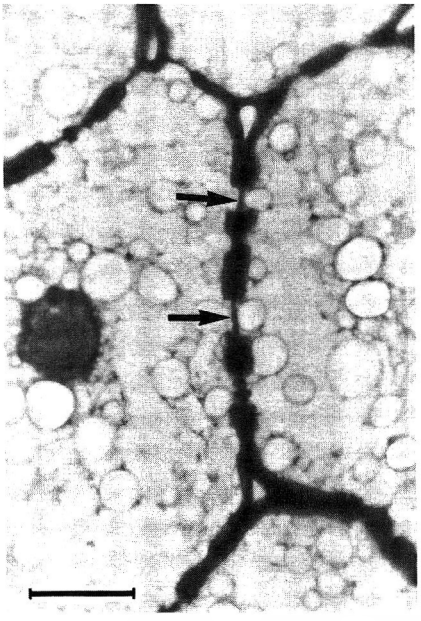

Fig. 2—Light photomicrograph of the parenchyma cell wall pitting (arrows) in the unpressed cotyledon stained with toluidine blue. Marker: 20μ.

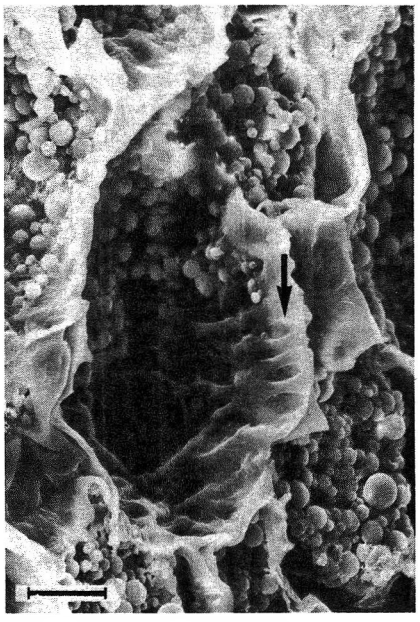

Fig. 3—Scanning electron photomicrograph of the parenchyma cell wall pitting (arrow) in the unpressed cotyledon. Marker: 20μ.

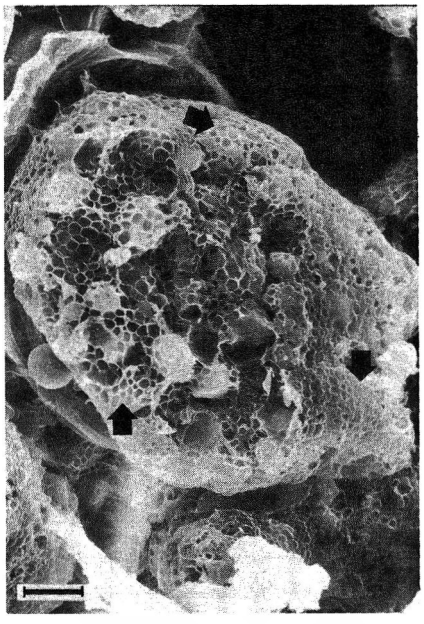

Fig. 4—Scanning electron photomicrograph of the entire cellular contents of a parenchyma cell of the unpressed cotyledon. Note the numerous 1–2 μ spherosome ghosts (arrows) surrounding the larger starch and aleurone grains. Marker: 20μ.

Woodroof and Leahy (1940) stated that the greater part of the embryo is made up of rather large, almost isodiametric parenchyma cells with pitted walls and small but distinct intercellular spaces. The pitted walls of the resting seed parenchyma cells have conspicuous depressions which give the walls a beaded appearance in sectional view with the light microscope (Fig. 2). These depressions have an elliptical to ovoid shape as seen with the scanning electron microscope (Fig. 3). The depressions in the walls of resting peanut seed parenchyma cells have been described by previous workers (Woodroof and Leahy, 1940; Vaughan, 1970; Yatsu, 1981).

The major subcellular organelles of the parenchyma cells are spherosomes (oil reserve bodies), aleurone grains, and starch grains. The transmission electron microscope has been used by Jacks et al. (1967) and Neucere and Hensarling (1973) to characterize the spherosomes as particles about 1.0–2.0 microns in diameter bounded by a limiting membrane. Jacks et al. (1967) determined the composition of spherosomes to be 98.1% total lipid and 1.9% nonlipid residue. After OsO_4 fixation, the spherosomes appear as electron-dense bodies surrounded by electron-dense membranes when observed with the transmission electron microscope. We found that spherosomal ghosts were easily observed with the scanning electron microscope (Fig. 4), but that starch grains were more readily distinguished from aleurone grains with the light microscope (Fig. 5). The almost spherical starch and aleurone grains range in size from 2–10 microns in diameter. After treatment with 1% acid fuchsin stain, the aleurone grains stain a deep pink color, and the starch grains remain colorless.

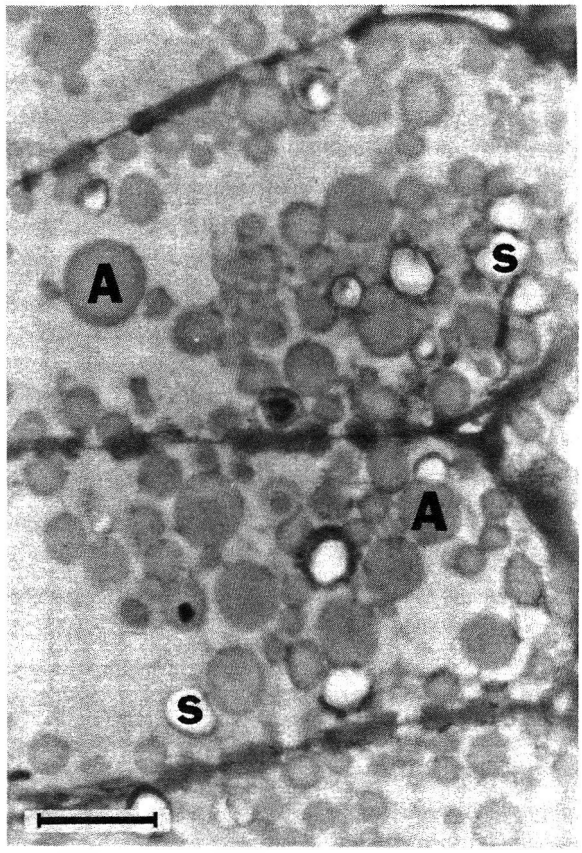

Fig. 5—Light photomicrograph of parenchyma cells of the unpressed cotyledon stained with toluidine blue and acid fuchsin. Compare the unstained starch grains (S) with the stained aleurone grains (A). Marker: 20μ.

Pressed peanut

Hydraulic pressing ruptures most of the spherosomes and creates a compact mass of aleurone grains, starch grains, and residual oil and cytoplasmic components within each cell. Distinct spherosomes can no longer be seen (Fig. 6). Neucere and Hensarling (1973) found that the pressing of peanut seed cotyledon tissue induced slight cellular aberrations and distention of membranes surrounding aleurone grains, but that starch grains and cell walls appeared unaltered. In the present study of pressed peanut seed tissue, we observed the unaltered appearance of the starch grains and the distention of membranes surrounding

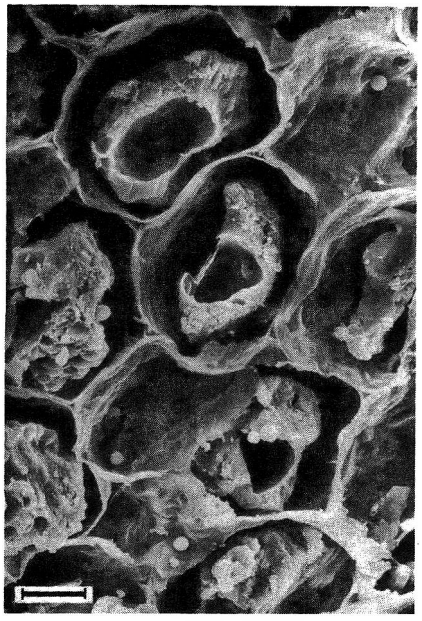

Fig. 6—Scanning electron photomicrograph of parenchyma cells of the pressed cotyledon. Note the absence of the majority of the distinct spherosomes. Marker: 20μ.

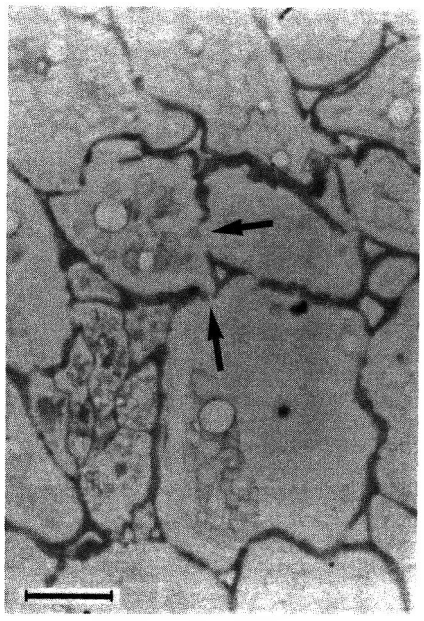

Fig. 7—Light photomicrograph of the pressed cotyledon stained with toluidine blue. Note the small, broken regions (arrows) of the cell walls and the compactness of the cellular contents. Marker: 20μ.

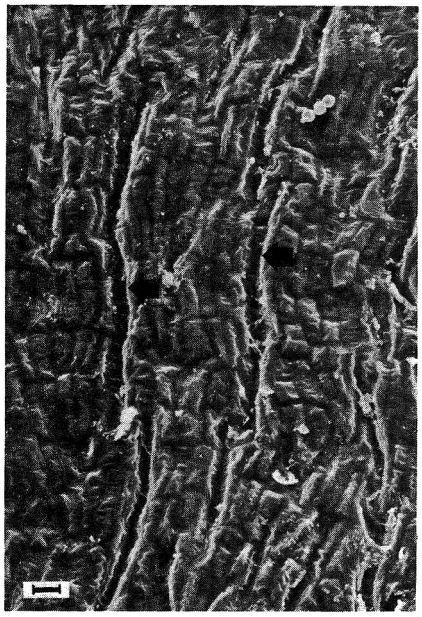

Fig. 8—Scanning electron photomicrograph of the rounded, outer surface of the pressed cotyledon. Note the absence of stomata and large fissures (arrows) along the epidermal cells cause by pressing. Marker: 20μ.

the aleurone grains, but unlike Neucere and Hensarling (1973), we observed that some cell walls were unaffected by pressing, while the majority of the cells had broken wall regions after pressing.

We believe that the broken cell wall regions which appear after pressing correspond to the depressions in the cotyledon cell wall of unpressed peanut. Esau (1965) stated that wall depressions previously referred to as pits may often be the sites of plasmadesmata, the tiny strand-like structures of cytoplasm that interconnect the living protoplasts of adjacent cells. Yatsu (1981) hypothesized that these depressions in the cell wall might impart structural strength (not unlike a keystone arch) and devised a crushing test to test this hypothesis. The results of his crushing test were inconclusive.

Our restuls indicate that these depressions, which are actually thin areas in the cell wall, are actually weak structural points. The small, broken cell wall regions appear to correspond to the thinner areas of the pitted wall (Fig. 7). Perhaps different peanut cultivars with different degrees of wall pitting, offer different resistances to release of oil during the pressing process. In addition to the broken wall areas of individual cells, we observed that the surface of the pressed cotyledon has numerous previously unreported fissures (Fig. 8), which may be involved in the release of oil during hydraulic pressing.

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Pectin-Protein Interaction in Tomato Products

NORIHISA TAKADA and PHILIP E. NELSON

ABSTRACT

The pH influenced the viscosity of a model system of tomato product which consisted of citrus pectin and bovine serum albumin. The viscosity change was completely reversible depending on the pH. The same pH effect was observed in tomato products. Since the amino acid compositions of proteins suggest that the electrostatic property of water-soluble protein fraction from tomato juice can be similar to that of bovine serum albumin, the comparison of pH effects indicates that pectin-protein interaction may be involved in consistency of tomato products. Denaturation of protein may result in a decrease in the serum viscosity during processing.

INTRODUCTION

SEVERAL COMPONENTS contribute to the consistency of tomato products. Kertesz and Loconti (1944) showed that gross viscosity of tomato juice had a variation depending on suspended solid particles and serum viscosity and that pectin material was an important factor. The critical contribution of pectin substances to the consistency of tomato product has been suggested by many researchers (McColloch and Kertesz, 1949; McColloch et al., 1950; Luh et al., 1954; Robinson et al., 1956). On the other hand, proteins in tomato products have been considered as a weak contributor to the consistency even though Williams and Bevenue (1954) reported that the alcohol insoluble solids contained about 17% protein. Foda and McCollum (1970) reported that proteolytic enzyme treatment showed a relatively small loss of consistency of tomato juice but that cellulase greatly decreased it. They insisted that the high molecular polymers associated with the insoluble solids contributed to the consistency of tomato juice. Brown and Stein (1977) also suggested that interactions among cellulose, pectins and proteins affected the rheological characteristics of tomato juice. However, their pronase treatment showed an increase in the consistency of tomato juice while pectinase and cellulase decreased it. Bartolome (1971) found a weak correlation between protein content and juice consistency. In spite of these efforts there has not been a clear understanding of the role of proteins in tomato consistency.

Our previous study (Takada, 1981) showed that serum viscosity of tomato concentrates, after adjusting the brix to the same level, decreased during concentration. However, it was also found that the water-soluble pectin content increased and the molecular weight of the pectins did not show a significant change during concentration. This observation could not be explained sufficiently by a generally accepted concept that pectin is the main component affecting the serum viscosity and that serum viscosity change is due to the degradation of pectin. Therefore, this study was conducted to consider the role of protein as a possible explanation of the above phenomenon.

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MATERIALS & METHODS

Model system for pectin-protein interaction

Bovine albumin (Fraction V, Sigma Chemical Co.) and pectin from citrus fruits (methoxy content 6.45%, Sigma Chemical Co.) were used to prepare a model system similar to the protein and pectin found in tomatoes. Bovine albumin was dissolved in 50 ml of 40 mM citrate sodium phosphate (McIlvaine) buffer solution at various pH's to a final concentration of 1.6% at room temperature. Then the pectin was added to a final concentration of 0.6%. After complete dissolution, 5.0 ml was added to an Ostwald viscometer to measure the viscosity at 25°C. In a study to observe the reversibility of the pH effect, 1M NaOH or 1M HCl solution was used to shift the pH to alkaline or acidic pH, respectively. Different ion strengths were obtained using the five levels of strength of citrate-sodium phosphate buffer at pH 4.35. The ion strength, μ, was calculated using the following equation:

$$\mu = 1/2[Na^+)*1^2+(HPO_4^=)*2^2+(H^+)*1^2+(Citrate^-)*1^2+ (Citrate^=)*2^2+(Citrate^{\equiv})*3^2]$$

where each ion concentration was calculated based onthe dissociation constant at pH 4.35.

Adjustment of pH of tomato products

Tomato puree at 10° Brix from Knox cultivar and the puree diluted to juice at 5.5° Brix were used in this experiment. tomato juice (original pH 4.21) was divided into 8 fractions of 210 ml each. The pH of each fraction was adjusted to various pH with 6N HCl or 6N NaOH solution. Six normal NaCl solution was used to compensate the ion-strength of all samples. After holding at 8°C overnight, viscosity was measured with a Libby's viscometer at 24°C. The pH of all samples were then readjusted to pH 4.20 using the same procedure as described above. The viscosity was measured again after 3 hr.

The adjustment of the pH of the puree was similar to that for juice except that 160g of the puree was used for each pH treatment. A Stormer viscosimeter was used to measure the gross viscosity of puree at 20°C as described by Gould (1953). The driving weight was 318g and 100 revolutions of the cylinder in the sample were timed with a stopwatch.

Precipitate Weight Ratio of tomato puree was calculated as described by Takada and Nelson (1983).

Protein determnation

Tomato puree from Knox and C-28 cultivars was centrifuged at 12,800 x *g* for 30 min at 4°C to obtain the serum fraction and the insoluble fraction. The insoluble fraction was added to two volumes of ethanol, mixed for 30 min, and filtered through Whatman No. 1 filter paper. The residue on the paper was then washed with hot 80% ethanol several times until the filtrate became colorless. The washed residue was suspended with a small amount of water and freeze-dried. The serum fraction was added to 80% ethanol after filtering and concentrating with a rotary evaporator. The washing and drying conditions were the same as those for the insoluble fraction. The nitrogen content in the freeze-dried insoluble fraction and serum fraction were determined by the Kjeldahl method. The conversion factor, 6.25, was used to calculate protein content from the nitrogen content.

Amino acid composition of soluble protein fraction

Tomato juice (approximately 400g) was centrifuged at 6,000 x *g* for 25 min at 4°C to obtain the serum fraction. After filtering through Whatman filter paper #1, the serum was combined with trichloroacetic acid to a final concentration of 20%. The resulting

precipitate was collected by centrifugation as described above and then dialyzed against water for 2 days. The dialyzate was freeze-dried resulting in a protein powder. The protein was hydrolyzed with 6N HCl at 145°C for 4 hr and the amino acid composition was analyzed with an amino acid analyzer (Beckman, Model 119CL).

RESULTS & DISCUSSION

A MODEL SYSTEM consisting of pectin and protein at the same concentration as those found in tomato products at 10° Brix showed a drastic change in the viscosity depending on pH (Fig. 1). Since pectin or albumin alone did not show such a phenomenon, the result indicates that the viscosity change is not attributed to an intramolecular interaction in protein nor pectin but a type of interaction between the two components. The maximum viscosity was obtained at pH 4.20, which is the average pH of tomato juice or tomato concentrates. Viscosity changes as a result of the pH shift from acidic to neutral and neutral to acidic were found as shown in Fig. 2. Furthermore, the interaction was not disturbed by the change of ion-strength in a range 0.01–1.00; the ion-strength of tomato juice is about 0.1 (Fig. 3). While cross bonding of divalent ions is well known, we avoided that reaction in this study. These results indicate that the viscosity change of the model system is attributed to the reversible electrostatic complex formation of pectin and protein at a specific range of pH, but not to weak hydrogen bonds between them.

A similar pattern of response of viscosity to pH change was found in tomato juice diluted from the 10° Brix puree (Fig. 4) with the maximum viscosity being obtained at pH 4.40. Since this pH effect disappeared after re-adjusting the pH to its original value, it is strongly suggested that a reversible electrostatic interaction of pectin and protein occurred in tomato juice. Fig. 5 shows that the same effect occurred

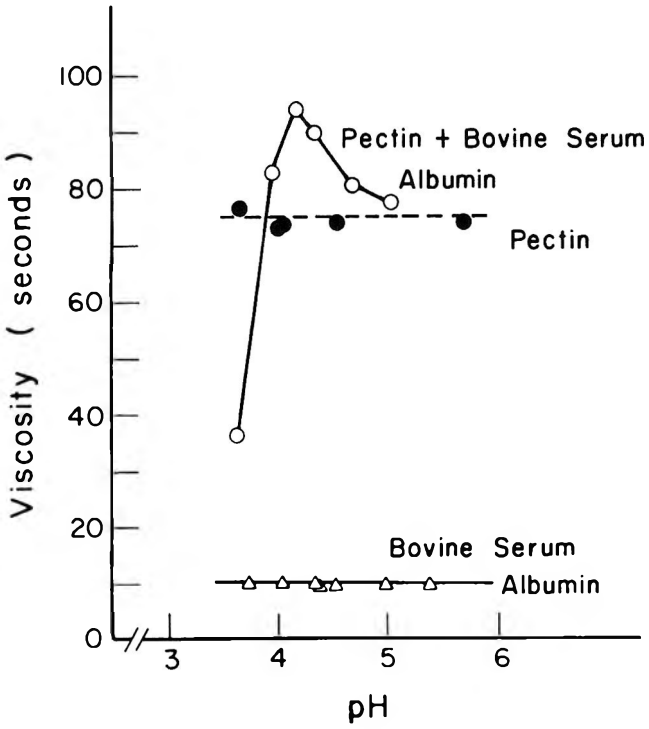

Fig. 1—Interaction of pectin with protein at various pH.

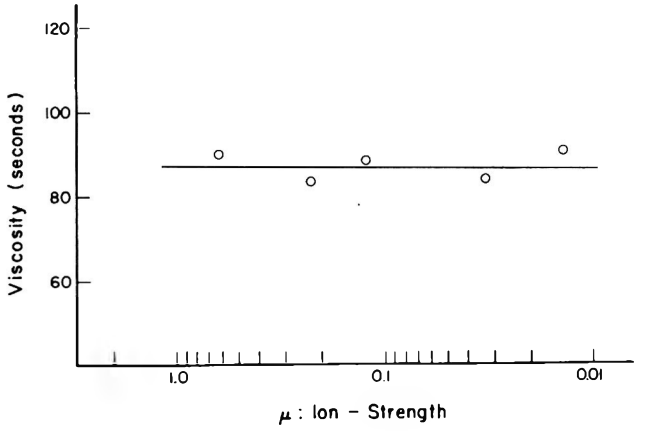

Fig. 3—Interaction of pectin with protein at various ion strength.

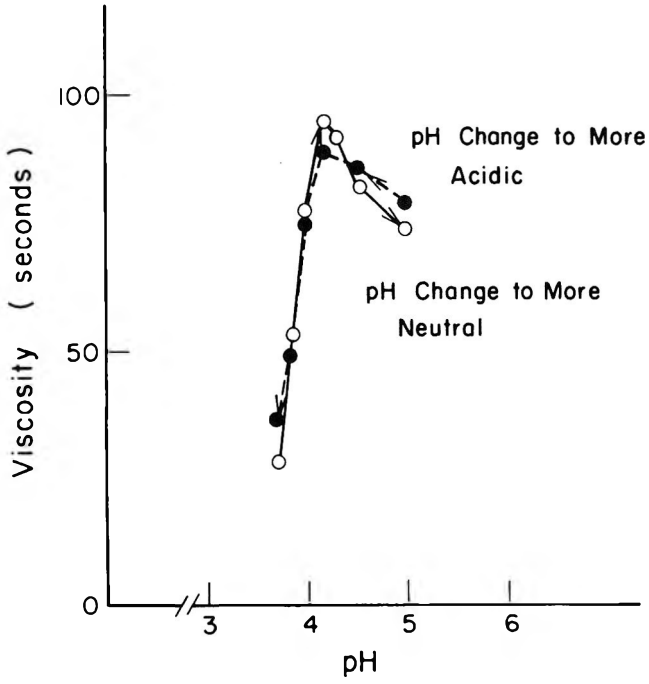

Fig. 2—Reversibility of protein-pectin complex formation at room temperature (Pectin-BSA model).

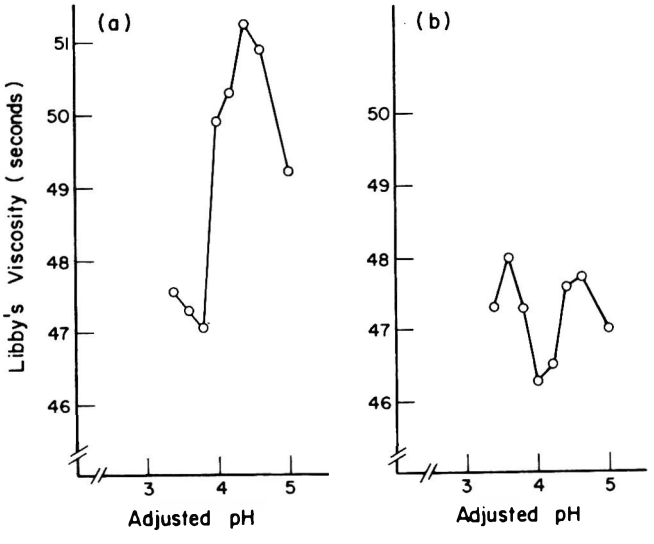

Fig. 4—Effect of pH on consistency of tomato juice: (a) After adjusting the pH from the original pH 4.2 to acidic or alkaline pH, then (b) After returning the pH from the adjusted pH to the original again.

in tomato puree. However, while the pH-dependent responses of viscosity of the serum fraction increased, the rheological property of the water-insoluble fraction (precipitate weight ratio) decreased. The serum showed a similar change in viscosity to that of gross viscosity even though the maximum values were present at a slightly different pH. Similar responses may be due to the fact that the consistency measured with the Stormer viscosimeter is greatly affected by the serum viscosity. Since the serum contains proteins at over 10% (Table 1) and the majority of the pectin, a pH change may result in a different interaction or a change in the friction between them.

The pH effect on the rheological property of water-insoluble solids was measured with the aid of the precipitate weight ratio which was found to be one of the important factors determining the consistency of tomato products (Takada and Nelson, 1983). The precipitate weight ratio was lowest around the natural pH of tomato products. Therefore, the pH change may result in higher consistency of tomato products if the consistency was determined by a consistometer which is greatly influenced by the insoluble fraction. Although the mechanism of the pH effect on the insoluble fraction is not known, it is speculated that the pectins associated with the cellulose fibrils in the cell walls make tight complexes or fascicles with proteins, resulting in a strong interaction or network around the cell surface.

The pectin-protein interaction in tomato products could be explained by the amphoteric property of the protein. Although pectins do not have a well-defined dissociation constant, the pKa is reported to vary from 3.0–5.0 (Deuel and Stutz, 1958). If the tomato protein has an isoelectric point similar to that of bovine albumin, 4.7–4.9 (Young, 1963), there might be some difference between the pKa of the pectin and the isoelectric point of the protein. If this is the case, pectin and protein in tomato products may be electrostatically attracted to each other or repulsed, as illustrated in Fig. 6, because the electrostatic charges of pectins and proteins may be opposite or similar depending on the pH. Since amino acid composition of soluble protein fraction in tomato juice is similar to that of bovine serum albumin (Table 2), the apparent isoelectric point of the soluble proteins could be at a range of 4.7–4.9. Therefore, it is strongly suggested that the above hypothetical pectin-protein interaction may occur in tomato products.

Wagner and Miers (1967) reported that lower pH resulted in higher consistency of tomato juice through inactivation of pectin degrading enzymes during its preparation. On the other hand, Crean (1969) investigated the direct effect of pH on juice consistency and reported that pH influence could be explained by physical changes in the cell wall materials and pectins because the extractability of pectin did not change at the pH range used. The effect was quite reversible and the consistency could be completely restored even after re-adjusting the pH. Dougherty and Nelson (1974) also showed that lowering the pH of tomato juice resulted in lower consistency. However, they reported that this pH effect was not reversible. In order to understand these different observations, one must consider the processing conditions.

Prolonged heating during concentration might change the characteristics of the complex of pectin and protein in tomato products. Since the natural pH of tomato products is within a range 4.2–4.5, the interaction of pectin with protein can be present throughout the processing. Therefore, severe heating or prolonged heating may denature the protein and stabilize its complex with pectin resulting in an irreversible complex formation. In fact, tomato puree diluted from higher solids such as 20° Brix did not show a change in consistency with a change in the pH. Imeson et al. (1977) separated a pectate-bovine serum albumin complex by Sephadex G-100 gel filtration after heating at 70°C for 15 min, suggesting that relatively mild heating may be sufficient to stabilize the complex. Furthermore, since the pKa of pectin is greatly influenced by the meth-

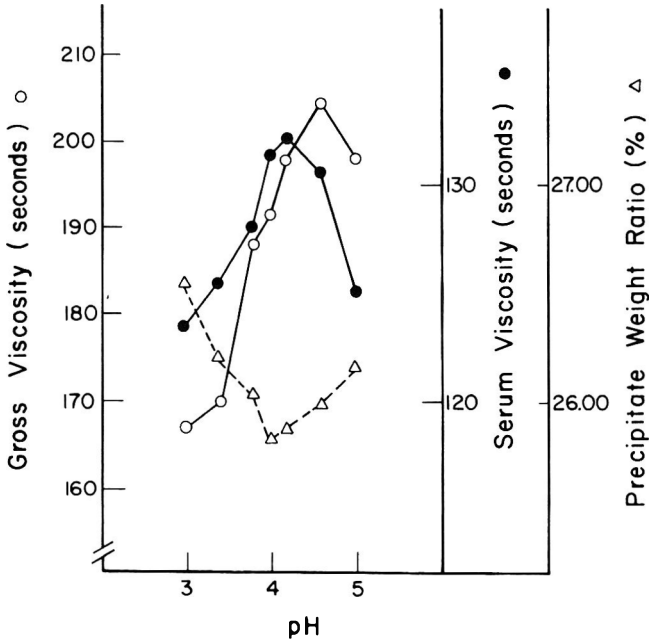

Fig. 5—Effect of pH on the consistency, serum viscosity and precipitate weight ratio of tomato puree.

Table 1—Protein content in tomato puree

Cultivar	Protein content in dried matter (%)	
	Serum fraction	Insoluble fraction
Knox	14.84	19.63
Campbell-28	10.14	22.89

Table 2—Amino acid composition of bovine serum albumin and water-soluble proteins from tomato juice

	Molar ratio of amino acid	
	Bovine serum albumin[a]	Tomato water soluble proteins
Arginine	3.98	3.75
Histidine	3.05	2.00
Lysine	10.32	6.67
Glumatic acid	12.21	11.73
Aspartic acid	9.62	12.71
Glycine	2.82	9.02
Alanine	8.21	7.17
Valine	5.87	6.53
Leucine	11.03	10.21
Isoleucine	2.35	5.68
Proline	4.81	6.22
Phenylalanine	4.69	4.08
Serine	4.69	4.02
Threonine	5.75	5.70
Tyrosine	3.29	3.29
Hydroxyproline	0.0	–
Tryptophan	0.35	–
Cystine		
Cystine (1/2)	6.33	0.00
Methionine	0.00	1.22

[a] Stein and Mohr (1949)

oxyl-content, some processing conditions such as hot break or cold break may change the pH to maximize the interaction between pectin and protein in tomato products.

Interaction of protein with polysaccharides has been studied to modify a protein food such as a milk product or plant protein concentrate (Ledward, 1979; Lin, 1977). Imeson et al. (1978) used pectate from orange peel albedo to recover proteins from blood plasma. They showed a pattern in protein recovery against pH which corresponded to the results shown in our study. Lin (1977) reported that the stabilizing ability of K-carageenan in casein solution was dependent on the pH. A 100% stability of protein suspension was obtained above pH 5.0. Our results reported here showed a similar phenomenon occurs in natural products such as tomatoes.

Vegetables became firmer in acidic pH condition. Goto et al. (1969) compared the degrading characteristics of pectin at neutral and acidic pH and concluded that the softening of vegetables upon boiling in alkaline solution or firmness in vinegar was due to the different stability of pectin. However, the pectin-protein interaction in such a phenomenon may have to be considered because it is known that the cell membranes of plants produce a rigid structure because of protein and pectin in addition to cellulose.

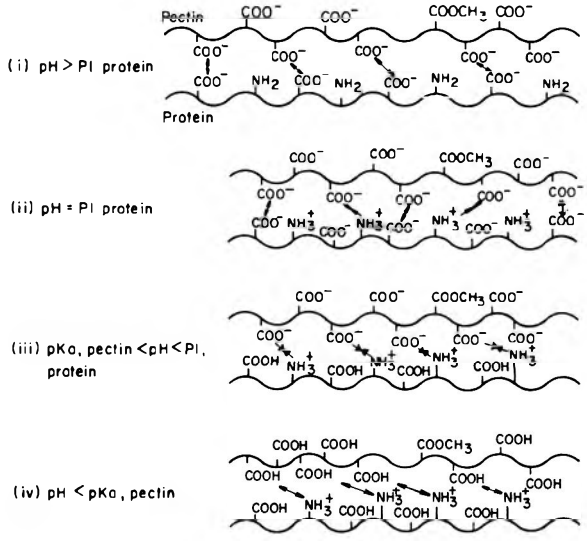

Fig. 6—Suspected schematic model of pectin-protein interaction in tomato product.

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Characterization of Soluble and Bound Peroxidases in Green Asparagus

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ABSTRACT

Soluble and ionically bound peroxidases were extracted from green asparagus with 0.05M sodium phosphate (pH 7.0) and the same buffer containing 1.0M NaCl, respectively. The two forms of peroxidase have been purified 237 and 53 fold, respectively, through ammonium sulphate fractionation, and successive chromatography on Sephacryl S-200 and ConA Sepharose 4B columns. Eleven isoenzymes with different pI values were detected from the soluble form using isoelectric focusing and eight from the ionically bound form. The two forms of perooxidase showed a similar optimum pH range of 4.2–5.0 using three kinds of hydrogen donor with different buffers. The optimum temperature of the two peroxidase forms at pH 4.5 was around 50°C. Heat inactivation of both forms at 70° and 90°C was observed to be biphasic.

INTRODUCTION

PEROXIDASE is widely distributed in higher plants. The subject has been reviewed by Maehly (1955), Paul (1963), Saunders et al. (1964), Whitaker (1972), Scott (1975), Burnette (1977) and Vamos-Vigyazo (1981). Joslyn and Bedford (1940) and Joslyn (1949) reported on the effect of scalding condition on flavor retention and enzyme activity in asparagus. Winter (1969) reported on the behavior of peroxidase in asparagus during blanching. He indicated that the rate of destruction of peroxidase in asparagus was mainly dependent on the particle size and rate of heat transfer. Haard et al. (1974) reported that the activities of peroxidase recovered from the butt, mid and tip sections of freshly harvested asparagus spears were different. They also reported on the differences of isoperoxidase distribution in the three sections and on the influence of exogenous ethylene on isoperoxidase change. Peroxidase has been shown to exist in plant tissues in soluble, ionically bound and covalently bound forms (Haard and Tobin, 1971; Haard, 1973; Haard and Marshall, 1976; Gkinis and Fennema, 1978; Lee, 1973; Kahn et al., 1981). Very little is known about the properties of purified peroxidase from asparagus.

This paper describes procedures for isolating and purifying soluble and ionically bound peroxidases from asparagus. Some enzymatic properties of the two forms of peroxidase are also presented.

MATERIALS & METHODS

Green asparagus

Fresh asparagus spears (*Asparagus officinalis* L., UC 157 variety) were harvested from the University farm in Davis. They were cut 15 cm from the tips. Spear diameters were measured at the cut end which ranged from 1–2 cm. The asparagus spears were washed, drained and frozen in a blast freezer at −50°C, then sealed in nylon bags and stored at −25°C.

Extraction of crude peroxidase

Extraction of crude peroxidase from green asparagus spears was performed with some mofidication of the procedure described by Gkinis and Fennema (1978). The details are as follows:

Unless otherwise stated, the extraction and purification procedures were carried out at 4°C. The frozen spears were thawed and cut into small pieces. 1000g asparagus pieces were blended for 2 min in a Waring Blendor with 1000 ml 0.05M sodium phosphate (pH 7.0) and 50g PVP (polyvinylpolypyrolidone). The pulp was homogenized for 1 min in a Virtis homogenizer, and then squeezed through six layers of cheesecloth. The residue was saved for further extraction. The extract was centrifuged at 18,000 × *g* for 30 min. The supernatant was designated as soluble peroxidase.

The residue from the asparagus pulp was washed thoroughly with deionized water until the washings were free from peroxidase activity. The washed residue was dispersed in 1500 ml 0.05 M sodium phosphate (pH 7.0) containing 1M NaCl, stirred overnight, then squeezed through six layers of cheesecloth. The extract was centrifuged at 18,000 × *g* for 30 min. The supernatant was designated as ionically bound peroxidase.

Purification

The soluble and ionically bound peroxidase extracts were treated separately with ammonium sulphate to reach 90% saturation at 0°C, and then centrifuged at 18,000 × *g* for 30 min. The precipitates were dissolved in the respective buffers used for extracting the soluble and ionically bound peroxidases. The two ammonium sulphate fractions were dialyzed against 0.05M sodium acetate (pH 3.8) for 16 hr. The buffer was changed after dialysis for 8 hr. The dialyzates were centrifuged at 18,000 × *g* for 30 min. The precipitates were discarded and the supernatants were concentrated by ultrafiltration in an Amicon ultrafiltration cell equipped with a YM-10 membrane. The concentrated enzyme solutions were dialyzed against 0.05M sodium phosphate (pH 6.0) containing 0.8M NaCl.

The concentrated enzyme solutions were applied to a sephacryl S-200 (Pharmacia Fine Chemicals) column (2.6 × 100 cm) equilibrated with 0.05M sodium phosphate (pH 6.0) containing 0.8M NaCl, and eluted with the same buffer. The flow rate was 30 ml/hr. Each fraction (6.5 ml) was monitored for protein absorbance at 280 nm in a Perkin Elmer 575 spectrophotometer, and also for peroxidase activity (see below). The soluble peroxidase solution was divided into two parts, and then applied to the column successively. The fractions containing peroxidase activity were combined and concentrated by ultrafiltration, and then dialyzed against 0.05M Tris-HCl (pH 7.4) containing 0.2M NaCl. The dialyzates were applied to a ConA-Sepharose 4B (Pharmacia Fine Chemicals) column (1.6 × 40 cm) equilibrated with 0.05M Tris-HCl (pH 7.4) containing 0.2M NaCl. The column was washed with 1.5 bed volumes of starting buffer, and then eluted with 0.05M Tris-HCl (pH 7.4) containing 1.0M NaCl and 0.3M α-Methyl-D-Mannoside. The volume of each fraction was 4 ml. The flow rate was maintained at 10 ml/hr. Protein content and peroxidase activity in each fraction was monitored. The fractions containing peroxidase activity were collected and dialyzed against deionized water. The dialyzates were freeze-dried and then stored in a freezer for further study.

Peroxidase assay

Peroxidase activity was assayed spectrophotometrically at 470 nm according to the method of Kahn et al. (1981) with some modification. The assay mixture contained 0.225 ml 0.3% H_2O_2, 0.225 ml 1% guaiacol in water and 2.5 ml 0.2M sodium acetate (pH 4.5). The reaction was initiated at 30°C after addition of 0.01–0.02 ml enzyme solution. Preliminary trials showed that the optimal pH of this reaction system was around 4.5. A Perkin Elmer Model 575 spectrophotometer equipped with thermostatical cell compartment and recorder was used to monitor absorbance

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change with time. Enzyme activity was calculated from the linear portion of the curve. One unit of peroxidase activity is defined as 1 optical density increment/min at 470 nm.

Protein content

Protein content was determined by the Bio-Rad protein assay method (Bio-Rad Laboratories, 1979). Bovine plasma gamma globulin was used as a protein standard.

Optimum pH

Three types of hydrogen donor in various buffers were used to determine the optimum pH of the peroxidase as follows:

(a) The optimum pH of the peroxidase was determined using guaiacol as hydrogen donor over a pH range of 3.6–5.6 with 0.2M acetate buffer, and of 3.0–6.6 with McIlvaine citrate-phosphate buffer.

(b) A modification of the method of Vetter et al. (1958) was also used for assaying peroxidase activity using o-phenylenediamine as hydrogen donor. The reaction mixture consisted of 0.2 ml 0.3% H_2O_2, 0.1 ml 1% o-phenylenediamine in 95% ethanol and 2.6 ml citrate-phosphate buffer over pH range 3.0–6.6. The mixture was equilibrated at 30°C, and then 0.01 ml enzyme solution was added. The change in absorbance with time was recorded at 430 nm. The peroxidase activity was calculated as before.

(c) A third method, a modification of the procedure described in the Worthington Enzyme Mannul (1972) was used for determining optimum pH of peroxidase at 30°C with o-dianisidine as hydrogen donor. The assay mixture consisted of 0.03 ml 0.3% H_2O_2, 0.025 ml 1% o-dianisidine in methyl alcohol, 2.9 ml citrate-phosphate buffer over pH range 3.0–6.6, and 0.01 ml enzyme solution. The change in absorbance with time was recorded at 460 nm.

Optimum temperature

The optimum temperature for asparagus peroxidase was determined by assyaing the activity at 30–60°C with increments at 5°C. The reaction mixture was the same as described in the "peroxidase assay" section.

Heat treatment

The procedure described by Wang and Dimarco (1972) was followed with some modification. Pyrex glass tubes of 15 cm long, 3 mm outer diameter and 0.6 mm wall thickness were sealed at one end. Asparagus peroxidase dissolved in 0.05 M acetate buffer (pH 5.0) was introduced to the glass tube with a capillary pipet. The glass tube containing the enzyme solution was preheated to 45°C in a constant temperature bath, and then transferred to another bath at 70°C or 90°C. After definite heating time was attained, the glass tube was cooled rapidly in an ice bath. Enzyme activity was assayed with o-phenylenediamine as hydrogen donor as described earlier.

Isoelectric focusing in polyacrylamide gel

Isoelectric focusing of asparagus peroxidase was performed according to the procedure described in the booklet, "Polyacrylamide Gel Electrophoresis-Laboratory technique" (Pharmacia Fine Chemicals, 1980) with some modification. The size of gel was 10 x 0.5 cm I.D. Total monomer concentration was 7.5% and methylene bisacrylamide constituted 3% of the total monomer. Then the ampholine reagent (pH range 3–10) (Pharmacia Fine Chemicals) was added to form a gel with a final concentration of 1 part in 15 parts (v/v). Ten percent of glycerol (v/v) was added to improve the consistency of gel (Righetti and Drysdale, 1973). The top and bottom chambers were filled with cathode electrolyte (0.01M ethylenediamine) and anode electrolyte (0.01M iminodiacetic acid), respectively. The enzyme sample dissolved in the 1 in 15 solution of the ampholine containing 15% sucrose was applied to the top part of the gel after prefocusing. For enzyme staining one unit of enzyme was used for each sample, and for protein staining 150 microgram of enzyme sample was applied for each one. Enzyme was focused at constant voltage (500v) and at 4°C for 9.5 hr. The procedure for enzyme staining was performed according to the method of Lee (1973), except that the gel was incubated for only 10 min. The staining solution contained a 0.03% H_2O_2, 0.05% benzidine and 0.2M acetate buffer (pH 4.5). The procedure described in the booklet as indicated above was used for protein staining. The blank gel (without the samples) was cut into 20 slices of equal length. Each slice was extracted with 0.8 ml 0.01M KCl solution for 6 hr. The pH of each solution was measured with a combined microelectrode to determine the pH gradient along the length of the gel.

SDS-Polyacrylamide gel electrophoresis

The sodium dodecylsulphate-polyacrylamide gel electrophoresis was performed following the Laemmli procedure (Laemmi and Favre, 1973) for molecular weight estimation except that 12% and 4% total monomer concentrations were used for separating and stacking gels, respectively. The SDS gel electrophoresis standards from Bio-Rad laboratories consist of lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin and phosphorylase B with molecular weight ranging from 14,300–96,000 daltons. The molecular weight range of asparagus peroxidase was obtained by comparing the mobilities of protein bands with standards.

Table 1—Purification of Soluble Peroxidase from Asparagus

Procedure	Volume (ml)	Activity units/ml	Total units	Protein content mg/ml	Total protein (mg)	Specific activity units/mg of protein
Crude extract	1390	1.80	2502	5.93	8242	0.304
Ammonium sulfate fractionation	320	5.75	1840	13.3	4256	0.432
Change buffer to 0.05M acetate buffer (pH 3.8)	433	4.1	1775	1.01	437	4.06
Sephacryl S-200 column	145	9.5	1378	0.837	121	11.4
Concanavalin-A Sepharose column	8	106	848	1.46	11.7	72.5

Table 2—Purification of ionically bound peroxidase from asparagus

Procedure	Volume (ml)	Activity units/ml	Total units	Protein content mg/ml	Total protein (mg)	Specific activity units/mg of protein
Crude extract	1500	3.1	4650	0.740	1110	4.19
Ammonium sulfate fractionation	132	32	4224	6.67	880	4.80
Change buffer to 0.05M acetate buffer (pH 3.8)	186	21	3906	1.24	231	17.1
Sephacryl S-200 column	68	46.4	3155	0.741	50.4	62.6
Concanavalin-A Sepharose column	8	198	1584	0.865	6.92	229

RESULTS & DISCUSSION

Purification of asparagus peroxidase

Results on purification of asparagus peroxidase are summarized in Tables 1 and 2. Both soluble and ionically bound peroxidases were stable in 0.05M acetate buffer pH 3.8. After ammonium sulfate fractionation, the enzyme solutions were dialyzed against 0.05m acetate buffer (pH 3.8). More than 96% of soluble enzyme activity and 92% of ionically bound enzyme activity remained in solutions while 90% and 74% of the proteins were precipitated. The specific activities of the soluble and ionically bound peroxidases increased significantly. A Sephacryl S-200 column (2.6 x 100 cm) was used for purification of the peroxidase by gel filtration chromatography. The elution profiles of soluble and ionically bound peroxidases on Sepharcyl S-200 column are shown in Fig. 1 and 2, respectively. Both forms of enzyme were eluted as a single activity peak around the similar elution volume (292.5 ml and 299 ml, respectively). Peroxidases from a variety of plants have been known to be glycoproteins. Maehly (1955) reported that the content of carbohydrates in horseradish peroxidase was 18.4%. Darbyshire (1973) also studied the glycoprotein nature of peroxidase from pea roots. ConcanavalinA (ConA) is a lectin isolated from jack bean meal which can bind carbohydrate molecules containing α-D-mannopyranosyl, α-D-glucopyranosyl and sterically analogous residues (Goldstein et al., 1965). Brattain et al. (1976) studied horseradish peroxidase (HRP) of various degrees of purity using a Sepharose-bound ConA column (2.5 x 25 cm). The Rz values (A_{403}/A_{275}) of HRP were increased from 0.62, 1.20 and 2.45 to 2.78, 2.95 and 3.08, respectively. From results shown in Fig. 3 and 4, it was seen that most parts of soluble and ionically bound peroxidases were adsorbed on ConA-Sepharose 4B, and most unbounded proteins were washed out by the starting buffer. The specific activities of the purified peroxidase were increased 237 fold for the soluble peroxidase

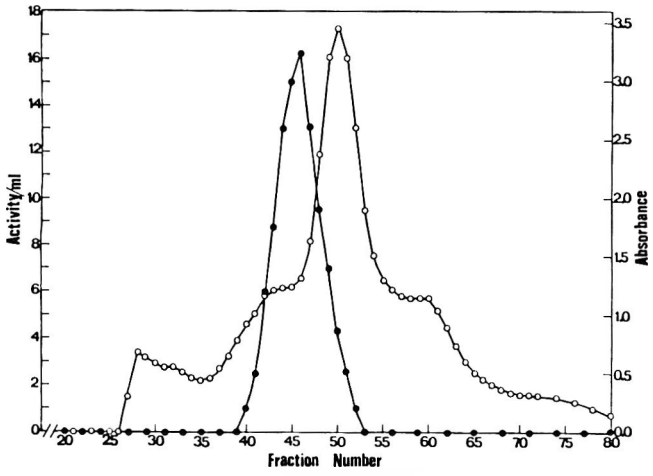

Fig. 1—Sephacryl S-200 chromatography of soluble asparagus peroxidase. The sample was divided into two parts for applying to the column twice. The column (2.6 x 100 cm) was equilibrated and eluted with 0.05M sodium phosphate (pH 6.0) containing 0.8M NaCl. The volume of each fraction was 6.5 ml. Absorbance (○) was measured at 280 nm and peroxidase activity (●) was assayed as described in the text.

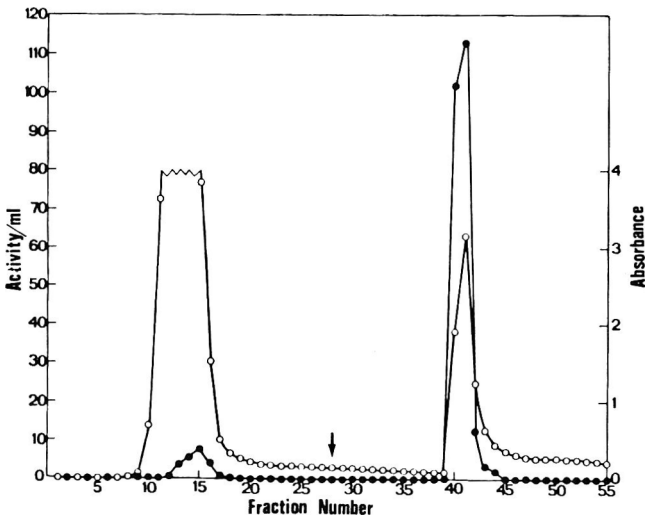

Fig. 3—ConA-Sepharose 4B chromatography of soluble asparagus peroxidase after Sephacryl S-200 chromatography. The column (1.5 x 40 cm) was equilibrated with 0.05M Tris-HCl (pH 7.4) containing 0.2M NaCl. At the point indicated by the arrow elution was started with the same buffer containing 0.3M α-Methyl-D-Mannoside and 1.0M NaCl. The volume of each fraction was 4 ml. Absorbance (○) was measured at 280 nm and peroxidase activity (●) was assayed as described in the text.

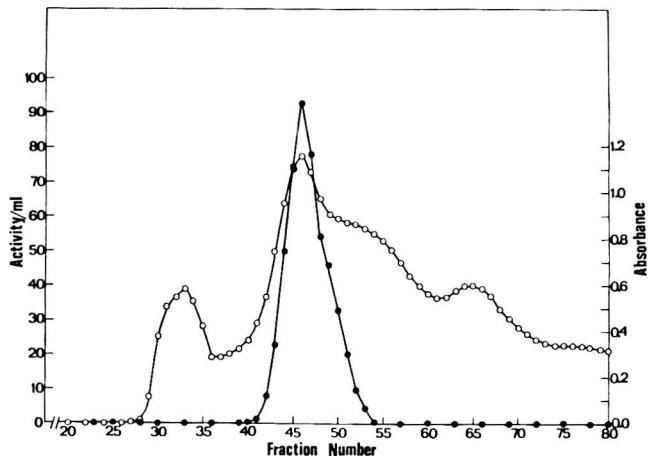

Fig. 2—Sephacryl S-200 chromatography of ionically bound asparagus peroxidase. The sample was applied once only. Other conditions were same as those shown in Fig. 1.

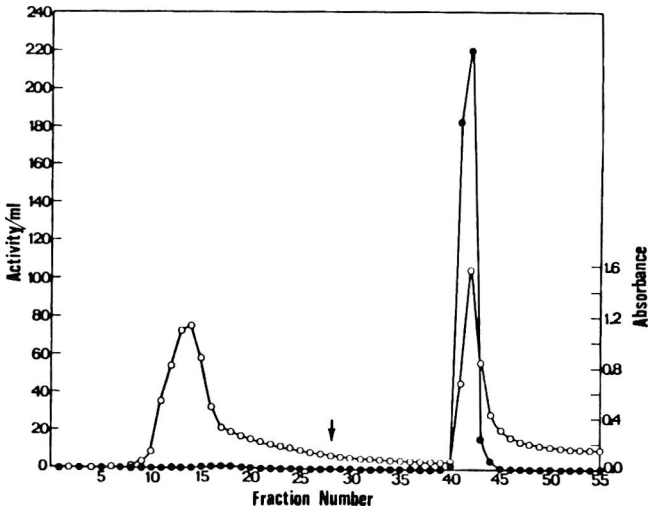

Fig. 4—ConA-Sepharose 4B chromatogrpahy of ionically bound asparagus peroxidase after Sephacryl S-200 chromatography. The conditions were same as those shown in Fig. 3.

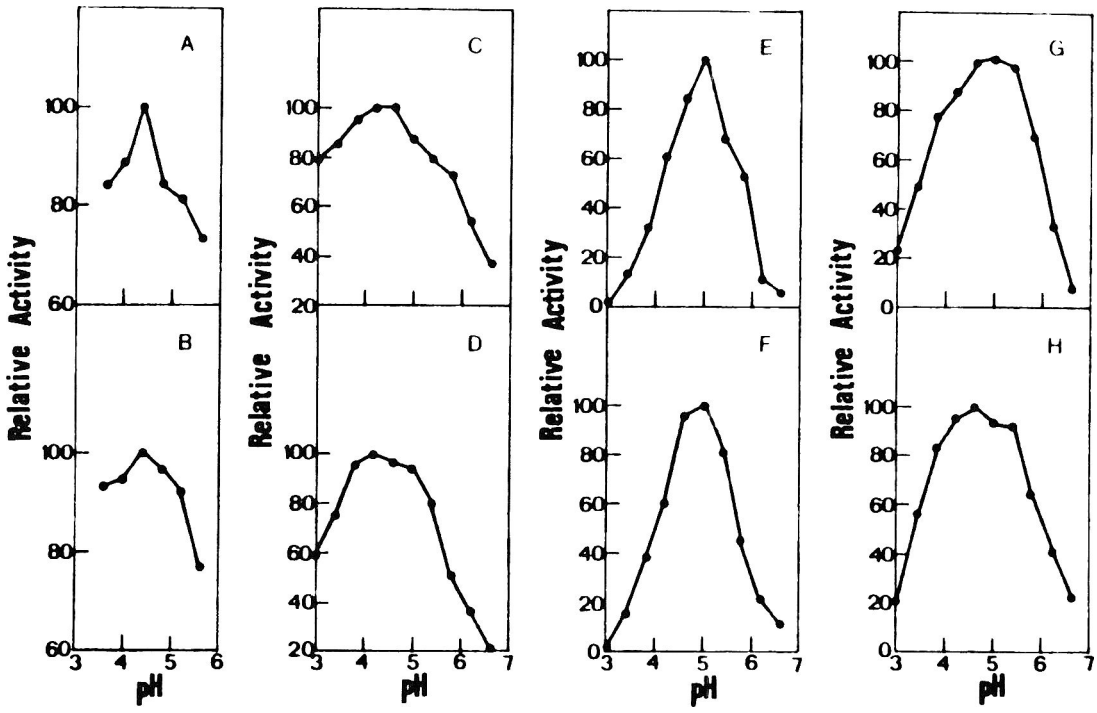

Fig. 5—Activity of soluble and ionically bound peroxidases from asparagus as a function of pH.

A. Soluble peroxidase, buffer: acetate, hydrogen donor: guaiacol.
B. Bound peroxidase, buffer: acetate, hydrogen donor: guaiacol.
C. Soluble peroxidase, buffer: citrate-phosphate, hydrogen donor: guaiacol.
D. Bound peroxidase, buffer: citrate-phosphate, hydrogen donor: guaiacol.
E. Soluble peroxidase, buffer: citrate-phosphate, hydrogen donor: o-phenylenediamine.
F. Bound peroxidase, buffer: citrate-phosphate, hydrogen donor: o-phenylenediamine.
G. Soluble peroxidase, buffer: citrate-phosphate, hydrogen donor: o-dianisidine.
H. Bound peroxidase, buffer: citrate-phosphate, hydrogen donor: o-dianisidine.

and 53 fold for the ionically bound enzyme. The specific activity of the ionically bound form was about three times as high as that of the soluble form because most of the extractable proteins remained in the crude extract of the soluble peroxidase. The Rz values of the soluble and ionically bound peroxidases were 0.25 and 0.59, respectively. Shannon et al. (1966) reported that the Rz values for isoenzymes of horseradish peroxidase ranged from 2.5–4.19. Although the ratio of absorbance at 403 to 275 nm was shown to be different among peroxidases from different sources (Whitaker, 1972), the Rz values of the asparagus peroxidase obtained here indicated that the soluble and bound forms of enzyme were partially purified.

Optimum pH

The optimum pH of soluble and ionically bound peroxidases with different hydrogen donors and buffers ranged from 4.2–5.0 (Fig. 5). The optimum pH of both soluble and ionically bound peroxidases increased with different hydrogen donors in order of guaiacol, o-dianisidine and o-phenylenediamine. On comparing with the data collected by Vamos-Vigyazo (1981), the optimum pH of peroxidase from asparagus is similar to that of green bean (5.0), potato (5.0–5.4) and pineapple (4.2).

Optimum temperature

The activity of both forms increased with increase in temperature from 30°C up to 50°C (Fig. 6). When the temperature was higher than 50°C, the enzyme became unstable, and the plot of absorbance vs time for enzyme assay exhibited a nonlinear relationship. At temperatures above 50°C, it was difficult to determine enzyme activity accurately according to the initial speed of the enzyme reaction.

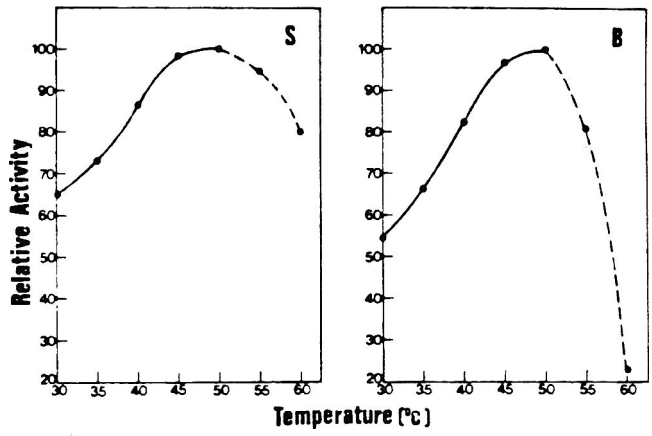

Fig. 6—Activity of soluble (S) and ionically bound (B) peroxidases from asparagus as a function of temperature. Dotted line indicates that peroxidase becomes unstable at high temperature.

Joslyn and Bedford (1940) reported that peroxidase activity in asparagus tissue did not chane appreciably with the temperature of scalding in the interval, 20–60°C, but it was little affected by heating at 55°C. The peroxidase used in the present investigation was less heat-stable than the crude enzyme in the asparagus tissue.

Heat stability

The effect of heating soluble and ionically bound peroxidases at 70°C and 90°C (pH 5.0) on enzyme inactivation

is presnted in Fig. 7. The percent residual activity on a log scale was plotted against time. The curves showed the heat inactivation of the two forms of peroxidase at two different temperatures to be diphasic. According to Yamamoto et al. (1962), the initial line represented inactivation of heat-labile peroxidase and the second the heat-resistant enzyme. Following their method of extrapolating the heat-resistant curve to zero time, it was found that at 70°C about 12% of the total activity existed in the heat-resistant form for soluble peroxidase and 31% for the ionically bound enzyme. When the heat treatment temperature increased to 90°C, the heat resistant form reduced to 3%

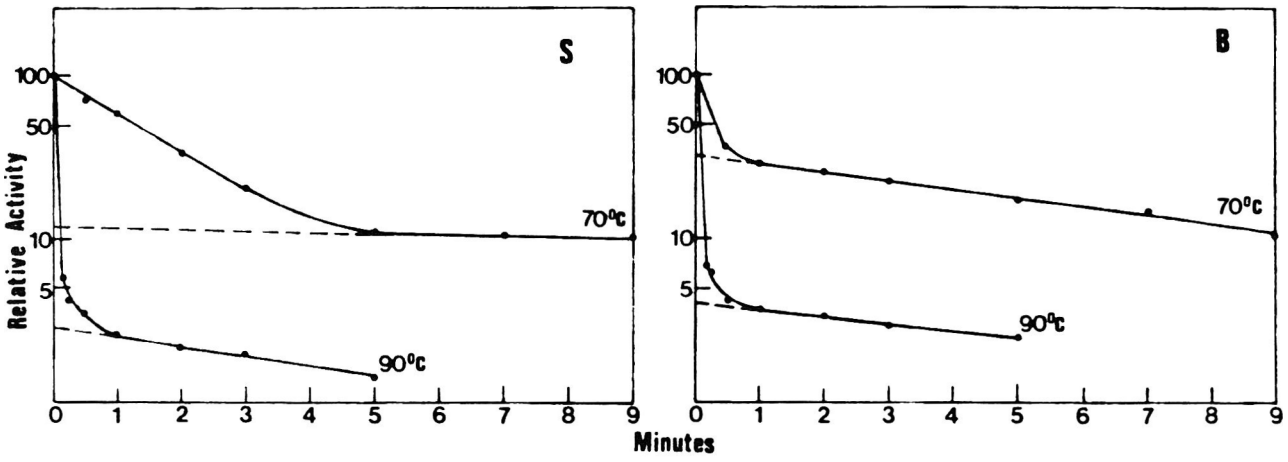

Fig. 7—Heat stability of soluble (S) and ionically bound (B) peroxidases from asparagus at 70° C and 90° C.

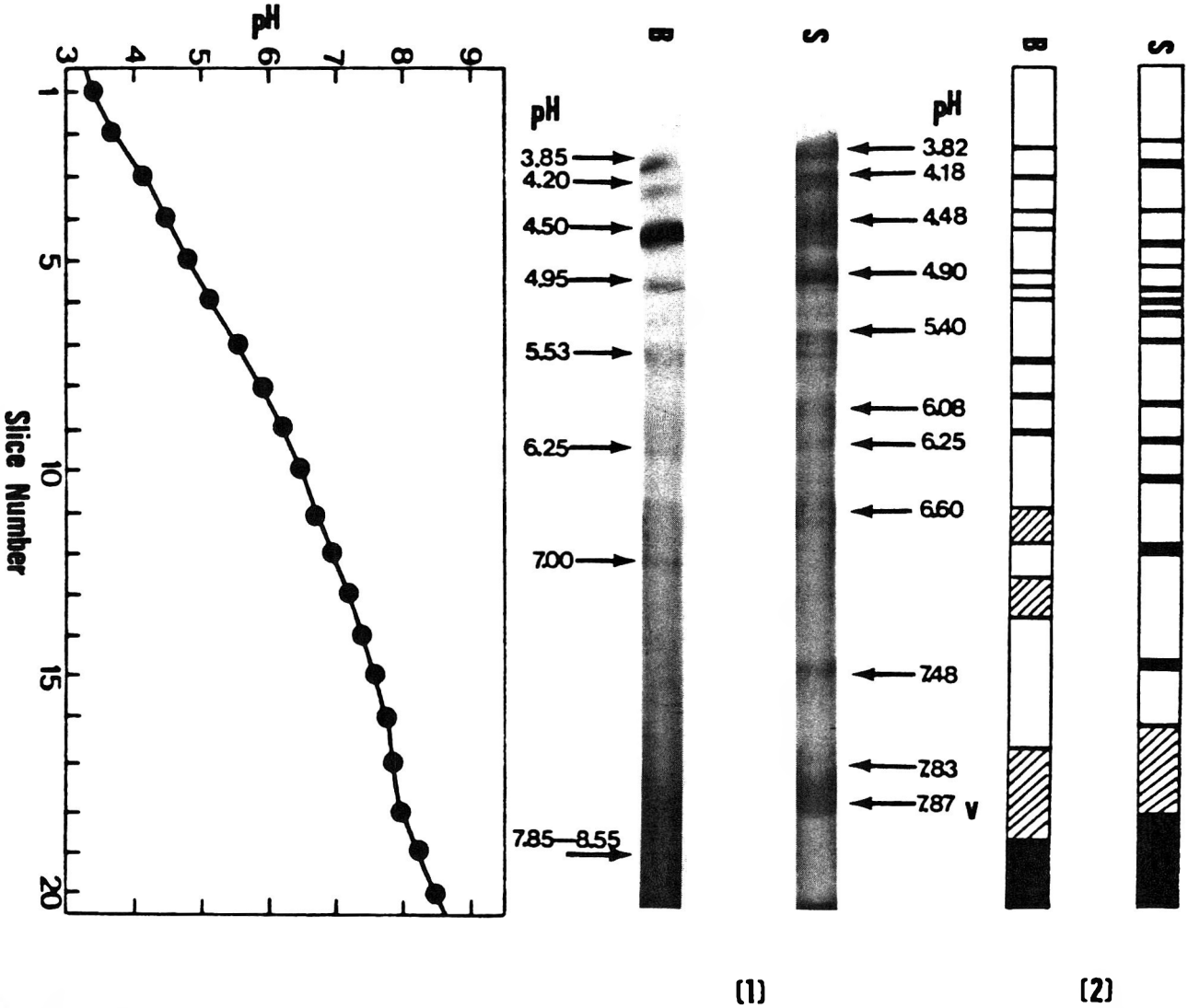

Fig. 8—Isoelectric focusing of the soluble and bound forms of asparagus peroxidase in 7.5% acrylamide gels containing ampholine (pH 3—10) with a final concentration of one part in fifteen parts (v/v). The amount of enzyme applied in each gel was as follows: one unit of activity for enzyme staining (1) and 150 microgram of enzyme sample for protein staining (2). S = soluble and B = ionically bound peroxidase. Electric focusing time: 9.5 hr at constant voltage (500v).

and 4%, respectively. It was noted that about 65% of total activity of bound form was inactive after 30 sec of heat treatment at 70°C, and only 32% was lost for soluble form. This indicated that the heat-labile fraction from bound form was more unstable than that from soluble form. The similar difference also could be seen in Fig. 6. The activity of bound form decreased much faster than that of soluble form at temperatures exceeding 55°C (pH 4.5). According to Winter (1969) the destruction speed of peroxidase in asparagus during blanching in boiling water followed the first order kinetics. The heat-resistant form in asparagus peroxidase was not noted. The difference in heat treatment of the samples might explain the discrepancy.

Isoelectric focusing in polyacrylamide gel

The soluble and ionically bound peroxidases were focused in polyacrylamide gels containing ampholine (pH range 3–10). The results after enzyme and protein staining are shown in Fig. 8. The pH gradient along the gel is shown below the gels in the same figure. At least eleven isoenzymes of soluble peroxidase were observed over the whole gel. There were eight bands in the ionically bound peroxidase. Although some isoenzymes in the two forms of peroxidase have similar pI values, the different patterns can be seen by comparing the bands of the main isoenzymes between the two gels. From pI 3.82–4.90 there were four strong isoenzyme bands present in soluble peroxidase, but only one band (pI 4.50) was predominant in the ionically bound peroxidase. At the other end of the gel, one strong and one weak isoenzyme band of soluble peroxidase was observed with pI 7.83 and 7.87, respectively. For ionically bound peroxidase, one wide and deep band existed in the pH range 7.85–8.55. It was possible that some isoenzymes with pI higher than 8.55 did not enter the gels. In the middle of both gels, the activities of the isoenzymes were rather weak, and some isoenzymes were difficult to be confirmed from the picture. It has been known that plant peroxidase consists of a high number of isoenzymes. Delincee and Radola (1970) reported that eight isoenzymes were separated from a more purified horseradish peroxidase using thin layer isoelectric focusing with ampholine of pH range 3–10, but the number of the isoenzymes was increased to more than 20 from a less purified sample. On comparing the gels of protein staining with the gels of enzyme staining, the protein bands were more than the isoenzyme bands for both soluble and ionically bound forms. So the enzyme samples studied here were only partially purified. The experiments presented here were repeated several times and the patterns of the asparagus peroxidase isoenzymes were reproducible.

Molecular weight

The result of SDS-polyacrylamide gel electrophoresis using a Laemmli discontinuous buffer system is shown in Fig. 9. The three gels represent molecular weight standards (A), soluble (S), and ionically bound peroxidase (B). The positions of most protein bands of the partially purified enzyme were between soybean trypsin inhibitor (MW 21,000 daltons) and Bovine serum albumin (MW 68,000 daltons). The range of the molecular weight of the main protein bands on the gel for soluble peroxidase sample was from 33,000–48,000 daltons. In the case of ionically bound peroxidase sample, the range was from 34,000 to 47,000 daltons. According to Vamos-Vigyazo (1981), the values of the molecular weight of peroxidase from various fruits and vegetables ranged from 30,000 to 54,000 daltons. the results obtained here are near to this value.

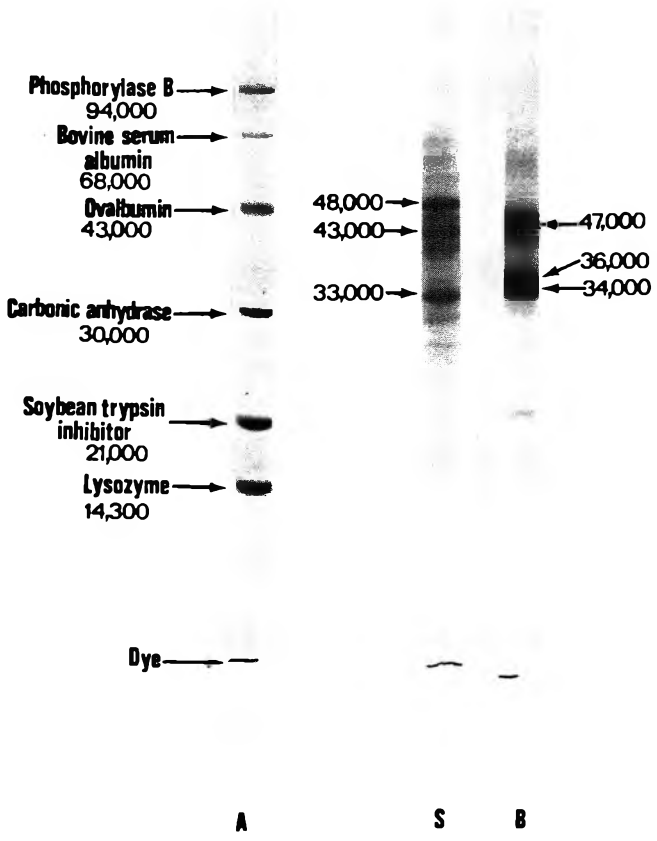

Fig. 9–SDS-polyacrylamide gel electrophoresis of soluble and bound forms of asparagus peroxidase. The conditions were described as in the text. Gel A: molecular weight standards; Gel S: soluble peroxidase; Gel B: bound peroxidase. The estimated molecular weights of protein bands are indicated by numerical values.

CONCLUSION

BOTH SOLUBLE and ionically bound peroxidases from green asparagus consist of numerous isoenzymes. The two enzyme forms have different isoenzyme patterns, but the difference is not considerable, and they show slightly different properties.

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Comparison of Four Methods for Determining Protease Activity in Milk

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ABSTRACT

Four methods (absorbance at 280 nm; the Lowry method; the fluorescamine method, and the trinitrobenzenesulfonic acid method) for determining hydrolysis of milk proteins were compared. Each method was applied to the trichloracetic acid soluble fraction of milk protein, which had been digested with trypsin for various periods of time. Detectability was measured as the ratio between standard error of estimate and slope calculated from the linear regression analysis of Deming for cases when both variables were subject to error. Although it was nondimensional, the detectability thus calculated was simple and reliable for comparing assay methods which were based on different analytical principles. Detectability as well as the detection limit measured according to Schwerdtfeger showed that, of the methods compared, the fluorescamine method was most reliable and sensitive.

INTRODUCTION

AS A CONSEQUENCE of changing socio-economic conditions and production methods, there is a trend toward a longer period of time elapsing between milking and processing of milk. These extended periods of time permit the growth of psychrotrophic bacteria in raw milk (Cousin, 1982). Proteases produced by many strains of psychrotrophic *Pseudomonas* species are extremely heat resistant (Adams et al., 1976) and have been shown to decrease the shelf-life of dairy products (Richter et al., 1979).

The Hull method (Hull, 1947) has been used to measure proteolytic activity in milk (Adams et al., 1975; Gebre-Egziabher et al., 1980; Richter et al., 1979). This colorimetric method measures the acid-soluble tyrosine and tryptophan released during proteolysis. Cogan (1977) indicated that the measurement of proteolysis by means of the increase in trichloroacetic acid (TCA) soluble tyrosine and tryptophan has some disadvantages since little TCA-soluble tyrosine and tryptophan may be released during proteolysis. The need for a rapid, sensitive and reliable method for determining proteolysis has been stressed (Law, 1979; Richter et al., 1979).

Juffs (1973) evaluated the Lowry modification of the Folin procedure (Lowry et al., 1951), expressing proteolysis in terms of tyrosine concentration. Alichanidis and Andrews (1977) studied the proteolytic activity of the extracellular protease produced by a *Pseudomonas* strain using a modification of the Lowry method. Richardson and Te Whaiti (1978) used absorbance at 280 nm (A_{280}) to assay protease activity. Trinitrobenzenesulfonic acid (TNBS), a nonspecific amino acid reagent, was used to measure proteolysis in milk (McKellar, 1981). Snoeren et al. (1979) and Snoeren and Both (1981) determined the nitrogen content in different fractions as ammonia by a rapid colorimetric procedure (Koops et al., 1975). Griffiths et al. (1981) used a protease assay based on the ability of a variety of proteases to release a blue dye covalently attached to denatured collagen. Starch gel and polyacrylamide gel electrophoresis have been used to estimate degradation of casein components in raw milk (Juffs, 1975; Law et al., 1979). Chism et al. (1979) developed a sensitive assay for proteases based on the reaction of amino groups of TCA-soluble peptides and amino acids with fluorescamine. A micromethod, using fluorescamine, for the assay of proteolysis was developed by Castell et al. (1979). Fluorescamine reacts with free amino groups of amino acids and peptides liberated during proteolysis.

Although there have been many papers published for assaying proteolysis, there are only a few papers in which objective and statistically reliable comparison of different methods are described. The papers to compare different methods reported by Schwabe (1973) and by Kas and Rauch (1982) are all without reliable supporting data. Schwerdtfeger (1977) compared the TNBS, Folin, turbidity, radial diffusion on gel and the anilinonaphthalene sulfonate fluorescence methods, using a detection limit determined as the minimum amount of the purest proteases detectable. However, the detection limit measured may be dependent on the analytical conditions, especially incubation conditions. The method is also tedious since the amount of proteases has to be changed until F-value becomes significant ($p < 0.01$; $n = 4$).

The object of this study was to evaluate four methods for measuring protein hydrolysis in milk in terms of their reliability and detectability. The methods compared were: absorbance at 280 nm (Juffs, 1973); the Lowry method (Lowry et al., 1951); a modification of the microfluorometric method of Castell et al. (1979); and the TNBS method (McKellar, 1981). Detectability calculated from the linear regression analysis of Deming (Wakkers et al., 1975) in addition to the detection limit defined by Schwerdtfeger (1977) were used for reliable comparison.

MATERIALS & METHODS

FLUORESCAMINE (Fluram) was obtained from Chemical Dynamics Corp. (South Plainfield, NJ). Trinitrobenzenesulfonic acid was obtained from Eastman Kodak (Rochester, NY) and Folin-Ciocalteau phenol reagent from Fisher Scientific (Fair Lawn, NJ). Trypsin (Type XI, diphenyl carbomyl chloride treated, from bovine pancreas) was obtained from Sigma Chemical Company (St. Louis, MO). All chemicals and reagents were of reagent grade.

Comparison of the protease assay methods

To assay the effect of trypsin on milk proteins, 2.5 ml of 0.02M phosphate buffer (pH 6.6) containing trypsin (24.1 g/ml) were added to 150 ml of 10% (w/v) reconstituted skim milk (RSM) in 0.2M phosphate buffer (pH 6.6). The reaction was run at 40°C. At various time intervals (1–7 min), 5.0 ml of the reaction mixture were withdrawn and the reaction stopped by the addition of an equal volume of 24% (w/v) TCA. The mixture was centrifuged (10,000 x *g*, 5 min) and the supernatant filtered (Whatman No. 4). The filtrate was assayed with the following methods. For some samples it was necessary to dilute with 12% (w/v) TCA to yield on-scale readings.

Absorbance at 280 nm

Absorabnce of the filtrates, which were diluted when necessary (usually diluted 5 times), was determined with a Unicam SP-800B Spectrophotometer (Pye Unicam Ltd., Cambridge, England).

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Lowry method

Filtrate (1.0 ml) was mixed with 4.0 ml of the alkaline copper tartrate reagent as described by Lowry et al. (1951). After 10 min, 0.5 ml of Folin-Ciocalteau reagent, 1.0N in acidity, was added. After incubation at room temperature for 30 min, absorbance was measured at 700 nm with a Unicam SP-800B Spectrophotometer.

Fluorescamine method

To 0.1 ml of filtrate, 0.3 ml of 3M K_2HPO_4 was added followed by 0.15 ml of 0.03% (w/v) fluorescamine in acetone. The mixture was immediately mixed. To adjust the volume to cuvette size, 3.0 ml of distilled water were added. Fluorescence ($\lambda_{excitation}$ = 395 nm; $\lambda_{emission}$ = 480 nm) was measured with an Aminco Bowman 4-8202 spectrofluorometer (American Instrument Co., Inc., Silver Spring, MD).

Trinitrobenzenesulfonic acid method

Filtrate (0.2 ml) was mixed with 2.0 ml of 0.2M $NaBO_3$ buffer (pH 9.2) and 1.0 ml of 4.0 mM TNBS. After incubation at room temperature for 30 min, 1.0 ml of 2.0M NaH_2PO_4 containing 18 mM Na_2SO_3 was added. Absorbance, at 420 nm, was measured with a Unicam SP-800B spectrophotometer.

Statistical comparison of the protease assay methods

Data, in terms of absorbance (from A_{280}, Lowry and TNBS methods) and fluorescence intensities (from the fluorescamine method), were pooled. Deming's method (Wakkers et al., 1975), using a program written for a Monroe 1880–88 programmable calculator, was employed. Data obtained at various time intervals with one proteolysis assay method (e.g. fluorometric) were compared with the data for the same time intervals from another proteolysis assay method (e.g. TNBS). This analysis produced six regression lines (each regression compared two methods). With this program, the standard error of estimate and the slope of the regression line were calculated. The detectability of each proteolysis assay method was expressed as the standard error of estimate of a particular assay method divided by its slope ratio.

For each pair of methods for measuring different hydrolysis products (i.e. amino groups and aromatic groups), the data transformation of Fujii and Nakai (1980) was applied for linearization using a transformation equation of $y^B = ax$. Then, Deming's method was applied for comparison of the methods using y^B vs x as variables.

Determination of the detection limit of the TNBS and fluorescamine methods

The method according to Schwerdtfeger (1977) was followed. To 10 ml of 0.2M borate buffer (pH 7.4) containing 0.05% $CaCl_2$ and 1.0 ml of 10% RSM, 10 ml of trypsin at various concentrations were added. The mixture was incubated at 35°C. After 24 hr, the reaction was terminated by the addition of TCA (3.5% final concentration). The mixture was centrifuged (10,000 x *g*, 5 min) and the supernatant filtered (Whatman No. 4). The filtrate was assayed with the fluorescamine method and the TNBS method as described by Schwerdtfeger (1974). The detection limit was defined as the trypsin concentration required to yield a significant difference ($P < 0.01$; $n = 4$) between samples before and after incubation (Schwerdtfeger, 1977).

RESULTS & DISCUSSION

THE RESULTS of comparison of the methods for determining protease activity are shown in Fig. 1, 2 and 3. Relationships between the fluorescamine method vs the TNBS method (Fig. 1), and the Lowry method vs the A_{280} method (Fig. 3) appear to be linear. This is reasonable since the first pair is both for determining amino groups while the second pair is both for aromatic groups.

On the contrary, the relationship between the TNBS method and the Lowry method (Fig. 2) does not appear to be linear. Similar nonlinear relationships were observed between the TNBS method and the A_{280} method, the fluorescamine method and the Lowry emthod, and the fluorescamine method and the A_{280} method. This curvilinearity may be a consequence of different hydrolysis products being detected by each method. For these pairs of methods the plot was linearized first, then the methods were compared by the Deming's method. Linearization considerably improved the detectability of the Lowry method but not for the other methods (Table 1) probably because of considerable deviation of the Lowry method from linearity.

Table 1 shows that the fluorescamine method was more reliable than the other three methods (smaller standard deviation of the random errors in the method). The TNBS method was more reliable than the Lowry method or A_{280} method as a measure of the degree of hydrolysis of milk proteins. The absorbance at 280 nm was more reliable than the Lowry method.

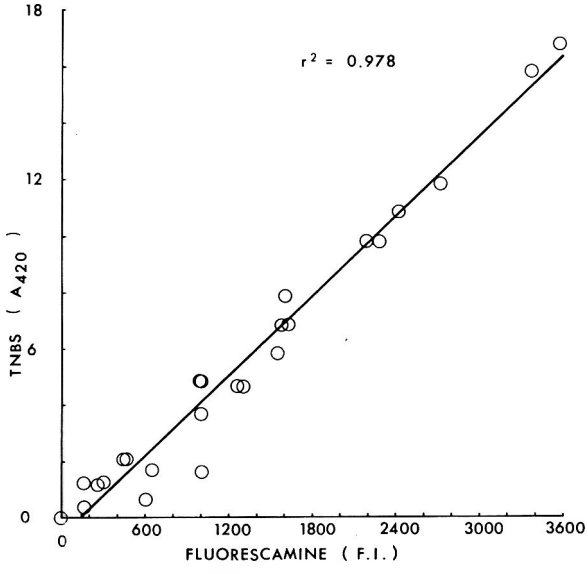

Fig. 1—Comparison of the fluorescamine method with the TNBS method as a measure of protein hydrolysis by Deming's method. Absorbance and fluorescence intensity are expressed as the apparent absorbance and fluorescence intensity of the undiluted solution.

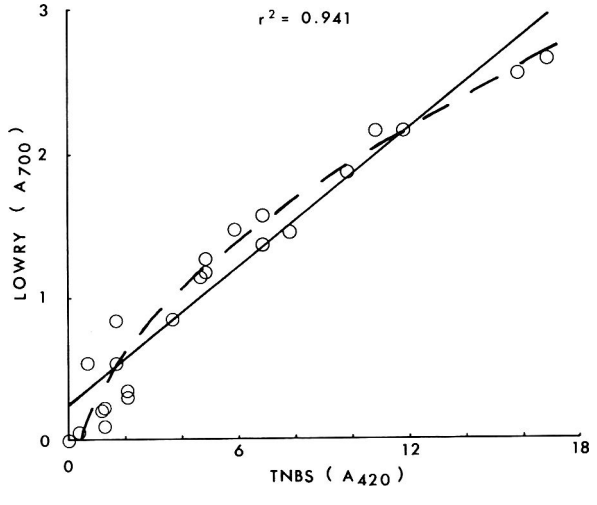

Fig. 2—Comparison of the TNBS method with the Lowry method as a measure of protein hydrolysis by Deming's method. Absorbances are expressed as the apparent absorbances of the undiluted solution. The broken line represents the transformed data from the Lowry method vs. the TNBS method.

Schwerdtfeger (1977) claimed that the TNBS method (Schwerdtfeger, 1974) could detect trypsin (Merck) activity at a level as low as 3 ng/ml. However, using the same analytical conditions but with a trypsin from a different source, we could detect the trypsin activity at a level of 260 ng/ml (P = 0.005, n = 4). This difference in sensitivity between our result and Schwerdtfeger's findings (1977) may be caused by the difference in the purity of the trypsins used. In comparison, the fluorescamine method we proposed could detect trypsin activity at a concentration of 35 ng/ml (P = 0.003, n = 4) according to the method of Schwerdtfeger (1974).

Calculation of the degree of dilution of TCA filtrate prior to measurement of absorbance and fluorescence intensity yielded values of 2.0, 1.82, 0.28, and 0.48 ml of TCA filtrate per 10 ml final diluted volume for the A_{280}, Lowry, fluorescamine and TNBS methods, respectively. This means the fluorescamine method needs the smallest sample amount for the best precision.

As indicated already, the two methods for comparing different assay methods yielded some different results. When the detection limit of Schwerdtfeger (1977) was used, the fluorescamine method was 7.4 times (260/35) more sensitive than the TNBS method. On the other hand, the comparison of detectability used in this study showed that the fluorescamine method was 2.6 times (1.5 in Table 1 multiplied by the sample ratio 0.48/0.28) more sensitive than the TNBS method. This difference in the sensitivity may be due to the difference in the incubation conditions (24 hr at 35°C vs 7 min at 40°C) and different parameters used (detection limit for the method of Schwerdtfeger vs. detectability used in this study).

The four methods chosen were those that could be used in most laboratories and which have been used by various research groups to detect proteolysis in milk and milk products. The microfluorometric method (Castell et al., 1979) was chosen over other published fluorometric methods because the blanks had a lower inherent fluorescence and less fluorescamine was used in this method. Phosphate buffer was chosen to raise the pH of the TCA filtrate to 8.5 because it was shown to be better than borate buffer for this purpose (Stein et al., 1974). The rate of reaction between fluorescamine and primary amines and the fluorescence intensity of the resulting fluorophor depend on pH, solvent composition, temperature and reagent composition (De Bernardo et al., 1974).

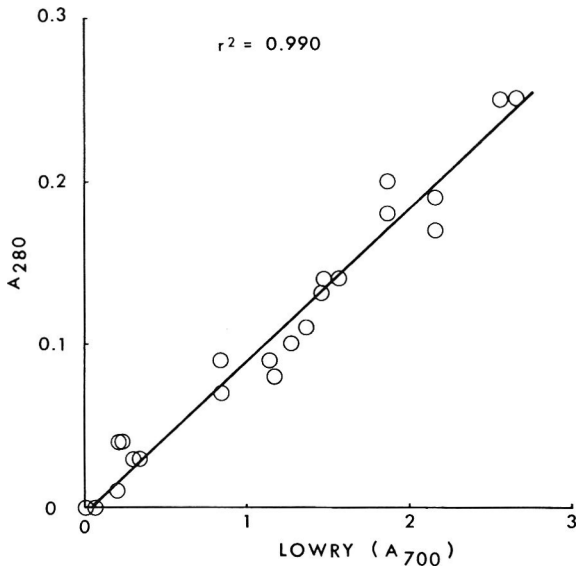

Fig. 3—Comparison of the Lowry method with the A_{280} method as a measure of protein hydrolysis by Deming's method. Absorbances are expressed as the apparent absorbances of the undiluted solution.

Comparison of methods for estimation of protein hydrolysis is difficult. First, the compounds being analyzed are different; aromatic amino acids (A_{280}, Lowry) vs amino groups (fluorometric, TNBS). Second, the instruments used are different; spectrofluorometer (fluorometric) vs spectrophotometer (A_{280}, Lowry, TNBS). When the working principle of the methods is different, direct comparison of the increase in fluoroscent intensity and the increase in absorbance is not appropriate (Schwabe, 1973). Since there is no error-free or referee method, direct application of regression analysis to compare the result of one method vs. the result of another method is not totally justified.

A sophisticated method using principal component analysis was suggested by Newell (1981) for comparing analytical methods when an error-free or referee method is unavailable. The reliability of this method, however, is doubtful when the analyses are for undefined objects, such as degree of hydrolysis.

Deming's method minimizes the weighted sum of squares:

$$S = \Sigma[(x_1 - \bar{X}_i)^2 + (y_i - \bar{Y}_i)^2 \lambda] \quad (1)$$

instead of

$$S = \Sigma[(x_i - \bar{X}_i)^2 + (y_i - \bar{Y}_i)^2] \quad (2)$$

in the classical linear regression procedure, where $\bar{X}_i$ and $\bar{Y}_i$ be the estimates for X_i and Y_i; λ is a 'weight':

$$\lambda = V(\epsilon)/V(\delta) \quad (3)$$

where $V(\epsilon)$ and $V(\delta)$ are the variances corresponding to x and y. Therefore, Demings procedure is a weighted linear regression analysis.

The "detectability" 2N/S described by Massart et al. (1978) was used in this study, where N and S are the average noise amplitude (roughly equals 4δ: standard error of estimate calculated was used instead) and sensitivity [the slope ratio of the statistical procedure of Deming described by Wakkers et al. (1975)]. The sensitivity was used for comparison of two analytical methods which are subject to random errors. Since N and S are both expressed by either absorbance or fluorescence intensity units, the parameter 2N/S cancels out difference in instrument as well as difference in compound analyzed. As Holguin and Nakai (1980) reported, the slope of the Deming plot reflects the selectivity of the analytical method and the selectivity relates compounds being analyzed.

Table 1—Detectability of the fluorescamine method (Fluo), the TNBS method, the Lowry method and the absorption at 280 nm (A_{280}) as estimated by Deming's procedure[a]

	Fluo	TNBS	Lowry	A_{280}
Direct comparison				
Fluo and TNBS	98 (1.0)	155 (1.58)		
Fluo and Lowry	46 (1.0)		192 (4.17)	
Fluo and A_{280}	71 (1.0)			189 (2.63)
TNBS and Lowry		0.45 (1.58)	1.22 (4.28)	
TNBS and A_{280}		0.64 (1.58)		1.01 (2.38)
Lowry and A_{280}			0.14 (4.17)	0.08 (2.38)
(average)	(1.0)	(1.6)	(4.2)	(2.5)
After linearization				
Fluo and Lowry	47 (1.0)		132 (2.81)	
Fluo and A_{280}	64 (1.0)			158 (2.47)
TNBS and Lowry		0.37 (1.73)	0.60 (2.81)	
TNBS and A_{280}		0.45 (1.28)		0.87 (2.47)
(average)	(1.0)	(1.5)	(2.8)	(2.5)

[a] The figures in parentheses are calculated for direct comparison of detectability by assigning 1.0 to the fluorescamine method.

The method of Schwerdtfeger (1977) has an advantage, by which the detection limit can be expressed as the minimum amount of enzymes that can be detected. Accordingly, a long incubation time is required for maximizing the sensitivity of assays. However, a drawback of this method is limited data points for statistical computation. To increase the number of data points, many more different concentrations of enzymes should be used for assay.

Our method is convenient for comparison since many data points can be readily collected by changing the incubation time of the same reaction mixture using one enzyme concentration. Therefore, more reliable data can be easily obtained, although the obtained data are nondimensional and thus difficult to convert to more realistic parameters, e.g. amount of amino acids or enzymes. Considering these situations, an appropriate method for comparison should be chosen or, if necessary, both methods should be employed to draw the most reasonable conclusion.

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Measurement of Glycosylated Vitamin B6 in Foods

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ABSTRACT

To examine the levels of glycosylated vitamin B6 in 22 foods, each food was stirred for 2 hr at pH 6.8 and then incubated for 2 hr at pH 5.0 and 37°C with β-glucosidase (60 units per g food). Vitamin B6 content of foods was measured microbiologically before and after enzyme treatment as well as with acid hydrolysis. Animal products contained no measurable amount of glycosylated B6. Grains and legumes generally had a high level of this bound form (6–57% of the total vitamin B6). Of the fruits analyzed, orange juice had the highest level of glycosylated vitamin B6 (47%). Among fresh vegetables studied, raw carrots had the highest level (51%). For broccoli and cauliflower, the glycosylated value was higher for the processed food as compared to the raw food.

INTRODUCTION

NATURAL SOURCES of vitamin B6 contain the vitamin mainly in a bound form as evidenced by the need for vigorous acid hydrolysis prior to microbiological assay (Siegel et al., 1943; Rubin et al., 1947). Conjugates of vitamin B6 of unknown structure have been reported in rice bran and urine (Scudi et al., 1942; Scudi, 1942). Subsequently, rice bran was found to contain a water-soluble pyridoxine conjugate of a low molecular weight which is not precipitated by protein precipitants, is absorbed by acid clay and eluted mostly as unbound pyridoxine (Scudi, 1942). It was later shown that vitamin B6 in rice bran concentrate is conjugated with glucose in a 1:1 ratio (Yasumoto et al., 1977). Also, vitamin B6 in orange juice has been found to be bound to a nonprotein compound with a molecular weight less than 3500 daltons (Nelson et al., 1977). The exact structure of this conjugated form of vitamin B6 has not been determined. Conjugated forms of pyridoxine and glucose (pyridoxine β-glucoside) have been synthesized by incubation of cellobiose and pyridoxine in the presence of the wheat bran β-glucosidase (cellobiase). The two resultant derivatives have been purified and identified as 4′-(β-glucosyl) pyridoxine and 5′-(β-glucosyl) pyridoxine. Treatment of both conjugates with almond β-glucosidase results in release of glucose and pyridoxine (Suzuki et al., 1979). Recently, the occurrence of a particulate enzyme in seedlings of podded pea (*Pisum sativum* L. cv. Kinusaya), which catalyzed the transfer of D-glucose from UDP-glucose to the 5′-, but not 4′-, hydroxyl group of pyridoxine, has been reported (Tadera et al., 1982).

The main objectives of this study were to determine if β-glucosidase treatment of foods could be used to determine the amount of vitamin B6 which may be present as a glycosidic linkage in foods and to determine the amount of such a bound form of vitamin B6 in several commonly consumed foods.

MATERIALS & METHODS

Food samples

The following foods were selected to represent different food groups: cooked ground beef (approximately 20% fat), canned (oil packed) tuna, raw and baked chicken, skim milk, pressure cooked soybeans, pressure cooked navy beans, peanut butter (made from 100% Spanish peanuts), whole wheat flour, whole wheat bread (made from whole wheat flour), wheat bran, frozen corn, cooked white long grain rice, rice bran, bananas, avocados, canned peaches, freshly-squeezed orange juice, frozen orange juice concentrate, untoasted filberts, raw and canned green beans, raw and frozen broccoli, raw and frozen cauliflower, and raw carrots. With the exception of oranges, raw and processed foods were obtained from the same lot of food directly at the processing plant. Those foods that were frozen were thawed at room temperature (23–25°C) and the thawed food tested without further heating.

Enzyme treatment of foods

The optimum concentration of β-glucosidase and time of incubation to be used to test each food was determined using frozen orange juice concentrate. This food was used since in preliminary studies it was found to contain a substantial amount of vitamin B6 presumed to be bound to glucose. Le els of 15, 30, 60, 90 and 120 units of β-glucosidase (β-D-glucoside glucohydrolase from almonds, Sigma Chemical Company, St. Louis, MO) were incubated with 1g of orange juice concentrate for 2 hr and the amount of vitamin B6 released was measured microbiologically (AOAC, 1980). The enzyme used had a reported activity of 6 units per mg protein (one unit will liberate 1.0 μmole of glucose from salicin per minute at pH 5.0 and 37°C). A level of enzyme beyond 30 units did not increase the amount of vitamin B6 released. In addition to orange juice, rice bran was also used to test the optimum concentration of enzyme. The results of this study also showed that the level of enzyme beyond 30 units per gram did not result in any additional release of the bound form of vitamin B6 from rice bran. The enzyme was tested for phosphatase activity and it did not show any activity with pure pyridoxal-5′-phosphate as a substrate. The enzyme does not appear to have protease activity since we did not observe the release of any bound form of vitamin B6 from animal products. To determine the optimum time for the reaction, 1g of orange juice concentrate and 60 units β-glucosidase were incubated for time intervals of 1/2, 1, 2, 3 and 4 hr and the amount of vitamin B6 released was measured microbiologically. Incubation for longer than 2 hr did not increase the amount of vitamin B6 released. The optimum pH for this enzyme is at pH 5 and this pH was used for all foods (Heyworth and Walker, 1962).

Since pyridoxal 5′-phosphate is a cofactor for many enzymes, pepsin and pancreatin were tested to see if these enzymes released vitamin B6 which might be bound to protein. We treated beef and cooked soybeans with either 100 mg of pepsin (porcine stomach mucosa, Sigma Chemical Co., St. Louis, MO) at pH 2.0 and 37°C for 18 hr or 100 mg of pancreatin (porcine pancreas, Sigma Chemical Co., St. Louis, MO) at pH 6.8 for 1 hr at 37°C. In neither situation could we detect any release of vitamin B6 as compared to the nonenzyme treated condition. As described below and in the results, these two foods were also treated with β-glucosidase. There was no vitamin B6 released from beef by the treatment of this enzyme, but there was vitamin B6 released from soybeans. Based on these data and work of Yasumoto et al. (1977) and Suzuki et al. (1979), the vitamin B6 released from foods treated with β-glucosidase will be referred to as glycosylated vitamin B6.

After the optimum concentration and incubation time were established, foods from different categories were treated by the following procedure to determine the glycosylated vitamin B6 content. One-hundred to 150g of each food were ground in a blender until a homogenous mixture was obtained. One gram of this was then added to each of duplicate flasks containing 100 ml of 0.1M phosphate buffer, pH 6.8. To control mold growth, 25 mg of thymol were added to each flask. The contents of the flask were

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stirred for 2 hr at room temperature (22–25°C). This 2-hr initial stirring in phosphate buffer was done to further break-up the food material and obtain a homogenous mixture for the subsequent enzyme treatment. A pH of 6.8 was selected to reflect the pH of the intestine. The pH was then adjusted to 5.0 with 1N HCl and to one of the duplicate flasks, 60 units β-D-glucosidase were added. From this stage on, all flasks were incubated at 37°C for 2 hr in a shaking water bath (GCA/Precision Scientific Company) at 40 cycles/min. Enzyme action was stopped by addition of 10 ml of 1N HCl to all flasks followed by steaming for 5 min. This acidification and steaming process by itself did not result in any release of bound vitamin B6 using rice bran (a food high in vitamin B6) as a test food. The pH was adjusted to 4.5 using 6N KOH, the volume diluted to 250 ml, and the mixture filtered through Whatman No. 1 filter paper. The filtrate was further diluted as necessary and the vitamin B6 activity was measured by microbiological assay using *Saccharomyces uvarum*. In addition, the total vitamin B6 content of each food was measured following acid hydrolysis of a 2g sample (AOAC, 1980). The enzyme source used contained no measurable vitamin B6. Recovery of pyridoxine added to orange juice and carried through each of the procedures for total vitamin B6, nonenzyme treated and enzyme treated assays averaged 97.6 ± 12.0% (n=6 samples). Glucose levels in the foods before and after enzyme treatment were not determined.

Calculation of % glycosylated vitamin B6 in each sample was a follows: % glycosylated vitamin B6 = (G – F)/T x 100. Where: G = mg B6/g of sample treated with β-glucosidase; F = mg B6/g of sample without enzyme; and T = mg B6/g of sample as determined by microbiological assay of the acid hydrolyzed sample.

To assess which of the B6 vitamers was conjugated with glucose, the levels of the three B6 vitamers, pyridoxine, pyridoxal and pyridoxamine, were determined in orange juice and peanut butter before and after the 2 hr treatment with β-glucosidase. The three vitamers were separated on an ion exchange resin (Dowex AG50W-X8 [K^+] 100-200 mesh) (AOAC, 1980).

RESULTS & DISCUSSION

THE TOTAL, nonconjugated and glycosylated levels of vitamin B6 in the 22 foods are shown in Table 1. There was

Table 1—Vitamin B6 and glycosylated vitamin B6 content of different foods

Food	Total vitamin B6[a]	Nonconjugated Vitamin B6[a]		Glycosylated Vitamin B6[a]		Sum[e]
	μg/100g		%[b]	μg/100g	%	
VEGETABLES						
Broccoli, raw	168 ± 0.8[c]	140 ± 4	84	n.d.[d]	–	84
Broccoli, frozen	119 ± 13	48 ± 2	23	78 ± 10	65	88
Cauliflower, raw	156 ± 0.8	148 ± 0.2	95	9 ± 0.5	5	100
Cauliflower, frozen	84 ± 7	20 ± 4	23	69 ± 8	82	105
Green beans, raw	60	51	85	6	10	95
Green beans, canned	28 ± 2	16 ± 0.1	56	8 ± 0.1	28	84
Carrots, raw	170	75	44	87	51	95
FRUITS						
Bananas	313 ± 6	308 ± 31	98	10 ± 14	3	101
Avocados, fresh	443 ± 4	221 ± 1	50	15 ± 6	3	53
Orange juice, fresh	43 ± 0.1	18 ± 0.5	42	16 ± 0.6	37	79
Orange juice, conc	165 ± 2	54 ± 1	33	78 ± 0.8	47	80
Peaches, canned	9 ± 0.3	7 ± 0.5	71	2 ± 0.1	21	92
NUTS						
Filberts, raw	587 ± 15	707 ± 16	120	26 ± 29	4	124
Almonds, raw	86	69	81	n.d.	0	81
GRAINS						
Corn, frozen	88 ± 14	38 ± 9	44	6 ± 1	6	50
Rice (white), cooked	138 ± 2	50 ± 2	37	19 ± 1	14	51
Rice bran	3515 ± 84	600 ± 6	17	153 ± 30	4	21
Whole wheat bread	169 ± 2	69 ± 1	40	29 ± 3	17	57
Wheat bran	903 ± 1	117 ± 3	13	326 ± 17	36	49
Whole wheat flour	265	129	48	29	11	59
LEGUMES						
Navy beans, cooked	381 ± 35	143 ± 6	37	159 ± 9	42	79
Peanut butter	302 ± 21	49 ± 5	16	54 ± 11	18	34
Soybeans, cooked	627 ± 11	130 ± 4	21	357 ± 4	57	78
ANIMAL PRODUCTS						
Beef, ground, cooked	263	83	31	n.d.	–	31
Tuna, canned	316	158	50	n.d.	–	50
Chicken						
Breast, raw	700	454	65	n.d.	–	65
Breast, cooked	684	316	46	n.d.		46
Leg, raw	388	176	45	n.d.		45
Leg, cooked	306	150	49	n.d.		49
Milk, skim	5 ± 1	4 ± 0.3	79	n.d.		79

[a] Total vitamin B6 refers to the amount of vitamin B6 measured microbiologically after acid hydrolysis. Nonconjugated vitamin B6 is the amount measured in a sample that was mixed with 0.1M phosphate buffer for 2 hr. Glycosylated vitamin B6 refers to the difference between the enzyme treated value and the free vitamin B6 value. All values are as pyridoxine equivalents per 100 g of food.

[b] The percent of nonconjugated and glycosylated forms was calculated by dividing the amount of free or glycosylated forms by the total vitamin B6 content of each corresponding food.

[c] The values listed are means ± standard deviation for duplicate samples. Values without a standard deviation are based on a single analysis.

[d] n.d. = not detected by the enzyme treatment.

[e] Sum of the % nonconjugated and glycosylated vitamin B6 as a percentage of the total vitamin B6.

a loss of 53%, 46% and 29% in total vitamin B6 when comparing the raw to the processed foods from the same lots of green beans, cauliflower and broccoli, respectively. However, this is in agreement with Schroeder (1971), who did not do actual analyses but reviewed work of others and calculated losses of 57–77% for canned vegetables and 37–56% for frozen vegetables. These values calculated by Schroeder were not necessarily from the same lot. Our values should be considered in view of the fact that we did not measure water uptake or loss which could influence the values when expressed on a per 100g basis.

In Table 1 the term "nonconjugated vitamin B6" refers to the amount of vitamin B6 from a sample which had been mixed with 0.1M phosphate buffer pH 6.8 for 2 hr, was neither acid-hydrolyzed nor enzyme-treated, but was further incubated at pH 5.0 for 2 hr. Glycosylated vitamin B6 refers to the difference in vitamin B6 between the enzyme treated sample and the nonconjugated vitamin for the paired sample. The results (Table 1) show that animal products, including skim milk, contained no measurable glycosylated vitamin B6. Filberts, bananas and avocados contained very small amounts compared to soybeans, navy beans, orange juice concentrate, wheat bran and rice bran. Almonds, the food from which the β-glucosidase enzyme is extracted, contained no glycosylated vitamin B6. When raw vegetables were compared to the processed forms (frozen broccoli and cauliflower), the percent of glycosylated vitamin B6 was found to increase with processing. Raw foods may contain some β-glucosidase activity and this activity might release the glycosylated vitamin B6 in the condition of this assay. In contrast, the enzyme could be denatured during processing. This may be the reason for the increase in the level of glycosylated vitamin B6 observed for the processed foods. To address this possibility, 1g of raw cauliflower was mixed with 1g of frozen cauliflower and carried through the assay procedure. No enzyme was added to this mixture of foods. Using the value obtained from this assay and those values from nonenzyme and enzyme treated samples for the individual foods, it was calculated that 52% of the glycosylated vitamin B6 in the frozen cauliflower was released in the presence of added raw cauliflower. This suggests that there is some enzyme activity in the raw food and that the enzyme is denatured during processing. The increase seen in the percent of glycosylated vitamin B6 in canned green beans, compared to the raw green beans was small and might be due to the loss of total vitamin B6 content during processing. It is also important to point out that there was only one value for the raw green beans as well as for certain other foods. In the case of the animal products the values for the total vitamin B6 generally agreed with published values (Orr, 1969), and there was consistently no glycosylated vitamin B6 detected. The total vitamin B6 content of the foods presented in Table 1 agree with the published values (Orr, 1969); however, there are some differences which may be due to varietal differences, stage of harvest, processing conditions, handling process either after harvest or processing, sample preparation and interlaboratory variations. There were major differences between the total vitamin B6 content of frozen corn, frozen cauliflower, canned peaches and bananas of the reported values and our values. Our values for these foods were lower than the reported ones. The increase in glycosylated vitamin B6 in frozen orange juice concentrate as compared to the freshly-squeezed orange juice was minimal. It should be pointed out that the freshly-squeezed orange juice and the concentrate were not from the same lot.

The three forms of vitamin B6, pyridoxal, pyridoxine and pyridoxamine, were measured in peanut butter and orange juice, both with and without enzyme treatment. β-glycosidase treatment resulted in an increased level of pyridoxine in both orange juice and peanut butter as compared to the level in the nonenzyme treated foods (Table 2). Thus it appears that pyridoxine was the predominate form bound to glucose in these two foods. *Saccharomyces uvarum* is known to show little or no growth in the presence of the phosphorylated forms of vitamin B6 as compared to the unphosphorylated forms. The relative growth for pyridoxal 5′-phosphate is 2% and for pyridoxamine phosphate is less than 0.4% (Morris et al., 1959). since there was an increase in vitamin B6 content of the enzyme treated sample which can be measured microbiologically, it appears that the conjugated form is nonphosphorylated. Further support for this is indicated by a lack of phosphatase activity of the enzyme when pyridoxal 5′-phosphate was used as a substrate and incubated for 2 hr in the pesence of 60 units of glucosidase enzyme. This is in agreement with findings on rice bran concentrate from which 5′-O(β-D-glucopyranosyl) pyridoxine was isolated (Yasumoto et al., 1977). These authors did not quantitate the amount in the rice bran. Vitamin B6 in orange juice has been reported to be bound to a compound with a molecular weight less than 3500 daltons (Nelson et al., 1977). This latter compound might be glucose, since in our study we found that 47% of vitamin B6 in orange juice concentrate was in the glycosylated form.

As shown in Table 1, the sum of the free and glycosylated form was less than the total for several foods tested. Exceptions to this were most vegetables, bananas and peaches, foods for which the sum was close to or equal to the total B6 (a mean ±SD of 94 ± 7% for these nine values). This raises the question of what other forms of vitamin B6 may be present in some of these foods. One likely explanation would be as the phosphrylated forms. There also may be as yet unidentified forms, or vitamin B6 might be bound to amino acids or proteins. Some of the reactions of vitamin B6 with amino acids and proteins are summarized as follows: (a) vitamin B6 is reported to form a Schiff base with amino acids (Matsuo, 1957); (b) pyridoxal and pyridoxal-5′-phosphate have been reported to bind to protein in a

Table 2—Level of the three B6 vitamers in frozen orange juice concentrate and peanut butter before and after β-glucosidase treatment

Food	Pyridoxine		Pyridoxal		Pyridoxamine	
	Before	After	Before	After	Before	After
			μg/100 g			
Orange juice conc	10.10 ± 0.07[a]	71.4 ± 0.2	9.6 ± 0.1	15.7 ± 0.1	16.6 ± 0.3	16.1 ± 0.7
Peanut butter	14.4 ± 0.2	50.6 ± 1.4	14.7 ± 0.7	16.2 ± 2.4	15.6 ± 0.4	15.0 ± 1.7

[a] Values for each form were derived from standard curves for the respective forms. Each value is the mean of duplicate samples. The levels of each of the three B6 vitamers were determined on 12 g of sample before and after β-glucosidase treatment for 2 hr using the procedure described in the text. Separation of the three B6 vitamers was done on an ion exchange resin (Dowex AG50Wx8 [K^+] 100-200 mesh) (AOAC, 1980). Values for vitamin B6 (expressed as pyridoxine equivalents) for samples which were not acid hydrolyzed and not subjected to ion-exchange chromatography were 49.0 ± 0.5, 118 ± 3.0, 53 ± 2.0 and 95 ± 2.0 μg/100 g for the orange juice without enzyme (nonconjugated), orange juice plus enzyme, peanut butter without enzyme (nonconjugated) and peanut butter plus enzyme, respectively.

food model system as a Schiff base, substituted aldamine and pyridoxal amino complex (Gregory and Kir, 1977) and during storage of a low moisture food at 37°C for 128 days, half of the pyridoxal-5'-phosphate was found to be bound to protein as ε-pyridoxyllysine complexes (Gregory and Kirk, 1978); (c) a reaction of sulfhydryl groups of milk serum proteins and pyridoxal occurs in evaporated sterilized milk, resulting in the formation of bis-4-pyridoxal, a compound with low B6 activity (Bernhart et al., 1960; Wendt and Bernhart, 1960).

Interestingly, with filberts the amount of free vitamin B6 was higher than the total amount of vitamin B6. One reason for this might be that other components in the filberts interact with the free forms of vitamin B6 during acid hydrolysis, a process which is required to assay total B6. This process is not used for free vitamin B6 determination. There may also be a growth stimulating factor for the yeast in filberts that is destroyed by heat during hydrolysis. We found that recovery values of added pyridoxine to the acid-hydrolyzed treated, nonenzyme treated and enzyme treated assays for filberts were 86%, 122% and 125%, respectively. This is in line with heat inactivation of a growth promoting factor. Recovery data for orange juice did not show this trend.

The significance of our findings is confirmation of a conjugated form of vitamin B6 in a variety of plant foods and its absence in animal foods. The nutritional importance of this compound is not yet clear. One area of nutrition where this form of vitamin B6 may be of significance is that of bioavailability. The bioavailability of vitamin B6 from orange juice has been determined in humans using a triple lumen technique. Based on samples taken at points along a 30 cm segment of the intestinal lumen, the uptake of vitamin B6 from orange juice and from a synthetic solution containing a mixture of three forms of vitamin B6 was 35% and 65%, respectively, of the amount contained in the original mixture infused into the lumen (Nelson et al., 1976). In our study, 47% of the vitamin B6 in orange juice was found to be bound to glucose. It has been reported that vitamin B6 from whole wheat bread is less available than from white bread or white bread supplemented with vitamin B6 (Leklem et al., 1980). In this study we found 36% of vitamin B6 in wheat bran is bound to glucose. Preliminary reports of other studies in our laboratory suggest a relationship between the amount of glycosylated vitamin B6 and its bioavailability from the food. From studies in humans, vitamin B6 in tuna was more available than that in peanut butter (Kabir et al., 1982). The amount of glycosylated vitamin B6 in tuna and peanut butter was 0 and 18%, respectively. Vitamin B6 in bananas and filberts was more available than in soybeans (Gonzalez, 1982). The amount of glycosylated vitamin B6 in bananas, filberts and soybean was 3%, 4% and 57%, respectively.

In summary, plant foods contain a bound form of vitamin B6 which appears to be a glycosylated form of vitamin B6. The level of this bound form of vitamin B6 seems to increase during processing, based on the limited number of foods studied.

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Iron-Enriched Bread: Interaction Effect of Protein Quality and Copper on Iron Bioavailability

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ABSTRACT

These studies examined the bioavailability of iron in iron-enriched breads. The test breads also contained added protein in the form of gluten, soy or nonfat dry milk. Bioavailability was assessed based on net hemoglobin synthesis in rats initially made iron deficient and then fed bread-based diets containing 38.5 ppm iron (NRC requirement for rat, 35 ppm) and 3.75, 5.00, or 6.25 ppm copper (NRC requirement for rat, 5 ppm). More hemoglobin was synthesized on milk-supplemented (83 mg/mg iron) and on soy-supplemented (85 mg/mg iron) bread diets than on gluten-supplemented (59 mg/mg iron) bread diets. Copper appeared to have no influence on hemoglobin regeneration or on hepatic iron accumulation under the conditions of this experiment.

INTRODUCTION

IN THE UNITED STATES, most of the commercially produced white bread and a number of variety breads are traditionally enriched with iron. This nonheme (inorganic) iron is less well absorbed than heme iron (Hallberg and Rossander, 1982a, b). A recent study (Resurreccion et al., 1981) showed a negative correlation between hemoglobin concentration and the concentration of iron in the liver of rats fed iron-enriched breakfast cereals. These researchers suggested that nutrients other than iron may be of critical importance in the utilization and distribution of iron in animals fed cereal diets. In cereal-based foods, fiber, phytate, proteins, and certain micronutrients are often reported to reduce iron absorption/utilization (Erdman, 1981). On the other hand, the role of ascorbic acid and meat in enhancing absorption/utilization of nonheme iron is well recognized (Hallberg and Rossander, 1982a, b), as is the effect of dietary protein sufficiency (Macdougall et al., 1982). However, less is known of the effect of protein quality, and its possible interaction with copper, on iron utilization. This was examined in present studies using the rat as the experimental model and iron-enriched bread with protein added from various sources as the test material.

MATERIALS & METHODS

Test breads and diets

Three types of pan breads were made using gluten, defatted soy flour, or nonfat dry milk as the source of added protein (Table 1). These protein sources differed in amino acid profile and, thus, in protein quality with gluten being a low quality protein, soy a medium quality protein, and NFD milk high in protein quality. Protein sources were added to provide the same amount of protein in test breads (Table 1) and resultant test diets (Table 2).

All breads were made using the same white wheat flour. Thus, a portion of the protein in the form of gluten in test breads originated from a common source. Dried and finely ground breads were used to formulate isoproteinous test diets (Table 2). Ferrous sulfate, a highly bioavailable iron source (Ranhotra et al., 1971), was used to enrich breads. With consideration of inherent iron, ferrous sulfate was added in amounts that provided an average of 3.33 mg of iron/100g flour-protein source mixture. The resultant amount falls within the current flour enrichment standards (Food and Nutrition Board, 1974). However, finely ground breads contained 3.95 mg iron/100g (39.5 ppm). Therefore, some iron originated from bread ingredients other than protein source and ferrous sulfate.

Breads provided 34.3 ppm iron in the diet (Table 2). Slight adjustments in the moisture content of finely ground breads were made to keep the amount of bread in test diets as well as the iron contributed by bread constant. Thus, all test diets contained 38.5 ppm iron, a level slightly in excess of the proposed requirement (NRC, 1978) of 35 ppm for the rats. Diets were adequate in all other nutrients. Bread provided 13.5 to 13.7% of the protein in the test diets (Table 2).

Copper in the form of copper sulfate was added to the test diets to provide three levels – 75%, 100%, or 125% of the proposed requirement (NRC, 1978) of 0.5 mg/100g for the rat – for each bread type.

Studies on the human requirements for copper have shown that diet provides 75–100% of the estimated 2.0 mg daily need (Sandstead, 1982). Thus, both iron and copper were provided at or about the physiological need level typical of the human situation.

Animals

Weanling male rats of the Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually in mesh-bottom stainless steel cages in a controlled environment. To improve iron absorption, all animals were first made slightly anemic through feeding of a low iron diet (Table 2). After 10 days on this diet, average hemoglobin (Hb) levels dropped from 11.2 g/dl to 9.3 g/dl. At this point, rats were randomly assigned to test diets (9 rats/diet) for iron repletion (Hb regeneration) studies which lasted 20 days. Diet and distilled deionized water were supplied *ad libitum* throughout the study. Body weight and diet intakes were recorded. Hemoglobin and hematocrit (Hct) measurements were made at 5-day intervals for 20 days. On day 20, blood was withdrawn by heart puncture from anesthetized rats which were then sacrificed. Livers were removed and frozen.

Table 1—Bread formula, straight dough procedure

Ingredients	Protein source[a]		
	Gluten	Soy+gluten	Milk+gluten
Wheat flour,[b] g	100	100	100
Compressed yeast, g	3	3	3
Yeast food,[c] g	0.5	0.5	0.5
NaCl, g	2	2	2
Sucrose, g	6	6	6
Shortening, g	3	3	3
Gluten,[d] g	9.94	–	–
Soy flour,[e] g	–	15.00	–
Nonfat dry milk,[f] g	–	–	29.01
Ferrous sulfate,[g] g	0.0094	0.0087	0.0123
Protein, %	14.7	14.7	14.7

[a] Added (gluten, soy or milk) and naturally occurring (gluten) in wheat flour.
[b] Patent white wheat flour containing 11.43% protein (N x 5.7) as gluten.
[c] Contained monocalcium phosphate, calcium sulfate, ammonium chloride, salt, potassium bromate, and flour.
[d] Vital wheat gluten from ITT Paniplus Co., Olathe, KS containing 69.6% protein (N x 5.7).
[e] Defatted soy flour from ADM Co., Decatur, IL containing 51.1% protein (N x 6.25).
[f] From Land O'Lakes, Minneapolis, MN containing 33.5% protein (N x 6.25).
[g] Fine powder from Mallinckrodt Co., St. Louis, MO containing 31.8% iron.

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Analytical

Protein (Kjeldahl) in wheat flour, protein sources, and experimental diets was determined by the standard AACC method (1977). Iron and copper in test materials and in serum and liver were determined by atomic absorption spectrophotometry (Model 251, Instrumentation Laboratories, Inc., Wilmington, MA). Hb was determined on tail blood by the cyanmethemoglobin method (Crosby et al., 1954) and Hct by microcapillary centrifugation (4 min) using fire polished heparinized microhematocrit tubes. Gain in Hb was calculated based on blood volume of rats (taken as 6.7 ml/100g body wt.) and on Hb concentrations. The data were subjected to appropriate statistical analysis (Snedecor, 1956).

RESULTS & DISCUSSION

Growth and diet efficiency

Irrespective of the dietary copper level, all rats fed gluten-supplemented diets (diets A-C) performed poorly, averaging only 25–31g weight gain in 20 days (Table 3). This, in part, resulted from their low diet intakes compared to rats fed soy- and milk-supplemented diets (diets D-I), but also from their poor diet utilization efficiencies. Diet:gain ratios on diets A-C exceeded 6 in contrast to values of about 3 on diets D-I. Apparently, this reflected the unfavorable amino acid profile of gluten-supplemented diets. Based on the growth response and diet intakes of rats as well as the diet:gain ratios, soy appeared to have improved the amino acid profile of bread protein substantially to a point that approached that achieved with milk (diets D-F vs. diets G-I). The exact profile was not determined to substantiate this, however. Thus, although proteins of different qualities were initially selected, quality differences for soy- and milk-supplemented breads vanished due to complimentary amino acid effects. This appeared to affect the liver weights (Table 3) in a manner similar to that for body weights.

Hemoglobin and hematocrit regeneration

Regeneration of Hb and Hct was most rapid during the first 5 days that the rats were fed bread diets (Table 4). Hct values, in fact, approached the normal range during this period. On the other hand, Hb values were well below the normal range suggesting a macrocytic hypochromic anemic state. After the initial 5 days, Hct values hardly improved, in most cases dropping by day 20 when the experiment was ended. This suggested that Hct measurements may not be suitable for assessing iron bioavailabilities in this type of study. Although it progessively slowed, Hb regeneration continued until day 20. At day 20, Hb values measured about 13 g/dl, which was still somewhat below

Table 2—Composition of experimental diets

	Test diet (bread-based)									
	Gluten			Soy + gluten			Milk + gluten			Low iron diet
	A	B	C	D	E	F	G	H	I	
Composition										
Bread, g	86.8	86.8	86.8	86.8	86.8	86.8	86.8	86.8	86.8	–
Casein, g	–	–	–	–	–	–	–	–	–	20.0
dl-methionine, g	–	–	–	–	–	–	–	–	–	0.1
Sucrose, g	–	–	–	–	–	–	–	–	–	66.7
Other[a], g	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
Content										
Protein[b], %	13.6	13.6	13.6	13.7	13.7	13.7	13.5	13.5	13.5	17.8
Iron[c], ppm	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	4.2
Copper, mg/100g										
From copper sulfate	0.112	0.237	0.362	0.002	0.127	0.252	0.181	0.306	0.431	0.500
Total	0.375	0.500	0.625	0.375	0.500	0.625	0.375	0.500	0.625	0.500

[a] Vitamin diet fortification mixture from ICN Pharmaceuticals, 2.2; trace mineral mix (contained Zn, 1.2 mg; Mn, 5.0 mg; Mg, 40 mg; and I, 0.015 mg in sucrose base), 1.0; corn oil, 5.0; NaCl, 0.5; KCl, 0.5; monosodium phosphate, 2.0; and calcium carbonate, 2.0.
[b] N x 5.7 for gluten bread; N x 6.25 for nongluten protein sources in bread.
[c] From bread, 34.3 ppm; from other diet ingredients, 4.2 ppm.

Table 3—Responses of rats to different bread diets[a]

	Protein source in bread diets								
	Gluten			Soy + gluten			Milk + gluten		
Diet	A	B	C	D	E	F	G	H	I
Body wt. gain, g	31±7	27±5	25±6	93±10	90±10	91±8	80±9	81±13	76±10
Diet intake, g	200±17	188±19	186±26	292±18	285±25	291±22	270±16	275±21	274±29
Iron intake, mg	7.69±0.68	7.12±0.62	7.18±0.99	11.24±0.71	10.98±0.96	11.20±0.84	10.41±0.60	10.59±0.80	10.67±1.03
Diet: gain, ratio	6.67±1.00	7.17±0.89	7.59±1.17	3.14±0.21	3.20±0.20	3.20±0.15	3.40±0.33	3.46±0.37	3.61±0.27
Liver									
Weight[b], g	3.12±0.39	2.80±0.33	2.69±0.31	4.91±0.50	4.65±0.63	4.82±0.53	4.16±0.50	4.43±0.42	4.30±0.54
Iron μg/g	151±25	155±21	149±26	125±23	140±38	87±14	122±21	101±15	105±13
Total iron, μg	476±119	430±93	396±83	627±145	661±215	427±76	511±110	454±94	450±82
Copper, μg/g	8.1±0.7	5.9±0.7	5.0±1.4	4.7±0.6	5.0±0.5	6.3±0.5	5.3±0.8	5.3±0.1	5.9±1.4
Total copper, μg	25.6±2.2	16.2±2.7	13.2±3.9	23.5±4.0	23.9±4.7	31.2±4.8	22.0±4.3	23.8±2.3	25.1±5.5
Serum									
Iron, μg/dl	164±9	174±29	182±22	168±8	157±10	120±8	217±13	143±7	218±11
Copper, μg/dl	145±6	162±11	151±15	144±17	115±12	151±13	151±10	130±14	124±10

[a] Values are avg (8—9 rats/diet) ± SD
[b] Wet weight.

the normal level. The Hb regeneration patterns differed significantly ($P < 0.05$) among diets, but Hb levels did not increase with dietary copper levels (Table 4, Fig. 1).

When the expansion of blood volume of test animals or their iron intakes are not considered, use of Hb concentration values to assess bioavailabilities can be quite misleading. To minimize, if not completely eliminate, this source of error, Hb concentration (regeneration) was calculated in terms of Hb gain, g Hb/mg iron consumed. This was done at 5-day intervals (Table 4) and for the entire 20-day period (Fig. 1). Such calculations again revealed the rapid initial Hb regeneration when the body's need for iron was most intense. Most revealing, however, was the distinction that developed between gluten-supplemented and soy- and milk supplemented diets in their ability to promote Hb synthesis. With a few isolated exceptions, more Hb was formed per mg of iron consumed on soy- and milk-supplemented breads than on gluten-supplemented breads at all intervals measured (Table 4). Clearly, iron bioavailability improved with protein quality. This is well illustrated in Fig. 1. When average Hb gain on soy-supplemented breads is considered as 100%, the gain on milk-supplemented breads was 97.3%, and that on gluten-supplemented breads only 69.1%. Since Hb gains on soy- and milk-supplemented diets were about the same (Fig. 1), protein, fiber or phytate in soy probably did not adversely influence iron absorption. Most likely little phytate was present in the bread since substantial phytate hydrolysis occurs during breadmaking (Ranhotra et al., 1974). The contribution of fiber from soy was also probably insignificant, since calculations showed the diet to contain no more than 1% fiber (neutral-detergent fiber).

Protein quality appeared to have no impact on copper utilization. The role of copper in Hb synthesis is well established, but under conditions of marginal deficiencies or excesses, as in the present studies, copper appeared not to be a critical factor in Hb synthesis. In adolescent females fed different protein diets, Gregor et al. (1978) found no difference in copper absorption and consequently in Hb levels when copper intake was adequate (2.9 mg/day). Only when the copper deficiency was severe, did Hb levels, plasma copper levels, and liver copper stores appear to be drastically reduced (Anonymous, 1982).

Serum iron and copper

Both serum iron and copper values were in the normal range and although these measurements varied significantly ($P < 0.01$) between diets (Table 3), no pattern emerged to suggest relationship of serum iron and copper values with Hb synthesized.

Liver iron and copper

Rats fed soy- and milk-supplemented diets consumed significantly ($P < 0.01$) more iron than rats fed gluten-supplemented diets (Table 3); they also accumulated more total iron in their livers. Rats fed gluten-supplemented diets showed a tendency to accumulate more liver iron when expressed on per g liver basis. However, on all diets, liver iron stores represented no more than 4–6% of the total iron consumed in 20 days. Thus, in the present studies, protein quality affected iron utilization but it did not affect the hepatic iron stores. Dietary copper also did not affect hepatic iron stores. Increased hepatic iron accumulation seemed to occur only when the diet was severely deficient in copper, less than 1 ppm (Williams et al., 1981; Bozian et al., 1982), but not under conditions of marginal deficiencies. Excessive iron intakes, as provided in studies by Resurreccion et al. (1981), may be another factor inducing hepatic iron accumulation. Liver copper levels, in the present studies, were in the normal range on all diets (Table 3) and appeared unrelated to the efficiency of iron utilization.

Thus, when a high quality protein is added to bread (or is consumed with bread) either for functional or nutri-

—Continued on page 1435

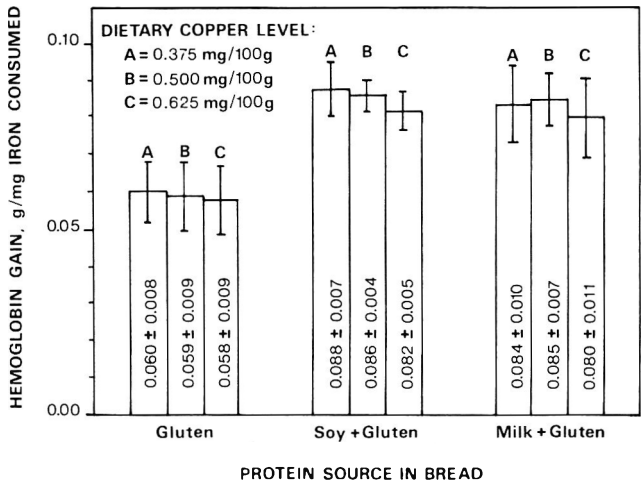

Fig. 1—Hemoglobin gain in rats fed bread-based diets for 20 days. Values within bars are mean ± SD.

Table 4—Hematologic response in rats fed different bread diets[a]

Diet	Protein source in bread: Gluten A	Gluten B	Gluten C	Soy+Gluten D	Soy+Gluten E	Soy+Gluten F	Milk+Gluten G	Milk+Gluten H	Milk+Gluten I
Hemoglobin, g/dl									
Day 0	9.30±0.62	9.31±0.69	9.32±0.50	9.30±1.34	9.31±0.82	9.31±0.89	9.30±1.24	9.30±0.64	9.28±2.12
Day 5	11.42±0.80	12.20±0.43	12.16±0.33	11.54±0.78	11.54±0.82	12.02±2.03	11.76±0.54	11.91±0.61	11.43±1.23
Day 10	12.96±0.86	12.69±1.09	12.67±0.98	12.59±0.73	12.67±0.65	11.66±0.66	12.44±0.85	11.86±0.71	11.53±0.79
Day 15	13.02±0.73	12.97±0.57	12.91±0.58	12.37±0.62	12.93±0.82	11.58±0.58	11.27±0.38	13.15±0.47	12.48±0.54
Day 20	13.51±0.78	13.54±0.79	13.42±0.61	13.07±0.47	13.11±0.47	12.67±0.57	13.11±0.85	13.29±0.61	13.17±0.39
Hematocrit, %									
Day 0	39.6±4.9	38.7±4.7	38.6±3.5	34.7±3.5	35.9±2.5	39.1±4.0	37.7±3.2	38.1±2.3	38.9±6.7
Day 5	50.8±5.3	50.4±4.3	51.6±6.7	49.2±3.6	49.7±4.0	48.3±3.7	48.7±3.6	49.2±1.0	49.6±4.9
Day 10	50.2±1.7	50.2±2.3	49.4±1.2	49.7±3.0	50.9±3.3	48.3±1.7	49.4±1.4	46.9±3.0	48.8±3.2
Day 15	54.2±2.6	53.3±4.6	52.2±2.6	52.1±5.3	50.8±3.4	49.2±1.8	48.9±1.8	50.0±2.5	50.1±2.2
Day 20	49.4±2.5	51.1±3.6	48.1±2.8	48.6±3.1	48.7±1.9	46.8±2.2	47.0±3.4	47.8±2.0	47.7±1.4
Hemoglobin gain[b], g/mg iron									
Day 0–5	0.081±0.038	0.119±0.040	0.112±0.026	0.120±0.036	0.120±0.037	0.123±0.054	0.119±0.032	0.110±0.028	0.106±0.054
Day 6–10	0.076±0.019	0.052±0.024	0.052±0.018	0.093±0.013	0.098±0.024	0.067±0.026	0.087±0.015	0.073±0.023	0.068±0.026
Day 11–15	0.041±0.020	0.041±0.026	0.042±0.029	0.068±0.032	0.076±0.024	0.062±0.016	0.035±0.023	0.096±0.018	0.078±0.023
Day 16–20	0.052±0.012	0.047±0.022	0.046±0.020	0.087±0.029	0.070±0.024	0.094±0.011	0.109±0.025	0.070±0.015	0.080±0.016

[a] Values are avg (8–9 rats/diet) ± SD.
[b] Based on blood volume (6.7 ml/100g; tabular values) of rats and their Hb concentrations on days considered.

Effects of Dissolved Free Oxygen on the Volatile Compounds of Oil

DAVID B. MIN and JYE WEN

ABSTRACT

The effects of 2.5, 4.5, 6.5, and 8.5 ppm dissolved free oxygen on volatile compounds formation and dissolved free oxygen disappearance in oils during storage were studied. The amount of compounds formed in oil containing 8.5 ppm was double that in oil containing 2.5 ppm oxygen. The rate of oxygen disappearance in oil containing 8.5 ppm was three times as fast as that in oil containing 2.5 ppm oxygen. The rates of dissolved oxygen disappearance and formation of volatile compounds were significantly different for the four levels of dissolved oxygen ($P < 0.05$). As the initial oxygen concentration in oil increased, the rate of oxygen disappearance and the amount of compounds formed increased. The results indicate that the formation of volatile compounds in oil during storage can be minimized by lowering the oxygen content.

INTRODUCTION

ONE OF THE MOST important qualities of oil is flavor. Well processed fresh oil has a very bland flavor, but the quality of oil deteriorates during storage, even at room temperature, because of the formation of undesirable volatile compounds. It has been reported that these volatile compounds are formed as a result of reactions between the oil and dissolved oxygen (Lundburg, 1962; Labuza et al., 1969; Frankel, 1980; Gray, 1980). There are numerous articles on the effects of different processes and additives on the oxidative stability of oils, but there is little information available on the effects of different levels of dissolved free oxygen on the formation of volatile compounds and oxidative stability. This study reports the effects of different levels of dissolved free oxygen on the oxidative stability of oil during storage.

MATERIALS & METHODS

Sample preparation

Refined, bleached, and deodorized soybean oil was obtained from Capital City Products (Columbus, OH). Fresh oil containing 10.3 ppm dissolved oxygen was transferred to a 2 liter round bottom flask and evacuated using a Welch Duo-Seal Vacuum Pump at about 1 mm mercury vacuum for periods varying from 1 to 6 hr to obtain samples containing 2.5, 4.5, 6.5, and 8.5 ppm dissolved oxygen. The oils were transferred to 50 ml Erlenmeyer flasks which were completely filled and air-tightly stoppered with rubber stoppers. The samples were stored in a forced draft air oven at 55°C to maintain uniform temperature.

Dissolved free oxygen measurement

The content of dissolved oxygen in oil was measured with a Beckman Model 0260 Oxygen Analyzer (Min et al., 1983).

Measurement and identification of volatile compounds

The volatile compounds in the oil were isolated and separated (Min, 1981; Min et al., 1983). The volatile compounds were quantitated using gas chromatography (H.P. 5880A Gas Chromatograph equipped with an electronic integrator). The volatile compounds were identified only by comparison of the G.C. retention times with those of known compounds.

Statistical analysis

The effects of initial oxygen contents on the volatile compounds formed in oil during storage were analyzed by Duncan's Multiple Range Test (SAS, 1979).

RESULTS & DISCUSSION

Effect of initial dissolved free oxygen content on oxygen disappearance rate

Oxygen content of the four samples containing different initial levels of oxygen during storage is shown in Table 1. Practically all of the dissolved oxygen disappeared within 48 hr of storage regardless of the initial content. The rates of oxygen disappearance in the oils during 48 hr of storage were determined by linear regression analyses from the data in Table 1. The equations for predicting free oxygen remaining in the oil containing 2.5, 4.5, 6.5, and 8.5 ppm oxygen were $y = 2.36 - 0.0491x$, $y = 3.89 - 0.858x$, $y = 5.62 - 0.126x$, and $y = 7.36 - 0.162x$, respectively, where y is the free oxygen content in ppm and x is the storage time in hours at 55°C. The oxygen disappearance rates in oil containing 2.5 ppm and 8.5 ppm were 0.0491 ppm/hr and 0.162 ppm/hr, respectively.

Correlation coefficients (r) between the amount of oxygen disappearing and the storage period were better than 0.93 indicating that lower oxidation rates occur at lower concentrations of dissolved oxygen. Statistical analysis indicated that the effects of initial oxygen levels on the rates of dissolved oxygen disappearance during storage were significantly different ($P < 0.05$). The coefficient of variation (C.V.) of the analysis of six oil samples containing 2.5 ppm oxygen was 5.0%. These results indicate that the reproducibility of free oxygen measurement was satisfactory.

Table 1—Dissolved oxygen and volatile contents in the oils containing 2.5, 4.5, 6.5 and 8.5 ppm initial oxygen during storage

Storage time (hr)	Dissolved Oxygen (ppm) 1	2	3	4[b]	Volatile Contents[a] 1	2	3	4[b]
0	2.5	4.5	6.5	8.5	0.30	0.30	0.30	0.30
12	1.9	2.6	3.9	4.6	—	—	—	—
24	0.7	1.0	1.4	2.8	0.44	0.51	0.63	1.3
36	0.6	0.7	1.0	1.1	1.1	1.6	2.1	3.9
48	0.2	0.3	0.4	0.5	1.8	2.0	2.7	4.2
72	0.0	0.0	0.0	0.0	1.9	2.4	3.3	4.8
96	0.0	0.0	0.0	0.0	2.3	2.9	4.2	4.9
120	0.0	0.0	0.0	0.0	2.8	3.7	4.6	5.5
144	0.0	0.0	0.0	0.0	2.9	4.0	5.0	5.6
168	0.0	0.0	0.0	0.0	3.0	4.1	5.1	5.6

[a] Volatile content was determined by measuring the G.C. peak areas using H.P. 5880 electronic integrator.
[b] Samples 1, 2, 3, and 4 are the oils containing 2.5, 4.5, 6.5, and 8.5 ppm initial oxygen, respectively.

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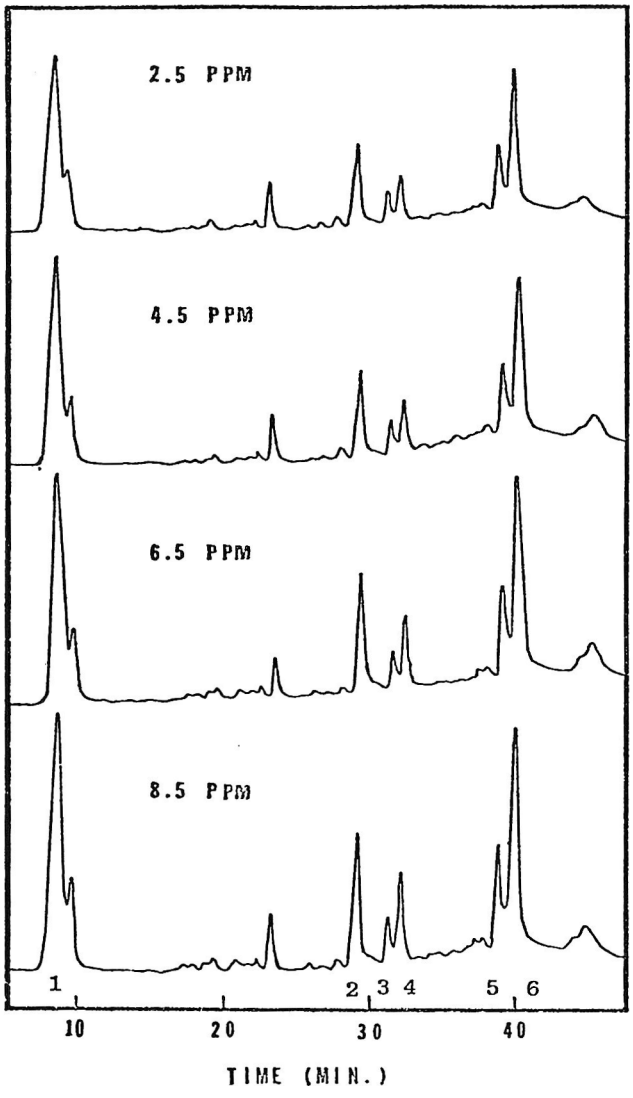

Fig. 1—Effects of different initial levels of dissolved free oxygen on the formation of volatile compounds in oil after 96 hr of storage (1) pentane; (2) 2-heptenal; (3) and (4) isomers of 2,4-heptadienal; (5) and (6) isomers of 2,4-decadienal).

Effect of initial dissolved free oxygen content on volatile compound formation

The effect of initial dissolved oxygen contents on the volatile compounds formed after 96 hr of storage at 55°C is shown in Fig. 1. The amount of volatile compounds increased with increasing initial oxygen content. The GC volatile compound profiles shown in Fig. 1 were similar to those previously reported in other studies (Dupuy et al., 1977; Jackson and Giacherio, 1977; Min, 1981) in which the major compounds were identified as autoxidation products of oil, namely pentane, 2-heptenal, and isomers of 2,4-heptadienal and 2,4-decadienal. Dupuy et al. (1977, 1978), Jackson and Giacherio (1977), Williams and Applewhite (1977), Warner et al. (1978), and Min (1981) reported that the flavor quality of soybean oil could be estimated by measuring the content of volatile compounds by gas chromatographic methods and that the flavor quality and the quantities of volatile compounds in oil were inversely related.

The amounts of volatile compounds formed in the oils increased as storage time increased and as the initial dissolved free oxygen content increased, so did the amounts of volatile compounds formed during storage (Table 1). The oil containing 8.5 ppm initial oxygen contained more than twice as much volatile compounds after 48 hr of storage as the oil with 2.5 ppm initial oxygen. Volatile compounds formation was most rapid between 24 and 48 hr of storage. The greater the rate of volatile compounds formation during the first 48 hr of storage agreed well with the greater rate of dissolved free oxygen disappearance during the first 48 hr of storage. Even though the dissolved oxygen almost disappeared after 48 hr of storage, the formation of volatile compounds continued as storage time increased beyond 48 hr. This may be due to the decomposition of oxidized nonvolatile compounds formed by the reaction between the oxygen and oil (Table 1).

Statistical analysis showed that the effect of initial dissolved free oxygen content on the formation of volatile compounds was significant at the 5% level.

In summary, the effects of different oxygen levels on the formation of volatile compounds and the oxidation rates of oil during storage indicate that the formation of volatile compounds in oil could be lessened by minimizing the dissolved free oxygen in oil.

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Effect of Iron Sources and Ascorbic Acid on the Chemical Profile of Iron in a Soy Protein Isolate

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ABSTRACT

The chemical changes in the iron profile of a commercial soy protein isolate resulting from ascorbic acid and iron fortification were examined. An in vitro method was devised to simulate gastrointestinal pH and an "enhancing factor" developed as a potential in vitro indicator of iron bioavailability. Ascorbic acid supplementation of soy protein produced substantial increases in soluble iron at pH 4 and 6. The solubilizing effect of ascorbic acid on iron was concentration and pH-dependent and contingent upon the exogenous iron source present. The exogenous iron entered a different chemical pool than the endogenous iron, thus raising questions about the concept of a nonheme pool at high levels of exogenous iron.

INTRODUCTION

SINCE THE LATE 1960's, the annual consumption of soy protein in the U.S. has increased to approximately 4–5 pounds per person (Bothwell et al., 1982; Soy Protein Council, 1982a). Soy proteins may be found in the form of flour, grits, textured proteins, concentrates or isolates. In particular, the soy isolates can be produced to exhibit different functional properties. Their wide range of uses include processed meats, analog products, snacks, coffee whiteners and infant formulas (Soy Protein Council, 1982b).

Information on the availability of iron from soy protein products has been contradictory. The results of Steinke and Hopkins (1978) using rats and of Rios et al. (1975) in humans showed that iron from soybeans was well absorbed. However, Cook et al. (1981) performed iron absorption studies in humans when fed full fat soy flour, textured soy flour, or isolated soy protein in a semi-purified meal. Their results indicated a pronounced inhibitory effect on the absorption of nonheme iron. In a separate investigation by the same laboratory (Morck et al., 1981), adult males were fed infant food supplements containing between 45% and 75% of the total protein as soy protein. Based on the low percentage iron absorption in these iron replete individuals, these authors suggested that the inhibition may have been due to the presence of soy protein.

In addition to soy protein, several dietary components are believed to affect iron absorption in either a positive or negative manner, and have been reviewed in detail (Bothwell et al., 1979; 1982; Morck and Cook, 1981).

Many investigators have demonstrated the importance of considering the ascorbic acid (asc) to iron ratio in both in vivo and in vitro studies (Bothwell et al., 1982; Lynch and Cook, 1980; Kojima et al., 1981). Increasing the asc concentration of a bean suspension from 0 to 10 mM (200:1 asc:iron), increased soluble iron from 10% to 50% (Kojima et al., 1981), and iron binding to wheat bran was completely inhibited by 60 mg of ascorbate (Camire and Clydesdale, 1982). Recently, Morck et al. (1982) demonstrated that asc added to a meal containing isolated soy protein, could partially reverse the inhibitory effect of soy protein on human iron absorption. A report prepared by the International Nutritional Anemia Consultative Group (Bothwell et al., 1982) indicated that the mean percent iron absorption from soy protein containing foods was increased in direct proportion to the molar ratio of asc to iron.

During the passage of chyme from the stomach to the duodenum, the pH gradually increases from approximately 2 to 6, owing to duodenal secretions (Bell et al., 1961; Langley et al., 1969; Rucer and Patton, 1966). Nojeim and Clydesdale (1981) tested the effect of pH on the degree of ionization of several iron sources added to various food systems. They found that pH could be used to predict the approximate amount and valence of ionic iron in these foods. The iron-binding capacities of major dietary fiber components (Camire and Clydesdale, 1981), and of pinto bean suspensions (Kojima et al., 1981) have also illustrated the importance of pH in determining the chemical form of iron. However, in addition to pH, the percent iron binding by soy protein has also been influenced by the iron source added, and the temperature and time of incubation (Nelson and Potter, 1979; 1980).

It was the intent of this research to correlate the chemical changes of iron in the soy protein isolate with the bioavailabilities observed in man. Such information might further the quest for technological methods of improving iron bioavailability.

MATERIALS & METHODS

Soy isolate

Ardex D, isolated soy protein, manufactured by the Archer Daniels Midland Company, Decatur, Illinois, was obtained courtesy of The Procter & Gamble Company (Cincinnati, OH), and stored in sealed plastic containers at ambient temperatures.

Iron sources

Ferrous sulfate heptahydrate ($FeSO_4$) and ferric chloride hexahydrate ($FeCl_3$) contained 20.1% and 20.7% iron, respectively, and were both obtained from Fisher Scientific Co. Both electrolytically reduced elemental iron (ERFe), consisting of 98.3% iron with 98% less than 44 microns, and hydrogen reduced elemental iron (HRFe), consisting of 96.9% iron with 97% less than 44 microns, were obtained courtesy of Glidden-Durkee (N.Y.). Ferric ortho phosphate (FOP) containing 29.1% iron, was obtained courtesy of Joseph Turner & Co. (N.J.).

Method

Four hundred ml of double distilled deionized water at 37°C were blended with 30g of soy protein isolate in a 500 ml erlenmeyer flask to produce a slurry. A Teflon-coated stirring bar was added to evaluate elemental iron and the temperature of the sample slurry was maintained at 37 ± 1°C for the entire time of each experiment. A two-liter beaker, filled with tap water at 37°C served as the water bath, and was placed on a Fisher Thermix Stirring Hotplate. All glassware was acid washed in concentrated HCl and rinsed in double distilled deionized water to remove contaminant iron.

Each soy isolate slurry was simultaneously incubated at pH 7 (endogenous pH), 2, 4, and 6, for 30, 30, 10, and 10 min, respectively, (Model 407A Orion Research Ionalyzer Specific Ion Meter). The sample pH was adjusted to 2.0 using concentrated HCl. At the end of the pH 2 incubation, sufficient Prep Tyrode buffer was added to produce 10% of the total volume, and to approximate the chemical environment found in the intestine. Prep Tyrode buffer

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contains the following ingredients diluted to 2 liters with double distilled deionized water: (grams) NaCl (16.0); KCl (0.4); $MgSO_4 \cdot 7H_2O$ (0.52); $NaH_2PO_4 \cdot 2H_2O$ (0.13); Glucose (2.0); $NaHCO_3$ (2.0); $CaCl_3$ (0.4); either NaN_3 (0.4) or chloramphenicol (0.02). Following the buffer, approximately 1 ml of NaOH (50% w/w) was required to increase the pH of the slurry to 4, and another ml was needed to produce the pH 6 slurry. At the end of the pH 6 incubation period, the magnetic stirring bar was removed from the slurry and placed in a 125 ml Erlenmeyer flask containing 20 ml concentrated HCl overnight. Elemental iron was determined by transferring the concentrated HCl into a 200 ml volumetric flask, diluting up to volume with double distilled deionized water, and measuring the iron by atomic absorption spectrophotometry (Perkin-Elmer Model 372 atomic absorption spectrophotometer). Nonelemental, soluble, and ionic iron analyses were performed after each incubation period as explained below.

Studies were performed on soy isolate alone, soy isolate plus approximately 120 mg L-ascorbic acid (asc), and soy isolate plus approximately 240 mg asc. The levels of added ascorbic acid (25:1 asc:iron, by weight) and exogenous iron (exogenous:endogenous 1:1) were similar to those employed by Morck et al. (1982). The effect of exogenous iron on the soy isolate iron profile was determined by adding an amount of iron equivalent to that found endogenously in 30g of the soy isolate, i.e., approximately 4.5 mg iron. The chemical iron profiles in the soy were also analyzed upon addition of both exogenous iron and 240 mg asc (asc:iron=25:1, by weight). The exogenous iron source and/or asc was added prior to the endogenous pH incubation.

Analysis

Nonelemental iron. Duplicate 10 ml aliquots of sample slurry were pipetted into separate 100 ml digestion flasks containing 20 ml concentrated HCl and three boiling chips. These samples were heated, allowed to boil for exactly 15 min, cooled, filtered through Whatman #1 filter paper, made up to volume with double distilled, deionized water, and the iron measured by atomic absorption spectrophotometer. A blank containing 10 ml double distilled deionized water in place of the 10 ml slurry was digested for the same period of time, cooled, and partially diluted with double distilled deionized water. Two-hundred and fifty microliters of a 1000 ppm Fisher iron standard were added to this blank to produce a 2.5 ppm iron solution. This digest blank then served as the iron standard for the nonelemental iron samples.

Total iron generally equalled nonelemental iron since no elemental iron was found in the soy isolate. However, elemental iron had to be accounted for when either ERFe or HRFe were added to the soy. In these cases, total iron was equivalent to nonelemental plus elemental iron.

Soluble iron. Two 30 ml aliquots (duplicates) of slurry were pipetted into each of two 50 ml tubes and centrifuged at a relative centrifugal force of 2,335g for 30 minutes (model K centrifuge, International Equipment Company). Each supernatant was decanted immediately into separate 30 ml beakers. Ten ml of supernatant from each beaker were added to 100 ml digest flasks containing 20 ml concentrated HCl and three boiling chips.

Samples were digested and analyzed in the same manner as the nonelemental iron samples. A digest blank, separate from that used for the nonelemental iron samples, was produced for the soluble iron samples since the latter were diluted to only one-half the volume, and therefore, twice the acid concentration, as the nonelemental iron samples.

Ionic iron. A few minor modifications of the original bathophenanthroline procedure (Lee and Clydesdale, 1979) were used in this study and deserve mention. In the present study, 1 ml of 1.0M pH 4 sodium acetate buffer was added to each 60 ml separatory flask. One ml of 10% hydroxylamine hydrochloride (a reducing agent) was added to the odd numbered flasks. The total volume for each flask was then increased to 14 ml with double distilled deionized water. Fifteen ml of 0.012% bathophenanthroline in 95% ethanol were added to each funnel. The reason for following this order of addition was to avoid interaction between the food and the buffer which would change the incubation pH prior to analysis (Platt and Clydesdale, 1983).

One ml aliquots of the soy isolate supernatant were pipetted into each of two separatory flasks, one containing reducing agent (ionic iron), and the other without (ferrous iron). The amount of ferric iron was calculated by difference (ionic-ferrous). The solutions were shaken for 5 sec, 10 ml of chloroform were added exactly 30 sec later, and the flasks reshaken. This precise timing was followed since Gorman and Clydesdale (1983) have found that bathophenanthroline has a higher binding constant for iron than asc. Therefore, when asc was present in the system the amount of ionic and ferrous iron detected actually increased over time.

Reagent blanks for ionic and ferrous iron were analyzed simultaneously with the samples. These included two separatory flasks, one with and one without reducing agent, with all reagents added except the sample supernatant. In addition to the reagent blank, an organic blank was produced by extracting the sample supernatant. In addition to the reagent blank, an organic blank was produced by extracting the sample (minus the bathophenanthroline) with 10 ml of chloroform. This blank would have accounted for any chemical specie which was both extracted into the organic layer and absorbed at 533 nm. Absorbance readings found in both blanks were subtracted from sample readings.

For each sample the extracted bathophenanthroline complex in the lower chloroform layer was drained into a 25 ml volumetric and diluted up to volume with 95% ethanol. The absorbance of each sample was measured in 1 cm glass curvettes at 533 nm using an Hitachi Perkin Elmer uv-vis spectrophotometer, Model 139.

Chemical iron profile in soy protein isolate with added asc

The experimental protocol was performed on the soy isolate alone, soy isolate plus approximately 120 mg asc, and soy isolate plus approximately 240 mg asc. The upper level of asc was included as a control for the soy isolate-asc-exogenous iron samples since the latter also contained 240 mg asc. The lower level of added asc produced approximately a 25:1 asc:iron ratio (by weight), and an 8:1 molar ratio.

Effect of exogenous iron source on soy isolate with and w/o asc

The effect of exogenous iron on the soy isolate iron profile was determined by adding an amount of iron equivalent to that found endogenously in 30 g of the soy isolate, i.e., approximately 4.5 mg iron.

The chemical iron profiles in the soy were also analyzed upon addition of both exogenous iron and 240 mg asc (asc:iron = 25:1, by weight).

Statistics

Data were analyzed using analysis of variance and the L.S.D. multiple test which compares all treatments with each other (Snedecor and Cochran, 1976).

RESULTS & DISCUSSION

THE METHOD USED in this study was devised to mimic the chemical environment of the gastrointestinal system. In rats, the intraluminal pH in the intestine does not change immediately from very acidic to slightly alkaline, (Forth and Rummel, 1973).

In humans, while the pH of the duodenal secretions may be 7.0–7.5, depending upon the meal, the pH of the human intestinal content is actually as low as 4.5–6.5, and only gradually rises to 7.3 in the ileum (Bell et al., 1961; Langley et al., 1969. Rucer and Patton, 1966). Since the majority of iron absorption occurs in the duodenum, this study attempted to simulate the approximate temperature (37°C) and pH of this intestinal segment at pH 4 and 6.

Since no significant differences in percent soluble, ionic or complexed iron was detected between incubation times of 10 and 30 min, the former time was used for pH 4 and 6.

Effect of added iron on soy isolate iron profile

The effect of the simulated gastrointestinal procedure on the chemical distribution of iron in the soluble phase is represented graphically in Fig. 1, 2 and 3. It is interesting to note that samples with added iron but without asc were all very similar in chemical iron profiles as compared to the control (Fig. 1, C).

The percent soluble, ionic, and complexed iron in the soy isolate was directly influenced by pH. As expected, the percent ionic iron was inversely related to pH of the

solution. The greatest percent ionic iron was inversely related to pH of the solution. The greatest percent ionic iron was noted at pH 2, whereas at pH 6 and 7, very small amounts, if any, were in this form. However, the total soluble iron (ionic and soluble complexed iron) did not follow the same pattern as the ionic iron in the asc-free samples.

After the addition of an exogenous iron source, the percent soluble iron which remained at pH 2 was essentially one-half that found in the control (9.0–11.9% vs 19.3%). Since the total iron in these systems was approximately double that in the control, the actual amount of soluble iron was practically the same. At pH 4 the amount of soluble iron was even smaller than at pH 2, and at no point produced greater than 6% of the total iron, regardless of the iron source. This suggests that even the more soluble iron sources (e.g., ferrous sulfate and ferric chloride) were insolubilized (bound) by the soy protein isolate, and also indicates that the exogenous iron in this experiment did not enter the same chemical pool as the endogenous.

The insolubility of the iron in these systems may be accounted for by two different mechanisms which are asc and pH dependent. At pH 4, without asc, the iron was most insoluble. This was probably due to the fact that free iron in solution may be bound to the protein which in turn became insoluble at its isoelectric point (pH 4.5). At pH 2, the reactive side chains of amino groups would tend to become protonated which would decrease their affinity for iron cations thus reducing complexation and solubilization at this pH. At pH 6 the same side chains would have a negative charge and tend to complex and solubilize the iron cations. It should be noted that such changes in solubility with pH may not be linear over the entire pH range nor may they be applicable to both exogenous and endogenous iron or other soy products such as bran, flour, or concentrate. That is, at pH 4.5 there is an isoelectric problem which may not exist at pH 5.0 or above. For instance, Clydesdale and Camire (1983) found an increase in total soluble iron when defatted soy flour was incubated at pH 5.0 rather than 6.8. However, it should be remembered that soy flour contains about 50% carbohydrate, much of it in the form of fiber,which has been shown repeatedly to release minerals in a soluble form as the pH is lowered (Camire and Clydesdale, 1981; Reinhold et al., 1981).

Without asc, the same solubilization pattern was noted for samples with both endogenous and exogenous iron at pH 4. However, the exogenous iron was rendered more insoluble at pH 2 than the endogenous. The endogenous iron may be bound to protein and remain somewhat soluble at pH 2 and 6, whereas the exogenous iron might bind to some other component, such as monoferric phytate, which is soluble at neutral pH but insoluble at acid pH (Morris and Ellis, 1976). With asc, this may not happen due to iron-asc complexes such as ferric ascorbate which are soluble and stable from acid to neutral pH (Gorman and Clydesdale, 1983; Conrad and Schade, 1968).

The percent soluble iron at both the endogenous pH and pH 6 for samples with added iron but without asc, reflected the relative solubilities of each iron source used. Without added asc, the percent soluble iron at pH 6 ranged from 21–24% with the exceptions of the FOP (13%) and the $FeSO_4$ (33%) systems. This iron was composed 95–100% of a soluble complexed form, presumably with soy protein. By definition, complexed iron in this study was that iron which remained bound to a ligand after 30 sec in the presence of bathophenanthroline.

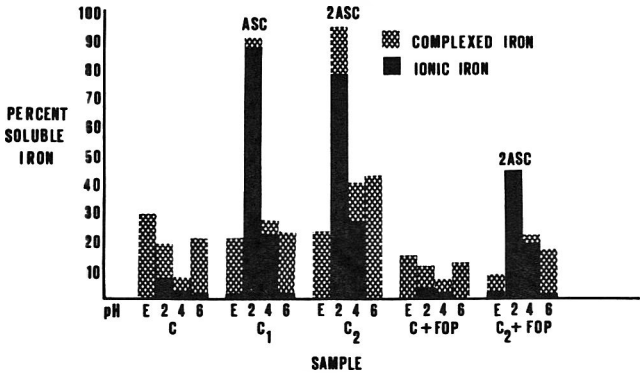

Fig. 1—Percent complexed, ionic, and soluble iron in the soy protein isolate slurry (C), C plus 120 mg ascorbic acid (C_1), or C plus 240 mg ascorbic acid (C_2), with and without 4.5 mg iron as ferric ortho phosphate (FOP) at endogenous pH (E), pH 2, 4, and 6.

Effect of ascorbic acid on soy isolate iron profile

Sufficient asc was added to the control in order to produce approximately a 25:1 and a 50:1 ratio of asc:iron by weight. The addition of asc to the soy isolate (Fig. 1, C_1) significantly increased the soluble and ionic iron at both the pH 2 and 4 levels ($p < 0.01$), but no difference was noted at the pH 6 level. A further addition of asc (Fig. 1,

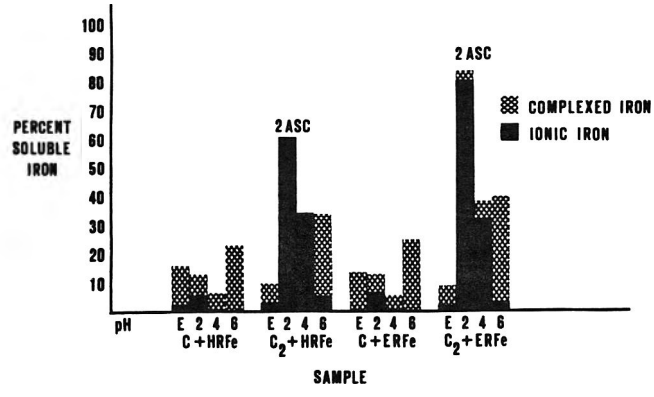

Fig. 2—Percent complexed, ionic, and soluble iron in the soy protein isolate slurry (C) or C plus 240 mg ascorbic acid (C_2), with or without 4.5 mg iron as hydrogen reduced elemental iron (HRFe) or electrolytically reduced elemental iron (ERFe) at endogenous pH (E), pH 2, 4, and 6.

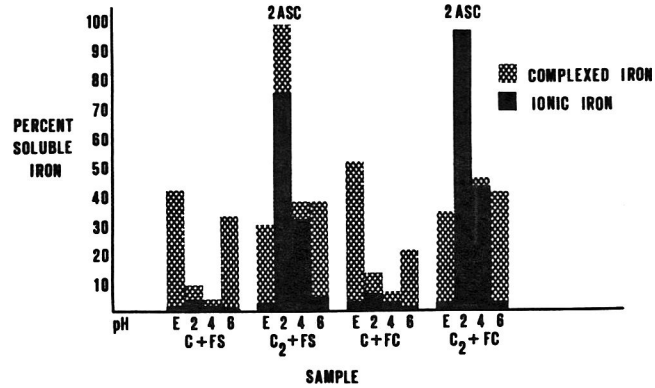

Fig. 3—Percent complexed, ionic, and soluble iron in the soy protein isolate slurry (C) or C plus 240 mg ascorbic acid (C_2), with or without 4.5 mg iron as ferrous sulfate heptahydrate (FS) or ferric chloride hexahydrate (FC) at endogenous pH (E), pH 2, 4, and 6.

C_2) produced statistically significant increases over the C_1 sample values in the following iron forms: percent soluble iron at pH 2 ($p < 0.05$), 4 ($p < 0.05$), and 6 ($p < 0.01$); percent complexed iron at pH 2 and 6 ($p < 0.01$); and percent ionic iron at pH 2 ($p < 0.05$). These increases in soluble iron would indicate that asc, and not iron, was the limiting factor in the soy isolate at the levels used, in contrast to the results of Kojima et al. (1981) with a pinto bean system.

Combined effects of asc and exogenous iron on the chemical profile

Ferric chloride, although not approved as an iron fortificant in the U.S., was included in this study since it has been used as an exogenous iron source in human absorption experiments. Morck et al. (1982) observed greater than a fivefold increase in iron absorption in normal men when 100 mg asc were added to an isolated soy protein meal containing one-half of the total iron from the soy protein, and the other half from ferric chloride. To mimic their system and study the effects of asc on the iron chemistry in soy, 4.5 mg of iron were added as $FeCl_3$ and sufficient asc was added to produce a 25:1 asc:iron ratio. The results indicate significant increases in soluble iron when asc was added to the $FeCl_3$ system (Fig. 3, C_2 + FC) at pH 2, 4, and 6 as compared to the system without asc ($p < 0.01$). At pH 2 and 4, greater than 95% of the soluble iron existed as ionic and essentially all of the ionic was in the ferrous state.

At pH 6, the percent soluble iron doubled upon addition of asc, but greater than 90% of this iron was in the complexed state. It was logical to assume that the additional iron analyzed as complexed was actually bound to asc, since this component was the only difference between the two systems. Based on the pH of this system (6.0) and on previous investigations (Gorman and Clydesdale, 1983), the complex formed was most probably ferric-asc, rather than ferrous-asc.

Qualitatively, the increase in iron absorption noted by Morck et al., (1982) may be partially explained by the increase in soluble iron at pH 2, 4, and 6. At these pH values, the ratios of percent soluble iron determined with asc to without asc, were 8.3, 8.3, and 2.0, respectively, versus a 5.69-fold increase in iron availability in human subjects. Therefore, the ratios of percent soluble iron noted cannot quantitatively explain the increase in bioavailability.

Nelson and Potter (1980) have demonstrated that although the percent soluble iron was very similar between ferrous and ferric soy isolate protein complexes, the ferric was found to be significantly lower in bioavailability than the ferrous complex. This indicates that while the percent soluble iron was important, the significance of the ionic form ws even greater.

In addition to $FeCl_3$, this study employed four iron sources commonly used to fortify foods. One way of expressing the effects of asc on the exogenous iron systems was to calculate the "enhancing factors" for the percent soluble iron (Table 1). This tends to remove effects due to absolute changes in iron concentration at various pH values and focuses on the effect of asc at a given pH, allowing more valid comparisons between different pH values. Enhancing factor was defined as the ratio of the percent soluble iron detected with asc present to the percent soluble iron w/o asc present. These values have been calculated and are shown in Table 1. For three of the five systems ($FeSO_4$, ERFe, and $FeCl_3$) the enhancing factors between pH 2 and 4 were the same or very close, while the FOP and HRFe systems had enhancing factors within about one. An enhancing factor of 1.0 would indicate no affect of asc on iron solubility in soy protein.

At pH 6, drastic decreases in the enhancing factors to 1.2–2.0 were noted for all samples. The fact that maximum enhancement occurs below pH 6 agrees with the findings of Kojima et al. (1981), who demonstrated that asc was maximally effective in solubilizing iron in a bean suspension between pH 1.5 and 5.

The systems containing 240 mg asc had substantially greater percentages of soluble iron at pH 4, and slightly larger percentages at pH 6, than the same systems w/o asc. The increases in soluble iron at intestinal pH values may explain observed iron availability increases in man. Asc produced 27–43% soluble iron at pH 4 in the form of ionic iron, with greater than 95% of this in the ferrous state. One exception was the addition of FOP and asc to the soy (Fig. 1, C_2 + FOP) which produced substantially less soluble (22%) and ionic (18%) iron than even the soy-asc sample without added iron (Fig. 1, C_2). Similar decreases for FOP were noted at pH 6.

All samples without asc invariably contained less than 3% ferrous iron at either pH 4 or 6. These data suggest that asc was acting by both solubilizing and reducing some of the iron from both endogenous and exogenous sources. However, as previously mentioned, the quantitation of ionic iron using the bathophenanthroline compound in the presence of asc, must be interpreted with caution, due to the larger binding constant of bathophenanthroline for iron and, therefore, its ability to pull iron away from asc. Hence, it was just as possible that the ferrous iron analyzed was originally in an iron-asc complex. Titrations of asc and iron solutions with base have been used successfully to analyze such a complex (Gorman and Clydesdale, 1983) in model systems, but would probably have limitations in a more complex system such as soy isolate.

The control sample containing 240 mg asc (Fig. 1, C_2) had 95%, 40% and 42% of the total endogenous iron solubilized from the soy protein at pH values of 2, 4, and 6, respectively. If an exogenous iron source interacted with the soy protein in the same manner as the endogenous iron, then identical chemical profiles should have been observed. $FeSO_4$, $FeCl_3$ and ERFe samples with asc had slight but statistically significant increases ($p < 0.05$) in soluble iron over the control (Fig. 1, C_2) at pH 4 and 6, whereas HRFe and FOP systems exhibited significantly lower percent soluble iron than the control at the same pH values. This indicates that the exogenous iron entered a different chemical pool than endogenous iron, thus raising questions about the nonheme pool concept at high levels of exogenous iron.

In summary, this study offers plausible chemical explanations for the observed increases in human iron absorption from a soy protein isolate meal containing asc (Morck et al., 1982). Since the meals consumed by the iron absorption subjects contained corn syrup solids and corn oil in

Table 1—A comparison of enhancing factors[a] at different pH values for samples with and without exogenous iron

Sample[b]	Enhancing factors[a]		
	pH 2	pH 4	pH 6
C_1[c]	4.5	4.5	1.1
C_2[d]	4.9	6.9	2.0
C_2 + $FeSO_4$	10.9	10.9	1.2
C_2 + FOP	4.5	3.6	1.3
C_2 + HRFe	5.1	6.2	1.5
C_2 + ERFe	7.1	7.4	1.6
C_2 + $FeCl_3$	8.3	8.3	2.0

[a] Enhancing factor is the ratio of the percent soluble iron detected with ascorbic acid present to the percent soluble iron without ascorbic acid present.
[b] $FeSO_4$ = ferrous sulfate heptahydrate; FOP = ferric ortho phosphate; HRFe = hydrogen reduced elemental iron; ERFe = electrolytically reduced elemental iron; $FeCl_3$ = ferric chloride hexahydrate.
[c] C_1 = Control plus 120 mg ascorbic acid.
[d] C_2 = Control plus 240 mg ascorbic acid.

addition to soy protein, direct comparisons to the in vitro results of asc on iron from the soy protein alone would not be valid. However, due to the presumed inhibitory effect of soy on iron it was important to test this component apart from the others.

Since iron solubility is a prerequisite for iron availability, the substantial increases in soluble iron noted in this study at intestinal pH values (4 and 6) as a result of asc supplementation offers a partial explanation for the increased iron absorption in man. The solubilizing effect of asc on iron from the soy protein isolate was pH-dependent; contingent upon the exogenous iron source present; and positively correlated with the concentration of asc. By understanding the chemical behavior of iron in the presence of competing ligands such as soy protein and asc, a greater knowledge of the factors affecting iron availability in man will ultimately be acquired. This in time will hopefully lead to the development of technological intervention techniques in foods to increase iron bioavailability.

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tional reasons or both, the utilization of bread iron seems to improve substantially.

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Rheology Models for Pseudoplastic Soy Systems Based on Water Binding

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ABSTRACT

The objectives were to (1) determine the effects of sucrose, glucose, sodium chloride and soy soluble carbohydrate on flow characteristics of suspensions of polymers, and (2) investigate the relationship between these effects and the quantity of water associated with polymer and solute in the suspension. Polymers were defatted soy flour, soybean sodium proteinate and soybean cell wall material. A strong linear relationship was developed between the ratio of polymer and polymer bound water to solute and solute bound water (P/S) and flow characteristics. The models developed from these relationships held for soy flours containing both polymer and solute. Suspensions with high P/S showed a high degree of pseudoplastic behavior.

INTRODUCTION

THE EFFECTS OF SOLUTES on flow characteristics of polymer suspensions have been investigated by many researchers. Hermansson (1975) found that addition of sodium chloride reduced viscosity and increased the degree of non-Newtonian behavior for soy protein, whey protein, and caseinate. Fleming et al. (1974) showed viscosity reduction for slurries of soybean concentrates and isolates when sodium chloride was added to the slurries. Catsimpoolas and Meyer (1970) reported reduction in gel viscosity of soybean globulins when sodium chloride was added; this reduction occurred at all temperatures. Finally, Ehninger and Pratt (1974) found that sucrose and dextrose also reduced viscosity of soy protein gels.

These authors generally considered that high viscosity of suspensions was caused by water uptake by the polymers. In distilled water the dissociation of quaternary structure and strong intramolecular repulsive forces result in molecular expansion and water uptake. Sodium chloride, because of its ionic nature in water, was thought to neutralize the intramolecular repulsive forces and stabilize the quaternary structure. Thus, it reduces viscosity by preventing molecular expansion and water uptake. Sucrose and dextrose were thought to reduce viscosity by nature of their affinity for water. In polymer systems containing these solutes, water uptake by the polymer is reduced when the water becomes associated with the solute instead of the polymer. However, this explanation was not substantiated by quantifying the water associated with both the polymer and solute.

Lang and Steinberg (1980, 1981) developed mass balance concepts which described the water relations in mixtures. With these concepts, if the water activity (a_w) of a suspension of polymers and solutes is known, the quantity of water associated with each material in the suspension can be calculated. Therefore, the objective of this study was to investigate the relationship between flow characteristics of a polymer suspension and the quantity of water associated with the polymer.

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MATERIALS & METHODS

Materials

Soybean components. Defatted soy flour (Nutrisoy 7B flour with minimal heat treatment) was obtained from Archer-Daniels-Midland Company, (Decatur, IL). From dispersions of this flour, soy sodium proteinate, soybean cell wall material and soybean soluble carbohydrate were fractionated by centrifugation, manipulation of pH and freeze drying (Urbanski et al., 1982a).

Solutes. Sucrose, analytical reagent crystals, Mallinckrodt Inc. (Paris, KY). Glucose, D-glucose, dextrose anhydrous, granular analytical reagent, J.T. Baker Chemical Co. (Phillipsburg, NJ). Sodium chloride; analytical reagent, Mallinckrodt Inc. (Paris, KY).

Moisture determination

Moisture was determined in duplicate by a modification of the vacuum oven method number 14.003 (AOAC, 1980). With this modification, samples were heated for 24 hr at 60°C instead of 98–110°C.

Protein content

Nitrogen content was determined in duplicate by the micro-Kjeldahl method (AOAC, 1980) and was multiplied by 6.25 to obtain crude protein content.

Sorption isotherms

Materials described above at known moisture contents were equilibrated in duplicate over several saturated salt slushes of known water activities. Equilibration was done in proximity equilibration cells as described by Lang et al. (1981). Weight gain upon equilibration was used to calculate the equilibrium moisture content at that water activity. These data were fitted to the Smith equation which gives a linear sorption isotherm (Lang and Steinberg, 1981). Regression analysis gave the slope and intercept of this line. These are shown in Table 1.

Rheological measurements

Suspensions were prepared by mechanically stirring mixtures of the freeze-dried component, the dry solute and distilled water. All suspensions were stirred for 10 min in a 50 ml beaker prior to measurement. A Haake Rotovisco Model RV-3 rotational viscometer (Haake Inc., Saddlebrook, NJ) as described by Urbanski et al. (1982b) was used for measuring the shear stress-shear rate relationship of all suspensions. For each mixture, duplicate suspensions were prepared and a shear stress-shear rate curve was determined for each. For all mixtures investigated, plots of log stress vs log rate showed correlation coefficients above 0.99. Therefore, it was concluded that all samples had flow behavior adequately described by the power law $\tau = a\dot{\gamma}^b$ (Scott-Blair, 1970), where τ = shear stress, $\dot{\gamma}$ = shear rate, a = consistency coefficient and b = flow behavior index. The intercept of this double logarithmic plot gives log a while the slope of the plot is b.

RESULTS & DISCUSSION

Sample calculations

Water activity of the suspension. Water activity of suspensions was calculated from the Lang and Steinberg (1981) equation:

$$\log (1-a_w) = \frac{MW - \Sigma(a_i\, w_i)}{\Sigma(b_i\, w_i)}$$

where: M = moisture content of mixture, g water/g solid; W = total dry matter of the mixture, g solid/g total; a_i = intercept of Smith plot for the ingredient; b_i = slope of Smith plot for the ingredient; w_i = dry matter of each ingredient, g solid/g total.

For a 25% w.b. suspension of proteinate in water to which was added 75g dry sucrose/100g suspension (Urbanski et al., 1982b):

Composition of suspension:
0.4286g sucrose
0.4286g water
0.1428g proteinate
1.000g total

$$M = \frac{0.4286 \text{ g water}}{0.4286 \text{ g sucrose} + 0.1428 \text{ g proteinate}}$$

$$= 0.7500 \text{ g water/g solid}$$

$$W = \frac{0.4286 \text{ g sucrose} + 0.1428 \text{ g proteinate}}{1.000 \text{ g total}}$$

$$= 0.5714 \text{ g solid/g total}$$

Other values are as follows:

	Sucrose	Proteinate
a_i (from Table 1)	−0.594	−0.098
b_i (from Table 1)	−1.257	−0.460
w_i (from composition)	0.4286	0.1428
$a_i w_i$ (calculated)	−0.2546	−0.0140
$b_i w_i$ (calculated)	−0.5388	−0.0657

Therefore:

$$\log(1-a_w) = \frac{0.4286 - (-0.2686)}{-0.6045} = -1.153$$

and $a_w = 0.930$.

For this mixture the a_w measured was 0.925. This represents a discrepancy of only +0.54% from the calculated value. When calculated values for water activity were compared with determined water activities for the other suspensions the error never exceeded ±2.0% (Urbanski et al., 1983).

Calculation of component equilibrium moisture contents. With the calculated a_w and with a_i and b_i values from Table 1, the equilibrium moisture content of each component in the sample suspension can be calculated by rearranging the Smith equation (Lang and Steinberg, 1981):

$$M = b_i \log(1-a_w) + a_i$$

For proteinate (polymer):

$$M = -0.460 \log(1-0.930) + (-0.098) = 0.433\text{g water/g polymer}$$

For sucrose (solute):

$$M = -1.257 \log(1-0.930) + (-0.594) = 0.858\text{g water/g solute}$$

Table 1—Smith plot parameters for polymers and solutes

	Intercept a_i	Slope b_i
Soy sodium proteinate	−0.098	−0.460
Soy cell wall material	−0.150	−0.575
Sucrose	−0.594	−1.257
Glucose	−0.706	−1.811
Sodium chloride	−5.112	−12.586
Defatted soy flour	−0.153	−0.590
Soy soluble carbohydrate	−0.894	−1.908

Ratio of polymer and polymer water to solute and solute water (P/S).

$$P/S = \frac{\text{g polymer} + \text{g polymer water}}{\text{g solute} + \text{g solute water}}$$

where: polymer = 0.1428g protein; polymer water = 0.1428g polymer x 0.433 g water/g polymer = 0.0618g water; solute = 0.4286g sucrose; solute water = 0.4286g solute x 0.858g water/g solute = 0.3677g water.

Therefore,

$$P/S = \frac{0.1428 + 0.0618}{0.4286 + 0.3677} = 0.257.$$

The calculated water content for the two components was 0.0618g + 0.3677g = 0.4295g. The experimental water content was 0.4286g. This represents an error of only +0.21%.

Consistency coefficients

The consistency coefficient ("a" value from the power law equation) of each suspension was determined from stress-rate curves (Urbanski et al., 1982b). There was a total of 46 suspensions divided as follows: 14 suspensions of soy sodium proteinate and sucrose; 8 of soy cell wall material and sucrose; 8 of soy sodium proteinate and sodium chloride; 6 of soy sodium proteinate and glucose; 4 of defatted soy flour and sucrose; 4 of soy sodium proteinate, sucrose and soy soluble carbohydrate; 2 of soy sodium proteinate, sucrose and salt.

The relationship between consistency coefficient and P/S is shown in Fig. 1. There was a linear increase in "a" value from 10 to 600 as P/S increased from 0.15 to 0.75. Linear regression analysis of the 46 points showed a correlation coefficient of 0.993. It was concluded that, for the materials here, there was a strong linear relationship between the ratio of polymer water to solute water in the suspension and the consistency coefficient of that suspension.

The broad range of "a" values in Fig. 1 may be interpreted as follows: For suspensions with high P/S ratios, most of the total suspension is comprised of the polymer and the water bound to it. In these suspensions, the swollen polymer particles rub against each other causing high internal friction so that consistency coefficients are high. For suspensions of low P/S, most of the total system is comprised of the solute and its water. In these suspensions, the polymer with its bound water is suspended in a relatively large amount of solute solution which provides lubrication so that internal friction and, consequently, consistency coefficients are low.

Flow behavior indices

Fig. 2 illustrates the relationship between flow behavior indices ("b" values from the power law equation) (Urbanski et al., 1982b) of 44 suspensions and their P/S. Regression analysis showed a linear correlation coefficient of 0.922. Therefore, for the materials tested, "b" values decreased linearly from 0.65 to 0.35 with increasing P/S from 0.2 to 0.7. For suspensions with high P/S, most of the total suspension is comprised of polymer and bound water. The flow behavior of such a suspension would resemble that of suspensions of hydrated polymer, which are known to be highly pseudoplastic. With decreasing P/S, more of the suspension is comprised of solute solution so that the suspension exhibits a behavior closer to that of a solute solution, which is Newtonian.

Rheology models based on water binding

Fig. 1 and 2 gave the following models, respectively:

$$a = 1023\,(P/S) - 155.9$$
$$b = -0.5473\,(P/S) + 0.7536$$

Thus, if the P/S ratio can be calculated, it may be used to calculate "a" and "b" values which, in turn, characterize the entire shear stress-shear rate curve for that soybean suspension.

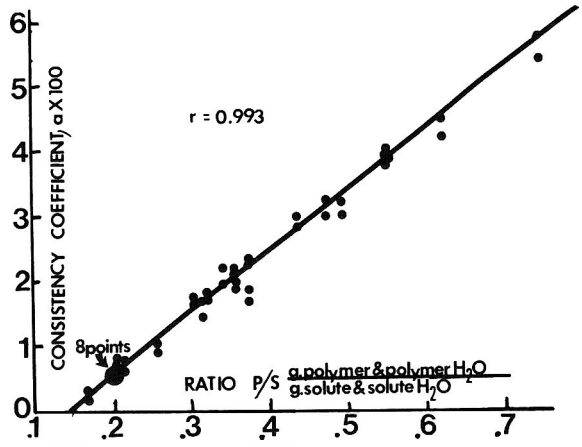

Fig. 1—Regression line for relationship between consistency coefficient and polymer to solute water ratio.

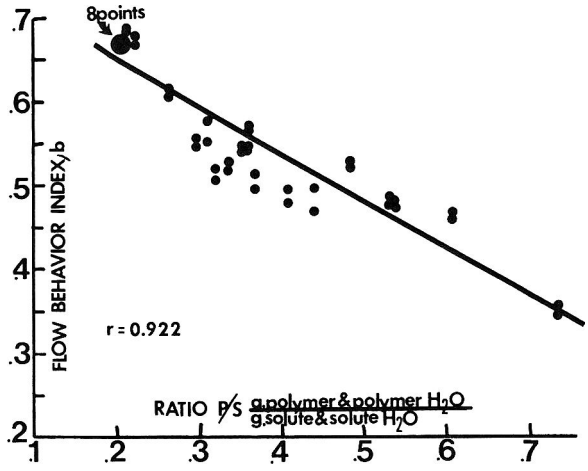

Fig. 2—Regression line for relationship between flow behavior index and polymer to solute water ratio.

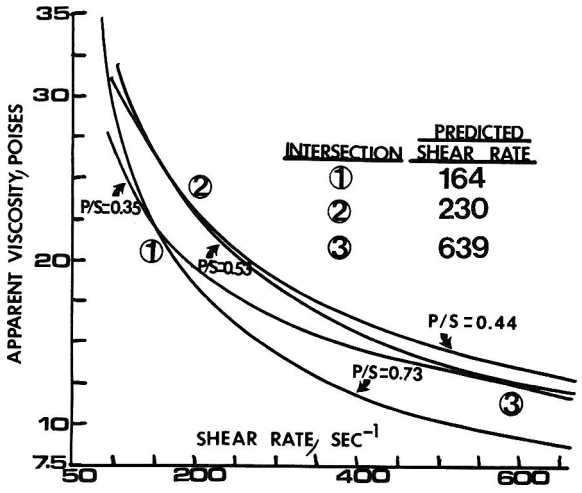

Fig. 3—Predicted and experimental intersections of curves describing decreased apparent viscosity with increasing shear rate for four suspensions of various P/S.

Validation of the models for flours

One of the suspensions evaluated contained 0.286g sucrose, 0.464g water and 0.250g defatted soy flour. Using the parameters for these materials in Table 1, the calculated a_w of the suspension was 0.95. P/S was calculated to be 0.6866. From the models, a = 546 and b = 0.378. However, the mean experimental values for this suspension were 318 for "a" and 0.525 for "b". This represents an error of +71.7% for the "a" value and −28.0% for the "b" value. The conclusion to be drawn is that the models do not hold when defatted soy flour is considered as a homogeneous polymer.

However, it must be considered that defatted soy flour itself is a mixture of polymers and solutes. It contains, on a dry basis, 54% protein, 31% cell wall material and 15% soluble carbohydrate (Smith and Circle, 1978). According to these values, the experimental suspension actually contained 0.286g sucrose, 0.464g water, 0.038g soluble carbohydrate, 0.135g protein and 0.078g cell wall material. Using the parameters in Table 1 for these materials, the calculated a_w of the mixture was 0.95. This is the same as the a_w calculated above considering the defatted soy flour as a single entity. This coincidence of calculated a_w validates

Table 2—Apparent viscosities at seven shear rates for four polymer-solute mixtures as predicted by the model and experimentally determined

Shear rate sec^{-1}	Apparent viscosity, cp: Exp. value, mean	Apparent viscosity, cp: Predicted from the Model	Percent error, $100 \times \frac{\text{Pred.-Exp.}}{\text{Exp.}}$
	P/S = 0.7315		
	(Glucose 0.131g, Water 0.652g, Protein 0.217g)		
90.7	3183	3204	+0.66
181.4	2032	2046	+0.69
272.1	1565	1574	+0.58
362.8	1297	1307	+0.77
453.5	1117	1131	+1.25
544.2	998	1005	+0.70
725.6	828	835	+0.85
	P/S = 0.5320		
	(Sucrose 0.200g, Water 0.640g, Cell Wall Material 0.160g)		
90.7	3557	3433	−3.50
181.4	2569	2364	−7.98
272.1	2112	1901	−9.99
362.8	1794	1628	−9.25
453.5	1571	1444	−8.08
544.2	1443	1309	−9.29
725.6	1204	1121	−6.89
	P/S = 0.4398		
	(Glucose 0.231g, Water 0.577g, Protein 0.192g)		
90.7	3410	3273	−4.02
181.4	2362	2336	−1.10
272.1	1905	1917	+0.63
362.8	1635	1666	+1.90
453.5	1453	1495	+2.89
544.2	1319	1368	+3.71
725.6	1133	1189	+4.94
	P/S = 0.3535		
	(Sucrose 0.333g, Water 0.500g, Protein 0.167g)		
90.7	2728	2835	+3.92
181.4	2032	2090	+2.85
272.1	1680	1748	+4.05
362.8	1499	1540	+2.74
453.5	1351	1396	+3.33
544.2	1259	1289	+2.38
725.6	1064	1135	+6.67

the distribution of defatted soy flour into its components. Because the soluble carbohydrate is solute in nature, it should be added to the denominator of P/S along with the water it binds at the calculated a_w. The protein and cell wall material and the water they bind would be the only materials considered in the numerator of P/S. With these considerations, P/S was calculated as 0.4807.

From the model, this ratio gives a consistency coefficient of 336 and a flow behavior index of 0.491. This represents an error of only 5.7% above the mean experimental "a" value of 318 and only 6.5% below the mean experimental "b" value of 0.525. This exercise demonstrates the sensitivity of the model for ingredients containing both polymer and solute constituents.

Apparent viscosity

Shear rates encountered in pouring, in the mouth and when pumping liquid food systems are drastically different and the shear rate at which apparent viscosity is measured should simulate the shear rate of particular interest. For pseudoplastic suspensions, viscosity decreases with increasing shear rate. Therefore, viscosity determined at a given shear rate is referred to as apparent viscosity.

Since the above models for "a" and "b" values allow an estimation of the entire shear stress-shear rate curve, an apparent viscosity can be estimated at any shear rate. Table 2 compares experimental apparent viscosities and those predicted from the models at seven different shear rates for four suspensions of different P/S. The error was below 1% when only glucose and protein were present at P/S = 0.73 and approached 10% for the sucrose-cell wall material system at P/S = 0.53. The average error for 46 suspensions, each measured at 7 shear rates, was only 3.9%.

Points of equal apparent viscosity

The experimentally determined apparent viscosities for the four suspensions in Table 2 are plotted against the shear rate of measurement in Fig. 3. The lines intersect at certain shear rates. At these shear rates the two suspensions have the same apparent viscosity. The importance of these points is that the two suspensions show the same viscosity at this shear rate but none other. Rearrangement of the power law equation allows prediction of the shear rate where shear stresses are equal. Since apparent viscosity (μ) is equal to shear stress (τ)/shear rate ($\dot{\gamma}$), this is also the shear rate where apparent viscosities are equal. For the two curves,

$$\tau_1 = a_1 \dot{\gamma}_1^{\,b_1} \text{ and } \tau_2 = a_2 \dot{\gamma}_2^{\,b_2}$$

At intersection of the two flow curves:

$$\tau_1 = \tau_2 \text{ and } \dot{\gamma}_1 = \dot{\gamma}_2$$

Thus,

$$(b_1 - b_2)\log \dot{\gamma} = \log a_2 - \log a_1$$

and

$$\log \dot{\gamma} = \frac{\log a_2/a_1}{b_1 - b_2}$$

For the four P/S ratios in Fig. 3, "a" and "b" values were calculated for the model and used to predict the shear rate where apparent viscosities were equal. These predicted values compared favorably with the graphical intersections as shown in Fig. 3.

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Differences in the Lipid Composition of Fresh Water Prawn *(Macrobrachium rosenbergii)* and Marine Shrimp

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ABSTRACT

Total lipid content of fresh water prawn (FWP, *Macrobrachium rosenbergii*) was found to be greater than that of marine shrimp (3.18 vs 1.33%). This was due to the much higher levels of triglycerides in FWP as compared to marine shrimp. The $\omega 6$ polyunsaturated fatty acids (PUFA) predominated in FWP lipids, whereas the $\omega 3$ PUFA predominated in marine shrimp, primarily due to the greater concentration of linoleic acid in FWP lipids (16.3 vs 2.9%). The triglycerides of both species contained considerable amounts of PUFA and FWP has a high triglyceride content. This may contribute, in part, to the shorter shelf-life of *Macrobrachium* as compared to marine shrimp.

INTRODUCTION

CULTURING FRESH WATER PRAWNS (*Macrobrachium rosenbergii*) has become profitable in Hawaii (Hanson and Goodwin, 1977) and its production is expected to increase substantially in the future. *Macrobrachium* requires temperatures above 13°C and areas of the continental U.S. with wet, subtropical climates, such as Louisiana, have the potential to be major locations for the commercial production of fresh water prawns. Successful commercialization of freshwater prawns will depend not only on culturing techniques, but also on maintenance of edible quality of the harvested product, which is an important factor in determining consumer acceptance.

It has been reported that raw whole prawns kept on ice begin to soften and deteriorate after 3–4 days (Nip and Moy, 1979). Iced marine shrimp, on the other hand, have a much longer shelf life. Nip et al. (1981) have suggested that this may be due to difference in the amino acid composition of insoluble collagen in the abdominal tissues of machrobrachium and marine shrimp. Sidwell (1977) and Sidwell et al. (1974) have reported a higher total fat content in *Macrobrachium* (2%) than in marine shrimp (1.1%). This indicates that an additional reason for the differences in the stability of the two species may exist, as autooxidation of unsaturated fatty acids leads to the development of off odors and flavors.

Bottino et al. (1979) found no significant changes in the fatty acid composition of marine brown shrimp (*Penaeus aztecus*) held on ice or frozen storage for 18 days. The stability of frozen *Machrobrachium* was investigated by Hale and Waters (1981), who, based on taste panel ratings, estimated the storage life to be about 7 months for whole prawns and 10 months for tails. The difference was attributed to the action of digestive enzymes in the whole prawn. Reddy et al. (1981) determined total lipid and fatty acid composition in *Macrobrachium* and detected no objectionable flavor during six months of frozen storage. These studies, however, have not determined the composition of the different lipid classes of *Macrobrachium* as compared to marine shrimp. The purpose of this study, therefore, was (1) to determine the relative amounts of total lipids, triglycerides, phospholipids and cholesterol in *Macrobrachium* and marine shrimp, (2) to determine the fatty acid composition of the lipid classes, as well as the total lipids and (3) to evaluate the stability of fatty acids in *Macrobrachium* held on ice for 7 days.

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MATERIALS & METHODS

LIVE FRESH WATER PRAWNS were obtained from the Louisiana State University Experiment Station Aquaculture farm. Total lipids were immediately extracted from 5–6 whole prawns, after trimming the antennae, by the method of Bligh and Dyer (1959). The rest were packed in crushed ice and stored in the cooler. This procedure was repeated with 5–6 iced prawns, every day for a week. total lipids were also extracted from 5–6 marine shrimp (*Penaeus aztecus*) obtained from a trawler, near Grand Isle, LA.

Total lipids were quantitated by the AOAC (1970) method after extraction of lipids byy the method of Bligh and Dyer (1959). Phospholipid phosphorus was determined by the modification of Bartlett (1959); triglyceride content by the method of Fletcher (1968), using 9-*trans*-12-*trans* linoleate as the standard; and total cholesterol was determined by a modification of Sperry's (Sperry and Webb, 1950) and Zak's (Zak et al., 1954) method. Prawn and shrimp lipids (10–20 μl) were saponified with ethanolic potassium hydroxide. The free cholesterol was then extracted with petroleum ether and precipitated as the digitonide. The precipitate was dissolved in glacial acetic acid, allowed to react with the color reagent, and the absorbance at 560 mμ was determined, to obtain concentration of total cholesterol.

Total lipids were fractionated by thin layer chromatography (TLC, Silica Gel G, Kontes, Vineland, NJ) using diethyl ether/petroleum ether/acetic acid (30:70:1, v/v/v). The phospholipid and triglyceride fractions were scraped off and eluted using 20 ml of chloroform/methanol/acetic acid (1:1;0.2, v/v/v). Total lipids, phospholipids and triglycerides were saponified and methylated by the method of morrison and Smith (1964) using boron trifluoride-methanol. The fatty acid composition of the lipids was determined by gas liquid chromatography as described in a previous publication (Hwang and Kinsella, 1979). Individual fatty acids were identified using methyl esters of standard fatty acids from Nu-Chek Prep (Elysian, MN) and standard mixtures from Supelco, Inc. (Bellefonte, PA 16823).

RESULTS & DISCUSSION

THE AMOUNTS of total lipids, triglycerides, phospholipids (as phosphorus) and total cholesterol are shown in Table 1. Total lipid content of *Macrobrachium* was significantly greater than that of marine shrimp ($p < 0.01$), and this difference was reflected in the higher levels of triglyceride in *Macrobrachium* ($p < 0.01$). The lipid content of marine shrimp (1.33%) obtained in this study, is in agreement with the value (1.1%) reported by Sidwell et al. (1974) and that (1.9%) reported by Bonnet et al. (1974). However, the lipid content of *Macrobrachium* (3.2%) is higher than that (2%) reported by Sidwell (1977) or that (1.63%) reported by Reddy et al. (1981). These workers analyzed only the tails, and while the composition of seafood can vary with animal age, size, diet, location, and season of catch (Sidwell, 1977), the higher lipid content obtained in this study indicates that whole prawns have a higher lipid content than prawn tails or shrimp and therefore are likely to deteriorate faster.

Table 2 shows the fatty acid composition of total lipids, phospholipids and triglycerides in *Machrobrachium* and marine shrimp. *Macrobrachium* had greater amounts of 18:2ω6, 16:0 and 18:1ω9 and lesser amounts of the long chain polyunsaturated fatty acids (PUFA) of the linolenic acid (ω3) family. In fact, in all the *Macrobrachium* lipid classes examined, the content of ω6 PUFA exceeded that of ω3 PUFA, which are the major PUFA in the lipids of most aquatic animals. In marine shrimp, on the other hand, the ω3 PUFA predominated. Ackman (1974) has pointed out that cultivated fish such as catfish and carp may have higher levels of 18:2ω6, as they are usually fed diets containing plant materials, often supplemented with vegetable oils. The prawns used in this study were not fed any special diet. In contrast to the triglycerides of domestic animals, the triglycerides of both species contained considerable amounts of PUFA. *Macrobrachium* has a very high triglyceride content (Table 1) containing 32.8% PUFA (Table 2) and therefore contains greater amounts of PUFA, which could shorten its shelf life, as autooxidation of PUFA is an important factor affecting edible quality.

The fatty acid composition of prawn total lipids and phospholipids during storage on ice for 7 days is given in Tables 3 and 4. There is an increase in the percentage of PUFA in phospholipids, but there is no change in the percentage of PUFA in total lipids. This is probably because the amount of phospholipids in the total lipid is too small to affect fatty acid composition of total lipid. Bottino et al. (1979) also found no change in amounts of PUFA in marine brown shrimp frozen for 18 days. However, Reddy et al. (1981) found decreases in total amounts (mg/100g flesh) of saturated, monounsaturated and polyunsaturated fatty acids in *Macrobrachium* during 6 months of frozen storage.

Table 1—Lipid composition of freshwater prawn and marine shrimp

	Fresh water prawn	Marine shrimp	
Number of observations[a]	7	2	p
Total Lipids (%)	3.18 ± 0.38[b]	1.32 ± 0.02	<0.01
Triglycerides			
mg/g fat	729.56 ± 187.12	212.84 ± 54.50	<0.01
mg/g body weight	23.05 ± 5.86	2.82 ± 0.78	<0.01
Phospholipids (as phorphorus)			
mg/g fat	12.65 ± 1.98	20.66 ± 0.87	<0.01
mg/g body weight	0.40 ± 0.04	0.28 ± 0.02	
Total Cholesterol			
mg/g fat	35.74 ± 6.18	67.70 ± 10.08	<0.01
mg/g body weight	1.13 ± 0.20	0.90 ± 0.12	

[a] Each observation consisted of a sample of 5—6 animals
[b] Mean ± S.D.

CONCLUSIONS

THIS STUDY on the lipid composition of *Macrobrachium* and marine shrimp has shown that *Macrobrachium* has a higher lipid content than marine shrimp, primarily due to a greater triglyceride content. It has also shown that the ω6 PUFA predominate in *Macrobrachium* lipids, whereas the ω3 PUFA predominate in shrimp lipids. Finally, it has been shown that the triglycerides of both species contain considerable amounts of PUFA. Since *Macrobrachium* has a

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Table 2—Fatty acid composition of prawn and shrimp lipids[a]

	Fresh water prawn			Marine shrimp		
Fatty Acid	TL	PL	TG	TL	PL	TG
16:0	26.0[b]	22.1[c]	28.7[d]	17.6[b]	23.7[c]	27.3[d]
16:1ω9	6.4	4.8	5.5	13.5	11.1	8.3
18:0	9.8	14.6	12.6	9.3	13.0	19.5
18:1ω9	28.8	25.0	24.2	14.9	13.8	13.5
18:2ω6	16.3	13.2	12.2	2.9	3.0	2.2
18:3ω6+20:1ω9	0.7	0.5	0.9	2.6	1.8	5.3
18:3ω3	1.9	1.5	1.4	1.5	1.3	–
20:2ω6	1.0	0.8	1.0	1.7	1.4	6.4
20:3ω6+22:1ω9	0.1	0.1	0.1	0.2	0.1	–
20:4ω6	2.7	5.2	1.1	6.4	5.5	1.2
20:5ω3	3.7	8.6	0.4	15.5	13.8	4.0
22:4ω6	0.2	–	3.0	0.8	0.8	–
22:5ω6	0.2	0.3	–	1.2	0.7	0.1
22:5ω3	0.2	0.2	1.8	1.5	1.2	9.9
22:6ω3	2.1	3.0	7.2	10.3	8.7	2.4
Total PUFA	28.3	32.8	28.1	41.8	36.4	26.2
Total ω6 PUFA	20.4	19.5	17.3	13.0	11.4	9.9
Total ω3 PUFA	7.9	13.3	10.8	28.8	25.0	16.3

[a] TL = Total Lipid; PL = Phospholipid; TG = Triglyceride
[b] Percent of total fatty acid
[c] Percent of phospholipid fatty acid
[d] Percent of triglyceride fatty acid

Table 3—Changes in the fatty acid composition of prawn total lipids during storage on ice (as percent of total fatty acids)

	Length of storage (days)						
Fatty acid	0	1	2	3	4	5	6
16:0	27.0	27.7	26.8	23.6	18.6	23.0	26.1
16:1ω9	6.2	6.7	6.8	7.3	4.5	5.9	6.1
18:0	10.3	10.0	10.1	10.4	12.0	11.3	10.7
18:1ω9	28.1	28.1	28.4	27.3	31.1	30.0	28.2
18:2ω6	15.8	14.7	14.7	14.5	16.3	17.1	16.0
18:3ω6+20:1ω9	0.7	0.7	0.7	0.9	1.5	1.1	0.8
18:3ω3	1.7	1.7	2.4	2.5	1.9	1.6	1.7
20:2ω6	1.0	0.9	1.0	1.6	1.7	1.2	1.0
20:3ω6+22:1ω9	0.1	0.1	0.1	1.6	0.1	–	0.1
20:4ω6	2.1	2.5	3.0	2.8	4.3	2.7	3.1
20:5ω3	3.5	3.6	3.7	4.2	4.0	3.6	3.9
22:4ω6	0.5	0.6	0.2	0.2	0.1	0.2	0.1
22:5ω6	0.1	0.2	0.1	0.6	0.3	0.5	0.2
22:5ω3	0.2	0.1	0.2	0.2	0.4	0.1	0.1
22:6ω3	2.7	2.3	1.9	2.9	1.4	2.1	1.6
Total PUFA	27.6	26.6	27.2	29.5	30.4	29.1	27.7
Total ω6 PUFA	19.5	18.9	19.0	19.7	22.7	21.7	20.4
Total ω3 PUFA	8.1	7.7	8.2	9.8	7.7	7.4	7.3

Table 4—Changes in fatty acid composition of prawn phospholipids during storage on ice (as percent of phospholipid fatty acids)

	Length of storage (days)						
Fatty acid	0	1	2	3	4	5	6
16:0	25.4	21.9	21.9	22.2	22.3	22.2	22.4
16:1ω9	6.1	3.8	5.7	3.8	6.1	4.3	5.2
18:0	11.0	15.8	14.2	14.7	12.1	11.1	11.1
18:1ω9	29.8	25.2	26.3	24.7	22.8	22.1	21.9
18:2ω6	16.2	13.8	14.5	13.1	13.8	15.6	14.7
18:3ω6+20:1ω9	0.9	0.5	0.4	0.5	0.3	0.3	0.3
18:3ω3	1.4	1.4	1.2	1.5	1.7	1.4	1.8
20:2ω6	1.0	1.0	0.8	0.9	0.7	0.7	0.7
20:3ω6+22:1ω9	–	–	–	–	–	–	–
20:4ω6	2.4	5.8	4.6	5.4	6.7	6.5	6.4
20:5ω3	3.5	7.8	7.5	8.9	9.4	11.4	11.7
22:4ω6	0.1	0.3	0.2	0.4	0.2	0.1	0.5
22:5ω6	0.1	0.2	0.3	0.3	0.3	0.3	0.2
22:5ω3	0.4	–	0.4	0.4	0.4	0.7	–
22:6ω3	1.6	3.1	2.6	3.6	3.4	3.2	3.7
Total PUFA	26.7	33.4	32.1	34.5	36.6	39.9	39.7
Total ω6 PUFA	19.8	21.1	20.4	20.1	21.7	23.2	22.5
Total ω3 PUFA	6.9	12.3	11.7	14.4	14.9	16.7	17.2

Effect of Age, Sex and Strain on the Fatty Acid Composition of Goose Muscle and Depot Fats

D. W. FRIEND, J. K. G. KRAMER, and A. FORTIN

ABSTRACT

The fatty acid composition of muscle and depot fats from four strains of geese was determined by GLC analysis. Differences due to sex and age of goose were not significant ($P < 0.01$). The major fatty acids of depot fat were oleic (54%), palmitic (22%), linoleic (11%) and stearic (8%). Muscle contained from 4.7–9.6% fat composed mainly of oleic (42%), palmitic (25%), linoleic (14%) and stearic (12%) acids. The muscle fat content of Pilgrim and Hungarian geese was less than that of the Crossbred ($P < 0.01$) and of the Chinese strain.

INTRODUCTION

FEW STUDIES have been made in North America of the lipids of goose tissue. Fristrom and Weihrauch (1976) reviewed the post-1960 literature; it was sparse and the data presented in the report were considered to be provisional only. The total fat content of goose flesh was 7.1g per 100g edible portion but it was 34% in the flesh-plus-skin. Of the saturated fatty acids, palmitic acid predominated and of the unsaturated fatty acids, oleic was the major constituent. The effects of strain of goose, nutrition and type of fat depot on fatty acid composition has received only scant consideration (Markiewicz et al., 1979; Martincic and Fulgosi, 1978; Molnar, 1972) and is without confirmation. The objective of the present study was to determine differences in fatty acid composition between internal and muscle fat from male and female geese of four strains at each of three ages.

MATERIALS & METHODS

FIFTEEN male and 15 female birds from each of four strains of geese, namely, Chinese, Hungarian, Pilgrim and Crossbred (a synthetic strain derived from the three other strains) were used. They were the product of three hatches during May and June 1980 and had been fed successively, starter (1–3 wk), grower (4–9 wk) then finisher (10 wk to slaughter) diets of which corn and soybean meal were the main components, together with wheat shorts, wheat middlings, limestone, calcium phosphate, salt and a vitamin-trace mineral premix. The birds were in grass paddocks during the grower and finisher stages and received silage when grass was in short supply.

All birds were killed and dressed the same day in a fully equipped poultry processing plant, details of which have been described by Leger and Gowe (1981). When the geese were cut for carcass quality measurements and appraisal (Fortin et al., 1983) samples of depot (abdominal, leaf) fat and breast muscle (m. pectoralis) were taken. These samples were kept in nitrogen-purged, vinyl screw-capped glass vials and stored at −20°C until needed for analysis.

A sample of 30–35 mg depot fat was put in a 15 ml tube (teflon lined screw-cap); 1 ml chloroform- methanol (2:1, v/v) was added and allowed to stand for at least 30 min. Then 5 ml of 5% HCl-methanol (m/m) was added to this lipid mixture.

A sample of about 1g frozen muscle tissue was weighed accurately then pulverized at dry ice temperature (Kramer and Hulan, 1978). The powdered sample was added to 25 ml chloroform-methanol (2:1, v/v) kept at 0°C. The mixture was dispersed lightly with a Virtis 45 homogenizer and allowed to stand at room temperature for 1 hr, then filtered quantitatively through a Whatman No. 4 filter paper. The filtrate was dried in a rotary evaporator under high vacuum and weighed to give total lipid per sample. The filter paper and residue were air dried; total nitrogen was determined by the Kjeldahl procedure (AOAC, 1980) and values adjusted for nitrogen content of the filter paper. Total protein content was calculated from nitrogen content by a conversion factor of 6.25. An aliquot of the total lipids was transesterified with HCl-methanol to prepare the methyl esters.

Samples of the goose finisher diet in approximately 1g quantities were homogenized with 75 ml of chloroform-methanol (2:1, v/v) for 5 min. The flask contents and two 15 ml rinsings were passed through a Whatman No. 41H filter paper, then dried under vacuum using a rotary evaporator. The total lipids were determined gravimetrically; an aliquot of the extract was transesterified with HCl-methanol for 45 min.

All methyl ester samples were extracted twice with 3 ml aliquots of glass distilled hexane after adding 0.4 ml distilled water to the tube. The hexane extractions were reduced to 0.1 ml with a stream of nitrogen and purified on thin-layer plates coated with 0.25 mm silica gel G. The plates were developed using the solvent 1,2-dichloroethane to separate dimethyl acetates from methyl esters (Winterfeld and Debuch, 1966). The methyl ester band was visualized by long-wave UV light after spraying the plate with rhodamine B in methanol. The band was transferred by vacuum to a Pasteur pipette containing a solvent-washed glass wool plug. The esters were eluted with about 3 ml glass distilled hexane which was then removed with a stream of nitrogen. Carbon disulphide was added and the sample transferred to 1 ml autosampler vials for subsequent analysis by gas-liquid chromatography(GLC).

A Hewlet-Packard 5830 gas chromatograph equipped with a flame ionization detector was used for GLC analysis. The methyl esters were separated on a flexible, fused silica column (25 m length, 0.2 mm ID) coated with Carbowax 20 M (Hewlet-Packard Ltd., Ottawa, Ontario). Injection and detector temperatures were 250°C; column temperature, 170°C; carrier and septum purge gas, helium; make-up gas (29 ml/min), nitrogen; and head pressure 30 psig helium. Methyl esters were identified by using authentic standards (Nu Chek Prep. Inc., Elysian, MN); the peaks were quantitated by digital integration. A single determination on each fat sample was considered sufficient to detect differences due to experimental treatments (Salmon and O'Neil, 1973).

Statistical examination of all methyl ester data involved analyses of variance for a 4 (strain) x 3 (age) x 2 (sex) factorial experiment. A probability level of 1% was selected for significance.

RESULTS & DISCUSSION

THE FAT CONTENT of the finisher diet was 2.4% (24 mg/g diet) of which the major component fatty acid was linoleic (52%) followed by oleic (25%) and palmitic (20%). The high percentage of linoleic acid reflected the high corn content (62% by weight) of the diet. When interpreting the fatty acid data associated with the fat depot relative to age, some differences might be coincident with changes in diet; for example, the starter diet fed up to 21 days of age, contained 2% dehydrated alfalfa. At this age the geese were given access to grass paddocks and at 70 days changed from a grower to a finisher diet. Furthermore, in Canada, geese are usually slaughtered between 16 and 24 wk of age, but in this study the geese were between 24.7 and 27.7 wk.

The total lipid content of the muscle for the four strains of geese is shown in Table 1. There was no age and sex effect, but differences occurred among strains. The Pilgrim and Hungarian geese were similar in muscle fat

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Table 1—Mean lipid and protein content of goose muscle

Sex	Age[a] (days)	Strain			
		Chinese	Crossbred	Hungarian	Pilgrim
		mg Lipid/g wet muscle			
Male	173	64.4[b]	73.6	52.0	52.3
	180	79.6	87.7	60.2	54.3
	194	71.7	76.2	61.6	61.1
Female	173	55.6	95.5	46.5	85.3
	180	74.3	82.7	58.8	59.7
	194	77.1	94.9	66.0	73.1
Mean[c]		70.4^{xy}	85.1^{y}	57.5^{x}	64.3^{x}
		mg Protein/g wet muscle			
Male	173	204	201	206	202
	180	213	208	210	212
	194	205	203	203	203
Female	173	192	207	199	197
	180	215	211	215	209
	194	199	192	201	215
Mean[c]		205^{xy}	204^{xy}	206^{xy}	206^{xy}

[a] Hatched at different dates, but all killed the same day.
[b] All values are means of 5 geese.
[c] Standard error of strain means = 3.88 (lipid) and 2.50 (protein). Means with no common superscript (x,y) differ at $P < 0.01$

content which was less ($P < 0.01$) than that of the Crossbred, while the Chinese strain was intermediate in fat content. The Chinese strain has a higher level of egg production than either the Hungarian or Pilgrim goose, both regarded more as meat producing strains. Differences between strains, in the fat content of goose muscle, is also seen in published reports such as that of Niewiarowicz et al. (1971) who found a fat content of 1.88% for the breast muscle of Pomorska geese at 168 days of age. This level is about half of that found in the Hungarian and Pilgrim strains in our study. Fristrom and Weihrauch (1976) on the other hand, reported a level of 7% fat for goose flesh which is similar to that found in our Chinese and Crossbred strains.

Values for total protein content of the muscle are also shown in Table 1, but differences were not significant for any of the variables examined.

The fatty acid composition of goose muscle fat is shown in Table 2. There were no age and sex differences but the relative concentrations of most of the fatty acids were significantly different among strains. The reason for this was likely due to the relative abundance of triglycerides and phospholipids and their characteristic fatty acid composition. The strains (Hungarian and Pilgrim) with a lower concentration of triglycerides had a relatively higher concentration of fatty acids characteristic of phospholipids, such as 20:4 n-6 and the C22 polyunsaturated fatty acids (PUFA). Conversely, the strains (Chinese and Crossbred) with a higher triglyceride concentration had the characteristically low C18 pattern.

Strain differences in concentrations of 16:0, 16:1, 18:1 and 18:2 appeared to be dependent upon factors unrelated to the proportion of triglyceride and phospholipid because these fatty acids were found equally in both the lipid groups. The relatively low level of C22 PUFA in goose muscle fat (0.2 to 0.3%) is similar to that found in chicken (0.5%) and pig (1.2%) muscle fat though lower than in monkey (2.5%) and rat (5.7%) muscle tissue (Kramer et al., 1978).

The only other values for goose muscle fat with which to compare ours, are those provided by Fristrom and Weihrauch (1976) for raw, goose flesh containing 7% total lipid which, from extrapolation give values of 45% oleic, 21% palmitic, 3% palmitoleic, 8% linoleic and 5% stearic acid. The first

Table 2—Mean fatty acid composition of muscle fat from four strains of geese

Fatty acid[a]	Age (days)	Strains				Significance[c]	
		Chinese	Cross-bred	Hun-garian	Pil-grim	LSD[b]	Strain
		Weight percentage of fatty acids					
14:0	173	0.3	0.3	0.3	0.3		
	180	0.3	0.3	0.3	0.3	0.1	3.40
	194	0.3	0.3	0.3	0.3		
16:0	173	26.2	24.8	24.3	24.1		
	180	25.8	24.6	24.4	22.9	2.5	7.4**
	194	24.9	24.5	24.4	24.6		
16:1	173	2.8	2.9	2.4	3.0		
	180	2.5	2.9	2.4	2.5	0.7	8.4**
	194	2.6	2.9	2.3	2.9		
18:0	173	10.8	10.4	12.8	11.4		
	180	11.2	10.6	12.9	12.6	2.2	19.4**
	194	11.6	10.8	13.9	11.7		
18:1	173	41.2	44.5	41.4	43.2		
	180	40.4	44.0	42.0	42.1	4.5	9.9**
	194	40.9	43.6	38.3	41.5		
18:2	173	14.1	12.8	13.2	12.7		
	180	14.8	12.8	13.0	13.7	2.5	7.1**
	194	15.1	13.1	14.6	13.5		
18:3	173	0.9	0.9	1.0	0.8		
	180	1.1	1.0	1.0	1.0	0.7	2.9
	194	1.1	1.0	1.6	1.1		
20:4	173	2.5	2.5	3.6	3.4		
	180	2.8	2.8	3.0	3.8	1.2	10.8**
	194	2.6	2.8	3.5	3.4		
C22[d]	173	0.2	0.2	0.3	0.3		
	180	0.2	0.2	0.3	0.4	0.2	11.8
	194	0.1	0.2	0.3	0.3		

[a] The major fatty acids are listed; minor amounts of 19:0, 20:5, and 24:0 are not shown.
[b] Least significant difference ($P < 0.01$), determined from pooled standard error.
[c] The F values shown assume a fixed treatment effects model; **$p < 0.01$
[d] The sum of 22:4 n-6, 22:5 n-6, 22:5 n-3 and 22:6 n-3.

three values correspond well with ours but the latter two are about half the magnitude.

The major fatty acids of the depot fat were 18:1 (54%), 16:0 (22%), 18:2 (11%) and 18:0 (8%) acid (Table 3). The order and magnitude of these fatty acids agreed closely with those reported by Fristrom and Wiehrauch (1976) and by Markiewicz et al. (1979). Differences in percentage fatty acids between the sexes were not significant but among ages, 18:2 increased while 16:0 decreased with advancing age. The differences due to age, although not large, were reasonably consistent.

Although Markiewicz et al. (1979) observed no significant differences in fatty acid composition of subcutaneous, abdominal and suet fat among six strains of 24-wk old geese, strain differences were evident in the present study, particularly the major fatty acids of the Chinese and Pilgrim geese. Oleic acid was lower and 16:0 and 18:2 were higher in the Chinese than in the Pilgrim geese. Stearic acid, however, while not differing between these two strains, was higher than that observed for either the Crossbred or the Hungarian geese.

A comparison of the fatty acid composition of depot fat from several species is shown in Table 4. The fatty acid profile for duck and goose fat is almost identical, and these two species differ only slightly from the chicken, turkey and the pig. These latter species contain less 18:1 and more saturated fatty acids (14:0, 16:0 and 18:0) in the depot fat. The chicken and turkey, however, have more 18:3. The fat from ruminants shows a markedly different profile because

Table 3—Mean fatty acid composition of depot fat from four strains

Fatty acid	Age[a] (days)	Strains				LSD[b]	Significance[c]	
		Chinese	Cross-bred	Hun-garian	Pil-grim		Age	Strain
14:0	173	0.5	0.4	0.4	0.3			
	180	0.4	0.3	0.3	0.3	0.1	8.3**	11.8**
	194	0.4	0.4	0.3	0.3			
16:0	173	28.6	26.7	26.9	23.3			
	180	25.7	24.2	23.0	21.0	3.7	23.0**	15.0**
	194	23.6	24.2	23.6	22.3			
16:1	173	2.8	2.9	2.3	2.1			
	180	2.4	2.6	2.2	2.0	0.7	2.8	11.5**
	194	2.4	2.6	2.4	2.1			
18:0	173	8.2	7.2	7.8	7.6			
	180	8.2	7.2	7.7	8.2	1.4	0.6	6.9**
	194	8.0	7.6	6.8	8.2			
18:1	173	49.2	53.1	53.0	57.6			
	180	51.2	54.9	55.7	56.7	4.4	3.3	23.1**
	194	51.4	54.5	53.0	54.6			
18:2	173	10.2	9.2	8.9	8.6			
	180	11.4	10.0	10.0	10.8	2.1	35.2**	10.7**
	194	13.1	10.0	12.0	11.5			
18:3	173	0.4	0.6	0.6	0.4			
	180	0.7	0.9	0.9	0.9	0.6	20.7	8.1**
	194	0.9	0.7	1.6	0.8			

[a] The major fatty acids are listed; minor amounts of 20:0, 20:1, 20:2 and 20:4 are not shown.
[b] Least significant difference ($P < 0.01$) determined from the pooled standard error.
[c] The F values shown assume a fixed treatment effects model; **$P < 0.01$.

of the intervention by rumen micro-organisms; the level of 18:0 is much higher and only about 1–3% of 18:2 is present.

The results of this study corroborate the sparse information available from other sources and provide further evidence of differences in fatty acid composition of fat from muscle and depot tissue of four strains of geese. Such information is important when evaluating the nutritional and food characteristics of the goose for the consumer.

Table 4—Comparison between fatty acid composition of goose depot fat and that from other sources

Fat source	Fatty acids						
	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Goose[a]	0.0	24.2	3.6	5.6	51.5	8.8	0.0
Goose[b]	0.4	22.5	2.4	7.7	53.7	10.5	0.8
Duck[a]	0.6	22.1	5.4	4.0	50.3	11.9	0.8
Duck[c]	0.2	21.4	4.8	5.6	52.8	14.3	0.6
Chicken[d]	1.2	26.6	4.0	8.8	39.5	13.3	1.9
Turkey[e]	1.4	28.0	6.7	9.9	28.5	21.2	1.9
Pig[f]	1.2	22.7	6.8	10.0	45.2	14.4	—[g]
Sheep[h]	2.4	26.8	1.7	21.9	40.5	1.1	0.5
Beef[i]	3.2	25.9	3.3	26.1	34.1	2.6	0.3

[a] Fristrom and Weihrauch (1976)
[b] Present study
[c] Pereira and Stadelman (1976)
[d] Pereira et al. (1976)
[e] Salmon and O'Neil (1973)
[f] Friend and Cunningham (1967)
[g] Not detected
[h] Duncan and Garton (1967)
[i] Sibbald and Kramer (1980)

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Evaluation of Iron Binding Compounds as Inhibitors of Gas and Toxin Production by *Clostridium botulinum* in Ground Pork

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ABSTRACT

Iron binding systems (heme iron binding gases alone or with iron binding salts) were evaluated for antibotulinal activity in ground pork. Compared with meat systems containing nitrite or nitrite plus supplemental iron compounds, carbon monoxide (CO) was not antibotulinal. Nitric oxide (NO) treated meats did swell slower, but nitrite was also found in these systems. Thus, neither CO nor NO would be suitable substitutes for sodium nitrite in meat curing. Addition of ferric chloride or myoglobin decreased the antibotulinal effectiveness of nitrite, but samples containing nitrite plus ethylenediaminetetraacetate (EDTA) or denatured nitrosylated myoglobin (NOMb) swelled slower. Supplemental iron compounds probably decreased residual nitrite levels in the product, thus permitting botulinal growth, rather than directly stimulating growth by providing iron as an external nutrient.

INTRODUCTION

THERE IS INTEREST in developing substitutes for sodium nitrite in meat curing, since under conditions of high heat such as occurs in frying bacon, residual nitrite may react with certain amines to produce carcinogenic nitrosamines (Howard et al., 1973; Kemp, 1974; Wasserman et al., 1978). A logical procedure to find a suitable nitrite substitute is to examine compounds which may have a similar mechanism in meat curing. In addition to typical color and flavor, protection against growth and toxin production by *C. botulinum* is essential. The exact mechanism of botulinal inhibition by nitrite is not fully understood. Tompkin et al. (1978a) postulated that nitrite inhibits botulinal organisms by either reacting with iron outside the cells thus reducing the iron available for botulinal growth, or by inactivating iron containing enzyme(s) within the botulinal cell itself. Van Roon and Olsman (1977) and Tompkin et al. (1978a, 1979) have shown that added iron stimulates botulinal growth. The stimulatory effect appears to be specific to iron (Tompkin et al., 1978a; 1979).

If botulinal growth is indeed dependent upon iron in the medium, other iron binding systems may also have antibotulinal activity, and therefore may have potential as substitutes for nitrite in meat curing.

Thus the purpose of these experiments are twofold; to test the antibotulinal potential of nitric oxide (NO) and carbon monoxide (CO), both of which are heme-iron binding gases, alone or with the free iron chelating agents, tetrasodium ethylenediaminetetraacetate (EDTA), sodium phytate, or sodium oxalate, and secondarily, to evaluate the extent to which other iron compounds, such as added myoglobin or nitrosomyoglobin may affect botulinal growth in a cured meat product. Phytate, oxalate, and EDTA are not permitted by the FDA as additives to meat products. These iron chelating agents might be expected to impair absorption of iron and other divalent cations, if added to meat products. However, phytate and oxalate are already natural constitutents of many foods of plant origin, and the possibility of their antibotulinal effects warrants their evaluation in meat curing systems.

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MATERIALS & METHODS

Experiment 1

Fresh ground pork leg was blended in about 1000g batches in a large stainless steel Waring Blendor with 2.5% sodium chloride, 0.5% dextrose, other ingredients as needed for the particular treatment, and about 10% deionized water. Sodium nitrite, used in formulating the control treatment, was added in water resulting in 156 mg/kg meat (156 ppm). Sodium phytate, sodium oxalate, or EDTA, as needed, was added at 250 ppm meat.

For gas-treated samples, the blender lid was modified by addition of a stainless steel nozzle. The stainless steel blender containing blended meat was evacuated via the nozzle immediately before introducing the heme binding gas. Also added to the lid was a stainless steel male connector, attached through stainless steel 1/8 inch internal diameter tubing to the gas cylinder. The gas flow from the gas cylinder was monitored by a flowmeter equipped with a stainless steel ball. All blending was done under the hood as a safety measure when using these gases. For nitric oxide (NO) treated samples, introduction of NO for 30 sec at 400 cc/min during blending provided 1 kg product with good cured color and a residual nitrite content of 150–160 ppm immediately after cooking. Blending the meat in the presence of carbon monoxide (CO) for one minute at 450 cc/min was sufficient for a bright pink color of 1 kg of the uncooked product. Upon cooking, however, the meat turned brown. Subsequent CO treatments therefore involved exposing meats to CO for 3 minutes at the same flow rate, to insure adequate formation of carboxymyoglobin.

The meat emulsions were then inoculated with the spore suspension, consisting of *Clostridium botulinum* type A and B spores in equal numbers at a concentration sufficient to give 100 spores/g final product (Christiansen et al., 1973). *Clostridium botulinum* type A #19397 and type B #17843 spores were obtained from the American Type Culture Collection, Rockville, MD. It was assumed that uninoculated product at day 0 contained little or no botulinal spores. This was later confirmed on similar product (Mettanant et al., 1983).

After blending for an additional 90 sec to distribute the spores, 80g portions of meat emulsion were packaged in polyethylene vacuum bags, vacuum sealed, double bagged, and again vacuum sealed. The details of our procedure have been previously described (Collinge, 1981; Collinge et al., 1981; Vahabzadeh, 1982). About 35 sample bags were prepared for each treatment. In most inoculated pack experiments, swelling has been followed in canned, rather than vacuum packaged product. However, Greenberg et al. (1966) described the use of plastic "anaerobic pouches" for isolation of *C. botulinum* spores from fresh meats.

After packaging, the product was cooked in a water bath at 70°C for 30 min, cooled in an ice water bath for 12–15 min, and then abused by storage at 27°C as previously described by Tompkin et al., 1978a, b, c). Bags were removed from the incubator for chemical and microbiological analyses at specified intervals or if the bag swelled.

Experiment 2

Fresh ground pork leg was blended and formulated as described in Experiment 1. Myoglobin or nitrosomyoglobin, as needed for particular treatments, was added at 2.16 g/kg meat, which was equivalent in iron content to 20 ppm iron as ferric chloride. Ferric chloride and EDTA, as needed, were added at 20 and 250 ppm meat, respectively.

Denatured nitrosomyoglobin was prepared as follows; 6g lyophilized horse heart myoglobin (Sigma, St. Louis, MO) were solubilized in about 300 ml of a 1000 ppm solution of nitrite (as sodium nitrite). About 1.5g of ascorbic acid was added, and the mixture was heated with occasional stirring for 20 min in a boiling water bath. Ascorbic acid was used to hasten development and stabilize the color of the pigment (Kramlich et al., 1973), although normally the sodium salt is used. Nitrosomyoglobin was precipitated by addition of a small amount of sodium chloride (less than 1g/6g myoglobin), while the solution was still hot. The precipitate was recovered by centrifugation at 6000 x *g* for 15 min, then resuspended in distilled water. The centrifugation and resuspension steps were repeated 4 more times to wash out residual nitrite, sodium chloride, and ascorbic acid, before the precipitated denatured nitrosomyoglobin was added to the ground meat. The nitrosylated myoglobin was about 74–87% nitrosylated, as determined by the procedure of Hornsey (1956). Also, a distinct color change was observed as the myoglobin solution converted from brown to the reddish-pink nitrosylated product.

Chemical analyses

Nitrite concentration was determined spectrophotometrically (AOAC, 1980). Total iron was determined by atomic absorption spectroscopy (Farmer et al., 1977). Total and nitroso heme iron were determined spectrophotometrically using the procedure of Hornsey (1956). Assays for botulinal toxin were conducted as described by Kautter and Lynt (1978). Type A and B antitoxins were obtained from the Center for Disease Control (CDC, Atlanta, GA), mixed in appropriate concentrations, and injected together, rather than separately, to minimize handling and consequent stress for the protected mice. The number of viable botulinal organisms in swollen, nontoxic bags from Experiment 1 was determined by the most probable number (MPN) technique (FDA, 1978). The modified peptone colloid broth was prepared as described by Greenberg et al. (1966).

Statistical analyses

Data for number of swollen bags, nitrite, nitroso and total pigments were analyzed by two-way analysis of variance with and without covariates. Least significant difference values were also computed (Ostle and Mensing, 1975).

RESULTS

Experiment 1

Significant differences ($p = 0.05$) in the number of swollen bags were observed among treatments. All 25 bags of the control treatment, formulated with only salt and dextrose, were swollen by day 6 of storage (Fig. 1a). Samples treated with CO also swelled significantly more rapidly than

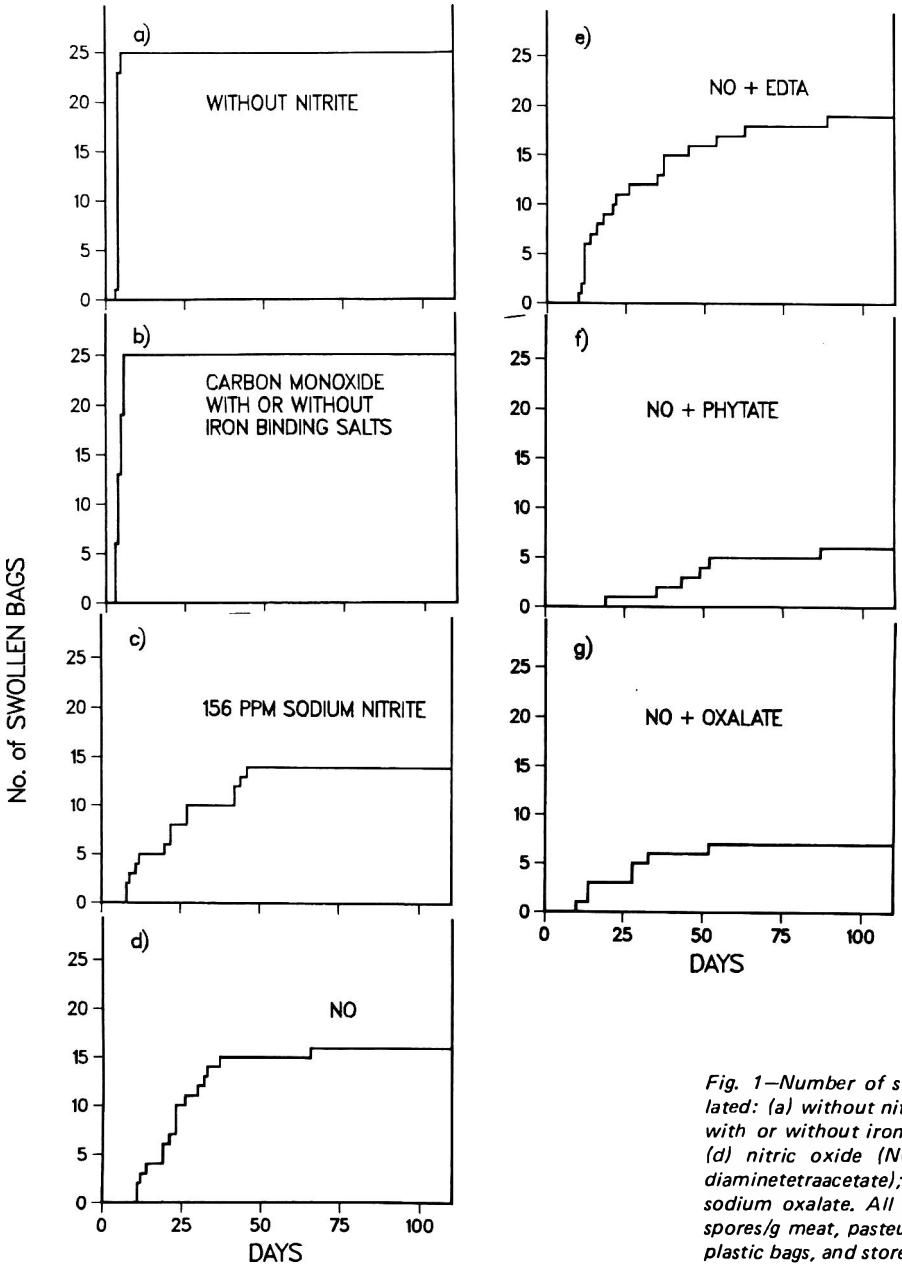

Fig. 1—Number of swollen bags with time in meat product formulated: (a) without nitrite, or formulated with; (b) carbon monoxide, with or without iron chelating agents; (c) 156 ppm sodium nitrite; (d) nitric oxide (NO), (e) NO + EDTA (tetrasodium ethylenediaminetetraacetate); (f) NO + sodium phytate; and (g) NO + sodium oxalate. All were then inoculated with 100 C. botulinum spores/g meat, pasteurized at 70° C for 30 min, vacuum packaged in plastic bags, and stored at 27° C.

did other samples. The addition of iron binding salts along with CO did not result in botulinal inhibition (Fig. 1b). All CO-treated samples were swollen by day 6 of storage at 27°C.

Formulating meat with sodium nitrite or nitric oxide decreased the number of swollen bags (Fig. 1c-g). The product formulated with sodium nitrite did not begin swelling until day 8 of storage, and 11 bags remained unswollen after 110 days storage (Fig. 1c). The meat treated with NO or NO and EDTA (Fig. 1d, e) exhibited a swelling pattern very similar to that of the sodium nitrite treated samples. Meat formulated with NO + oxalate or phytate (Fig. 1f, g), however swelled significantly more slowly than did the other NO or sodium nitrite treated samples. For both treatments, at least 18 of 25 bags were unswollen after 110 days storage. In addition, both treatments also had much higher nitrite levels immediately after cooking than other samples formulated with NO. Nitrite levels after cooking were 143 and 130 ppm for NO + oxalate and NO + phytate, respectively, compared to 82 and 56 ppm for NO and NO + EDTA treated samples, respectively. Thus, it is likely that the lower number of swollen bags observed in the NO + oxalate or NO + phytate treated samples were due to their higher content of nitrite and related compounds, rather than to any specific antibotulinal effects of oxalate and phytate.

Experiment 2

The addition of 156 ppm sodium nitrite to meat samples (Fig. 2a) decreased the swelling rate, compared to that of inoculated samples without sodium nitrite (Fig. 1a). When myoglobin or ferric chloride was added to meat samples (Fig. 2b, 2c, respectively), the samples swelled faster. For example, all 25 bags of myoglobin and ferric chloride treated samples were swollen on day 43 and 47 of storage, respectively, while the control samples treated with 156 ppm sodium nitrite were not all swollen until day 62 of storage.

The addition of EDTA (Fig. 2d, f, g) greatly decreased the number of swollen bags, compared to the sodium nitrite treated control samples. For example, when samples were formulated with 250 ppm EDTA and 156 ppm sodium nitrite, only 1 bag swelled during the storage period (Fig.

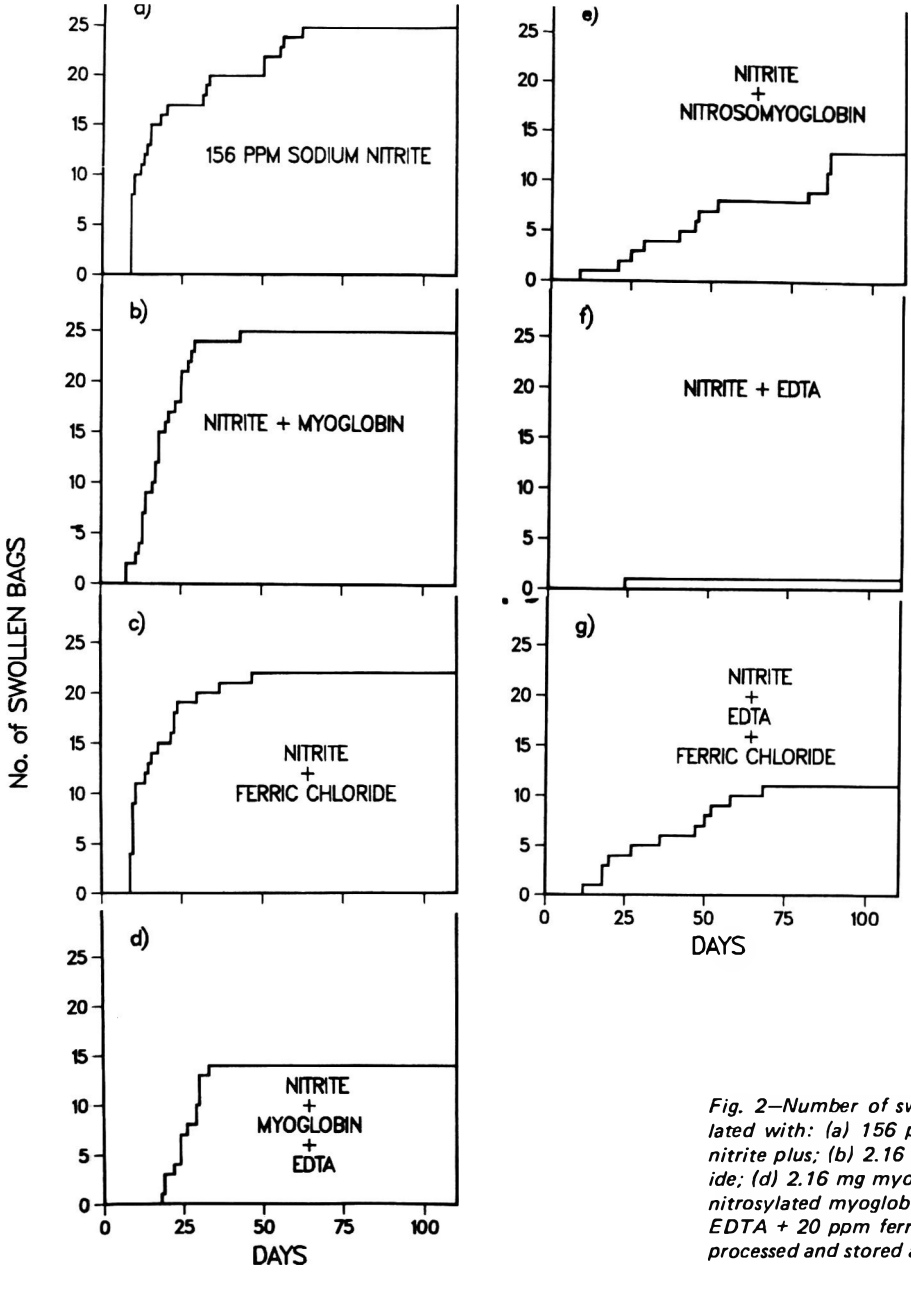

Fig. 2—Number of swollen bags with time in meat product formulated with: (a) 156 ppm sodium nitrite, or with 156 ppm sodium nitrite plus; (b) 2.16 mg myoglobin/g meat; (c) 20 ppm ferric chloride; (d) 2.16 mg myoglobin/g meat + 200 ppm EDTA; (e) 2.16 mg nitrosylated myoglobin/g meat; (f) 250 ppm EDTA; or (g) 250 ppm EDTA + 20 ppm ferric chloride. All samples were then inoculated, processed and stored as described in Fig. 1.

2f). Samples formulated with myoglobin or ferric chloride (Fig. 2 d, g) did swell faster than similar samples formulated with only sodium nitrite and EDTA (Fig. 2f).

Samples containing EDTA had somewhat higher residual nitrite levels. For example, after 10 days of storage at 27°C, samples treated with sodium nitrite and EDTA alone (Fig. 2f), or with myoglobin (Fig. 2d) or ferric chloride (Fig. 2g) had residual nitrite levels of 10, 26, and 11 ppm, respectively, while all other samples had residual nitrite levels below 5 ppm. Thus, EDTA may slow the rate of nitrite depletion in an abused meat product, therby slowing botulinal growth, although more work is needed to confirm this possibility.

The addition of denatured, pre-nitrosylated myoglobin to meat before cooking (Fig. 2e) greatly decreased the number of swollen bags. Only 13 of the 25 bags were swollen at the end of the storage period, while all control samples formulated with 156 ppm sodium nitrite were swollen by day 62 of storage (Fig. 2a). Both treatments had about 100 ppm nitrite immediately after cooking. The pre-nitrosylated myoglobin was washed thoroughly to remove free nitrite before it was added to the meat. Subsequent preparations of denatured, nitrosylated hemoglobin, however, were found to contain about 300 ppm sodium nitrite, as measured by standard procedures. Thus, the denatured, nitrosylated myoglobin, added at a level of 2.16 g/kg meat, could have contributed about 0.65 ppm nitrite to the meat. This level of nitrite would probably not be sufficient to account for the lower number of swollen bags observed in the treatment prepared with denatured, nitrosylated myoglobin.

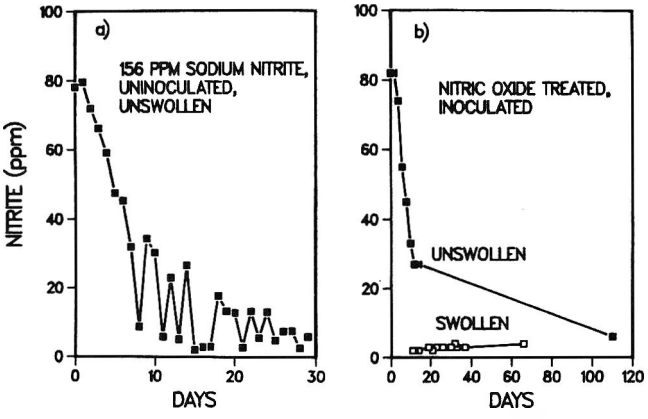

Fig. 3—Nitrite depletion over time in: (a) an uninoculated meat product formulated with 156 ppm sodium nitrite, or (b) a meat product exposed to nitric oxide gas, then inoculated with 100 C. botulinum spores/g meat. Both treatments were cooked at 70°C for 30 min, vacuum packaged, then stored at 27°C. Note that uninoculated bags (part a) did not swell during the 30 day storage period.

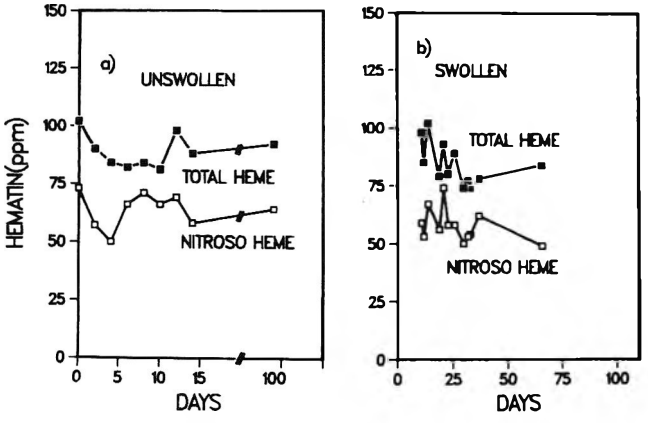

Fig. 4—Nitroso and total heme pigment levels over time in: (a) unswollen, or (b) swollen bags of meat product exposed to nitric oxide gas, and then inoculated, processed and stored as described for Fig. 1.

Chemical analysis

In most treatments, residual nitrite decreased only slightly due to cooking. However, nitrite levels decreased rapidly during storage at 27°C, reaching levels below 20 ppm sodium nitrite after 14 days. The nitrite levels of unswollen bags of uninoculated meat product formulated with 156 ppm sodium nitrite varied considerably (Fig. 3a). This variation may partly explain why not all bags of a treatment swelled on the same day. A typical nitrite depletion curve for sample treated with NO gas is shown in Fig. 3b. Residual nitrite in swollen bags from all treatments was below 10 ppm. However, some bags that remained unswollen after 110 days storage also contained residual nitrite levels below 10 ppm. The residual nitrite level of swollen bags was significantly lower ($P = 0.05$) than that of unswollen bags, when averaged for all treatments in Experiment 1 (Table 1).

Total and nitroso pigment levels did not change appreciably during storage at 27°C, as illustrated in Fig. 4 for NO treated samples. Furthermore, there was no significant difference ($p = 0.05$) in the level of nitroso and total heme pigments among swollen vs unswollen bags (Table 1). Two way analysis of covariance confirmed that there was no significant change in the nitroso pigment contents of samples with storage time (Table 2). However, total pigment values were slightly but significantly ($p = 0.05$) lower in both swollen and unswollen samples stored for longer times (Table 2).

Total pigment values were higher in samples that had received added myoglobin, as expected. All samples with added myoglobin contained 150–180 ppm total heme pigment, compared to 80–100 ppm total heme pigment in all other samples. Similarly, the samples containing denatured nitrosylated myoglobin had nitroso pigment values in both swollen and unswollen bags usually exceeding 110 ppm, compared to values of about 50 ppm in samples without added myoglobin or nitrosomyoglobin.

Most (76%) of the swollen bags in experiment 1 contained botulinal toxin, as determined by mouse bioassay (Kautter and Lynt, 1978). All of the control samples without nitrite contained toxin. For both the CO and NO treated samples, four of the five swollen bags tested were toxic. Of control samples with added nitrite, NO + phytate, or NO + oxalate, however, only two, one, and two bags of 5-bag samples were toxic, respectively. In Experiment 2, only 64% of the swollen bags were toxic. All of the samples treated with sodium nitrite or nitrite + ferric chloride were toxic. Samples treated with nitrite + myoglobin, nitrite + myoglobin + EDTA, nitrite + denatured, nitrosylated myoglobin, nitrite + EDTA, or nitrite + EDTA + ferric chloride had 4, 3, 0, 0, and 1 toxic bags out of 5-bag samples, respective-

Table 1—Comparison of estimated means of selected variables by swelling (only treatments from experiment 1 containing sodium nitrite or nitric oxide)

	Nitrite (ppm)	NO-heme pigment (ppm)	Total heme pigment (ppm)	% Conversion (NO-heme/total heme x 100)
Unswollen bags	47.00[a]	44.09[a]	68.00[a]	64.36[a]
Swollen bags	3.40[b]	43.94[a]	66.30[a]	66.21[a]

[a,b]Values in the same column with the same letter superscript are not significantly different at p = 0.05 (LSD Test).

Table 2—Two-way analysis of covariance of selected variables in treatments from experiment 1 containing sodium nitrite or nitric oxide

Source of variation	d.f.	Nitrite M.S.	Nitrite F-ratio	NO-heme pigment M.S.	NO-heme pigment F-ratio	Total heme pigment M.S.	Total heme pigment F-ratio	% Conversion (NO-heme/total heme x 100) M.S.	% Conversion (NO-heme/total heme x 100) F-ratio
Treatment	4	267.84	0.48	2216.95	64.40*	2486.72	50.93*	445.90	5.86*
Swelling	1	6211.23	11.09*	1.57	0.05	35.47	0.73	40.90	0.54
Treatment x Swelling	4	679.31	1.21	98.22	2.85*	109.63	2.25	56.52	0.74
Time	1	4609.21	8.23*	1.21	0.04	20415	4.18*	240.02	3.15
Error	90	559.96		34.42		48.83		76.09	

* Significant at p = 0.05.

ly. Thus, swelling was not always associated with the presence of botulinal toxin.

Most probable number (MPN) estimation of botulinal population size in swollen, but nontoxic bags confirmed that most nontoxic bags did contain viable botulinal organisms (Table 3). Colonies isolated on anaerobic egg agar (Leininger, 1976) from swollen, nontoxic bags were also Gram-stained to assist in the detection of botulinal cells. Gram positive cocci were found in 4 of the 12 nontoxic swollen bags from Experiment 1 (Table 3). In addition, the pH of samples containing Gram positive cocci was generally 5.5 or lower, and botulinal populations were low.

All of the samples that contained Gram positive rods also had a high botulinal population level, as determined by MPN (Table 3). While it is possible that the Gram positive rods were *C. perfringens* cells, some of the rods were swollen, characteristic of endospore development in *C. botulinum*. Thus, most of the swollen, nontoxic bags did likely contain botulinal cells. Toxin production in these samples may have been depressed, possibly due to acid production by competing organisms. Townsend et al. (1954) found that the lowest pH for toxin production by *C. botulinum* varied from pH 4.84 to pH 5.44, depending upon the type of food. The minimum pH for toxin production in pork and beans was 4.93.

Table 3—Most Probable Number (MPN) estimation of C. botulinum total and spore count, microscopic characteristics of colonies isolated on anaerobic egg agar, and pH of swollen, nontoxic samples

Treatment	Sample number	MPN Index Per g: Total cell count	MPN Index Per g: Spore count[a]	Gram stain	pH
$NaNO_2$	2-1	43	3	G+, cocci	5.10
	2-13	240	4	G+, cocci	4.96
	2-24	>2400	3	G+, rod	5.22
NO	3-2	>2400	>2400	G+, rod	5.66
NO + Phytate	5-4	3	3		5.23
	5-5	>2400	1100	G+, rod	5.92
	5-10	>2400	>2400	G+, rod	5.98
	5-24	>2400	240	G+, cocci	5.86
NO + Oxalate	6-10	3	3	G+, cocci	5.53
	6-12	>2400	>2400	G+, rod	5.85
	6-21	>2400	>2400	G+, rod	5.92
CO	7-26	>2400	4	G+, long rod	5.65

[a] To determine spore count, the diluted extracts were heated at 80°C for 15 min to kill vegetative cells. Thus, any subsequent growth in these tubes during incubation was due to the presence of spores.

DISCUSSION

NO BOTULINAL INHIBITION was observed in CO treated meats, with or without addition of iron chelating agents. The samples all swelled rapidly, and were highly toxic. The CO was probably driven from the product during cooking, since the meat turned brown. A bright red color was observed when the emulsion was first exposed to CO, due to formation of carboxymyoglobin (Watts, 1954), which has a visible spectrum very similar to that of oxymyoglobin (Livingston and Brown, 1981). The brown color of the cooked product was probably due to the formation of denatured metmyoglobin, the brown pigment of cooked meat (Livingston and Brown, 1981). Gee and Brown (1978) reported that the rate of loss of carboxymyoglobin in uncooked, ground meat exposed to a CO containing atmosphere was about equal to the rate of metmyoglobin formation. Using ^{14}CO, Watts et al. (1978) reported an 85% reduction in the ^{14}Carbon content of ground meat after cooking. Even had the CO been retained, it may not have had antibotulinal activity, judging by its inability to inhibit activity of bacterial iron-containing enzymes. Mortenson et al. (1963) found that CO did not inhibit the activity of ferredoxin or pyruvic dehydrogenase in *C. pasteurianum*.

Definite botulinal inhibition was observed in NO treated samples, both with NO alone or plus iron chelating salts. Samples treated with NO + phytate or oxalate were more antibotulinal than NO alone, suggesting that oxalate and phytate were acting synergistically with NO to inhibit botulinal organisms. This was probably not the case, however. Although all NO treated samples were exposed to NO under the same conditions, the gassing of meat samples is more difficult to control than simply adding a measured amount of sodium nitrite solution, as was the case for the control samples. The samples treated with NO + oxalate or phytate may have received more NO, since a higher level of residual nitrite was measured immediately after cooking, probably accounting for the increase botulinal inhibition observed in these samples compared with those treated with NO or NO + EDTA.

Nitrite in water may be readily and reversibly converted into nitrous acid (HNO_2), NO, HNO_3, or other compounds, depending upon the oxidation-reduction state of the system (Shank et al., 1962). Ingram (1974) stated that "nitrite represents one step in a biological oxidation-reduction chain, potentially reversible and extending from nitrate to ammonia." Thus, it is not surprising that residual nitrite and related compounds may be detected in a meat system treated with NO. Therefore, NO would not have potential as a nitrite substitute in meat curing, since nitrite itself is still formed in the product. In fact, the antibotulinal effects observed in the NO treated meats were probably dependent upon conversion of NO into related compounds. Shank et al. (1962) found that NO itself has low antibacterial properties, while nitrous acid was much more bacteriostatic.

The addition of myoglobin did increase the total number of swollen bags, but not to the extent previously reported following the addition of hemoglobin to a similar canned meat product (Tompkin et al., 1978b). Tompkin et al. (1978b) concluded that the addition of hemoglobin reduced

the residual nitrite in the product, leaving less available to inhibit spore outgrowth in temperature abused product. Tompkin et al. (1978c) postulated that adding iron salts stimulated swelling by a different mechanism, i.e., by directly providing a critical nutrient needed by the newly germinated cell for its further development and growth, since iron chelating agents acted synergistically with nitrite to inhibit botulinal growth. Benedict (1980) similarly postulated that nitrite may react either directly on the botulinal cell to cause destruction of cellular enzymes, membranes, or nucleic acids, or indirectly, outside the cell, by destroying or chelating external vital nutrients.

Since heme iron in meat is a good iron source for mammalian systems (Martinez-Torres and Layrisse, 1971; Mahoney et al., 1979), heme iron in hemoglobin might similarly serve as an iron source for microbial growth. This could partially account for the stimulation of botulinal growth observed by Tompkin et al. (1978b) when hemoglobin was added to meat samples, or in meats naturally higher in heme pigments (Tompkin et al., 1978a). The slight stimulatory effect of added myoglobin on degree of swelling observed in this study does tend to confirm the observations of Tompkin et al., (1978a). It does not indicate, however, whether: (a) the myoglobin addition reduced residual nitrite, leading to swelling, or (b) heme iron served as a microbial source of iron, stimulating growth and leading to swelling. When EDTA and myoglobin were both added to cured meat product (Fig. 2d), the degree of swelling was reduced compared to that associated with myoglobin alone (Fig. 2b). Since EDTA cannot chelate heme iron, the lower number of swollen bags observed in the system with EDTA and myoglobin suggests that myoglobin iron was not stimulatory to botulinal growth. It is more likely, therefore, that adding myoglobin or hemoglobin to meat products reduces their residual nitrite levels, (thereby permitting enhanced botulinal growth) rather than directly serving as a source of nutrient iron.

In this study, treatments formulated with sodium nitrite and EDTA (Fig. 2f) were more inhibitory than had been previously reported by Tompkin et al. (1978c). It is possible that EDTA more effectively complexed meat iron, since the plastic bags used in this study would not contribute iron, as might be the case for tin plate of cans. Olsman (1974) showed that EDTA slowed the rate of nitrite depletion in model comminuted beef product. He concluded that nitrite loss was affected by metal traces that could be bound by EDTA, including Fe, Cu, and Sn, the latter originating from the tinplate of the can. He further concluded that, since nitrite breakdown is a reductive process, these metal traces behave as electron carriers.

The reduced number of swollen bags in samples containing denatured, prenitrosylated myoglobin was quite surprising. If the heme iron was complexed with NO, and therefore unavailable for use as a bacterial nutrient, this treatment should have exhibited about the same rate of swelling as the control sample, which had been treated with only nitrite. The inhibition observed in the samples with extra prenitrosylated myoglobin suggests that some inhibitory compound was formed during the nitrosylation procedure. Subsequent testing of similarly denatured, nitrosylated hemoglobin showed that some nitrite remained with the nitrosylated myoglobin, and that it could not be removed with washing. Although the free nitrite added with the myoglobin would not significantly increase the nitrite content of the product, additional protein bound nitrite was probably also formed during the pre-nitrosylation procedure. Several papers (Mirna, 1974; Olsman, 1977a, b; Woolford et al., 1976) have previously reported the formation of protein bound nitrite in cured meat systems. Mirna (1974) described a procedure in which bound nitrite was the content of nitrite detectable with Griess reagent after cleavage of nitroso compounds with Hg^{++}, minus the free nitrite. Olsman (1977a) pointed out that the protein bound nitrite should be considered, just as free nitrite, as a reservoir for nitrosation, and therefore as perhaps having antibotulinal properties. Van Roon and Olsman (1977) showed that protein-bound nitrite does have antibotulinal properties. S-nitrosocysteine was also shown to be antibotulinal, and, when it was added to pork product, residual free nitrite was released (Van Roon, 1980).

Thus, nitrite can react with protein sites other than the heme of myoglobin. Its reaction with free amino groups is the basis of the Van Slyke reaction (Benedict, 1980). Its reaction with free sulfhydryls can produce compounds such as S-nitrosocysteine, which can reversibly release free nitrite, which in turn could have antibotulinal effects in cured meat. This is the probable explanation for the reduced rate of swelling observed in the present study when pre-nitrosylated myoglobin was added to the meat.

Olsman and Krol (1972) showed that addition of small amounts of Fe^{++} to a heated beef product increased the rate of nitrite depletion. Van Roon (1974) pointed out that iron, being a transition metal, is capable of forming coordination complexes with NO and cysteine (McDonald et al., 1965; Woolum et al., 1968). Danner et al. (1973) have been issued a patent based on the fact that adding small amounts of ferrous iron (Fe^{++}) to cured meats enhances and stabilizes the cured meat color. Thus, there is ample evidence that nitrite or its metabolites can react directly with free ferrous iron.

In summary, there appear to be two divergent explanations for the observation that adding iron reduces the antibotulinal effectiveness of nitrite in cured meat products. Tompkin et al. (1978a, c) argued that the newly germinated botulinal organisms need iron as a nutrient, and cured meat systems were somewhat deficient in iron, due to binding with nitrite. Alternatively, Olsman (1974) suggested that ferrous iron traces reduced residual nitrite in the product. The results of the present study suggest that the available iron content of cured meat systems is not the factor limiting botulinal growth. First, not all meat iron is nitrosylated. Second, the amount of nitroso pigment did not decrease, even in swollen bags. Third, the iron requirement of botulinal organisms, if any, is probably quite small. Shankar and Bard (1951) found that *C. perfringens* required only 2 micrograms Fe^{++} per ml medium for optimum growth. Furthermore, some microorganisms that do require iron have been shown to have very efficient systems for satisfying their requirement, including the production of iron transport substances (siderophores) that have a greater affinity than EDTA for iron (Emery, 1982).

CONCLUSIONS

IN CONCLUSION, CO alone or with iron chelating agents did not have antibotulinal properties in cured meat. Binding available meat iron with these compounds in an attempt to create iron deficiency conditions for the germinating cell did not prevent growth of *C. botulinum*.

Botulinal growth, as indicated by the number of swollen bags, was reduced in meat samples treated with sodium nitrite or NO. Since nitrite was formed in NO-treated meats, NO would not be a practical substitute for sodium nitrite in meat curing. Botulinal inhibition was even greater in some cases when EDTA and nitrite was present, but reduced somewhat if ferric chloride or myoglobin was added. The iron associated stimulation of the swelling rate of cured meat products is probably due to depletion of residual nitrite in the product, rather than directly supplementing the iron requirement of growing botulinal cells. Nitrite or related compounds probably inhibit iron containing enzymes such as ferredoxin within the vegetative

botulinal cell (Tompkin et al., 1978a; Woods et al., 1981), rather than indirectly slowing growth by complexing iron in the medium (Tompkin et al., 1978a).

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Factors Affecting the N-Nitrosothiazolidine Content of Bacon

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ABSTRACT

The analysis of raw and cooked bacon for the presence of N-nitrosothiazolidine (NTHZ), a recently identified nitrosamine in bacon, indicated that the levels found were unrelated to residual nitrite or refrigerated storage. Raw bacon contained higher levels of NTHZ than either fried, baked, or broiled bacon. Since the total amount of NTHZ in whole raw bacon was higher than that found in fried bacon and its drippings combined, it was concluded that NTHZ was not formed during frying as is N-nitrosopyrrolidine. The presence of NTHZ in bacon appears to be associated with smokehouse processing.

INTRODUCTION

N-NITROSOTHIAZOLIDINE (NTHZ) was recently identified in fried bacon (Kimoto et al., 1982) analyzed by the mineral oil distillation-Thermal Energy Analyzer procedure adopted by the AOAC in 1982 as a screening procedure for N-nitrosopyrrolidine. N-Nitrosothiazolidine has also been reported by Gray et al. (1982); however, details on the method of isolation were not described. Since the mineral oil distillation procedure formed nitrosamines during analysis (Hotchkiss et al., 1980; Pensabene et al., 1982), we developed a dual column extraction method that does not artifactually produce nitrosamines as a result of the analytical method (Pensabene and Fiddler, 1982). NTHZ was isolated by this procedure and subsequently confirmed by gas chromatography-mass spectrometry (Kimoto et al., 1982).

N-Nitrosothiazolidine has been reported to be a direct acting mutagen in the Ames Salmonella Test (Mihara and Shibamoto, 1980; Sekizawa and Shibamoto, 1980). Both N-nitrosothiomorpholine, the six-membered homolog of NTHZ (Garcia et al., 1970), and nitroso-1,3-oxazolidine, its oxygen analog (Wiessler and Schmahl, 1976), were found to be carcinogenic to rats. Therefore, it is likely that NTHZ might also be carcinogenic, although this has not yet been determined.

Occasionally, fried bacon sample analyzed in our laboratory and by the Food Safety and Inspection Service (FSIS) were found to contain NTHZ. Therefore, the various factors influencing the formation of this nitrosamine in bacon were investigated, and the results of these studies are reported herein.

MATERIALS & METHODS

Materials

N-Nitrosothiomorpholine (NTMOR) and NTHZ were synthesized from their corresponding amines and sodium nitrite, and purified by fractional vacuum distillation as described previously (Pensabene et al., 1972). A complete list of reagents needed for determining NTHZ in raw and fried bacon were reported elsewhere (Pensabene and Fiddler, 1982). Bacon samples, cured and nitrite-free bellies, cured pork butts, and ham were obtained from local processors or purchased from local retail stores. Liquid smoke samples were obtained from FSIS or from a local processor. All other reagents were purchased from commercial suppliers and used without further purification.

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Bacon processing

Pork bellies were stitch-pumped with a commercial cure to approximately 10% of their green weight to achieve added target levels of 2% NaCl, 0.25% sugar, 120 ppm sodium nitrite, and 550 ppm sodium erythorbate in the finished product. The pumped bellies were split in half lengthwise and each half processed into bacon in one of three smokehouses. In House No. 1 (commercial), the bellies were sprayed first with a commercial liquid smoke solution and then placed in a gas-fired smokehouse in which slab wood (sawmill trimmings) was used for smoke generation. The bellies were kept in the house with no air exchange for 12–14 hr until the internal temperature of the bellies reached 60°C. House No. 2 (commercial) was heated by electricity. Bellies were kept in the house for 1/2 hr at 54°C, sprayed with liquid smoke, heated for 1 hr at 54°C, resprayed with liquid smoke, heated for two more hours at 54°C, followed by 3–4 hr at 66°C until the bellies reached an internal temperature of 60°C. House No. 3 (laboratory) was a gas-heated smokehouse in which the processing schedule was: 1 hr at 38°C, 1 hr at 49°C, 5 hr at 57°C, and 1/2 hr at 66°C, with medium to heavy sawdust smoke introduced after the drying period (first 2 hr) and a constant air exchange exhaust. The bacon attained an internal temperature of 59°C at the end of the processing schedule.

Bacon sampling, frying, and handling

The raw bacon was ground and mixed thoroughly prior to analysis. Samples (300g) of the comminuted bacon were either fried in a preheated (177°C) electric pan for 6 min, 9 min, or 12 min, baked for 13 min in a preheated oven set at 204°C, or broiled 1.6–2.4 cm below a 305°C source for 5 min. The edible portion and rendered drippings were retained for nitrosamine analysis. For the storage study, bacon was held in a refrigerator maintained at 5°C. For the processing time study, half the samples were removed from House No. 1 after 6 hr; the others after 12 hr.

Sodium nitrite analysis

Residual sodium nitrite content was determined in 10g of cured meat sample prior to cooking by the Griess-Saltzman procedure as modified by Fiddler (1977).

Nitrosamine analysis

Unless noted, all cured meat samples were analyzed for NTHZ prior to cooking (raw). The complete details of the procedure for analysis of NTHZ in raw and fried bacon have been described elsewhere (Pensabene and Fiddler, 1982). The standard deviation for the repeatability of the method was determined to be 1.20 ppb when NTHZ concentration was corrected for the recovery of the internal standard. The average recovery and standard deviation of the 10 ppb NTMOR internal standard was 93.3% ± 6.0%. The procedure for the determination of NTHZ in bacon drippings was similar to that reported for NTHZ in cured meats except for the following: 10 ml of hexane was added to 10g bacon drippings in a 50-ml beaker followed by the NTMOR internal standard. The sample was transferred to the alumina column, the beaker rinsed twice with 4 ml hexane, then the procedure continued as described for cured meats. To test for artifactual nitrosamine formation, 10 ppm morpholine was added to bacon drippings obtained from bacon that had a residual $NaNO_2$ of 38 ppm before frying. No N-nitrosomorpholine was detected after analysis. The average recovery and

standard deviation of the 10 ppb NTMOR internal standard was 81.8% ± 6.3%. All NTHZ values reported were corrected for the recovery of NTMOR internal standard in each individual sample. "N.D." denotes "none detected" or <1 ppb, the minimum level of reliable measurement based on the gas chromatography-Thermal Energy Analyzer system (gc-TEA) response (Pensabene and Fiddler, 1982).

Mass spectrometer confirmation

All samples over 10 ppb were confirmed by gas chromatography-mass spectrometry (Kimoto et al., 1982).

Liquid smoke samples

To 50g liquid smoke concentrate was added 50 ml H_2O and 100 ml DCM. The solution was kept at room temperature for 72 hr, then the DCM was removed from the mixture. The DCM was extracted once with 50 ml 5N NaOH, dried by passing through anhydrous sodium sulfate into a Kuderna-Danish flask, and concentrated to 1–4 ml. The concentrated DCM extract was added to a flask containing 10 ml of 0.2N NaOH and 25 ml mineral oil, and vacuum distilled to 130°C at 0.5 mm Hg. The aqueous distillate was extracted 3 x with 50 ml DCM each, the combined DCM washed once with 25 ml 5N NaOH, dried, and concentrated to 1 ml for gc-TEA analysis of NTHZ.

RESULTS & DISCUSSION

SINCE RESIDUAL NITRITE is known to decrease during bacon storage (Herring, 1973), and a positive correlation exists with N-nitrosopyrrolidine after frying (Pensabene et al., 1979) we conducted a similar storage study for NTHZ. Refrigerated storage of the bacon for 35 days did not affect the level of NTHZ found, although the mean residual nitrite content decreased from 12 ppm to 1 ppm.

When NTHZ was absent in uncooked bacon, none was detected after frying under the standard conditions (177°C for 6 min). Six samples of commercial bacon were cooked under various conditions (Table 1). NTHZ was present in four of the six irrespective of whether they were fried, baked, or broiled. These samples also contained NTHZ in the raw bacon. In the two samples containing no NTHZ in the raw material, no NTHZ formed. All raw bacon samples contained residual nitrite ranging from 13–36 ppm. When 10 ppb NTHZ was added to nitrite-free bacon and heated at 185°C for 6 min in either an open or closed Wheaton flask, no significant decrease in NTHZ was found, indicating it was not destroyed by heating under bacon frying conditions. Development of a method for NTHZ determination in bacon drippings permitted a comparison of NTHZ values in raw and fried bacon and the drippings (Table 2). In the five samples where NTHZ was not detected in the raw bacon, none was detected in the corresponding fried bacon or its drippings. Comparison of the NTHZ levels in raw bacon with that in combined fried bacon and drippings showed a higher level in raw bacon. Therefore, NTHZ was not formed during frying, baking, or broiling, but was already present in the raw bacon. This is in contrast to the two volatile nitrosamines, N-nitrosodimethylamine and N-nitrosopyrrolidine, which are formed during frying and are absent in the uncooked product (Fazio et al., 1973; Sen et al., 1973).

NTHZ was consistently found in bacon samples produced by one manufacturer who used a gas-fired, wood-burning smokehouse (House No. 1), suggesting that NTHZ may be formed during the processing procedure. In a preliminary experiment, NTHZ was not found in five cured, unprocessed bellies, but was present in the bellies after smokehouse treatment, again indicating that NTHZ was formed during processing. To determine whether processing time in House No. 1 had an effect on NTHZ formation, six cured bellies were split in half lengthwise to minimize differences due to composition, processed, and analyzed for NTHZ. NTHZ levels were 46% higher and $NaNO_2$ levels were 33% lower after 12 hr compared to 6 hr processing. When cured meat products were processed in Houses 1 and 2, NTHZ levels were consistently higher in products processed in the gas-fired, wood-burning house (House No. 1), even though NTHZ was detected in much lower concentrations in the products processed with liquid smoke in the electrically heated house (Table 3). Also, the role of nitrite in the formation of NTHZ is not clear, since the NTHZ content appeared to be independent of the amount of sodium nitrite present. This was particularly apparent in the case of nitrite-free bacon where little nitrite was present, but 3.9 ppb NTHZ was found after treatment in House No. 1. This contrasted with the 20.3 and 34.1 ppb NTHZ found in cured pork butts containing comparable low concentrations of sodium nitrite.

Green bellies were pumped, halved, and processed separately in Houses 1 and 3, which were both gas-fired, but differed in the source of smoke (liquid plus wood vs sawdust, respectively). NTHZ was detected only in bacon processed in House No. 1. This may be due to the higher uncontrolled smokehouse temperature and higher humidity resulting from limited air exchange in House No. 1 or from the liquid smoke sprayed on the bellies prior to processing. NTHZ was detected in 10 samples of liquid smoke, including the one used in Houses 1 and 2, in concentrations ranging from 1.1–58.7 ppb, with an average of 22.5 ppb, indicating that these solutions may contribute to the presence of NTHZ on the product. Bacon was then prepared using bellies that were split into thirds: one-third was processed normally (liquid + wood smoke); one-third only with wood smoke; and one-third only with liquid smoke, each prepared separately in House No. 1. The results (Table 4) indicated that liquid smoke infrequently, but only to a minor extent, contributed to NTHZ formation, perhaps due to the lower concentration of smoke components present

Table 1—N-Nitrosothiazolidine formation in bacon cooked under various conditions

Sample no.	Residual $NaNO_2$ (ppm)	N-Nitrosothiazolidine (ppb)					
			Frying time (min)[a]				
		Raw bacon	6	9	12	Bake[b]	Broil[c]
1	36	11.4	10.5	10.5	7.4	8.9	10.0
2	13	4.7	3.8	3.8	2.5	3.7	3.7
3	29	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4	36	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5	34	4.1	3.1	2.0	2.4	4.7	4.1
6	13	5.1	4.8	3.5	3.2	4.4	3.4

Cooking conditions: [a]177°C; [b]204°C for 13 min; [c]1.6–2.4 cm below source (305°C) for 5 min.

Table 2—N-Nitrosothiazolidine in raw bacon, its fried product, and drippings

Sample no.	N-Nitrosothiazolidine (ppb)		
	Raw	Fried[a,b]	Drippings[b]
1–5	N.D.	N.D.	N.D.
6	6.3	1.8	2.5
7	5.6	1.3	1.7
8	7.5	1.6	2.0
9	4.5	1.2	0.8
10	3.8	2.0	1.6
11	4.2	1.3	1.9
12	2.6	0.9	0.6
13	4.9	1.5	1.1
14	2.4	0.9	0.8

[a] Fried at 177°C for 6 min
[b] Based on 34% fried and 31% drippings yield

Table 3—Effect of smokehouse on N-nitrosothiazolidine formation

Sample no.	Product type	N-Nitrosothiazolidine (ppb) House 1[a]	N-Nitrosothiazolidine (ppb) House 2[b]	Residual $NaNO_2$ (ppm) House 1	Residual $NaNO_2$ (ppm) House 2
1	Bacon	13.8	2.6	17	12
2	Bacon	13.7	3.4	11	15
3	Bacon	13.5	3.8	13	9
4	Nitrite-free bacon	3.9	N.D.	3	2
5	Pork butt	29.4	4.5	29	5
6	Pork butt	34.1	7.8	3	5
7	Pork butt	20.3	7.2	2	3
8	Ham	10.8	—	25	—

[a] Gas-fired, wood + liquid smoke
[b] Electric, liquid smoke

Table 4—Effect of liquid smoke on N-nitrosothiazolidine formation in bacon[a]

Sample no.	N-Nitrosothiazolidine, ppb Wood + liquid	Wood only	Liquid only
1	6.7	7.5	N.D.
2	6.2	10.6	N.D.
3	6.3	7.1	1.3
4[b]	N.D.	N.D.	N.D.
5[b]	6.1	4.8	3.3
6[b]	3.6	3.4	3.5

[a] House No. 1
[b] Nitrite-free bellies

in comparison with those obtained by the more lengthy smoking process generated by the wood over a 12-hr period. The presence of NTHZ in the samples tested appeared to be associated with wood smoke itself, and the source of nitrosation species may not only be the residual nitrite in the cured meat product, but also the nitrogen oxides in the wood smoke and/or gas used in heating.

Experiments were then conducted to determine whether NTHZ formation occurred only on the surface of bacon after it was processed. In the samples analyzed to date, the NTHZ content was found to be higher on the exterior than in the less exposed interior portion of the bacon.

In conclusion, the results of these experiments indicate that the presence of NTHZ in bacon appears to be associated with the smoking step in smokehouse processing. The results also indicate that NTHZ can be present in cured meat products other than bacon, suggesting that bacon does not have a unique composition that would favor formation of this nitrosamine. Whether NTHZ is formed during wood smoking and is deposited on the meat during processing, or whether one or more of the smoke components react with meat constituents to form the nitrosamine is not known. Further research in this area is in progress.

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Note: Nitrosamines are potential carcinogens. Exercise care in handling these materials.

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Inhibition of Lactic Acid Bacteria by Herbs

LAURA L. ZAIKA, JOHN C. KISSINGER, and AARON E. WASSERMAN

ABSTRACT

Increasing concentrations (0.5–8g/liter) of oregano, rosemary, sage, and thyme progressively delayed growth and acid production by *Lactobacillus plantarum* and *Pediococcus acidilactici* in a liquid medium. After the bacteriostatic activity was overcome, all four herbs strongly stimulated acid production. The relative inhibitory effect of the herbs toward both microorganisms was oregano >> rosemary = sage > thyme. *L. plantarum* was more resistant than *P. acidilactici* to the toxic effect of the herbs. Organisms from cultures exhibiting delayed fermentation in the presence of sublethal concentrations of an herb, when subcultured into fresh media containing identical herb concentrations, initiated fermentation without delay, indicating development of resistance to the herb's effect. Moreover, bacteria which had acquired a resistance to one herb were also resistant to the other three herbs.

INTRODUCTION

ANTIMICROBIAL PROPERTIES of spices such as cinnamon, clove, and mustard have been extensively studied. Less attention has been devoted to investigations of antimicrobial properties of the leafy spices or herbs such as oregano, rosemary, sage, and thyme. Beuchat (1976) reported that oregano and thyme were highly toxic to *Vibrio parahaemolyticus* when present in growth media at a concentration of 0.5%. Shelef et al. (1980) found that rosemary and sage inhibited Gram-positive bacteria to a greater extent than Gram-negative bacteria. Julseth and Deibel (1974) reported that the growth of *Salmonella* in pre-enrichment cultures was inhibited in the presence of oregano. Llewellyn et al. (1981) found that thyme and oregano inhibited growth and aflatoxin production by three toxigenic *Aspergillus* strains, whereas rosemary supported good growth and aflatoxin production. Corran and Edgar (1933) reported that herbs, among them thyme and rosemary, did not inhibit yeast fermentation. Dold and Knapp (1948) considered sage and thyme to be ineffective against a number of pathogenic bacteria.

Past investigatons have indicated that the antimicrobial factor of spices resides in the essential oil and/or oleoresin fraction. The essential oils of oregano, rosemary, sage, and thyme have been reported to have antibacterial (Collier and Nitta, 1930; Kellner and Kober, 1954; Maruzzella and Sicurella, 1960) and antifungal (Maruzzella and Liguori, 1958; Maruzzella, 1960) activity. Of the four herbs, the essential oils of oregano and thyme were generally found to be the most inhibitory and were considered among the most active of a large number of tested spice and herb essential oils.

Most of the reported studies on the antimicrobial activity of spices and herbs involved pathogenic microorganisms, and only a few reports involving lactic acid bacteria are available. These reports suggest that lactic acid bacteria are relatively resistant to the toxic effects of spices (Karaioannoglou et al., 1977; Salzer et al., 1977; Shelef et al., 1980; Park et al., 1980) or their essential oils (Molina and Merzari, 1949; Anderson et al., 1953; Shcherbanovskii et al., 1973). Previous observations indicate that Lactacel MC starter culture (*Lactobacillus plantarum* and *Pediococcus cerevisiae*), used for preparation of fermented sausages, was relatively resistant to the antimicrobial action of 19 spices and herbs (Kissinger and Zaika, 1978; Zaika and Kissinger, 1979a, b). in most cases, the starter culture bacteria grew in a liquid medium containing up to 12g/liter spice; however, the microorganisms were severely inhibited by a few spices, notably oregano and clove. It was observed that growth in the presence of a variety of spices was accompanied by enhanced acid production relative to that in the unspiced control.

Previous investigations (Zaika and Kissinger, 1981) have indicated that while *L. plantarum* and *P. cerevisiae* can be completely inhibited by appropriate concentrations of oregano, the microorganisms can acquire resistance to the toxic effects by first being exposed to sublethal concentrations of the herb. One of the objectives of the present work was to compare viability and acid production by *L. plantarum* and *P. acidilactici* in the presence of the botanically-related herbs of the family *Labiatae* (oregano, rosemary, sage, and thyme), to determine if the starter bacteria could acquire resistance to the inhibitory effects of the other members of the *Labiatae*, and if resistance to one herb imparted resistance to the others.

MATERIALS & METHODS

Microorganisms

Frozen cultures of *Lactobacillus plantarum* (Lactacel 804, Microlife Technics, Sarasota, FL) and *Pediococcus acidilactici* (Lactacel, Microlife Technics) were used throughout the study. According to the supplier (personal communication), these are single-strain cultures.

Liquid medium

The fermentation medium was prepared by dissolving 3g beef extract (Difco), 5g tryptone (Difco), 20g sucrose, and 20g glucose in 1 liter of distilled water. The pH of the medium was adjusted to 6.5 with 6N H_2SO_4 (giving a post-sterilization pH of 5.7–6.3). Aliquots (250 ml) of the medium were dispensed into 500-ml Erlenmeyer flasks and sterilized for 15 min at 15 psi.

Herbs

All herbs used were commercially dried and ground. Commercially sterilized rosemary, sage, and thyme were obtained from Griffith Laboratories, Inc., Union, NJ. Oregano and oregano essential oil were obtained from Penn Herb Company, Philadelphia, PA. Oregano was sterilized with ethylene oxide in a Cryotherm Portable Sterilizer, series 8040 (American Sterilizer Company, Erie, PA). All herbs used in the experiment contained less than 100 organisms/g as determined by total aerobic plate counts.

Fermentation

Herbs were added aseptically to the flasks of sterile medium to provide concentrations of 0.5, 1, 2, 4, or 8g/liter. All flasks were inoculated with 2.5 ml of the thawed commercial starter culture diluted with 0.1% peptone water such that the initial bacterial population in the flasks ranged from $10^2 - 10^4$ cells/ml. The flasks were

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incubated statically at 35°C for up to 7 days. Samples for bacterial counts and titratable acidity were taken at 24-hr intervals. Additional experiments were conducted using 3g/liter oregano and 8g/liter thyme, rosemary, and sage. For experiments with oregano essential oil, solutions (1 ml) of the oil in ethanol were added to the flasks containing medium to provide concentrations of 40 to 400 ppm. Ethanol (1 ml) was added to the control.

Titratable acidity

A 25 ml portion of each sample was centrifuged at 20200 X *g* for 15 min at 5°C. Ten ml of the supernatant, diluted with 50 ml of distilled water, were titrated with 0.1N NaOH to pH 7.0 with the aid of a Fisher Accumet Model 325 pH meter equipped with a Corning combination pH electrode. The titratable acidity was expressed in terms of ml of 0.1N NaOH/10 ml medium. The titratable acidities of uninoculated media were 0.33–0.65 ml.

Enumeration of bacteria

Bacterial counts were determined by conventional pour plate techniques using APT agar (Difco). Plates were incubated aerobically for 48 hr at 35°C.

Adaptation to herbs

A modification of a method developed previously (Zaika and Kissinger, 1981) was used to test for adaptation. Starter culture was inoculated into media containing either 3g/liter oregano, 8g/liter rosemary, 8g/liter sage, or 8g/liter thyme. When acid production began after a delay of several days, bacteria from the spice-containing samples were inoculated into medium containing the same spice or one of the other test spices.

RESULTS & DISCUSSION

Effect of herbs on starter cultures

The effect of oregano, rosemary, sage, and thyme on growth and acid production by *L. plantarum* is shown in Fig. 1A-1D, respectively. For the sake of clarity of the graphs, not all of the herb concentrations tested (0.5–8g/liter) are shown in the figures. Addition of 0.5g/liter oregano to the medium did not affect growth of the microorganism, but did increase acid production by 2.5-fold. Increasing the oregano concentration to 2 and 4g/liter resulted in a delay in bacterial growth. Some bactericidal activity was evident during early stages of fermentation in cultures containing 2 and 4g/liter oregano and acid production was delayed until the inhibition of growth was overcome. After 7 days of fermentation the titratable acidity of the medium containing 4g/liter oregano was 2.5 times that of the control medium. Increasing the oregano concentration to 8g/liter was bactericidal to *L. plantarum*.

The effect of rosemary, sage, and thyme on growth and acid production by *L. plantarum* was similar to that observed for oregano, except that those herbs were not as inhibitory. The bacteria survived, grew, and produced acid in media containing 8g/liter rosemary, sage, or thyme. The relative inhibitory activity of the herbs was oregano >> rosemary = sage > thyme. In all cases increasing concentrations of an herb caused a progressive delay in bacterial growth and acid production. However, after a growth lag,

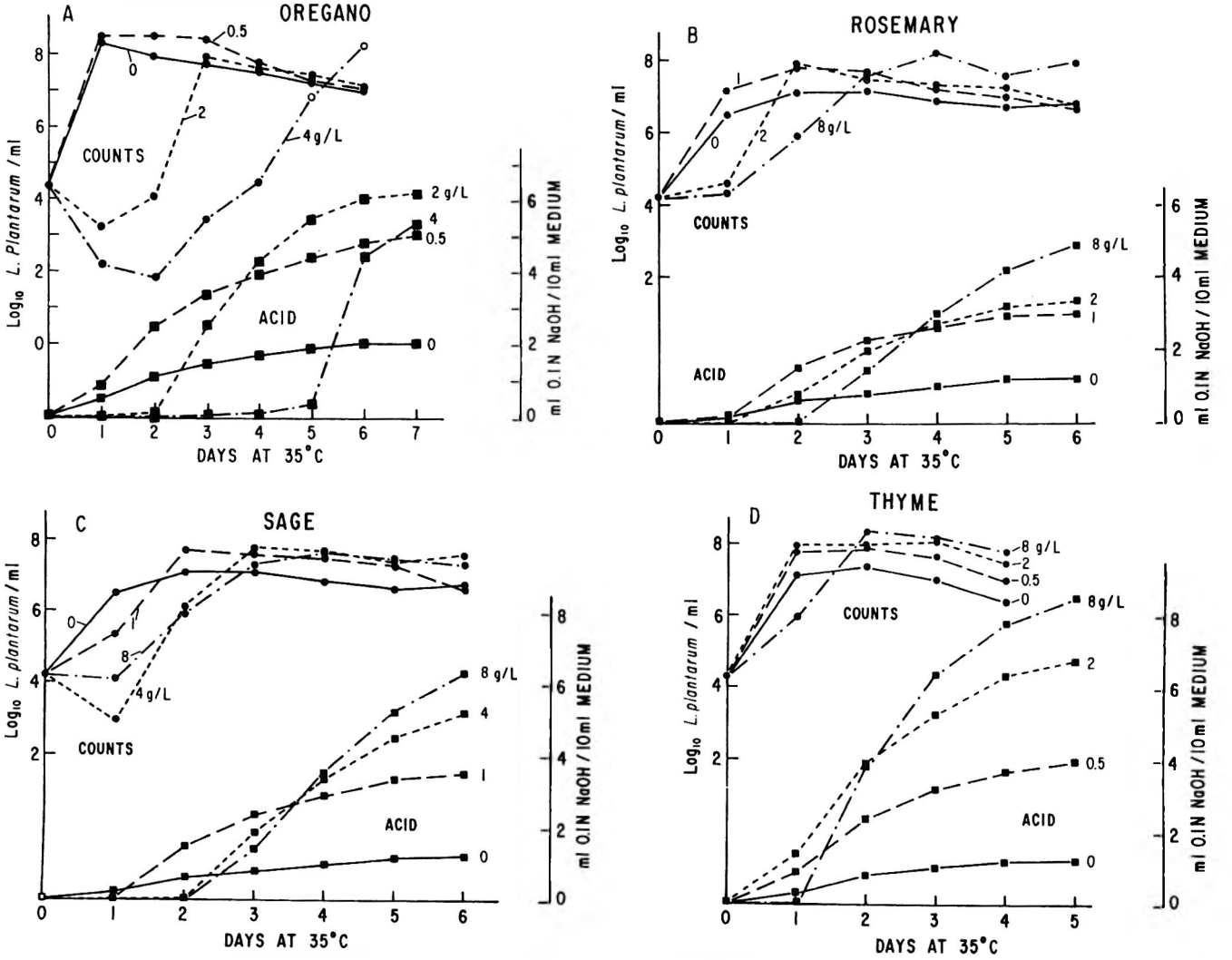

Fig. 1—Effect of herbs on growth and acid production by L. plantarum: oregano (A), rosemary (B), sage (C), thyme (D).

acid production increased with increase in herb concentration. Other experiments demonstrated that addition of 8g/liter thyme, sage, and rosemary resulted in titratable acidity values 7, 5, and 4 times greater, respectively, than those of control cultures after 6 days of fermentation, and acid production in the presence of 3g/liter oregano generally was three to four times higher than in the controls after 6 days.

The effect of the four herbs on growth and acid production by *P. acidilactici* (Fig. 2A-2D) was similar to that noted for *L. plantarum* (Fig. 1A-1D). *P. acidilactici* was more sensitive than *L. plantarum* to the toxic effect of high concentrations of the herbs. While acid production by *P. acidilactici* was stimulated in the presence of the herbs, the degree of stimulation was not as great as that observed for *L. plantarum*. The relative inhibitory activity of the herbs toward *P. acidilactici* was oregano >> rosemary = sage > thyme. In cultures containing 0.5g/liter oregano bacterial growth was not affected, while titratable acidity was doubled; however, a concentration of 4g/liter (data not shown) was bactericidal. *P. acidilactici* initially declined in numbers in media containing 4 and 8g/liter rosemary and for similar concentrations of sage, but later grew and produced acid. Significant inhibition of *P. acidilactici* by thyme was observed only at the 8g/liter level. Rosemary, sage, and thyme appeared to stimulate acid production by *P. acidilactici* to a similar extent. Titratable acidity values after 5 days in media containing 8g/liter of the herbs were approximately three times higher than in control cultures.

Essential oils of the botanically related members of the *Labiatae* family have a number of constituents in common (Rhyu, 1979). The oils of oregano and thyme both contain carvacrol and thymol as major constituents. Antimicrobial activity for both compounds has been reported (Katayama and Nagai, 1960; Kellner and Kober, 1955). Other constituents of the essential oils of the four herbs studied (such as p-cymene, 1,8-cineole, d-linalool, thujone, and α-terpineol) have been reported to exhibit antimicrobial activity (Kellner and Kober, 1955).

Although the composition of oregano and thyme oils may be similar, the inhibitory activity of oregano observed in the present work was much greater than that of thyme. This difference may be due to the essential oil content of the two herbs. According to Shankaracharya and Natarajan (1971), good commercial samples of the herbs should have the following essential oil content: oregano 4% (minimum), rosemary 0.3–2.0%, sage 1.5–3.0%, and thyme 1.5–2.5%.

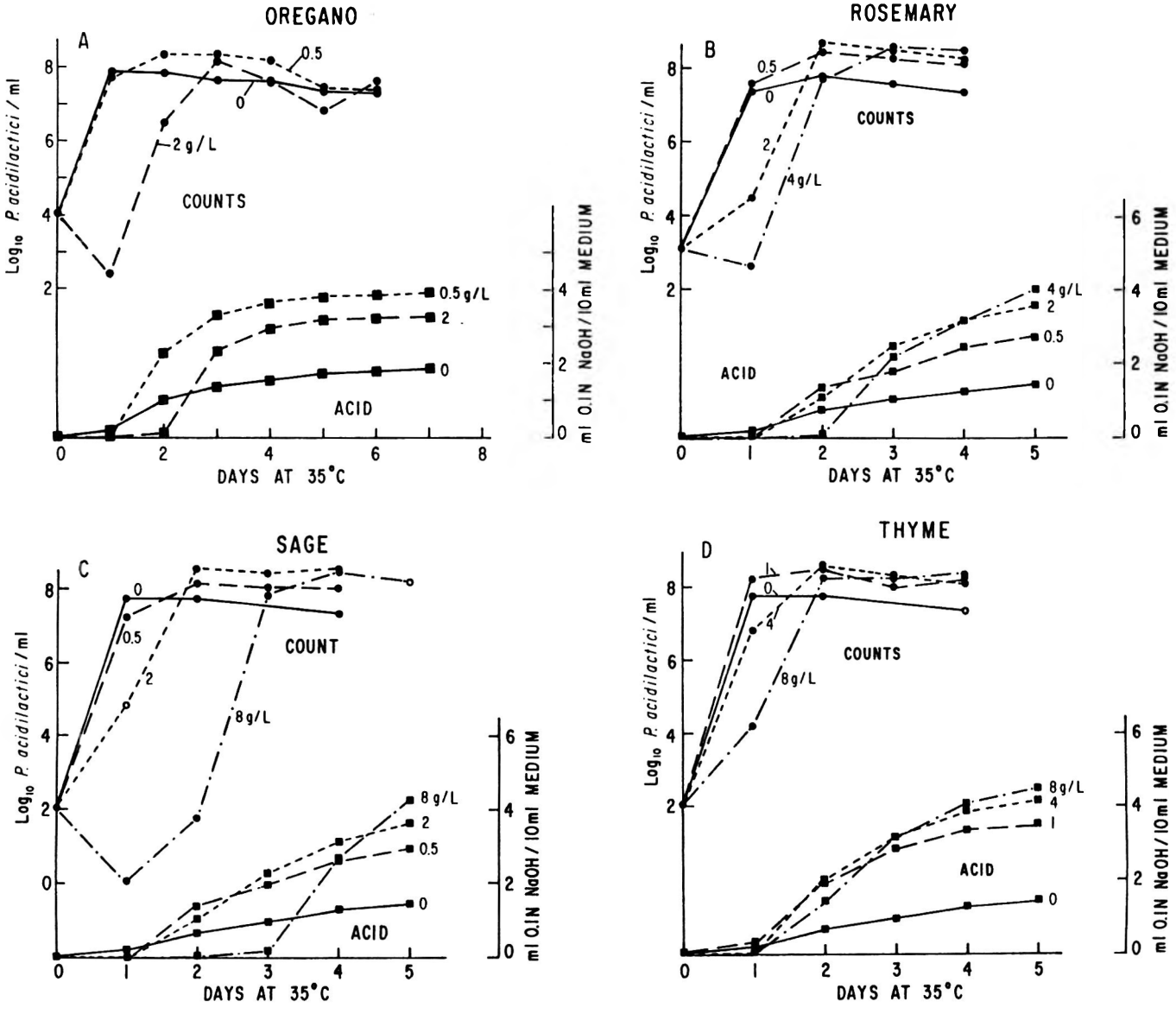

Fig. 2—Effect of herbs on growth and acid production by P. acidilactici: oregano (A), rosemary (B), sage (C), thyme (D).

Adaptation to herbs

A sequential subculturing scheme (Fig. 3) was used to test for adaptation of the starter bacteria to the toxic effects of the herbs. When *L. plantarum* was inoculated into media containing sublethal concentrations of oregano (3g/liter) or thyme (8g/liter), growth and acid production (Fig. 4A, B) were delayed in a manner as previously noted. After the bacterial population in the medium containing oregano or thyme increased to 10^8 cells/ml, cells were transferred to fresh media containing the same concentrations of the herbs (Fig. 4C, D). In this case, no inhibition of growth due to the presence of the herbs was evident. In fact, the herbs appeared to stimulate growth, possibly due to additional nutrients or cofactors supplied by the herbs. Cells grown in the presence of one herb acquired resistance to the other. Similar experiments with *P. acidilactici* indicate that this organism can also acquire resistance to the toxic effects of oregano, rosemary, sage, and thyme when exposed to sublethal concentrations of these herbs. For example, in the presence of 8g/liter rosemary or 8g/liter sage, bacterial counts decreased considerably before growth began (Fig. 5A). After 3 days of incubation, when the bacterial population reached 10^8 cells/ml, bacteria from the rosemary-containing medium were subcultured into fresh media containing the same concentrations of rosemary and sage. As shown in Fig. 5B, *P. acidilactici* acquired resistance to inhibition not only to rosemary, but to sage also.

Additional experiments using adaptation and challenge combinations of oregano, rosemary, sage, and thyme indicated that both starter cultures can acquire multiple resistance to the inhibitory effects of various herbs. In all cases, acid production by the adapted cultures was strongly stimulated by the presence of an herb. Adaptation of the bacteria also allowed them to tolerate herb levels normally bactericidal. For example, *L. plantarum*, previously grown in the presence of 3g/liter oregano, was able to survive, grow, and produce acid when subcultured into a medium containing 8g/liter oregano, a concentration normally bactericidal (Fig. 6).

When the starter cultures were incubated in media containing various concentrations of oregano essential oil, little if any inhibition of growth and acid production was noted in the presence of 40 ppm oregano oil, while levels >200 ppm were bactericidal to both organisms. As in the case of the herb, bacteria exposed to sublethal concentrations of oregano oil were able to overcome the inhibition and to develop resistance to the toxic effect of oregano oil or oregano. It should be noted that the essential oil did not enhance acid production by the bacteria.

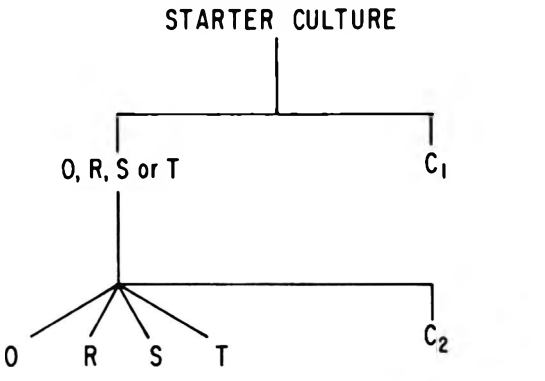

Fig. 3—Sequential subculturing of starter culture bactera exposed to herbs. C_1, C_2 = control medium; O, R, S, T = medium containing oregano, rosemary, sage, and thyme, respectively.

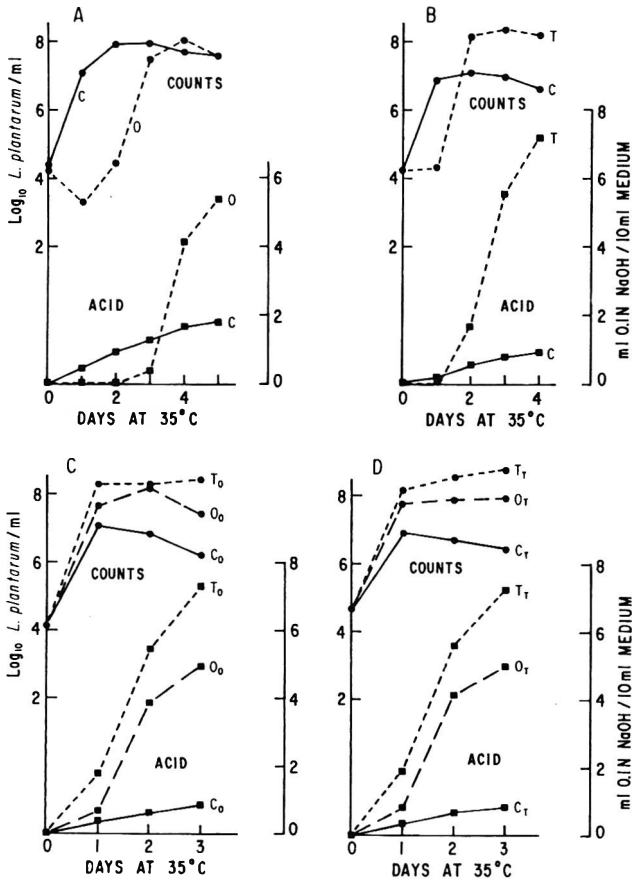

Fig. 4—Growth and acid production by L. plantarum: (A) unadapted cells cultured in control medium, C, and in medium containing 3g/liter oregano, O. (B) unadapted cells cultured in control medium, C, and in medium containing 8g/liter thyme, T. (C) adapted cells subcultured from 0 after 3 days; C_O = control medium; O_O = medium containing 3g/liter oregano; T_O = medium containing 8g/liter thyme. (D) adapted cells subcultured from T after 2 days; C_T = control medium; O_T = medium containing 3g/liter oregano; T_T = medium containing 8g/liter thyme.

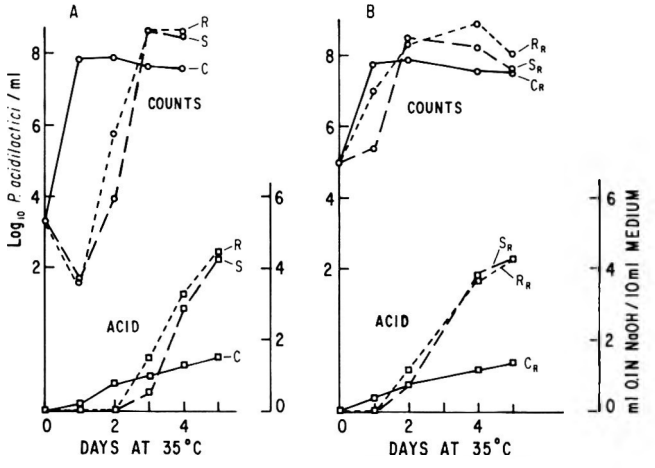

Fig. 5—Growth and acid production by P. acidilactici: (A) unadapted cells cultured in control medium, C, in medium containing 8g/liter rosemary, R, and in medium containing 8g/liter sage, S. (B) adapted cells subcultured from R after 3 days; C_R = control medium; R_R = medium containing 8g/liter rosemary; S_R = medium containing 8g/liter sage.

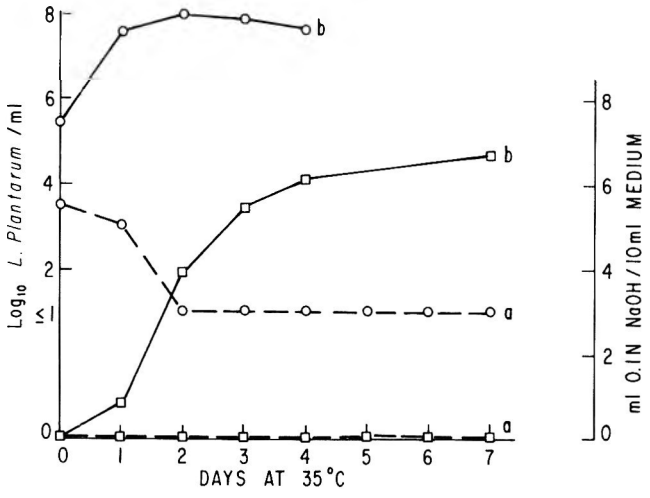

Fig. 6—Growth and acid production by L. plantarum in media containing 8g/liter oregano: (a) before adaptation, (b) after adaptation, subcultured from medium containing 3g/liter oregano after 3 days. ○ = bacterial count; □ = titratable acidity.

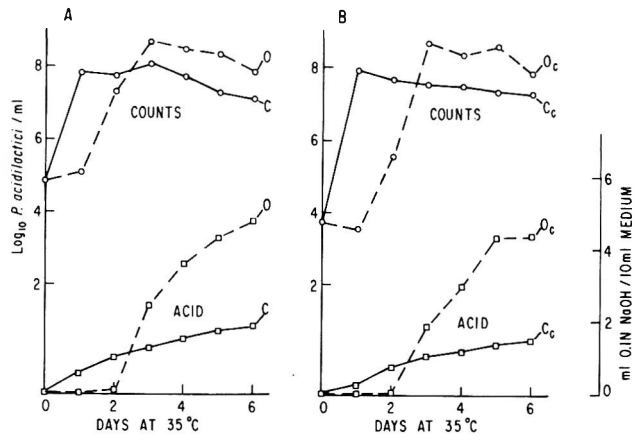

Fig. 7—Growth and acid production by P. acidilactici: (A) inoculum from thawed commercial culture. C = control medium; O = medium containing 3g/liter oregano. (B) inoculum from broth medium, C, after 3 days at 35°C. C_C = control medium; O_C = medium containing 3g/liter oregano.

The possibility was considered that adaptation may be the result of recovery of the starter organisms from injury due to frozen storage. A comparison was made of inocula prepared from the thawed commercial starter with and without a preliminary culturing in the liquid medium. The results for *P. acidilactici* inoculated into control media and media containing 3g/liter oregano, shown in Fig. 7, were similar for both types of inoculum. In both cases, growth and acid production were delayed to a similar extent in the presence of oregano. This and other experiments indicated that injury of the starter bacteria due to freezing was not an important factor in their adaptaton to herbs.

The mechanism by which the starter cultures acquire resistance awaits future research, as does the mechanism of their inhibition by spices and their components. The possibility that other food-borne microorganisms could respond to the inhibitory effect of spices in a similar way should be examined.

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

A New Consistency Method for Tomato Products: The Precipitate Weight Ratio

NORIHISA TAKADA and PHILIP E. NELSON

ABSTRACT

The precipitate weight ratio (PPT) which refers to the weight ratio of precipitate to the initial sample weight after centrifugation was found to be highly correlated with the Bostwick consistency and the efflux viscosity of tomato products. Lower correlation coefficients were found for products containing added ingredients. However, the PPT may be a unique and reliable parameter to evaluate the consistency of tomato products before formulation.

INTRODUCTION

THE RHEOLOGICAL PROPERTIES of tomato products are attributed to their high molecular components (Foda and McCollum, 1970). The quantitative evaluation of high molecular components has been accomplished with pectin contents, water-insoluble solids, alcohol-insoluble solids, etc. (Luh et al., 1954; York et al., 1967; Bartoleme, 1971; Stevens and Paulson, 1976). Alcohol-insoluble solids contents is reported to be a reliable index for textural quality of tomatoes (Brown and Stein, 1977), tomato juice (Janoria and Rhodes, 1974; Janoria et al., 1975), and tomato paste (McColloch et al., 1950). Recently, Marsh et al. (1980) studied the compositional contribution of tomatoes to Bostwick consistency of tomato concentrates reporting that the variation in Bostwick consistency was related to the ratio of water-insoluble solids to total solids content and to the viscosity of serum of clarified juice. These methods quantitatively determine the high molecular components but do not include the physical or qualitative properties.

Kertesz and Loconti (1944) showed that the gross viscosity of tomato juice depended on the suspended solid particles and serum viscosity. Whittenberger and Nutting (1957) similarly reported that the flesh cells of tomato were the principal structural element in tomato juice and that an abundance of cell walls and pectins was necessary to obtain maximum consistency. Furthermore, the shape or configuration of cell fragments was recognized as an important rheological factor (Hand et al., 1955; Whittenberger and Nutting, 1957).

Miers et al., (1970) measured the precipitate weight of tomato juice after centrifugation. However, they did not discuss the significance of this factor. The same principle was used to measure the water-holding capacity of vegetable foodstuffs by McConnell et al. (1974). Becker et al. (1972) called the weight of the precipitate after centrifugation at 15,000 rpm for 30 min as cake weight and reported that the cake weight of tomato juice varied with the degree of mechanical treatment.

These studies strongly suggest that the physical or qualitative properties of water-insoluble solids should be considered in order to understand the rheological property of tomato products. However, there has not been a method to evaluate the quality of water-insoluble components.

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This study was undertaken to investigate factors which would relate to the consistency of tomato products.

MATERIALS & METHODS

Preparation of tomato concentrates

Each 50-lb lot of tomatoes from 21 cultivars was crushed through a 1.375 in. screen and quickly heated to boiling temperature in an open steam jacketed kettle. The hot macerate was passed through a 0.023 in. screen to remove seeds and skins. The extract was concentrated with a steam jacketed, vacuum kettle at 60–65°C. The concentrates at different brix levels were packed in enameled cans and cooled in ice water.

Commercial tomato puree, paste, and catsup were purchased from local stores.

Determination of precipitate weight ratio

Approximately 40g of sample were accurately weighed into a 50 ml preweighed glass centrifuge tube. The sample was centrifuged at 12,880 x *g* for 30 min at 4°C. After centrifugation the supernatant was poured off. The tube was drained for 3 min to remove the supernatant from the precipitate. Samples were replicated four times. The precipitate with the tube was then reweighed accurately and the precipitate weight ratio was calculated by using the following equation:

$$PPT\% = \frac{(\text{Precipitate + tube weight}) - (\text{tube weight})}{(\text{Initial sample + tube weight}) - (\text{tube weight})} \times 100$$

Determination of consistency

Bostwick consistency was measured with a Bostwick consistometer at 20°C.

Efflux viscosity was measured with a Libby's viscometer at 23.9°C.

RESULTS & DISCUSSION

FIG. 1 SHOWS there is a linear relationship between logarithmic precipitate weight ratio and logarithmic centrifugation force. This relationship can be expressed by the following mathematical model:

$$\text{Log(Precipitate weight ratio)} = 0 + 1^{*\text{Log(G)}}$$

where G is centrifugation force, 0 is a constant, and 1 is a slope of this line. Since the slopes of three experimentally obtained lines from three different tomato puree samples were almost the same, the item $1^{*\text{Log(G)}}$ was dependent on the centrifugation force only. Therefore, if the centrifugation force is fixed, the precipitate weight ratio varies with 0 which is a constant for a particular sample. In other words, it is theoretically possible to compare the precipitate weight ratio at any given centrifugation force. This could be the reason why McConnell et al. (1974) and Jauregui et al. (1981) failed to find the most suitable centrifugation force to measure the water-holding capacity of vegetable constituents and muscle food. Secondly, low centrifugal forces less than 2,240 x *g* resulted in the formation of a loosely packed precipitate which sometimes collapsed when the supernatant was removed. A centrifugal force of 12,800 x *g* was chosen for these studies because it yields a compact precipitate and this force can be obtained by various types of centrifuges which are commonly used.

In order to determine the relationship between Bostwick consistency and precipitate weight ratio of various tomato products, approximately 170 samples were analyzed (each replicated 4X). They included 21 commercial tomato puree or paste samples of which processing methods and cultivars of the raw tomatoes were unknown, 11 single strength tomato juice samples, and 139 tomato concentrate samples prepared from 21 cultivars in our laboratory. The Brix levels varied from approximately 5–18°. In spite of various cultivars, Brix levels and processing methods, the tomato products showed a very high correlation between precipitate weight ratio and Bostwick consistency as shown in Fig. 2. The Bostwick consistency was expressed by a quadratic equation of precipitate weight ratio. This result strongly suggests that a certain level of precipitate weight ratio or a certain level of water-holding capacity of water-insoluble components is required to obtain a given Bostwick consistency, no matter what processing or cultivar is used. For instance, if a processor desired a tomato puree with Bostwick consistency 9.0 cm, one could calculate the necessary precipitate weight ratio by using the following equation:

$$PPT = 33.128 - \frac{\text{Bostwick value}}{0.0382} - 59.293$$

where PPT is precipitate weight ratio. The precipitate weight ratio value for Bostwick consistency 9.0 cm is 19.8. This value would be required for a product to obtain this particular level of consistency.

The precipitate weight ratio was also found to be highly correlated with the Libby's viscosity of tomato juice as shown in Fig. 3. The r^2 value was 0.997. The precipitate weight ratio necessary to obtain a certain Libby's viscosity can be calculated by using the following equation:

$$PPT = 21.12\left(1 - \frac{15.311}{\text{Libby's viscosity}}\right)$$

As shown in Fig. 4, the influence of precipitate weight ratio on the Bostwick consistency of catsup is less than that of other tomato products. Bostwick consistency changes of tomato concentrate from 8.0 cm to 4.0 cm required only a 5% increase in the precipitate weight ratio. But the catsup

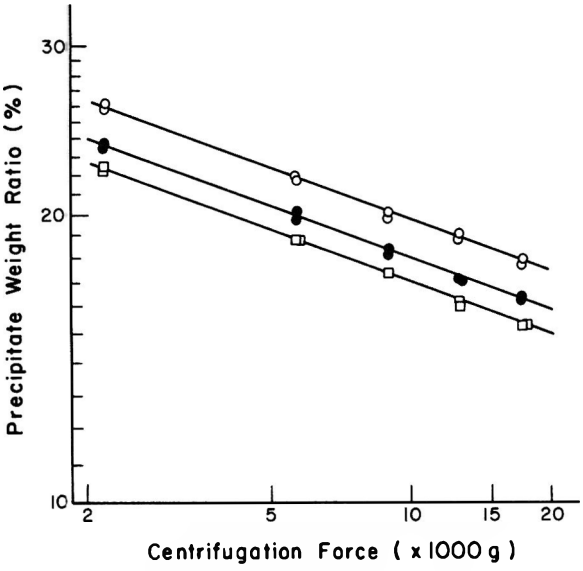

Fig. 1—Effects of centrifugation force on precipitate weight ratio of three different tomato concentrates.

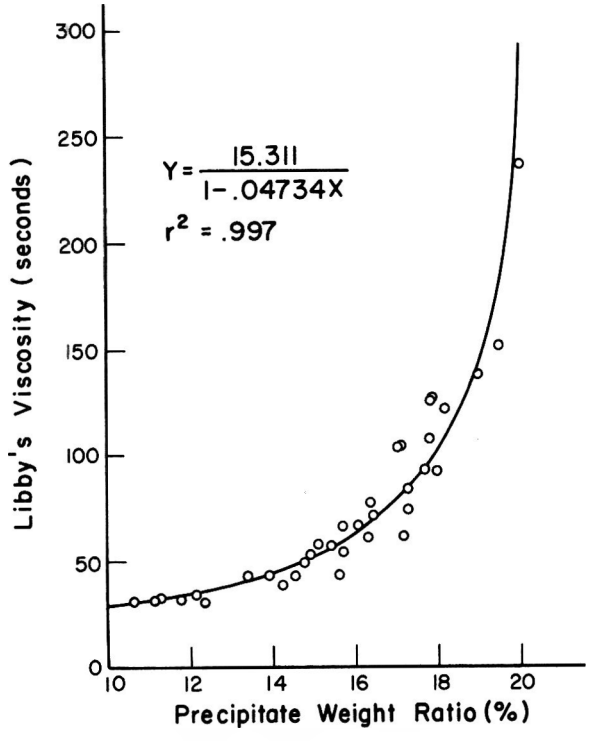

Fig. 3—Effect of precipitate weight ratio on the Libby's viscosity of tomato juice.

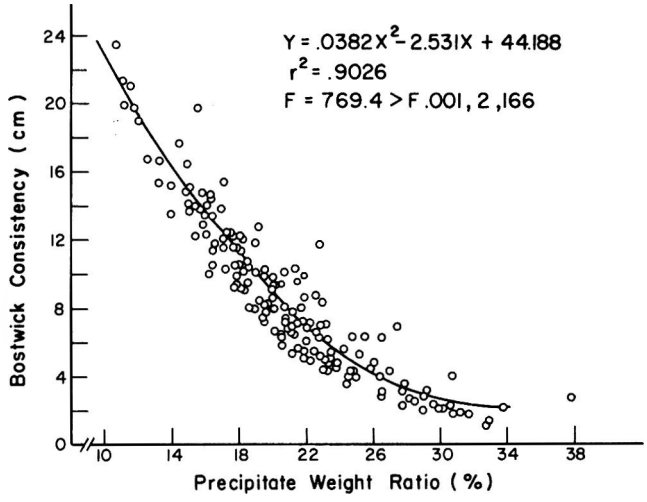

Fig. 2—Effect of precipitate weight ratio on the Bostwick consistency of tomato concentrates from various sources.

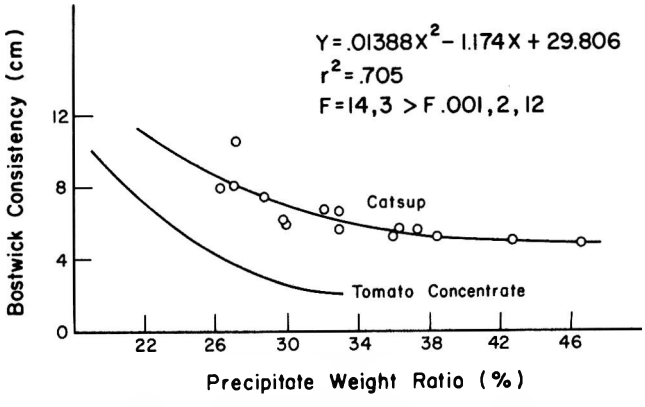

Fig. 4—Effect of precipitate weight ratio on the Bostwick consistency of commercial tomato catsup.

samples required more than 10% increase. This may be due to the fact that a low pH and a high ion strength of catsup decreases the physical function of the water-insoluble solids.

These observations indicate that the precipitate weight ratio can be a fundamental factor contributing to the consistency of tomato products. The precipitated weight ratio would vary with not only the quantity of water insoluble solids in tomato products, but also with their physical properties. Tomato cultivar, tomato maturity, break conditions, pulping conditions, etc., will mainly affect the quantity of water-insoluble solids in tomato products. On the other hand, extracting conditions, concentration, homogenization, milling, addition of some ingredients, etc., will cause change in the physical properties of the water-holding capacity of the water-insoluble solids. In the case of tomato catsup, for instance, all of the above processing steps might influence the precipitate weight ratio in various ways.

Our results strongly suggest that the precipitate weight ratio can be used to evaluate the physical property or water-holding capacity of the water-insoluble solids of tomato products.

Tomato breeders and processors now use different methods to measure for consistency. This creates a difficulty in communication because of a lack of the same consistency standard. However, they will now be able to utilize the same standard by using the precipitate weight ratio since it can be utilized for both juice and concentrates.

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high triglyceride content, it contains large amounts of PUFA, which may cause it to have a shorter shelf life.

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Sunflower Hull Flour as a Potential Dietary Fiber Supplement

M. L. DREHER and G. PADMANABAN

ABSTRACT

Confectionery hull flour (CHF) and oilseed hull flour (OHF) were evaluated as potential dietary fiber supplements. Analyses included: proximate analyses, neutral detergent fiber, acid detergent fiber, acid detergent lignin and buffered acid detergent fiber, water- and oil-holding capacity, pH, cation exchange capacity, emulsifying activity (EA), Gardner color values, and sensory evaluation. CHF (90.7% NDF) was slightly higher in dietary fiber than OHF (83.4%). Sunflower hull flours had a relatively high cation exchange capacity, equivalent to lettuce. Sunflower hull flours were highly variable in color and EA. There was no significant difference in aroma of muffins made with hull flour or wheat bran but differences did exist in appearance, flavor and texture. Sunflower hull flour showed potential as a dietary fiber supplement but its exact physiological effects have not yet been established.

INTRODUCTION

AN INCREASE in dietary fiber is being encouraged as a potential preventive or corrective measure to several noninfectious diseases such as diverticulosis, diabetes, coronary heart disease, colon cancer and constipation (Burkitt et al., 1974; Trowell, 1978; IFT, 1979).

Dietary fiber is not a single entity (IFT, 1979). Dietary fiber is generally accepted as any plant component which is not digested by mammalian enzymes (Trowell, 1976). Under this definition dietary fiber includes cellulose, hemicellulose, lignin, pectins, gums, mucilages, and indigestible starch and protein (Hellendoorn, 1981). Since the chemical components and physical characteristics of fiber sources vary widely, a variety of physiological responses can occur. For example, cellulose containing fibers appear to decrease stool transit time and soluble fibers appear to increase stool transit time (Anderson and Chen, 1979). In addition, gel-forming fibers alter the patterns of glucose uptake and may be hypocholesterolemic (Cummings, 1978).

Several sources of dietary fiber such as purified cellulose, wheat bran and pea hulls have been incorporated into food products as fiber supplements (IFT, 1979; Sosulski and Cadden, 1982). In addition, peanut hulls have been extensively evaluated as a potential fiber additive (Childs and Abajian, 1976; Collins and Post, 1981). Also, several crops are currently being grown in the upper midwest (flax, mustard and sunflower) which have hulls high in potential food grade dietary fiber (Sosulski and Cadden, 1982).

According to information from the U.S. Dept. of Agriculture, 4.7 million acres were planted in sunflowers in the United States in 1982, up 28% from the previous year and second only to the record 5.6 million acres planted in 1979 (McDonald, 1982). Hulls represent a large portion of the sunflower seed and improved dehulling processes are increasing the amount of sunflower hulls available as animal feed, energy source, or potential human fiber source (National Sunflower Council, 1982). Finally the low density of sunflower seed hulls, which according to Wan et al. (1979) ranges from 0.24–0.42g/ml, may present storage or disposal problems if expanded uses are not found. The purpose of this study was to evaluate both confectionery and oilseed sunflower hull flour as potential sources of dietary fiber.

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MATERIALS & METHODS

Preparation of fiber supplements and muffins

Sunflower seed hulls from confectionery (Interstate Seed Co., Fargo, ND) and oilseed (Cargill, Inc., Riverside, ND) types were obtained. Hulls were cleaned by hand and rinsed in water followed by air drying. The percentage hull of each type of sunflower seed was determined by hand dehulling 100 seeds. Hull flour was prepared by grinding the hulls in a Wiley Mill to pass through 60 and 80 mesh screens (confectionery hull flour, CHF; oilseed hull flour, OHF). A portion of the hulls was toasted at 150°C for 1 hr.

Untoasted sunflower seed hull flours and Miller's wheat bran flour (commercially available hard red spring wheat bran) ground to −60 mesh were added to a honey wheat muffin formulation (Table 1). Each muffin was adjusted to 46g of moist batter. All muffins were baked at 350°F (177°C) under uniform conditions.

Composition

The following chemical analyses were made in triplicate on 60 mesh hull flours. Proximate analyses of hull flours included moisture, crude protein (N X 6.25), crude fat, and crude fiber (AACC, 1962; AOAC, 1980). The dietary fiber content and the composition of the fiber was determined by detergent fractionation (Goering and Van Soest, 1970) as neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Crude fiber was determined by the AOAC 1980 method. The percentage of nitrogen-free extract (NFE) was determined by subtracting the percentage moisture, crude protein, crude fat, and crude fiber from 100. Hemicellulose was calculated as the difference between NDF and ADF. Cellulose was estimated by the amount of ADF residue dissolved in 72% sulfuric acid during the ADL method. The acid detergent solution was buffered at pH 2 as described by Baker (1977) and used to determine the amount of lignocellulose referred to as buffered acid detergent fiber (BADF).

Water-holding, oil-holding and cation exchange capacities

The following analyses were made in triplicate on 80 mesh hull flour. Water-holding capacity (WHC) and oil-holding capacity (OHC) were determined at 38° and 66°C for 30, 90 and 150 min by the method of Collins and Post (1981). The cation exchange capacity (CEC) of the acetone dried flour was determined by the methods of Helfferich (1962) and Childs and Abajian (1976) and reported as meq Na/g dry flour.

pH and color measurements

The pH of a mixture of 1g flour and 50 ml dionized water was measured by pH meter (Collins and Post, 1981).

A Gardner Instruments XL-23 colorimeter with a white standard plate was used to measure the color of toasted and untoasted flours. The flour was held in a cuvette for reading the L, a and b values.

Emulsifying activity

Emulsifying activity (EA) was evaluated by the method of Deshpande et al. (1982). Seven percent (w/v, db) aqueous dispersions of the 80 mesh flours were prepared. Twenty ml of dispersion was mixed with 20 ml of vegetable cooking oil and blended in a Waring Blendor for 5 min at a speed setting of 6. An aliquot was

centrifuged at 3,000 rpm for 5 min. EA was expressed as the percentage of the total mixture that remained emulsified after centrifugation.

Cholorgenic acid and anthocyanins

Sunflower seed hull flours were dried at 75°C for two hours. For chlorogenic acid extraction, 50 mg of each hull type was accurately weighed into a 125 ml Erlenmeyer flask. Seventy ml of 70% methanol was added and the sample was shaken for two hours (Fleming and Sosulski, 1977). Each sample was filtered through a Whatman #4 filter paper and quantitatively transferred into a 100 ml volumetric flask. The chlorogenic acid concentration was determined with a Waters Associates high performance liquid chromatography (HPLC) system including a U6K injector, both a 6000A and M45 solvent delivery system, Model 400 absorbance detector set at 313 nm and a Data Module. The separation conditions included a micro-Bondapak C_{18} column (3.9 mm i.e. 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. Calculation:

$$\% \text{ Chlorogenic acid} = \frac{\mu g/ml \times 100 \text{ ml}}{1000 \times \text{mg sample (dry weight)}} \times 100$$

For anthocyanin determination, sunflower hulls were extracted with 95% ethanol: 1.5N HCl (85:15) by a series of blending and percolation steps. The total concentration of anthocyanins was determined by the method of Fuleki and Francis (1968) using the extinction coefficient for cranberry anthocyanins.

Sensory evaluation

Fresh, warm muffins were evaluated by a panel of seven untrained members for appearance, aroma, flavor and texture. The 9-point hedonic rating scale (9-like extremely to 1-dislike extremely) described by Amerine et al. (1965) and Johnston (1979) was used to rate each attribute. The panel members consisted of faculty, staff and graduate students. The tests were performed in partitioned booths with fluorescent lighting. Samples were randomly coded and served individually with water. The sensory data were statistically evaluated by analysis of variance ($P \leq 0.05$) and Duncan's multiple-range test (Steel and Torrie, 1980).

Table 1—Sunflower hull flour and wheat bran muffin formulations

Ingredient	(g)
All-purpose flour	160
Whole wheat flour	66.0
Baking powder	9.00
NaCl	3.00
One large egg	55.0
Whole milk	175
Honey	160
Sunflower oil	50.0
Fiber source (CHF, OHF or wheat bran flour)	22.0 (3% w/w level)

Table 2—Proximate analysis of flour prepared from sunflower hull flour

Composition, %	Confectionery hull flour	Oilseed hull flour
Moisture	6.3	6.4
Crude protein	3.9	5.9
Crude oil	1.3	2.1
Ash	2.6	3.1
Crude fiber	65.2	62.4
Nitrogen free extract (NFE)	20.7	20.1

Table 3—Fiber components of flour prepared from sunflower hull flour[a]

Composition, %	Confectionary hull flour	Oilseed hull flour
ADF:lignocellulose	71.6	65.8
BADF:lignocellulose	88.8	83.4
NDF:Cell wall material	90.7	83.4
ADL:lignin	18.5	23.3
NDF-ADF:hemicellulose	19.1	17.6
ADF-ADL:cellulose	53.1	42.5
H:C:L Ratio[b]	20:59:21	28:51:21

[a] Calculated on a dry weight basis
[b] Hemicellulose:cellulose:lignin

RESULTS & DISCUSSION

THE PROXIMATE ANALYSES of the sunflower hull flours are shown in Table 2. Of the seed evaluated, the confectionery and oilseed contained 45.5% and 25.0% hull, respectively. The amount of moisture, ash, crude fiber and NFE of the two hull types differed only slightly. The amount of crude protein and oil were more variable. These values agreed with published values for sunflower seed hulls (Cancalon, 1971; Wan et al., 1979). Element analyses of the ash were not done because of the probable low availability and the wide variability in levels due to type and fertility of the soil in which the sunflower seeds were grown.

The levels of the different sunflower hull fiber fractions are shown in Table 3. Sunflower hulls are a concentrated source of dietary fiber. CHF and OHF, with their phytomelanin layers intact, had 90.7% and 83.4% NDF, respectively. These values were lower than the 97.0% NDF value for sunflower hull flour with the phytomelanin layer removed as reported by Sosulski and Cadden (1982). CHF and OHF had the same ratio of lignin but the CHF had a higher ratio of cellulose and a lower ratio of hemicellulose than the OHF. The iodine test for starch indicated no starch present in the sunflower hulls. Both types of sunflower hull flours contained more ADF, BADF, NDF and cellulose; equal hemicellulose; and less ADL than peanut hull flour (Collins and Post, 1981). The AACC white wheat bran contained approximately 8.9% crude fiber, 11.9% ADF and 40.2% NDF. Sunflower hulls have several similarities with cellulose (Avicel PH 10) in fiber content and particle shape, which may have an influence on their functionality (Sosulski and Cadden, 1982).

The WHC and OHC for the hull flours are shown in Table 4. The flours acted essentially the same at all temperatures and times, although the data was highly variable. No difference in WHC was noted between CHF and OHF. OHF was found to have a higher OHC than CHF. Both sunflower hull flours have higher WHC and OHC than the values previously reported for peanut hull flours (Collins and Post, 1981). Sunflower hulls also had higher OHC than flax hulls, mustard hulls, pea hulls, cellulose and wheat bran (Sosulski

Table 4—Water and oil holding capacity of flour from sunflower hull flour

Material	Soaking time, min	Temperature, °C 38	66
Water-holding capacity		------- g water/g flour -------	
Confectionery	30	3.95 ± 0.15	3.91 ± 0.20
	90	3.77 ± 0.21	4.10 ± 0.10
	150	3.87 ± 0.05	4.02 ± 0.05
Oilseed	30	3.76 ± 0.10	3.98 ± 0.12
	90	3.84 ± 0.17	4.39 ± 0.25
	150	3.94 ± 0.12	3.81 ± 0.18
Oil-holding capacity		--------- g oil/g flour ---------	
Confectionery	30	2.83 ± 0.20	3.09 ± 0.13
	90	2.83 ± 0.20	2.78 ± 0.18
	150	2.60 ± 0.14	2.34 ± 0.15
Oilseed	30	3.34 ± 0.05	3.41 ± 0.14
	90	3.20 ± 0.10	3.09 ± 0.05
	150	2.97 ± 0.18	3.18 ± 0.10

Table 5—Gardner color values for flour prepared from toasted and untoasted sunflower hull flour

Treatment	Confectionery hull flour	Oilseed hull flour
	Gardner L	
Untoasted	52.5	30.9
Toasted	51.7	34.4
	Gardner a	
Untoasted	0.1	1.2
Toasted	1.9	1.8
	Gardner b	
Untoasted	10.8	6.5
Toasted	15.2	8.5

and Cadden, 1982) so that the consumption of sunflower hull flour may help as a good stool bulking agent to help relieve constipation. In rats, Sosulski and Cadden (1982) have shown WHC to be positively correlated with fecal moisture, weight and volume but negatively associated with the apparent digestibility measurements. Sosulski and Cadden (1982) have shown sunflower hull fed rats to have significantly different fecal properties than customary fiber sources such as wheat bran. In their study, sunflower hull flour containing diets were shown to result in significantly lower fecal moisture, fat and density values and a higher serum cholesterol level than wheat bran containing diets. Thus, the practical potential for sunflower hull flour as a beneficial food supplement has not been established yet.

The cation exchange capacity is the number of counter ion equivalents in a specified amount of material (meq/g dry material) (Helfferich, 1962). The CEC values for the CHF and OHF were similar to each other; for confectionery hull flour, 3.57 meq/g and for oilseed hull flour, 3.40 meq/g. The CECs for the sunflower hull flours are similar to that of lettuce (3.1 meq/g) and twice as high as the CEC of peanut hull flour (1.55 meq/g) (McConnell et al., 1974; Childs and Abajian, 1976).

The gross energy content for the sunflower seed hulls was not determined because of the low digestibility of the fibrous components in monogastric animals. Thus, the Atwater coefficients of digestibility for protein (1.82), fat (8.27) and carbohydrate (2.35) of wheat bran were used to approximate the actual caloric content (Watt and Merrill, 1963; Collins and Post, 1981). The amount of digestible carbohydrate was determined by subtracting percentage crude protein, ash, crude fat and NDF from 100. The estimated available caloric content was determined by multiplying the amount of each component times the respective coefficient. The calculated value for CHF was 0.21 kcal/g and OHF was 0.41 kcal/g as compared to 0.52 kcal/g for peanut hull flour using the same method.

The pH and emulsifying activity (EA) of both sunflower hull flours were determined. The pHs of the sunflower hull flours were similar with CHF 6.3 and OHF 6.0. The EA of the sunflower hull flours were extremely different with CHF and OHF having EA of 27.5% and 72.5% respectively. This correlated with the OHC values and may be attributed to the higher protein content of the OHF.

The Gardner color values for the sunflower hull flours are shown in Table 5. The CHF was lighter (higher L value), contained less red (lower a value) and more yellow (higher b value) than the OHF. Toasting the hulls produced a more red and more yellow flour in both types of hulls. Toasting the CHF produced a darker flour and toasting the OHF produced a lighter flour. The color of the CHF may be described as light green and that of OHF as a dark green. The green coloration may not be a problem because of the processing techniques of purification and bleaching to produce a more acceptable color (Halpern, 1981).

Table 6—Sensory evaluation of muffins containing sunflower hull flour[a,b]

Muffins	Appearance	Aroma	Flavor	Texture
Control[c]	7.5a	6.6a	6.6a	6.8a
Confectionery hulls	5.5b	6.3a	5.8b	4.3b
Oilseed hulls	3.6c	6.2a	5.4b	3.2c

[a] Values in an entire column followed by a common letter are not significantly different at $P \leq 0.05$ (a through c)
[b] Hedonic scale: 9 = like extremely; 1 = dislike extremely.
[c] Miller's Wheat Bran.

Several minor components of sunflower hulls need to be discussed. Chlorogenic acid and anthocyanin content of the sunflower hull flour were determined in each flour. Chlorogenic acid is a major polyphenolic compound found in sunflower seeds (Sabir et al., 1974; Dorrell, 1976). At alkaline pH levels, it casues a dark green discoloration of food products. The chlorogenic acid content of the CHF was 0.15% and that of the OHF was 0.19% which was approximately 10% of the chlorogenic acid level of the kernel. Anthocyanins have been shown to occur in several genotypes of purple sunflower seeds (Vaccari et al., 1981; Fox and Dreher, 1982). No anthocyanins were found in the CHF and OHF evaluated. According to Casper et al. (1981), sunflower seeds have the added advantage of being extremely low in aflatoxin.

The sensory analyses data for sunflower hull and wheat bran flour muffins are shown in Table 6. Sunflower hull flour muffins scored significantly lower in appearance, flavor and texture than the wheat bran flour muffins. No significant difference in aroma was noted between the sunflower and wheat bran muffins. Within the sunflower hull muffins, CHF muffins scored significantly higher in appearance and texture than the OHF muffins, but there was no significant difference in flavor.

CONCLUSIONS

THE DATA PRESENTED in this study indicate that sunflower hull flours have potential as human dietary fiber supplements, but more evaluation is required. The compositional analyses showed sunflower hull flours to have high levels (83.4–90.7%) of cell wall material (NDF) which is composed mainly of cellulose, lignin and hemicellulose. The results of the various capacity tests showed OHF to have satisfactory functionality as a fiber additive. It had a relatively high CEC which needs further evaluation under in vivo conditions. The sunflower hull flours, especially that from oilseeds, may require purification or bleaching to produce a more acceptable color. Sensory testing of sunflower hull flours in muffins indicated no significant difference in aroma when compared to wheat bran containing muffins but differences did exist in appearance, flavor and texture. In all sensory attributes the CHF rated higher than the OHF.

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New Media for Enumeration and Detection of *Clostridium sporogenes* (PA3679) Spores

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ABSTRACT

Modified PA3679 agar (MPA3679A) was formulated as a plating medium to provide improved recovery of *Clostridium sporogenes* Putrefactive Anaerobe (PA) 3679 spores. A comparative study of MPA3679A and two currently used plating media, Yeast extract agar (YEA), and Peptone trypticase agar (PTA), showed that MPA3679A was superior for the recovery of heat activated and heat stressed spores. Further, when this medium was altered for use as an inoculated pack subculture medium, recovery of severely heat stressed spores was superior to that in the previously recommended PA3679 agar, also known as T-Best.

INTRODUCTION

ESSENTIAL TO THE CANNING of low acid foods is the destruction of *Clostridium botulinum* spores. A theoretically safe process for any product can be calculated using Ball's formulae (Ball, 1923, 1928; Ball and Olson, 1957), thermal death time parameters for *C. botulinum* and heat penetration data for the product and process in question. For new products and processes the theoretical process may be confirmed by an inoculated pack.

The organism most frequently used for conducting inoculated packs in low acid foods is *C. sporogenes*. Spores of this organism have a similar z-value (slope of the thermal death time curve) to that of *C. botulinum* and are more heat-resistant (Esty and Meyer, 1922). A thermal process that produces a 5 log (5-D) reduction of *C. sporogenes* spores produces more than a 12 log (12-D) reduction in the spores of *C. botulinum*. Therefore, the increased heat resistance of *C. sporogenes* over that of *C. botulinum* provides a built-in safety factor when using *C. sporogenes* in inoculated pack studies. Additionally, *C. sporogenes* is nonpathogenic and can be used safely for conducting inoculated packs in canneries. The strain of *C. sporogenes* most commonly used by the canning industry is Putrefactive Anaerobe (PA) 3679 which was isolated by the National Food Processors Association (NFPA).

Concentrated suspensions of PA3679 spores are supplied to member companies in the canning industry by the NFPA. The medium recommended to confirm NFPA's spore counts was Andersen's Pork Pea Infusion (APPI) using the Most Probable Number (MPN) technique (Andersen, 1951; Bernard, 1983). As alternatives to APPI and the MPN method, several anaerobic plating media have been developed for enumeration of PA3679 spores (Wynne et al., 1955; Polvino and Bernard, 1982; Frank and Campbell, 1955; Hauschild and Hilsheimer, 1977; Pflug et al., 1979; Wheaton and Pratt, 1961; Andersen, 1951; Odlaug and Pflug, 1977).

Improvement in subculture methods and especially improvement in the subculture medium for recovering injured spores from inoculated pack tests would increase the accuracy of establishing a safe process. Thus, a subculture medium which consistantly provides better recovery of injured PA3679 spores from inoculated packs would result in more meaningful data.

This study describes two new anaerobic media. The first one is a plating medium which is superior to Yeast extract agar and Peptone trypticase agar for enumerating heat activated and heat stressed PA3679 spores. The second replaces PA3679 agar as a subculture medium for inoculated test packs.

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MATERIALS & METHODS

Spore suspensions

Spores of *C. sporogenes* PA3679 were prepared by NFPA using beef heart infusion broth (Anon., 1968). Following centrifugation, a portion of the supernatant was removed. PA3679 spores were received from NFPA suspended in the mother liquor.

Recovery media

A basal medium for Modified PA3679 agar (MPA3679A) was prepared by adding 10g of Tryptone (Difco), 2g of soluble starch (Difco), 2g of K_2HPO_4 (Fisher), 2g of yeast extract (Difco) and 15g of Bacto-agar (Difco) to 1000 ml of distilled water and autoclaving for 15 min at 121°C. Two milliliters of a sterile sodium thioglycollate (Difco), sodium bicarbonate (Fisher) and lysozyme solution (TBL) were aseptically pipetted into 100 ml of the tempered basal medium before pouring plates. The components of the TBL solution were prepared as follows: (1) 3.3g of sodium thioglycollate were added to 20 ml of distilled water and autoclaved for 15 min at 121°C; (2) 2.5g of sodium bicarbonate were added to 27.5 ml of distilled water and filter sterilized; and (3) 0.1 mg/ml lysozyme (78,000 units/mg, Chemalog) solution was prepared in distilled water and filter sterilized. The TBL solution was prepared by combining all of parts (1) and (2) with 2.5 ml of part (3). Solutions were prepared fresh on the day of each experiment.

A basal medium for Modified PA3679 semisolid agar (MPA-3679SA) was prepared as above except that 3g of Bacto-agar and 800 ml of distilled water were used. Two ml of a sterile sodium thioglycollate, sodium bicarbonate, dextrose (Difco) and lysozyme solution (TBDL) were aseptically added to 8 ml of tempered basal medium in an 18 x 150 mm metal capped tube prior to subculturing. The components of the TBDL solution were prepared as follows: (1) 0.88g of sodium thioglycollate was added to 75 ml of distilled water and autoclaved for 15 min at 121°C; (2) 1.88g of dextrose and 0.63g of sodium bicarbonate were dissolved in 50 ml of distilled water and filter sterilized; and (3) 0.1 mg/ml lysozyme solution was prepared in distilled water and filter sterilized. The TBDL solution was prepared by combining all of parts (1) and (2) with 0.63 ml of part (3). Solutions were used the same day of preparation. Following subculturing each tube was overlayed with 3–4 ml of sterile molten 2% agar.

Modified trypticase agar (MTA) was prepared by adding 20g of Trypticase peptone (BBL), 10g of Phytone peptone (BBL), 0.5g of sodium thioglycollate, 3g of K_2HPO_4 and 15g of Bacto-agar to 900 ml distilled water and autoclaving for 15 min at 121°C. A 10% solution of soluble starch in distilled water was prepared and sterilized for 15 min at 121°C. Ten ml of starch solution were aseptically added to 90 ml of tempered medium.

Yeast extract agar (YEA; Pflug et al., 1979) was prepared by adding 10g of yeast extract, 1.0g of soluble starch, 2g of K_2HPO_4 and 15g of Bacto-agar to 1000 ml of distilled water and autoclaving for 15 min at 121°C. Before pouring plates, the following components were aseptically added to 300 ml of tempered medium: 3.75 ml of a 10% sodium thioglycollate solution, 3.75 ml of a 40% dextrose solution and 7.5 ml of a 4% sodium bicarbonate solution.

The dextrose and sodium bicarbonate solutions were sterilized by membrane filtration. The sodium thioglycollate solution was sterilized by autoclaving for 15 min at 121°C.

PA3679 agar (PA3679A), also known as T-Best agar (Wheaton and Pratt. 1961), was prepared by adding 10g of Tryptone (Difco), 1g of dextrose, 3g of beef extract (Difco), 1g of soluble starch, 1g of yeast extract, 1.25g of K_2HPO_4, 0.5g of sodium thioglycollate and 16g of Bacto-agar (Difco) to 1000 ml of distilled water. Ten milliliters of medium were dispensed in each 18 x 150 mm metal capped tube and autoclaved for 15 min at 121°C.

Peptone trypticase agar (Polvino and Bernard, 1982) was prepared by adding 50g of Trypticase peptone, 5g of Bacto peptone (Difco), 1g of sodium thioglycollate and 15g of Bacto-agar to 1000 ml of distilled water and autoclaving for 15 min at 121°C. Before pouring plates, 1.6 ml of a filter sterilized 10% solution of sodium bicarbonate were added to 100 ml of tempered medium.

Sorensen's M/15 phosphate buffer, pH 7.0, was prepared by adding 38.9 ml of a stock solution of M/15 monopotassium phosphate (KH_2PO_4) to 61.1 ml of a stock solution of M/15 disodium phosphate (Na_2HPO_4) and autoclaving for 15 min at 121°C.

Heat activation

One ml of a spore suspension containing approximately 1 x 10^4 spores was added to a 20 x 150 mm screw-capped test tube containing 9 ml of Sorensen's M/15 phosphate buffer solution (pH 7.0). The tube and its contents were immersed in boiling water for 8 min, then immediately placed into an ice water bath. The suspension was prepared in a quantity sufficient to inoculate all plates and tubes and was plated or subcultured within 1 hr of heat-activation.

Heat stress

Approximately 5 x 10^5 spores in 0.05 ml of mother liquor plus 1.95 ml Sorensen's M/15 phosphate buffer solution (pH 7.0) were placed in a 16 x 125 mm screw-capped test tube. To achieve a 99.9% or more reduction in spore number and to assure spore injury, the spores were exposed to a temperature of 115.5°C (240°F) for 5.00 min plus a 0.93 min heating lag in a miniature retort. Following exposure, the tubes were immersed in an ice bath. In one series of trials, thermally stressed spores prepared in this manner were used as a model system for testing the efficacy of MPA3679SA as a subculture medium. Suspensions were plated or subcultured within 1 hr.

MPN studies

Sufficient spores were heat stressed for each study to inoculate five 18 x 150 mm metal capped tubes in each of duplicate 5 tube MPN series (Anon., 1975) to compare MPA3679SA with PA3679A. Each tube of MPA3679SA was overlayed with 3 ml of sterile molten 2% agar. All tubes were incubated at 32°C for up to 21 days.

Inoculated pack simulation

Nine products, including pasta and a variety of soups containing seafood, chicken, mushrooms and vegetables were used to simulate inoculated packs and subsequent subculturing. Each product was blended for 2 min in a Waring Commercial Blender after which 5g were pipetted into each of fifty 16 x 125 mm screw-capped tubes. The product in each tube was inoculated with 0.1 ml of a spore suspension to a final concentration of 1 x 10^4 spores/tube. Eight replicate tubes were heated in miniature retorts at 115.5°C at each of six increasing periods of time such that at least one exposure time yielded survival and at least one exposure time yielded no survival. Spores in control tubes were heat activated by heating for 8 min at 100°C. Following rapid cooling, the tubes from each time increment were randomly divided into two groups. One-half the tubes were subcultured with MPA3679SA while the remaining half were subcultured with PA3679A. The tubes were slowly inverted several times to disperse product throughout the medium. Extended cooling of tubes of MPA3679SA was necessary to allow complete hardening of the agar. Tubes were overlayed with 3 ml of sterile 2% agar and incubated at 32°C for up to 21 days.

Plating studies

Plates were incubated inverted in an anaerobic atmosphere using a BBL H_2 + CO_2 gas generator in a Gaspak Jar. Unless otherwise stated, the plates were incubated at 35°C for 48 hr prior to counting.

Statistical analysis

Statistical analyses were performed using a standard analysis of variance, Duncan's multiple range test, or student's t-test.

RESULTS & DISCUSSION

HISTORICALLY, the MPN technique has been the method of choice in the canning industry for counting both heat activated and heat stressed PA3679 spores. While used extensively, the MPN method is cumbersome, time consuming, slow in yielding results, imprecise and provides poor reproducibility. A joint study conducted by Campbell Soup Company and NFPA indicated that anaerobic plating using MTA for recovery of heat activated spores was easier and quicker than MPN using APPI and yielded reliable results as well as better recovery (unpublished data).

However, although MTA was a good medium for the recovery of heat activated spores, it performed poorly when compared to PA3679A for recovery of heat-stressed spores. PA3679A is commonly used by the canning industry as a subculture medium for the recovery of heat stressed spores from low-acid foods in inoculated pack tests. As such, it was considered a good base upon which to formulate an improved anaerobic plating medium which would provide good recovery of both heat activated and heat stressed spores.

A major difference between MTA and PA3679A is the absence and presence of beef extract, respectively. Recovery of heat activated and heat stressed spores was influenced by the beef extract (Fig. 1). As the concentration of beef extract in PA3679A was increased from none to 0.45%, the recovery of heat stressed spores increased. Conversely, the presence of beef extract had an inhibitory effect on the recovery of heat activated spores. As the concentration of beef extract in the medium increased, the

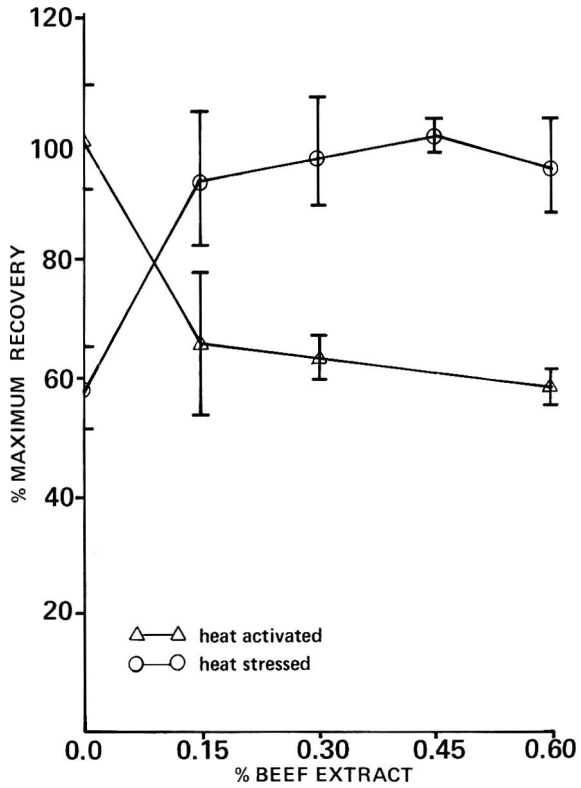

Fig. 1—Effect of beef extract in PA3679A on recovery of heat activated and heat stressed spores. Points represent mean of triplicate plates. Bars represent ± 1 standard deviation.

inhibitory effect upon the recovery of heat activated spores increased. Similar results were found when beef extract was added to MTA (data not shown).

The effect of beef extract was further demonstrated using spores subjected to various degrees of thermal treatment at 115.5°C (Fig. 2). This study showed decreasing inhibition by beef extract upon the recovery of spores exposed to increasing time increments of mild thermal treatment up to 1.3 min, as well as its increasing stimulation on the recovery of spores exposed to more severe thermal stress. Because of its inhibitory effect on the recovery of heat activated spores, beef extract was omitted from MPA3679A.

Lysozyme has been shown to improve the recovery of *Clostridium perfringens* and *C. botulinum* subjected to severe thermal stress (Adams, 1974; Alderton et al., 1974; Hauschild and Hilsheimer, 1977), thus, the effect of incorporating lysozyme into MPA3679A was determined. Lysozyme in MPA3679A (0.1 μg/ml) increased the recovery of heat stressed spores by 20–25% when compared to enumeration in the medium without lysozyme, and >30% when compared to enumeration in YEA. Unlike beef extract, lysozyme at 0.1 μg/ml did not inhibit the recovery of heat activated spores when used in MPA3679A, although concentrations in excess of 0.1 μg/ml medium were shown to progressively suppress recovery of heat stressed and heat activated spores.

Sodium bicarbonate, known to improve recovery of *Clostridium* species (Wynne et al., 1955), markedly improved the recovery rate of heat activated and heat stressed PA3679 spores when it was included in MPA3679A. Like lysozyme, sodium bicarbonate was found to depress the recovery of heat-stressed spores when used in quantities greater than 0.1%. For example, increasing the concentration of sodium bicarbonate in the medium from 0.1% to 0.25% caused a 35% decrease in recovery of heat-stressed spores. Dextrose, on the other hand, did not significantly influence the recovery of either heat activated or heat stressed spores. This is in contrast with previous reports which found a stimulatory effect by dextrose on recovery of heat stressed PA3679 spores (Pflug et al., 1979; Wheaton and Pratt, 1961).

To determine optimum concentrations of soluble starch,

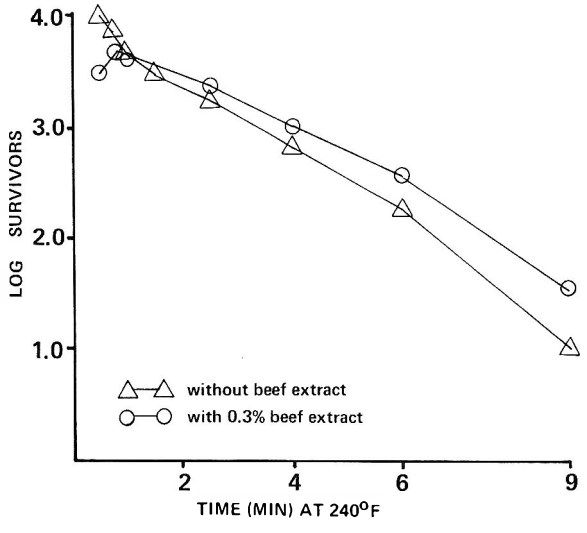

Fig. 2—Effect of beef extract on recovery of PA3679 spores subjected to varying degrees of thermal treatment. Points indicate mean of quadruple replicate plates. Points at time ≤ 0.75 min and ≥ 2.5 min were significantly different ($p < 0.05$) as determined by Student's t-test.

potassium phosphate and yeast extract, MPA3679A was prepared with varying concentrations of these ingredients at less than and more than they occur in PA3679A. Increasing the quantity of these components from that used in PA3679A to 2g of each per liter of MPA3679A reproducibly improved its overall effectiveness in the recovery of heat activated and heat stressed spores (data not shown).

PTA and YEA are anaerobic plating media widely used to recover and enumerate heat activated and heat stressed putrefactive anaerobic spores, respectively (Polvino and Bernard, 1982). YEA is also used for heat-shocked spores. Data comparing MPA3679A, MTA, PA3679A, YEA and PTA for the recovery of heat activated and heat stressed PA3679 spores are summarized in Table 1. The recovery of heat activated spores was significantly greater in MPA-3679A than in YEA, PTA or PA3679A ($p < 0.05$); the difference between recovery in MPA3679A and MTA was not statistically significant. MPA3679A permitted enumeration of heat activated spores following incubation at 37.5°C for 24 hr. Continued incubation up to 96 hr did not significantly increase recovery of heat-activated spores. Other media required a minimum of 48 hr incubation for maximum recovery. The conspicuously poor recovery of heat activated spores in PA3679A as compared with the other test media may be due to the presence of beef extract, which is present only in PA3679A.

MPA3679A also was found to be significantly better ($p < 0.05$) than the other media for enumerating heat stressed spores (Table 1). The differences between MPA-3679A and YEA, PTA and MTA in the recovery of heat stressed spores were greater than those obtained from the same media in the recovery of heat activated spores. This suggested that some of the spores had suffered sub-lethal damage or injury leading to a greater dependency upon or sensitivity to a medium constituent following severe thermal stress. Unlike recovery of heat activated spores, optimum recovery and enumeration of heat stressed spores required an incubation time of not less than 96 hr at 32°C. The YEA plates were difficult to count after 48 hr, and especially so after 72 and 96 hr due to considerable disruption of the agar by gas. This was not a problem with MPA3679A, MTA, PTA or PA3679A. Further, it may be that the presence of beef extract in PA3679A at the recommended level of 0.3% accounted, in part at least, for its superiority to MTA for the recovery of heat stressed spores.

The formula for APPI contains pork and pea infusions which are tedious and time consuming to prepare and often vary from batch to batch. Because of ease of preparation and reproducibility, PA3679A has been used in place of APPI as a subculture medium. The superiority of MPA-3679A as a plating medium for the recovery of heat stressed spores suggested that it also may be best as a subculture medium for inoculated product tests of low acid foods. The inclusion of dextrose and the deletion of 80% of the agar increased its superiority to PA3679A (data not shown).

Table 1—Comparison of media for recovery of heat activated and heat stressed PA3679 spores

Medium	Heat activated		Heat stressed	
MPA3679A	100%[a]	a[b]	100%	a
MTA	92%	ab	16%	d
PTA	90%	b	0.5%	e
YEA	84%	b	44%	b
PA3679A	21%	c	31%	c

[a] Count for MPA3679A was set at 100%; other counts expressed as % thereof (results based upon 8 plate counts per medium)
[b] Media with the same letter were not significantly different when analyzed by Duncan's Multiple Range Test

An unfortunate aspect of the subculturing procedure is the necessity of pipetting product into medium in a ratio of 1:2, making the medium opaque due to the presence of the subcultured food. This opacity prevents observation of colonies within the medium and growth cannot be detected without production of gas and concomitant splitting of the agar. Studies showed that up to five PA3679 colonies could grow in a single tube of PA3679A without obvious production of gas. However, rapid and consistant spread of motile vegetative cells throughout MPA3679SA with separation of the agar plug from the medium due to gas production and the resultant distinctive and characteristic putrefactive odor provided sufficient indication of growth in the medium. Using a model system in which thermally stressed spores were subcultured into MPA3679SA and PA3679A, without the addition of food product, it was shown that MPA3679SA was equal to or better than PA3679A in 9 of 10 trials when tubes of PA3679A containing colonies not producing gas were counted as positive. However, MPA-3679SA was better than PA3679A in all trials when only tubes in which gas was produced were counted as positive (Table 2).

Replacement of PA3679A with MPA3679SA for subculturing low acid food inoculated packs required testing with product. Nine food products were used in simulated inoculated packs to compare these media for recovery of thermally injured spores. In eight out of nine tests, spores exposed to severe thermal stress were found to grow out and produce gas in MPA3679SA one time increment above that evident for PA3679A. The exception provided equal results. In three of the simulated inoculated packs, product alone also was overlayed with 2% agar without benefit of subculturing; growth was evident in only one product. These results suggest that some products provide relatively poor environments for germination and outgrowth of thermally injured spores when compared to the results of those same products subcultured in MPA3679SA.

Table 2—Comparison of PA3679A and MPA3679SA as subculture media for the recovery of heat stressed spores by the MPN method

	PA3679A			MPA3679SA		
Trial[a]	Counts[b] (± gas)	Counts[c] (+ gas)	Tubes[d] (− gas)	Counts[b] (± gas)	Counts[c] (+ gas)	Tubes[d] (− gas)
1	540	240	2_2	540	540	0_0
2	540	70	4_{14}	920	920	0_0
3	1600	220	3_5	920	920	0_0
4	540	140	2_8	2400	2400	0_0
5	920	110	4_{11}	2200	1700	1_1
6	920	540	1_1	3500	3500	0_0
7	240	79	2_6	1600	1600	0_0
8	700	170	3_6	5400	5400	0_0
9	540	70	4_8	1700	1700	0_0
10	350	350	0_0	920	920	0_0

[a] Trials represent 10 nonreplicate tests in model system, using 5 tube MPN series.
[b] MPN counts based on tubes demonstrating growth, with or without obvious gas production
[c] Counts based only on tubes with obvious gas production
[d] Number of tubes without gas production (subscript equals total number of colonies in nongas producing tubes)

Comparing the recovery of subcultured heat stressed spores incubated at 30°C, 32°C and 37.5°C showed that maximum count was attained at 32°C. In some cases, at that temperature positive subcultures were recorded beyond 7 days. Consequently, the incubation time was increased from 7 days to 14 days at 32°C.

MPA3679A is a medium for the improved recovery of heat activated and heat stressed PA3679 spores. Although there was a significant increase in the recovery of heat activated PA3679 spores using MPA3679A as compared to some available media, the increase in heat stressed spore recovery provides even greater improvement over existing formulations. Further, as a subculture medium MPA-3679SA not only gives rapid recovery, but demonstrates better and more consistant recovery than the currently used PA3679A. Thus, by providing conditions for the growth of thermally injured spores, which would otherwise have been assumed nonviable in a less complete medium, MPA3679A provides an additional margin of safety when used for subculturing inoculated pack test products.

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Textural and Rheological Properties of Cooked Potatoes

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ABSTRACT

Cooked potato samples of different cultivars and specific gravities were analyzed for textural and rheological properties by sensory evaluation, texture profile analysis (TPA) and stress relaxation tests. Mealiness was correlated with the product of cohesiveness and adhesiveness, but not with other TPA parameters. Stress relaxation tests of cooked potatoes yielded a Maxwell model consisting of two viscous and three elastic elements. One of the elastic moduli was correlated with mealiness. Hardness by sensory evaluation correlated well with hardness and fracturability by TPA, and with the three elastic moduli in the relaxation model.

INTRODUCTION

THE TEXTURE OF POTATO has been a subject of interest for several decades. Cooked potato texture was classified by Salamon (1926) into four types: floury, close, waxy and soapy. "Floury" is usually referred to as "mealy" in the literature. It is generally agreed upon that mealiness is the most important aspect of cooked potato texture (Unrau and Nylund, 1957; Smith and Davis, 1963; Warren and Woodman, 1974). Bettelheim and Sterling (1955a, b) described mealiness as a dry and granular feel on the tongue. According to Burton (1966), a mealy potato may retain its form during cooking but is easily broken down. Generally, mealiness is associated with cell separation upon chewing. Although extensive studies have been done on factors affecting potato texture (Greenwood et al., 1952; Bettelheim and Sterling, 1955a, 1955b; Kuhn et al., 1959; Linehan and Hughes, 1969; Keijbets and Vaal, 1974; Warren et al., 1975; Reeve, 1977; Nonaka, 1980), the causes and physical basis of mealiness in cooked potatoes are still unclear (Warren and Woodman, 1974; Ridley et al., 1981).

Texture of cooked potatoes is usually evaluated using sensory panel (Davis and Dixon, 1976; Collison et al., 1980; Ridley et al., 1981; True and Work, 1981). Attempts have been made to develop objective methods for measuring potato texture, especially mealiness. Early studies have shown correlation between mealiness of cooked potatoes and relative viscosities (Unrau and Nylund, 1957; Kuhn et al., 1959). Le Tourneau et al. (1962) developed a cooking test to evaluate sloughing of cooked potatoes. DeMan (1969) determined texture of cooked potatoes with the Kramer Shear press and a puncture tester but did not conduct any sensory evaluation. In 1971, Schmidt and Ahmed studied the effect of peeling methods on textural properties of cooked potatoes using the texture profile analysis (TPA) (Bourne, 1968). They did not, however, correlate the TPA parameters with sensory data. In 1972, Woodman and Warren developed a method to assess mouth-feel (mealiness) of canned potatoes using a Kramer shear press with the grid extrusion cell. Hughes et al. (1975) used the Wolodkewitsh tenderometer to measure the compressive strength of cooked potato disks. More recently, Iritani et al. (1977) developed a technique to determine cooking ability by assessing time-to-breakdown of different portions of potato tubers. Despite the extensive studies, a satisfactory method for evaluating potato texture, in particular mealiness, still remains to be developed.

Relatively little research has been conducted on rheological properties of cooked potatoes. Schmidt and Ahmed (1972) studied the influence of peeling methods, varieties and storage conditions on the apparent elastic properties of processed and unprocessed potatoes. However, they did not relate the elastic and textural properties of processed potatoes. Recently Davis et al. (1983) demonstrated that the stress relaxation response for cooked potatoes can be represented by a generalized Maxwell model consisting of three elastic and two viscous elements. The effects of cooking times, specific gravities, varieties and group lots on the viscous and elastic elements of the model were investigated. Their results indicated that these elastic and viscous properties may be related to texture.

The purpose of this study was to evaluate texture and stress relaxation properties of cooked potatoes and to correlate objective and subjective assessments of potato texture.

MATERIALS & METHODS

Potato samples

Kennebec, Russet Burbank, White Rose, White California, Red and Seedling A 503-42 potatoes were used in this study. The first three varieties and Seedling A 503-42 were grown at the Washington State University Othello Field Station. Seedling A 503-42 was out of the University of Idaho breeding program and has not yet been released as a variety. White California and Red were unknown varieties purchased in lots of 30 kg at a local grocery store.

Medium size tubers were used except for Russet Burbank where medium and small sizes were selected (Table 1). The tubers were washed, separated into specific gravity groups with brine solution, washed again to remove the brine and air dried at room temperature. They were packed in polyethylene bags and stored at 5°C and 65% RH for no more than 15 days before analysis.

To reduce the variability in the cooked potato samples, three groups of ten Russet Burbank potatoes of medium size (6.2 cm diameter) with specific gravities of 1.085, 1.095, and 1.100 were used to determine the coefficient of variation of fracturability as determined by texture profile analysis (TPA). The whole tuber was divided cross sectionally into four rows and longitudinally into three columns (Fig. 1A). Two cylindrical samples (1x1 cm) were taken from each row and each column, excluding the core region. Based on the coefficient of variation, row two was chosen as the sampling location.

Sample preparation

Tuber samples were taken out of the refrigerator 24 hr before all experiments. Using a No. 9 cork borer, four cylindrical samples (1 cm diameter) were taken from row two (Fig. 1B) and trimmed to a height of 1 cm with a specially constructed apparatus. Two samples were used for sensory evaluation, one for TPA, and one for stress relaxation.

The cylindrical samples were rinsed and cooked in distilled water

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with a potato-to-water ratio of about 1:4. The cooking device consisted of a 250 ml Erlenmeyer flask and a wire mesh with 0.16 cm openings at the bottom of the flask to avoid direct contact of the samples with the bottom. Attached to the Erlenmeyer was a Liebig glass condenser with circulating cold water to keep the water from boiling off during cooking. This was done to minimize variability of the water-to-potato ratio (Zaehringer et al., 1963). For the first three training sessions, the potatoes were cooked for 5 minutes. Later the samples were cooked until one of the pieces began to slough. Sloughed samples were discarded. The cooking times were determined separately for each of the six groups of potatoes.

After cooking, the samples were drained and kept in polyethylene bags to avoid dehydration. The TPA, relaxation test and sensory evaluation of the cooked samples were conducted within 60, 120 and 75 minutes, respectively, after preparation.

Sensory evaluation

Twelve graduate students from the Dept. of Food Science & Technology, Washington State Univ., participated in the sensory evaluation. Five training sessions were carried out over a period of seven days. During the training sessions, the texture profiling method was introduced (Civille and Szczesniak, 1973). Cooked potato samples of different cultivars and with different specific gravities and cooking times were evaluated, and the terminology to express potato texture was discussed. It was decided that only hardness and mealiness would be evaluated. Russet Burbank (1.100 sp. gr.) and White California (1.070) potatoes were selected as mealy and gummy references based on the preliminary results and panel recommendation.

In the fifth (last) training session, the panelists were screened for their ability to evaluate mealiness and hardness using triangle tests. Two of the panelists did poorly in the triangle tests. They were kept in the taste panel but their data were not included in the analysis.

Following the last training session, three taste panel sessions were conducted in a period of five days. A completely balanced design was used for the graphical rating scale (Sidel and Stone, 1976). Eight sets of cooked potato samples were served to the panelists at room temperature. Each set contained two samples of the same treatment. The eight sets of samples included references for mealy (Russet Burbank sp. gr. 1.100) and gummy (White California) potatoes. The panelists were instructed to indicate the degree of hardness and mealiness (or gumminess) on the appropriate scales. The same samples were used to evaluate both hardness and mealiness. The type of potatoes, specific gravities and cooking times used for the three taste panel sessions are shown in Table 1.

Texture profile analysis (TPA) test

The TPA test was performed using an Instron Model 1350 Servohydraulic Testing Machine (Instron Corporation, Canton, MA) according to Bourne (1978; 1980). Since it was difficult to prepare cylindrical samples with perfectly flat ends, it was necessary to compensate for any unevenness of the two ends by preloading the sample with 0.5 percent of the total load. Bourne (1967) reported a similar method for overcoming the effect of small imperfections in shape of standard pieces. The crosshead speed was 1.89 cm/min and the deformation was 75% of the original length. Thirty replicates were tested for each of the eight treatments. The seven textural parameters as modified by Szczesniak (1975) were determined from each curve.

Table 1—Specific gravities, weights and diameters of potato tubers

Potato	Specific gravity	Weight[a] (g)	Diameter[a] (cm)	Cooking time (min)
White Rose	1.105	223 ± 38.7	6.6 ± 0.61	7.5
Kennebec	1.100	201 ± 32.0	6.8 ± 0.33	6.25
Russet Burbank	1.090	188 ± 38.1	6.2 ± 0.47	8.75
A 503-42	1.100	204 ± 45.8	7.6 ± 0.67	6.0
Russet Burbank	1.075	99 ± 14.3	4.8 ± 0.52	10.5
Red	1.075	243 ± 20.7	7.4 ± 0.27	19.5
Russet Burbank	1.100	188 ± 38.1	6.2 ± 0.47	6.25
White California	1.070	179 ± 41.54	6.0 ± 0.50	20.0

[a] Each value is the mean ± standard deviation for 20 samples.

Stress relaxation test

The stress relaxation test was performed using the Instron Model 1350 Servohydraulic Testing Machine. The uneveness of the two ends of the sample was compensated by preloading it with 0.5% of the total load. The crosshead speed was 50 cm/min and the strain was 10%. Thirty replicate samples were tested for each treatment. Data points from the relaxation curves were entered into an Amdahl/470 V8 CPV Computer for nonlinear regression analysis using the NLIN program from the Statistical Analysis System (SAS, 1979).

Statistical analysis

Computer programs from the Statistical Analysis System (SAS, 1979) were used to analyze the data. The significance of the sensory evaluation, TPA and relaxation tests was estimated by Analysis of Variance (ANOVA) and the Duncan's multiple range test (DUNCAN) procedures (Steel and Torrie, 1980). The correlation coefficients (CORR) procedure was used to establish the relationship between the most significant parameters obtained by the three methods.

RESULTS & DISCUSSION

Sensory evaluation

During the training sessions, the panelists were asked to describe the texture of cooked potatoes in their own words. Table 2 shows a list of 28 terms suggested by the panel. The terminology was discussed and the panelists agreed that the terms "mealy" and "gummy" were the most appropriate to describe potato texture. They defined mealiness as a sensation of granular consistency, easy to break into small parts or crumbs. The term gummy was related to cohesiveness and adhesiveness.

Since it was very difficult to establish a proper cooking time for the potatoes, the samples were cooked until the first sample in a batch started sloughing, or disintegrating. This time was arbitrarily chosen as the cooking time for the potato. Table 3 shows that the cooking time for the Red potato (19.5 min) was approximately three times as long as the cooking time for the Seedling A 503-42 (6 min). This is not surprising since the Red potato is considered a "hard"

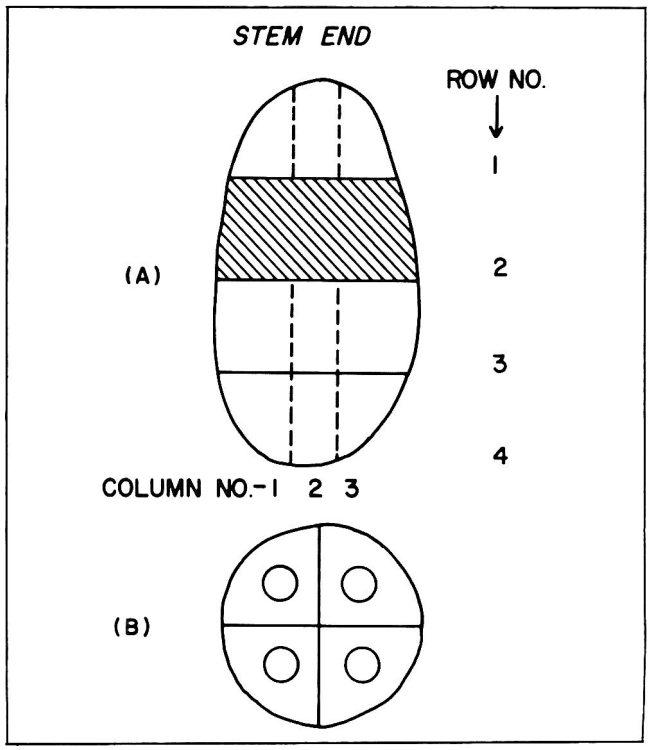

Fig. 1—Diagrams showing method of core sampling of a potato tuber. (A) Row and column numbers. (B) Cross-section area of row showing four core samples.

Table 2—Terms used by taste panelists to describe the textural characteristics of cooked potatoes

Term	Frequency	Term	Frequency
Grainy	18	Sheared	2
Smooth	14	Brittle	1
Mealy	11	Cohesive	1
Gummy	11	Compact	1
Crisp(y)	7	Dry	1
Raw	7	Friable	1
Break (easy to)	4	Hard	1
Crumbly	3	Pasty	1
Chewy	3	Rubbery	1
Mushy	3	Slippery	1
Fibrous	2	Soft	1
Fracturable	2	Swallow	1
Moist	2	Tender	1
Oily	2	Tough	1

Table 3—Gumminess and hardness of cooked potatoes determined by sensory evaluation[a]

Potatoes	Specific gravity	Cooking time (min)	Mealiness[b]	Hardness[c]
White Rose	1.105	7.5	8.05 a	4.02 b
Kennebec	1.100	6.25	6.99 b	5.99 c
Russet Burbank	1.090	8.75	6.82 bc	3.81 b
A 503-42	1.100	6.0	6.00 c	4.35 b
Russet Burbank	1.075	10.5	4.97 d	5.05 bc
Red	1.075	19.5	1.65 e	0.89 a

[a] Means (n = 30) within each column followed by the same letter are not statistically different ($P < 0.05$).
[b] Mealiness rating: 0 = gummy, 10 = mealy.
[c] Hardness rating: 0 = soft, 10 = hard.

cooker because of its low specific gravity. The hard cooking is probably caused by low water-soluble pectin content and high concentration of hemicellulose and acid-soluble pectin (Reeve, 1977).

Table 3 shows that low specific gravity potatoes such as the Red potatoes (sp gr 1.075) were generally more gummy or less mealy than high specific gravity potatoes such as White Rose (sp gr 1.105). Also, Russet Burbank potatoes with specific gravity of 1.090 were more mealy than those with specific gravity of 1.075. An early study by Unrau and Nylund (1957) showed that within a variety, potatoes with higher specific gravity were more mealy. They also found that potatoes of different varieties but with the same specific gravity may differ in mealiness. Other reports have also associated mealiness with specific gravity of potato tubers (Bettelheim and Sterling, 1955a; Kuhn et al., 1959; Young, 1962; Murphy et al., 1969).

Hardness is affected by cooking time, variety and specific gravity of the potatoes. Although White Rose, Russet Burbank (sp gr 1.090 and 1.075) and A 503-42 had different cooking times (6.0–10.5 min), they were not significantly different in hardness. However, the mealiness scores of these four potatoes were significantly different ($P < 0.05$). Therefore, hardness was not directly associated with the mealiness of cooked potatoes. Probably because of the especially long cooking time (19.5 min), California Red had the softest texture. Red was different from the other potatoes in that it was very resistant to sloughing. It was also the gummiest of the potatoes used in this study. Gumminess or stickiness of potatoes has been associated with exuded gelatinized starch from ruptured cells of the potato tissue (Reeve, 1977).

Texture profile analysis

A typical TPA curve for cooked potato samples is shown in Fig. 2. The negative peak during the unloading of the first stroke was relatively small, indicating limited adhesiveness of the sample. The TPA parameters of the cooked potatoes are summarized in Table 4. The fracturability value was highest for Kennebec and lowest for Red. This is in agreement with the hardness rating by the taste panel (Table 3). A comparison of the TPA fracturability and hardness values in Table 4 and sensory hardness in Table 3 suggests that fracturability was a more sensitive indicator of hardness assessed by sensory evaluation.

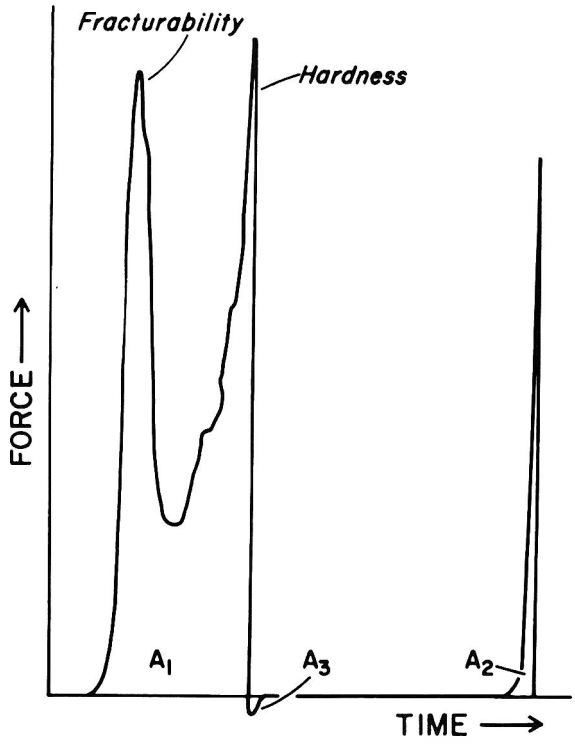

Fig. 2—A typical texture profile analysis (TPA) curve of cooked potatoes. A_1, A_2 and A_3 indicate areas.

Based on the sensory evaluation data (Table 3), Red was the gummiest potato and Russet Burbank with specific gravity of 1.075 was second in gumminess. However, TPA indicated that Red was the most cohesive and Russet Burbank (sp gr 1.075) was the least cohesive (Table 4). Therefore, cohesiveness alone did not seem to be a good indicator of gumminess, or mealiness. Similarly, adhesiveness was not indicative of gumminess of cooked potatoes.

According to the TPA, there was no significant difference in gumminess among the six potatoes. This is contrary to the taste panel result. Therefore, the usefulness of the TPA gumminess parameter in evaluating potato texture is doubtful. Although springiness is considered as a primary textural parameter related to elasticity (Szczesniak, 1975), it was not sensitive enough to detect any difference among the six potatoes. This parameter did not seem to be useful in describing the textural properties of potatoes. The same was true for chewiness since it was calculated as the product of gumminess and springiness.

Stress relaxation

A typical stress relaxation curve for a cooked potato sample is presented in Fig. 3. Nonlinear regression analysis of the stress relaxation data indicated that the relaxation curve can be fitted with an equation consisting of three components, namely, an equilibrium stress and two exponential decay terms. The following equation represents the stress relaxation behavior of the cooked potato samples:

$$\sigma(t) = \epsilon_0(E_0 + E_1 e^{-t/T_1} + E_2 e^{-t/T_2}) \qquad (1)$$

Table 4—Texture profile analysis parameters of cooked potatoes determined by the Instron Universal Testing Machine[a]

Potato	Specific gravity	Fracturability (Newton)	Hardness (Newton)	Cohesiveness	Adhesiveness (Joules)	Springiness (Meter)	Gumminess (Newton)	Chewiness (Joules)
White Rose	1.105	14.1 bc	16.6 b	0.075 b	0.017 ab	0.0075 a	1.3 a	0.010 ab
Kennebec	1.100	18.0 c	18.2 b	0.073 ab	0.016 ab	0.0085 a	1.4 a	0.012 b
Russet Burbank	1.090	17.5 bc	18.0 b	0.077 b	0.016 ab	0.0078 a	1.4 a	0.011 ab
A 503-42	1.100	12.8 b	14.1 ab	0.080 b	0.012 a	0.0069 a	1.2 a	0.008 ab
Russet Burbank	1.075	17.1 bc	19.4 b	0.067 a	0.022 bc	0.0064 a	1.3 a	0.009 ab
Red	1.075	4.3 a	10.1 a	0.100 c	0.023 c	0.0061 a	1.0 a	0.006 a

[a] Means (n = 30) within each column followed by the same letter are not statistically different ($P < 0.05$).

Table 5—Stress relaxation parameters of cooked potatoes[a]

Potato	Specific gravity	E_0 (Pa)	E_1 (Pa)	E_2 (Pa)	η_1 (Pa-sec)	η_2 (Pa-sec)
White Rose	1.105	4.45×10^5b	7.25×10^5bc	2.60×10^5bc	1.57×10^6a	2.52×10^4c
Kennebec	1.100	7.42×10^5d	9.39×10^5d	3.77×10^5d	1.77×10^6a	3.05×10^4c
Russet Burbank	1.090	5.76×10^5c	7.41×10^5bc	2.95×10^5c	1.50×10^6a	2.54×10^4c
A 503-42	1.100	4.14×10^5b	6.35×10^5b	2.16×10^5b	1.37×10^6a	1.85×10^4b
Russet Burbank	1.075	4.70×10^5b	7.65×10^5c	3.69×10^5d	1.31×10^6a	2.69×10^4c
Red	1.075	1.14×10^5a	2.74×10^5a	0.85×10^5a	0.62×10^6a	0.91×10^4a

[a] Means (n = 30) within each column followed by the same letter are not significantly different ($P < 0.05$).

where σ = stress; t = time; ϵ_0 = constant strain; $T_1 = \eta_1/E_1$, $T_2 = \eta_2/E_2$; η_1, η_2 = viscosities; E_0, E_1, E_2 = elastic moduli.

The above equation can be represented by a generalized Maxwell model (Mohsenin, 1970) consisting of three elastic elements (springs) and two viscous elements (dashpots) as demonstrated in Figure 4. The two Maxwell elements, each consisting of a spring and a dashpot in series, correspond to the two exponential terms in Eq. (1). The lone elastic element corresponds to the equilibrium modulus (E_0) at infinite time. This fitted model is in agreement with the findings of Davis et al. (1983).

The three elastic moduli (E_0, E_1, E_2) were largest for Kennebec and smallest for Red. This agrees with the sensory evaluation data which show Kennebec to be the hardest and Red the softest among the six groups of potatoes (Table 3). Comparison of the data in Tables 3 and 5 indicates that the elastic moduli were closely related to sensory hardness of the potato samples. It is generally recognized that elastic modulus is highly correlated with hardness of food materials (Finney, 1972).

There was no significant difference among the η_1 values of all the potato samples. Although the η_2 values were significantly different for some of the potatoes, it was not sensitive enough to detect any differences in mealiness among White Rose and Russet Burbank (sp gr 1.090 and 1.075). Since gumminess is associated with stickiness, it is somewhat surprising to find no correlation between the viscous elements of the model and gumminess. Previous studies showed that mealiness of cooked potatoes was correlated with relative viscosities determined by Brabender Amylograph (Unrau and Nylund, 1957) and Brookfield Viscometer (Kuhn et al., 1959). A possible explanation for the lack of sensitivities of η_1 and η_2 to textural difference is that initially all the stress applied to the system goes to the elastic elements and only a secondary reaction pertains to the viscous elements.

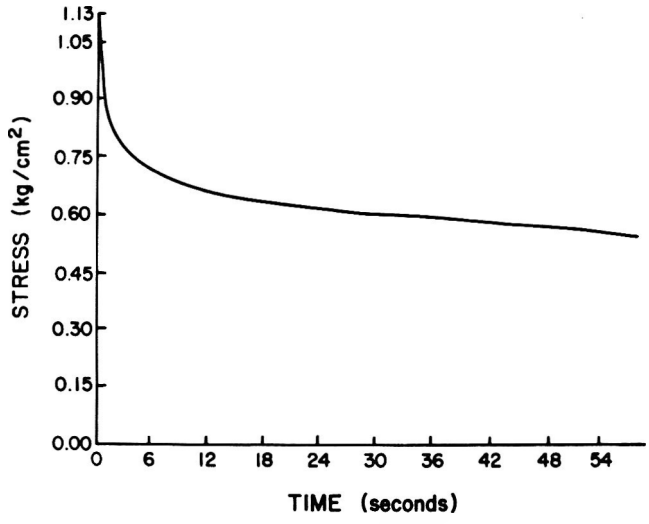

Fig. 3—A typical stress relaxation curve of cooked potatoes.

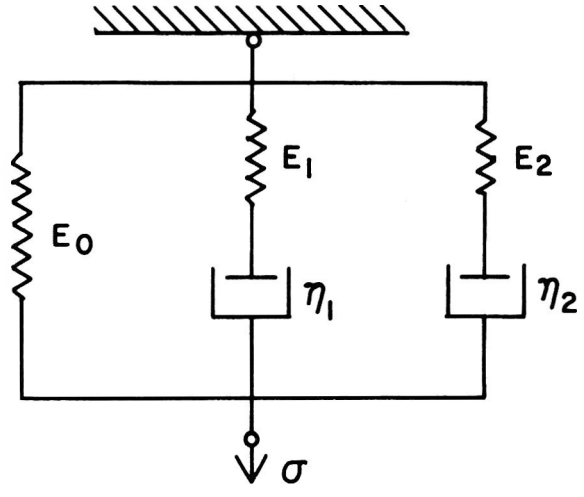

Fig. 4—Five-element Maxwell model representation for cooked potatoes.

Correlations

Correlations between TPA parameters and taste panel scores of cooked potatoes are summarized in Table 6. Hardness rating by sensory evaluation was more highly correlated with fracturability (r = 0.907) than with hardness obtained by TPA (r = 0.851). A similar finding was also reported by other researchers on cheese (Lee et al., 1978).

Table 6—Correlation coefficients between TPA parameters and taste panel scores

Sensory property	Fracturability	TPA Hardness	Cohesiveness	Adhesiveness	Coh. x Adh.
Mealiness	N.S.	N.S.	N.S.	N.S.	−0.87*
Hardness	0.91*	0.85*	−0.91*	N.S.	−0.80*

* $P < 0.05$; N.S. = Not Significant.

Table 7—Correlation coefficients between rheological properties and TPA parameters or taste panel scores

Textural parameters	E_0	E_1	E_2	$E_0+E_1+E_2$	η_2	T_2
Sensory						
Mealiness	N.S.	0.83*	N.S.	N.S.	N.S.	N.S.
Hardness	0.90*	0.96**	0.93**	0.95**	0.91*	−0.86*
TPA						
Fracturability	0.93**	0.96**	0.96**	0.97**	0.96**	−0.84*
Hardness	0.84*	0.91*	0.97**	0.92*	0.96**	−0.84*
Cohesiveness	N.S.	−0.91*	−0.94**	−0.87*	−0.93**	0.86*

*$P < 0.05$; **$P < 0.01$; N.S. = Not Significant.

There was no significant correlation between gumminess determined by TPA and mealiness score by sensory evaluation. Therefore, gumminess by TPA is not a reliable indicator in assessing gumminess or mealiness of cooked potatoes. Although the mealiness score was not significantly correlated with either adhesiveness or cohesiveness, it was correlated with the product of these two TPA parameters. This is not unexpected since the panelists associated gummy potatoes with cohesive and adhesive potatoes during the training sessions. Cohesiveness was negatively correlated with hardness scores by sensory evaluation, meaning that softer cooked potatoes were more cohesive. A previous study of canned potato texture by Woodman and Warren (1972) showed that mealiness was correlated with extrusive force ($r = 0.61$). However, TPA hardness or fracturability was not significantly correlated with mealiness in the present study. Sensory data (Table 3) also showed no direct relationship between hardness and mealiness. Our results indicated that hardness and mealiness were two independent attributes of potato texture. Since compressive, puncture and extrusive forces largely reflect firmness or hardness of a food material (Mohsenin, 1970), their usefulness in assessing mealiness of cooked potatoes is questionable.

Table 7 shows that the three elastic moduli were highly correlated with hardness ($P < 0.01$). This is expected since hardness and cohesiveness are related to elasticity (Sherman, 1969). Mealiness score, which is correlated with the product of cohesiveness and adhesiveness, is also significantly correlated with E_1. Relaxation times were negatively correlated with both hardness determined by TPA and hardness score by taste panel, indicating that it took a longer time for stress to relax in soft potatoes than in hard potatoes. The viscous element η_2 is positively correlated with hardness score by taste panel, and with fracturability and hardness determined by TPA. This suggests that viscosity, or internal resistance of the potato tissue to flow, is higher for hard potatoes than for soft potatoes.

In conclusion, hardness of cooked potatoes by sensory evaluation correlated well with the TPA fracturability and hardness, and with the elastic elements of the stress relaxation model. Mealiness of cooked potatoes was found to correlate with the product of cohesiveness and adhesiveness by TPA, and with one of the elastic elements (E_1) obtained by stress relaxation tests at 5% significance level. Because of the limited number of samples used in this study, further work is needed to confirm the usefulness of TPA and stress relaxation test in determining mealiness in cooked potatoes.

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Effect of Baking and Frying on Nutritive Value of Potatoes: Minerals

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ABSTRACT

The effect of conventional baking and frying on the mineral content of Katahdin, Chipbelle and Rosa varieties of potatoes was investigated. The cortex and pith tissues of the tubers were used. Conventional baking reduced the cortical content of potassium, phosphorus, and iron by 10–13%, 4–21%, and 19–31% respectively. In all three varieties, movement of potassium, phosphorus, and iron toward the interior tissues was demonstrated during conventional baking although varieties varied widely in their mineral content. Conventional baking increased the pith content of potassium (14–23%), phosphorus (2–9%), and iron (2–8%). Frying decreased significantly all the minerals in both the cortical and pith areas with the major loss occurring in the cortical area (10–45%).

INTRODUCTION

POTATOES, a major vegetable crop in many parts of the world and a staple food for man, are consumed by a high proportion of the population in the United States with an annual per capita consumption of 115–120 lb (USDA, 1982). Potatoes provide practically all essential dietary factors including a high quality protein, minerals, and essential vitamins (Woodward and Tally, 1953). True et al. (1978, 1979) concluded that a 150g serving of fresh potatoes furnishes 10% of the U.S. Recommended Dietary Allowance for iodine, 8% for copper and magnesium, 6% for phosphorus, and 2% for iron and zinc.

Wide variations in mineral content of potatoes have been attributed to differences in soil type, mineral content of the soil and varietal differences (Augustin, 1975). In addition, the mineral contents of cortex and pith tissues within the same tubers also differ. Generally higher concentrations of minerals are found in the outer cortical region. The retention of minerals following cooking of the two tissues is also important. Macklon and Dekock (1967) and Johnston et al. (1970) reported that progressing from the outer epidermis towards the pith there are decreases in magnesium, phosphorus, potassium and calcium. In recent years 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. Therefore, it is important to determine the effect of cooking on the nutritive value of the different sections of potatoes rather than on the whole tuber.

True et al. (1979) found that cooking had a negligible effect on mineral content of potato flesh regardless of whether the potatoes were boiled with or without skin or baked in a conventional or microwave oven. Augustin et al. (1979), however, observed that whether raw or cooked, and regardless of the cooking methods used, potato peel contained significantly higher amounts of ash, crude fiber, protein and riboflavin and less thiamin than the corresponding flesh.

This study was undertaken in order to determine the effect of baking and frying on the mineral content of different sections of potatoes including the peel.

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MATERIALS & METHODS

POTATOES of the Katahdin and Chipbelle varieties were grown at the Cornell Vegetable Research Farm at Riverhead, Long Island, NY and Rosa was grown at the Cornell Research Farm at Freeville, NY during the 1981 growing season. The tubers were stored for 4 months at 5°C before analysis.

Tubers of medium size and uniform shape were selected from each variety in order to limit variations resulting from size differences. Thirty potatoes from each variety were baked at 204°C in two conventional ovens (15 in each oven) for 1 hr. All potatoes were allowed to cool but were still warm when each was separated into cortex (including the periderm) and pith sections. Equal sampling of the bud and stem ends of the tubers were included in both the cortex and pith sections. The ratio of cortex to pith tissue varies from variety to variety but generally the cortex area comprises approximately 40% of the whole tuber by weight. In some commercial operations the cortex tissue including the peel is baked and frozen before frying.

Therefore, in this study a procedure similar to that used in commercial practice was followed. The baked cortex tissue was frozen and fried in 100% vegetable oil at 177°C for 3–4 min using a Sunbeam Fryer. Wedges of raw pith were fried in a similar fashion. All tissues were frozen, lyophilized in a Stokes freeze-dryer, ground in a Wiley Mill through a 40 mesh screen, except the fried tissues which were ground using mortar and pestle. The powders were stored under nitrogen until analyzed. Results are reported on a dry weight (D.W.) basis in order to eliminate differences in dehydration which may have occurred during cooking. Duplicate determinations were made on each treatment.

Determination of minerals

Freeze-dried potato powder was analyzed for mineral content by atomic emission spectroscopy using an inductively coupled plasma system as described by Fassel and Kniseley (1974). Duplicate determinations were made on each treatment.

Statistical analysis

Completely randomized design was employed, and statistical significance of the data was determined using three factor analysis of variance with protected LSD test described by Steel and Torrie (1980).

RESULTS & DISCUSSION

Macrominerals

Baking. The major macrominerals present in potatoes include potassium, phosphorus, calcium and magnesium. Conventional baking of Katahdin potatoes resulted in significant losses of potassium ($p < 0.01$) and phosphorus ($p < 0.05$) contents from the cortex tissue and a significant increase in potassium ($p < 0.01$) and phosphorus ($p < 0.05$) in the pith tissue (Fig. 1). Similar trends were observed for Chipbelle and Rosa cultivars (Fig. 2 and 3). Such a compositional change suggests that potassium and phosphorus transfer from cortex to pith tissue. This is in agreement with compositional changes observed in potatoes for nitrogenous constituents as well as potassium and iron during conventional baking (Klein and Mondy, 1981). Klein and Mondy (1981) suggested that the higher potassium concentration in the pith tissue is probably due to dehydration and cellular damage of cortex tissue on prolonged heating which could cause a diffusion gradient for the movement of potassium from cortex to pith.

In all three varieties no significant difference in the quantity of calcium and magnesium in cortex and pith tissues was found between raw and conventionally-baked potatoes (Fig. 4 and Table 1).

Frying. Both cortex and pith tissue showed significant reduction in potassium and phosphorus ($p < 0.01$) following frying, but the decrease was greater in cortex than pith tissue (Fig. 1). Chipbelle and Rosa cultivars showed the same trends (Figs. 2 and 3). Following frying calcium and magnesium were significantly ($p < 0.01$) reduced in cortex and pith tissues (Fig. 4 and Table 1). These decreases may have been due to either the increased cell separation of the cooked tissues at high temperatures (Fedec et al., 1977) or the leaching of minerals into fat medium in the dissolved form in droplets of water "blasted" out by the steam, or perhaps both of these factors. The fat medium was not analysed for minerals.

The cortex tissue was significantly higher ($p < 0.05$) than the pith in potassium, phosphorus, calcium and magnesium contents for all three varieties. Significant varietal differences ($p < 0.05$) in phosphorus and calcium content of raw tissue were observed (Fig. 3 and Table 1). Katahdin and Chipbelle varieties were significantly higher ($p < 0.05$) than Rosa in phosphorus and calcium contents. This was true for both cortex and pith tissues.

Microminerals

Baking. The effect of cooking on the micromineral content of the Katahdin cultivar is given in Table 2. Iron and aluminum were present in greater quantities in cortex than pith tissue (Fig. 5). However, conventional baking resulted in a significant reduction ($p < 0.01$) of iron and aluminum in cortical tissue. A significant increase ($p < 0.05$) in iron content was observed in the pith tissue. This apparent movement of minerals toward the interior is in agreement with the findings observed for nitrogenous constituents (Klein and Mondy, 1981). Levitt and Todd (1952) found

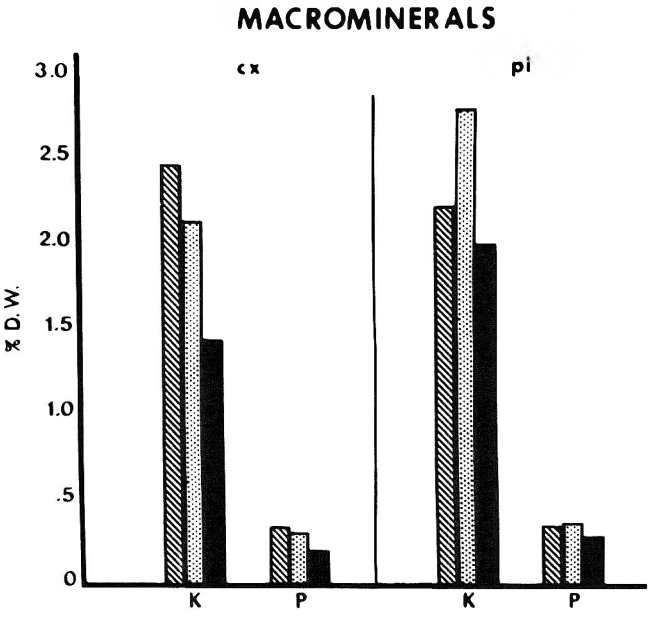

Fig. 1—Effect of baking and frying on the potassium and phosphorus content (& D.W.) of cortex and pith tissue of Katahdin potatoes: ▨ Fresh, ▩ Baked, ■ Fried.

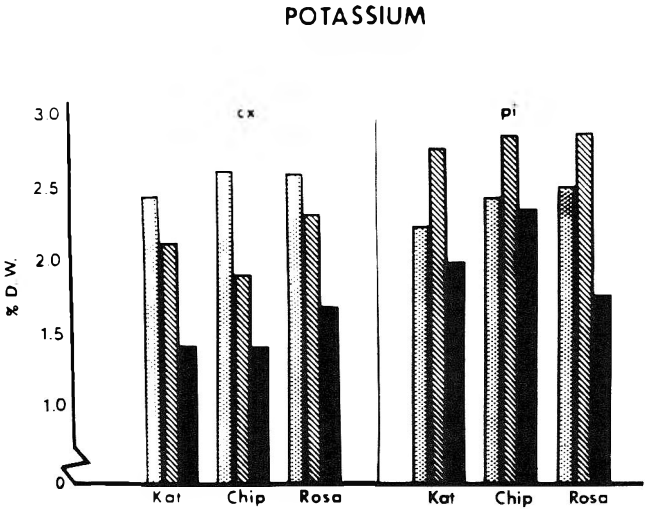

Fig. 2—Comparison of potassium content (% D.W.) in cortex and pith tissue of Katahdin, Chipbelle, and Rosa potatoes after conventional baking and frying: ▨ Fresh, ▩ Baked, ■ Fried.

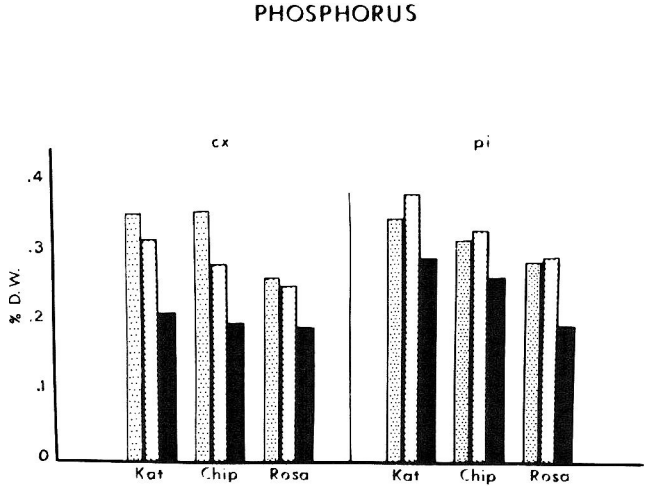

Fig. 3—Comparison of phosphorus content (% D.W.) in cortex and pith tissue of Katahdin, Chipbelle, and Rosa potatoes after conventional baking and frying: ▨ Fresh, ▩ Baked, ■ Fried.

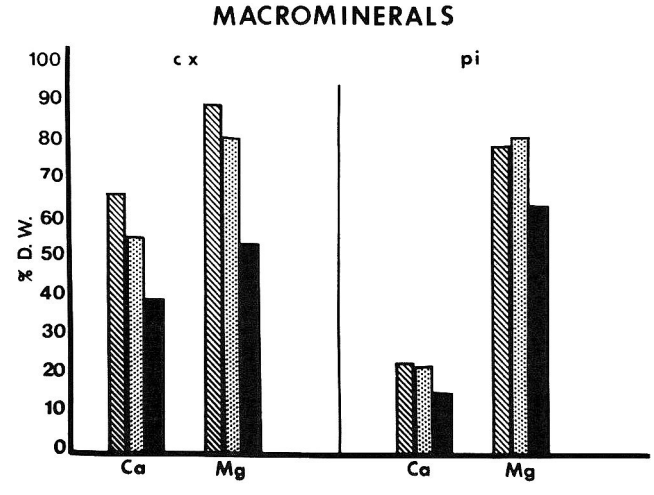

Fig. 4—Effect of baking and frying on the calcium and magnesium content (% D.W.) of cortex and pith tissue of Katahdin potatoes: ▨ Fresh, ▩ Baked, ■ Fried.

that iron, copper and zinc are present in potatoes as protein complex and the increased iron in the pith area following baking may have resulted from protein degration in the cortex area. Movement of iron towards the interior results from cooking. All three varieties showed the similar trend (Fig. 6). The copper, arsenic, zinc, and manganese contents of cortex tissue were reduced slightly following conventional baking (Fig. 7 and Table 2). Even though there was a slight increase in copper and zinc in the pith tissue these were not significant. No significant changes were observed for arsenic and manganese in pith tissue.

Frying. Frying significantly ($p < 0.05$) reduced the cortical content of iron, aluminum, manganese, copper, and zinc in Katahdin potatoes. Pith tissue showed significant decreases ($p < 0.01$) in iron, copper, zinc, arsenic and manganese ($p < 0.05$) (Fig. 5, 7, and Table 2). Chipbelle and Rosa cultivars showed the same trend. These changes cannot be explained at the present time, but indicate a need for further research.

The cortex tissue in the fresh tissue of all three varieties was significantly ($p < 0.05$) higher in iron, aluminum, copper, and manganese than the pith tissue (Table 3). Significant ($p < 0.05$) varietal differences in cortical iron

Table 1—Influence of conventional baking and frying on the macromineral content (% D.W.) of potatoes[a]

Macromineral and Variety	Raw	Conventional baking	Frying
		Cortex	
Calcium			
Katahdin	0.067 ± 0.008	0.056 ± 0.003	0.040 ± 0.002
Chipbelle	0.053 ± 0.001	0.058 ± 0.001	0.042 ± 0.004
Rosa	0.033 ± 0.001	0.032 ± 0.001	0.023 ± 0.001
Magnesium			
Katahdin	0.090 ± 0.001	0.081 ± 0.001	0.054 ± 0.001
Chipbelle	0.150 ± 0.000	0.084 ± 0.001	0.058 ± 0.008
Rosa	0.091 ± 0.001	0.087 ± 0.001	0.066 ± 0.004
		Pith	
Calcium			
Katahdin	0.024 ± 0.000	0.023 ± 0.001	0.016 ± 0.003
Chipbelle	0.020 ± 0.006	0.027 ± 0.007	0.022 ± 0.001
Rosa	0.018 ± 0.000	0.017 ± 0.000	0.011 ± 0.000
Magnesium			
Katahdin	0.079 ± 0.001	0.081 ± 0.000	0.064 ± 0.011
Chipbelle	0.088 ± 0.013	0.092 ± 0.012	0.068 ± 0.003
Rosa	0.092 ± 0.001	0.096 ± 0.001	0.062 ± 0.000

[a] Data are reported as an average of two values ± S.D.

Table 2—Influence of conventional baking and frying on the micromineral (ppm D.W.) of Katahdin potatoes[a]

Micromineral	Raw	Conventional baking	Frying
		Cortex	
Iron	70.8 ± 2.83	48.4 ± 2.12	40.9 ± 0.78
Copper	12.6 ± 1.91	12.0 ± 0.28	9.3 ± 1.89
Arsenic	15.9 ± 2.47	14.7 ± 0.21	14.3 ± 3.46
Aluminum	96.7 ± 3.32	61.5 ± 3.46	53.6 ± 1.84
Maganese	5.5 ± 0.91	5.1 ± 0.21	3.8 ± 0.06
Zinc	17.7 ± 1.27	17.6 ± 0.14	12.8 ± 1.20
		Pith	
Iron	20.0 ± 0.64	21.6 ± 0.64	15.0 ± 0.10
Copper	8.7 ± 0.18	9.6 ± 0.29	3.8 ± 0.90
Arsenic	13.8 ± 0.85	14.0 ± 0.71	6.4 ± 3.86
Aluminum	12.6 ± 2.55	9.5 ± 0.64	9.2 ± 1.97
Manganese	5.6 ± 0.11	5.4 ± 0.06	3.6 ± 0.01
Zinc	17.1 ± 0.28	20.4 ± 1.63	13.7 ± 0.09

[a] Data are reported as an average of two values ± S.D.

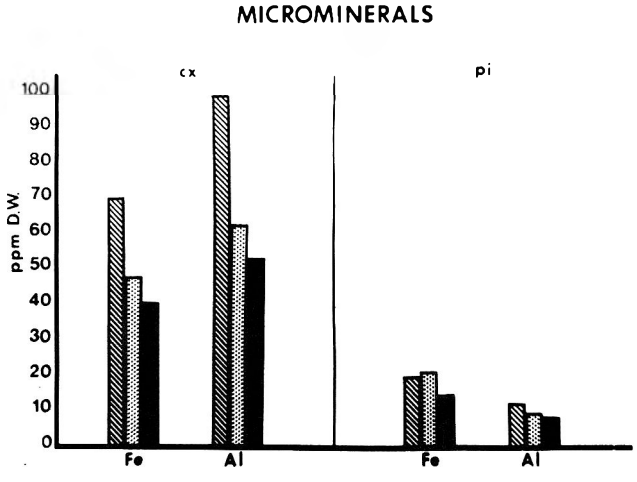

Fig. 5—Effect of baking and frying on the iron and aluminum content (ppm D.W.) of cortex and pith tissue of Katahdin potatoes: □ Fresh, ▨ Baked, ■ Fried.

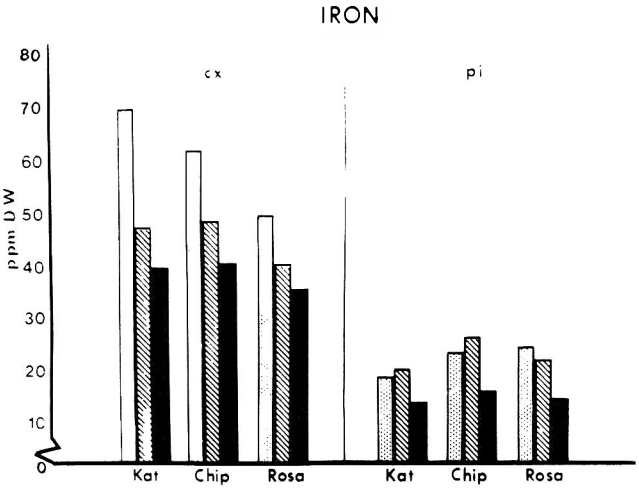

Fig. 6—Comparison of iron content (ppm D.W.) in cortex and pith tissue of Katahdin, Chipbelle, and Rosa potatoes after baking and frying: □ Fresh, ▨ Baked, ■ Fried.

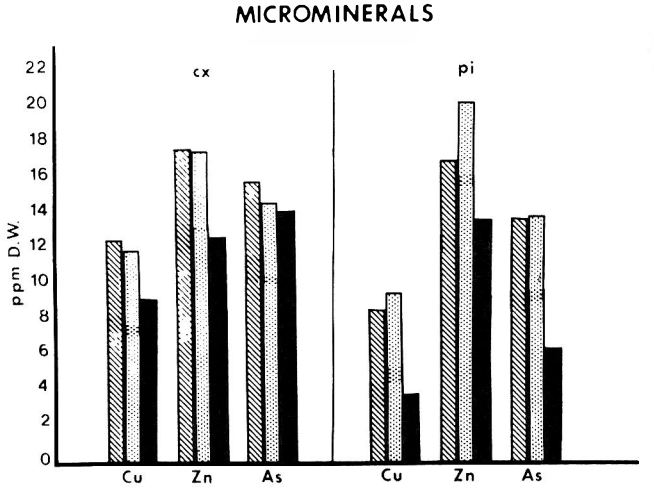

Fig. 7—Effect of baking and drying on the copper, zinc and arsenic content (ppm D.W.) of cortex and pith tissue of Katahdin potatoes: ▨ Fresh, ▨ Baked, ■ Fried.

Table 3—Comparison of micromineral contents (ppm D.W.) in cortex and pith tissue of fresh Katahdin, Chipbelle and Rosa varieties[a]

Microminerals	Katahdin	Chipbelle	Rosa
		Cortex	
Iron	70.8 ± 2.83	64.4 ± 0.64	51.2 ± 2.97
Aluminum	96.7 ± 3.32	76.5 ± 0.49	35.0 ± 0.85
Copper	12.6 ± 1.91	11.6 ± 0.14	6.7 ± 0.06
Manganese	5.5 ± 0.91	10.6 ± 0.14	7.6 ± 0.83
		Pith	
Iron	20.0 ± 0.64	23.6 ± 1.91	26.0 ± 0.42
Aluminum	12.6 ± 2.55	9.4 ± 2.16	3.2 ± 0.19
Copper	8.7 ± 0.18	8.2 ± 0.07	6.6 ± 0.16
Manganese	5.7 ± 0.11	5.6 ± 0.42	6.5 ± 0.18

[a] Data are reported as an average of two values ± S.D.

and aluminum contents were observed (Table 3). Iron and aluminum contents in the cortical tissue were much greater in Katahdin and in decreasing order Chipbelle and Rosa. This may be attributed to differences in soil type, mineral content of the soil, and varietal differences of potatoes. The distribution of iron in cortex and pith tissues varied among varieties. Cortex:pith ratio of iron for Katahdin was 3.5:1), Chipbelle (2.7:1), and Rosa (1.9:1).

CONCLUSION

CONVENTIONAL BAKING resulted in the migration of potassium, phosphorus, and iron from cortex to pith areas of the tuber. The mineral content of the turber tissue was significantly reduced by frying and this effect was greater for cortex than pith tissue. The mineral content of potato tubers varied widely with variety, and considerable loss of minerals occurred with frying. With baking the transfer of minerals from one section to another occurred.

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Control of Enzymatic Browning in Processed Mushrooms (*Agaricus bisporus*)

JEFFERY DODD McCORD and ARUN KILARA

ABSTRACT

Control of polyphenol oxidase (E.C. 1.14.18) activity by the use of citric acid was investigated. The enzyme was inactivated at pH 4.0 and was stable to 10 min exposures at 25°C in the pH range 4.0–8.0. At pH 6.5 the enzyme was active at 45°C but not at 70°C and thermal inactivation followed pseudo first-order kientics. At pH 6.5 the activation energy (E_a) for enzyme inactivation was 41.1 Kcal/mole while at pH 3.5 two rate constants and hence two values for E_a were observed. Between 0–5 min E_a for inactivation of polyphenol oxidase was 8.7 Kcal/mole and >5 min Ea was 21.8 Kcal/mole.

INTRODUCTION

POLYPHENOL OXIDASE (PPO) (E.C.1.14.18) is believed to be ubiquitous in the plant kingdom. Its presence in fruits and vegetables has recently been reviewed (Va'Mos-Vigyazo, 1981). Enzymatic browing is an indirect result of PPO action (Walker, 1975). While enzymatic browning may be desirable in products such as black tea, Sultana raisins and prunes, it is highly undesirable in most other food products. In mushrooms, for example, PPO has been reported to utilize catechol, 3,4-dihydroxyphenylalanine (L-DOPA), dopamine, D-DOPA, L-adrenaline, D-adrenaline, L-noradrenaline and D-noradrenaline as substrates for melanin formation (Harrison et al., 1967). Additionally, chlorogenic acid has also been reported to be a substrate for mushroom PPO and pH optima with catechol and p-cresol substrates have been reported to be 5.5–7.0 and 6.0–7.0, respectively (Dawson and Mager, 1962). Thermostability of PPO and its isoenzymes varies with the source of the enzyme, but, generally exposure to temperature in the 70–90°C range results in destruction of catalytic activity (Va'Mos-Vigyazo, 1981). Inhibitors of PPO can be classified as compounds acting primarily on the enzyme and compounds reacting with reaction products and/or substrates (Va'Mos-Vigyazo, 1981). The edible, cultivated mushroom, *Agaricus bisporus* (Lange) Sing, contains both active and latent forms of PPO activities which increase during post-harvest storage (Murr and Morris, 1975). Discoloration was also observed to be directly proportional to enzyme activity (Murr and Morris, 1975). Biphasic inactivation of PPO during the drying of mushrooms at 50°C has been observed (Zhuk and Tsapalova, 1974). When mushrooms were dried at 70°C, however, inactivation of PPO was monophasic. In experiments conducted at The Pennsylvania State University, it was observed that blanching mushrooms in 0.05M citric acid pH 4.5 or vacuum hydration of mushrooms in 0.05M citric acid pH 4.5 prior to conventional thermal processing resulted in an appreciable improvement in the color of the processed mushrooms (Beelman et al., 1981 unpublished results). Inclusion of EDTA in the canning brine at an equilibrium concentration of 250 mg/kg also contributed to the control of thermophilic spoilage of canned mushrooms. This paper attempts to explain the mechanisms which may be operative in the destruction of PPO in mushrooms processed with citric acid.

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MATERIALS & METHODS

MUSHROOM POLYPHENOL OXIDASE (PPO) (2,000–4,000 units/mg) was obtained from Sigma Chemical Company (St. Louis, MO). Other reagents and chemicals were purchased from Fisher Chemical Company (Pittsburgh, PA).

Determination of enzyme activity

Activity of PPO with L-tyrosine as the substrate was determined by the assay procedure described in Worthington Enzyme Manual (Anonymous, 1977). One unit of PPO activity was defined as the change in absorbance (ΔA) at 280 nm of 0.001/min at 25°C and pH 6.5. Assays were performed in a Gilford Model 250 spectrophotometer equipped with a 241-A automatic cuvette positioner, 2530-wavelength scanner, 2527-thermoprogrammer, 6046 analog multiplexer and 6051 recorder.

pH optimum and stability

Optimum pH for activity was determined by assaying PPO at different pH values ranging from 2.0–10.0 (in 0.5 pH unit increments) using 0.5M potassium phosphate buffer. Enzyme stability at various pH values were determined by incubating PPO solutions (0.33 mg PPO/ml) at 25°C in 0.005M potassium phosphate buffer with pH values ranging from 2.0–10.0 (at 0.5 pH unit increments) for 10 min. At the end of the incubation period 1.0 ml of 0.5M potassium phosphate pH 6.5 was added to each aliquot to attain an equlibrium pH of 6.5 prior to assay for enzyme activity.

Thermal stability

Aliquots of a solution of PPO (0.33 mg/ml) in 0.5M potassium phosphate buffer, pH 6.5 were held at various temperatures ranging from 25–70°C (in 5°C increments) for 10-min periods prior to cooling and assaying at 25°C.

Determination of activation energy

Activation energy (E_a) needed to inactivate tyrosinase was determined at pH 6.5 and 3.5 using 0.1M phosphate buffer and 0.1M citric acid, respectively. Temperatures of 50, 55 and 60°C were used for experiments at pH 6.5 with incubation periods of 1, 3, 5, 7, 10 and 15 min. At pH 3.5 the enzyme solution was made up in 0.1M citric acid and samples were incubated at 20, 25 and 35°C for 1, 3, 5, 7, 10 and 15 min. After incubation, enzyme activity was assayed at 25°C. Assuming pseudo first-order reaction kinetics, rate constants for inactivation of PPO at pH 6.5 and 3.5 were calculated and the calculated rate constants were used to derive E_a, half-lives (t ½) and Q_{10} values as recommended by Labuza (1972).

Thermal denaturation

For thermal denaturation studies, 150 μl aliquot of PPO (4 mg/ml), 100 μl glass distilled water and 250 μl of either 0.5M KH_2PO_4 buffer pH 6.5, or 0.1M citric acid pH 3.5, or 0.1M citric acid pH 3.5 containing 250 mg/liter ethylendiaminetetraacetate (EDTA) were combined to obtain a total volume of 500 μl. The temperature of this solution was increased at the rate of 1°C/min using the thermoprogrammer and absorbance at 280 nm of the enzyme solution was recorded as a function of temperature. Enzyme activity at points of inflection was determined.

Nonenzymatic reactions

To study the fate of reaction products produced by PPO the enzyme was immobilized on agarose as per methods described by

Kilara et al. (1977). The immobilized PPO was brought in contact with 1 ml of 1 mM tyrosine in 1M KH_2PO_4 buffer pH 6.5. The enzyme was filtered from the reaction mixture after 5 min period and the mixture was scanned in a spectrophotometer between 200 and 600 nm at 10-min intervals over a total period of 1 hr. Next the reaction times between enzyme and substrate solutions were varied for 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0, and 15 min. The reaction mixture after contact with the enzyme was filtered into quartz cuvettes containing 1 ml of either 0.1M KH_2PO_4, pH 6.5, or 0.1M citric acid pH 3.5, or 0.1M citric acid pH 6.5 and absorbance was monitored at 308 nm. Additionally the reaction products were scanned between 230 and 360 nm.

Isolation of substrates from mushrooms

Fresh mushrooms obtained from the Mushroom Research Center of The Pennsylvania State University were blended in a Waring Blendor for 1.5 min under nitrogen in 200 ml of deaerated 80% isopropanol. The homogenate was centrifuged at 2600 x *g* for 5 min and then at 9500 x *g* for 15 min in a Beckman J2-21 refrigerated centrifuge using the JA-14 rotor. The supernatant was decanted and stored in the refrigerator. The pellet was resuspended in 200 ml of deaerated isopropanol and blended for 1.5 min followed by centrifugation. The supernatants were combined and concentrated in vacuo at 30°C to one-tenth the original volume. The concentrate was filtered through a Gelman 0.45 μm membrane filter. The filtrate was used a crude preparation of PPO substrates obtained from mushrooms in place of 1 mM tyrosine in assays.

RESULTS & DISCUSSION

THE PHYSICOCHEMICAL PROPERTIES of PPO were investigated under a variety of conditions in an attempt to explain the observed improvement in the color of processed mushrooms after either vacuum hydration in citric acid or blanching in citric acid. The relative activity of PPO as a function of pH assay (Fig. 1) indicated that optimal activity was at pH 7.0 and declined very rapidly between pH 7.0 and 4.0. On the alkaline side of the observed pH optimum, activity decreased more gradually. These observations are in agreement with values published in the literature (Anon., 1977; Barman, 1969). The enzyme was stable to 10 min exposures between pH 4 and 9 as indicated by retention of >80% activity. The natural pH of mushroom tissue post-harvest has been reported to be pH 6.3 (Kuhn and Beelman, 1971). If this pH can be lowered to below pH 3.5, then the enzymes may be substantially inactivated. Such a reduction in pH during conventional mushroom canning operations can be attained either during washing and soaking steps, vacuum hydration or blanching operations. In pilot plant trials with 2 kg batches the tissue was washed and soaked in water at pH 3.5 and no improvement in color was observed, whereas when the pH was lowered in the vacuum hydration or blanching operations, substantial improvement in color over nonacidified controls was observed (unpublished data). Therefore, it appeared that not only was it necessary to lower tissue pH but it was also essential for the acid to penetrate membranes compartmentalizing PPO and hence to lower the pH in the microenvironment of PPO *in situ*.

When stability of PPO after 10 min exposure to different temperatures was investigated at pH 6.5, the enzyme remained fully active up to 45°C. Between 45°C and 70°C a gradual decline in activity was observed and above 70°C no activity was detectable (Fig. 2). In preliminary experiments it was observed at pH 3.5 and 40°C PPO was inactivated rapidly. Therefore inactivation of PPO at the two pH values could not be directly compared. For that reason, it was decided to determine activation energies for the inactivation of PPO at pH 6.5 and pH 3.5. Data collected at pH 6.5 could be assumed to follow first-order kinetics since plots of residual activity as a function of time were linear for 50, 55 and 60°C. Data collected at pH 3.5 and at 20, 25, and 35°C showed that inactivation of PPO was a two stage process in which the first stage occurred 0–5 min and the second stage occurred at greater than 5 min. The order of the reaction could thus not be determined. If the inactivation was considered to be a two phase process, then each phase obeyed pseudo first-order kinetics. The reaction rate constants calculated by linear regression for all experiments is shown in Table 1. The correlation coefficients indicate that over the temperature range studied for both pH 6.5 and 3.5 the kinetics could be assumed to be consistent with pseudo-first order reactions.

The biphasic nature of the plots for rate constants at pH 3.5 can be explained if two different mechanisms of inactivation (i.e., pH and thermal) were operative under these experimental conditions. Acidic pH could result in protonation of free carboxyl groups in the enzyme molecule thereby neutralizing negative charges on the enzyme molecule and leading to electrostatic repulsive forces due to the positively charged amino groups on the enzyme molecule. Electrostatic

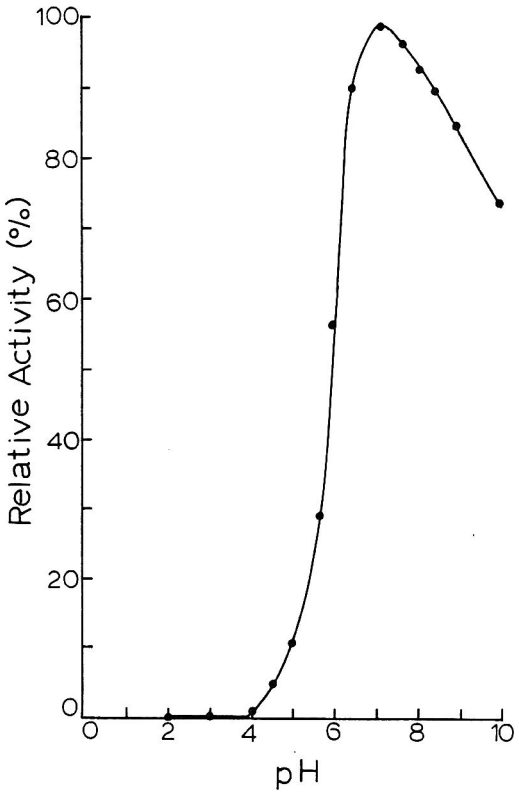

Fig. 1—Effect of pH on polyphenol oxidase activity.

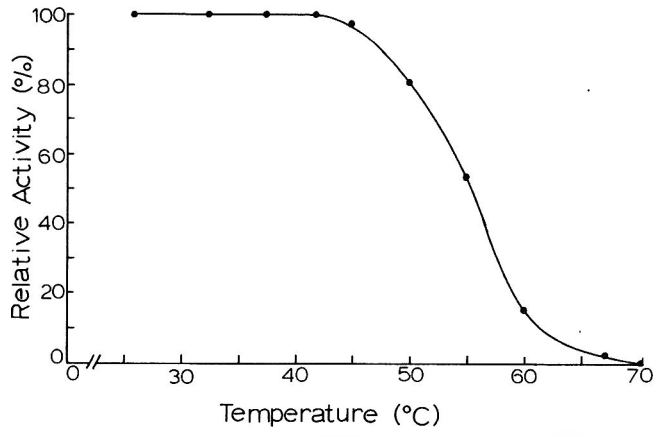

Fig. 2—Thermal stability of polyphenol oxidase. The enzyme was held at various temperatures for 10 min prior to cooling and assay at 25°C.

repulsive forces can cause a partial disruption of tertiary structure of the enzyme as suggested by Mallete et al. (1948). Such disruption may account for the rapid loss of activity in the 0–5 min time span at pH 3.5. The remaining activity may be more gradually lost due to the input of thermal energy.

Activation energies (E_a) determined by regression analysis of reaction rate constants and the resultant Arrhenius plot (Fig. 3) revealed that E_a for inactivation of PPO at pH 6.5 was 41.4 Kcal/mole whereas E_a for the 0–5 min, pH 3.5 condition was 8.7 Kcal/mole and >5 min at pH 3.5 was 21.8 Kcal/mole. These E_a values help to substantiate the above hypothesis for the biphasic plots obtained at pH 3.5. As pointed out by Labuza (1972) the higher the value of E_a the greater is that substance's sensitivity to increase in temperature. Half-life or time needed to reduce enzyme activity by 50% was calculated using the rate constants for inactivation (Table 2). For example, a PPO solution at pH 6.5 and 60°C will need 41.3 min to undergo eight half-lives corresponding to a 99.6% loss of activity. In contrast, the same enzyme at pH 3.5 and 60°C requires only 11 min to undergo eight half-lives. The increase in the rate of inactivation for each 10°C rise in temperature, also known as Q_{10} value, was calculated from E_a values and is shown in Table 3. The Q_{10} values were extrapolated to 50°C and 60°C for pH 3.5. The Q_{10} at pH 3.5 was 1.5–2.7 whereas Q_{10} at pH 6.5 was 6.6. This further substantiates that the biphasic rate constant plots at pH 3.5 are indeed due to two different mechanisms operating to inactive PPO. Increased hydrogen ion concentration (lower pH) is not very temperature dependent and can be considered to be an independent factor in the inactivation of PPO. Increase in 280 nm absorbance is a manifestation of the exposure of aromatic amino acid residue as the enzyme molecule uncoils. Tracings obtained for the thermal denaturation of PPO (Fig. 4) showed that in tracing A, PPO at pH 6.5 enzyme structure is stable to 50°C but above 50°C the tertiary structure of this protein is rapidly disrupted as indicated by the increase in 280 nm absorbance. In tracing B, PPO at pH 3.5 in 0.1M citric acid, the absorbance increased sharply until 27°C and then at slower rates at temperature >27°C. Once again, the above data support the explanation that at pH 3.5 a biphasic destruction of enzyme activity is occurring. In tracing C the solution contained 250 mg/liter EDTA in addition to 0.1M citric acid pH 3.5. Inclusion of the chelating agent stabilized the teritary structure of PPO to denaturation the scan was similar to that observed at pH 6.5. PPO is a copper-containing metalloenzyme and EDTA is capable of chelating copper at pH 3.5 (West and Sykes, 1960). This tended to negate pH-induced disruption of the enzyme molecule. Inclusion of a similar concentration of EDTA at pH 6.5 resulted in a tracing, superimposible on tracing A.

Table 1—Rate constants (k) for the inactivation of polyphenol oxidase

Temp (°C)	k	r[a]	1nk
pH 6.5			
50	0.019	0.83	−3.945
55	0.054	0.99	−2.925
60	0.134	0.99	−2.007
pH 3.5[b]			
20 (0–5 min)	0.109	0.94	−2.216
(> 5 min)	0.006	0.86	−5.159
25 (0–5 min)	0.139	0.95	−1.974
(> 5 min)	0.010	0.96	−4.572
30 (0–5 min)	0.226	0.89	−1.484
(> min)	0.035	0.98	−3.340

[a] Correlation coefficient for the least square lines. All r values were significant at p = 0.05.
[b] At pH 3.5 a biphasic inactivation curve was observed with the first phase being 0—5 min and the second phase >5 min.

Table 2—Half-life ($t_{1/2}$) values for polyphenol oxidase calculated from rate constants for the inactivation of polyphenol oxidase

Temp (°C)	Half-life (min) Phosphate buffer pH 6.5	Citric acid pH 3.5 (0–5 min)	Citric acid pH 3.5 > (5 min)
20	–[a]	6.36	121.57
25	–	4.98	67.28
30	–	–	35.66
35	–	3.06	19.8
40	–	–	11.14
45	–	–	6.44[b]
50	35.83	–	3.80[b]
55	12.91	–	2.24[b]
60	5.16	–	1.37[b]
70	0.92[b]	–	0.52[b]
80	0.17[b]	–	0.21[b]

[a] — = Values were not determined.
[b] Values were extrapolated.

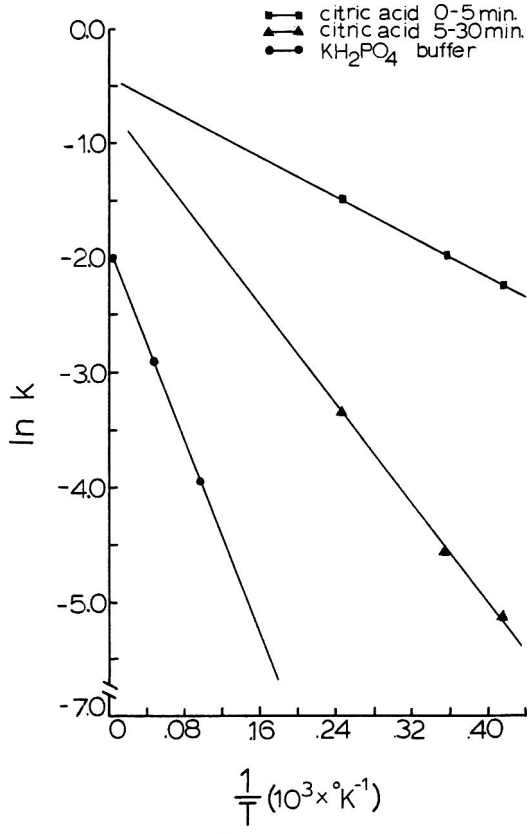

Fig. 3—Arrhenius plots for the inactivation of polyphenol oxidase.

Table 3—Calculated Q_{10} values for polyphenol oxidase at pH 6.5 and 3.5

E_a (Kcal/mole)	pH	Temperature coefficient (Q_{10}) 25–35°C	50–60°C
41.4	6.5	–	6.6
8.7 (0–5 min)	3.5	1.6	1.5[a]
21.8 (<5 min)	3.5	3.3	2.7[a]

[a] Values extrapolated

Since changes in absorbance at 280 nm need not necessarily parallel changes in the activity of the enzyme, PPO activity at points of inflection on the three tracings was determined (Table 4). Activity of PPO decreased with increase in absorbance for tracing A (Fig. 4) and greatest loss in activity occurred at the point of inflection (54°C). A 4°C rise in temperature from 50 to 54°C resulted in a corresponding loss in enzyme activity of 32%. In tracings B and C (Fig. 4) in contrast to tracing A, the greatest observed loss in PPO activity occurred at much lower temperatures. When the temperature increased from 15 to 34°C losses in activity of 64% in tracing B and 54% in tracing C (Fig. 4) were observed.

In all these experiments purified mushroom PPO and a pure synthetic substrate, tyrosine, were used. Utilizing this system and by varying other parameters it was hypothesized that citric acid at pH 3.5 inactivates PPO by mechanism other than thermal denaturation. Enzymatic steps in enzymatic browning are terminated very early in the chain of reactions leading to melanin formation. Many of these reactions are autocatalytic and nonenzymatic (Bulock and Harley-Mason, 1951). Therefore, it was not clear whether the improvement of color of mushrooms could be attributed entirely to destruction of PPO. It is also possible that the nonenzymatic steps were inhibited at pH 3.5 by 0.1M citric acid. Immobilized PPO preparation proved useful in inhibiting the enzymatic reaction and then eliminating the enzyme from the reaction. In this manner the nonenzymatic reactions could be studied after the initiation of the enzymatic phases of the reactions. Absorbance scans over a period of 1 hr indicated the disappearance of a component absorbing at 308 nm (Fig. 5). At 0 min when nonenzymatic reactions had not occurred a component absorbing at 308 nm was present whereas at 60 min, when nonenzymatic reactions would have been substantially complete, this component had diminished. Therefore, increase in absorbance at 308 nm could be used to monitor a build up of intermediates in the nonenzymatic phases leading to melanin formation. Nonenzymatic activity as measured by this method was studied as a function of pH, time and reaction buffer and results are shown in Table 5. At any given time of reaction an approximately 10-fold increase in the nonenzymatic reaction can be observed in phosphate buffer at pH 6.5 when compared to pH 3.5 in citric acid buffer. Perhaps the lower pH inhibits nonenzymatic reaction. When citric acid at pH 6.5 was used the nonenzymatic reaction

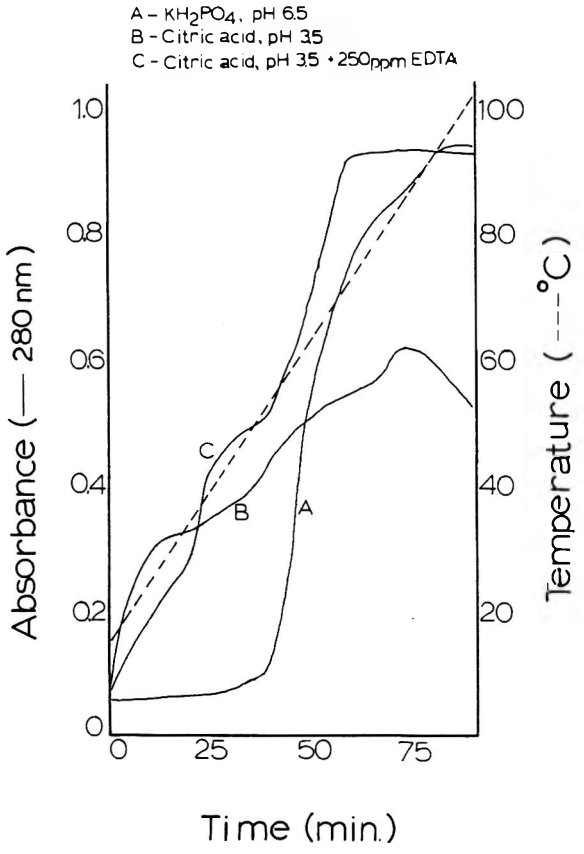

Fig. 4—Effect of pH on thermal denaturation of polyphenol oxidase.

Table 4—Relative activity of polyphenol oxidase on points of inflection on the denaturation curve

Temp (°C)	Phosphate buffer pH 6.5	Citric acid pH 3.5	Citric acid pH 3.5, 250 ppm EDTA
15	100	100	–
31	–[a]	36	–
34	–	–	46.0
35	87	–	–
42.5	–	20	–
45	–	–	25.1
52	70	–	–
54	–	3.0	–
56	37.5	–	–
61	–	–	2.2

[a] — denotes values were not measured

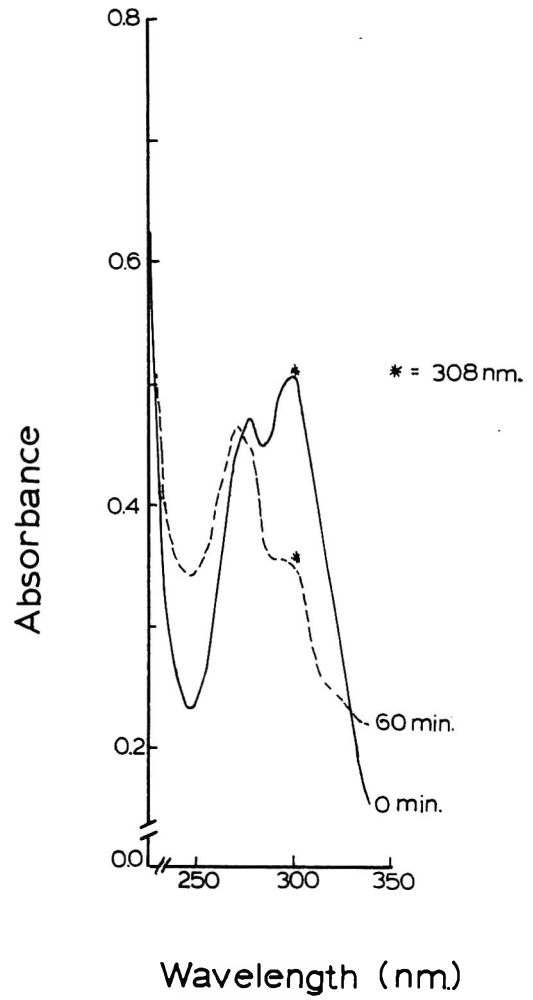

Fig. 5—Absorbance characteristics of products of reaction of L-tyrosine and polyphenol oxidase after 0 and 60 min.

was found to be substantially lower than at pH 3.5. Hence, reduction in the nonenzymatic phase of the reactions was considered to be uninfluenced by pH but was influenced by the inate properties of citric acid. Another point to consider from Table 5 is that, as the time of contact of tyrosine with immobilized PPO was increased, the reaction rate of the nonenzymatic phases of the browning reaction also increased and hence implicating a build-up of intermediates. Using available knowledge, the type of compound that absorbs at 308 nm could not be ascertained (McCord, 1982).

If substrates isolated from mushrooms were used in place of L-tyrosine it was observed that lowering pH from 6.5 to 3.5 resulted in a 96.6% reduction in PPO activity, whereas with tyrosine as a substrate, a comparable acidification resulted in only a 50% loss in PPO activity (Table 6). The inference can be made that lowering pH of mushroom tissue reduces PPO activity more drastically than in the model system tested.

Table 5—Observed velocities[a] of nonenzymatic reactions when subjected to different treatments

Time of reaction (Min)	KH_2PO_4 pH 6.5	Citric acid pH 3.5	Citric acid pH 6.5
0.5	0.50	0.00	0.00
1.0	0.57	0.08	0.00
1.5	1.08	0.15	0.00
2.0	1.55	0.15	0.04
3.0	2.66	0.29	0.18
5.0	4.00	0.42	0.39
10.0	5.85	0.91	0.60
15.0	6.50	1.09	0.87

[a] Velocity = decrease in absorbance at 308nm (−ΔA) per minute x 100 or (−ΔA/min x 100)

Table 6—Comparison of polyphenol oxidase activity with different substrates at pH 6.5 and pH 3.5

pH (buffer)	Substrate: Isolated from mushroom	Substrate: L-tyrosine
	% Relative activity	
6.5 (phosphate)	100	100
3.5 (citric)	3.4	50

SUMMARY & CONCLUSIONS

ONE OF THE SEVERAL PROBLEMS domestic mushroom canners have in competing with imports is the dark color of domestic products. Foreign products generally possess a lighter and brighter color and lowered production costs; thus, it is increasingly difficult for the domestic processor to compete in the market place. This study assessed the ability of citric acid to inhibit PPO induced browing in processed mushrooms. The study involved the investigation of enzymatic and nonenzymatic aspects of melanin formation. Citric acid at pH 3.5 was found to be effective in improving the color of mushrooms. Several conclusions can be drawn from the results of this study. First, low pH was responsible for the loss of activity in PPO. Second, the pH and thermal mechanisms of inactivation were independent of one another. Last, the inhibition of nonenzymatic activity was shown to be caused by the acidulant, citric acid, and pH per se.

A number of implications derived from these results can be applied to mushroom processing. First acidification of water used in vacuum hydration or blanching operations should result in improving the color of processed mushrooms. Second, the use of citric acid as the acidulant has the dual effect of inhibiting both enzymatic (by lowered pH) and nonenzymatic activity (by citric molecule). This means that discoloration can be inhibited partially even if the enzyme is not rapidly inactivated. Last, it may be possible to lower blanch water temperatures and still obtain inactivation of the enzyme when citric acid is used as a processing aid. These implications have been verified in pilot plant and commercial cannery trials and the preliminary results support the validity of these conclusions.

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High Vacuum Flame Sterilized Fruits: Storage Study on Sliced Clingstone Peaches, Sliced Bartlett Pears, and Diced Fruit

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ABSTRACT

Slices of four major commercial clingstone peach varieties, slices of Bartlett pears from major growing areas, and mixtures of diced peaches and pears were successfully vacuum packed without the conventional covering syrup or water. For selected packs, the fruits were sweetened to the desired cutout °Brix value with concentrated syrup. The high vacuum flame sterilized packs (HVFS) required only 7.5 min total heating for pear and peach slices and 5.5 min for diced fruit to achieve biological stability. Because of the minimal process, all HVFS fruits retained more of the original flavor attributes and texture of the fresh fruit through 18 months storage than did the conventional packs. Omission of the covering liquid, combined with the necessary blanching treatment, allowed packing of 1/3 more fruit into a standard size can.

INTRODUCTION

HIGH VACUUM FLAME STERILIZATION (HVFS) of fruits has been the subject of intense research and has been shown to yield a canned product that is closer to fresh than is the conventionally canned product (O'Mahony et al., 1981). Carroad et al. (1980) showed that the process, measured on a pilot scale flame sterilizer, used 30% less energy per pound of fruit processed than conventional procedures. In the HVFS process, fruits are packed with little or no water, deaerated to high vacuum levels, and given a minimal thermal treatment, thus providing conditions that enhance the attributes of canned fruits (Carroad et al., 1980, Heil et al., 1983).

Heil et al. (1983) developed commercially feasible HVFS processes for sliced clingstone peaches and Bartlett pears. However, to complete the study and expedite commercial consideration of the process, it remained to be shown that major commercial varieties of clingstone peaches, Bartlett pears from major growing areas and fruit cocktail would lend themselves to HVFS processing and subsequent storage. A mixture of diced clingstone peaches and Bartlett pears was packed and evaluated in lieu of fruit cocktail, of which diced peaches and pears are major constituents (40 ± 10% and 35 ± 10%, respectively). The evaluation was to be carried out on the basis of pack composition and quality factors of color, flavor and texture.

MATERIALS & METHODS

Raw material selection and handling

Varieties of California clingstone peaches which represent a large percentage of the commercial peach pack and cover most of the harvest season were selected. The varieties included 7,7-52, Evert, Halford, Starn, and Corona. Corona was used only in the diced fruit packs.

All peach varieties were grown near Yuba City, California. The individual orchards were selected to exclude diseased and stressed orchards, to minimize transportation and to fit the canning schedule. At canning maturity the peaches were commercially hand harvested into bins and arrived at the University processing laboratory between 12 noon and 2 p.m. on the day of harvest.

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Simulating commercial handling, yet within the limitation of can sizes and equipment, comparatively large, small, green and overripe peaches were discarded by hand as they were transferred to smaller containers. The peaches between 2.5 in. and 2.75 in. diameter were placed in 40°F storage until processing the following day.

Sized and sorted Bartlett pears from Washington, Oregon and California were shipped to Davis during August, 1979. The pears were cold stored until Sept. 13–14, 1979. Prior to ripening, the pears pressure tested between 15 and 19 psi, using a mechanical force gauge with a 3/8 in. tip on the cut surface. This method is commercially used to indicate maturity. Ripening at 68°F and 85% relative humidity was monitored by pressure testing samples of 20 pears. Values on the day of packing are in Table 1.

Pitting and peeling

Peaches were torque pitted and lye peeled, cup down, with 1.5–2% sodium hydroxide at 215–217°F. Exposure to lye was 60 sec. After the peach halves were rinsed to remove the lye, they were resorted, further eliminating overripe, green, and blemished fruit (excessive scars, insect damage, etc).

Pears were live-knife peeled, cored and halved. Halves were rinsed, core residues trimmed as needed, and the excessively defective halves (over-ripe, green, bruised, discolored, etc.) were discarded.

Slicing

Fruit halves were sliced from stem to blossom end in a three-blade slicer, yielding four slices per half. The slices were sorted, eliminating defective and small pieces. Acceptable slices were randomly divided for conventional and high vacuum canning.

Diced peaches and pears

Bartlett pears for the diced pack came from the Sacramento River area in California and were ripened 3 days at 68°F and 85% relative humidity, then live-knife peeled, cored and halved, as described above.

Pitted, peeled Corona peach halves and peeled, cored pear halves were mechanically diced in a ratio of 4:1. Dice size was 1/2 in. x 1/2 in. x 3/8 in. Smaller pieces and slivers were partly eliminated by screening the fruit discharged from the dicer.

Conventional (control) packs

The conventional packs consisted of fruit packed in water and fruit packed in a syrup of concentration calculated to give 18°Brix cutout value. All controls were packed in 401 x 411 plain tin cans with enameled ends. Each can was filled with 19.5 oz peach slices or 19 oz pear slices or diced fruit and covered with water or syrup mixed from a 73°B stock of commercially provided mixture of 50% sucrose (Type 0), 40% corn syrup (62DE) and 10% high fructose corn syrup. The cans were vacuum closed under 15–20 in. vacuum, cooked at 210°F in a rotary atmospheric cooker for 25 min (peaches) or 20 min (pears and diced fruit), and water cooled 24 min in a rotary atmospheric cooler. These processes are used commercially in rotary atmospheric cooker/coolers.

Table 1—Firmness of pears on the day of processing, pressure tested with 3/8 in. tip on peeled surface

Pear growing area	Avg. Firmness (psi)	Fruit size[a]
Washington State	2.1 ± 0.4	150
California	2.1 ± 0.5	135
Oregon	2.5 ± 0.4	135

[a] Relative commercial measure which states the number of fruit needed to fill a standard box. Smaller values indicate larger fruit.

Ideally, 303 x 406 cans which were used for the HVFS packs should have been used as controls. However, the only commercial pack that could be produced at our research facility was in 401 x 411 cans, which are commonly used in commercial practice. While actual weights in 401 x 411 cans were greater than would be the case for 303 x 406 cans, liquid-to-fruit ratios and quality factors were representative of commercial packs and processes regardless of can size.

High vacuum flame sterilized packs

Blanching. The release of noncondensible gases from fruit tissues during processing is accompanied by shrinkage of the fruit. To circumvent shrinkage in the can, the fruits were preheated and partly deaerated by blanching in steam prior to filling into the cans. The blanching temperature and time were selected based on heat penetration data to achieve at least 185°F at the center of the fruit particles (Heil et al., 1983).

Single can quantities of peach slices were weighed and fed into the blancher. Blanching losses were determined by weighing 5 individual 1 lb samples of each variety both before and after blanching.

Filling. Cans were hot filled with fruit discharged from the blancher. When no syrup was used (natural pack), the blanched equivalent (less spillage) of 16 oz raw slices was filled into each 303 x 406 plain tin can with enameled ends. For packs with syrup, approximately 2 oz of 73°B syrup stock was volumetrically measured into each can and covered with the blanched equivalent of 14 oz raw fruit. Diced fruits were filled without weighing because fill weights were adequately uniform when judged from apparent fill of containers.

Deaeration. Lids were clinched on the hot filled cans using a loose first seaming operation. The clinch was tight enough to withstand some internal pressure associated with the generation of steam, but loose enough to permit the discharge of gases.

The clinched cans were flame deaerated, first on an angular deaerator for 1 min, then on a vertical deaerator for 2.5 min. On the angular deaerator, cans were slanted 30° from vertical and rotated at 60 rpm over three individually-controlled 6 in. long burners positioned beneath the cans. Flame intensity was controlled with a pressure regulator. Half-way through the angular deaeration section, steam and some liquid were visibly discharging from around the clinched lid.

At the end of the angular deaerator, cans dropped 12 in. onto a low-intensity vertical deaerator where deaeration continued over low flame while the cans traveled in an upright position, without agitation, in an environment above 300°F. The flame in the vertical deaerator was controlled with a pressure gauge and a flow meter (Fisher-Porter 1/8-16/G-5/81). The vertical deaerator was covered, and the transfer mechanism from the vertical deaerator to the closing machine was shrouded. Can temperatures were maintained with an atmosphere of superheated steam (>212°F) until double seaming was completed.

Processing. Because the lethal effects of thermal treatments are additive, the effects of blanching and deaeration were given proper consideration in achieving the necessary thermal process (Heil et al., 1983). Since thermocouple readings of can temperatures at the slowest heating point exceeded 195°F during flame deaeration, further flame sterilization of the closed cans was not necessary (Lopez, 1975). The cans were water cooled to <100°F. To date, in more than 6000 cans packed, no spoilage of any type has occurred.

Storage. Processed samples were warehoused at ambient temperature. Outside temperature fluctuated between 40–80°F in the spring and fall, from 25–50°F in the winter and from 60–110°F in the summer. No daily temperature record was kept.

Table 2—Average blanching losses according to the time/temperature treatments given

Variety	Time (min)	Blancher temp (°F)	Weight loss (%)
7, 7-52 Peaches	4	205	4.2
Evert Peaches	4	205	5.0
Halford Peaches	4	205	4.9
Starn Peaches	4	205	5.4
Diced Fruit	2	200	9.9[a]
Bartlett Pears	4	200	6.4

[a] Diced Corona peaches and diced Bartlett pears (4:1), losses include slivers and other small fruit fragments.

Laboratory analyses

Net weight is the total contents (oz) of a can including water or syrup.

Drained weight is the weight of fruit (oz) remaining after the contents of a can were drained 2 min on a No. 8 sieve, as described in the USDA procedures (Judge and Sons, 1981).

Vacuum in the cans (in. Hg) was measured by piercing the can with a vacuum gauge prior to opening.

Slices in each can were counted. The average counts were adjusted to slices per pound of raw fruit filled.

Instrumental texture analyses. An instrumental texture study was conducted in parallel with the sensory evaluation of firmness on the day of packing (O'Mahony et al., 1981). Parameters of firmness were measured on the fresh peach slices on the day of packing and on processed fruit on the day of packing and after various intervals of storage. Instrumental analysis for peaches used the OTMS (Ottawa Texture Measuring System) cell adapted for blade extrusion with seven blades. In each replication 300g fruit and 40 cm/min crosshead speed were used in the Instron Model 1122 (Schweingruber et al., 1981).

Since the Instron was not available for the initial evaluation of pear texture, firmness of the raw material and the processed samples was measured using a mechanical force gauge with a 3/8 in. tip. The values reported are the pressures in psi needed to puncture the peeled surface of the fruit. This method is common in commercial practice. Due to small particle size, diced fruit firmness was not objectively measured.

Color change with storage time was monitored with the Hunter-Lab Color/Difference Meter D25D2, with "A" head, using the 1/2 in. aperture with condenser lenses. The instrument was standardized on the white tile (C2-1060) and L, a_L, b_L values were measured on the peeled surface of the canned slices.

The HunterLab tristimulus color values were converted to brightness indexes for peaches where

$$BI = 0.01L\,(5.715b_L - L)$$

and to yellowness indexes for pears where

$$YI = \frac{71.53a_L + 178.82b_L}{L}$$

(Gardner, 1976). The BI values were found to correspond to observed brightness ranking of color, and were not affected as much by ripeness and solids levels as the individual L, a_L and b_L values, which were difficult to interpret. The yellowness index (YI) was used for pears because YI is generally a good indicator of yellowing and aging in light colored, storage-sensitive products. No color measurements were made on diced fruit because individual pieces were too small to test.

Flavor and odor were subjectively evaluated as described in the U.S. Standards for Grades of Canned Clingstone Peaches/Pears (Judge and Sons, 1981).

pH was measured with standard calomel-glass electrodes and a Beckman Zeromatic SS-3 pH meter standardized with pH 4.01 and pH 7.00 buffers.

°Brix or soluble solids concentration in °B was determined at 20°C using the sugar scale of a Zeiss-Opton temperature-controlled refractometer.

Titratable acidity was determined by titrating weighed samples of serum with 0.1N NaOH to the pH equivalent of phenolphthalein end point. Results are reported in milliequivalents of acid per 100g sample.

The pH, °Brix and titratable acidity were measured on the liquid recovered when drained weights were determined. Especially in vacuum packed diced peaches and pears, little or no liquid separated, and the processed fruit was pressed to release enough liquid for analysis. Data represent averaged values of six cans ± one standard deviation.

RESULTS & DISCUSSION

BLANCHING needed for enzyme inactivation and deaeration was performed prior to filling. This allowed most of the shrinkage caused by heating to occur outside the can so that proper fill of container could be achieved. Blanch-

Table 3—Comparative physical and chemical evaluation of high vacuum flame processed and conventionally processed peach slices after 1 and 18 months of storage

	Storage time (mo)	Vacuum pack Natural 303 x 406 cans	Conventional pack in water 401 x 411 cans	Vacuum pack w/syrup 303 x 406 cans	Conventional pack in syrup 401 x 411 cans
			7,7-52		
Net weight (oz)	1	15.1 ± 0.5	27.5 ± 0.2	15.7 ± 0.2	29.1 ± 0.1
	18	15.3 ± 0.4	27.6 ± 0.3	15.4 ± 0.7	28.8 ± 0.3
Drained weight (oz)	1	14.5 ± 0.4	18.9 ± 0.3	12.9 ± 0.8	18.8 ± 0.3
	18	14.8 ± 0.4	18.8 ± 0.3	14.0 ± 0.6	19.0 ± 0.3
Vacuum (in. Hg)	1	23.7 ± 1.5	14.3 ± 1.2	23.7 ± 1.5	13.0 ± 0
	18	21.3 ± 1.2	13.3 ± 0.5	23.8 ± 1.2	11.2 ± 5.5
Slices per pound	1	24.4 ± 6.6	19.4 ± 3.4	14.9 ± 1.1	23.0 ± 2.5
	18	18.6 ± 3.2	22.2 ± 4.8	21.5 ± 6.7	18.1 ± 1.9
Brightness index	1	38.1	39.2	33.9	32.1
	18	38.7	35.5	30.0	31.7
pH distribution	1	3.82 ± 0.04	3.95 ± 0	3.88 ± 0.03	3.86 ± 0.06
	18	3.78 ± 0.04	4.01 ± 0.04	3.92 ± 0.06	3.92 ± 0.03
°Brix	1	9.4 ± 0.6	7.0 ± 0.6	18.6 ± 0.6	17.4 ± 0
	18	10.2 ± 0.6	6.3 ± 0.3	18.0 ± 0.3	18.0 ± 0.4
Titratable acidity (meq/100g)	1	6.4 ± 0.5	4.1 ± 0.3	5.0 ± 0.2	3.9 ± 0.1
	18	7.5 ± 0.3	4.1 ± 0.3	4.8 ± 0.5	4.2 ± 0.2
			EVERT		
Net weight (oz)	1	15.4 ± 0.1	27.7 ± 0.1	15.2 ± 1.0	29.0 ± 0.3
	18	14.9 ± 0.3	27.8 ± 0.1	15.3 ± 0.2	29.0 ± 0.1
Drained weight (oz)	1	14.6 ± 0.1	18.4 ± 0.4	13.2 ±1.0	17.7 ± 0.1
	18	14.9 ± 0.3	18.3 ± 0.2	14.7 ± 0.1	18.8 ± 0.2
Vacuum (in. Hg)	1	24.3 ± 0.6	14.7 ± 0.6	25.7 ± 1.2	14.3 ± 1.2
	18	23.3 ± 0.5	13.0 ± 2.2	23.7 ± 0.5	11.2 ± 1.5
Slices per pound	1	25.2 ± 3.3	24.0 ± 2.6	27.1 ± 6.3	29.8 ± 2.5
	18	35.1 ± 5.8	27.5 ± 2.5	35.4 ± 9.6	30.6 ± 3.0
Brightness index	1	34.2	39.2	32.8	30.9
	18	37.9	37.4	31.0	30.4
pH distribution	1	3.70 ± 0.03	3.80 ± 0.05	3.75 ± 0.04	3.78 ± 0.03
	18	3.78 ± 0.05	3.83 ± 0.03	3.79 ± 0.03	3.82 ± 0.02
°Brix	1	10.1 ± 0.4	8.2 ± 0.5	17.1 ± 0.5	17.7 ± 0.1
	18	10.5 ± 0.1	7.6 ± 0.5	16.9 ± 0.6	17.5 ± 0.2
Titratable acidity (meq/100g)	1	7.5 ± 0.2	5.1 ± 0.3	6.2 ± 0.4	4.8 ± 0.3
	18	7.6 ± 0.3	4.9 ± 0.3	6.5 ± 0.5	4.9 ± 0.3
			HALFORD		
Net weight (oz)	1	15.4 ± 0.6	28.6 ± 0.1	15.3 ± 0.5	30.3 ± 0.2
	18	15.0 ± 0.2	28.7 ± 0.2	15.0 ± 0.4	29.9 ± 0.1
Drained weight (oz)	1	14.8 ± 0.8	18.4 ± 0.2	13.2 ± 0.8	17.8 ± 0.2
	18	14.2 ± 0.2	18.3 ± 0.2	13.3 ± 0.5	17.9 ± 0.1
Vacuum (in. Hg)	1	23.3 ± 1.5	11.0 ± 0	25.5 ± 2.9	9.7 ± 0.6
	18	22.0 ± 1.1	8.2 ± 1.7	20.7 ± 0.5	9.7 ± 1.2
Slices per pound	1	23.2 ± 7.8	not available	23.3 ± 2.2	not available
	18	21.3 ± 2.7	24.0 ± 9.1	21.1 ± 5.5	17.6 ± 2.7
Brightness index	1	35.1	37.5	29.9	30.0
	18	36.6	37.6	33.8	28.8
pH distribution	1	3.79 ± 0.01	3.93 ± 0.01	3.85 ± 0.04	3.83 ± 0.01
	18	3.78 ± 0.02	3.93 ± 0.05	3.79 ± 0.04	3.82 ± 0.04
°Brix	1	8.9 ± 0.8	6.4 ± 0.2	18.6 ± 1.3	18.0 ± 1.2
	18	8.6 ± 0.5	6.1 ± 0.5	17.8 ± 0.4	18.3 ± 0.4
Titratable acidity (meq/100g)	1	7.1 ± 0.2	4.5 ± 0.2	5.6 ± 0.1	4.8 ± 0.4
	18	7.9 ± 0.3	4.8 ± 0.4	6.6 ± 0.5	5.5 ± 0.3
			STARN		
Net weight (oz)	1	14.9 ± 0.8	28.9 ± 0.1	15.3 ± 0.3	29.5 ± 0.2
	18	14.7 ± 0.5	28.8 ± 0.1	15.4 ± 0.4	29.3 ± 0.2
Drained weight (oz)	1	14.3 ± 0.9	18.3 ± 0.2	13.5 ± 0.4	17.3 ± 0.3
	18	13.9 ± 0.4	17.8 ± 0.1	13.8 ± 0.4	17.6 ± 0.3

continued

Table 3 continued

	Storage time (mo)	Vacuum pack Natural 303 x 406 cans	Conventional pack in water 401 x 411 cans	Vacuum pack w/syrup 303 x 406 cans	Conventional pack in syrup 401 x 411 cans
Vacuum (in. Hg)	1	23.5 ± 1.8	6.7 ± 0.6	24.7 ± 0.6	9.0 ± 1.0
	18	22.7 ± 1.2	7.7 ± 0.8	23.0 ± 0.6	9.3 ± 1.0
Slices per pound	1	20.7 ± 4.1	13.9 ± 1.6	18.6 ± 0.7	15.8 ± 2.6
	18	20.9 ± 7.9	16.2 ± 2.0	17.5 ± 2.2	14.9 ± 1.4
Brightness index	1	39.0	38.1	31.3	34.1
	18	36.7	32.6	30.9	31.1
pH distribution	1	3.73 ± 0.03	3.73 ± 0.01	3.72 ± 0	3.79 ± 0.02
	18	3.74 ± 0.03	3.74 ± 0.04	3.72 ± 0.04	3.69 ± 0.02
°Brix	1	8.8 ± 0.3	6.7 ± 0.3	17.8 ± 0.2	17.6 ± 0.5
	18	9.4 ± 0.5	6.0 ± 0.5	18.0 ± 0.5	18.0 ± 0.5
Titratable acidity (meq/100g)	1	7.0 ± 0.3	5.1 ± 0.4	6.1 ± 0.1	4.5 ± 0.2
	18	7.7 ± 0.3	5.3 ± 0.4	6.8 ± 0.4	5.2 ± 0.3

[a] Results indicate mean ± one standard deviation.

ing losses for peach slices, diced fruit and pear slices (Table 2) show small varietal differences which could disappear if measured on larger samples. For peach and pear slices the blanch losses are serum extracted during heating. The losses experienced with diced fruit included physical loss of fragmented fruit small enough to fall through the mesh of the conveyor belt. While no attempt was made to recover the extracted serum, it seems feasible to recover and return it to the pack to enhance flame deaeration and the juiciness of the pack. Diced fruit fragments, on the other hand, may be screened by proper equipment prior to blanching or handled on tighter mesh belts to reduce the loss.

Peaches

Analytical data for the four peach varieties are summarized by varieties in Table 3. Average net weights were higher for sweetened packs, both vacuum and conventional, than for unsweetened packs. This is due to the volume of added syrup and the density difference between water and syrup. Differences between 1 and 18 month values reflect initial packing variations.

For unsweetened peaches, a comparison of fruit fill weights and net weights calculated as % fruit content in the pack showed that high vacuum packed peaches contained 100% fruit. The commercial fruit fill weight of 303 x 406 cans was 10.5 oz, which was far less than that offered in HVFS packs.

Sweetened high vacuum packed peaches had approximately 2 oz of 73°Brix syrup added per can to increase sweetness; this added mass was reflected in the difference between fruit fill and net weights, so that the fruit content of the pack was reduced to 87.5%. In exploratory studies where granulated sugar (100% sucrose) was used, differences between net and fruit fill weights were closer to those found in unsweetened packs, because the sucrose added was not diluted by water.

In conventional packs, the difference between net and fruit fill weights represented added water or syrup. Using a fill weight of 19.5 oz fruit and a net weight of 29 oz, the fruit constituted only 67.2% of the pack.

Some variations of vacuum in the HVFS products relate to fill variations; cans with higher net weight may show lower vacuum. After 18 months of storage, nearly all packs exhibited some decrease in average vacuum. Whether this decrease was due to sample variation, experimental error, reaction between fruit and can, or the ultimate release of residual noncondensible gases from the fruit tissues is a subject for further investigation.

Knowing the relative fruit size often helps in interpreting the analytical data. More slices per pound corresponds to smaller slices and, therefore, smaller fruit. Use of larger fruit is generally beneficial with respect to firmness, color measurements, flavor (°B, titratable acidity) and aroma. In about 2/3 of the comparisons, conventional syrup and water packs contained larger average fruit size than the vacuum packs. Even though this favored the comparative position of the conventional packs, the HVFS peaches remained firmer after processing than their conventionally processed counterparts. Sweetened HVFS peach slices were usually firmer than those packed without sweetener. Syrup affected the conventional packs in a similar manner.

Comparative firmnesses of different varieties of fresh and canned peach slices, as measured by the OTMS blade extrusion method on the day of packing, are shown in Fig. 1. The fresh slices were firmest, and varietal differences were great. Sensory evaluation confirmed the instrumental findings in that HVFS peaches were ranked closer to fresh than were the conventional packs (O'Mahony et al., 1981).

Fig. 2 illustrates changes in firmness through 18 months storage of natural and sweetened HVFS and conventional water and syrup packed Halford peach slices. Both water and syrup controls indicated a firming trend through storage, whereas the high vacuum packs showed an initial drop in firmness (at 6 mo.) after which they fluctuated with the seasons through the remainder of the storage study. Some fluctuation in firmness was due to can-to-can variation of initial firmness of the fruit. With a larger sample size than used here, the firmness of natural HVFS peach slices could parallel the firmness of HVFS syrup packed peach slices. Some softening in HVFS packs was reasonable as the fruit pieces equilibrated in the high vacuum in the can. Additional softening with time may occur in all four packs; however, in preliminary experiments, through 5 years of storage, the HVFS peaches remained unmistakably firmer than the conventionally processed controls. The effects of storage temperature fluctuation are not clear, although a pattern is beginning to emerge showing less firmness measured after the winter (6 and 18) than after summer (0 and 12) months of the year.

Changes in firmness of the different varieties as affected by storage time are shown in Fig. 3 for natural HVFS peach slices. The different varieties responded similarly to storage. All four varieties showed an initial drop in firmness (6 mo.) followed by some firming (12 mo.) and then variable softening (18 mo.). Some variations were probably due to seasonal changes and some to differences among samples.

Color of canned peach slices in terms of brightness indexes is given in Table 3 for both early (1 mo.) and final

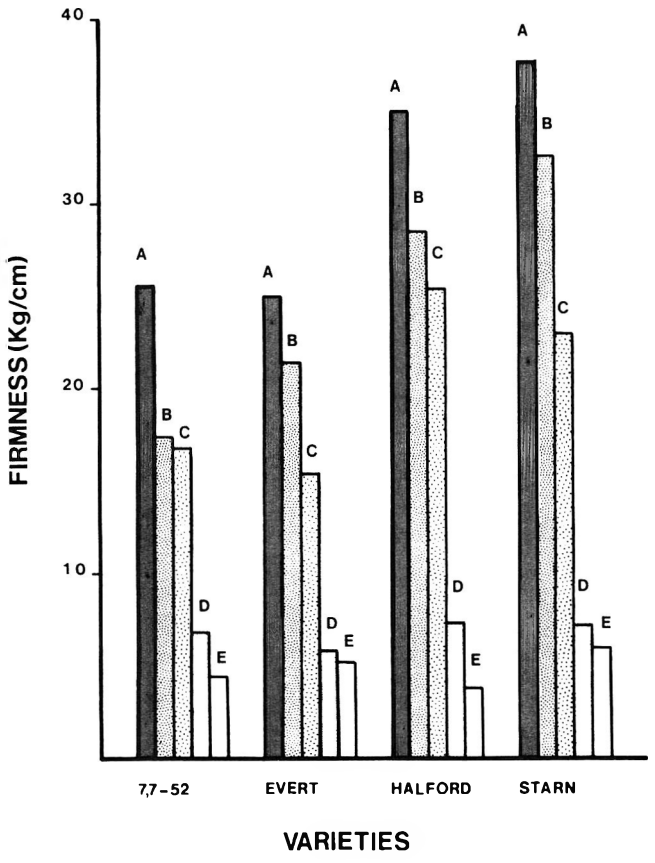

Fig. 1—Relative firmness of canned and fresh peach slices evaluated by the OTM System on the day of canning. (A) Fresh peach slices; (B) HVFS peach slices in syrup; (C) HVFS natural pack; (D) control pack in syrup; (E) control pack in water.

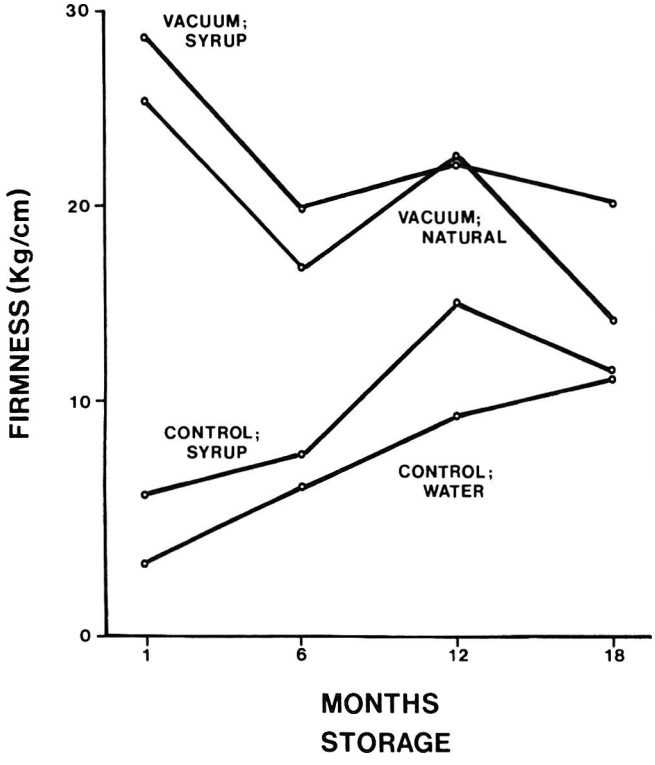

Fig. 2—Effect of process and storage time on firmness (OTMS Blade Extrusion) of Halford peach slices.

(18 mo.) cutouts. Brightness of conventionally water- and syrup-packed peach slices decreased, whereas brightness of HVFS peach slices did not change systematically with storage. While there were shifts among varieties, the brightness indexes remained within the range of the initial evaluations, as shown by Heil et al. (1983).

Similarly, by subjective observations, HVFS peach slices of all reported varieties were generally more translucent than those conventionally packed. This is attributed to deaeration, which made HVFS fruit initially appear more intensely orange-yellow than either fresh or conventionally packed slices. Sweetened packs, both HVFS and conventional, appeared more intensely orange-yellow than unsweetened packs. This was attributed to differences in soluble solids levels rather than to the amber colored syrup, because a similar tendency was observed with granulated sucrose which added no color. The difference between unsweetened HVFS and conventional water packs gradually decreased, but the difference between syrup and unsweetened packs became more pronounced with time. Since drained weights did not decrease in syrup packs, loss of moisture from the fruit to the syrup by osmosis is not indicated to be a reason.

In commercially packed fruits, it is common to have variations in ripeness within the limits of acceptable maturity. Thus, it was not unusual that the pH varied among samples, because this parameter is sensitive to fluctuations in ripeness of the individual peaches. Often the influence of ripeness overshadowed the expected increase in pH due to dilution in conventional packs (Chang, 1969). Slight changes in pH were observed through storage. However, the changes were random and no trend with storage was apparent.

Soluble solids or °Brix values, though influenced by ripeness, were much more significantly affected by dilution. The effect of adding 1/3 water in conventional water packs was clearly demonstrated by the difference in Brix values between unsweetened HVFS packs and conventional water packs. Within a given pack, fluctuations in Brix values through storage were probably due to sample variation.

Titratable acidity (% acidity) levels between processes also indicated, to a lesser degree than Brix, the impact of

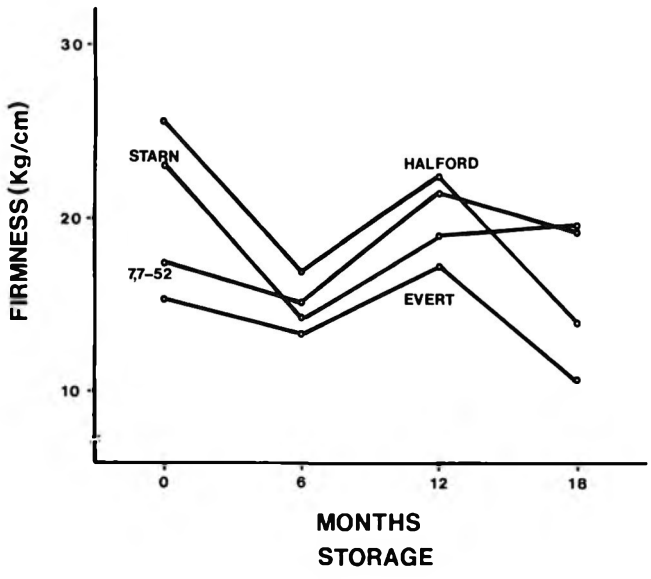

Fig. 3—Effect of peach vareity and storage time on firmness (OTMS Blade Extrusion) of natural, high vacuum flame sterilized peach slices.

Table 4—Comparative physical and chemical evaluation of high vacuum flame processed and conventionally processed Bartlett pear slices after 1 and 18 months of storage

	Storage time (mo)	Vacuum pack Natural 303 x 406 cans	Conventional pack in water 401 x 411 cans	Vacuum pack w/syrup 303 x 406 cans	Conventional pack in syrup 401 x 411 cans
		WASHINGTON STATE			
Net weight (oz)	1	15.5 ± 0.2	28.6 ± 0.3	16.2 ± 0.7	29.6 ± 0.3
	18	15.5 ± 0.2	28.9 ± 0.2	16.1 ± 0.8	29.9 ± 0.2
Drained weight (oz)	1	14.0 ± 0.4	17.6 ± 0.1	13.9 ± 0.8	18.4 ± 0.2
	18	14.3 ± 0.3	17.9 ± 0.4	14.5 ± 0.9	18.1 ± 0.3
Vacuum (in. Hg)	1	24.0 ± 1.0	7.5 ± 0.5	22.3 ± 0.6	9.0 ± 1.0
	18	21.7 ± 0.8	6.2 ± 1.0	21.5 ± 2.0	7.8 ± 1.6
Slices per pound	1	38.5 ± 1.2	–	45.7 ± 2.4	–
	18	39.4 ± 1.8	36.6 ± 4.4	41.1 ± 6.3	35.2 ± 2.8
Firmness (psi)	1	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
	18	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.3	0.3 ± 0.1
Color (YI)	1	19.2 ± 5.4	22.4 ± 8.1	17.7 ± 3.1	23.0 ± 4.7
	18	25.2 ± 3.6	25.3 ± 5.1	25.5 ± 4.9	28.1 ± 3.7
pH distribution	1	3.78 ± 0.04	3.88 ± 0.01	3.77 ± 0.02	3.85 ± 0.05
	18	3.72 ± 0.03	3.79 ± 0.02	3.69 ± 0.02	3.78 ± 0.02
°Brix	1	11.2 ± 0.2	7.6 ± 0.3	18.1 ± 0.3	18.1 ± 0.2
	18	11.1 ± 0.2	7.7 ± 0.2	18.1 ± 0.3	17.9 ± 0.1
Titratable acidity (meq/100g)	1	4.5 ± 0.1	2.9 ± 0.1	4.1 ± 0.5	2.9 ± 0.1
	18	5.0 ± 0.1	3.2 ± 0.1	4.4 ± 0.1	3.1 ± 0.1
		CALIFORNIA			
Net weight (oz)	1	15.8 ± 0.2	28.1 ± 0.1	16.2 ± 0.8	29.9 ± 0.1
	18	14.7 ± 1.0	28.5 ± 0.5	16.4 ± 0.5	28.7 ± 2.0
Drained weight (oz)	1	14.6 ± 0.3	17.9 ± 0.3	14.4 ± 1.1	18.8 ± 0.2
	18	13.5 ± 1.1	17.7 ± 0.5	15.0 ± 0.6	18.3 ± 0.3
Vacuum (in. Hg)	1	22.2 ± 0.3	9.7 ± 0.6	20.3 ± 1.2	8.3 ± 0.6
	18	22.2 ± 2.2	7.8 ± 2.0	18.7 ± 1.2	8.3 ± 2.6
Slices per pound	1	31.3 ± 3.6	–	32.2 ± 2.9	–
	18	31.8 ± 2.2	28.8 ± 1.5	32.3 ± 1.7	28.9 ± 1.9
Firmness (psi)	1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.3	0.3 ± 0.1
	18	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.3	0.2 ± 0.1
Color (YI)	1	15.0 ± 4.5	15.4 ± 2.6	14.1 ± 3.0	17.3 ± 4.8
	18	23.2 ± 5.3	24.2 ± 7.1	23.2 ± 3.9	22.6 ± 3.0
pH distribution	1	3.96 ± 0.03	4.08 ± 0.01	3.99 ± 0.00	4.03 ± 0.03
	18	3.88 ± 0.03	4.01 ± 0.02	3.86 ± 0.04	3.95 ± 0.03
°Brix	1	11.3 ± 0.1	7.9 ± 0.2	17.9 ± 0.3	17.5 ± 0.2
	18	11.3 ± 0.2	7.5 ± 0.2	17.9 ± 0.2	17.4 ± 1.1
Titratable acidity (meq/100g)	1	3.7 ± 0.1	2.4 ± 0.1	3.3 ± 0.1	2.3 ± 0.1
	18	4.0 ± 0.1	2.5 ± 0.1	3.9 ± 0.1	2.8 ± 0.3
		OREGON			
Net weight (oz)	1	14.9 ± 0.5	28.4 ± 0.1	16.5 ± 0.1	29.4 ± 0.2
	18	14.8 ± 0.5	28.5 ± 0.1	16.1 ± 0.7	29.6 ± 0.2
Drained weight (oz)	1	13.3 ± 0.7	17.6 ± 0.1	14.0 ± 0.1	17.5 ± 0.2
	18	13.7 ± 0.6	17.7 ± 0.2	14.3 ± 0.9	17.7 ± 0.2
Vacuum (in. Hg)	1	23.3 ± 1.5	9.3 ± 0.6	21.3 ± 1.2	12.7 ± 0.6
	18	23.5 ± 0.8	7.8 ± 0.8	21.0 ± 1.0	8.3 ± 0.8
Slices per pound	1	28.7 ± 3.4	–	28.2 ± 1.4	–
	18	33.0 ± 2.0	31.2 ± 2.9	29.4 ± 2.9	33.4 ± 3.8
Firmness (psi)	1	0.6 ± 0.3	0.6 ± 0.3	1.0 ± 0.9	0.5 ± 0.1
	18	0.6 ± 0.3	0.4 ± 0.1	1.6 ± 0.8	0.5 ± 0.1
Color (YI)	1	18.5 ± 4.1	19.7 ± 4.2	15.2 ± 3.9	18.2 ± 3.5
	18	24.8 ± 4.5	22.6 ± 4.2	25.5 ± 5.9	25.1 ± 3.9
pH distribution	1	3.97 ± 0.01	4.14 ± 0.01	4.00 ± 0.01	4.06 ± 0.05
	18	3.95 ± 0.02	4.05 ± 0.03	3.98 ± 0.00	3.95 ± 0.04
°Brix	1	10.5 ± 0.1	7.2 ± 0.1	18.0 ± 0.4	17.6 ± 0.2
	18	10.5 ± 0.2	7.0 ± 0.1	17.9 ± 0.4	17.8 ± 0.1
Titratable acidity (meq/100g)	1	4.0 ± 0.1	2.5 ± 0.1	3.5 ± 0.2	2.5 ± 0.1
	18	4.2 ± 0.1	2.6 ± 0.1	3.6 ± 0.2	2.6 ± 0.1

dilution. Again, changes between the 1 and 18 mo. values were random; thus storage time did not appear to have a strong influence on titratable acidity.

Bartlett pears

Results of the storage study on sliced pears follow trends which are similar, if not identical, to those of the sliced peaches. Discussion of results for pears will be limited to observations which were either unique to pears or were different from those on peaches.

The dessert quality of canned pear slices can be markedly influenced by fruit size and stage of fruit ripeness measured in terms of firmness at the time of canning. The number of slices per pound (Table 4) clearly reflected the differences in fruit size which were indicated in Table 1. Although the Washington State pears were smaller than the others, they had comparable or better Brix values than the larger pears (Table 4).

The firmness of raw pears from the three growing areas appeared to differ only slightly; however in the canned products, Oregon pears remained firmer than the others, indicating a nonlinear relationship between raw and subsequently canned pear textures.

Penetration firmness data on canned slices showed only small differences between growing areas. However, the average values for each area were established by the firmness of the fresh fruits at the time of canning. In general, firmness decreased only slightly through storage. The firmer initial texture of Oregon pears affected the net weights of the natural packs because the firmer fruit slices were not as pliable and fewer slices fit into each can.

Some variations of vacuum observed in the HVFS packs may relate to fill variations in that cans with higher net weight occasionally showed lower vacuum because the vacuum gauge was obstructed by pieces of fruit. Vacuum changes may also occur from the H_2-producing reaction between some pears and the metal container. Work is planned using gas chromatography to examine this possibility.

To reduce the complexity of interpreting the tristimulus color values, yellowness indexes (YI) were used to show both direction and magnitude of color change in a single value. Initially, vacuum packs were observed to be brighter (lower YI) than either the water or syrup controls. After equilibration (18 mo.), the difference in color diminished, with all packs exhibiting an increase in YI values. The color of all packs converged after 18 mo. Color of the HVFS pears remained competitive with the controls.

Sporadic pinking of individual pear slices from all three states was observed in all four packs. Literature and experience offer two possible causes. One is that pinking relates to conditions inherent to some fruit, and the other is that the pears were over-processed (Boggess, 1974 ; Chandler and Clegg, 1970). Since all the canned pears received comparable processes within a pack and the problem was sporadic, over-processing can be eliminated as the cause of pinking.

The pH varied among packs because this parameter is sensitive to fluctuations in ripeness of the individual pears. Addition of water and syrup to the control packs generally caused the pH to increase slightly. However, through storage, the pH of all packs decreased which may indicate fruit-container reaction. As pH and titratable acidity are inversely related, an increase in titratable acidity was observed in all packs. These changes, though not experienced with peaches, were in agreement with observations by Chang, 1969).

The soluble solids values paralleled those for peaches. The decrease in Brix values from the unsweetened vacuum packs to the conventional water packs illustrates the effect of adding 1/3 water in conventional water packs. The impact of dilution can be seen by comparing the titratable acidity levels between processes.

Diced peaches and pears

Objective evaluation of diced fruit was limited by particle size (Table 5).

Color, flavor and texture were subjectively evaluated, and both followed the trends for peaches and pears. The shape of the diced pears remained intact, without erosion of the edges or sloughing of tissues. This indicated that the diced fruit mixture was successfully HVFS processed. Since diced pears are more fragile than clingstone peaches, cherries or pineapple bits, only the behavior of grapes in HVFS processing needs testing before it can be concluded that fruit cocktail will lend itself to the process.

General observations

Omission of covering liquid in HVFS processing offers several benefits. In addition to the possible energy savings (Carroad et al., 1980), elimination of the covering liquid combined with precise heat processing would also result in better retention of nutrients (Seet et al., 1983) and flavor attributes. Although no evaluation of specific nutrient retention was made in this study, soluble solids and % acidity are reasonable indexes of both gross nutritive worth and

Table 5—Comparative evaluation of high vacuum flame processed and conventionally processed diced fruit after 1 and 18 months of storage.

	Storage time (mo)	Vacuum pack natural 303x406 cans	Conventional pack in water 401x411 cans	Vacuum pack w/syrup 303x406 cans	Conventional pack in syrup 401x411 cans
		DICED PEACHES AND PEARS			
Net weight (oz)	1	15.4 ± 0.3	28.0 ± 0.1	17.0 ± 0.1	29.6 ± 0.1
	18	15.4 ± 0.5	27.9 ± 0.2	16.3 ± 0.3	29.6 ± 0.2
Drained weight (oz)	1	15.4 ± 0.3	18.5 ± 0	17.0 ± 0.1	18.9 ± 0.3
	18	15.3 ± 0.5	18.3 ± 0.2	16.1 ± 0.3	18.1 ± 0.6
Vacuum (in. Hg)	1	21.8 ± 1.0	10.0 ± 0	18.3 ± 0.6	9.3 ± 0.6
	18	21.8 ± 2.6	10.3 ± 0.4	21.3 ± 1.8	9.0 ± 1.1
pH distribution	1	3.68 ± 0.01	3.75 ± 0.01	3.66 ± 0.01	3.75 ± 0.06
	18	3.72 ± 0.01	3.83 ± 0.04	3.70 ± 0.01	3.80 ± 0.03
°Brix	1	10.7 ± 0.1	7.5 ± 0.1	17.2 ± 0.2	17.8 ± 0.2
	18	10.6 ± 0.4	7.2 ± 0.1	17.1 ± 0.5	17.7 ± 0.1
Titratable acidity (meq/100g)	1	7.2 ± 0.1	4.8 ± 0.2	6.2 ± 0.1	4.8 ± 0.1
	18	8.0 ± 0.3	4.8 ± 0.2	7.0 ± 0.2	5.1 ± 0.1

Table 6—Comparison of flavor attributes of canned fruits in terms of retention of soluble solids (°B) in unsweetened canned fruits, and % acidity to °Brix ratio of fruits packed in syrup

Variety	Vacuum pack natural (% Soluble solids/Fresh)	Conventional pack in water (% Solubles solids/Fresh)	Vacuum pack natural[a] (% Acid/°Brix)	Vacuum pack w/syrup (% Acid/°Brix)	Conventional pack in syrup (% Acid/°Brix)
7, 7-52 Peaches	100	67.9	0.71	0.27	0.23
Evert Peaches	100	76.7	0.73	0.37	0.28
Halford Peaches	100	71.4	0.86	0.34	0.28
Starn Peaches	100	69.8	0.81	0.36	0.27
Washington Pears	100	68.6	0.43	0.23	0.17
California Pears	100	68.1	0.34	0.20	0.15
Oregon Pears	100	67.6	0.39	0.20	0.14
Diced Fruit	100	69.0	0.71	0.38	0.28

[a] % Acid/°Brix ratios of natural vacuum packed fruits include any changes caused by the application of heat to the fresh fruits.

flavor attributes. Comparative retentions of soluble solids (°Brix) and % acidity are summarized in Table 6. Based on °Brix measurements of the raw fruits, natural HVFS packs retained 100% of soluble solids present in the fresh fruit. Although blanching extracted some serum, the loss was not selective for the solids present in the serum, and steam condensed on the fruit was removed through deaeration; thus no change in °Brix occurred. The addition of water to conventional water packs decreased the % soluble solids in proportion to the amount of water added, allowing for some variation in the actual °Brix of the fresh fruit used in the pack. Dilution of soluble solids (°Brix) by the added water was offset when syrup was added to the conventional syrup packs. However, dilution of natural acidity in conventional syrup packed fruits made them taste sweeter and less fruity than the undiluted HVFS packed sweetened fruits at comparable cutout °Brix value. Values of natural fruit acidity in the HVFS and conventional syrup packs are expressed, for comparison, as a ratio of % acidity/°Brix (Table 6). The reported differences in % acidity/°Brix, however small, were easily detected, and in scientifically designed comparative sensory evaluations, the closer-to-fresh flavor of HVFS peaches has been confirmed by O'Mahony et al. (1981).

CONCLUSIONS

SINCE DIFFERENCES in color, texture and flavor attributes in terms of °Brix and titratable acidity are immediately recognized in canned foods, this work was intended to compare these easily perceived factors in high vacuum flame sterilized (HVFS) and conventionally packed peaches and pears. The data and success of the HVFS packs show that with high vacuum and omission of the conventionally used covering water or syrup, a dessert quality pack which retains more of the natural attributes of fruits can be obtained without addition of sweeteners. The pack would offer a natural option in the can, especially to those consumers who restrict the use of sweeteners.

The typical HVFS process for peaches and pears produced high vacuum and biological stability, using a 4 min steam blanch, 3.5 min heating in the can with deaeration, followed by cooling. The gentle process minimized thermal degradation, which is evident in the improved color and texture of the canned fruits. Omission of the covering liquid increased the amount of fruit that could be packed into the can thus saving container costs. Omission of the covering liquid also minimized dilution of nutrients, aroma, and flavor as shown in this work with closer-to-natural average values of °Brix and titratable acidity. The advantage of this is shown by work done by O'Mahony et al. (1981), showing sensory advantages for HVFS packs of peach slices. Although nutrient retention is usually not a consideration in peaches, Seet et al. (1983) indicate superior nutritional retention in HVFS packed tuna, as compared to conventional packs.

However, with over 6,000 cans experimentally packed without failure using high vacuum flame sterilization procedures and with the initial high quality being maintained for more than 18 months of storage so far, the process can be considered a successful and attractive alternative to conventional preservation of sliced peaches, pears and diced fruit.

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Effects of Rennet Treatment and Water Content on Thermal Conductivity of Skim Milk

TOMOSHIGE HORI

ABSTRACT

Effective thermal conductivities of skim milk and skim milk curd over a moisture range of 60–95% were measured by the hot wire method. The measured thermal conductivity of skim milk decreased significantly with change in structure through rennet treatment at 30.0°C; the intrinsic thermal conductivity of solids content was determined on the basis of the series model to be 0.346 W/mK for skim milk and 0.338 W/mK for skim milk curd. The relationship between the water content and effective thermal conductivity of the specimens was described by using a single parabolic formula. The deviations of empirical values based on this formula related to the experimental ones were 0.31 ± 0.30%.

INTRODUCTION

THERMAL CONDUCTIVITIES of foods were categorized inadequately along with rather low accuracy of measurements, although Kastaropoulos (1971) and Vachon et al. (1972) collected 275 publications concerning this property (Sweat, 1975); e.g., the standard deviations of the thermal conductivity of selected fruits and vegetables ranged from 2–12% (Sweat, 1974). The data with these deviations are acceptable only for industrial use. For scientific use, however, this accuracy is considerably lower.

Measurements of thermal conductivities of liquids require conditions under which the absence of convection can be ensured. The presence of convection gives rise to spuriously large values of the thermal conductivity. Detection of the onset of convection during actual measurements can most readily be accomplished by using unsteady-state techniques (Horrocks and McLaughlin, 1963). The absence of convection can be checked by departures from linearity of the ln(time) vs temperature plots (Fig. 1).

The unsteady-state hot wire method first suggested by Scheiermacher (1888), however, had little use in the actual measurements of thermal conductivity. This limited application was because there were no general procedures to determine the optimal conditions such as the diameter of hot wire, and electric current for heating the wire (Nagashima et al., 1977). The problem of recording time-dependent temperatures was present as well (Horrocks and McLaughlin, 1963). Moreover, the specimens must be homogeneous, because this method was established for homogeneous systems.

Fortunately, protein gels were homogeneous when heat conduction was considered (Kong et al., 1980). Both skim milk and skim milk curd are considered to be homogeneous, because the size of casein micelles in these milks (30–300 nm) (Knoop, 1972; Freeman and Mangino, 1981; Mangino and Freeman, 1981; Kalab et al., 1982) is small enough to consider these milks to be homogeneous on the basis of the Kerrisk (1971) index for homogeneity. Moreover, skim milk curd formed a network-type of gel with the same structure in all dimensions (Green et al., 1978). Thus, the adverse effect on the accuracy for measuring the thermal conductivity of skim milk and skim milk curd can be eliminated when the hot wire method was applied.

In general, physical properties reflect both component and structure of a given object. Riedel (1949) presented the empirical correlation for the thermal conductivity of liquid foods such as sugar solution, fruits juices and milk. Fernandez-Martin and Montes (1972) obtained the experimental regression formulas of thermal conductivity for the three sets of milk. These correlation and formulas led to a linear-in-concentration expression of thermal conductivity when temperature was constant.

A series layer model was applicable to protein gels. Yano et al. (1981) examined the applicability of four heat conduction models such as parallel, series, Hamilton and Crosser (1962), and Kunii and Smith (1960) model. Out of these four models, only the series model was applicable to soy protein curds.

Kong et al. (1982b) indicated that the series model best described such gels of gelatin, egg albumin, wheat gluten and milk casein; this model was acceptable especially for hydrophobic proteins. Moreover, Kong et al. (1982a) estimated an intrinsic thermal conductivity of wet gelatin (value 0.38 W/mK) from the empirical parabolic formula of Filippov (1968) for organic solutions rather than using the models of heterogeneous systems.

The major objective of this research is to characterize the thermal conductivity behavior of skim milk and skim milk curd relative to the component and structural change through rennet treatment on the basis of accurate absolute determinations of thermal conductivity.

MATERIALS & METHODS

Sample

Skim milks and skim milk curds were used as the specimens. The spray-dried skim milk [protein, 36.1% db (dry base); fat, 0.8% db] was reconstituted with water (30°C) to obtain the skim milk specimens. The weight fraction of water content (X_w^w) of these specimens lay in the range between 0.60 and 0.95.

The skim milk specimens were treated with natural rennet. A cheese-making rennet from calf stomach was added (0.340% db) to these specimens. Thus, the skim milk curd specimens were coagulated for 30 min at 30.0 ± 0.1°C. The rennetting was made to attain no change in water content of the curd specimens.

Distilled water was used as the control.

Thermal conductivity measurements

The effective thermal conductivity (λe, W/mK) of the specimens (30.0 ± 0.1°C) was measured by the hot wire method. The development of temperature was observed in an electrically heated (0.7A in direct electrical current) platinum wire (0.1 mmΦ x 130 mm) immersed axially in the specimen (1000 cm^3) in a cylindrical flask (90 mmΦ x 180 mm) made of glass. The specimen was initially in thermal equilibrium. The increasing temperature with time in the central portion (approximately 90 mm) of the platinum wire was measured ten times automatically at regular intervals (60 msec) during heating the wire for 1.2 sec in each measurement, and subsequently the λe values of the specimens were determined by:

$$\lambda e = \left(\frac{Q}{4\pi}\right)/\left(\frac{d\,\Delta\,\theta}{d\,\ln t}\right) \qquad (1)$$

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where Q is heat generated in the wire (W/m); $\Delta\theta$ is temperature of the wire with reference to the initial temperature (K); and t is time (sec) after heating started (Horrocks and McLaughlin, 1963). The replicate number of λe determinations and the waiting time between the replication were five times and 30 sec. The method of least squares was applied to calculate the $d(\Delta\theta)/d(\ln t)$ value in Eq. (1).

The temperature and time measurements were carried out with 0.044% and 0.17% accuracy, respectively, by using the data acquisition and control unit (model 3497A from Hewlett Packard Co., California) directly connected to the desk top computer (model 9845T from H.P. Co.). The response lags in the instrument used (approximately 25 msec) were calibrated to minimize the error in data collected. The electrical current was supplied with 0.014% accuracy to attain constant heat generation in the wire by using the direct electrical current standard (model 2853/2862 from Yokogawa Electric Works, Ltd., Tokyo, Japan).

Volume fraction measurements

The densities of skim milk specimens were measured with a pycnometer (100 cm^3) at 30.0°C. The measurements were repeated three times. The density of solids content (ρs, kg/m^3) was determined to be 1.441×10^3 kg/m^3 by extrapolating to zero water content from the measured densities. The ρs value was postulated to be constant during rennet treatment.

The volume fractions of water (X_w^v) and of solids content (X_s^v) were calculated from the weight fractions by:

$$X_w^v = \frac{(X_w^w/\rho w)}{(X_w^w/\rho w) + (X_s^w/\rho s)} \quad (2)$$

and

$$X_w^v + X_s^v = 1 \quad (3)$$

where X_s^w is weight fraction of solids content and ρw is the density of water (kg/m^3).

Proton pulsed NMR measurements

The spin-lattice (T_1, sec) and spin-spin (T_2, sec) relaxation times of hydrogen nuclei in the free water of skim milk specimens were measured by using the proton pulsed NMR analyzer (model PR103 from the Praxis Corp., Texas). The radio frequency and the static magnetic field of this analyzer were 10.78 MHz and 0.251T (2510 gauss).

The T_1 of free water was measured with a repeated 90°–90° pulse sequence or the spin-locking method (Look and Lowe, 1966). For T_2 measurements of free water, a repeated 90°–180° pulse sequence or the spin-echo method (Hahn, 1950) was applied. The NMR measurements of the skim milk specimens were made at 30 ± 1°C. The NMR signals were integrated 108 times in each measurement.

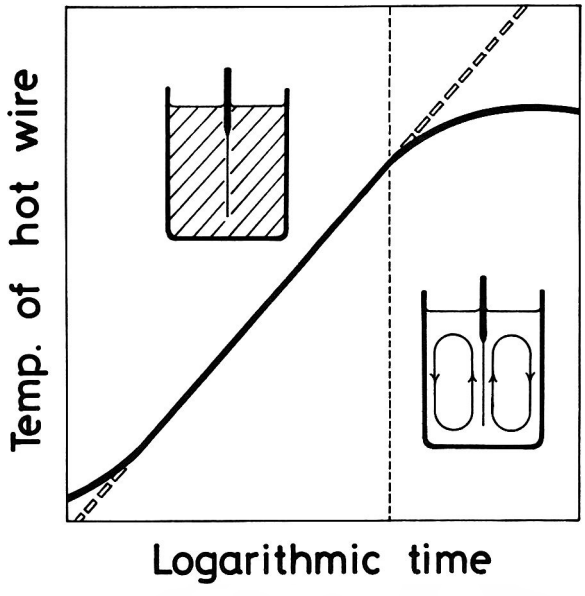

Fig. 1—Illustrative effect of the onset of convection current on the temperature of line heat source during actual measurement by the hot wire method.

RESULTS & DISCUSSION

THE STANDARD DEVIATIONS of λe values of the specimens determined by the hot wire method were fairly small. The deviations ranged from 0.2–0.5%, and consequently the present data were applicable for scientific use (Table 1).

The thermal conductivity of skim milk specimens decreased significantly with decreasing water content, and with the rennet treatment. The λe of most concentrated (X_w^w = 0.60) skim milk and skim milk curd decreased by about 21.5% and 22.8%, respectively, compared to water. The analysis of variance proved that the effects of both water content and rennet treatment on the measured λe values were significant ($p < 0.001$) (Table 2). The population means of λe decreased by 0.018 W/mK (2.9% compared to the mean values) for every 0.05 decrease of X_w^w. As opposed to this fairly large decrease with X_w^w, the decrease due to the coagulation was only 0.003 W/mK (0.6%) (Fig. 2).

The present research is an advancement in the scientific literature, because the quantitative analysis of λe above discussed on the basis of accurate values of measured λe indicated that the λe of skim milk over a moisture range 60–95% was actually considered to be constant during the rennetting. This result is useful for automatic control of the heat-sensitive coagulation process in cheese manufacturing. In general, thermal conductivity must be considered, when determining the length of time that a heating or cooling process will take (Polley et al., 1980).

The present author believes that a small increase of MW (molecular weight) of casein micelles due to the rennet treatment resulted in the rather small decrease of λe. Dalyleish et al. (1981) indicated that the MW of differently sized casein micelles in the order of 10^8–10^9 increased by about three times after coagulation by rennet for 1 hr. In addition, the casein content in the specimens ranged from about 1.4–10.8% wet base; the ratio of casein to total protein content being assumed as 0.75. Hence, the 0.6% decrease of λe with the rennetting was reasonable.

Table 1—Effective thermal conductivity of skim milk and skim milk curd determined by the hot wire method at 30.0°C.

	Skim milk		Skim milk curd	
X_w^w	λe, W/mK	Standard deviation, %	λe, W/mK	Standard deviation, %
1.00	(0.6231	0.28)	–	–
0.95	0.6107	0.29	0.6102	0.35
0.90	0.5959	0.48	0.5958	0.39
0.85	0.5787	0.41	0.5730	0.24
0.80	0.5667	0.16	0.5626	0.28
0.75	0.5429	0.30	0.5406	0.45
0.70	0.5275	0.17	0.5266	0.27
0.65	0.5074	0.21	0.5040	0.31
0.60	0.4888	0.54	0.4813	0.22

Table 2—Analysis of variance of measured effective thermal conductivity values of skim milk and skim milk curd at 30.0°C.

Factor	d.f.	V	F
Water content	7	1.9226×10^{-2}	4091.29***
Rennet-treatment	1	2.0098×10^{-4}	42.77***
Error	71	4.6992×10^{-6}	
Total	79	1.7103×10^{-3}	

***Significant ($p < 0.001$)

The intrinsic thermal conductivity of solids content (λi, W/mK) decreased with change in structure of skim milk specimens through rennet treatment. The λi values were determined to be 0.346 W/mK for skim milk and 0.338 W/mK for skim milk curd by using the following series model, in which the λi value was postulated to be independent of water content:

$$\lambda e = \frac{1}{(X_w^v/\lambda w) + (X_s^v/\lambda i)} \quad (4)$$

where λw is the thermal conductivity of water. The λi decreased with coagulation (value 0.008 W/mK or 2.3% compared to λi) was almost three times larger than the case of λe of whole specimens discussed above.

The series model was applicable to the skim milk and skim milk curd in relation to scientific use. The deviations of the mean values of measured λe relative to the best fit curves based on the series model were 0.94 ± 0.58%, 1.02 ± 0.64% and 0.98 ± 0.59%, for skim milk, skim milk curd or both (Fig. 3). These sufficiently small deviations showed

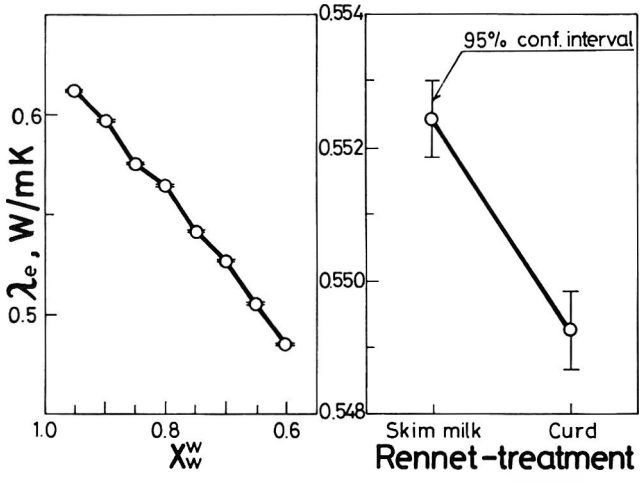

Fig. 2—Population means of measured effective thermal conductivity of skim milk and skim milk curd at 30.0°C.

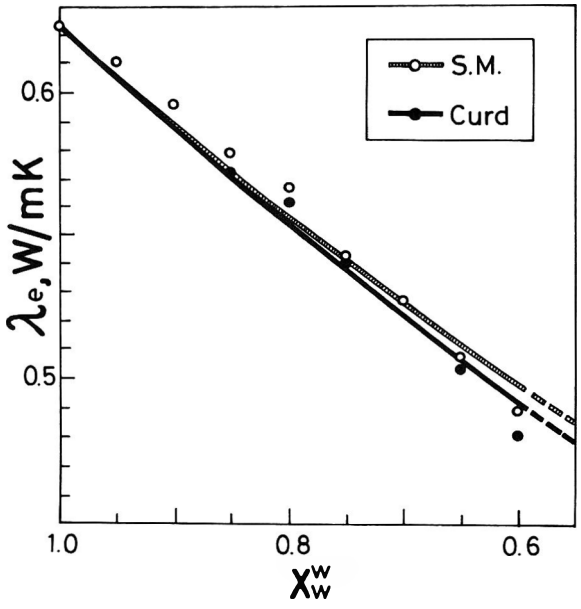

Fig. 3—Regression curves for measured effective thermal conductivity of skim milk and skim milk curd at 30.0°C based on the series model.

that the series model which best described hydrophobic protein gels (Yano et al., 1981; Kong et al., 1980, 1982a, 1982b) was applicable for analyzing the sensitivity of thermal conductivity to structural change of skim milk with rennet treatment. Hence, the estimated λi values above can be acceptable not only for industrial use but also for scientific use.

A single parabolic formula described the concentration dependence of measured λe data as well. Despite the good fitness of the slight concave curves based on the series model (Fig. 3), the present λe data suggested that convex curves will better describe the measured relationship between λe and X_w^w than concave curves. In addition, the reliability of parabolic formula was justified by Kong et al. (1982a) who estimated the reasonable λi of wet gelatin (value 0.38 W/mK) on the basis of the Filippov formula:

$$\lambda e = \lambda w X_w^w + \lambda s X_s^w - \beta X_w^w X_s^w |\lambda w - \lambda s| \quad (5)$$

where λs is effective thermal conductivity of solids content and β is the numerical constant which ranged from 0.3–0.7 for aqueous organic solutions. The β value was actually independent of temperature (Filippov, 1968). The substitution of estimated λi values into λs of Eq. (5) yielded optimal β value to be 0.223 for skim milk and 0.294 for skim milk curd. The least squares method was applied for this calculation. The deviations of the regression in this case were 0.94 ± 0.61% for both skim milk and skim milk curd.

The relationship between λs and X_w^w was estimated on the basis of the Filippov formula. Suppose Eq. (5) correctly describes the thermal conductivity behavior of skim milk and skim milk curd, the effect of water content on λs can be characterized when Eq. (5) is rewritten as:

$$\lambda s = \frac{\lambda e + \lambda w X_w^w (\beta X_s^w - 1)}{X_s^w (1 + \beta X_w^w)} \approx f(X_w^w) \quad (6)$$

Thus, the concentration dependence of λs was estimated as follows by applying the least squares method:

$$\lambda s = 0.342 - 0.202 X_w^w + 0.294 (X_w^w)^2 \quad (7)$$

for skim milk, and

$$\lambda s = 0.410 - 0.386 X_w^w + 0.416 (X_w^w)^2 \quad (8)$$

for skim milk curd.

The Filippov formula better described the convex relationship between λe and X_w^w than the series model. Substitution of optimal β values and empirical λs values based on Eq. (7) or Eq. (8) into Eq. (5) led to considerably better fitted regression curves (Fig. 4). The deviations of empirical values related to experimental ones were only 0.20 ± 0.22% for skim milk and 0.41 ± 0.34% for skim milk curd (Table 3). The 0.31 ± 0.30% deviations for both skim milk and skim milk curd were similar to the accuracy of present measurements by the hot wire method (Table 1). Hence, the parabolic formula was more applicable to the present data than the series model in regards to the accuracy of regression.

The state of water suggested that the interaction effect between the solids and water content in relation to the concentration dependence of measured λe values. The NMR properties are, in general, useful to help explain the mechanism related to the water content. These properties show not only how much water is present in foods but also to what degree the water in foods is immobilized. The T_1 of skim milk specimens indicated a significant concave relationship to X_w^w, while a slight convex curve was measured for T_2 (Fig. 5). The higher correlation between λe and T_2 (r^2 = 0.9912) than that between λe and T_1 (r^2 = 0.7891) suggested that the interaction effect on the thermal conductivity behavior of skim milk specimens was significant, because T_2 is related to the interaction between the solids and water content.

The present data were different from the literature data. The λe values of the present experiment were higher above 80% water content and were lower below 75% water content compared to the empirical values of Fernandez-Martin and Montes (1972).

The calibration of both present and literature data characterized the difference between these data. Nagasaka and Nagashima (1980) presented the following formula of thermal conductivity of liquid water at atmospheric pressure:

$$\lambda w = 0.5617 + 2.005 \times 10^{-3}\,\theta - 8.49 \times 10^{-6}\,\theta^2 \quad (9)$$

where θ is the temperature of water (°C). The general formula which yielded the internationally accepted standard values of thermal conductivity of water (Kestin, 1978) can be rewritten as in Eq. (9). Hence, the recommended value of λw at 30.0°C was 0.6142 W/mK. Thus, those data were calibrated by:

$$\lambda c = \lambda m \frac{\lambda wl}{\lambda wm} \quad (10)$$

where λc and λm are calibrated and measured thermal conductivity; and λwl and λwm are literature and measured values of λw. As a result of calibration, the present values were lower than the literature ones at any water content (Fig. 6).

The major potential source of these deviations appeared to be the difference of the samples used. The present experiment was made by using the reconstituted skim milks, whereas Fernandez-Martin and Montes (1972) prepared vacuum concentrated skim milks. Another source of the deviations was the effect of the convection during actual measurement, because the presence of convection gives rise to spuriously large values of the thermal conductivity. Although they discussed that the absence of convection was proved in the case of water calibration tests, this calibration by using water may not be applicable to skim milk samples because of differences in rheological properties. The present method, on the other hand, can detect the onset of convection during each actual measurement.

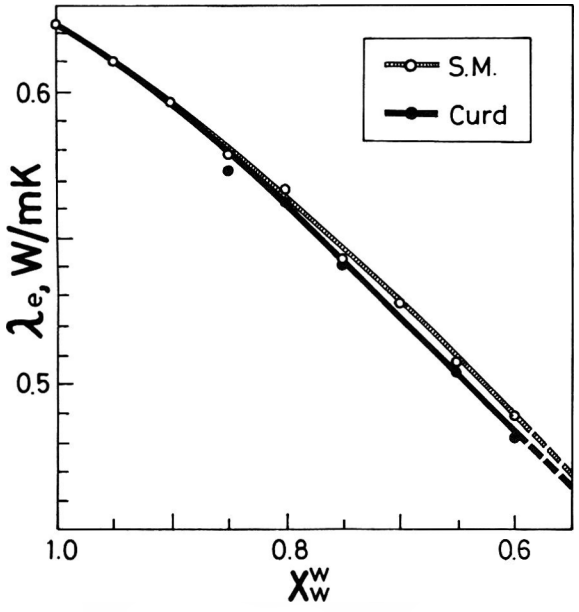

Fig. 4—Regression curves for measured effective thermal conductivity of skim milk and skim milk curd at 30.0°C based on the Filippov model; the concentration dependence of λs being assumed.

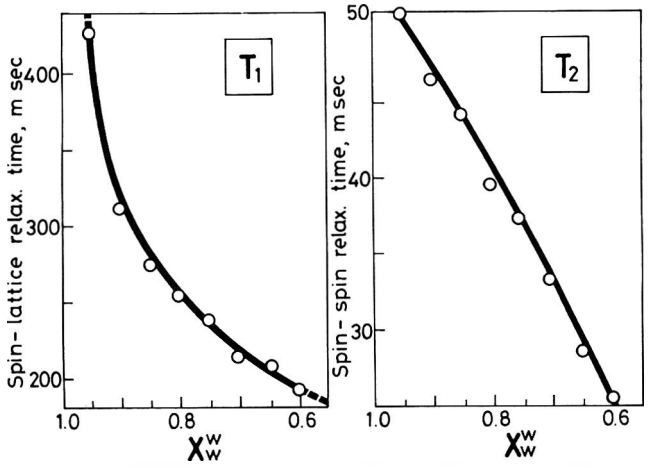

Fig. 5—Relationship between water content and pulsed NMR properties of free water in skim milk at 30°C.

CONCLUSIONS

THE INTRINSIC thermal conductivity of solids content was determined on the basis of the series model to be 0.346 W/mK for skim milk and 0.338 W/mK for skim milk curd, while a parabolic formula better described the rela-

Table 3—Empirical effective thermal conductivity of skim milk and skim milk curd at 30.0°C based on the Filippov formula, and deviations of empirical values related to experimental mean values.

	Skim milk		Skim milk curd	
X_w^w	λe, W/mK	Deviation, %	λe, W/mK	Deviation, %
0.95	0.6105	0.03	0.6101	0.02
0.90	0.5962	0.04	0.5949	0.15
0.85	0.5803	0.27	0.5781	0.88
0.80	0.5632	0.62	0.5601	0.45
0.75	0.5452	0.43	0.5413	0.13
0.70	0.5266	0.16	0.5222	0.84
0.65	0.5077	0.07	0.5031	0.17
0.60	0.4888	0.00	0.4845	0.67

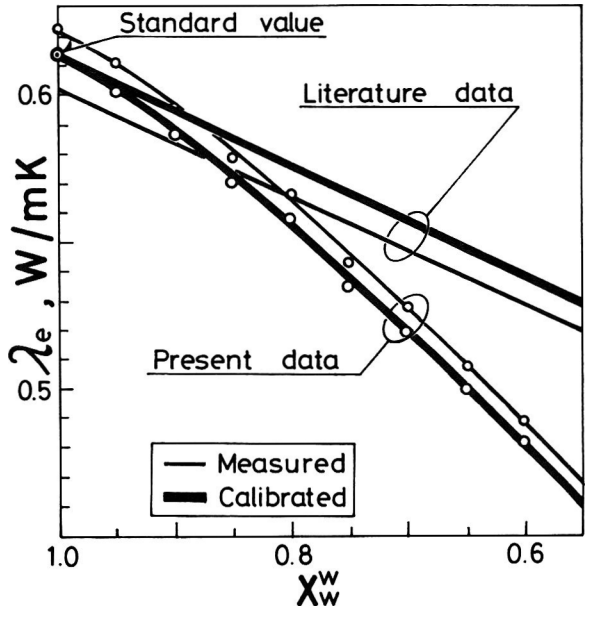

Fig. 6—Deviations of the present values of effective thermal conductivity of skim milk at 30.0°C related to the literature values; all values being calibrated on the basis of the standard value of water.

tionship between the effective thermal conductivity and water content in the specimens than the series model; the deviations of empirical values related to experimental ones being 0.31 ± 0.30%.

NOMENCLATURE

- λe Effective thermal conductivity, W/mK
- λi Intrinsic thermal conductivity of solids content, W/mK
- λs Effective thermal conductivity of solids content, W/mK
- λw Thermal conductivity of water, W/mK
- λc Calibrated thermal conductivity, W/mK
- λm Measured thermal conductivity, W/mK
- λwl Literature value of thermal conductivity of water, W/mK
- λwm Measured value of thermal conductivity of water, W/mK
- X_w^w Weight fraction of water content, –
- X_s^w Weight fraction of solids content, –
- X_w^v Volume fraction of water content, –
- X_s^v Volume fraction of solids content, –
- Q Heat generated in the wire, W/m
- $\Delta\theta$ Temperature of the wire with reference to the initial temperature, K
- θ Temperature of water, °C
- t Time, sec
- ρs Density of solids content, kg/m^3
- ρw Density of water, kg/m^3
- r^2 Square of statistical coefficient of correlation, –
- p Statistical significance level, –
- β Numerical constant in the Filippov formula, –
- T_1 Spin-lattice relaxation time, sec
- T_2 Spin-spin relaxation time, sec

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Shrinkage, Porosity and Bulk Density of Foodstuffs at Changing Moisture Contents

J. E. LOZANO, E. ROTSTEIN and M. J. URBICAIN

ABSTRACT

Dependable data on bulk density, volumetric shrinkage due to water loss and porosity are needed to model processes such as drying, packaging and storing. Experimental data are presented for all three properties. It is possible to model the water-loss-based bulk shrinkage coefficient to obtain a predictive equation based on composition of the foodstuff. From this, a generalized correlation is obtained which predicts bulk shrinkage coefficient knowing only the initial moisture content of the food. Porosities for the foodstuffs considered can be predicted through suitable correlations, but there is no generalized equation spanning all foods.

INTRODUCTION

MANY FOOD PROCESSING OPERATIONS can be successfully designed or studied on the basis of sound engineering modelling. There is a family of operations which involve the interaction between a water containing foodstuff and water in the surrounding atmosphere at the prevailing temperature and pressure, such as drying, packaging, storing and other similar operations. Modeling in these cases is based on properly setting up the heat and mass transfer equations, coupling them by means of a suitable hypothesis (usually, local equilibrium) and solving the resulting system of differential equations. This approach requires a knowledge of transport properties in general and porosity in particular.

Representative heat and mass transfer equations and the role porosity play in them, have been recently outlined by Lozano et al. (1980). When these equations are used, there is need to account for the change in porosity and the overall shrinkage of the samples as they lose moisture. One possible approach is the use of a fictitious length z (Román et al., 1982), so that every change of real length Δx can be transformed into the change of fictitious length through the expression:

$$\Delta z = \frac{\rho_b}{(1 + x)\,\rho}\,\Delta x \qquad (1)$$

where ρ_b is the bulk density of the sample and ρ is defined below:

$$\rho = \left(\frac{\rho_b}{1-\epsilon}\right)_{X=0} \qquad (2)$$

The above model requires data on porosity and bulk density as a function of moisture content.

An interesting potential application of data on volume change as a function of moisture content results from the drying theory recently put forward by Whitaker (1980). Cellular tissue making up the solid foodstuff may be regarded as a multiphase system and, by making use of the transport theorem and the averaging theorem, the macroscopic transport equations are developed. In this case the interphase velocity w becomes important. The transport theorem is:

$$\frac{d}{dt}\int_{V_{b(t)}} \psi\, dV = \int_{V_{b(t)}} \frac{\partial \psi}{\partial t}\, dV + \int_{A_{b(t)}} \psi\, w \cdot n\, dA \qquad (3)$$

for the case $\psi = 1$ it follows:

$$\frac{d\, s_b}{dt} = \frac{1}{V_{b,o}} \int_{A_{b(t)}} w \cdot n\, dA \qquad (4)$$

where s_b is the bulk shrinkage coefficient (Lozano et al., 1980):

$$s_b = \frac{V_{b(X)}}{V_{b,o}} \qquad (5)$$

Thus, in this approach it is necessary to know or to be able to predict the change of s_b as a function of moisture content. This type of information is also needed when simplified effective diffusivity models are used (Charm, 1978).

There are very few data on porosity, bulk density and bulk shrinkage coefficient as a function of moisture content. Kilpatrick et al. (1955) studied volume shrinkage of potatoes and other vegetables as drying proceeds. Charm (1978) reported on volumetric contraction of meat and potatoes. Chirife (1969) provided data for apples and potatoes. Görling (1958) studied shrinkage during the drying of macaroni. Suzuki et al. (1976) investigated the shrinkage in dehydration of root vegetables. Shrinkage and porosity of apple tissue at different moisture contents were reported by Lozano et al. (1980).

It would be useful to use this information as a basis to obtain general predictive correlations. Görling (1958), Chirife (1969) and Charm (1978) have used the following analogy of the thermal expansion equation;

$$\frac{\Delta V_b}{V_{b,o}} = \alpha\, \Delta X \qquad (6)$$

In this case, there is need to obtain the specific linear shrinkage coefficient value for each foodstuff and α is not valid over the entire range of X.

Kilpatrick et al. (1955) suggested an equation valid for the early stages of drying. It requires prior knowledge of the initial moisture content and the density of the dried material.

Suzuki et al. (1976) developed three equations which apply to three different drying models: uniform drying, core drying, and semicore drying. The first model results in two alternate equations: one needs data for equilibrium moisture contents and bulk density, while the other requires the initial moisture content and bulk density of the material. The second and third model need the initial and equilibrium values for moisture and bulk density. Suzuki's and Kilpatrick's equations are summarized in Table 1 to facilitate comparison with the one developed in the present work.

The objective of this paper is to present data on bulk density, porosity, and bulk shrinkage coefficients for several foods, as they change in moisture contents. The data are correlated to simplify further use. Both in the case of

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the bulk shrinkage coefficient and porosity an attempt was made to obtain predictive correlations based as composition. The attempt was particularly successful in the case of s_b, which could be predicted over the entire moisture range with only a knowledge of the initial moisture content of the food.

MATERIALS & METHODS

THE FOODS SELECTED were pears, carrots, potatoes, sweet potatoes, and garlic. Typical compositions are shown in Table 2 (Watt and Merrill, (1963). Except for garlic the samples were cut from the fresh product in the shape of cylinders, 1 cm in diameter, 4 cm long. Two sets of garlic samples were prepared: whole pieces or slices obtained cutting one piece in half along the longest axis. In the case of carrots the cylinders were cut in an angle with respect to the longest axis, so that the sample had balanced amounts of the inner core and the external phloem and parenchymatic tissue. Drying was carried out in a conventional pilot air drier, with a mesh wire tray and through circulation of air. Air velocity was 1 m/sec in all cases. For foodstuffs containing starch the dry bulk temperature was 40 ± 1°C, relative humidity 35%. For other foodstuffs, 60 ± 1°C and 30%. After drying, all samples were tightly wrapped in polyethylene film and placed into a thermostatic chamber for 24 hr, to homogenize water and temperature profiles across the sample.

Table 1—Previous models for bulk shrinkage coefficient prediction

Model	Equation	
Kilpatrick et al (1955)	$s_b = \frac{X + 0.8}{X_o + 0.8}$	(7)
Suzuki et al, (1976)		
Uniform drying (a)	$s_b = \frac{X + a}{X_o + a}$	(8)
	$a = X_e \left(\frac{1}{\rho_{b,e}} - 1\right) + \frac{1}{\rho_{b,e}}$	
(b)	$s_b = bX + c$	(9)
	$b = \frac{\rho_{b,o}}{(X_o + 1)}$	
	$c = 1 + b - \rho_{b,o}$	
Core drying model	$s_b = K\frac{X}{X_o} + 1$	(10)
	$K = 1 - \frac{(X_e + 1)\rho_{b,o}}{(X_o + 1)\rho_e} \frac{X_o}{X_o - X_e}$	
semicore drying model	$s_b = r\frac{X}{X_o} + n'$	(11)
	$r = \frac{X_o(1 - \xi)}{X_o - X_e - z(\xi X_o - X_e + \xi - 1)}$	
	$\xi = \frac{(X_e + 1)\rho_{b,o}}{(X_o + 1)\rho_{b,e}}$	
	$z = \frac{\rho_{b,ec} - (1 - X)\rho_{b,e}}{\rho_{b,o}}$	
	$n' = \frac{\xi X_o - X_e - z(\xi X_o - X_e + \xi - 1)}{X_o - X_e - z(X_o - X_e + \xi - 1)}$	

Table 2—Typical composition of foodstuffs studied

Foodstuff	X_o (kg/kg)	X_{sg} (kg/kg)	X_{st} (kg/kg)	X_{cw} (kg/kg)
Carrot	7.55	0.64	–	0.36
Garlic	2.28	0.80	–	0.20
Pear	7.03	0.83	–	0.17
Potato	4.50	0.15	0.80	0.05
Sweet potato	2.55	0.22	0.75	0.03

The procedures for measurement of bulk density and particle density were the same as reported in a previous contribution (Lozano et al., 1980).

Calculations

Porosity, ϵ, was calculated on the basis of the density measurements:

$$\epsilon = \frac{\rho_p - \rho_b}{\rho_p} \tag{12}$$

where ρ_p, the particle density, is based on the particle volume excluding all internal pores which are connected to the surrounding air space and ρ_b is the bulk particle density which is based on the particle volume including these internal pores. The density ρ_p was determined by measuring the particle volume in a pneumatic psycrometer in which the compressed air volume included the volume of air inside the open internal pores and ρ_b was determined by using buoyant force to measure the volume of water displaced by the particle when immersed.

The bulk volume at each moisture content was calculated from the bulk density and the corresponding sample weight W:

$$V_b = \frac{W}{\rho_b} \tag{13}$$

RESULTS & DISCUSSION

FIG. 1 SHOWS bulk density vs moisture content for the foodstuffs studied. It can be seen that there is no homogeneity of behavior. Bulk density increased in carrots and pears with decreasing moisture content, while in the case of potato, sweet potato and garlic it increased up to a certain moisture content and then decreased with decreasing moisture content. Particle density (Fig. 2) for all foods but carrots increased with decreasing moisture contents. For carrots it increased to $X/X_o = 0.075$ and then decreased as X tended to zero.

The bulk and particle densities can be correlated with an equation of the type:

$$\rho = h + \ell\frac{X}{X_o} + p\exp\left(-q\frac{X}{X_o}\right) \tag{14}$$

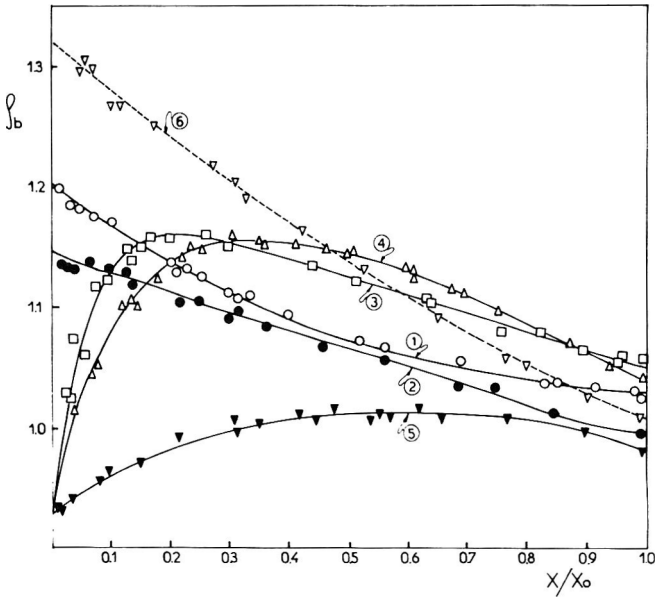

Fig. 1—Bulk density vs moisture content (Units of ρ_p are 10^3 kg/m^3). (1) Carrot; (2) Pear; (3) Potato; (4) Sweet Potato; (5) Garlic (whole pieces); (6) Garlic (sliced).

Table 3 shows the corresponding values of the constants and the coefficient of determination r^2 obtained from applying at least square nonlinear parameter algorithm (Marquardt, 1963). The one exception is the particle density of sweet potato for which the fit of Eq (14) is poor and the following correlation is more adequate ($r^2 = 0.97$):

$$(\rho_p)_{sp} = 1.553 - 4.954\frac{X}{X_o} + 4.630\left(\frac{X}{X_o}\right) 1.051 \qquad (15)$$

Note that carrots are the only ones showing the peculiar result of decreasing ρ_p in the last stages of drying. Since the porosity measured is that due to open pores, the radical drop in measured porosity appears to be due to a closing off of these pores, as shown by apples by Lozano et al. (1980). The dotted line in Fig. 2 projects the change in ρ_p that would have occurred if the pores had remained open.

Porosity values as a function of moisture content were calculated using Eq (12). The results are plotted in Fig. 3 for carrots and pears and in Fig. 4 for potatoes, sweet potatoes and garlic. The corresponding coefficients for Eq (14) are shown in Table 3. The shape of the other porosity lines indicate that the structural changes in the other foodstuffs are of a different nature.

Fig. 5 shows the change in bulk shrinkage coefficients of the foodstuffs, as a result of drying. The bulk shrinkage coefficient is plotted as a function of moisture content. With the exception of carrots and pears, the slope of s_b becomes noticeably less steep for $X/X_o < 0.15$. This is important because it indicates that all linear predictive equations for s_b will fail to cover the entire range $0 < X/X_o < 1$. Not less important is the fact that the range $0 < X/X_o < 0.15$ is very significant in modelling drying operations. For instance, for potato (Crapiste and Rotstein, 1982) it corresponds to $X = 0.68$, a value which is in equilibrium with an atmosphere of relative humidity $\phi > 0.9$. In other words, this range of X at which there is a change of slope in s_b is the one where most of the modeling and drying simulation is done. As a result, the ability to predict values of s_b within this range should be a required feature of the models under consideration. Fig. 6 indicates that this is accomplished by the new correlation.

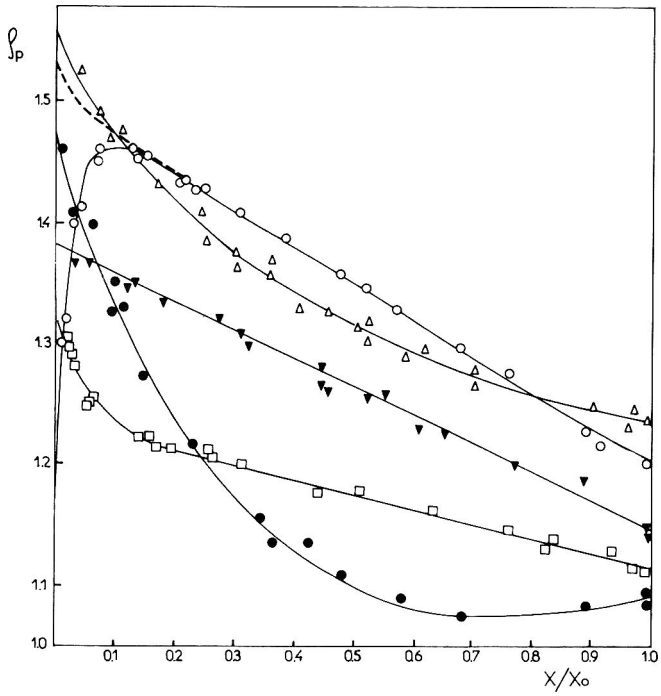

Fig. 2—Particle density as a function of moisture content (Units of ρ_p are 10^3 kg/m^3). (1) Carrot; (2) Pear; (3) Potato; (4) Sweet potato; (5) Garlic.

Table 3—Constants and coefficient of determination corresponding to Eq (14)

Foodstuff	density	h	ℓ	p	q	r^2
Carrot	bulk	0.984	0	0.224	1.800	0.97
	particle	1.497	−0.294	−0.253	39.793	0.96
	particle (true porosity)	1.497	−0.294	0.033	36.820	0.97
Pear	bulk	1.251	−0.153	−0.107	1.33×10^{-6}	0.97
	particle	0.832	0.220	0.632	2.775	0.97
Potato	bulk	1.202	−0.148	0.259	15.507	0.96
	particle	1.234	−0.117	0.085	19.040	0.97
Sweet potato	bulk	1.266	−0.219	−0.319	6.700	0.96
	bulk (whole piece)	3.260	1.172	−2.325	−0.395	0.96
Garlic	bulk (sliced)	1.130	−0.567	0.187	−0.866	0.95
	particle	2.694	0	−1.316	−0.1638	0.95

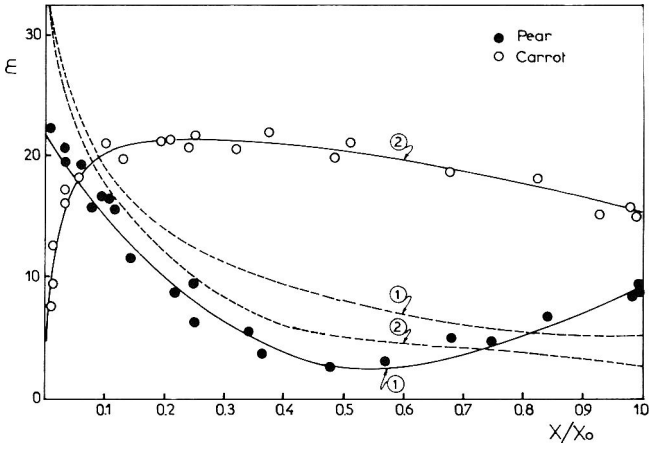

Fig. 3—Porosity dependence on moisture content: experimental (full line) and predicted (dotted line) data. (1) Pear; (2) Carrot.

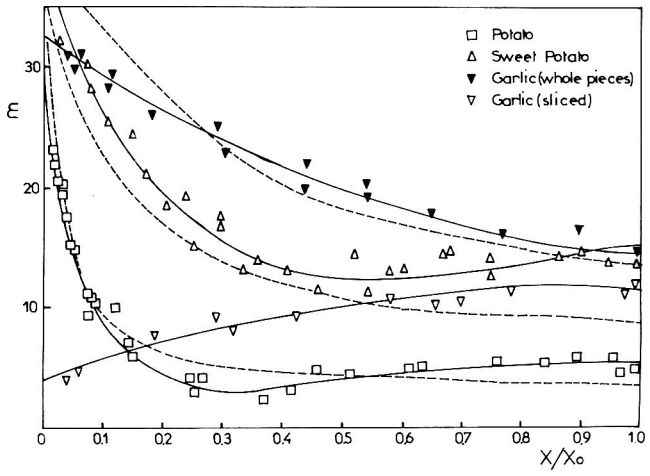

Fig. 4—Porosity as a function of moisture content. Experimental (full line) and predicted (dotted line) data. (1) Potato; (2) Sweet potato; (3) Garlic.

Volume shrinkage modeling

It appears as desirable to be able to predict volume shrinkage of a foodstuff without having to measure the property itself. This can be accomplished provided composition data are available such as that shown in Table 2, i.e. amount of the main constituents per unit weight of dry matter. Since the focus is on volume, the other information required is density of the same constituents.

For the foodstuffs under consideration, the main non-water constituents are sugar, starch and the cell wall materials. Cellular membrane material can be neglected because of its small contribution to the total weight. The weight fraction of the above constituents results from a material balance on a representative sample and it can be calculated from the following expressions:

$$\chi_{sg} = \frac{\sum_j m_{sg,j}}{\sum_j m_{sg,j} + \sum_i d_i} \quad (16)$$

$$\chi_{cw} = \frac{d_{cw}}{\sum_j m_{sg,j} + \sum_i d_i} \quad (17)$$

$$\chi_{st} = \frac{d_{st}}{\sum_j m_{sg,j} + \sum_i d_i} \quad (18)$$

where $m_{sg,j}$ is the amount of sugar j and d_i the amount of nonsugar dry matter i involved in the material balance. Note that the total amount of dry matter, m_d, is:

$$m_d = \sum_j m_{sg,j} + \sum_i d_i \quad (19)$$

and:

$$\chi_{sg} + \chi_{cw} + \chi_{st} = 1 \quad (20)$$

Density of sugar solutions has been reported by Honing (1953). Assuming water is mainly present as a solvent for the sugars, considering that sugar solutions tend to reach high degrees of oversaturation and they approach asymptotically the density of solid sugar, Eq (21) can be used over the entire range of moisture content:

$$\rho_{sn}(X) = 0.997 \exp\left(0.413 \frac{\chi_{sg}}{X + \chi_{sg}}\right) \quad (21)$$

The other intervening densities are regarded as constant, neglecting swelling due to water sorption. The corresponding values (Kirk-Othmer, 1964) are:

$$\rho_{st} = 1.64 \times 10^3 \text{ kg/m}^3$$

$$\rho_{cw} = 1.55 \times 10^3 \text{ kg/m}^3$$

By systematic addition of volume contribution of constituents, it is possible to predict the bulk shrinkage coefficient with the following expression:

$$s_b = |\beta \frac{X}{X_o} + \gamma(X)|\xi \quad (22)$$

where:

$$\beta = [1 + \frac{\chi_{sg}}{X_o} + \frac{\rho_{sn,o}}{X_o} M]^{-1} \quad (23)$$

$$\gamma = \frac{(\chi_{sg} + \rho_{sn}(X) M)\beta}{X_o} \quad (24)$$

$$M = \frac{\chi_{cw}}{\rho_{cw}} + \frac{\chi_{st}}{\rho_{st}} \quad (25)$$

$$\xi = \frac{1 - \epsilon(X_o)\ \rho_{sn}(X_o)}{1 - \epsilon(X)\ \rho_{sn}(X)} \quad (26)$$

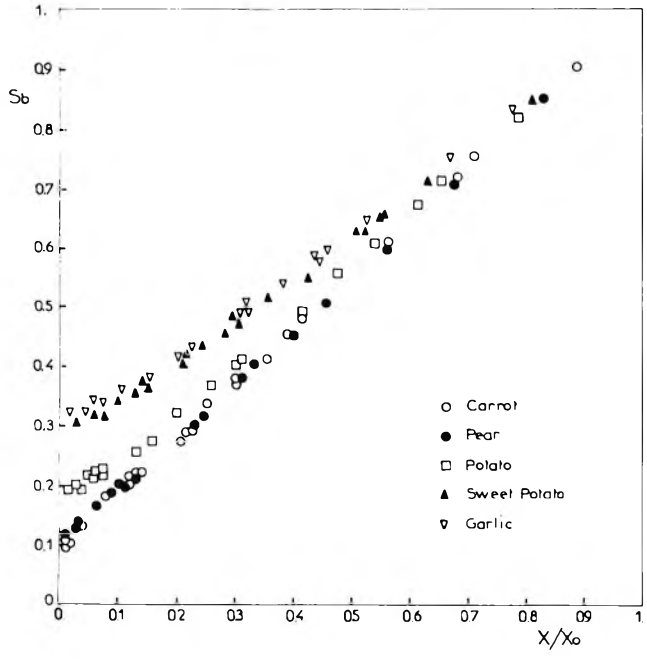

Fig. 5—Bulk shrinkage coefficient of different foodstuffs as a function of moisture content.

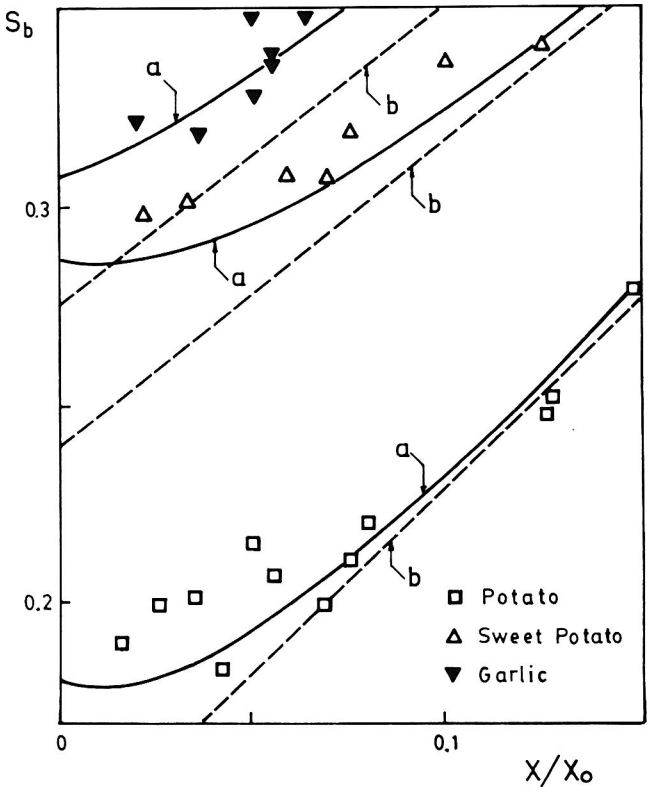

Fig. 6—Prediction of bulk shrinkage coefficient at low moisture contents. (a) Eq (22); (b) linear extrapolation of Eq (22) through $0.2 < X/X_o \leq 1$.

The pore volume was added through use of porosity values. In the case of carrots total porosity, as described above, was considered.

Eq (22) requires a knowledge of porosities to predict bulk volumes and as such it is not very useful. Fortunately, sample calculations show that: $\xi \rightarrow 1$ in the range; $0.01 < (X/X_o) < 1$.

Thus, Eq (11) can be approximated by:

$$s_b = \beta \frac{X}{X_o} + \gamma (X) \tag{27}$$

Expanding Eq (21) in the form of a power series and substituting it in Eq (22), it is possible to write s_b as a direct function of moisture content:

$$s_b = B + C\frac{X}{X_o} + D\left(\frac{\chi_{sg}}{X + \chi_{sg}}\right) + F\left(\frac{\chi_{sg}}{X + \chi_{sg}}\right)^2 \tag{28}$$

where:

$$B = \frac{0.997\, M + \chi_{sg}}{X_o + \chi_{sg} + M\, \rho_{sn,o}} \tag{29}$$

$$C = \left(1 + \frac{\chi_{sg}}{X_o} + \frac{\rho_{sn,o}}{X_o} M\right)^{-1} \tag{30}$$

$$D = \left(\frac{X_o + \chi_{sg}}{M} + \rho_{sn,o}\right)^{-1} \tag{31}$$

$$F = 0.085 \tag{32}$$

The error in s_b with respect to Eq (27) is less than 1% for $X = 0$, which is the most unfavourable case.

Fig. 6 shows in more detail the behavior of Eq (22) at low moisture contents ($0 < X/X_o < 0.15$). It can be seen that the proposed correlation does show the significant decrease in the rate of change of s_b, while a linear extrapolation would fail to do so.

While Suzuki's and Kilpatrick's equation fit the s_b data reasonably well at high X/X_o values, they fit the data less well than the presently proposed s_b vs X/X_o correlation when $X/X_o < 0.15$. Moreover, they fail to indicate the curvature in s_b vs X/X_o which is encountered when $X/X_o < 0.15$. Fig. 7 shows the case of sweet potato, to illustrate this point.

Having shown that Eq (27) is a good predictive correlation for foodstuffs where χ_{sg}, M and X_o are known, a reasonable task is to see whether a more general expression can be built on its basis so as to predict the bulk shrinkage coefficient starting from less information about the foodstuff. Fig. 8 shows that the final value of the bulk shrinkage coefficient at $X = 0$ for the different foods is a function of X_o. The representative correlation, with $r^2 = 0.991$, is:

$$s_{b,f} = \frac{0.966}{X_o + 0.796} \tag{33}$$

Fig. 5 shows that the s_b vs (X/X_o) lines are ordered in a particular fashion. The ordering is such that smaller s_b at $X/X_o = 0$ corresponds to larger initial moisture content. The separation between lines increases as X/X_o decreases. It follows that all s_b points could be made to coincide in one by defining a function of $[1 - (X/X_o)]$. A suitable function to accomplish this end is:

$$s_b = 0.161 + 0.816\frac{X}{X_o} + 0.022 \exp\left(\frac{0.018}{X + 0.025}\right) + f\left(1 - \frac{X}{X_o}\right) \tag{34}$$

where f is related to the bulk shrinkage coefficient at $X = X_o$, Eq (31), through the equation:

$$f = 0.209 - s_{b,f} \tag{35}$$

Fig. 9 is a plot of the experimental values adjusted as suggested in Eq (34), $s_b - f[1 - (X/X_o)]$, as a function of X/X_o. It also shows the lines corresponding to Eq (34). It can be seen that the fit of experimental values to the correlation is quite satisfactory, the coefficient of determi-

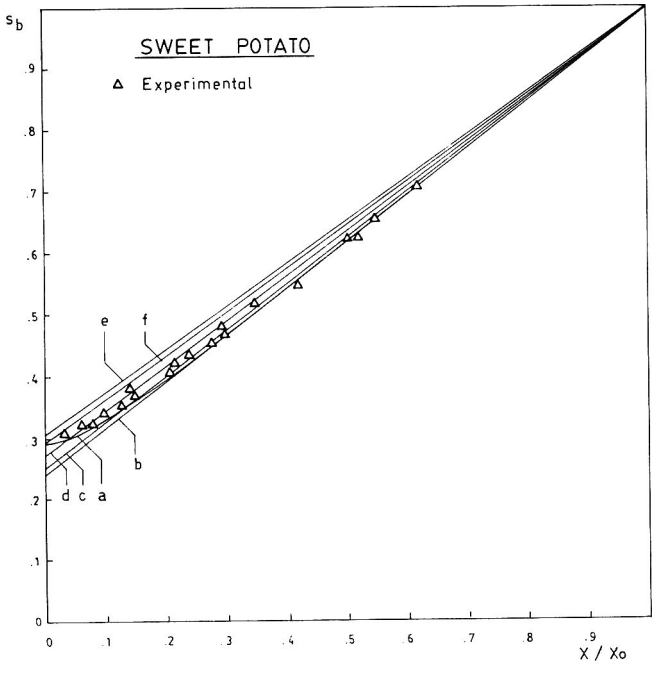

Fig. 7—Bulk shrinkage coefficient of sweet potato as a function of moisture content. (a) Eq (22); (b) Eq (7); (c) Eq (8); (d) Eq (9); (e) Eq (10); (f) Eq (11).

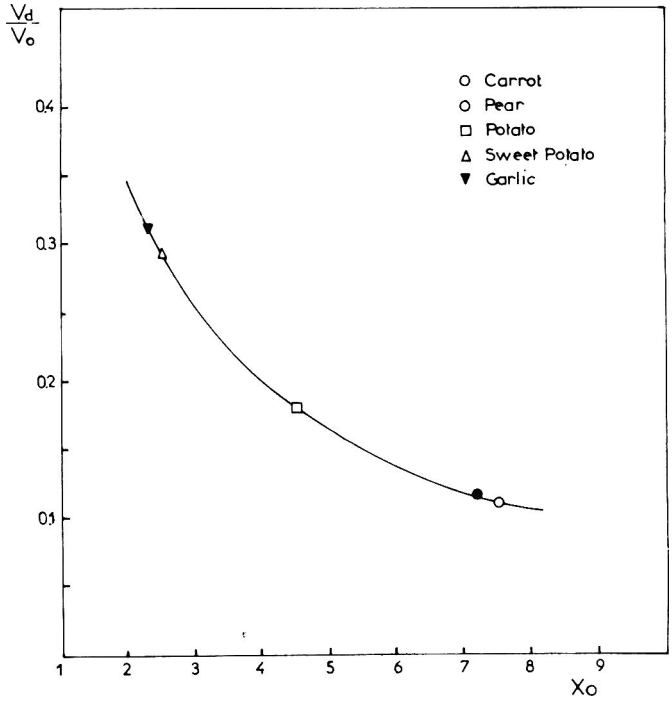

Fig. 8—Bulk shrinkage coefficient of different foodstuffs at X = 0.

nation being $r^2 = 0.97$. Data for onions from other authors (Mazza and Lemagner, 1980), plotted in the same figure, also fit the proposed correlation well. Thus, it can be stated that Eq (33), (34) and (35) provide the means to predict s_b accurately in the entire range $0 < X < X_o$ for a variety of foods, requiring only a knowledge of the fresh food moisture content.

The generalization cannot be extended with equal success to the modeling of porosity. It is possible to write Eq (12) in terms of the constituents as:

$$\epsilon = 1 - \frac{(X + 1)}{(X_o + 1)} \rho_{b,o} \frac{1}{s_b \rho_b} \qquad (36)$$

The initial bulk density can be approximated to an average $\rho_{b,o} = 1.019 \times 10^3$ kg/m^3, which represents any of the foodstuffs tested with less than 3% error. A way to generalize s_b has been discussed above. But the difficulty appears when considering ρ_p (X), which varies widely in value and behavior from one foodstuff to the other, as shown in Fig. 2. An attempt to obtain a predictive equation on the basis of water content and composition, results in:

$$\epsilon = 1 - \frac{(M + \frac{\chi_{sg}}{\rho_{sg}} + \frac{X}{\rho_{sn}})\rho_{b,o}}{(X_o + 1)\, s_b} \qquad (37)$$

As shown in Fig. 4, the results provide an approximation for garlic, sweet potato and potato; the prediction of values for pear is poor and it fails to predict the porosity of carrots with changing X (Fig. 3).

A valid question is how sensitive the data are to different drying conditions and sample shape. Data by Kilpatrick et al. (1955), Suzuki et al. (1976), and Mazza and Lemagner (1980) are quite close to the data reported here. All authors used conventional air drying. Kilpatrick et al. (1955) did not report sample shape or drying conditions, although they referred to tunnel drying. Suzuki et al. (1976) used 40°C dry bulb temperature and 30% relative humidity and air at 0.6–0.7 m/sec. Mazza and Lemagner (1980) used 40.5–60°C, an unreported relative humidity and 0.30–0.55 m/sec. Thus, as long as it is conventional air drying and changes in drying conditions are not too drastic, the results are valid. As far as the authors know, there are no similar data available for other drying procedures. As to the influence of sample shape, this paper reports data corresponding to cylinders 1 cm in diameter, 4 cm long and, in the case of garlic, there are data in addition corresponding to slicing the original cylinder. Suzuki et al. (1976) used 1 in. cubes; Mazza and Lemagner (1980) dried onion slices. The implication is that the correlations suggested are not sensitive to shape.

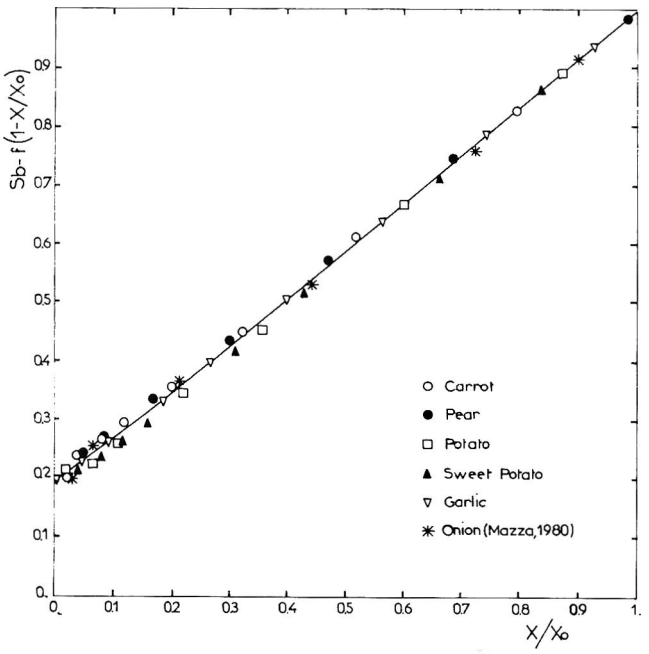

Fig. 9—Shrinkage coefficient generalized correlation.

Notation

a = constant defined in Eq (8)
A = area enveloping volume V, m^2
b = constant defined in Eq (9)
B = constant defined in Eq (22)
c = constant defined in Eq (9)
C = constant defined in Eq (23)
d = mass of nonsugar constituent, kg
D = constant defined in Eq (24)
f = adjusting parameter, defined in Eq (28)
F = constant defined in Eq (25)
h = constant defined in eq (9)
K = constant defined in Eq (10)
ℓ = constant defined in Eq (9)
m = mass of constituent, kg
M = constant defined in Eq (20)
n = outward normal vector, m
n′ = constant defined in Eq (11)
p = constant defined in Eq (9)
q = constant defined in Eq (9)
r = constant defined in Eq (11)
s = shrinkage coefficient, m^3/m^3
t = time, s
x = length, m
X = moisture content, kg/kg dry matter
V = volume, m^3
w = interphase velocity, m/s
W = wet sample weight, kg
z = fictitious length, m

α = specific linear shrinkage coefficient
β = constant defined in Eq (18)
γ = constant defined in Eq (19)
ϵ = porosity, m^3/m^3
ξ = relationship defined by Eq (21)
ρ = density, kg/m^3
ϕ = relative humidity
χ = constituent concentration, kg/kg dry matter
ψ = typical transport property

Subscripts

b = bulk
cw = cell wall material
d = dry matter
e = equilibrium
f = final
o = initial
p = particle
sg = sugar
sn = sugar solution
sp = sweet potato
st = starch

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—Continued on page 1553

Date Bars Fortified with Soy Protein Isolate and Dry Skim Milk

W. N. SAWAYA, J. K. KHALIL, W. J. SAFI, and H. A. KHATCHADOURIAN

ABSTRACT

Fortification of plain and chocolate coated date bars with soy protein isolate (SPI) and dry skim milk (DSM) in different proportions (1.5% SPI + 10.5% DSM; 3.0% SPI + 7.0% DSM and 4.5% SPI + 3.5% DSM) resulted in an increase of protein, fat, fiber, ash, Na, K, Ca, Mg, P, Zn, and all the essential amino acids. The chemical score increased from 48 to a range of 83–95. The in vitro protein digestibility (IVPD) values (77.8–81.8%) and calculated protein efficiency ratios (C-PERs) of the fortified bars (2.40–2.47) were close to the IVPD (90.0%) and C-PER (2.50) of ANRC-casein. In general, sensory evaluation indicated no difference in the control and fortified date bars, which were stored up to six months. 1.5% SPI + 10.5% DSM was the optimum level of supplementation in terms of nutritive benefit.

INTRODUCTION

DATES (*Phoenix dactylifera* L.) are the most important food crop in many countries of the Middle East. The Kingdom of Saudi Arabia, for example, is considered to be one of the major producers of dates in the world with an annual production of over 300,000 tons (Anonymous, 1978). Dates are extremely popular in Saudi Arabia for their delicious taste and good storability which extends their availability throughout the year. The large surplus quantities of dates available in the Kingdom and their popularity among the whole population justifies the possibility of utilizing dates in diversified food formulations which are compatible with the local taste and habits and which might be marketable in the international sector. Several studies have been carried out to incorporate dates in different food products (Zim et al., 1981; Mohammad and Ahmad, 1981; Khatchadourian et al., 1983). However, dates are considered rich in calories with 80% carbohydrates and relatively low in protein content (about 3%). Therefore, one approach for the improvement of their nutritional quality could be to supplement dates with inexpensive high-protein ingredients that can lead to final products which are high in both protein and carbohydrates. The addition of animal proteins to foods to fortify their proteins qualitatively and/or quantitatively offers the most versatile method of fortification. However, the increasing costs and the shortage of animal proteins, especially in many developing countries, has initiated a search for new protein sources that can partially or completely replace the animal proteins in foods. Recently, increased emphasis was placed on formulating blends of dairy and vegetable proteins that display a variety of functional properties and retain the nutritional value of milk proteins (Nichols and Cheryan, 1982). Dry skim milk (DSM), single cell proteins (SCP) and soy proteins (SP) have shown promise as successful high-protein ingredients in the fortification of different foods for human consumption (Bressani and Marenco, 1963; Senti, 1969; Hallab et al, 1974; Bressani et al, 1979). Kamel and Kramer (1977) fortified dates with single-cell proteins and dry skim milk and prepared high-protein date bars. The use of SCP in foods, is, however, limited to a certain level because of its high nucleic acid content.

The objective of the present study was to fortify dates with soy-protein isolate and dry skim milk for the purpose of preparing enriched date bars which would be popular especially among children.

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MATERIALS & METHODS

Date bar processing

Tamr dates of the soft Ruzeiz variety and almond kernels were purchased from the local market. 'Argo' corn starch was obtained from CPC International, Inc. (New Jersey), dry skim milk (DSM) (37% protein) from Arinco (Denmark), and soy-protein isolate (SPI, Supro-620, 90% protein) from Ralston Purina (St. Louis, MO). The date bars were processed at the Date Pilot Plant, Hofuf, Saudi Arabia. The air-dried dates were first washed with an automatic belt-driven water sprayer and the water was removed by sieving. The cleaned dates were soaked in potable water to a moisture content of 22–23%. They were then automatically pitted and macerated to produce a paste. The paste was ground and mixed thoroughly with starch and chopped almonds in a Hobart Mixer to obtain a homogenous mixture for the preparation of control date bars (B_1). A mixture of DSM and SPI was added in different proportions to produce bars containing 1.5% SPI + 10.5% DSM (B_2), 3.0% SPI + 7.0% DSM (B_3) and 4.5% SPI + 3.5% DSM (B_4), (W/W), each formulated to contain about 10% protein. The protein ingredients were included at the expense of corn starch. These four mixtures were used to prepare the desired date bars each 4 cm long and 2 cm thick. Part of the bars from each lot were hand dipped in milk chocolate to give two series of products, plain date bars and chocolate date bars. All the bars were allowed to air dry, wrapped in cellophane and packed in small cartons. They were then stored at 7°C and 25°C for 6 months. The final recipe for the various date bars is shown in Table 1.

Chemical analyses

Proximate analysis for moisture, crude protein (N x 6.25), crude fat, crude fiber, ash and carbohydrates (by difference) was carried according to standard procedures outlined in AOAC (1975). Food energy was calculated by multiplying carbohydrates, fat and protein contents by 4, 9 and 4 kcal/g, respectively.

Analyses for nine nutritionally important mineral elements, Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn were done by ashing about 2g

Table 1—Recipes for the unsupplemented control and protein supplemented date bars

Ingredients (g)	Date bars[a]			
	Unsupp. control (B_1)	Supplemented date bars		
		(B_2)	(B_3)	(B_4)
Date paste	70	70	70	70
Almonds	11	11	11	11
Corn starch	19	7	9	11
Soy protein isolate (SPI)	–	1.5	3.0	4.5
Dry Skim Milk (DSM)	–	10.5	7.0	3.5
Total (g)	100	100	100	100

[a] Two categories, plain and chocolate-coated date bars were produced.

sample and dissolving the ash in 5 ml of 20% HCl and making the volume to 25 ml with deionized water. Na and K were determined with a flame photometer (Beckman, Klina flame). Ca, Mg, Fe, Cu, Zn and Mn were determined with an atomic absorption spectrophotometer (Perkin-Elmer, 603). Phosphorus was determined spectro-photometrically with Spectronic-20 (Bausch and Lomb, Belgium) by the method of Watanabe and Olsen (1965). The final dilution for Ca and Mg contained 1% lanthanum to overcome interferences, especially by phosphates.

For the amino acid analysis, the fat-free and freeze-dried samples were hydrolysed with 6N HCl for 24 hr at 110°C (Moore and Stein, 1963). The S-containing amino acids were obtained using a performic pretreatment of samples followed by hydrolysis of the samples with 6N HCl as before (Moore, 1963). Tryptophan was released using an alkaline hydrolysis with sodium hydroxide (Hugli and Moore, 1972). All the hydrolysates were analysed on an amino acid analyser (Beckman, 120C). Amino acid scores were calculated by dividing the contents of the essential amino acids in the test proteins by the contents of the same amino acids in the reference pattern (FAO/WHO, 1973).

In vitro protein digestibility (IVPD) and calculated protein efficiency ratio (C-PER)

The in vitro digestibility of the proteins were determined using the multienzyme automatic recording technique of Hsu et al. (1977) with the modification suggested by Satterlee et al. (1979). The C-PER was determined from the IVPD, and the essential amino acid composition as described by Satterlee et al. (1979).

Sensory evaluation

All the date bars were presented to a panel of 12 semi-trained judges selected from colleagues at the Research Center. The test was an 'affective' type test where the preference for taste, texture and overall acceptability of the products was evaluated by a 9-point hedonic scale where a score of 9 represented 'like extremely' and 1 'dislike extremely'. The tests were carried out in a taste panel room using partitioned booths. The products were presented to the panelists on trays which contained the control as well as the test products for each category of treatment. The data so obtained were analyzed by the analysis of variance method (Snedecor and Cochran, 1980). The F-values were tested for their significance at the 5% level of probability.

Table 2—Chemical composition[a] of date bars (dry basis)

Nutrient	Date bars[b] + B_1 (control)		B_2		B_3		B_4	
	P	C	P	C	P	C	P	C
Moisture %	19.6	15.6	16.6	14.0	17.6	15.6	17.3	14.7
Protein (N x 6.25) %	5.3	4.9	12.1	10.8	11.5	10.7	11.8	11.0
Fat %	6.1	9.8	6.9	13.5	7.1	13.3	6.5	13.9
Fiber %	2.2	1.9	5.4	4.3	4.6	4.2	3.4	2.7
Ash %	1.8	1.8	3.3	2.4	2.5	2.5	2.3	2.1
NFE[c] %	84.6	81.6	72.3	69.0	74.3	69.3	76.0	70.3
Na mg/100g	40	49	195	94	106	92	87	79
K mg/100g	568	550	884	628	825	692	622	557
Ca mg/100g	87	110	207	211	149	184	137	146
P mg/100g	168	159	328	267	261	244	249	229
Mg mg/100g	73	70	94	82	87	80	82	73
Fe mg/100g	6.2	5.3	4.2	3.9	5.5	5.1	6.2	4.8
Zn mg/100g	1.2	1.2	1.2	1.2	1.3	1.3	1.3	1.3
Cu mg/100g	0.5	0.4	0.5	0.3	0.5	0.5	0.8	0.6
Mn mg/100g	0.5	0.4	0.4	0.4	0.5	0.4	0.5	0.4
Food energy (kcal/100g)	415	434	400	441	407	440	410	450

[a] Means of duplicate determinations (variation = <5%).
[b] For B_1-B_4 refer to Table 1. P indicates plain and C indicates chocolate bar.
[c] NFE = Nitrogen free extract (carbohydrates).

RESULTS & DISCUSSION

Chemical composition

The chemical composition of Ruzeiz variety of dates used in the present study has been reported from this laboratory (Sawaya et al., 1983). The dates contained 3.1% protein, 0.3% fat, 4.2% fiber and 2.6% ash on dry basis. The almond kernels contained 18.5% protein, 5.8% fat, 2.67% fiber and 3.5% ash. The inclusion of 11% almond kernels in the plain date bar (control) increased the protein content by approximately two fold and practically all the fat content was contributed by almonds. Since almonds are liked and consumed with dates locally, they were included in the basic formulation. The results of the chemical composition of date bars are shown in Table 2. The control plain date bar (B_1) contained the lowest protein content (5.3%) which increased by more than 100% in the fortified date bars (B_2–B_4) as a result of SPI and DSM addition in different proportions. The fat content showed a comparatively smaller increase (7–16%), but the crude fiber increased by 55–145% and ash by 28–83% in the fortified plain bars. Although carbohydrate contents of the fortified date bars decreased slightly, their energy contents were generally higher than the control, mainly due to higher amounts of fat in the fortified bars. The inclusion of milk chocolate showed a slight dilution effect on the protein, crude fiber and ash contents of the fortified date bars, due to lower concentrations of these nutrients in milk chocolate as compared to the high protein ingredients (Watt and Merrill, 1963). On the other hand, the fat contents of the chocolate bars were higher than those of the corresponding plain date bars because of a higher fat content of milk chocolate in comparison to the protein supplements. Similarly, the data in Table 2 would indicate that the concentrations of all the mineral elements studied increased as a resut of fortification with the high protein ingredients. Sodium increased by 118–388%, potassium 10–56%, calcium 57–138%, magnesium 12–29%, phosphorus 48–95% and zinc 3–10%. These increases were achieved because both SPI and DSM were better sources of these elements than dates (Watt and Merrill, 1963). The amount of iron in the control plain and chocolate bars was unexpectedly higher than that in the fortified bars. The reason for this is not clear and might be due to some contamination during the preparation of this batch of bars. In the fortified bars, however, as SPI increased in bars relative to DSM (B_3–B_4), the iron content increased, probably due to higher Fe content of the SPI relative to DSM, which is known to have a very low iron content. The amounts of copper and manganese in all the bars did not show a large variation. The mineral contents of the plain bars, in general, were reduced slightly, on a percentage basis, when dipped in milk chocolate, because milk chocolate contained lower amounts of these minerals compared to SPI + DSM (Watt and Merrill, 1963). Chocolate bars, however, were found to contain higher amounts of calcium than plain bars.

When expressed in terms of the Recommended Dietary Allowances (RDAs) of the Food and Nutrition Board, NRC/NAS (USA) (Anonymous, 1980), 100g of the moisture-free control date bars and fortified bars would furnish 11–14% and 17–26% of the RDA for Ca, 20–21% and 29–41% for P, 28–29% and 29–38% for Mg, 53–62% and 39–62% for Fe, 12% and 12–13% for Zn, 16–20% and 12–32% of the daily requirement for Cu, and 13–17% and 13–17% for Mn, respectively, for children up to 10 years of age.

Amino acid composition, IVPD and C-PER

The essential amino acid composition, IVPD and C-PER values of the various date bars are reported in Table 3. Ly-

sine, threonine, isoleucine and leucine were the only essential amino acids that fell short of the FAO/WHO pattern (1973) in the unsupplemented control date bar (plain). Fortification with the high protein ingredients (SPI + DSM) increased the contents of these amino acids to adequate levels. Milk chocolate also showed a beneficial effect on these amino acids. In general, the amino acid profiles of the fortified bars were more balanced than the control date bar. The leucine to isoleucine ratio in all the date bars was also favorable and compared well with that of FAO/WHO pattern (1973). The chemical score of the protein in plain date bar (control) was 48, with lysine, threonine (chemical score = 77) and isoleucine (chemical score = 88) being the first, second and third limiting amino acids, respectively. The chocolate date bar (control) showed a higher chemical score of 71 with lysine as the first limiting amino acid, suggesting that milk chocolate considerably improved the lysine deficiency. However, milk chocolate did not increase the protein content, hence the need for the addition of protein ingredients was obvious. The data indicated that, from a practical standpoint, lysine was the only essential amino acid showing a critical deficiency in the date bars. Fortification of the date bar resulted in an increase of lysine content and, hence, an increase in the chemical score from 48 to 83–95 for the fortified bars (B_2–B_4). The increase in the chemical score by approximately twofold, coupled, with a similar increase in the protein content, significantly improved both the protein quality and quantity of the fortified bars over that of the unfortified bars.

IVPD and C-PER

The IVPD and C-PER values of the various date bars indicated an improvement in the digestibility and C-PER values of the plain date bar (control) when supplemented with different proportions of high-protein ingredients. The digestibility of the control date bar improved from 74.8% (B_1) to a range value of 77.8–81.8% in the fortified bars (B_2–B_4), an increase of 4–9%. The C-PER of the plain date bar (control) significantly increased from 1.24 to a range of 2.40–2.41 for the fortified bars (100% increase) relative to a casein value of 2.50. Milk chocolate also had a beneficial effect on both the digestibility and C-PER values of the plain date bars.

The data on digestibility and C-PER further confirmed the observed increases in the essential amino acids and the chemical score of the fortified bars and the subsequent improvement in the protein nutritional quality of the fortified bars thus approaching that of casein.

Sensory evaluation

Results of the mean sensory scores for taste, texture and overall acceptability for the plain date bars (Table 4) and chocolate date bars (Table 5) showed that the inclusion of SPI + DSM in different proportions did not affect significantly ($P > 0.05$) the acceptability of both the plain and chocolate date bars. Similarly, storage of both the plain and chocolate date bars up to 6 months at 7°C and 25°C did not influence significantly ($P > 0.05$) the overall acceptability of these bars compared to their respective control bars. A comparison of the mean scores of both the control plain date bar (B_1-plain) and control chocolate date bar (B_1-chocolate) for the three characteristics evaluated showed no significant difference at the 5% level of probability, although the chocolate bars were generally scored higher than the plain bars.

Even though the overall acceptability scores of all the date bars (plain and chocolate) were not significantly different from their respective control bars, the taste score of B_2-plain, stored for 3 months at 7°C, was significantly ($P < 0.05$) lower than that of the control at the same storage period and temperature (Table 4). This may be due to the subjective differences shown by some of the panelists because this quality deterioration effect was not observed in other plain date bars stored at the same temperature for the same period of time, as well as in those stored at 7°C and 25°C for a higher period of time (6 months). Likewise, the texture scores of B_3-chocolate (Table 5), stored at 7°C and 25°C for 6 months, were also significantly lower than the score at the initial time. However, these scores were not found significantly different ($P > 0.05$) from the texture scores of the control (B_1) stored under identical conditions. Evidently, as the sensory evaluation for texture did not reveal differences, it can be accepted that the functional properties were not changed significantly as a result of the addition of the supplements.

CONCLUSIONS

BASED ON THE RESULTS in this study, it can be stated that the addition of SPI + DSM in proportions shown above can enhance the protein quality and quantity of date bars without any deleterious effect on their acceptability. A level of 1.5% SPI + 10.5 DSM is the optimum level for supplementation in terms of the nutritive benefits such as the increase in the protein quantity and quality that can be derived from supplementation of date bars. Such a product is anticipated to augment the efforts in the date producing countries to update the nutritional status by introducing a more nutritious food for the pre-school and

Table 3—Essential amino acid composition, chemical score, IVPD and C-PER of date bars[a]

	B_1 (control)		B_2		B_3		B_4	
	Plain	Chocolate	Plain	Chocolate	Plain	Chocolate	Plain	Chocolate
Isoleucine	3.50	4.24	4.49	4.49	4.46	4.66	4.18	4.42
Leucine	6.80	8.00	8.47	8.64	8.37	8.65	7.95	8.23
Lysine	2.66[b]	3.90[b]	4.58[b]	5.25[b]	4.77[b]	5.21	4.82[b]	5.22
Methionine (M)	1.22	1.28	2.52	2.36	2.10	2.12	2.14	2.01
Cystine (C)	2.29	2.12	1.47	1.19	1.39	1.13	1.44	1.19
M + C	3.51	3.40	3.99	3.45	3.49	3.25[b]	3.58	3.20[b]
Phenylalanine (P)	4.49	4.78	4.97	5.22	5.26	5.13	5.32	5.07
Tyrosine (T)	2.17	3.40	3.51	3.86	3.60	3.72	3.29	3.45
P + T	6.66	8.18	8.48	9.08	8.86	8.85	8.61	8.52
Tryptophan	1.12	1.01	1.12	1.11	1.06	1.01	1.08	1.07
Valine	6.96	6.29	5.65	5.57	5.56	5.63	4.94	5.27
Chemical score[c]	48	71	83	95	87	93	88	91
IVPD	74.8	75.7	77.8	79.3	80.4	79.4	81.8	81.1
C-PER	1.24	1.89	2.41	2.47	2.41	2.43	2.40	2.43

[a] Average of duplicate determinations.
[b] First limiting amino acid.
[c] Based on FAO/WHO (1973) reference pattern.

Table 4—Sensory evaluation scores of plain date bars[a] at different times and temperatures

Storage conditions	Plain date bars											
	B_1 (Control)			B_2			B_3			B_4		
	Taste	Texture	Overall	Taste	Texture	Overall	Taste	Texture	Overall	Taste	Texture	Overall
Initial (zero time)	6.9	6.9	6.8	6.9	7.0	6.9	6.9	6.9	6.6	6.5	6.8	6.5
1 Month												
7°C	7.0	6.8	7.1	6.7	6.8	6.8	6.8	6.8	7.0	6.5	6.9	6.8
25°C	7.1	6.5	6.7	6.8	7.0	6.7	6.8	6.9	7.0	6.8	7.0	6.7
3 Months												
7°C	6.9	6.4	6.5	5.8[b]	6.4	6.2	6.6	6.5	6.5	6.9	6.8	6.8
25°C	7.2	6.7	6.6	6.5	6.3	6.1	6.8	6.8	6.6	6.7	6.9	6.7
6 Months												
7°C	6.4	6.7	6.2	6.1	6.7	6.4	6.1	6.1	5.7	6.3	5.9	6.0
25°C	6.3	5.8	6.0	6.1	6.6	6.2	6.6	6.6	6.0	6.1	6.3	6.2

[a] For B_1—B_4 refer to Table 1. Results are the means of 12 semi-trained judges.
[b] Significantly different ($P < 0.05$) value from the control (B_1) at the same temperature and time of storage (horizontal comparison).

Table 5—Sensory evaluation scores of chocolate date bars[a] at different times and temperatures.

Storage conditions	Chocolate date bars											
	B_1 (Control)			B_2			B_3			B_4		
	Taste	Texture	Overall	Taste	Texture	Overall	Taste	Texture	Overall	Taste	Texture	Overall
Initial (zero time)	7.3	7.4	7.3	7.3	7.3	7.2	7.4	7.6	6.4	7.1	7.2	6.7
1 Month												
7°C	7.9	7.8	7.8	7.8	7.5	7.8	7.2	7.6	7.2	7.0	6.8	7.0
25°C	7.2	6.8	7.0	7.4	7.5	7.5	7.3	7.1	7.2	7.1	6.9	7.0
3 Months												
7°C	6.8	6.9	6.7	6.4	7.0	7.0	7.2	7.3	6.8	6.6	6.9	6.6
25°C	7.0	7.0	6.8	7.0	6.3	6.5	6.6	6.8	6.5	6.6	7.1	6.5
6 Months												
7°C	6.3	6.4	6.5	6.8	6.8	6.8	6.7	6.0[b]	6.3	6.7	6.2	6.5
25°C	6.9	6.6	6.6	6.8	6.8	6.8	6.8	6.3[b]	6.5	6.1	6.3	6.2

[a] For B_1—B_4 refer to Table 1. Results are the means of 12 semi-trained judges.
[b] Significantly different ($P < 0.05$) values from the initial scores (vertical comparison).

school-age children. The development of high protein date bars might find potential in opening a new channel for introducing date-based products in the international market. Chocolate date bars like other chocolate-based products, are usually transported and stored under refrigeration, hence their nutritive value is not expected to be affected much during storage and distribution.

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Tubular Heat Exchanger Fouling by Milk During Ultra High Temperature Processing

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ABSTRACT

The effect of milk fouling deposits on the heat transfer rate of a tubular heat exchanger was investigated for varying process heat treatments. Product input and discharge temperatures were maintained constant while steam temperature increased as deposits formed. Processing conditions included product entrance temperatures of 347.6 and 366.4°K for heater exit temperature of 410.7°K and product velocity of 1.58 m/s; 363.9 and 366.7 for 410.7 and 2.70; 364.6 for 410.7 and 3.25; 370.6 and 357.8 for 427.4 and 1.58; 378.5 for 427.4 and 2.70; and 379.5 and 376.3 for 427.4 and 3.25. Predictive expressions representing product deposition were determined for process variables, heating curve variables, and equivalent times and temperatures representing the thermal treatment. Product constituent losses during the fouling process and aspects of fouling kinetics were examined.

INTRODUCTION

MILK DEPOSITS on heated surfaces have long been recognized as a normal byproduct of milk processing. When product deposition (fouling) occurs on heat exchanger surfaces three problems occur. First, processing rates may be reduced. Deposit may restrict the flow of product or deposit may decrease the heat transfer rate to the extent that product flow rate must be reduced if processing temperature is to be maintained. Second, product quality is often impaired. And third, removal of the deposited materials is often a difficult part of the cleaning operation. This problem can be as serious as having to shut down to clean after only 2 hr of operation or having to flush with acid after 2 hr to extend the run time (Casey and Swientek, 1982). The formation of the deposits result in increased steam consumption and appearance of solid particles in the final product (Jelen, 1981). To minimize formation of the deposits heating media temperature is usually limited to a few degrees above product temperature (Farrall, 1963). Since heat exchange area must be increased if heating media temperature is reduced, this adds to the size and expense of the heating equipment. An additional technique known in industry to extend run time calls for lengthtening the preheat by adding a holding section to the preheater. Some processing plants have found it more economical to add a second sterilizer in parallel to their existing one than to shut processing completely down for cleaning (Jelen, 1981). In this arrangement one sterilizer is cleaned while the other continues the processing. These approaches are expensive practical answers to a difficult industry problem.

In an effort to understand the basic mechanism investigators have given attention to isolating product and process variables which are contributing factors to the deposition problem. Product variables that have been related are content (Gynning et al., 1958; Burton, 1961, 1968), pH (Gynning et al., 1958; Burton, 1965, 1968; Lalande and Corrieu, 1981; Claesson et al., 1974), seasonal variations (Burton, 1967), "age" of the milk (Burton, 1968), and ammonia concentration (Lalande and Corrieu, 1981). Compositional variations between milk-based products (flavored milks, ice cream mix, etc.) have been discussed because increased viscosities lead to an increased probability of non-uniform heating, low heat transfer rates and an increased product depositional rate (Jelen, 1981).

Single process variables have been associated with fouling rates for a variety of products. Among the most common process variables are product velocity (Bunchero and Gordon, 1960; Perry, 1963; Gynning et al., 1958; Thonie, 1958; Kern, 1966, Morgan et al., 1959; Gonionskiy et al., 1970; Crozier, 1982), exposure time (Burton, 1961), wall temperature (Morgan et al., 1959; Gonionskiy et al., 1970; Burton, 1968; Lalande and Corrieu, 1981), processing temperature (Thonie, 1958; Burton, 1961) and bulk fluid temperature (Morgan et al., 1959; Taborek et al., 1972; Kern, 1966).

Some researchers have combined process variables such as heating surface – product temperature difference (Thonie, 1959) and heating media – product temperature difference (Gynning et al., 1958; Gonionskiy et al., 1970; Kern, 1966). Others have made use of nondimensional groups such as the Reynolds number (Lund and Bixby, 1975). Dimensionless functions of average deposition temperature, hot temperature difference, average milk velocity and temperature of pasteurization have also been examined (Lalande and Corrieu, 1981). The heat exchanger wall surface has been shown to affect the deposition rate as noted by Kern (1966), Morgan et al. (1959), Taborek et al. (1972), and Ling and Lund (1978b).

Pretreatment has been shown to affect the rate of fouling. Bell and Sanders (1944), Morgan et al. (1959), Jelen (1981) and Ball (1977) have all noted that preheating product reduces the rate of increased resistance in the heat exchanger. Lund and Bixby (1975) demonstrated that by prefouling a heat exchanger with one species of foul deposit a reduction of deposit by other sources could be achieved.

With a variety of product and process variables shown to contribute to product deposition, development of basic mechanisms has been a major unresolved problem for researchers. Many of the variables or functions of variables are interrelated. Attempts to reduce contributing variables in statistical models have tended to reduce the usefulness of the model (Lalande and Corrieu, 1981). General considerations for a possible mechanism were proposed by Burton (1968). He suggested that two separate processes occur. First, the temperature effect produces a condition where milk solids are no longer in true solution. At this state they either absorb to a surface or aggregate. He proposed that the presence of an available surface would determine if the solids form deposit in the heat exchanger or sediment in the processed product. However, Burton (1968) noted that the literature was conflicting as to the relation of the degree of product deposition during processing to sediment formation during storage.

Having a site available for deposition may depend on many of the variables listed above. Crozier (1982) noted that if the stress at the wall exceeded the bond strength

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of the deposit the deposit will not form. Lalande and Corrieu (1981) investigated fouling kinetics during milk pasteurization with a plate heat exchanger. The fouling kinetics was identified as a half order autocatalytic reaction. Three main operational variables (pasteurization temperature, milk velocity and hot temperature difference) were used to formulate an empirical expression for the fouling rate. A calculated average deposition temperature alone was able to explain 79% of fouling rate variation. An Arrhenius plot of fouling rate versus average deposition temperature indicated an activation energy of fouling of 96 ± 21 kJ/mol. This low activation energy led them to dismiss protein denaturation as a rate controlling reaction for product deposition. However, Hegg and Larsson (1981) using ellipsometry techniques determined that protein denaturation is one important factor involved in the fouling of milk components in heat exchangers.

Since many of the product and process variables are dependent on one another conflicting conclusions have occurred when dependent variables have been treated as independent ones. Lack of adequately understanding the process is a major factor making discussion of fouling rate so difficult. Also many studies cited above refer to pasteurized temperatures while some refer to UHT ranges. Factors controlling the rate may be different in different temperature ranges.

The objective of this study was to investigate the depositional phenomenon of milk solids on the heated surface during UHT processing. Also it was the intent of this study to develop models for predicting run time and changes in the thermal effects (constituent losses) throughout the processing.

MATERIALS & METHODS

STANDARDIZED LOW FAT winter milks were processed in a Model XLV No-Bac Unitherm tubular heating UHT system (Cherry-Burrell, Cedar Rapids, Iowa). Temperatures at three locations were monitored at two or five minute intervals. These locations were the second heater product entrance, second heater product exit, and steam inlet to the second heater. The product entrance and exit temperatures for each condition were maintained constant. Steam temperature increased automatically as required to maintain the desired exit temperature. The second heater product exit temperature was set at 410.7 and 427.4°K. For each of these two temperatures product velocity was adjusted to 1.58, 2.70 and 3.25 m/s. Product temperature at the second heater entrance was held at a constant value for each run. These temperatures were 347.6 and 366.4°K for second heater product exit temperature of 410.7, and velocity of 1.58 m/s; 363.9 and 366.7 for 410.7 and 2.70; 364.6 for 410.7 and 3.25; 370.6 and 357.8 for 427.4 and 1.58; 378.5 for 427.4 and 2.70; and 379.5 and 376.3 for 427.4 and 3.25 m/s.

Standardized raw product was evaluated for several physical characteristics. Standard plate counts were determined as described by Foster et al. (1957). A Fisher Model 292 pH meter (Pittsburgh, PA) was utilized to determine pH. Fat and total solids percentages were evaluated using the Mojonnier test (Mojonnier Bros. Co., Chicago, IL). Dissolved oxygen concentration was determined by means of a YSI Model 51B oxygen meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). The meter was calibrated with aerated milk prior to testing. The hardness (as Ca CO_3) of the water used for sterilizing was also evaluated as described by Sawyer and McCarty (1967). The heat exchanger had an internal conventional finish of #4 or better (Engineering Department, Cherry-Burrell, Cedar Rapids, Iowa).

Each run was operated with a single pass of product operating with a maximum of 5300 liters of product available or to the limit of the steam supply (450°K). When the steam supply reached this level product temperature at the exit of the heat exchanger began to drop. This point in time was considered the limit of operation. Because of the volume of product and expense required, runs were not duplicated. Each run was made with a different batch of milk.

RESULTS & DISCUSSION

THE LOG of the standard plate count on the raw milk ranged from 3.58–5.94 with a mean ($\bar{x}$) = 4.67 and standard deviation (s) = 0.80. The standardized fat level tested $\bar{x}$ = 2.17%, s = 0.09. Total solids was $\bar{x}$ = 11.0%, s = 0.32. The pH of the raw product was tested to yield $\bar{x}$ = 6.66, s = 0.04. Dissolved oxygen content of the raw product was $\bar{x}$ = 6.08 ppm, s = 0.79. City water used for sterilizing was tested to be very soft (12 ppm hardness as Ca CO_3).

The constant heat input for each run was established as

$$q = \dot{m}\, c_p\, \Delta T \qquad (1)$$

where c_p was taken as 4.00×10^3 J/kg K (Fernandez-Martin, 1972).

The overall resistance to heat flow (Ri) was then determined by

$$Ri = \frac{1}{U} = \frac{A\,\Delta T_m}{q} \qquad (2)$$

with U being the overall heat transfer coefficient.

The fouling resistance (RF) was established as

$$RF = (Ri)\Big|_{t_r = t} - (Ri)\Big|_{t_r = 0} \qquad (3)$$

Multiple linear regression was utilized to evaluate single and/or multiple variables which relate as predictors to resistance in the heat exchanger. From the wide range of variables suggested from the literature a multitude of predictive equations were attempted. The predictor shown in Table 1 gave the lowest standard error and highest coefficient of determination (r^2). The variable $(1/v)^{0.8}$ was suggested as a velocity function from a Wilson plot (Anon, 1977) and represents the inverse of the dependence of the heat transfer coefficient on Reynolds number. Although many other dependencies on velocity were attempted, $(1/v)^{0.8}$ resulted with the smallest coefficient of variability. Utilizng these same variables of t_r, $(1/v)^{0.8}$, PT and

Table 1—Equations for predicting fouling based on monitored process variables in a tubular heat exchanger

Model	Predicted expression	r^2	Std. error of estimate
Fouling resistance m^2 Khr/MJ	RF = − 0.877 (X1)		0.0129
	+ 0.115 (X1) (X2)		0.0061
	+ 0.002 (X1) (X3)	0.97	2.9E-5
	+ 0.004 (X1) (X4)		0.0003
	− 0.009 (X1) (X2) (X4)		0.0005
Degree of K temperature rise for steam supply (°K/hr)	ΔT_s = −110.47 (X1)		1.547
	+ 11.29 (X1) (X2)		0.736
	+ 0.255 (X1) (X3)	0.96	0.003
	+ 0.810 (X1) (X4)		0.039
	− 1.432 (X1) (X2) (X4)		0.063

With: X1 = t_r; X2 = $\left(\frac{1}{v}\right)^{0.8}$; X3 = PT; X4 = $(\Delta T_m)_{t_r = 0}$

Range of validity: $0 \leq t_r \leq 8.7$ hr; $1.57 \leq v \leq 3.24$ m/sec; $410.7 \leq PT \leq 427.4$°K; $6.1 \leq \Delta T_m \leq 16.4$°K

$(\Delta T_m)_{t_r = 0}$ Table 1 also contains the correlation with the temperature change of the steam supply established as

$$\Delta T_s = (T_s)\Big|_{t_r = t} - (T_s)\Big|_{t_r = 0}. \quad (4)$$

This prediction expression correlates the temperature rise of the steam supply with run time. Length of run is then determined at the point where the steam generator's capacity is reached.

Although the expressions of Table 1 are good predictors they give few clues as to the mechanism of fouling. However, the four variables do constitute some definition of the heat treatment. Improved definition of the heat treatment given to the product may lead to a better understanding of the fouling mechanism One method of defining the heat treatment during heating is to define the heating curve. For each run and t_r the heating curve was established from

$$\text{Ln}\,(T_s - T_\theta) = \text{Ln}\,(T_s - T_o) - \left(\frac{UA}{\omega c_p}\right)\theta. \quad (5)$$

Table 2 depicts the standard errors and the coefficient of determination for predicted expressions for RF and ΔT_s. Dependent variables include the intercept and abscissa (θ) of Eq. (5) while the slope is dependent on the characteristics of the system. Combinations of these three basic variables along with t_r make up the other variables which comprise the nine terms. Within the range of validity the equations in Table 2 constitute good predictors of RF and ΔT_s. The equations in Table 2 better predict RF than do the variables in Table 1 because they better explain how the heating occurs from T_o to PT. The heating curve may rise rapidly and level off at or near T_s. This would constitute a high heat treatment. For a low heat treatment the curve may rise gradually from T_o and not reach PT until just before the end of the heat exchanger. For given T_o and PT this would correspond with a lower fouling rate. Increasing product velocities would alter the slope of the curve from a rapid riser to a gradual one but there would also be more product per unit time and $\Delta\Gamma_m$ would be altered. However, changing velocity may be the same as changing θ.

By using a method developed by Swartzel (1982) thermal reduction relationships (G values) were established for the heating section for each run and t_r as

$$G = \frac{1}{2.303N}\left[E_1\left(\frac{r}{1+b}\right) - E_1\left(\frac{r}{1+be^{-N\theta'}}\right)\right] - \frac{e^{-r}}{2.303N}\left[E_1\left(\frac{r}{1+b} - r\right) - E_1\left(\frac{r}{1+be^{-N\theta'}} - r\right)\right] \quad (6)$$

where $r = \frac{E_a}{RT_s}$ and $N = \frac{UA}{\omega c_p}$.

A different and distinct G for each different processing condition and t_r for each E_a selected was determined. The relationship G has also been defined for a holding section where product is held for a given time at a specified temperature as

$$G = \frac{t}{2.303}e^{-E_a/RT} \quad (7)$$

For each heating section for each selected E_a used in Eq. (6) an infinite number of time-temperature conditions would match the determined reduction level G. By plotting the determined Log (t) versus T for each E_a value for each heating condition a straight line results. Swartzel (1982) demonstrated that these lines representing different E_a values for a distinct indirect heating system all uniquely intersect at one point. The intersection of the E_a values represent the equivalent time and temperature where the heat treatment of the heating section is totally defined for determining reduction levels of any constituent. This is true since the intersection point is independent of the E_a value. Table 3 summarizes predicted expressions for RF and ΔT_s utilizing the equivalent times (t_E) and temperatures (T_E) established from Eq. (6) and (7). Interestingly only T_E and t_E at $t_r = 0$ and t_r are required to explain 96–97% of the variation. Since with a build-up of deposition the heating curve drops (indicated by arrow of t_r in Fig. 1), T_E and t_E correspondingly are reduced through t_r. The models in Table 3 indicate that milk fouling of tubular heat exchangers in the ultra high temperature range represent linear fouling where the fouling rate predominates over the removal rate. No induction period exists. Since fouling through t_r was linear, a rate equation was obtained by taking the first derivative of the two expressions with respect to t_r. Fig. 2 depicts these rate relations graphically.

Table 2—Equations for predicting fouling from heating curve variables for a tubular heat exchanger

Model	Predicted expression			r^2	Std. error of estimate
Fouling resistance m^2 Khr/MJ	RF =	53.80	(X1)		1.356
		− 27.19	(X1) (X2)		0.499
		− 25.69	(X1) (X3)		0.664
		0.24	(X1) (X4)	0.99	0.006
		28.32	(X1) (X5)		0.507
		3.46	(X1) (X6)		0.089
		− 0.0027	(X1) (X7)		6.2E-05
		0.36	(X1) (X2) (X4)		0.0083
		− 0.095	(X1) (X3) (X4)		0.0022
Degree K temperature rise for steam supply (°K/hr)	ΔT_s =	8870.5	(X1)		168.49
		−4057.5	(X1) (X2)		62.07
		−4297.1	(X1) (X3)		82.51
		+ 44.48	(X1) (X4)		0.700
		+4224.6	(X1) (X5)	0.99	62.95
		+ 581.36	(X1) (X6)		11.05
		− 0.425	(X1) (X7)		0.007
		+ 55.42	(X1) (X2) (X4)		1.0256
		− 16.53	(X1) (X3) (X4)		0.267

With: $X1 = t_r$; $X2 = \frac{UA}{\omega c_p}$; $X3 = \text{Ln}(T_s - T_o)$; $X4 = \theta$; $X5 = (X2)^2$; $X6 = (X3)^2$; $X7 = (X4)^2$

Range of validity: $0 \leq t_r \leq 8.7$ hr; $0.15 \leq X2 \leq 0.55$ sec^{-1}; $410.3 \leq T_s \leq 452.4$°K; $346.5 \leq T_o \leq 423.4$°K; $5.26 \leq \theta \leq 14.76$ sec.

By determining the equivalent time and temperature at $t_r = 0$ the rate of increase of RF and the rate of increase of ΔT_s may be easily determined. For a given T_E, d (RF)/d (t_r) increases with increasing t_E. However $d(\Delta T_s)/d(t_r)$ decreases with increasing t_E at constant T_E. The product is exposed to the higher temperatures for longer periods of time. This does result in an increase in the fouling resistance but as resistance increases the heating curve drops exposing product to the higher temperatures for less time. This process will continue until the PT at the end of the heating curve begins to drop. At this point T_s increases. The longer it takes to drop the heating curve the lower $d(\Delta T_s)/d(t_r)$. With an increase of T_E with constant t_E both $d(RF)/d(t_r)$ and $d(\Delta T_s)/d(t_r)$ increase.

Because an increase of RF lowers the heating curve, t_E and T_E decrease with t_r. Predicted expressions for the variation of t_E and T_E through t_r are shown in Table 3. The models demonstrate a slight difference between calculated t_E and T_E values at $t_r = 0$ and the actual values. The models were not fixed at the actual values at $t_r = 0$ because the rate of decay was slightly greater for the first few minutes of operation and better models for the entire process were possible with this treatment of the data. This decrease of t_E and T_E through t_r results in decreased heat treatment and reduces the constituent losses through the heat exchanger. To illustrate, F_o values can be calculated from

$$F_o = (t_E)_{min}\, 10^{(T_E - 394.1\ K)/10K} \qquad (8)$$

by substitution of t_E and T_E and conversion to Kelvin temperature from the basic lethal rate equation given by Pflug (1980). As an example, if the heating curve at $t_r = 0$ corresponded to $t_E = 11$ sec and $T_E = 420°K$, then F_o would be 71.32 min. If the steam generator capacity will only allow for a ΔT_s of 26°K then the system would have to be shut down after 6.75 hr or begin to lose PT ($d(\Delta T_s)/d(t_r) = 3.85°K/hr$). At $t_r = 6.75$ hr T_E would equal 416.11°K; $t_E = 7.85$ sec; and $F_o = 20.81$ min. If the generator capacity were greater, t_r could be extended as the exponential heating curve approached linearity. A substantial drop in the F_o value does occur. Using $z = 10°K$ over this wide temperature span is certainly in question. But as long as traditional methods of evaluating sterilization processes are in use and until better kinetic data are available in the literature, F_o values over this wide temperature span will suffice.

Attempts at determining a fouling E_a value have been made in the literature. However, this concept as demonstrated by Lalande and Corrieu (1981) and Ling and Lund (1978b) is questionable. The increase of resistance in both studies was determined from a run time basis whereas E_a

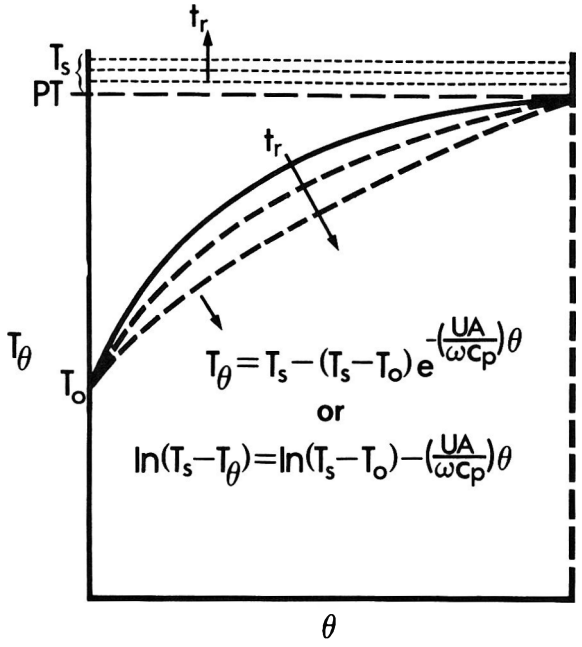

Fig. 1—Typical heating time-temperature curves for indirect UHT processing. The arrow direction of t_r indicates the direction that the heating curve moves from time zero (solid line) as run time continues (dashed lines).

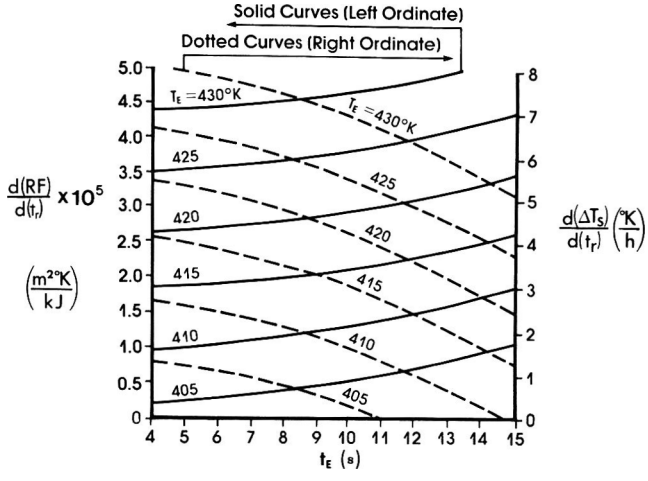

Fig. 2—Fouling resistance rate $d(RF)/d(t_r)$, and steam temperature difference rate of change $d(\Delta T_s)/d(t_r)$, as a function of equivalent time (t_E) and temperature (T_E) representing the thermal treatment in an indirect heat exchanger.

Table 3—Equations for predicting fouling based on equivalent times and temperatures of the heat treatment in a tubular heat exchanger

Model	Predicted expression	r^2	Std. error of estimate
Fouling resistance m^2 Khr/MJ	RF = −0.00156 (X1) (X2)		8.63E-5
	+3.86E-6 (X3) (X2)	0.97	2.10E-7
	+4.119E-5 (X4) (X2)		1.00E-5
Degree K temperature rise for steam supply (°K/hr)	ΔT_s = −0.2444 (X1) (X2)		0.0116
	+6.13E-4 (X3) (X2)	0.96	2.83E-5
	−0.0135 (X4) (X2)		1.54E-3
Equivalent time (sec)	t_E = −0.345 (X2)	0.98	0.084
	+ .9259 (X5)		0.025
Equivalent temperature (°K)	T_E = −0.451 (X2)	0.99	0.1069
	+ .998 (X1)		0.0007

With: X1 = $(T_E)_{t_r = 0}$, (°K); X2 = t_r, (hr); X3 = $(X1)^2$, $(°K)^2$; X4 = $(X5)^2$, sec^2; X5 = $(t_E)_{t_r = 0}$, sec

values are based on exposure time. The fact that only 79% of the variation could be explained using average deposition temperature or temperature of pasteurization indicates that these temperatures are only scale approximation of the total heat treatment of the product through the heat exchanger. This may also help explain why researchers do not agree on the extent of the effect of protein denaturation to increases of RF. As reviewed, the literature is conflicting with regard to the individual importance of the many reactions occurring during the fouling phenomenon. Each reaction would have its own E_a value which under varying conditions may become the rate controlling reaction.

In an effort to investigate this process further Ln (G) values from Eq. (6) were plotted versus E_a values for each run establishing thermal reduction levels. At an intersection (same E_a value) of two runs the thermal reduction levels (Ln G) are equal. If that E_a value corresponded to the E_a values associated with the fouling rate then the two runs would have similar fouling rates. The runs with similar fouling rates are the ones with lower T_E and t_E combinations. Their intersections are all at negative E_a values. The highest intersection point for all runs was E_a = 140 kJ/mol. Since all resistance rates were different it is logical to expect the E_a value associated with product deposit to be greater than 140 kJ/mol. As we increase the E_a value in Eq. (6) for the determination of Ln (G) the coefficient of determination becomes higher when modeling ln (G) versus the fouling resistance. This may be explained by the fanning out of the lines in a plot of Ln (G) versus E_a for the runs after the last intersection of 140 kJ/mol. The developed model for fouling resistance rate is $d(RF)/d(t_r) = -0.7173 (Ln\ G) - 0.007245 (Ln\ G)^2$ for Ln G evaluated at E_a = 335 kJ/mol where $r^2 = 0.95$ and standard errors are 0.0422 for the linear term and 0.00044 for the curvilinear term (units on $d(RF)/d(t_r) = m^2{}^\circ K/kJ \times 10^{-5}$). A good estimate of the fouling rate can be obtained from a single term, $(Ln\ G)_{t_{r=0}}$, utilizing a large E_a value for a tubular heating section. As surface conditions, shear stress at the wall, and product properties are varied attachment to available sites may be altered possibly leading to varied fouling rates for the same heat treatment. Availability of sites may control sediment formation in the finished product as proposed by Burton (1968). Additional work relating fouling rates with formation of sediment for varying surface, product and heater configurational conditions are under investigation.

NOMENCLATURE

Symbol	Quantity Represented	Units
A	Heat transfer surface area	m^2
b	$(T_o - T_s)/T_s$	Dimensionless
c	Specific heat	J/kg K
E_a	Activation energy	J/mol
$E_1(\alpha)$	$\int_\alpha^\infty \frac{e^{-t}}{t} dt$ (Function representing exponential integral)	–
F_o	Thermal death time for a microorganism at 394.11°K that is characterized by z = 10°K	min
G	Product constituent reduction relationship	sec
N	$(UA/C_p\ \omega_p)$	s^{-1}
$\dot{m}$	Mass flow rate	kg/hr
PT	Process temperature (desired temperature at the end of the heat exchanger)	K
q	Heat flux at the heat exchanger wall	W/m^2
R	Universal gas constant (8.314)	J/mol K
Ri	Overall resistance to heat flow	m^2 K hr/kJ
RF	Resistance due to fouling	m^2 K hr/kJ
r	E_a/RT_s	Dimensionless
$d(RF)/d(t_r)$	Rate of change of RF/unit hr	m^2K/kJ
t	Time	units of sec or hr
T	Temperature	°K
$d(\Delta T_s)/d(t_r)$	Rate of change of T_s/unit hr	°K/hr
U	Overall heat transfer coefficient	J/m^2 hr°K
v	Product velocity	m/sec
z	°K temperature change required to change the thermal death time by a factor of 10	°K
ω	Mass of flowing product in heat exchanger	kg
ΔT	Temperature difference	K
θ	Mean residence time through the heat exchangers, also used as a subscript	sec
θ'	Total mean residence time	sec

E	Equivalent
m	Log mean
0	Initial condition (t = 0)
p	Product
r	Run (when used with t refers to the time at some point into the run)
s	Steam

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Chemical and Sensory Assessment of Nonspawning Capelin (*Mallotus villosus*) Subjected to Long Term Frozen Storage

J. R. BOTTA, J. T. LAUDER, A. P. DOWNEY, and W. SAINT

ABSTRACT

Nonspawning capelin (*Mallotus villosus*) were processed immediately after being caught, after 5 days of iced storage or after being frozen at sea and later thawed. Fish from all variables were stored at −23°C for up to 21 months. Both pre-processing treatment and frozen storage time significantly ($P \leq 0.05$) affected all sensory variables except appearance. Regardless of treatment, the sensory quality was very stable during early and intermediate stages of frozen storage; capelin processed at sea was superior to that of the other two treatments. Appearance, dimethylamine, trimethylamine, moisture and thiobarbituric acid values were dependent upon an interaction between pre-processing treatment and frozen storage time. Hypoxanthine concentration had the greatest potential as an index of sensory quality.

INTRODUCTION

THE LONG TERM TOTAL allowable catch (TAC) of capelin on the Labrador and Grand Banks is estimated to be approximately 200,000 tons per year (Anon. 1981). Recently the largest use of Newfoundland caught capelin and the major reason for the fishery has been the sale of frozen roe or frozen capelin containing roe to Japan (Anon. 1982). Although spawning capelin are also suitable for human consumption in other than roe products, the spawning process may lead to quality defects (Anon. 1982; Botta and Shaw 1978; Jangaard 1974; Shaw and Botta 1975, 1977; Templeman 1948). Nonspawning capelin are not subject to these defects and have been reported to have an iced storage life superior to that of spawning capelin (Botta et al., 1978).

Any future utilization of nonspawning capelin by the Canadian processors would probably involve processing on land after chilled storage at sea or freezing at sea followed by thawing and processing ashore. Depending on the market conditions, the final frozen product could be stored for a substantial period of time.

The present investigation was undertaken to assess the combined effects of pre-processing treatment and length of frozen storage on the chemical and sensory characteristics of nonspawning capelin.

MATERIALS & METHODS

Raw materials

Nonspawning capelin were caught along the Northeast coast of Newfoundland and Labrador (51°19'N x 55°21'W) on Oct. 28, 1977. Processing consisted of beheading, gutting, washing and packaging the fish in 454g capacity waxed cardboard boxes, then freezing in an air blast freezer at −20°C. One lot of round nonspawning capelin received no treatment prior to processing and served as a control for the study. A second lot of round capelin was stored in ice (two parts fish to one part ice) for 5 days prior to processing. A third lot of round fish were, immediately after capture, packed in 7.5 kg blocks, air blast frozen at −20°C, stored at −23°C for 4 months and thawed in running tap water prior to processing. Immediately after processing all samples were stored at −23°C for up to 21 months. At periodic intervals, three samples for chemical assessment and four samples for sensory assessment were taken from each of the three treatment combinations, wrapped with an oxygen impermeable film and immediately transferred to −62°C until analyzed. Good quality control samples consisted of fish processed shortly after catching and upon reaching port immediately wrapped with an oxygen impermeable film and transferred to −62°C.

Sensory assessment

The samples were placed inside two 4.5 kg capacity polyethylene bags and thawed in water at 20°C for 2 hr. They were baked in aluminum trays covered with aluminum foil for 14 min at 230°C in a conventional oven, transferred to glass petri dishes (one fish per dish), and served using an electric warming tray. The evaluations were conducted in partitioned booths with daylight fluorescent lighting. The judges used room temprature distilled water for rinsing between samples. In general, samples were evaluated within 15 min of cooking.

An analytical panel of six judges (employees of other sections) all trained in differentiating appearance, flavor, odor, texture and overall acceptability of frozen nonspawning capelin, evaluated each sample using a 5-point descriptive scale (Table 1). Training consisted of 12 sessions using fresh, moderately degraded and slightly spoiled (during storage at −5°C) nonspawning capelin and continued until all the judges agreed with the terms shown in Table 1. A score of 5 indicated the highest quality whereas a score of 1 indicated the lowest and represented complete unacceptability for human consumption. The scores of the six judges were averaged for each sample giving four observations per pre-freezing treatment per storage time or 96 observations in all.

Chemical assessment

Dimethylamine (DMA) was determined by the copper dithiocarbamate procedures of Dyer and Mounsey (1945), trimethylamine (TMA) by the picric acid procedure of Dyer (1945) with potassium hydroxide as the alkalizing agent (Tozawa et al., 1971), thiobarbituric acid (TBA) value by the trichloracetic acid extraction method of Vyncke (1970), and moisture using the hot-air oven method described by AOAC (1975).

Hypoxanthine concentration was determined by the xanthine oxidase method of Jones et al. (1964) modified (Hiltz, private communication) by centrifuging (10,000 rpm for 20 min) at 0°C rather than filtering at 0°C, in order to shorten the time required to conduct an analysis.

All chemical analyses were conducted in duplicate on each of the three boxes of frozen capelin per pre-freezing treatment per storage time.

Statistical analyses

Analyses of variance were conducted on the chemical and sensory variables using a two-way analysis of variance with interaction of the pre-processing treatment and storage time means as the fixed main effects. If the pre-processing treatment x storage time interacton means were not significant ($P \leq 0.05$), the sums of squares and degrees of freedom were pooled with the error sums of squares and degrees of freedom, resulting in a two-way analysis of variance without interaction. If this analysis of variance indicated that either pre-processing treatment or storage time significantly ($P \leq 0.05$) affected the variable, the Studentized Range Test (Snedecor and Cochran, 1980) was conducted to determine differences within each main effect. Correlation coefficients were determined using the pre-processing treatment x storage time interaction means of the variables, remembering that a correlation coefficient indicates only

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an association and not a cause and effect relationship. Unless otherwise stated, "significant" means significant at the 5% level.

RESULTS & DISUSSIONS

Sensory assessment

The odor, flavor, texture and overall acceptability scores of round capelin stored at −23°C or in ice prior to processing were significantly lower than that of capelin receiving no treatment prior to processing (Table 2). The odor, flavor, texture and overall acceptability of capelin stored in ice prior to processing did not significantly differ from that of capelin stored at −23°C prior to processing (Table 2).

Although frozen storage time significantly affected overall acceptability of the samples, no significant change occurred until the samples had been stored for 9 months and these samples were not significantly different from those which had been stored 3 months, which, in turn, did not significantly differ from the samples stored for 21 months (Table 3). Somewhat similar trends were also observed with flavor, odor and texture storage time means (Table 3). The effect of preprocessing treatment and length of frozen storage time on appearance of the cooked samples was not clear, as there was a significant treatment x storage time interaction (Fig. 1). Examination of these interaction means revealed that, in general, the appearance means of samples receiving no pre-processing treatment were larger than those of the two treated samples (Fig. 1). The relationship between the appearance of the samples stored in ice prior to processing and that of samples stored at −23°C prior to processing was very unclear and no conclusions could be made (Fig. 1). Particularly with the treated samples, the effect of length of frozen storage time was also unclear (Fig. 1).

Chemical assessment

With all chemical variables analyzed, the effect of preprocessing treatment and length of frozen storage was not clear, as there was a signifiant pre-processing treatment x frozen storage time interaction with each variable (Fig. 2 and 3, Tables 4 and 5).

Examination of the interaction means for both DMA and TMA concentration revealed that these values were almost always very low (below 1.0 mg per 100g) (Table 4), an indication that the changes were of minor importance. In addition, with both variables, there were no consistent trends of these changes (Table 4). In general, the DMA and TMA values of the present study were within the

Table 1—Sensory assessment terms used to describe sensory quality of cooked non spawning capelin

Sensory variable	Numerical rating				
	5	4	3	2	1
Appearance	Desirable, silver sheen to skin, white flesh, sl oily	Mod desirable, sl loss of sheen, sl discoloration of flesh, sl oily	Sl desirable, v little sheen discolored flesh, sl bellyburn, sl oily	Sl undesirable, darker flesh, substnatial bellyburn, mod oily	Undesirable, no sheen, off-color, mushy, partially decomposed, oily
Flavor	Fresh capelin flavor, sl sweet, no off-flavor, sl oily	Almost neutral, sl sweet sl off-flavor sl oily	Almost neutral, sl stale, sl ammonical, sl aftertaste, sl oily	Mod oily ammonical flavor, sl bitter, stale aftertaste	Putrid bitter flavor, sl rancid, very stale, oily
Odor	Fresh capelin odor	Loss of fresh odor, almost neutral with sl off-odor	Sl stale, sl fishy, sl musty	Stale, fishy, musty, sl ammonical odor	Putrid, sl rancid, strong ammonical odor, very fishy
Testure	Moist, tender, flaky, juicy	Sl coarse, sl dry, sl stringy, sl tough	Coarse, dry stringy, tough	Sl mushy, sl oily, tough	Mushy, oily, sl decomposed, tough
Overall acceptability	Very good	Good	Fair	Slightly spoiled	Spoiled

Table 2—Treatment means and results of Studentized Range Test for sensory variables of nonspawning capelin stored at −23°C for up to 21 months[a]

Treatment prior to processing[b]	Sensory variable			
	Odor	Flavor	Texture	Overall acceptability
No treatment	3.62a	3.66a	3.65a	3.51a
Storage in ice for 5 days	3.24b	3.30b	3.23b	3.07b
Storage at −23°C for 4 months	3.30b	3.26b	3.31b	3.06b

[a] Means not sharing the same letter are significantly ($P \leq 0.05$) different from each other.
[b] n, for each treatment, = 32.

Table 3—Storage time means and results of Studentized Range Test for sensory variables of nonspawning capelin stored at −23°C for up to 21 months[a]

Months of storage[b]	Sensory variable			
	Odor	Flavor	Texture	Overall acceptability
0	3.81a	3.78a	3.81a	3.64a
3	3.72ab	3.67ab	3.68ab	3.40ab
6	3.57abc	3.64ab	3.58abc	3.35ab
9	3.12cd	3.26bc	3.25bcd	3.06b
12	3.17cd	3.28bc	3.17cd	3.01b
15	3.33bcd	3.31bc	3.29bcd	3.11b
18	3.11d	3.06c	3.08d	2.99b
21	3.28bcd	3.26bc	3.32bcd	3.13b

[a] Means not sharing the same letter are significantly ($P \leq 0.05$) different from each other
[b] n, for each storage time = 12

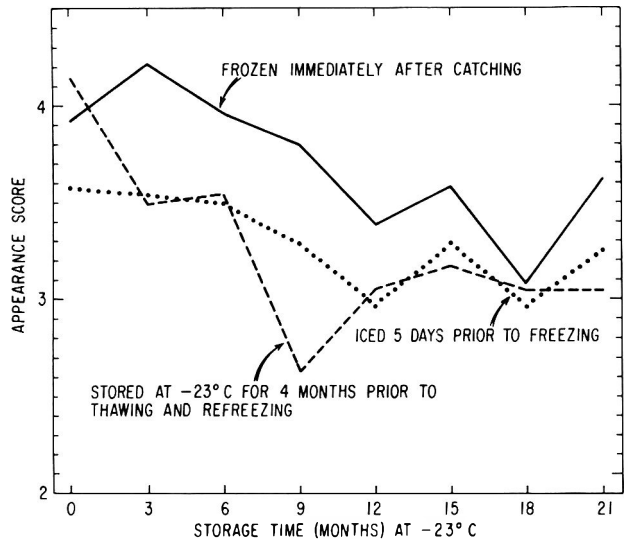

Fig. 1—Mean appearance score of nonspawning capelin stored at −23°C for up to 21 months.

range of those observed for frozen Newfoundland spawning capelin (Botta and Shaw, 1978; Shaw and Botta, 1977).

Inspection of the moisture interaction means revealed that the moisture content of the capelin processed without any prior treatment was relatively constant throughout frozen storage and somewhat lower than that of capelin stored in ice or frozen then thawed prior to processing (Table 5). The inital moisture levels were similar to that previously observed for nonspawning capelin (Botta et al., 1978). The mean moisture content of refrozen capelin was greater during the early stages of frozen storage than that of the other capelin, but during frozen storage, it decreased the most (Table 5). The moisture content of capelin stored in ice prior to processing was the most variable, but had an overall trend of little change (Table 5). The capelin appeared to gain moisture during both iced storage and water thawing which supports that previously observed for nonspawning capelin stored in ice (Botta et al., 1978).

Thiobarbituric acid (TBA) value interaction means were very variable and no meaningful trends emerged (Fig. 2).

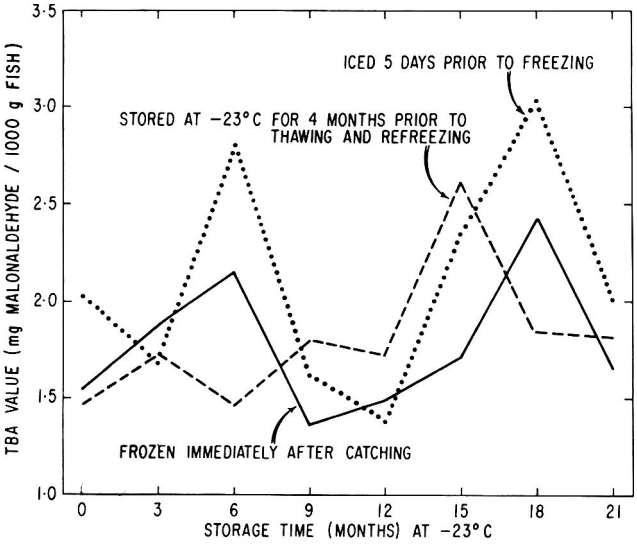

Fig. 2—Mean thiobarbituric acid (TBA) value of nonspawning capelin stored at −23°C for up to 21 months.

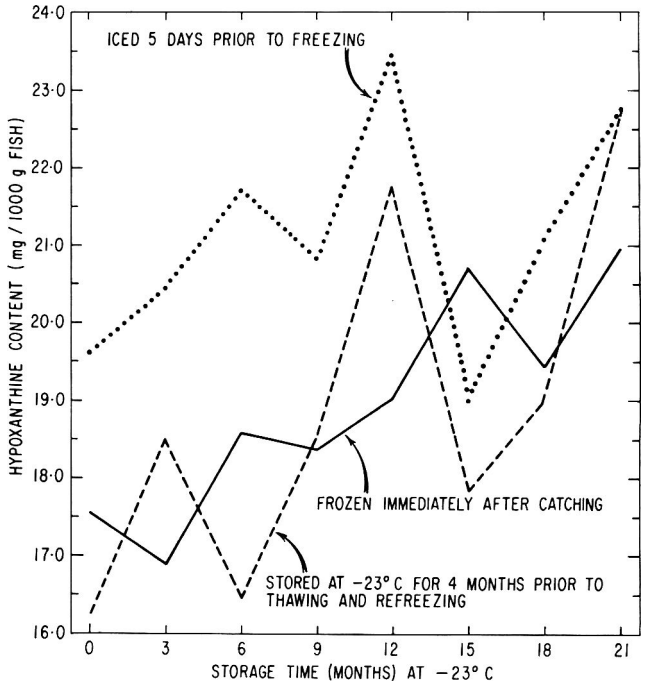

Fig. 3—Mean hypoxanthine concentration of nonspawning capelin stored at −23°C for up to 21 months.

Table 4—Pre-processing treatment x frozen storage time means for chemical variables for nonspawning capelin stored at −23°C for up to 21 months[a]

Months of storage	Mean DMA conc Mg DMA-N/100g muscle			Mean TMA conc Mg TMA-N/100g muscle		
	Stored at −23°C for 4 months prior to processing	No treatment prior to processing	Iced for 5 days prior to processing	Stored at −23°C for 4 months prior to processing	No treatment prior to processing	Iced for 5 days prior to processing
0	0.20	0.24	0.17	0.15	0.57	0.89
3	0.23	0.29	0.17	0.82	0.66	0.57
6	0.19	0.33	0.47	0.62	0.57	0.28
9	0.34	0.25	0.33	0.75	0.87	0.44
12	0.50	0.30	0.33	0.97	0.43	0.48
15	0.51	0.45	0.63	0.75	0.92	0.75
18	0.73	0.89	0.83	0.51	0.93	1.97
21	0.61	0.88	0.48	0.64	0.52	0.86

[a] n, for each mean, = 3.

In general, with capelin from the early stages of frozen storage, the present values were higher than those observed with mackerel stored at −18°C and at −28°C (Vyncke 1975). During the latter stages of frozen storage, a noticeable portion of the samples had TBA values greater than 2.0, a value regarded as the probable threshold limit for sensory detection of oxidative rancidity in fish (Connell, 1980). However, rancidity was not detected by the sensory evaluation panel. Similar disagreements have been observed with frozen spawning capelin (Shaw and Botta, 1977).

The hypoxanthine concentration of nonspawning capelin receiving no preprocessing treatment generally increased with frozen storage time (Fig. 3). This is similar to that observed with spawning capelin frozen immediately after catching (Shaw and Botta, 1977). The hypoxanthine content of samples receiving either preprocessing treatment varied so greatly among the different storage times that few meaningful trends were observed (Fig. 3). During the early and middle periods of frozen storage, the hypoxanthine content of samples whch had been iced five days prior to processing were noticeably higher than both the samples receiving no preprocessing treatment and the refrozen samples (Fig. 3). The hypoxanthine concentrations observed in the present investigation were within the range of those observed with frozen and refrozen spawning capelin (Botta and Shaw, 1978; Shaw and Botta, 1977) where it also increased in concentration during storage at −23°C. Hypoxanthine has also been observed to increase in concentration during frozen storage of other species (Connell and Howgate, 1969). Since comparatively little variability occurred with capelin whch were not treated prior to processing, the decreases in concentration observed with pretreated fish may be, at least partially, due to leaching of hypoxanthine from the muscle or bacterial oxidation during iced storage or water thawing (Kassemsarn et al., 1963).

Table 5—Pre-processing treatment x frozen storage time means of moisture content of nonspawning capelin sotred at −23°C for up to 21 months[a]

Months of storage	Mean Moisture Concentration (%)		
	Stored at −23°C for 4 months prior to processing	No treatment prior to processing	Iced for 5 days prior to processing
0	78.38	70.51	74.01
3	78.64	71.24	73.06
6	75.57	71.78	74.00
9	74.62	69.85	76.73
12	75.47	70.72	74.96
15	74.76	69.30	72.95
18	70.62	69.14	73.56
21	69.77	69.05	73.97

[a] n, for each mean, = 3.

CONCLUSIONS

IN GENERAL, sensory quality of nonspawning capelin was more affected by preprocessing treatment than by storage at −23°C. This was particularly true during the early and intermediate stages of frozen storage. Pre-processing treatment also substantially affected hypoxanthine and moisture content but, in both cases, length of frozen storage also very substantially affected both variables in samples from some of the treatments. Neither pre-processing treatment or length of frozen storage time substantially affected DMA or TMA concentration.

No one primary sensory variable (appearance, flavor, odor or texture) was a limiting factor concerning sensory quality. In fact, individually, each variable was not as readily affected by either pre-processing treatment or by frozen storage as was overall acceptability, suggesting a synergistic effect upon overall acceptability.

Although both pre-processing treatment and length of frozen storage significantly affected sensory quality, all samples were acceptable. Samples at zero month of frozen storage were rated fair/good whereas samples of 18 and 21 months of frozen storage were rated fair.

Although nonspawning capelin is a much fattier fish than spawning capelin (Botta et al., 1978), the present results indicate that the keeping quality of frozen nonspawning capelin is at least as good as that of spawning capelin (Shaw and Botta, 1977), and that the keeping quality of refrozen nonspawning capelin is moderately better than that of spawning capelin (Botta and Shaw, 1978).

Few of the chemical variables significantly correlated with most sensory variables (Table 6). The significant correlation coefficients (r) ranged from 0.420 − 0.566 giving coefficients of determination (r^2) of 0.176 − 0.320. Consequently, these chemical variables may be useful indicators of sensory quality but their practicality is questionable. Hypoxanthine concentration had the greatest potential as an index of sensory quality.

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Table 6—Correlation coefficients (r) of chemical variables versus sensory variables for nonspawning capelin[a]

Chemical variable	Sensory variable				
	Appearance	Odor	Flavor	Texture	Overall acceptability
Dimethylamine	−0.467*	−0.420*	−0.465*	−0.448*	−0.361[n.s.]
Hypoxanthine	−0.525**	−0.566**	−0.458*	−0.551**	−0.513*
Moisture	−0.076[n.s.]	−0.047[n.s.]	−0.093[n.s.]	−0.144[n.s.]	−0.236[n.s.]
Thiobartituric acid	−0.266[n.s.]	−0.288[n.s.]	−0.250[n.s.]	−0.316[n.s.]	−0.213[n.s.]
Trimethylamine	−0.357[n.s.]	−0.334[n.s.]	−0.317[n.s.]	−0.351[n.s.]	−0.244[n.s.]

[a] n = 24 in each case
* Significant at the 5% level ($P \leq 0.05$)
** Significant at the 1% level ($P \leq 0.01$)
n.s. Not significant at the 5% level ($P > 0.05$)

Ensilage Treatment of Crawfish Waste for Improvement of Astaxanthin Pigment Extraction

HUEI-MEI CHEN and SAMUEL P. MEYERS

ABSTRACT

Acid ensilage treatment preserves crawfish heat-processed waste under ambient temperature conditions with stabilization of the astaxanthin pigment present. Implementation of acid ensiling prior to pigment extraction increased concentration of the astaxanthin oil extract by 40–50%, and oil recovery by 10%. A twofold increase in free amino nitrogen, and a 70% reduction in exoskeleton calcium carbonate, were observed in crawfish silage (pH 4.2) from acid/enzymatic hydrolysis, compared with controls. A correlation was seen between solubilization of calcium carbonate and pigment release in relation to silage pH. The role of acid-resistant proteolytic microorganisms in breakdown of carotenoprotein complex is discussed. Application of ensilage process for ultimate commercial production of astaxanthin-enriched vegetable or fish oils from pigment rich crustacean wastes is postulated.

INTRODUCTION

PREVIOUS STUDIES have demonstrated the presence of significant concentrations of astaxanthin pigment in heat-processed waste from the Louisiana crawfish (*Procambarus clarkii*) industry (Meyers and Bligh, 1981; Chen and Meyers, 1982a). Biologically active characteristics of the crawfish waste and its oil-extracted pigment have been shown with aquaculturally raised rainbow trout (*Salmo gairdneri*), coho salmon (*Oncorhynchus kisutch*), the pearl gourami (*Trichogaster leeri*) and the American lobster (*Homarus americanus*) (Spinelli, 1982; Thibodeaux, 1981; D'Abramo et al., 1983). Furthermore, workers have demonstrated the value of crustacean meals as complementary pigment agents in broiler diets to impart desirable coloration to egg yolks in laying hens and quails (Nelson and Baptist, 1968; Chawan and Gerry, 1974).

The compositional profile of freshwater crawfish meal has been analyzed and reported to comprise 32.2% chitin-free protein, 4.9% ether extract, 29.0% ash, 14.2% crude fiber (chitin), 18.1% calcium, and 1.2% phosphorus (Lovell et al., 1968). Earlier investigations in our laboratories have indicated problems associated with the calcareous component of the crawfish waste in attaining energy-efficient extraction of the oil-soluble astaxanthin pigment. The susceptibility of this proteinaceous waste to putrefaction also requires application of an economically-effective preservation method for large scale utilization of the pigment and the resultant presscake. The industrially heat-dried crustacean meal contains relatively low concentrations of the astaxanthin pigment, thus necessitating inclusion rates in fish diets as high as 20% to impart desirable pigmentation (Saito and Regier, 1971). In addition, high levels of calcium may adversely affect the final nutritional value of such crustacean meals or substantially reduce its inclusion rate to <10% when formulated into aquatic or poultry diets as protein supplement (Lovell et al., 1968; Meyers and Rutledge, 1971).

Recently, employment of an acid-preservation (ensilage) technology, used in the Norwegian sea fishing industry for production of a stable liquefied fish protein, was demonstrated to stabilize the astaxanthin present in the shrimp waste. The digestability of astaxanthin pigment and its rate of accumulation in rainbow trout (*Salmo gairdneri*) muscle were significantly increased when fish were fed the silage diet instead of those from fresh or dried shrimp waste (Torrissen et al., 1981/1982). An ancillary benefit of the ensilage technique was that calcium salt and chitin shell constituents were dissolved at low pH. Treatments of crab waste with phosphoric acid has been used for preservation and improvement of the protein value of such waste and for provision of a desirable Ca/P ratio in the dehydrated meal, thus increasing its use in aquatic animal and poultry diets Williams and Miller, 1981). Besides, the addition of organic and/or inorganic acid minimizes bacterial spoilage and has a stimulatory effect on indigenous proteolytic enzyme activities (Tatterson and Windsor, 1974). Fish viscera silage, preserved by propionic acid at pH 4.3, was microbiologically stable for months at ambient temperatures, and also could be mixed with dry carbohydrate feed ingredients without putrefaction. Furthermore, increasing the temperature of fish viscera silage accelerated autolysis, with >90% of the total protein solubilized after 2–3 days at 30°C, following which most of the lipids could be removed by centrifugation (Gildberg and Raa, 1977).

Recently, production of fish silage for utilization of potential wastes and by-catch from fishery industries was reviewed by Raa and Gildberg (1982), and Asgard and Austreng (1981), in which biochemical, nutritional, and technological aspects of ensiling techniques for feed application were thoroughly discussed.

In view of increasing interest being shown in commercial availability of the natural astaxanthin from crawfish, this investigation was conducted to combine ensilage technology with oil extraction techniques for optimal pigment recovery. Compositional changes, i.e., calcium structure dissociation and protein solubilization, and microbiological activities in crawfish silage were analyzed to examine their relation to the degree of pigment release.

MATERIALS & METHODS

HEAT-PROCESSED CRAWFISH (*Procambarus clarkii*) peeling waste containing the intact cephalothorax, abdominal exoskeleton, and viscera was used. The waste was kept frozen at −20°C prior to analyses. The frozen intact waste was comminuted through a Sorval grinder and mixed with 1:4 or 1:1 (w/v) distilled water for free amino nitrogen and other analyses, respectively. The procedures for preparation of samples, followed by pigment extraction, are outlined in Fig. 1. The Feed Curb/Endox® liquid (Kemin Industries, Inc.), a mixture of organic/inorganic acids, mainly propionic acid, and the antioxidant Endox®, were used to inhibit microbial spoilage and minimize pigment degradation (Strøm et al., 1980; Chen and Meyers, 1982b). A short term incubation period at 40–45°C was evaluated for optimal hydrolysate production.

Biochemical analysis

Free amino nitrogen. A modified formol titrimetric method (Tongnual et al., 1981) was used to determine the degree of amino acid release, resulting from the action of endogenous enzymes and the acid treatment. Comminuted crawfish waste in distilled water (1:4 w/v) was prepared, and a 10-ml aliquot was mixed thoroughly

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with 0–20% various intermediate amounts of the Feed Curb/Endox liquid. The pH of each sample, with or without acid treatment, was adjusted to pH 8.0 (Corning digital 110 pH meter). A 15-ml portion of formaldehyde was added to each sample, thoroughly stirred, and titrated back to pH 8.0 using 0.02N NaOH. Milligrams of amino nitrogen in the substrate were calculated from milliliters of NaOH based on the formula of Harrow et al. (1955): 0.28 mg amino nitrogen = 1 ml of 0.02N NaOH. The results of acid hydrolysis were expressed as mg amino nitrogen/10 ml substrate.

Proteolytic activity. An experimental design for determination of proteolytic enzyme activity of crawfish trypsin and carboxypeptidase (Zwilling and Neurath, 1981) was adapted. During hydrolysis of casein by crawfish proteolytic enzymes, breakdown products, i.e., tyrosine and tryptophan, soluble in trichloroacetic acid are formed; the optical density of the resultant supernatant was determined spectrophotometrically at 280mμ (Bergmeyer, 1965).

Experimental sample (A) was composed of tris-HCl buffer (1.0 ml), 0.1M, pH 8.0; casein solution (2.0 ml), 1% in Tris-HCl buffer; 1.0 ml of substrate solution (1:1 v/v dilution of the hydrolysate), which was incubated at 40°C for 20 min., and with final addition of 5% trichloroacetic acid (6.0 ml) to the incubation mixture. Blank sample (B) was of similar composition except that trichloroacetic acid was added prior to substrate solution. The substrate solution was prepared by mixing comminuted waste with 1:1 (w/v) distilled H_2O and treated with 0–20% various intermediate amounts of Feed Curb/Endox®.

All final mixtures were centrifuged, and the optical density of supernatants were measured at 280 mμ with A against B. One enzyme unit was then defined as the increase of extinction value, ΔE(A–B), for 0.001/min under the given conditions (20 min incubation at 40°C, volume of incubation mixture:4 ml, after addition of trichloroacetic acid:10 ml). In order to stay within linear range, the extinction value (ΔE) of A against B did not exceed 0.800.

Solubilized calcium carbonate. The extent of solubilization of calcium carbonate shell structure after acid treatment was measured by a titrimetric method using tetrasodium ethylenediamine tetraacetate (EDTA) as the titrant (Diehl and Ellingboe, 1956). Calcein (fluorescein-methylene-iminodiacetic acid), 2% in 1N sodium hydroxide, was used as an indicator to give a clear end point. The hydrolysate, with or without Feed Curb/Endox® liquid treatment, was diluted to 1:200 (v/v), giving a final volume of 50 ml. To this was added 5 ml of 1N sodium hydroxide containing 1% sodium cyanide and 1–2 drops of 2% calcein indicator. The final solution was titrated with 0.02N EDTA until the color changed from yellow-green to brown. Vigorous stirring was necessary throughout the titration and the fluorescent light source should be eliminated.

To determine the residual crawfish shell calcium, 30 ml of concentrated HCl solution was added and thoroughly mixed with the hydrolysate. The mixture was incubated at 85–90°C, with intermittent stirring for 6 hr. Following centrifugation, the supernatant was diluted to 1:10 (v/v), giving a final volume of 50 ml. A solution of 5 ml 10N sodium hydroxide with 5% sodium cyanide and 3–4 drops of indicator were added. The titration was conducted under the same condition mentioned above, except with 0.1N EDTA solution as the titrant.

Standard curves of 0.02N and 0.1N EDTA titrant against analytical grade of calcium carbonate were used for computing the results.

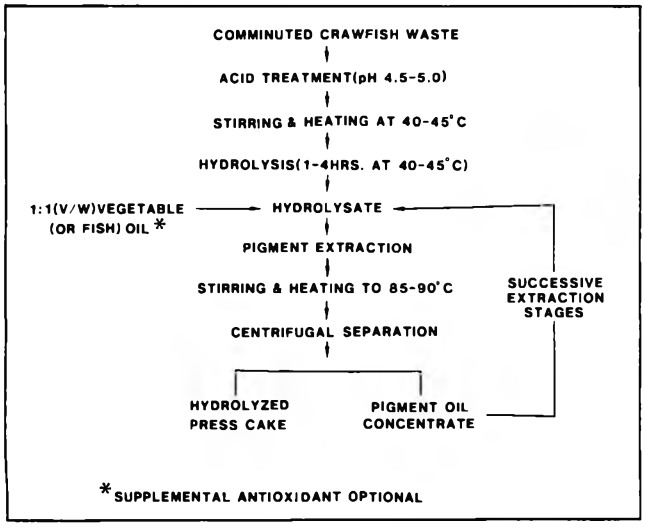

Fig. 1—Schematic of crustacean acid/enzymatic silage process and subsequent astaxanthin pigment extraction.

Microbiological assay

Proteolytic bacterial count. The effect of Feed Curb/Endox® on the proteolytic activities (enzymatic or bacterial) of hydrolyzed crawfish waste also was determined microbiologically. The 2% skim milk assay medium and pour plate method was employed (Collins and Lyne, 1970). All plates were incubated at 35°C and activity, expressed by a clearing zone at the colony periphery, was enumerated within 24–48 hr (Meyers and Ahearn, 1977).

In an ancillary investigation, antibiotics, including streptomycin sulfate, 200 μg/ml, and chlortetracycline hydrochloride, 50 μg/ml, were added to samples with or without Feed Curb/Endox® treatment. Some samples were heated to 100°C for 12 min to inhibit enzymatic and bacterial activities. All the samples were then incubated at 40–45°C for 1 or 2 hr, and the remaining viable proteolytic activities were examined microbiologically.

Enumerations of chitinoclastic microorganisms. The chitin used for determination of chitinase activity of hydrolyzed crawfish waste was prepared by dissolving 20g ball-milled chitin (Sigma) in 50 ml of concentrated HCl. The precipitated chitin was washed free of acid with distilled water and adjusted to a concentration of 10 mg/ml (Hood and Meyers, 1977). The final pH of the solution was adjusted to 7.0. The chitinase assay comprised a pour plate method comparable to that used in the proteolytic study. The plates were incubated at 29°C and the clearing zone at the colony periphery was observed within 7–10 days.

Astaxanthin pigment extraction

Astaxanthin concentration in hydrolyzed crawfish. The extractable astaxanthin in the hydrolyzed crawfish waste at different pH values was determined using a previously described soybean oil extraction procedure (Chen and Meyers, 1982a).

In an ancillary investigation, propionic acid and a 1:1 (v/v) mixture of propionic acid and formic acid were added to comminuted waste and oil-extractable astaxanthin was examined at pH values of 5.0, 4.5, and 4.0. The pigment content was extracted with soybean oil in a ratio of 2:5 (w/w) and the extraction followed the procedure mentioned above. It was noted that the acidic strength of 1 volume of formic acid in lowering the pH of the crustacean waste was equivalent to 3 volumes of propionic acid.

Astaxanthin stability in crawfish ensilage. To prepare ensiled samples, comminuted crawfish waste was thoroughly mixed with 10% and 20% Feed Curb/Endox® liquid and Feed Curb alone. The samples, including control, were stored at temperatures of –20°, 5°, and 20°C. Prior to storage, the initial amount of oil-extractable astaxanthin present was analyzed. At storage periods of 1, 2, 5, and 6 weeks, the ensiled crawfish waste was thoroughly agitated, incubated at 80°C for 30 min, and the amount of oil-extractable astaxanthin was analyzed. In addition, the pH value of each analyzed sample was measured.

Astaxanthin oil stability with acid treatment. The subsequent stability of the pigmented oil from the acid-treated samples was determined as follows. Comminuted waste was thoroughly mixed with 0–40% various intermediate amounts of Feed Curb/Endox®, incubated at 80°C for 30 min, and the oil-extractable astaxanthin was analyzed according to aforementioned procedures. The pigmented oil solution was stored in the dark in a plastic bottle at 10°C. The absorbance of the diluted (1:20, v/v) pigmented oil was measured spectrophotometrically at periods of 0, 2, 6, and 8 wk.

Statistical analysis

All data, four replicates for each observation or measurement, were subjected to analysis of variance using GLM procedure to study differences between acid treatments. Correlations between the degree of compositional changes and concentration of oil-extractable astaxanthin were also analyzed using CORR procedure in SAS computer system (Steel and Torrie, 1980). A randomized block design was applied in analysis of variance, and the calculated F value exceeding 5% tabular F was considered evidence of significant effect ($P < 0.05$).

RESULTS & DISCUSSION

Acid-ensiled crawfish waste pH

The corresponding pH values of acid-ensiled crawfish waste treated with different levels of Feed Curb/Endox®, and with 0–4 hr incubation periods at 40–45°C, are shown in Table 1. As noted, only 5% Feed Curb/Endox® was needed to reduce the pH from 8.3 to 4.8, while an additional 15% was required for further reduction to 4.2. The control sample, without acid treatment, exhibited microbiological spoilage during short-term incubation, with the pH value becoming neutral. All samples showed fluctuations in pH values, which may be indicative of chemical changes, or enzymatic and microbial activities, occurring in the hydrolysate.

Free amino nitrogen content and proteolytic enzyme activity

The free amino nitrogen content of the acid-ensiled crawfish waste is shown in Fig. 2, indicating the increasing amount of soluble proteinaceous materials formed under hydrolytic conditions. The hydrolysate gave more active proteolysis as the pH was lowered from the original, i.e., pH 8.6 for samples without incubation and pH 7.6 for samples with 1-hr incubation, to near pH 4.0. As shown, a twofold increase in free amino nitrogen concentration (4.0 vs 8.4 mg/10 ml substrate) due to acid hydrolysis was observed in samples with 1-hr incubation at 40°C. Accelerated proteolysis due to acid treatment also was seen with proteolytic enzyme activity (Fig. 3), although the activity became completely depressed as the pH of the silage approached 4.0. This is consistent with results obtained by Zwilling and Neurath (1981), indicating that crawfish trypsin, a serine protease, gradually loses activity at pH 5.0 and is irreversibly inactivated at pH 3.0. The optimal pH of crawfish proteolytic enzymes, i.e., trypsin, in the current study was near 6.0.

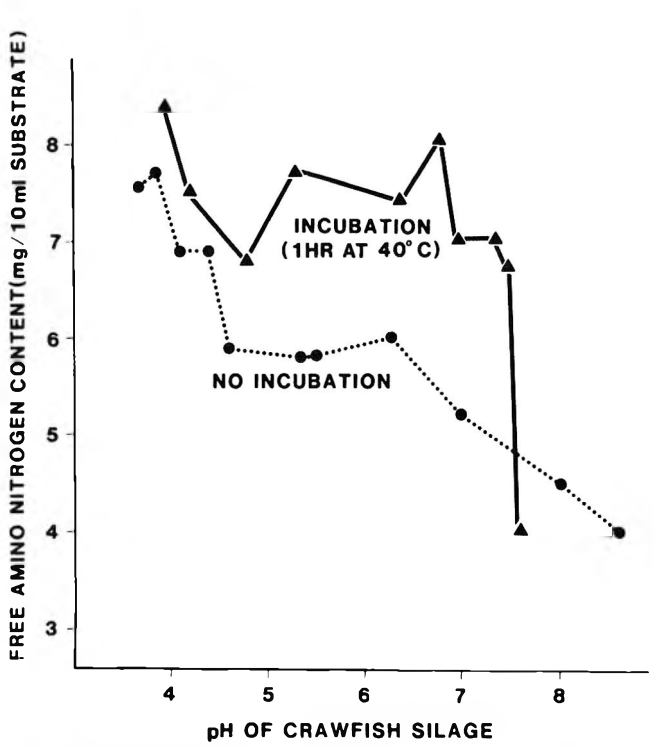

Fig. 2—Free amino nitrogen content of acid-ensiled crawfish waste in relation to silage pH.

Decalcification of crawfish exoskeleton

The effect of acid treatment on decalcification of crawfish carapace is given in Fig. 4. Approximately seventy percent of the total calcium content of the exoskeleton was solubilized as the pH of autolyzed crawfish silage was reduced from 8.3 to 4.0. The degree of calcium dissolution in crawfish silage due to acid treatment corresponds to the decalcification process developed by Rutledge (1971), in which 56–68% of skeletal calcium was removed from crab and crawfish meals. Use of such reduced calcareous dried silage, i.e., 3.3–5.5%, in dietary formulations for aquaculturally-raised salmonids, or for poultry diets, is desirable since problems of possible mineral imbalances are minimized (Meyers and Rutledge, 1971).

Proteolytic and chitinoclastic bacterial counts

The proteolytic and chitinoclastic microbial populations of acid-ensiled crawfish waste are shown in Fig. 5. The bacteriostatic effect of acid treatment (log count of proteolytic bacteria: 4.75) (Table 2) was almost equivalent to those of antibiotic (log count: 4.89) and heat treatments (log count: 4.64); an overall inactivation of proteolytic bacteria could not be achieved shortly after acid treatment. There were no marked changes in counts of proteolytic bacteria after 2-hr incubation for all treated samples. In addition, the

Table 1—pH of crawfish silage treated with Feed Curb/Endox® after different incubation periods

Feed Curb/ Endox liquid treated level (%)	pH Incubation period (hr) 0	2	4
0	8.3	7.4	7.2
0.3	7.1	7.1	6.9
0.5	6.8	7.0	6.8
0.6	6.6	6.9	6.8
1.0	6.3	6.7	6.7
1.3	6.1	6.6	6.7
2.5	5.8	6.1	6.3
5.0	4.8	5.0	5.1
10.0	4.5	4.5	4.5
15.0	4.3	4.3	4.3
20.0	4.2	4.1	4.1

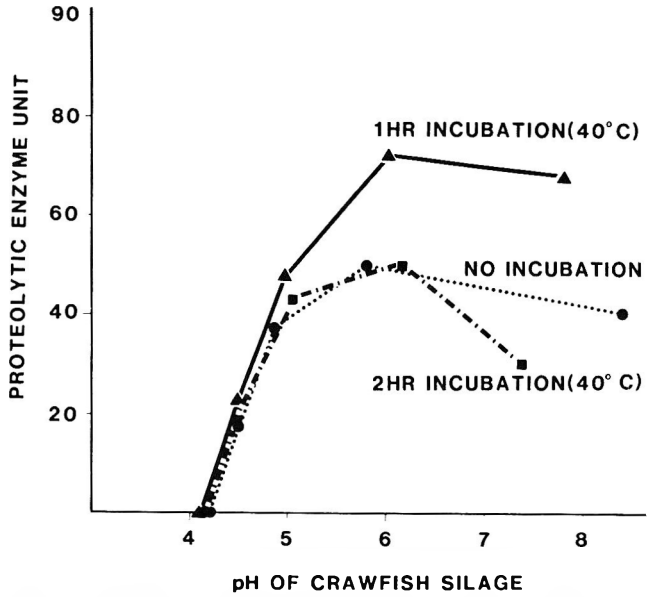

Fig. 3—Proteolytic enzyme activity of acid-ensiled crawfish waste of different pH and incubation time.

combination of acid with heat (100°C, 12 min) showed a slight synergistic effect on suppression of proteolytic bacteria count (log count: 4.22); there were no changes for the combination with antibiotics (log count: 4.87). In contrast, activities of chitinoclastic bacteria in the acid-ensiled samples were restrained completely under higher acidic conditions, i.e., pH 4.0. In general, chitinoclastic activities were much weaker than proteolytic processes.

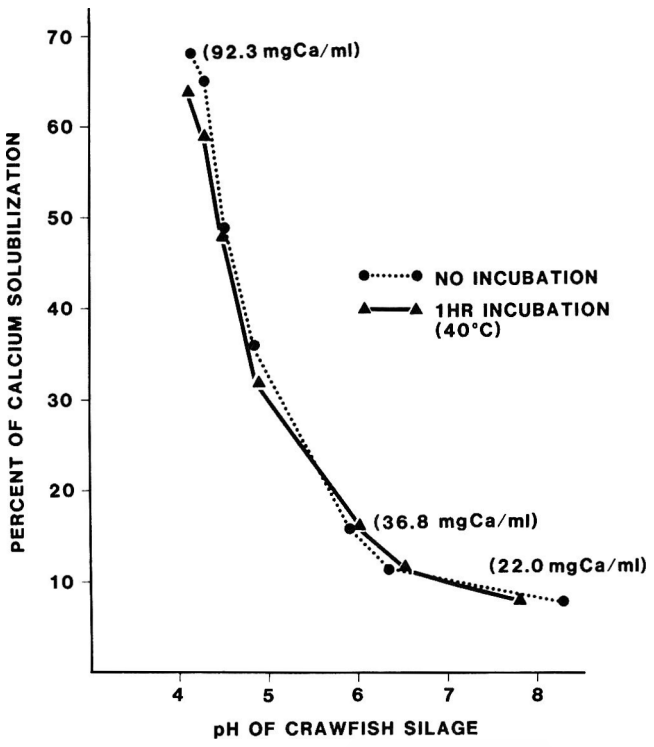

Fig. 4—Calcium solubilization in acid-ensiled crawfish waste (the figures in parentheses are the calcium concentrations in substrate solution).

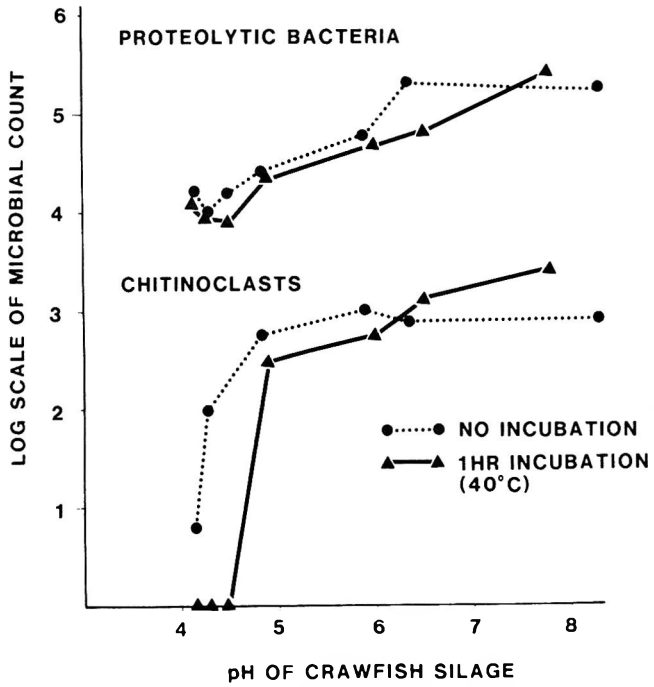

Fig. 5—Proteolytic and chitinoclastic populations of acid-ensiled crawfish waste as a function of pH and incubation time.

Astaxanthin pigment release and stability

The oil-extractable astaxanthin in acid hydrolyzed wastes, treated with Feed Curb/Endox® and organic acids, of different pH values was analyzed, with results shown in Fig. 6 and Table 3, respectively. As noted, astaxanthin was released and became more susceptible ($P < 0.05$) to the oil extractant at low pH, probably due to the effect of the acid on chemical bondings of astaxanthin with skeletal parts or with protein constituents (Fox, 1973; Cheesman et al., 1967). A comparable phenomenon also was reported by Torrissen et al. (1981/1982), showing approximately 50% additional pigment concentration (49.7 vs 73.5 mg/kg wet weight) extracted from shrimp silage (pH 4.0) stored at 4–5°C for 2 days. In the current study, a correlation ($\gamma = 0.80$, $p < 0.05$) was found between the degree of calcium structure solubilization and concentration of oil-extractable astaxanthin. This observation suggests that the natural deposition of astaxanthin pigment might be chemically bonded to calcium (bi)carbonate in crawfish exoskeleton

Table 2—Log (count) of proteolytic bacteria in incubated crawfish silage[a]

Treatment	Incubation period (hr) 1	2
Control	6.89[b]	7.13
5% Feed Curb/Endox liquid	4.87	5.00
20% Feed Curb/Endox liquid	4.75	4.89
Heat treatment	4.64	4.70
Heat treatment + 5% Feed Curb/Endox liquid	4.36	4.85
Heat treatment + 20% Feed Curb/Endox liquid	4.22	4.00
Antibiotics	4.89	5.02
Antibiotics + 5% Feed Curb/Endox liquid	4.82	5.00
Antibiotics + 20% Feed Curb/Endox liquid	4.87	4.82

[a] Based on formation of clearing zones
[b] Figures are average of four replicate analyses.

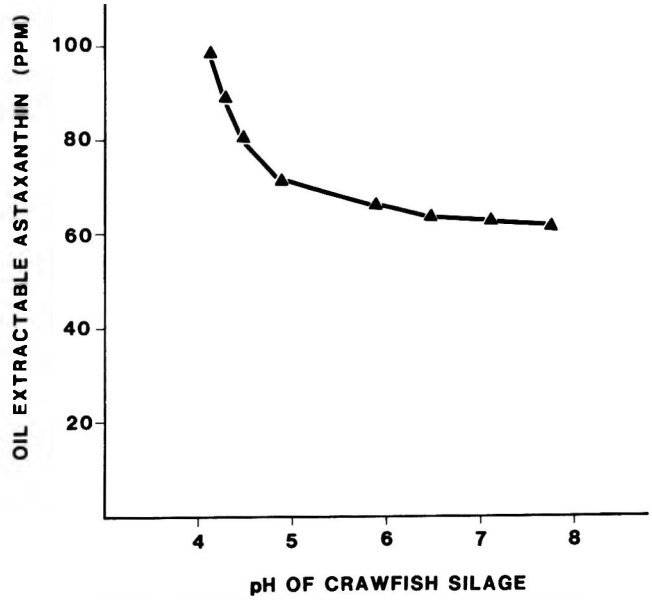

Fig. 6—Oil-extractable astaxanthin in acid-ensiled crawfish waste of different pH.

Table 3—Effect of various acids and pH on oil-extractable astaxanthin in acid-preserved crawfish waste

Treatment	pH	Astaxanthin conc (ppm)
Control	8.2	119
Propionic acid	5.0	133
	4.5	149
	4.0	144
Propionic acid:Formic acid (1:1, v/v)	5.0	123
	4.5	130
	3.7	203

as that in the skeleton of hydrocoral (Fox, 1972). Solubilization of the calcium structure in the crustacean shell appeared to have rendered materials containing astaxanthin more available for extraction. Torrissen et al. (1981/1982) used this phenomenon to explain the improved digestibility of astaxanthin in shrimp silage fed to rainbow trout (*Salmo gairdneri*) in which 71% digestibility was obtained compared with 45% from fresh or dried wastes.

The stability of astaxanthin in acid-preserved crawfish waste is of noteworthy consideration in employment of ensilage technology as an alternative method for large-scale preservation of the proteinaceous waste material. Crawfish ensiled waste showed an increase in pH value ($P < 0.05$) after a 1-month storage period, however, the changes were much less significant in samples with lower initial pH value, i.e., 4.7. Astaxanthin is quite stable in acid-ensiled waste (pH 4.7) teated with Feed Curb/Endox® at all storage temperatures, i.e., −20°, 5°, and especially at 20°C for up to 6 wk (Fig. 7 and 8). This result is consistent with that of Torrissen et al. (1981/1982), who demonstrated that astaxanthin pigment is stable in an acid silage of shrimp processing wastes at 4–5°C, with a slow conversion of astaxanthin diester to the corresponding monoester. In the present work, control samples without acid ensiling were completely spoiled under ambient and refrigeration temperatures in 1–2 days and 1–2 wk, respectively. Even under frozen storage, the astaxanthin pigment component of the waste was degraded, and only half of the initial level remained after 6 weeks. Clearly, ensilage is an inexpensive and effective method for preserving crawfish waste during the sample collection period prior to initiation of pigment extraction under ambient temperature.

The ensiled wastes gradually became liquefied with increased storage period, and a liquid product was produced as a result of enzymatic autolysis. The initial liquefaction process enhanced the stirring and heating stages during subsequent pigment extraction. As mentioned earlier, under the controlled enzymatic/ensilage process, 40–50% additional astaxanthin was concentrated in the extraction vehicle used. Furthermore, the acid added served as a protein structure modifying agent to precipitate protein and to eliminate the undesirable protein/water/oil emulsion problems associated with the oil/silage homogenate, following which oil recovery was increased by 10% in the subsequent centrifugation stage. This phenomenon may be further explained by the work of Potter et al. (1980), in which they demonstrated that maximal oil removal could be achieved by heating silage to approximately 90°C, comparable to our heat treatment during pigment extraction, before centrifugation. Significant stability of the pigmented oil extracted from the ensiled waste (pH 4) was seen over a 2-month period at 10°C.

As mentioned earlier, pH adjustment could cause protein breakdown, however, after heat treatment during pigment extraction, the soluble protein is coagulated and precipitated along with the presscake in the subsequent clarifying centrifugation stage. The recovered presscake is a proteinaceous crustacean meal with sufficiently lowered Ca/P ratio suitable for incorporation into aquacultural or poultry feedstuffs. Further work is being directed toward development of an overall commercial process, based on an initial pilot plant scale, for enzymatic digestion and pigment extraction. These efforts are designed to convert the traditionally discarded crawfish waste into products comprising a valuable oil-extracted red pigment, and a decalcified proteinaceous crawfish meal.

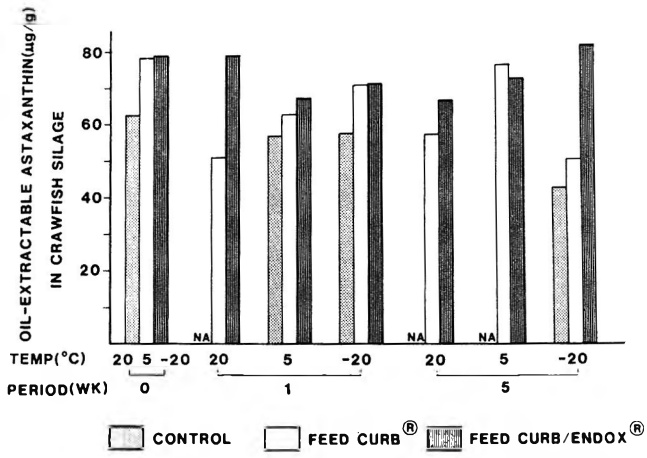

Fig. 7—Stability of astaxanthin in crawfish silage (initial pH 5.4).

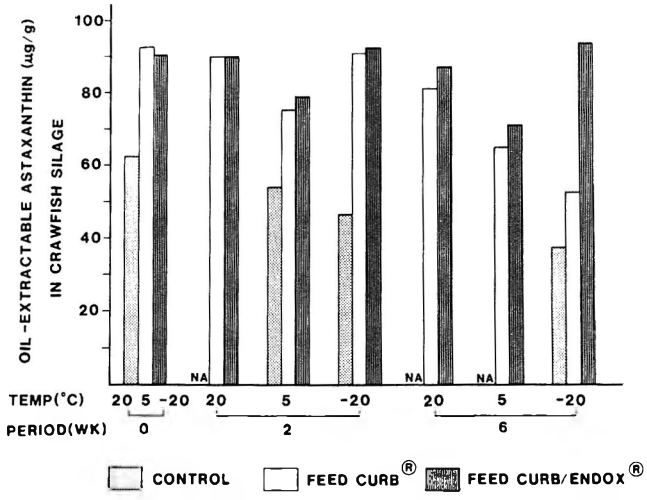

Fig. 8—Stability of astaxanthin in crawfish silage (initial pH 4.7).

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Quality Comparison of Thermoprocessed Fishery Products in Cans and Retortable Pouches

S. S. CHIA, R. C. BAKER, and J. H. HOTCHKISS

ABSTRACT

The physical, chemical and sensory properties of rainbow trout, pollock and shrimp processed at equal lethalities in cans and retortable pouches were compared at certain storage periods. Pouched rainbow trout, pollock and shrimp required respectively 34%, 32% and 37% less thermal processing time than the canned product. The pouched products had lower amounts of ammonia, trimethylamine and total carbonyl compounds than the canned products. The pouched products retained 17%, 9% and 15% more Vitamin B_1 than the canned products for rainbow trout, pollock and shrimp, respectively. Pouched products had a firmer texture and lighter color than the canned products. In sensory evaluations, the pouched products were scored higher, in most cases, for color, flavor and overall acceptability.

INTRODUCTION

THE RESEARCH EFFORTS of several workers (Hu et al., 1955; Pflug et al., 1963; Goldfarb, 1970; Thrope and Antherton, 1972; Tung et al., 1975; Lyon and Klose, 1981) have proven the technical and commercial feasibilities of using the retortable pouch for thermoprocessed foods. Because of the advantages of retortable pouches such as shelf stability, weight, storage space, ease of opening and preparation, reduced heat exposure resulting in improved quality and eventually packaging economy, several researchers have reported the desirability of using retortable pouches for various products.

Heidelbaugh and Karel (1970) utilized 0.5 mil polyester/5 mil aluminum foil/2 mil polyethylene, metal cans and two other plastic films as packaging materials to compare chemical and sensory characteristics of cranberry sauce, vegetable with bacon mixture and pork with pork broth. For cranberry sauce there was no significant difference in sensory scores or the destruction rate of ascorbic acid in the pouched and canned products after 5 wk of storage. Similar findings were reported for the vegetable with bacon mixture and the pork samples except that the pouched products showed a lower degree of oxidation measured by the thiobarbituric acid test (TBA) and peroxide values. Szczeblowski (1971) studied various foods in retortable pouches for military rations and found there were no significant changes in product quality over a 2-yr period at 21°C or even at 38°C for 12 months. Dymit (1973) reported that after 8 yr, shrimp in retortable pouches were superior in flavor and color to canned products. Tung et al. (1975) compared cream style corn in pouches and cans over a 6-month storage period. They concluded that the pouched product was significantly lighter in color but found no significant difference in overall acceptability or storage stability. Tung et al. (1977) showed that corn in butter sauce and other vegetable products processed in pouches were highly acceptable and had a normal storage stability. The corn product in pouches had a score of 77.7 out of 100 for overall acceptability, while a commercial frozen product was rated at about 50.

Greene (1979) showed that the shorter processing time of the retortable pouch was responsible for a greater retention of thiamin and riboflavin than canned sweet potato puree. He also found that the two packaging systems showed comparable stability characteristics for retaining nutrients during 24 wk of storage at room temperature. Gomez et al. (1980) compared mango slices processed in jars, boil-in-bag transparent pouches and retort pouches. They found that the retort pouched products retained quality better at 23°C and 10°C storage for 24 wk. Chen and George (1981) showed that green beans processed in cans retained slightly more ascorbic acid than in pouches. Similarly, metal cans offered better ascorbic acid retention during 11 wk of storage at 21°C. However, the flavor, texture, and overall acceptability of the pouched products were better than the can but the color of the canned beans was preferred.

Lyon and Klose (1981) studied heat effects on sensory properties of chicken meat processed in retortable pouches and cans. They conducted the study with the Quantitative Descriptive Analysis technique to evaluate the samples and found the retort pouched chicken meat after heat processing showed less shredding, less stringiness and was less fibrous than the canned products. They concluded that the retort pouch process offered a method for improving the texture of processed chicken meat from spent hen by adequately cooking to tenderize the meat but not overcooking it to the extent that the meat chunks were reduced to fibrous, shredded or stringy pieces.

Relatively few comparisons of the retortable pouch and the tin-plated can, in terms of processing times required and product quality through storage, have been reported in the seafood area. This study was therefore undertaken with the objective of comparing the relative physical, chemical and sensory properties of three fishery products packaged and processed in retort pouches and cans as well as the relative storage stability of the products.

MATERIALS & METHODS

RAINBOW TROUT and pollock arrived gutted and unfrozen; the shrimp arrived shelled and frozen. Special arrangements were made with each source to acquire product in the freshest condition possible. All products were carefully inspected upon arrival, immediately washed and the fin fish filleted. All samples were randomly divided into two batches: one for canning and the other for pouch processing. The fillets of rainbow trout and pollock were precooked on a steam tray with steam for 10 min while the shrimp were precooked for 4 min. After precooking, the fish and shrimp were hand-filled without added liquid (170g for fish and 150g for shrimp) into cans (303 x 117, American Can Co.) and retortable pouches (6.5 in. x 4 in., Reynolds Metals Co.). Residual air was removed from the pouch by vacuum prior to sealing. The cans were sealed with a Dixie Automatic Can Sealer. Both canned and pouched products were given a 10D process (z = 18F°) at a retort temperature of 250°F (121°C). The thermal processing times were determined by the methods of Herdon et al. (1968) using computer derived tables. The thermocouple was inserted at the center of the pouch and can. Time-temperature relationships were monitored by a strip-chart recorder (Honeywell, type 153) throughout the process. Cans were processed in a laboratory autoclave (Thermatic 60, Wilmot Castel

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Co.) and pouches in a modified pressure cooker (Presto Co., Model No. 21-B) under 100% steam. Nitrogen overpressure was added during cooling. Pouches were loaded into a restraining rack (vertical position) with spacing.

Processed canned and pouched samples were stored at room temperature (22°C). Test periods for rainbow trout and pollock were immediately after processing, 60 and 120 days of storage; and for shrimp, immediately after processing and 90 days of storage. At least three cans and pouches were chosen at random and triplicates were run for each analysis. The raw and precooked fish and shrimp were also analyzed to serve as controls.

Chemical and physical analyses were undertaken on the edible solids after draining off the cook-out. Ammonia was determined by the method of Fernandez-Flores and Salwin (1968). The method of Murray and Gibson (1972) was used to analyze the trimethylamine. Quantification of total carbonyls was carried out using the procedure of Mai (1978). TBA values were determined by the method of Lemon (1975). Vitamin B_1 was determined by the AOAC (1980) method.

Protein in the cook-out was determined by adding 20% trichloroacetic acid (TCA) and centrifugation for 10 min at 10,000 rpm at 2°C (Sorvall, SS34 rotor). The precipitate was weighed and the protein content of the precipitate was determined by Kjeldahl (AOAC, 1975). The cook-out volume was determined by draining the samples using a funnel containing a nylon screen (0.1 cm^2) and a graduated cylinder. Total solids of well mixed cook-out were determined by oven drying (105°C). Shear properties were determined using the Kramer Shear Press (Model 43HE) with a compression and shear cell. A maximum shear force (lb/g) was recorded using a texture gauge (Model TG4B, Food Technology Corp.). Changes in color were measured by the Hunter Color Difference Meter (Hunter Assoc. Inc.) equipped with a signal processor (Model D-25-2) and an optical sensor (Model D-25-AA).

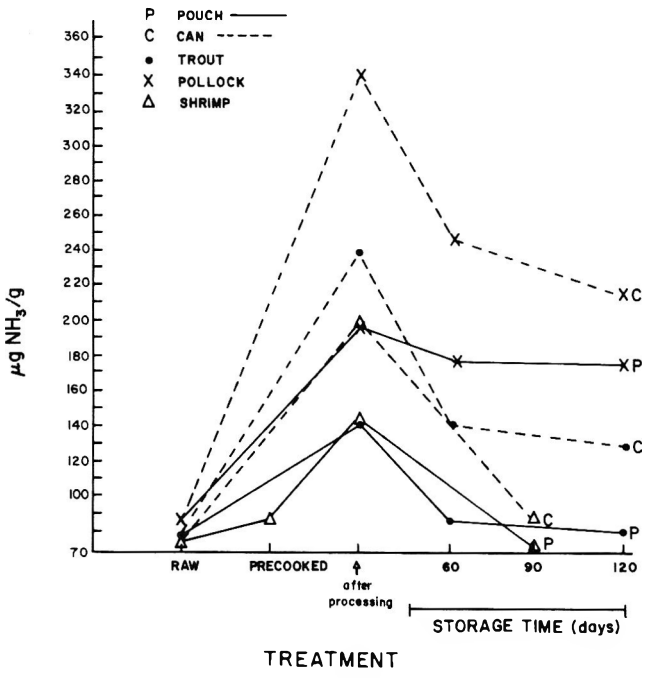

Fig. 1—Effect of processing and storage on ammonia content of rainbow trout, pollock and shrimp.

The sensory quality of the products was evaluated by a panel of eight untrained persons. The samples were served warm in a coded aluminum dish. Water and crackers were provided to restore taste sensitivity. The room was darkened so that the panelists would not let color bias their judgments. Panelists were asked to assign a score of 1 to 9 to each sample for aroma, flavor, texture, juiciness and overall acceptability, where 1 was least desirable and 9 most desirable, except for tenderness and juiciness, where 5 was optimum, 9 too tender or juicy and 1 too tough or dry. Panelists then moved to a lighted area and were asked to evaluate desirability of color. Raw scores obtained from the panel for each attribute were analyzed by a Randomized Complete Block Analysis of Variance (RCB-AOV) and an F test was performed at $P < 0.05$ and 0.1 to determine if difference between treatment means existed.

RESULTS & DISCUSSION

FOR CANNED TROUT AND POLLOCK, the processing time was 50 min. For canned shrimp the processing time was determined to be 38 minutes. The shorter processing time for shrimp was due to the smaller quantity used (150g vs 170g) and the differences in heat transfer parameters. For pouched trout, pollock and shrimp, the processing times were 33, 34 and 24 min, respectively. In comparison with canned products, the reduction of processing time for trout, pollock and shrimp was 34, 32 and 37%, respectively. The thin profile and the increased surface area of the retort pouches are responsible for the reduction in the heating time. There is no information available on the heat processing time for these three products in pouches. However, reduction of heating time using pouches has been reported by a number of workers (Thrope and Antherton, 1972; Saigo et al., 1974; Tung et al., 1975; Lampi, 1977; Przybyla, 1980).

Heat processing caused an increase in the amount of ammonia in all products (Fig. 1). This agreed with the work done on canned albacore (Tokunago, 1975), sardines (Ota and Nakamura, 1952) and herring (Hughes, 1959). Heat caused an increase in the rate of the breakdown of proteins, amino acids and other nitrogenous compounds such as nucleic acids and amines to free ammonia (Vyncke, 1970). Higher amounts of ammonia were observed in raw, pouched and canned pollock when compared to trout and shrimp. Canned samples had a higher amount of ammonia than the pouched samples for all three species tested (Fig. 1). However, the amount of ammonia in both canned and pouched products decreased with storage. With rainbow trout, there was a sharp decrease in ammonia in the initial 60 days storage period, and additional storage for 60 days produced little change. Both pollock and shrimp followed the same trend as trout during storage. The decrease in ammonia content for stored products could be due to the solubilization of ammonia from the flesh into the cook-out.

The amount of trimethylamine (TMA) in raw, canned and pouched pollock and shrimp was determined (Table 1). Although the significance of TMA is subject to controversy, it is generally thought to be a fairly good indicator of freshness in fish (Gould and Peters, 1971). However, shrimp generally contain much less TMA than raw fish in the Gadoid family (pollock) and TMA content may not be a good indicator of freshness for shrimp. In our work, the shrimp showed an increased amount of TMA in canned and pouched

Table 1—Effect of processing and storage on the trimethylamine (TMA) content of pollock and shrimp[a]

					Storage time (days)					
			After Processing		60		90		120	
Fish species	Raw	Precooked	C	P	C	P	C	P	C	P
Pollock	39.3	–	27.2	22.3	24.3	24.5	–	–	25.2	20.6
Shrimp	4.2	5.4	10.9	7.9	–	–	15.5	11.5	–	–

[a] Each value is the mean of at least three determinations; C = Canned sample; P = Pouched sample

products following heat processing. This increase in TMA agreed with the work of Hughes (1959) and Tokunago (1975). For pollock, there was a decrease in TMA content which was probably due to volatilization during the longer precooking with steam. Nevertheless, canned products which were heat processed for longer periods of time showed a higher amount of TMA than pouched samples. There was no significant change in TMA content in the canned and pouched pollock at 120 days of storage, but the shrimp showed a slight increase in TMA content at 90 days of storage.

The amount of carbonyl compounds including volatiles and nonvolatiles was measured for raw, canned and pouched fish and shrimp products (Table 2). The raw pollock and raw shrimp contained a higher concentration of carbonyl compounds than raw trout. Following heat processing, an increase in carbonyl content was observed both in the canned and pouched products of rainbow trout and shrimp. This is in agreement with Lovern (1970) who showed that cooking of fish increased the carbonyl compound content. The increase was greater in the canned products than in the pouched which was probably related to the longer heating of the cans. However, a decrease in carbonyls was observed in the processed pollock as compared to the raw pollock, thus it is difficult to conclude that heating solely caused an increase in carbonyl compounds. This may be related to loss of moisture and concomitant dissolution of carbonyls in the liquid. Nevertheless, the canned pollock showed a higher carbonyl content than the pouched.

Malonaldehyde content was determined by the thiobarbituric acid (TBA) procedure on raw, canned and pouched fish and shrimp. Heat processing resulted in a decrease in TBA values in the canned and pouched samples. For example, raw rainbow trout had a TBA value of 0.67 μmoles malonaldehyde/100g of tissue which decreased to 0.32 μmoles in canned samples after processing, approximately a 50% reduction. The pouched products showed a smaller reduction. Results with pollock and shrimp were similar. Sinnhuber and Yu (1958) also showed that heating of fish caused a rapid drop in TBA values. Higher values were observed in rainbow trout samples, probably due to the higher fat content. During storage, canned samples had a faster rate of increase in TBA values compared to the pouched samples. This is likely due to the presence of more head space or more residual oxygen in the can.

Vitamin B_1 (thiamin) content was measured using the raw and precooked canned and pouched samples (Table 3). Rainbow trout contained 0.18 mg of thiamin per 100g of raw flesh; the precooked samples contained 0.14 mg of thiamin showing a 22% reduction in Vitamin B_1 due to precooking. After heat processing, the canned and pouched samples retained about 50 and 67% of Vitamin B_1, respectively.

The storage studies of Vitamin B_1 of rainbow trout in cans and pouches showed that the pouched samples had about the same rate of loss of Vitamin B_1 as the canned samples during the 120 days of storage. The differences in Vitamin B_1 retention were due to the difference in heating time rather than the effect of storage. The results with pollock and shrimp showed a similar trend, but the relative values were different (Table 3).

There is very little data concerning the change in Vitamin B_1 content due to processing and storage on the specific species of fish studied. However, Komato et al. (1956) reported that canned mackerel and tuna showed no loss in riboflavin, niacin, and B_{12}, while a considerable amount of thiamin was lost. Higashi (1962) reported a loss of 70% of the thiamin in the canning of tuna. He also found that about 50% of the thiamin was lost after sterilization. During storage for 1, 3, and 5 months, it was found that the canned tuna retained 50.6, 43.9 and 40.3% of thiamin, respectively. The canning of swordfish resulted in a 75% loss of thiamin as reported by Lopez-Matas (1948). Gordon et al. (1979) showed a 40% reduction of thiamin content in cooked shrimp and 68% in canned shrimp.

The cook-out or "pot liquor" was analyzed for volume,

Table 2—Effect of processing and storage on total carbonyls content[a]

					Storage time (days)					
			After Processing		60		90		120	
Fish species	Raw	Precooked	C	P	C	P	C	P	C	P
					Total carbonyls content (μmole carbonyls/100g)					
Rainbow Trout	27.6	–	45.1	43.8	55.1	51.1	–	–	30.4	23.4
Pollock	69.5	–	42.1	39.8	68.8	57.3	–	–	70.4	62.4
Shrimp	41.8	43.2	60.6	57.3	–	–	39.1	33.0	–	–

[a] Each value is the mean of at least three determinations; C = Canned sample; P = Pouched sample

Table 3—Effect of processing and storage on vitamin B_1 content

					Storage time (days)					
			After processing		60		90		120	
Fish species	Raw	Precooked	C	P	C	P	C	P	C	P
					Vitamin B_1[a] (mg/100g wet)					
Rainbow trout	0.18	0.14	0.09	0.12	0.07	0.1	–	–	0.06	0.08
Pollock	0.17	–	0.09	0.11	0.06	0.08	–	–	0.04	0.06
Shrimp	0.02	0.017	0.004	0.007	–	–	0.003	0.005	–	–
					% Retention (based on raw material)					
Rainbow trout	100	77.8	50.0	66.7	38.9	55.6	–	–	33.3	44.4
Pollock	100	–	52.9	61.1	35.3	47.0	–	–	23.5	35.3
Shrimp	100	85	21.4	35.7	–	–	15.0	25.0	–	–
					% Retention (based on processed material)					
Rainbow trout	–	–	100	100	77.8	83.8	–	–	66.7	66.7
Pollock	–	–	100	100	66.7	72.7	–	–	44.4	54.5
Shrimp	–	–	100	100						

[a] Each value is the mean of at least three determinations. C = Canned sample; P = Pouched sample.

total solids and protein content. In all products, the volume of cook-out did not change with the method of processing or storage time. The solids content of the cook-out from the pouched and canned products was about the same for a given product up to 60 days of storage. The volume for the finfish was 19–28 ml, and for the shrimp 28–30 ml. However, longer storage periods (120 days) resulted in an increase in the solids and protein content of the cook-out. In general, the protein content of the cook-out from the canned products was higher (by 3–7% of dry solids) when compared to the pouched products.

In general, precooking and processing of all products increased firmness as shown in Fig. 2, 3 and 4. Trout (Fig. 2) and pollock (Fig. 3) had similar shear values when raw and both products increased to a similar firmness after processing and maintained these values during storage. Raw shrimp (Fig. 4) had considerably higher shear values than the finfish. Precooking and processing only slightly increased this firmness. All products demonstrated small increases in shear values during storage.

In all cases, the pouched fish products were firmer than the same products in cans. This was especially true for shrimp (Fig. 4). Canned shrimp decreased in shear values from 7.55 after precooking to 4.75 after processing. The pouched product decreased only slightly after processing and after 90 days storage increased to a value somewhat higher than the precooked shrimp. As would be expected, increased heat inputs for canned products results in a less firm, softer product.

Color measurements showed that the canned samples were generally darker in color than the pouched samples. Longer heat processing with the canned products may have caused an increase in browning reactions which were responsible for the darker color. Storage did not affect the color in the canned or pouched samples.

The sensory ratings of each product in cans were compared to the same product in pouches immediately after processing as well as during storage (Tables 4, 5 and 6). In general, statistically significant differences between the can and pouch were seen most often in flavor followed by color. In all cases where there was a significant difference in flavor or color, the pouch was judged more desirable than the same product processed in the can. We believe that these data support the physical and chemical data presented above and are a result of the lower heat input necessary for products processed in pouches.

The overall acceptability of the finfish products was judged significantly higher in the pouch in two of six tests. This failure to consistently rate one container over the other, even though there were substantial differences, may be due to the fact that the panelists evaluated acceptability in terms of past experiences with canned tuna. This was not the case with shrimp; in all cases shrimp in the pouch was

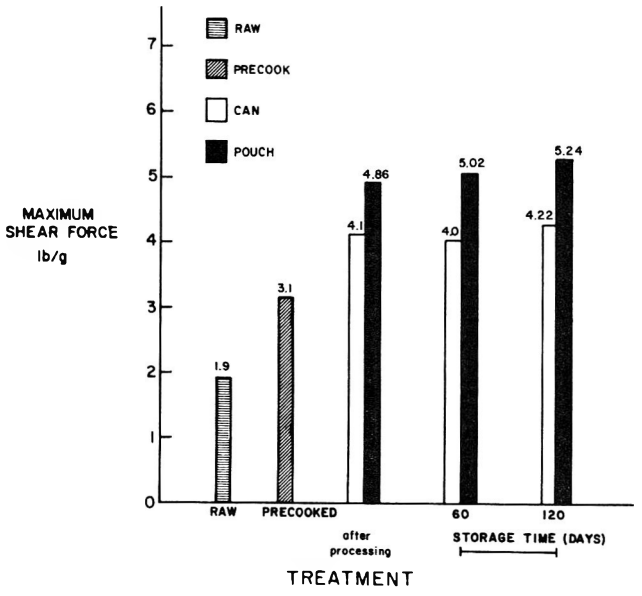

Fig. 2—Effect of processing and storage on shear values of rainbow trout.

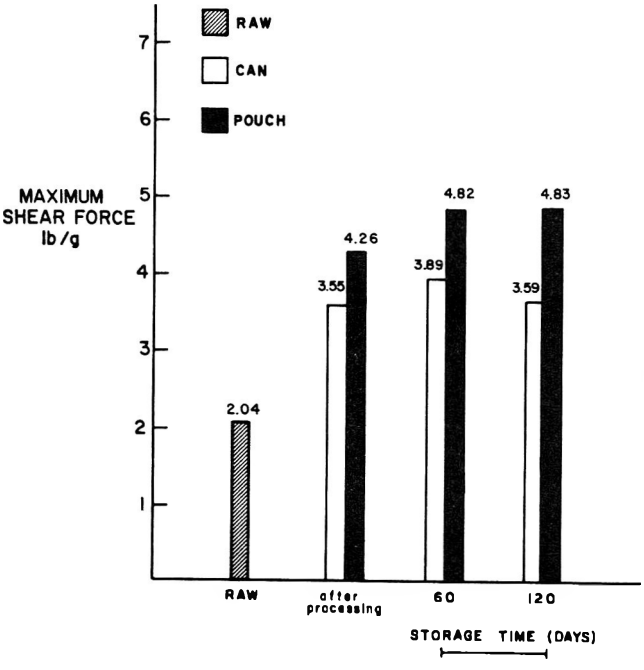

Fig. 3—Effect of processing and storage on shear values of pollock.

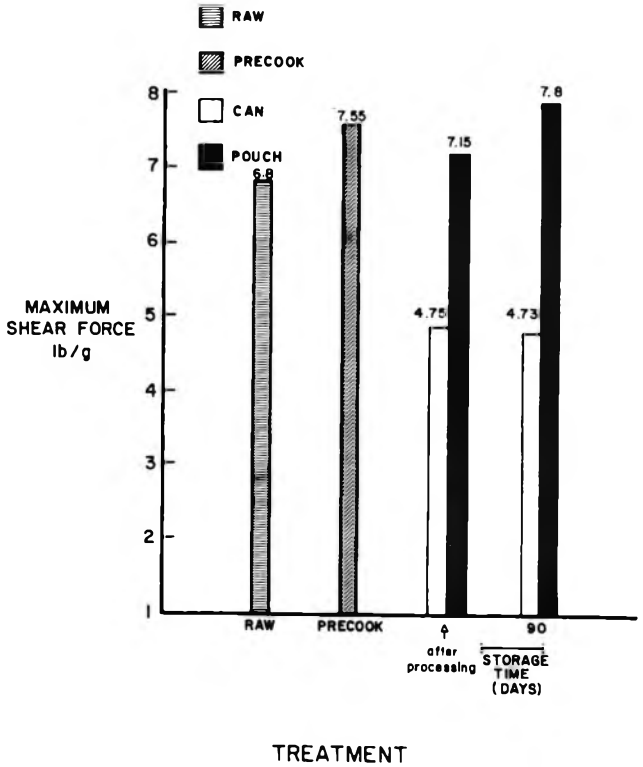

Fig. 4—Effect of processing and storage on shear values of shrimp.

Table 4—Effect of heat processing and storage on sensory evaluations of rainbow trout

Sensory Attributes	Mean scores[a,b] After processing C	After processing P	Storage time (days) 60 C	60 P	120 C	120 P
Color	6.79	6.86	6.0*	7.50*	6.25*	7.50*
Aroma	4.43	4.07	4.19	4.31	4.33	4.13
Flavor	6.57	6.64	5.19**	6.25**	5.81**	6.63**
Tenderness	6.36	5.93	5.19	5.0	5.25	5.0
Juiciness	4.93*	3.93*	3.44	3.38	4.13	3.50
Overall Acceptability	6.71	6.43	5.44**	6.38**	5.81	6.38

[a] Mean ratings using an evaluation scale where 1 is least desirable and 9 is the most desirable except for tenderness and juiciness where 5 is optimum, 9 is too tender or juicy and 1 is too tough or dry. C = Canned product; P = Pouched product.
[b] Differences between cans and pouches which received the same treatment and are underlined where statistically significant.
* $P < 0.05$.
** $P < 0.1$.

Table 5—Effect of heat processing and storage on sensory evaluations of pollock

Sensory attributes	Mean scores[a,b] After processing C	After processing P	Storage time (days) 60 C	60 P	120 C	120 P
Color	5.38	6.63	5.50	7.50	5.38	7.50
Aroma	4.38	4.31	4.56	3.88	5.0	5.31
Flavor	4.88	6.19	5.13	7.06	4.88	5.94
Tenderness	5.56	4.94	4.88	4.94	4.81	4.13
Juiciness	4.63	4.13	4.16	3.94	4.06	3.56
Overall acceptability	5.53	6.07	4.88	6.69	5.13	5.05

[a] Mean ratings using an evaluation scale where 1 is least desirable and 9 is most desirable except for tenderness and juiciness, where 5 is optimum, 9 is too tender or juicy and 1 too tough or dry. C = Canned product; P = Pouched product.
[b] Differences between cans and pouches which received the same treatment and are underlined were statistically significant ($P < 0.05$).

Table 6—Effect of heat processing and storage on sensory evaluations of shrimp

Sensory attributes	Mean scores[a,b] After processing C	After processing P	Storage time (days) 90 C	90 P
Color	5.50*	7.38*	6.13	6.63
Aroma	5.58**	6.50**	6.38	6.69
Flavor	5.75*	6.75*	5.86**	6.50**
Tenderness	5.81	5.5	5.81	5.56
Juiciness	4.63	5.06	4.44	4.63
Texture acceptability	5.69	6.06	5.05	6.06
Overall acceptability	5.63**	6.38**	5.50**	6.19**

[a] Mean ratings using an evaluation scale where 1 is least desirable and 9 is the most desirable except for tenderness and juiciness where 5 is optimum, 9 is too tender or juicy and 1 is too tough and dry. C = Canned product; P = Pouched product.
[b] Difference between cans and pouches which received the same treatment and are underlined were statistically significant.
* $P < 0.05$.
** $P < 0.1$.

rated more acceptable overall. The pouched shrimp were also judged to be more desirable in color and tenderness in most tests. The panelists did not detect significant differences in desirability for aroma, tenderness or juiciness in most cases, although for most products, the pouch scored higher. No consistent effects of storage could be found for any product or container.

CONCLUSIONS

IN CONCLUSION, this study pointed out that there is a significant reduction in the thermal processing time required to achieve an equal lethality for retortable pouched fishery products when compared to cans containing equal weights of product. This reduced processing time has important benefits. Heat sensitive vitamins are less liable to be destroyed or solubilized during processing; and there is less chance of developing heat damage which can reduce the overall quality of the food. Chemical, physical, and sensory information was obtained for both canned and pouched products. A comparison of this information showed that the retortable pouched products had better nutrient retention with respect to Vitamin B_1, were firmer in texture, and were lighter in color. In sensory evaluations, the panelists scored the pouched products higher, though not always significantly, in flavor and overall acceptability. The pouching process produced smaller amounts of off-flavor compounds (NH_3, TMA, carbonyls) than the canning process. And finally, pouched products had at least the same storage stability as canned products. We conclude that retortable pouching produces shelf-stable fishery products with a quality at least equal to and often better than that of canned products.

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Development of a Time-Temperature Integrator Indicator for Frozen Beef

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ABSTRACT

A time-temperature integrator-indicator for frozen beef, based on the diffusion of a dye in gel, was developed, considering as an essential condition that the energy of activation for this device shall coincide with the energy of activation of frozen food deterioration. Different experiments have been performed in order to measure the effective diffusion coefficient of the dye in the supporting medium at different temperatures and to establish the dependance on dye concentration; these results have been related to the rate of food deterioration.

INTRODUCTION

THE TEMPERATURE at which frozen food is maintained is one of the most important variables, which directly affects the length of time it preserves its quality, that is the storage life. Therefore, it is important to monitor the thermal abuses during the stages of transportation and distribution of frozen food.

Basically, there are three types of indicators (Byrne, 1976): Temperature indicators, Time-temperature integrators, Time-temperature integrator indicators.

Temperature indicators show by a change in color when a preselected temperature is reached; this device provides no information concerning how much time the system remained above the selected temperature, nor how far it rose above it (Schoen and Byrne, 1972; Hu, 1972; American Frozen Food Inst., 1977).

Time-temperature integrators begin to react as soon as they are activated and show a gradual change throughout their operative life, reaching their final point for a preselected combination of time and temperature (the final point is generally a change in color).

Time-temperature integrator-indicators are based on the advance of a colored line along a graduated scale; some begin to react when activated, while others are activated on reaching a given temperature. In both cases the higher the temperature to which it is exposed, the faster does the colored line advance along the scale. This type of device appears to be the most promising, as it provides quantitative information and, furthermore, accumulates effects of repeated exposure to variable temperatures.

This time-temperature integrator indicator is based on the diffusion of a dye in a supporting medium, so that the quantified advance over a graduated scale will be proportional to the loss in quality of the frozen food concerned.

The Q_{10} of a food product indicates the magnitude of the increase in the rate of deterioration when the temperature is increased by 10°C and is related to the activation energy of quality losses, Ea_f, by the following relation:

$$Q_{10\ food} = \exp\left[-\frac{Ea_f}{R}\left(\frac{1}{T+10} - \frac{1}{T}\right)\right] = \frac{\theta_T}{\theta_{T+10}} \qquad (1)$$

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where θ_T is the practical storage life at temperature T(PSL).

The Q_{10} of the indicaor, equivalent to the Q_{10} of the food, can be defined as the ratio between the time required for the colored line to advance a distance δ at a temperature T, and the time elapsed to reach the same distance at a temperature T + 10 (Kramer and Farquhar, 1976; Schubert, 1977).

The above definition takes into account that the time elapsed for the dye to cover a determined distance is proportional to the practical storage life of the food.

The purpose of this study was to develop a time-temperature integrator indicator for frozen beef in a temperature range between −5°C and −25°C considering as an essential conditon that the energy of activation of the time-temperature integrator indicator shall coincide with the activation energy of the frozen food deterioration.

MATERIALS & METHODS

Materials

The time tempeature integrator indicator was based on the diffusion of a dye (solution of methylene blue (MW = 355,89), conc. 10^{-3} g/ml in agar gel 1% W/V).

Determination of the effective diffusion coefficient

The color advance in the time-temperature integrator indicator is related to the diffusion coefficient of the dye in the supporting medium.

The effective diffusion coefficient of the methylene blue in the agar gel was measured at different temperatures, (−5°C, −10°C, −15°C, −20°C and −25°C) using cylindrical 2.3 cm diameter x 10 cm long tubes containing 10 ml and 20 ml of methylene blue solutions layered on 15 ml of gel that acts as a semi-infinite medium.

The concentration profiles of the dye were measured using a densitometric technique (Shimadzu Dual Wavelength TLC Scanner CS-910). At the same time, the dye concentration in the gel at the interface and that corresponding to the advance front of the colored line, were determined by a spectrophotometric technique, cutting thin sections of gel in the appropriate zones. A Shimadzu Digital UV150 spectrophotometer was used, at a wave length of 665 mμ.

The advance of the dye was quantified in terms of time for the different temperatures, using a graduated scale attached to the diffusion tubes.

RESULTS & DISCUSSION

Measurement of the diffusion coefficient and its dependence on the concentration

The methods for measuring the diffusion coefficeint can be grouped under two types (Vickerstaff, 1954; Crank, 1957; Crank and Park, 1968) depending on whether the flow of matter is stationary or transitory.

In the present study experiments were made in a semi-infinite medium, (nonstationary state) in which concentration distribution curves in terms of time were analyzed.

The solution of the diffusion equation with the appropriate initial and border condition leads to a theoretical profile of concentration versus distance. Comparison of the theoretical and experimental curves gives the desired value of the diffusion coefficient.

The diffusion of a substance A in a complex heterogeneous medium such as the gel, can be interpreted in a simplified form as the diffusion in a pseudobinary mixture formed by the dye (A) and the matrix (m), formulating the equations in terms of the effective diffusivity $D_{Am} = D$ that takes into account all the diffusional interactions that are very important when the polymer-disperse phase interface is of high area (Bird et al., 1960; Lightfoot, 1974).

The gel matrix is considered as one of the diffusion species, except that it is constrained to zero velocity and to near zero concentration gradient.

In the case of diffusion in polymers, the effective diffusion coefficient is in general highly dependent on the concentration of the substance that diffuses (Crank, 1957; Busk and Labuza, 1979); this fact should be taken into account in the statement of diffusion equations.

The equation governing unidirectional diffusion of a substance A in a nonstationary state, in terms of mass concentration [ρ_A = (mass of A)/(total volume)], in the absence of convective movement and chemical reaction, in a semiinfinite medium is:

$$\frac{\partial \rho_A}{\partial t} = \frac{\partial}{\partial x}\left(\rho D(\rho_A, T)\frac{\partial w_A}{\partial x}\right) \tag{2}$$

where w_A is the mass fraction of A.

Accepting that ρ is constant,

$$\frac{\partial \rho_A}{\partial t} = \frac{\partial}{\partial x}\left(D(\rho_A, T)\frac{\partial \rho_A}{\partial x}\right) \tag{3}$$

with conditions:

$$\begin{array}{lll} t = 0 & \rho_A = 0 & \forall x \\ t > 0 & \rho_A = \rho_{Ai} & x = 0 \\ & \rho_A = 0 & x \to \infty \end{array} \tag{4}$$

where ρ_{Ai} is the mass concentration of the dye in the gel at the interface.

The value of the effective diffusion coefficient D at a constant temperature and its dependence on ρ_A was obtained from the distribution of concentration observed experimentally at a given time and using a solution given by Crank (1957):

$$D\left|_{\rho_A = \rho_{A'}} = -\frac{1}{2t}\frac{dx}{d\rho_A}\right|_{\rho_A = \rho_{A'}} \int_0^{\rho_{A'}} x\, d\rho_A \tag{5}$$

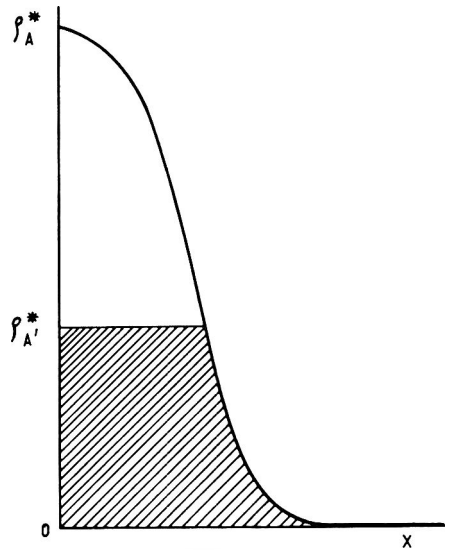

Fig. 1—Dimensionless concentration profile (ρ_A^* vs. distance). At $\rho_{A'}^*$, the diffusion coefficient is evaluated by the shaded area.

where $\rho_{A'}$ is any value of ρ_A between 0 and ρ_A.

Working with dimensionless dye concentration

$$\rho_A^* = \frac{\rho_A}{\rho_{Ai}} \tag{6}$$

the result is:

$$D\left|_{\rho_{A'}^*} = \frac{1}{2t}\frac{dx}{d\rho_A^*}\right|_{\rho_{A'}^*} \int_0^{\rho_{A'}^*} x\, d\rho_A^* \tag{7}$$

where the integral corresponds to the shaded area in Fig. 1.

For the described system the plane x = 0 corresponds to the dye-gel interface.

Fig. 2 shows one of the curves of dimensionless dye concentration versus distance obtained by means of the densitometric technique for T = −5°C, −10°C, −15°C, −20°C and −25°C, t = 16 days.

Applying Eq. (7) corresponding to the method proposed by Crank, it was possible to determine on those profiles the variation of the effective diffusion coefficeint with the concentration of the dye at different temperatures.

Fig. 3 shows the variation of D with ρ_A^* for some working temperatures; an increase of D with concentration ρ_A^* was observed according to the following relationship:

$$D = \frac{D_o}{1 - \alpha \rho_A^*} \tag{8}$$

the mean value being $\alpha = 0.9$ for the different working temperatures.

By extrapolation it was possible to obtain for each temperature the value of D_o corresponding to $\rho_A^* = 0$ (effective diffusion coefficient at infinite dilution). Experimental data are presented in Table 1 in the column corresponding to Crank's method; the results shown in the other column will be discussed in the section related to the activation energy of the indicator diffusional process.

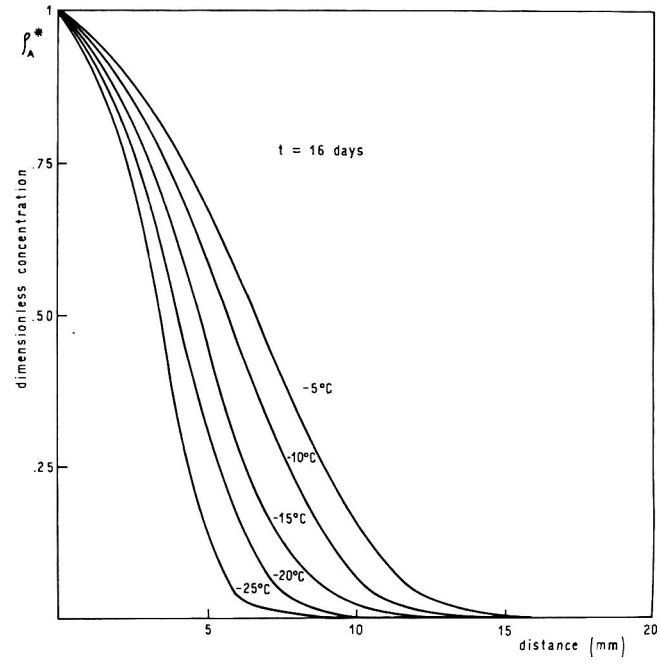

Fig. 2—Dimensionless concentration profiles of methylene blue in agar gel at different temperatures.

Thermodynamic interpretation for the variation of the effective diffusion coefficeint with concentration

The flux of A in the case of ordinary diffusion in a binary mixture can be expressed (Bird et al., 1960) as:

$$j_A = \frac{c^2}{\rho R T} M_A^2 M_B D_{AB} x_A \left[\frac{\partial}{\partial x_A}\left(\frac{\bar{G}_A}{M_A}\right)\right]_{T,P} \nabla x_A \quad (9)$$

since

$$d\bar{G}_S\,|_{T,P} = R T d \ln a_A \quad (10)$$

Eq. (9) becomes:

$$j_A = -\frac{c^2}{\rho} M_A M_B D_{AB} \left(\frac{\partial \ln a_A}{\partial \ln x_A}\right)_{T,P} \nabla x_A \quad (11)$$

This expression can be compared with others of the first Fick law which relates mass flow of A with molar fraction:

$$j_A = -\frac{c^2}{\rho} M_A M_B D_{AB} \nabla x_A \quad (12)$$

by comparing Eq. (12) with Eq. (11) the result is:

$$D_{AB} = D_{AB} \frac{\partial \ln a_A}{\partial \ln x_A} \quad (13)$$

then D_{AB} coincides with D_{AB} when solutions are ideal.

The equation can be applied to determine the effective diffusion coefficient in a pseudo binary mixture represented by the dye and the gel and considering the variation of the dye activity in the matrix with dye concentration, it is possible, theoretically, to calculate the desired functionality according to Eq. (13).

In a dyeing system the molecules can be adsorbed and migrate along the matrix surface. When a dye is adsorbed on specific sites in a monomolecular layer the activity of the substance may be represented by the expression of Atherton, (Crank and Park, 1968):

$$a_A = \frac{c_A}{s_A - \beta c_A} \quad (14)$$

where c_A is the concentration of dye in the polymer, s_A is the concentration of saturation, that is proportional to the total number of sites in the polymer and β is related to affinity of the dye for the matrix.

Considering that $x_A = c_A/c$ and applying Eq. (13) the following relationship is obtained:

$$D = \frac{D}{1 - \beta \frac{c_A}{s_A}} \quad (15)$$

This theoretical expression interprets the experimental variation of the effective diffusion coefficient with concentration presented in Eq. (8).

Activation energy of frozen beef deterioration

The time-temperature-tolerance relationships of frozen foods are not mathematical functions but empirical data subjected to large variability, particularly because of changes in the quality of raw material and type of packaging (Singh and Wang, 1977).

Khan and Lentz (1977) attempted to compare biochemical changes in frozen beef (quality index) with data obtained by taste panel tests to get the assestment of quality losses on objective bases.

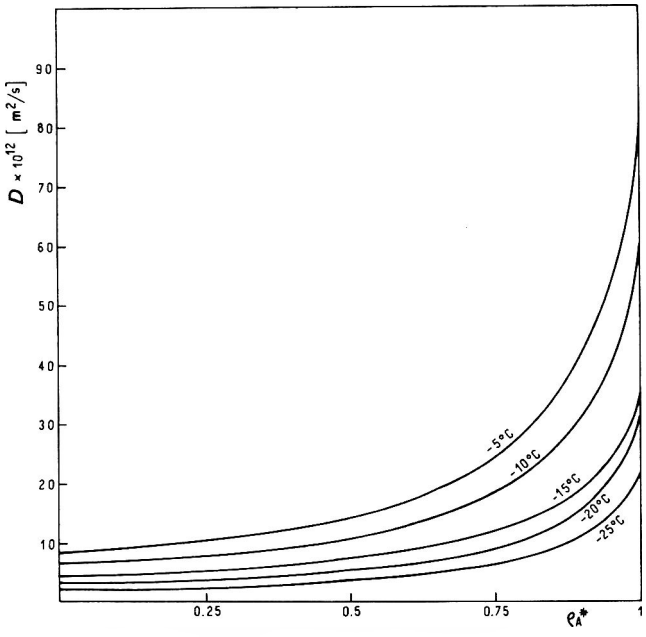

Fig. 3—The effective diffusion coefficient D as a function of dimensionless concentration. ($D = D_0/1 - \alpha \rho_A^*$).

Table 1—Comparison of the diffusion coefficients (D_0) of methylene blue in agar gel in terms of temperature obtained by two experimental methods

Temperature (°C)	$D_0 \times 10^{12}$ (m²/sec) Crank's method	Advancing lines method
− 5	8.0	8.0
−10	6.0	6.3
−15	4.0	4.2
−20	3.0	3.1
−25	2.0	2.1

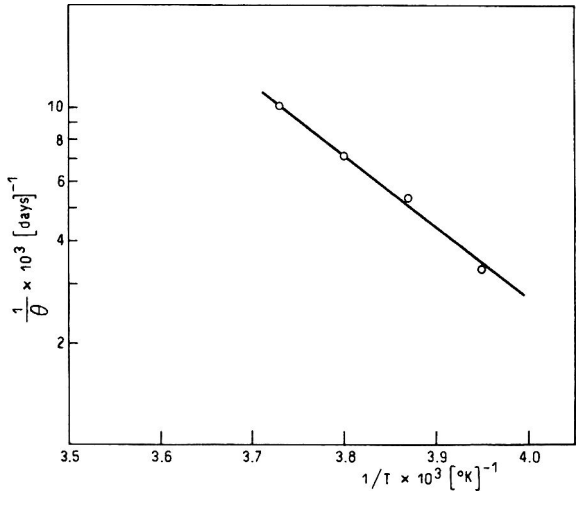

Fig. 4—Effect of storage temperature on frozen beef practical storage life.

Table 2—Practical storage life of beef on terms of temperature. (Source: International Institute of Refrigeration, 1964; Jul M., 1969)

θ = PSL (days)	Temperature (°C)
100	− 5
140	−10
185	−15
300	−20
600	−25

Numerous data on practical storage life (PSL) for frozen beef in terms of storage temperature can be extracted from the literature (International Institute of Refrigeration 1964, 1972; Van Arsdel et al., 1969); the values presented in Table 2 have been obtained from the International Institute of Refrigeration 1964, and Jul M., 1969.

Based on Eq. (1) the plot of log $1/\theta_T$ vs 1/T (Fig. 4) leads to the activation energy of frozen beef deterioration obtaining from the Arrhenius relationship a value Ea_f = 3.95 x 10^4 Joule/mol.

Eq. (1) indicates that Q_{10} is not constant over a wide range of storage temperature. Table 3 shows the values of Q_{10} for frozen beef in terms of temperature.

Activation energy of the indicator diffusional process

Q_{10} of the indicator was defined in terms of the time required for the dye to cover a distance δ at a temperature T with respect to the time required to cover the same distance at a temperature T + 10°C. For that reason it becomes necessary to measure the advance of the colored line in terms of time.

Assuming that diffusion occurs in a semiinfinite medium, the advance of the dye was plotted in Fig. 5 in terms of $\sqrt{t}$, keeping a linear relationship; no differences being recorded in the straight lines for the tested volumes of dye. When D varies with concentration according to Eq. (8), the theoretical solution of Eq. (3) is given by the curves of Fig. 6 in terms of ρ_A^* vs. $x/\sqrt{4D_o t}$.

This solution was obtained by Fujita (Crank, 1957) and the values on the curves correspond to $1/1-\alpha$, the parameter that gives the ratio between D at $\rho_A^* = 1$ and D when $\rho_A^* = 0$.

The experimental profile of dye dimensionless concentration ρ_A^* obtained by the densitometric technique at −5, −10, −15, −20, −25°C were compared with Fujita's theoretical curves; a satisfactory coincidence being noted for $1/1-\alpha = 10$. The plot of ρ_A^* in terms of $x/\sqrt{4D_o t}$ at −15°C is presented in Fig. 7.

Measurements of the dye concentration in the colored front ($x = \delta$) and in the interface ($x = 0$) show that these concentrations remain constant during all the experiences being:

$$\rho_A^* = 0.005 \text{ at } x = \delta_v \; \forall t$$

For this dimensionless concentration value, the theoretical curve of Fig. 6 with $1/1-\alpha = 10$ gives the following ratio:

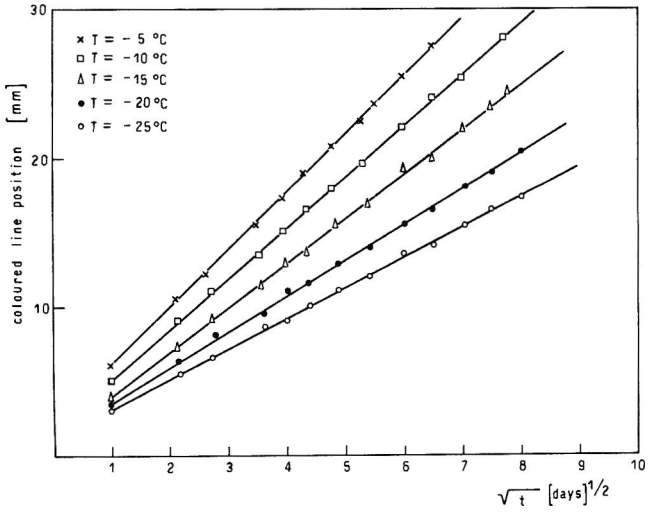

Fig. 5—Colored line position as a function of $t^{1/2}$.

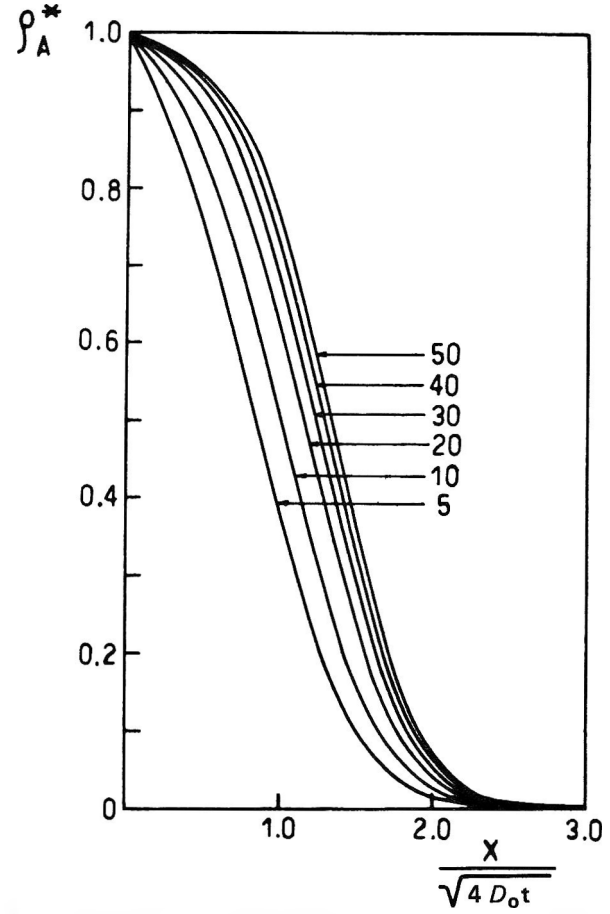

Fig. 6—Theoretical concentration-distance curves for $D = D_o/(1-\alpha \rho_A^*)$ (Crank, 1957).

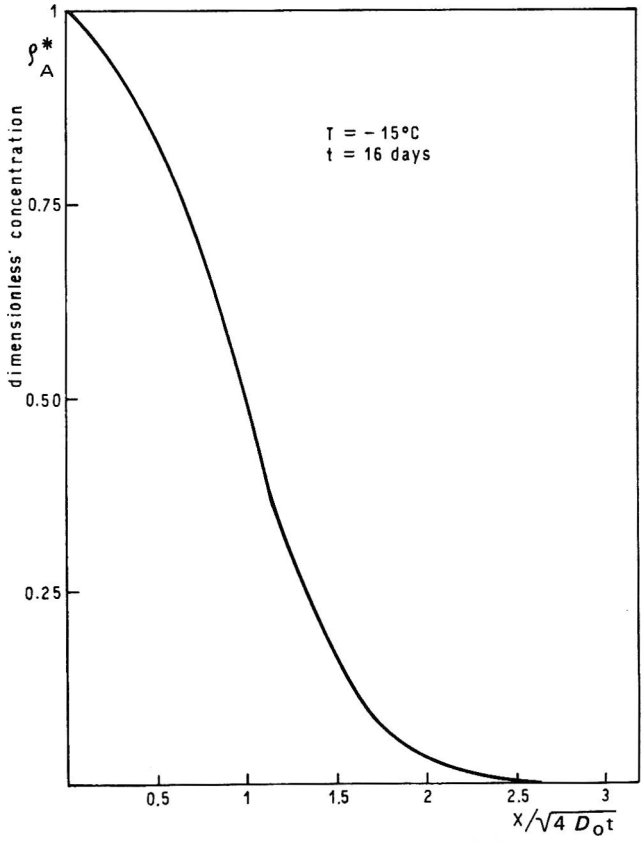

Fig. 7—Experimental concentration profile (ρ_A^* vs. $x/\sqrt{4 D_o t}$) for methylene blue in agar gel. T = −15°C, t = 16 days.

$$\frac{\delta}{\sqrt{4D_o t}} = 2.3 \qquad (16)$$

That is, the position of the colored line δ, is proportional to $\sqrt{D_o t}$ in a similar manner to that given by the solution of semi-infinite medium with D = cte, but differing in the numerical constant.

One way of verifying the values of D_o found experimentally with Crank's method is to use the values of slopes ξ of Fig. 5 (Method of the advancing lines) at different temperatures which correspond to the following relation:

$$\xi = 2.3\sqrt{4D_o} \qquad (17)$$

The values of D_o obtained by this method are also presented on Table 1 showing satisfactory coincidence between them.

Plotting log D_o versus 1/T the apparent activation energy of the dye diffusion in the gel Ea_d was obtained, considering that D_o has an Arrhenius type dependence with temperature (Crank and Park, 1968). Linear regression (correlation coefficient 0.99) leads to the apparent activation energy of dye diffusion $Ea_d = 3.86 \times 10^4$ joule/mol that is comparable to the activation energy of frozen beef deterioration.

According to the definition of Q_{10} of the indicator and taking into account Eq. (16) the following relation can be derived:

$$Q_{10}\ \text{indicator} = \frac{t_T}{t_T + 10} = \frac{D_o\,|T+10}{D_o\,|T} = e^{-\frac{Ea_d}{R}\left(\frac{1}{T+10} - \frac{1}{T}\right)} \qquad (18)$$

Values of Q_{10} obtained from Eq. (18) are presented in Table 4 as a function of temperature.

Table 3—Q_{10} of beef in terms of temperature range

Temperature range (°C)	Q_{10} of foodstuff
− 5°C to −15°C	1.99
−10°C to −20°C	2.04
−15°C to −25°C	2.10

Table 4—Q_{10} of the indicator in the range of working temperatures

Temperature range (°C)	Q_{10} of the Indicator
− 5 to −15	1.90
−10 to −20	2.03
−15 to −25	2.00

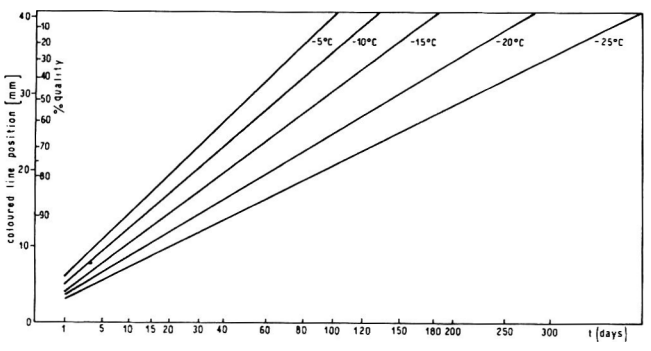

Fig. 8—Chart of the time temperature integrator indicator. Each curve on the chart has been determined by recording the movement of the color along a scale when exposed to a specific constant temperature. Conversion of the colored line position to percentage of remained practical storage life in the foodstuff.

The usefulness of the indicator is based on the fact that, through a simple reading, it permits determination of the percentage of practical storage life that the foodstuff retains.

In Fig. 8 the advance of the indicator colored line (mm) vs time (days) was plotted from the data of Fig. 5; in the same figure a scale in ordinates was included corresponding to the percentage of practical storage life that the foodstuff retains (% quality) defined as:

$$\%\ \text{quality} = \left(1 - \frac{t_T}{\theta_T}\right) \times 100 \qquad (19)$$

assuming a zero order kinetics where t_T is the storage time at temperature T and the values of practical storage life at different temperatures (θ_T) were obtained from Table 2.

Labuza (1979) demonstrated theoretically that for less than 50% of consumed storage life, zero and first order predictions of quality losses are comparable and emphasized the importance of a correct Q_{10} evaluation.

It can be observed in Fig. 8 that a parallelism exists between the advance of the indicator and the percentage of P.S.L. that remains in the food. The foodstuff expires when the position of the colored line reaches the value of 0% regardless of the temperature, although it corresponds to different exposition times.

The way to use the indicator together with the chart is easy:

(a) When the position of the colored line is known, the percentage of losses in storage life can be determined regardless of the time-temperature combination to which the foodstuff was submitted.
(b) Knowing how long the product has been handled out, the effective temperature to which it was exposed can be inferred.

The developed indicator was tested, performing experimental simulations of storage conditions with packages of beef samples that have been exposed to thermal step changes in freezing chambers with controlled temperature and, satisfactory results were obtained in the determination of the effective temperature.

CONCLUSIONS

Based on the diffusion of a dye in agar gel, a time-temperature integrator indicator has been developed whose activation energy coincides with that of frozen beef deterioraton in accordance with the purpose of the study.

The developed indicator simulates satisfactorily the deterioration of frozen beef and allows the determination of the percentage of losses in practical storage life of the foodstuff by means of a simple reading. Moreover, knowing the time that had elapsed from the activation of the indicator the effective temperature to which the product was exposed during its distribution can be established.

NOMENCLATURE

a_A	activity of species A
c	total molar concentration
c_A	concentration of A in the polymer
D	effective diffusivity
D_o	effective diffusion coefficient at infinite dilution
D_{AB}	binary diffusivity in terms of chemical potential
E_a	activation energy
G_A	Gibbs partial molar free energy
j_A	mass diffusional flux of A
M_A, M_B	molecular weights of species A and B
P	pression
Q_{10}	temperature quotient
R	gas constant
s_A	concentration of saturation
T	temperature

t time
w_A mass fraction of component A
x distance
x_A molar fraction of A

Greek letters

α parameter in Eq. 15
β parameter related to the affinity of the dye for the matrix
δ position of the colored front
ξ slope of lines in Fig. 5
ρ total mass concentration
ρ_A mass concentration of dye in the gel
$\rho_{A'}$ any value of ρ_A between 0 and ρ_A
θ practical storage life

Superscripts

* dimensionless value

Subscripts

d dye
f food
i interface value
T at temperature T

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Influence of Pre-Rigor Processing, Mechanical Tenderization, Tumbling Method and Processing Time on the Quality and Yield of Ham

ROBERT R. MOTYCKA and PETER J. BECHTEL

ABSTRACT

Eight market weight hogs with similar genetic and management background were conventionally slaughtered with the resulting sixteen pork carcass sides randomly assigned to treatments. Four treatments at 2 levels each (2^4 factorial design) included muscle condition [pre-rigor (hot) or post-rigor (chilled)], mechanical knife blade tenderization [no tenderization or tenderization], vacuum tumbling [continuous or intermittent], and the processing delay time between vacuum tumbling and further processing [no delay time or 20-hr delay]. Results indicated that pre-rigor processed meat tissue exhibited higher pH, water-holding capacity (WHC), and cooked yield characteristics. Mechanical knife blade tenderization consistently increased protein content of the uncooked meat exudate, cooked yield, and palatability characteristics. Tumbling method and processing time had little influence on either uncooked or cooked meat characteristics.

INTRODUCTION

CONSIDERABLE EFFORT has been put forth since 1970 in an attempt to gain an understanding of the physical, chemical and histological changes in meat associated with or as a result of the mechanical mixing action referred to as "massaging" or "tumbling." Such efforts by Siegel (1976), Theno (1977), Krause et al. (1978) and Ockerman et al. (1978) have focused on the changes in meat tissue due to massaging or tumbling and associated processing variables of salt, phosphate, fat and cooking temperature levels. Other research has evaluated the influence of tumbling method (continuous or intermittent) and time on cooking yield and quality characteristics of boneless ham (Ockerman et al., 1978; Krause et al., 1978; Gillett et al., 1981). Currently, industry reports reveal that a multitude of massaging and tumbling methods are being used (Weiss, 1974; Michels, 1976). Lack (1977) and Jacobson (1978) have indicated that industry utilizes continuous tumbling methods with the time of operation ranging from 20 min to 6 hr. Massaging via intermittent method for 15–20 min per hr for 18–24 hr periods is common. From these research and industry reports on various massaging and tumbling methods and time schedules, it is apparent that additional information is needed to clarify the essential method(s) and time period(s) required in the production of section and formed ham.

A relatively new ham processing innovation of mechanically disrupting the meat tissue structure prior to massaging or tumbling is gaining acceptance (Michels, 1976). This meat disruption process is referred to as "mechanical tenderization" or "masceration" depending on the type of machine used. The mechanical technique and severity of the meat tissue disruption varies due to different machine designs. This disruption of the meat tissue structure would seemingly improve tenderness and allow freer movement of intra- and extracellular materials resulting in improved uniformity in appearance and binding between meat sections. However, no information has been reported to substantiate such assumptions.

All the previously published studies involving tumbling or massaging have utilized post-rigor (chilled) meat. Postmortem changes in meat and their relationship to meat processing characteristics have been extensively studied and reviewed (Cassens, 1966; Newbold, 1966; Pearson, 1971). Sayre and Briskey (1963), Johnson and Hendrickson (1970) and Solomon (1979) have shown an increase in extractable salt soluble proteins from pre-rigor processed meat. Hamm (1978) has indicated that the increased water-holding capacity of pre-rigor processed beef can be preserved by the addition of salt. Both increases in extractable salt soluble proteins and water-holding capacity could aid in the binding of meat sections and improving yield in massaged or tumbled products.

Therefore, the objectives of this experiment were to: (1) access the influence of pre-rigor processed meat, mechanical tenderization, tumbling method and processing time on yield and quality of section and formed ham; and (2) identify possible relationships between the uncooked meat tissue characteristics and the subsequent cooked yield.

MATERIALS & METHODS

EIGHT MARKET WEIGHT HOGS with similar genetic and management background were conventionally slaughtered with the resulting sixteen pork carcass sides randomly assigned to one of sixteen treatments (Fig. 1) such that animal variation could be statistically accounted for (Box et al., 1978). Pre-rigor processed ham muscles (semimembranous and biceps femoris) were removed from the carcasses within 10 min after bleeding; whereas, post-rigor processed ham muscles remained intact and were chilled at 2 ± 2°C for 24 hr. The further processing sequence for both pre- and post-rigor ham muscles consisted of trimming, multineedle hand injection of the curing solution, mechanical knife blade tenderization, vacuum tumbling, a processing delay time interval, stuffing, cooking, and chilling. Due to difficulty in accurately separating the adductor muscle from the semimembranous muscle, the two muscles remained joined and were considered as a semimembranous muscle.

Trimming involved the removal of all visable subcutaneous and intermuscular fat and connective tissue. The individual muscles were multineedle hand injected (20% of initial weight) with the overflow and subsequent weapage of the curing solution collected and added back to the muscles at the time of tumbling. The curing solution contained 81.3% water, 12.4% salt, 3.7% dextrose, 2.5% phosphate mixture, and 0.08% sodium nitrite.

Mechanical tenderization

Mechanical tenderization was achieved with a Model IT 2 Belam Mechanical Tenderizer (Belam, Inc., Downers Grove, IL). This machine is equipped with two horizontal, parallel shafts to which 65 circular variegated knife blades per shaft are attached and spaced 5 mm apart. Each shaft rotates towards the other drawing meat sections down past the knife blades. The distance between the knife blades from each shaft is less than 1 cm. Therefore, meat sections receive cuts 5 mm apart and within 1 cm of being completely separated. In this experiment, individual muscles were tenderized via one pass between the knife blades such that cuts occurred perpendicular to the long axis of the muscle fibers.

Tumbling and cooking

Tumbling was accomplished with a Universal 190 Inject Star Tumbler (Globus Labs, Hackensack, NJ) operated at 28 rpm with a

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vacuum of 584–660 mm Hg. The meat was tumbled either continuous or intermittently at 2 ± 2°C with total tumbling revolutions (3360) and time under vacuum (4 hr) held constant. The intermittent tumbling cycle consisted of alternating rotation (15 min) and rest (15 min) periods. After tumbling, the meat was either sampled and cooked or stored for 20 hr at 2 ± 2°C prior to sampling and cooking. Total processing time from injection to cooking was either 4 or 24 hr. Random samples for meat tissue pH, water-holding capacity and exudate weight and composition were collected on each muscle prior to weighing and hand stuffing (two halves of each muscle) into size 6 easy peel, prestuck, fibrous casing (Tee-Pak, Danville, IL). The individually stuffed muscles were vacuum packaged (Kenfield Model C-14, International Kenfield, Park Ridge, IL) in a cooking pouch, cooked in a hot water bath (75 ± 2°C) for approximately 150 min until an internal temperature of 72 ± 2°C, and chilled to an internal temperature of 2 ± 2°C.

Cooking yield and sensory evaluation

After chilling, each muscle was removed from the cooking pouch and casing and allowed to drain for 10 min prior to weighing. Cooking yield was calculated:

$$\% \text{ Cooking Yield} = \frac{\text{Wt after cooking}}{\text{Wt before cooking}} \times 100$$

Visual evaluation of the cooked cut surface [3 slices (1.5 cm thick) with the first slice cut 4 cm from the meat section end] of each treatment was performed by a six-member experienced panel for shape uniformity, cure color distribution, cure color intensity and overall appearance according to a nine-point scalar test (9 – extremely desirable).

Palatability evaluations of a 2 cm^3 meat portion served at 2 ± 2°C were performed by a six-member experienced taste panel for flavor, tenderness, juiciness and overall satisfaction according to a nine-point hedonic scale (9 – extremely desirable).

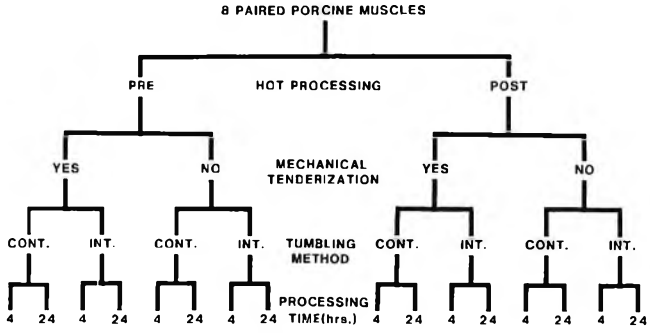

Fig. 1—Experimental design.

Water-holding capacity, pH, exudate weight and composition

The press technique of Wierbicki and Deatherage (1958) was used to measure the water-holding capacity of the processed uncooked meat. The meat tissue pH was determined in duplicate according to procedures by Koniecko (1979). Exudate weight per meat surface area was measured by applying a dried and pre-weighed Whatman No. 1 filter paper (12.5 cm diameter) to the meat surface. The filter paper was allowed to absorb the meat surface exudate until visually saturated. The filter paper plus exudate was weighed, dried for moisture determination and subsequently analyzed for for protein via macro-Kjeldahl method (AOAC, 1975).

Binding strength and hardness determination

Binding strength values were determined as described by Siegel (1976) using a Model TM Instron Universal Testing Machine (Instron Corp., Canton, MA). One slice (1.3 cm thick) was removed at a point 2.5 cm from the end of the meat section. The binding strength of each slice was determined by measuring the force (kg/cm^2) necessary to break the slice. Hardness values were determined as described by Bourne (1978) using an Instron Universal Testing Machine. Duplicate meat cores (2.5 cm diameter x 1.3 cm thick) were penetrated (1.0 cm) with a 0.6 cm diameter rod. Resistance to penetration was recorded as hardness (kg/cm^2).

Statistical analysis

Estimated treatment and interaction effects from a 2^4 factorial experiment with two factor interactions confounded with blocks equal animals were calculated according to Box et al. (1978). Correlation coefficients were determined by use of the Statistical Analysis System of Barr et al. (1976).

RESULTS & DISCUSSION

GILLETT ET AL. (1981) indicated the importance of plac-

Table 1—Means and standard errors for the effect of pre-rigor processing on yield, quality, meat tissue and exudate characteristics of cured, mechanically tenderized, tumbled and cooked pork semimembranous and biceps femoris muscle

Characteristic	Semimembranous			Biceps femoris		
	Pre-rigor	Post-rigor	S.E.	Pre-rigor	Post-rigor	S.E.
Yield (%)[a]	93.1**	91.6	0.44	91.0*	89.0	0.96
Appearance evaluation[b]						
Shape uniformity	6.6	6.7	0.40	5.3	4.9	0.57
Cure color intensity	5.6	5.9	0.34	6.1	6.2	0.25
Cure color distribution	5.9	5.4	0.48	5.3	5.2	0.16
Overall appearance	6.3	6.3	0.35	5.9	5.5	0.45
Palatability evaluation[c]						
Flavor	7.3	7.3	0.12	7.1	7.1	0.16
Tenderness	7.4	7.2	0.48	6.8**	6.0	0.18
Juiciness	6.9	6.9	0.17	6.8	6.5	0.26
Overall satisfaction	7.2	7.2	0.32	6.9*	6.5	0.14
Mechanical evaluation[d]						
Binding strength	0.22	0.23	0.016	0.25	0.24	0.012
Hardness	3.5	3.5	0.15	3.8	4.0	0.18
Meat tissue (before cooking)						
pH	6.4**	6.0	0.10	6.4**	6.0	0.13
WHC (% bound)	59.7*	48.4	4.93	55.9*	42.6	5.98
Exudate (before cooking)						
Weight (mg/cm^2)	11.6	12.3	1.52	13.3	12.7	1.81
Moisture (%)	85.7	85.9	0.50	85.6	86.4	0.59
Protein (%)	9.9	9.7	0.31	10.2	9.6	0.33

[a] Yield (%) = $\frac{\text{Wt of muscle after cooking}}{\text{Wt of muscle before cooking}} \times 100$

[b] Panel evaluation — Scalar test (9 = extremely desirable, 1 = extremely undesirable)

[c] Panel evaluation — Hedonic test (9 = extremely desirable, 1 = extremely undesirable)

[d] Measured on an Instron Universal Testing Machine in kg/cm^2

* Significant ($P < 0.05$)

** Highly significant ($P < 0.01$)

ing meat processing research with laboratory size equipment in proper perspective. Such influences as equipment capacity and design and the industrial environment are variables of noteworthy dimensions. However, the results from laboratory experimentation are deemed pertinent in developing processing concepts. In light of this perspective, the results of this study should be useful in the advancement of pre-rigor processing, mechanical tenderization and tumbling concepts related to the production of section and formed products.

Effect of pre-rigor processing

The influences of pre-rigor processing on yield, quality, meat tissue and exudate characteristics are shown in Table 1. Pre-rigor processed ham had significantly greater yield of 1.5% and 2.0% for the semimembranous ($P < 0.01$) and biceps femoris ($P < 0.05$) muscles, respectively. Slight, but significant, improvement in palatability characteristics of tenderness ($P < 0.01$) and overall satisfaction ($P < 0.05$) for the biceps femoris muscle was evident. Pre-rigor processed meat tissue (uncooked) consistently exhibited higher pH ($P < 0.01$) and water-holding capacity values ($P < 0.05$). These results support numerous previous reports on pre-rigor pH and water-binding characteristics (Bendall, 1960; Briskey, 1963; Hamm, 1978). The pre-rigor meat tissue's higher pH and water binding capacity would be expected to be associated with the resulting increased cooked yields. However, these values were not correlated (except biceps femoris pH) to cooked yields (Table 2). These results suggest the possibility of additional meat and/or processing characteristics contributing to the increase in cooked yields.

Effect of mechanical tenderization

Mechanical tenderization improved cooked yields ($P < 0.01$) in processed ham muscles (semimembranous and biceps femoris) by 2.1 and 3.6%, respectively (Table 3). All quality characteristics except cure color intensity, overall satisfaction and binding strength were increased in hams processed from the semimembranous muscle (Table 3). Mechanical tenderization improved all palatability characteristics, but reduced appearance characteristics [shape uniformity ($P < 0.05$) and overall appearance ($P < 0.05$)] of hams processed from the biceps femoris muscle. Exudate from mechanically tenderized meat tissue (Table 3) consistently exhibited increased protein levels ($P < 0.01$) which were positively correlated to cooked yields (Table 2). These results agree with Acton (1972a, b) and Theno (1977) in that the increased presence of surface proteins aid in reducing cooking loss. Also, the amount of extractable salt-soluble protein is increased by increasing the degree of muscle destruction. Muscle destruction via a mechanical tenderizer increases the availability of salt-soluble proteins for solubili-

Table 2—Correlation coefficients within muscles and between percent cooked yield and uncooked meat tissue characteristics

Uncooked meat tissue characteristics	Semimembranous					Biceps femoris				
	Observations	Pre-rigor processing	Post-rigor processing	Mechanical tenderization	No mechanical tenderization	Observations	Pre-rigor processing	Post-rigor processing	Mechanical tenderization	No mechanical tenderization
pH	8	0.17	−0.04			8	0.74*	0.40		
WHC	8	−0.32	0.10			8	0.58	0.25		
Exudate weight	8			0.36	−0.68	8			0.70*	−0.49
Exudate protein	8			0.86**	−0.73*	7			0.71*	−0.22

* Significant ($P < 0.05$)
** Highly significant ($P < 0.01$)

Table 3—Means and standard errors for the effect of mechanical tenderization on yield, quality, meat tissue and exudate characteristics of cured, tumbled and cooked pork semimembranous and biceps femoris muscles

Characteristics	Semimembranous			Biceps femoris		
	No tenderization	Tenderization	S.E.	No tenderization	Tenderization	S.E.
Yield[a]	91.3	93.4**	0.44	88.2	91.8**	0.96
Appearance evaluation[b]						
Shape uniformity	6.2	7.1*	0.40	5.8	4.4*	0.57
Cure color intensity	5.8	5.8	0.34	6.1	6.2	0.25
Cure color distribution	4.9	6.4**	0.48	5.4	5.1	0.16
Overall appearance	5.6	7.0**	0.35	6.3	5.1*	0.45
Palatability evaluation[c]						
Flavor	7.1	7.5**	0.12	6.9	7.3*	0.16
Tenderness	6.7	7.9*	0.48	5.8	7.1**	0.18
Juiciness	6.7	7.1*	0.17	6.2	7.1**	0.26
Overall satisfaction	7.0	7.5	0.32	6.2	7.2**	0.14
Mechanical evaluation[d]						
Binding strength	0.21	0.23	0.016	0.24	0.25	0.012
Hardness	3.9	3.2**	0.15	4.2	3.8*	0.18
Meat tissue (before cooking)						
pH	6.2	6.2	0.10	6.2	6.2	0.13
WHC (% bound)	56.5	51.6	4.93	45.8	52.6	5.98
Exudate (before cooking)						
Weight (mg/cm^2)	10.7	13.8*	1.52	11.6	14.4	1.81
Moisture (%)	87.5**	84.1	0.50	87.5**	84.6	0.59
Protein (%)	8.2	11.4**	0.31	8.2	11.5**	0.33

[a] Yield = $\frac{\text{Wt of muscle after cooking}}{\text{Wt of muscle before cooking}} \times 100$

[b] Panel evaluation — Scalar test (9 = extremely desirable, 1 = extremely undesirable)

[c] Panel evaluation — Hedonic test (9 = extremely desirable, 1 = extremely undesirable)

[d] Measured on an Instron Universal Testing Machine in kg/cm^2

* Significant ($P < 0.05$)

** Highly significant ($P < 0.01$)

zation. Tumbling provides the energy (impact) necessary for exudate formation. Both muscle destruction and energy (impact or frictional) potentiate the formation of a high protein exudate.

Disruption of meat tissue via a mechanical tenderizer could allow freer movement of intra- and extracellular material of tumbled meat resulting in improved binding between meat chunks. However, there was no difference

Table 4—Means and standard errors for the effect of tumbling method on yield, quality, meat tissue and exudate characteristics of cured, mechanically tenderized and cooked pork semimembranous and biceps femoris muscles

Characteristics	Semimembranous			Biceps femoris		
	C.T.[e]	I.T.[f]	S.E.	C.T.	I.T.	S.E.
Yield[a]	92.3	92.4	0.44	90.3	90.3	0.96
Appearance evaluation[b]						
Shape uniformity	6.5	6.7	0.40	4.9	5.3	0.57
Cure color intensity	5.8	5.7	0.34	5.8	6.4*	0.25
Cure color distribution	5.9	5.4	0.48	5.3	5.2	0.16
Overall appearance	6.4	6.4	0.35	5.7	5.7	0.45
Palatability evaluation[c]						
Flavor	7.3	7.3	0.12	7.0	7.2	0.16
Tenderness	7.3	7.3	0.48	6.3	6.5	0.18
Juiciness	7.0	6.7	0.17	6.8	6.5	0.26
Overall satisfaction	7.3	7.1	0.32	6.7	6.7	0.14
Mechanical evaluation[d]						
Binding strength	0.22	0.23	0.016	0.23	0.26*	0.012
Hardness	3.5	3.5	0.15	3.9	4.1	0.18
Meat tissue (before cooking)						
pH	6.2	6.1	0.10	6.2	6.2	0.13
WHC (% bound)	50.3	57.8	4.93	47.1	51.4	5.98
Exudate (before cooking)						
Weight (mg/cm^2)	12.1	11.8	1.52	12.4	13.7	1.81
Moisture (%)	86.2	85.4	0.50	86.0	86.1	0.59
Protein (%)	9.5	10.2*	0.31	10.1	9.7	0.33

[a] $\text{Yield} = \frac{\text{Wt of muscle after cooking}}{\text{Wt of muscle before cooking}} \times 100$

[b] Panel evaluation — Scalar test (9 = extremely desirable, 1 = extremely undesirable)

[c] Panel evaluation — Hedonic test (9 = extremely desirable, 1 = extremely undesirable)

[d] Measured on an Instron University Testing Machine in kg/cm^2

[e] Continuous tumbling: 28 rpm for 2 hr (3,360 revolutions) at 584—660 mm Hg vacuum and 2°C in Inject Star Tumbler (Model 190)

[f] Intermittent tumbling: 28 rpm (15 min on — 15 min off) for 4 hr (3,360 revolutions) at 584—660 mm Hg vacuum and 2°C in Inject Star Tumbler (Model 190)

* Significant ($P < 0.05$)

Table 5—Means and standard errors for the effect of processing time[a] on yield, quality, and exudate characteristics of cured, mechanically tenderized, tumbled and cooked pork semimembranous and biceps femoris muscles

Characteristics	Semimembranous			Biceps femoris		
	4 hr	24 hr	S.E.	4 hr	24 hr	S.E.
Yield[b]	92.3	92.4	0.44	90.3	90.3	0.96
Appearance evaluation[c]						
Shape uniformity	6.8	6.5	0.40	4.9	5.3	0.57
Cure color intensity	5.5	6.1	0.34	6.1	6.2	0.25
Cure color distribution	5.7	5.6	0.48	5.1	5.4	0.16
Overall appearance	6.1	6.5	0.35	5.8	5.7	0.45
Palatability evaluation[d]						
Flavor	7.3	7.3	0.12	7.1	7.1	0.16
Tenderness	7.0	7.6	0.48	6.2	6.6*	0.18
Juiciness	6.7	7.0	0.17	6.5	6.8	0.26
Overall satisfaction	7.0	7.3	0.32	6.6	6.8	0.14
Mechanical evaluation[e]						
Binding strength	0.22	0.23	0.016	0.23	0.26*	0.012
Hardness	3.4	3.6	0.15	4.1	3.9	0.18
Meat tissue (before cooking)						
pH	6.1	6.2	0.10	6.1	6.3	0.13
WHC (% bound)	53.7	54.4	4.93	48.8	49.7	5.98
Exudate (before cooking)						
Weight (mg/cm^2)	11.7	12.2	1.52	12.9	13.1	1.81
Moisture (%)	85.2	86.3*	0.50	86.1	85.9	0.59
Protein (%)	10.1	9.6	0.31	9.6	10.2	0.33

[a] Time includes injection, tenderization, tumbling and delay time prior to cooking.

[b] $\text{Yield} = \frac{\text{Wt of muscle after cooking}}{\text{Wt of muscle before cooking}} \times 100$

[c] Panel evaluation — Scalar test (9 = extremely desirable, 1 = extremely undesirable)

[d] Panel evaluation — Hedonic test (9 = extremely desirable, 1 = extremely undesirable)

[e] Measured on an Instron Universal Testing Machine in kg/cm^2

* Significant ($P < 0.05$)

detected in binding strength due to mechanical tenderization (Table 3). Siegel (1976) reported a binding strength of greater than 0.1 kg/cm^2 was necessary for the product to exhibit acceptable slicing characteristics. With mean scores for binding strength over 0.2 kg/cm^2, all product exhibited acceptable slicing characteristics.

Effects of tumbling method and processing time

The influences of tumbling method and processing time on yield, quality, meat tissue and exudate characteristics are shown in Tables 4 and 5, respectively. Tumbling method and processing time had no consistent influence on yield, quality, meat tissue and exudate characteristics. These results do not support Krause et al. (1978) that intermittent tumbling (10 min per hr for 18 hr) improves cooked yield or Weiss (1974) and Michels (1976) that holding periods of 1–3 days improve yields. It should be noted that Krause et al. (1978) used a 1:6 ratio of tumble (12 rpm) to rest cycle/hr with 2,160 total revolutions; whereas, a 1:2 ratio of tumble (28 rpm) to rest cycle/hr with 3,360 total revolutions was utilized in this study. Both studies do agree that quality characteristics are not influenced by tumbling method.

In conclusion, pre-rigor processed meat tissue (uncooked) consistently exhibited higher pH and water-holding capacity values with resulting increases in cooked yields. However, the pH and water-holding capacity values were not correlated to cooked yields. Mechanical tenderization consistently improved cooked yield and palatability characteristics of both muscles, but its influence on appearance characteristics was inconsistent. Increased exudate protein content from mechanical tenderized meat tissue was associated with improved cooked yields. There was no difference in binding strength between meat chunks due to mechanical tenderization. Tumbling method and processing time had little influence on yield, quality, meat tissue and exudate characteristics of cured, mechanically tenderized and cooked pork semimembranous and biceps femoris muscles.

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Microflora, Sensory and Exudate Changes of Vacuum- or Nitrogen-Packed Veal Chucks Under Different Storage Conditions

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ABSTRACT

Apart from increases in microflora number at −4°C under vacuum and at 0°C under nitrogen, there was in general no significant ($P > 0.05$) differences in numbers of lactobacilli, psychrotrophs, aerobes and anaerobes between vacuum- and nitrogen-packed veals during 49 days of storage at 3 and 7°C, regardless of dark and lighted displays. The initial psychrotrophs consisted primarily of *Pseudomonas putida* (> 72%) but by day 49 *Lactobacillus* spp. comprised at least 64% of the total in both atmospheres. Sensory and exudate data showed a highly significant ($P < 0.05$) difference due to the effect of storage time and temperature above 0°C. Nitrogen-packed veals exhibited a lower incidence of greening and exudate loss at 0, 3 and 7°C. Light generally had important effects on off-odor and exudate loss, irrespective of package types.

INTRODUCTION

ALTHOUGH VACUUM PACKAGING is currently a preferred means for controlling growth of common aerobic spoilage bacteria and thereby extending the shelf-life of meats, problems can arise because of pack leakage, purge loss, off-flavors/odors and discoloration (Cutaia and Ordal, 1964; Watts et al., 1966; Hodges et al., 1974).

Storage of meats in a modified gas atmosphere using CO_2, N_2, O_2, CO and a mixture of these gases has been suggested as an effective alternative to conventional vacuum-packaging to inhibit aerobic spoilage bacteria and delay metmyoglobin formation.

The inhibitory effect of vacuum packaging and CO_2 on aerobic gram-negative spoilage bacteria is well documented (Baran et al., 1970; Huffman et al., 1975; Silliker et al., 1977). However, prior investigation on the effect of nitrogen gas on microflora development of meats is not extensive and results have been contradictory: nitrogen gas was found to have no inhibitory effect on the microflora of packed fresh beef (Huffman, 1974; Taylor, 1972) but others found tht nitrogen atmosphere packaging was useful in the control of the microflora and in the extension of the shelf-life of lamb (Seideman et al., 1979) and pork chops (Spahl et al., 1981).

In two separate studies, sandwiches were contaminated with *Staphylococcus aureus* (Bennett and Amos, 1982) and *C. botulinum* (Kautter et al., 1981), packaged and stored in an atmosphere of nitrogen at normal refrigerator temperature and under temperature abuse conditions. Under all conditions of storage, spoilage in nitrogen packs was retarded when compared to inoculated samples stored in air. Unfortunately, no comparisons were made with vacuum-packaged samples. The finding of toxigenic samples in some temperature-abused but sensorially acceptable sandwiches prompted the suggestion that these products may be hazardous. Whether the nitrogen packed sandwiches are more hazardous than vacuum-packaged samples remains to be established. Steele and Stiles (1981) found that inoculated enteropathogens survived when vacuum-packed with ham slices and stored 30 days at 4°C. Pathogens later grew in temperature-abused sandwiches fabricated from the ham.

Simard et al. (1983a, b) reported that by storage of frankfurters in nitrogen rather than vacuum, yeast and mold growth was retarded and the apparent shelf-life was improved somewhat through better odor and color retention.

Partmann (1980) studied the effect of packaging in modified atmospheres containing air, CO_2 and a mixture of 20% CO_2 plus 80% N_2 on the shelf-life of beef steaks, veal, pork chops and chicken halves stored at 1°C. In general, shelf-life was increased from 2 to 8 wk when modified atmospheres were used, the best results being obtained with 100% CO_2. Mixed gas atmospheres (80% N_2 + 20% CO_2) also gave substantially improved shelf-life, but a comparison with vacuum-packed products was not made.

Hess et al. (1980) compared the 1°C shelf-life of fresh beef, veal and pork attainable in modified atmospheres (pure CO_2, 20% CO_2 + 80% O_2, 50% CO_2 + 50% N_2) with that in air and vacuum packages. Modified atmosphere packaging gave improved shelf-life over both vacuum and air permeable packaging. Although N_2 was used in some of their work, results were pooled and it was not apparent whether N_2 had been used with veal. Again, pure CO_2 gave the best shelf-life and major factors which influenced shelf-life positively were: low O_2 packaging film permeability, and low initial bacterial numbers. With the exception of these studies by Hess et al. (1980) and Partmann (1980) there has been no work reported on the use of nitrogen- or vacuum-packaging of veal.

The present study reports on the effects of various storage conditions on the microflora, sensory and exudate changes of vacuum- or nitrogen-packed veal chucks.

MATERIALS & METHODS

Samples and packaging

Fresh veal was purchased directly from the manufacturer (Abattoir Labbé, St-Georges-de-Beauce, Québec) within 24 hr of slaughtering and cut into cubes of approximately 5 cm. Veal cuts (150 ± 1g) were aseptically placed at 4°C in reduced gas permeable laminated packages (6 x 8½ in., Winpack Ltd., Winnipeg, Manitoba) with transmission rates of: O_2 (8 cc/m²/24 hr/4°C/100% RH); CO_2 (124 cc/m²/24 hr/25°C/100% RH); H_2O (18.6 cc/m²/24 hr/37°C/100% RH). Winpak laminate (PAE-R 2060) was composed of 60μ polyethylene laminated with 20μ nylon (PA).

Samples in one group were individually vacuum-packed at a vacuum of 73 cm Hg using a chamber-type, heat-seal packaging machine (Pemberton, C.A. Co., Toronto, Ontario). Samples in the other group were back flushed with 100% nitrogen and sealed in the conventional manner. Packages were randomly assigned to storage periods of up to 70 days at −4, 0, 3 and 7°C under fluorescent light (1030 lux) or dark display. Neon lamps were arranged at a distance of about 10 cm above the meat packages. The temperature in the vicinity of the bags was thus increased by about 1−1.5°C. For total darkness, the meat packages were arranged on racks with black plastic sheets. The samples were stored in four separate cold cham-

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bers having a size of 2.1 x 3.4 x 2.7 m under automatically controlled (±1°C) temperature and relative humidity (95 ± 5°C).

Microbiological tests

Sample preparation and enumeration of psychrotrophs, aerobes, anaerobes and lactobacilli are described in a previous report by Simard et al. (1983a). Bacterial strains were identified according to the methods published by Vanderzant and Nickelson (1969). Confirmation of bacterial identity was made at the Microbiology Dept., Quebec Dept. of Social Affairs, Ste-Anne-de-Bellevue, Québec.

Subjective evaluations

At the termination of each storage period, individual packages were aseptically opened after defrost periods (30 min) at room temperature (23°C) for the samples of −4°C but without defrost periods for the rest of samples. Small portions (approx. 1.5-cm cubes) of duplicate samples were evaluated under incandescent light (970 lux) at room temperatures by four panelists composed of food science staff members experienced in meat flavor assessment. Samples were presented to the panel in random order at room temperature in an open area of laboratory. Panelists were instructed to rate each sample on a 7-point scale (7 = extremely desirable; 1 = extremely undesirable) for appearance and odor, using a fresh reference sample. Odor was evaluated by sniffing vapors emanating from the sample source. Surface color attributes of veal chucks (including exudates) were further designated as slight or advanced greening.

Exudate loss evaluations

After the designated storage period, individual packages were reweighed, aseptically opened and the accompanying exudate volume was measured. Exudate loss volumes were scored using a 7-point scale: 1 (no detectable loss) = 0.0–0.5 ml; 2 = 0.5–1.5 ml; 3 = 1.5–2.0 ml; 4 = 2.0–2.5 ml; 5 = 2.5–4.0 ml; 6 = 4.0–5.0 ml; 7 (extreme loss) = >5.0 ml.

Statistical analysis

Bacteriological, sensory and exudate data were treated by using one-way variance analysis with Duncan's multiple range test (Steel and Torrie, 1960). Variance analysis in "split plot" design was used to establish the difference among the various treatments. Factorial analysis was also used to compare the effect of darkness and lighted display at four different temperatures.

RESULTS & DISCUSSION

Microflora changes

Tables 1, 2, 3 and 4 show the changes in numbers of psychrotrophic, lactobacilli, aerobic and anaerobic bacteria. The data in each of the four tables represent the averages of two separate experiments which were run in duplicate.

Table 1 shows that significant ($P < 0.05$) increases in numbers of psychrotrophs occurred after 14 days of storage even at −4°C, with the exception of vacuum-packed samples in the dark and nitrogen-packed samples in the light. A significant ($P < 0.05$) increase in their numbers was noted thereafter in both treatments at 0°C. Psychrotrophs in nitrogen-packed samples after 28 days at 0°C were significantly ($P < 0.05$) higher than those of comparable vacuum-packed samples.

Significant ($P < 0.05$) differences in the influence of packaging atmosphere were not evident as the storage tem-

Table 1—Psychrotrophic bacterial counts (log_{10}/g) of veal chucks arranged according to packaging treatment and storage interval[a,b]

Storage (days)	−4°C				0°C				3°C				7°C			
	Vacuum		N_2		Vacuum		N_2		Vacuum		N_2		Vacuum		N_2	
	R[c]	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
0	4.6b A	4.7a A	4.5a A	4.7a A	4.6a A	4.7a A	4.6a A	4.7a A	4.6a A	4.7a A	4.6a A	4.7a A	4.6a A	4.7a A	4.6a A	4.7a A
14	4.6b A	5.3b C	4.9ab AB	4.7a A	5.7b DE	5.8b DE	6.0b E	5.9b E	5.5b CD	5.2a BC	5.6b C	5.5b CD	9.2c H	9.0c GH	8.6c F	8.8b FG
28	4.4b A	5.7b CD	5.4bc BC	5.1ab B	6.0bc D	6.0b D	6.8c E	7.4c F	7.5c F	7.6b F	7.5c F	7.5c F	9.7d H	9.6d H	8.7c G	8.7b G
49	4.5b A	5.6b B	4.8a A	5.6b B	5.8bc B	6.3b C	7.4d D	8.0d E	8.1d EF	8.1bc EF	8.1d EFG	8.6d FGH	8.7b H	8.7b GH	9.0c H	8.7b H
70	4.1a A	5.2ab B	5.5c B	6.3c C	6.1c C	6.2b C	8.1e DE	8.0d DE	8.4d EF	8.3c EF	8.2d DEF	8.3d EF	8.8bc G	8.6b FG	7.8b D	8.8b G

[a] Means in the same column bearing a common postscript letter do not differ ($P > 0.05$).
[b] Means in the same row bearing a common subscript do not differ ($P > 0.05$).
[c] R ; darkness; S: lighted display.

Table 2—Aerobic counts (log_{10}/g) of veal chucks arranged according to packaging treatment and storage interval[a,b]

Storage (days)	−4°C				0°C				3°C				7°C			
	Vacuum		N_2		Vacuum		N_2		Vacuum		N_2		Vacuum		N_2	
	R[c]	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
0	4.8b A	4.7a A	3.8a A	4.9a A	4.8a A	4.7a A	3.8a A	4.9a A	4.5a A	4.6a A	3.8a A	4.8a A	4.5a A	4.6a A	3.8a A	4.8a A
14	4.0a A	5.6b CD	5.2b B	5.2a B	6.0b D	5.5b BC	5.3a BC	5.3a BC	6.4b E	6.4b E	5.9b D	6.4b E	8.0b F	9.0bc G	8.0b F	7.9b F
28	4.3ab A	5.3ab BC	5.4b BC	4.9a AB	6.2b DE	5.9c CD	6.6b EF	7.0b F	8.3c G	8.4c G	8.0c G	7.2c F	10.1e I	9.5c H	9.4c H	8.2bc G
49	4.4ab A	6.6c CD	6.2c C	5.4a B	5.3a B	6.4d C	7.3b DE	7.8c EF	8.3c FG	8.1c EF	7.9c EF	7.3cd DE	8.5c FG	8.6b FG	8.9c G	8.4c FG
70	3.9a A	5.8bc B	6.3c C	5.8a B	7.1c D	7.8e EF	8.3c FG	8.2c F	8.2c F	8.2c F	8.3c FG	7.5d E	8.9d H	8.7b GH	8.2b F	9.0d H

[a] Means in the same column bearing a common postscript letter do not differ ($P > 0.05$).
[b] Means in the same row bearing a common subscript do not differ ($P > 0.05$).
[c] R: darkness; S: lighted display.

perature increased from 0°C to 3–7°C. This is essentially similar to the pattern observed for beef chucks (Simard et al., 1983c) and ground beef (Lee et al., 1983a) at –4 and 0°C but the growth pattern of microflora in frankfurters was different at –4°C and 0°C, where the psychrotrophs remained relatively constant (<log 5.8) for up to 49 days (Simard et al., 1983a). The longer shelf-life of frankfurters was probably due to the inhibitory action of curing agents used during product formulation.

The highest counts of psychrotrophs were obtained after 14 days at 7°C and psychrotrophs tended to stabilize between log 8.6–9.7 per gram, regardless of the different treatments. Veal chucks were spoiled at levels of 10^8 cells/g.

Changes in the levels of aerobic and anaerobic bacteria in vacuum- or nitrogen-packed veal cuts stored under different conditions are presented in Tables 2 and 3, respectively. Except for a few instances (e.g. log 9.9 for anaerobes during 28 days of storage at 3°C), the pattern of aerobic and anaerobic bacterial growth was similar to that shown by the psychrotrophs. Since samples were packaged under vacuum or under nitrogen, it should be expected that the "predominant" flora could grow with or without oxygen. As with the psychrotrophs, few significant ($P > 0.05$) differences in numbers could be attributed to the influence of packaging type or illumination.

Use of either N_2 or vacuum packaging had no significantly ($P > 0.05$) different effect upon the extent of lactobacilli growth (Table 4). At 0°C, growth of lactobacilli was slightly more rapid under N_2. Elevation of temperature to 7°C had a most profound effect upon growth rate and peak cell populations were achieved within 14 days. This was essentially similar to the pattern noted for psychrotrophic, aerobic and anaerobic bacteria and was taken to indicate that during storage the principal bacteria on the veal chucks were facultatively mesophilic as well as psychrotrophic. This result is in general agreement with our previous results with frankfurters (Simard et al., 1983a), beef chucks (Simard et al., 1983c) and ground beef (Lee et al., 1983a, b). Seideman et al. (1976) also observed similar numbers of psychrotrophic, mesophilic, lactic and anaerobic bacteria from vacuum-packed beef cuts.

An examination of bacterial distribution on veal chucks in Table 5 showed that initially the dominant flora of both package samples consisted of *Pseudomonas putida* (72–87%) but by 28 days their number decreased to less than 17% and the dominant flora became *Lactobacillus* spp. (50–54%). By 49 days, *Pseudomonas* represented <5% of the bacterial types present. *Pseudomonas* have shown themselves to be sensitive indicators of oxygen exclusion in other experiments (Shaw and Nicol, 1969; Christopher et al., 1979) but inhibition by carbon dioxide produced by animal and bacterial cell respiration was probably also important (Roth and Clark, 1975) in reducing their numbers.

Following an initial delay in growth up to day 14 of storage, *Brochothrix thermosphacta* numbers increased significantly ($P < 0.05$) and similarly in all treatments (Table 5). This pattern of growth may be partly explained

Table 3–Anaerobe counts (log_{10}/g) of veal chucks arranged according to packaging treatment and storage interval[a,b]

Storage (days)	–4°C				0°C				3°C				7°C			
	Vacuum		N_2		Vacuum		N_2		Vacuum		N_2		Vacuum		N_2	
	R[c]	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
0	4.8b A	4.4a A	4.0a A	4.7a A	4.4a A	4.4a A	3.3a A	4.7a A	4.4a A	4.4a A	3.3a A	4.7a A	4.4a A	4.4a A	3.3a A	4.7a A
14	5.5c BCDE	5.6b CDE	4.9c AB	4.7a A	6.0b E	5.2b ABCD	5.6b CDE	5.8b DE	5.1b ABC	5.5b BCDE	6.0b E	5.3b BCD	9.3c G	9.1b G	8.2b F	8.3b F
28	4.3ab A	5.5b B	4.3b A	4.4a A	6.2b C	6.5c C	7.5c D	7.4c D	9.9d GH	8.2c E	7.5d D	7.3c D	10.2d H	9.5c G	9.0c F	8.2b E
49	4.6b A	5.6b B	5.6d B	4.6a A	6.6c C	6.9d C	7.8c D	8.6d FG	8.2c DE	8.1c DE	7.0c C	8.3d EF	8.4b EF	8.7b FG	9.0c G	8.2d EF
70	4.0a A	5.5b C	4.9c B	5.5b C	6.6c D	6.7cd DE	7.5c F	8.5d GH	8.2c G	8.2c G	7.3cd F	7.2c EF	8.9e HI	9.0b HI	8.8c HI	9.2c I

[a] Means in the same column bearing a common postscript letter do not differ ($P > 0.05$).
[b] Means in the same row bearing a common subscript do not differ ($P > 0.05$).
[c] R: darkness; S: lighted display.

Table 4–Lactobacilli counts (log_{10}/g) of veal chucks arranged according to packaging treatment and storage interval[a,b]

Storage (days)	–4°C				0°C				3°C				7°C			
	Vacuum		N_2		Vacuum		N_2		Vacuum		N_2		Vacuum		N_2	
	R[c]	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
0	4.0a A	3.9a A	3.8ab A	4.0a A	3.6a A	3.9a A	3.8a A	4.0a A	3.6a A	3.9a A	3.8a A	4.2a A	3.6a A	3.9a A	3.8a A	4.0a A
14	3.8a A	5.0ab B	4.0ab A	4.1a A	5.1c B	4.3a A	5.4b BC	5.8b C	5.5b BC	5.5b BC	5.6bc BC	5.2b B	9.5c F	8.4b E	7.7b D	8.6c E
28	4.1a AB	5.2b D	3.7a A	5.0c CD	4.6bc BCD	4.4a BC	5.8bc E	6.1b EF	6.3c EFG	6.8c G	5.1b D	6.6c FG	9.4c K	8.6bc J	8.2bc H	8.5bc I
49	3.5a A	5.9b C	4.0ab AB	4.5b B	4.3ab B	5.7b C	6.4c CD	7.0c DE	8.1d FGH	7.8d FG	6.2cd C	7.4d EF	8.6b H	8.5b GH	8.6c H	8.3b GH
70	3.3a A	5.3b C	4.2b B	5.4d C	6.3d D	6.4c D	7.7d EF	7.5c E	8.2d FGH	7.9d EFG	6.8d D	8.0d EFG	8.4b GHI	8.9c I	8.9c I	8.7c HI

[a] Means in the same column bearing a common postscript letter do not differ ($P > 0.05$).
[b] Means in the same row bearing a common subscript do not differ ($P > 0.05$).
[c] R: darkness; S: lighted display.

by the initial inhibitory influence of competing organisms (Collins-Thompson and Lopez, 1980) which, when fermentable carbohydrate is used tend to raise the meat pH to a level when lactate is no longer inhibitory toward *B. thermosphacta* (Grau, 1980).

It is possible that oxygen permeability of the packaging film as well as meat pH of meat (Campbell et al., 1979), the amount of surface fatty tissue (Shay and Egan, 1982) or substance availability (Newton and Gill, 1978) may have been responsible for the limited growth of *B. thermosphacta* on vacuum- or nitrogen-packed veal chucks.

During sample storage *Serratia liquefaciens*, *Micrococcus* spp. and *Streptococcus faecalis* constituted a minor portion of the total bacterial population.

The data in this study show that temperature is the most important parameter for growth of lactics and other organisms, as would be expected in view of the known optimal temperatures for growth of these microorganisms on meat. The temperature dependency of the bacteriostatic effect of protective gas has been confirmed by other workers (Adams and Huffman, 1972; Clark and Burki, 1972; Hess et al., 1980; Enfors and Molin, 1981). Enfors and Molin (1981) showed that the inhibitory effect of CO_2 on *Pseudomonas fragi* was increased with decreasing temperature, accompanied by the increased solubility of CO_2 with decreasing temperature.

With respect to the effect of light on the microflora, the number in vacuum packed veal stored under fluorescent light was significantly ($P < 0.05$) higher than in vacuum under dark. However, this difference was not significant ($P > 0.05$) at 0, 3 and 7°C. Although Marriott et al. (1967) found that lighted display at −1°C resulted in higher bacterial numbers than those in dark storage, light did not affect development of surface microorganisms in studies

Table 5—Percentage distribution of psychrotrophic bacteria on veal chucks stored at 0–3°C for 70 days arranged according to packaging treatment and storage interval

Storage (days)	Vacuum pack (dark)					Vacuum pack (light)				
	0	14	28	49	70	0	14	28	49	70
Microbial type										
Pseudomonas putida	79.5	61.8	16.7	2.1	2.3	71.7	59.8	15.0	1.9	4.5
Lactobacillus spp.	6.5	23.0	52.3	63.0	64.5	7.5	25.0	54.0	65.0	64.5
Brochothrix thermosphacta[a]	9.1	12.2	28.3	33.0	31.7	12.5	12.0	28.5	30.0	29.3
Streptococcus faecalis	4.9	–[b]	–	–	–	8.3	3.2	2.5	3.0	2.7
Serratia liquifaciens	–	3.0	2.7	1.9	1.5	–	–	–	–	–
Storage (days)	Nitrogen pack (dark)					Nitrogen pack (light)				
	0	14	28	49	70	0	14	28	49	70
Pseudomonas putida	79.2	55.9	12.5	3.2	3.4	86.6	52.2	13.0	3.0	1.2
Lactobacillus spp.	6.0	27.0	54.0	66.1	66.5	7.5	30.0	50.0	67.0	66.7
Brochothrix thermosphacta[a]	10.8	12.1	30.0	26.7	27.3	4.4	11.2	33.3	25.3	29.1
Micrococcus spp.	4.0	–	–	–	–	–	–	–	–	–
Serratia liquifaciens	–	5.0	3.5	4.0	3.8	–	6.6	3.7	4.7	3.0
Acinetobacter	–	–	–	–	–	1.5	–	–	–	–

[a] Formerly *Microbacterium thermosphactum*
[b] — Not detected

Table 6—Overall appearance scores of veal chucks arranged according to packaging treatment and storage interval[a,b]

Storage (days)	−4°C				0°C				3°C				7°C			
	Vacuum		N_2		Vacuum		N_2		Vacuum		N_2		Vacuum		N_2	
	R[c]	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
0	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A
14	7.0a A	6.7a B	7.0a A	7.0a A	5.0b C	6.0b B	7.0a A	6.0ab B	3.7b D	6.0b B	7.0a A	5.7b B	3.3b D	4.7b C	7.0a A	5.0b C
28	7.0a A	6.7a AB	7.0a A	6.7a AB	4.3c EF	6.0b BC	5.3b CD	5.7b CD	3.7b FG	6.0b BC	5.7b CD	5.0bc DE	3.0b G	3.7c FG	5.3b CD	4.3b EF
49	6.0b A	6.7a A	6.7a A	6.7a A	3.7d CDE	3.7c CDE	3.3c DEF	4.0c BCD	2.7c EFG	4.7c BC	5.0b B	4.0cd BCD	2.0c G	2.3d FG	4.3c BCD	4.7b BC
70	6.0b A	6.0a A	6.0a A	4.3b C	3.0e DEF	3.0d DEF	2.7c EF	3.7c CD	2.3c F	3.0d DEF	5.7b B	3.3d DE	1.0d G	1.3e G	3.3d DE	3.0c DEF

[a] Means, based on a 7-point scale (7: extremely desirable; 1: extremely undesirable) in the same column bearing a common postscript letter do no differ ($P > 0.05$).
[b] Means in the same row bearing a common subscript do not differ ($P > 0.05$).
[c] R: darkness; S: lighted display. - - - - - slight greening (including exudate); —— advanced greening (including exudate)

Table 7—Off-odor scores of veal chucks arranged according to packaging treatment and storage interval[a,b]

Storage (days)	−4°C				0°C				3°C				7°C			
	Vacuum		N_2		Vacuum		N_2		Vacuum		N_2		Vacuum		N_2	
	R[c]	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
0	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A	7.0a A
14	7.0a A	6.7a AB	7.0a A	7.0a A	6.0b BC	6.0b BC	6.7a AB	7.0a A	6.0b BC	4.7b D	6.0b BC	5.7b C	6.0b BC	3.7b E	6.0a BC	4.3b D
28	7.0a A	6.7a AB	6.7a AB	7.0a A	6.0b BC	6.0b BC	5.3b CD	4.0b EF	6.0b BC	4.7b DE	5.7b C	4.2c E	6.0b BC	3.3b FG	4.3b E	2.7c G
49	6.7a A	6.7a A	6.0ab AB	5.7b ABC	6.0b AB	4.7c CD	4.3c DE	2.0c H	5.0c BCD	3.3c EFG	4.7c CD	3.3d EFG	4.0c DEF	2.3c GH	3.0c FGH	2.0d H
70	6.3a A	6.0a A	5.0b B	3.3c CDE	3.7c CD	4.0d C	4.0c C	2.7c EFG	3.7d CD	3.0c DEF	3.7d CD	2.0c GH	2.3d FG	1.3d HI	2.3c FG	1.0e I

[a] Means based on a 7-point scale (7: extremely desirable; 1: extremely undesirable) in the same column bearing a common postscript letter do not differ ($P > 0.05$).
[b] Means in the same row bearing a common subscript do not differ ($P > 0.05$).
[c] R: darkness; S: lighted display.

Table 8—Exudate loss scores of veal chucks arranged according to packaging treatment and storage interval[a,b]

Storage (days)	−4°C				0°C				3°C				7°C			
	Vacuum		N_2		Vacuum		N_2		Vacuum		N_2		Vacuum		N_2	
	R[c]	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
0	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A	1.0a A
14	1.0a A	2.0b BC	1.0a A	1.0a A	2.0b BC	2.3b CD	1.0a A	1.0a A	2.0b BC	3.7b E	1.0a A	1.7a AB	2.7b D	4.3b F	1.3a A	2.0b BC
28	1.0a A	2.7c B	1.0a A	1.3a A	4.7c C	2.7d B	1.3a A	3.0b B	4.7c C	4.3b C	2.3b B	2.7b B	5.0c C	4.7bc C	3.0b B	4.3c C
49	1.0a A	2.0b AB	1.3a A	1.3a A	4.7c EFGH	4.7c EFGH	3.3c CD	4.0c DEF	5.7d H	4.3b DEFG	3.7c DE	2.7b BC	5.3c GH	5.3cd GH	5.3c GH	5.0d FGH
70	1.0a A	2.0b B	1.0a A	3.3b C	5.0c DEF	4.7c DE	2.3b B	4.3c D	5.7d FG	4.7b DE	3.3c C	5.3c EFG	6.0d G	6.0d G	4.7c DE	5.7e FG

[a] Means, based on a 6-point scale (1: 0.0—0.5 ml volume; 6: > 5.0 ml volume), in the same column bearing a common postscript letter do not differ ($P > 0.05$).
[b] Means in the same row bearing a common subscript do not differ ($P > 0.05$).
[c] R: darkness; S: lighted display.

of fresh meat by Kraft and Ayres (1954). Results from studies on frankfurters (Simard et al., 1983a), beef chucks (Simard et al., 1983c) and ground beef (Lee et al., 1983a) showed that light had no important influence on development of microflora.

Sensory changes

Table 6 represents overall appearance scores and the results indicate that except for veal chucks packed at −4°C, the overall appearance of samples from both package treatments tended to deteriorate as the storage time and temperature increased.

Veal chucks packed in vacuum generally exhibited more surface discoloration and greening (including exudate) at 3 and 7°C than those packed in nitrogen over the 70-day storage period. Vacuum-packaged samples under dark storage also tended to have slightly more discoloration at 3–7°C than those exposed to light – a finding which is similar to that seen with ground beef (Lee et al., 1983b). However, these results do not correspond with those obtained using frankfurters (Simard et al., 1983b) and beef chucks (Simard et al., 1983c) where light had a detectable effect on greening of vacuum- and nitrogen-packed samples.

Sharpe (1962) found that greening was more common in vacuum-packed sausages than other meats and was caused by lactobacilli and leuconostoc spp. Discoloration was seen in high pH beef (pH 6.2–6.7) and was found to be due to the sulfmyoglobin pigment formed by the reaction between myoglobin and H_2S produced by microorganisms such as *Alteromonas putrefaciens* and *Pseudomonas mephitica* (Nicol et al., 1970; Taylor and Shaw, 1977).

Shay and Egan (1982) recently observed the production of H_2S by a *Lactobacillus* spp. growing on meat. H_2S-producing *Lactobacillus casei* was also reported in cheddar cheese (Kristofferson and Nelson, 1955; Smith and Cunningham, 1962) though H_2S production by lactobacilli is very rare. In this laboratory, it has also been observed that some strains of lactobacilli are able to produce greening in meats and H_2S from different peptone media containing only few fermentable sugars (unpublished data).

Except for samples stored at −4°C, there was in general an increased incidence of off-odors in both package treatments as the storage period was extended or temperature increased (Table 7). Samples stored for longer than 28 days at 0, 3 and 7°C under light display tended to have more off-odors than those under dark. Although no definite trend was always observed on the effect of light in this study, a slight increase in surface temperature (1–1.5°C) by fluorescence rays may account for the increased microflora growth and for the greater detrimental changes in appearance, odor and exudate. Lactics, which normally lower the pH, do not usually produce free amino compounds that are the main putrefactive agents and thereby

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cause a sour rather than putrid odor (Ingram, 1963). However, the high incidence of off-odors was related to the number and composition of lactobacilli (Tables 4 and 5) and it was also closely associated with sour and slightly sulfide smells. A high level of lactobacilli found in fresh meats at any given time cannot therefore necessarily be regarded as an indication that the bacterial status of the product is good.

The results of exudate loss in Table 8 showed that nitrogen-packed veal chucks had significantly ($P < 0.05$) lower amounts of exudate compared to those vacuum-packed, particularly when stored at 0, 3 and 7°C.

Nitrogen packaging under light display seemed to release more exudate than those under dark storage – a finding which is similar to the experience with frankfurters (Simard et al., 1983b) and beef chucks (Simard et al., 1983c). Light, however, did not significantly ($P > 0.05$) affect the exudate loss from ground beef (Lee et al., 1983b). It is notable that there was no significant difference in numbers of organisms between these two display conditions. Extract release volume (ERV) which is related to exudate loss, in both ground pork and beef was influenced by the type of microflora and the changes in pH associated with the storage conditions (Riedel et al., 1967).

The storage of meats in high nitrogen atmospheres has been reported to result in a more swollen structure, increased water holding capacity and less extractability of sarcoplasmic and myofibrillar proteins (Belousov et al., 1973; Seideman et al., 1979). By comparison, Bastkowski et al. (1982) found that modified atmosphere stored steaks lost more moisture than vacuum-packed steaks but the study lasted only 9 days. Increased exudate losses were associated with a high incidence of greening (Table 6), similar to the previously reported results on frankfurters (Simard et al., 1983b), beef chucks (Simard et al., 1983c) and ground beef (Lee et al., 1983b).

During storage of veal and other meats in oxygen impermeable films, lactobacilli became a predominant part of the microflora and the spoilage of these various meats was a function of lactobacilli growth. Thus it is important to view the lactobacilli from both perspectives with their ability to preserve meat by homolactic fermentation and also to spoil it by heterolactic fermentation as seen in this study. In addition, it can be anticipated that lactic acid bacteria may cause spoilage as a result of amino acid metabolism in the absence of fermentable carbohydrates (Sharpe, 1962; Bulmash and Fulton, 1964). Consequently, the inhibition of lactobacilli growth appears to be an important criteria in the extension of shelf-life of various meats. Nevertheless, nitrogen gas packaging of various meats was useful to retard the discoloration and exudate losses.

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Comparison of Commercial Processing Method vs Hot-Deboning of Fresh Broilers on Nutrient Content of Breast Meat

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ABSTRACT

Conventional commercial processing of fresh broilers usually involves ice-water immersion chilling, which may cause some losses of water-soluble nutrients. This study was conducted to determine the effect of conventional chilling vs hot deboning on the nutrient content (proximate composition, riboflavin, niacin, vitamin B_6, P, Na, Mg, Ca and K) of breast meat from broilers. Significant differences were found between the two methods when the results of moisture, ash, P, K and Na were compared. No differences were found for other nutrients. A leaching mechanism for ash, mostly of P and K salts by the commercial process is suggested.

INTRODUCTION

COMMERCIAL PROCESSING of broilers involves evisceration and rapid chilling of eviscerated carcasses to less than 4°C internal temperature. The chilling step is important for reducing the body heat and extending shelf life of the processed birds.

Immersion chilling in ice-water slush is the most commonly used method employed in commercial poultry processing plants in the U.S. This type of chilling may take 30–50 min with or without agitation. The chilled carcasses may absorb the chilling water up to 12% of the carcass weight as permitted by the U.S. Dept. of Agriculture (USDA, 1976).

The microbiological and sensory quality aspects of the chilling process have been reviewed by Thomson et al. (1974) and Lillard (1982). Pippen and Klose (1955) found increased levels of Na and P in ash from water used to chill broiler carcasses. Hurley et al. (1958) reported increases in Ca, Na, P and K in chiller water as carcass time in the water was extended. It has been a concern of some food technologists that the conventional commercial process, especially the immersion chilling step, may affect other water-soluble nutrients of broiler meat.

A new approach for harvesting of broiler meat was proposed by Hamm (1982) and Hamm et al. (1982) which involves the rapid removal of meat from the noneviscerated carcass immediately after feather removal (hot-deboning). The present study compared the proximate composition, riboflavin, niacin, vitamin B_6, Ca, P, Na, K and Mg content of broiler breast meat from hot-deboned and commercially processed broilers.

MATERIALS & METHODS

Broilers

The broilers used in this study were 7-wk old Hubbard x Hubbard reared on a commercial research farm. Sixty-four male and a like number of female birds were randomly selected from the flock and one-half of each sex were sent to a commercial processing plant while the others were brought to our laboratory for the hot-deboning process. The average preslaughter weight was 1980g for the male and 1700g for the female.

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Commercial process

Birds were tagged with wingbands before processing for identification. They were processed by typical commercial poultry plant procedures: slaughtering and defeathering, evisceration, chilling in a prechiller at 15–21°C and then into an ice-water slush chiller with tumbling agitation at about 0°C. The chilled birds with an internal temperature below 4°C were placed in an ice chest and held for 24 hr in crushed ice before transporting to our laboratory. The breast meat was removed from the carcasses by the same technique used for the hot-deboned non-eviscerated birds, and placed individually in plastic bags.

Hot-deboning of noneviscerated birds

This process was carried out at our laboratory pilot plant. Live birds were slaughtered, scalded and defeathered by the techniques similar to those used commercially. After defeathering, the breast meat was removed from the hot carcasses (approx. 35°C) with no evisceration. Details of the procedures have been reported by Hamm (1982). The boneless skinless breast meat was immediately placed in plastic bags and rapidly cooled between 1° and 4°C before further handling.

Analytical methods

Ten breast meat samples (5 from each sex) for each processing technique, i.e., commercial processing and hot-deboning, were randomly chosen for nutrient analyses. Each sample (the left and right pectoralis major from the same bird) was blended in a food processor, aliquots drawn for immediate moisture, fat and protein analyses and the remainder stored in a glass jar with cap at −34°C until needed.

Moisture content was determined on fresh meat aliquots by the vacuum oven method at 100°C and ash was determined by muffle furnace incineration at 550°C to constant weight (AOAC, 1975). Fat was extracted from the raw tissue using the dry column technique of Maxwell et al. (1980) and protein was determined by the Kjeldahl method (AOAC, 1975).

Mineral content was determined by previously used procedures (Ang et al. 1982). Riboflavin was determined by a fluorometric method, and niacin and vitamin B_6 were measured by microbiological methods (AOAC, 1975). No separation of the individual forms of vitamin B_6 was performed. The total vitamin B_6 values are expressed as pyridoxine. A Polytron homogenizer, Type PT10/35, (Brinkmann Instruments, Westbury, NY) was used for tissue disintegration to prepare samples for the extraction of vitamins. All samples were run in duplicate for each analysis.

Statistical significance of the nutritional data were analyzed by ANOVA and linear regression method using a SAS program of Barr et al. (1979).

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION, and the riboflavin, niacin and vitamin B_6 content of the broiler breast meat are given in Table 1. Statistical analyses showed that significantly higher ($P < 0.01$) moisture content, but significantly lower ($P < 0.01$) ash content were found in the commercially processed broiler breast meat compared to hot-deboned breast. There was no difference due to sex or process on fat, protein, and the three vitamins analyzed on a wet weight or moisture-free basis. A sex x process interaction was found for niacin content on either basis. Moisture to protein ratios are also presented in Table 1, with the difference between the two processes being statistically significant.

Table 1—Proximate and vitamin analyses of broiler breast meat of commercially processed vs hot-deboned broilers, wet weight basis

Process	Bird sex	Bird live wt (g)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Riboflavin (mg/100g)	Vit. B_6 (mg/100g)	Niacin (mg/100g)	Moisture/ protein
Hot deboning	F	1760±51[a]	73.66±0.38	1.25±0.02	23.11±0.15	2.49±0.84	0.092±0.005	0.825±0.057	11.79±0.81	3.19±0.02
	M	2024±205	73.51±0.64	1.24±0.04	23.32±0.36	2.91±0.99	0.094±0.008	0.858±0.061	12.64±0.30	3.15±0.04
	$\bar{x}$	1892±198	73.58±0.50	1.24±0.03	23.21±0.28	2.70±0.89	0.093±0.006	0.842±0.058	12.22±0.81	3.17±0.03
Commercial	F	1776±240	74.47±0.71	1.11±0.02	22.92±0.45	1.91±0.69	0.087±0.009	0.814±0.061	12.58±0.64	3.25±0.06
	M	2163±199	74.09±0.48	1.06±0.02	22.74±0.59	2.72±0.89	0.085±0.015	0.833±0.042	11.38±0.33	3.25±0.09
	$\bar{x}$	1970±291	74.28±0.60	1.09±0.03	22.83±0.50	2.32±0.86	0.086±0.012	0.824±0.050	11.98±0.80	3.25±0.07
Statistics[b] between processes		NS	P<0.01	P<0.01	NS	NS	NS	NS	NS	P<0.01

[a] Mean ± standard deviation of five birds. Duplicate determinations were made for each nutrient of each bird.
[b] NS = no significant difference; P < 0.01 = significant at 1% level.

Table 2—Ash and mineral content of broilers processed by two methods, fat-free dry weight basis in breast meat

Process	Bird sex	Ash (%)	P	Mg	Na	Ca	K
			◄ — — — — — — — — — — — — — — — — mg/100g — — — — — — — — — — — — — — — — ►				
Hot-deboning	F	5.23±0.18[a]	1028±29	103±4	117±15	9.6±1.7	1472± 40
	M	5.25±0.24	1029±19	102±2	133±10	12.5±2.5	1505± 72
	$\bar{x}$	5.24±0.20	1029±23	102±3	125±15	11.0±2.5	1488± 58
Commercial	F	4.70±0.13	992±25	104±3	137± 7	13.8±5.9	1423± 34
	M	4.59±0.20	960±38	97±3	142± 5	15.6±8.7	1379±110
	$\bar{x}$	4.65±0.17	976±35	101±4	139± 7	14.7±7.0	1401± 80
Statistics[b] between processes		P<0.01	P<0.01	NS	P<0.01	NS	P<0.05

[a] Mean ± standard deviation of five bires. Duplicate determinations were made for each bird.
[b] NS = no significant difference, P < 0.01 and P < 0.05 = significant at 1 and 5% level, respectively.

The vitamin B_6 values found in this study were higher than those published in Agriculture Handbook 8-5 (0.55 mg/100g, CFEI, 1979). The difference could be due in part to the extraction method used. The Polytron homogenizer used might be more effective in preparing meat samples for extraction than a meat grinder or food processor which are frequently used.

Review of the literature has shown that water pick-up can be as high as 12% of the eviscerated whole bird after immersion chilling, and still be up to 7% after the shipping and storage (Kotula et al., 1960). However, most of the water was found in the skin and between the layer of meat and skin. Klose et al. (1960) reported very little water uptake of the breast meat by different chilling treatments. Nevertheless, the data did show a 0.6% increase (from 75.6 to 76.2%) of water content of the breast muscle when the eviscerated birds were chilled in ice-water slush for 30 min with tumbling. The present study showing a 0.5% difference agrees with those of previous workers. Higher moisture retention (2% gain), though, was reported by Lentz and Rooke (1958) for the outer layer breast muscle after 24 hr immersion chilling (from 74.6% to 76.7%).

The results of analyses of mineral content on a wet weight basis indicate some significant differences due to sex and processing method. Since the moisture content differed significantly between the processes and high variations were observed for fat content among birds, all results were converted to a fat-free dry weight basis for elimination of the dilution effect of moisture and fat. These results and their statistical analyses are shown in Table 2. Commercially processed birds appeared to contain less ash. Significantly less P and K were also detected in those birds as compared to those hot-deboned.

A slightly higher level of Na content was noted for the commercially processed breast meat. Since those broiler carcasses were stored in crushed ice for 24 hr after processing, the meat tissue could have absorbed some Na from the skin during that period.

For the calcium content, a higher numerical value was found in the commercially processed birds although the differences were not statistically significant, undoubtedly due to much greater variation noted in conventionally processed birds. Ang et al. (1982) suggested a migration mechanism of Ca from bone to meat tissue during chill holding of raw processed broilers. Possibly, some migration of Ca occurred during the ice-water chilling process. A sex difference in Mg and Na values and an interaction of sex with process for Na were observed for data on a fat-free dry weight basis. No explanation can be offered at this time.

Protein, fat, vitamin B_6, niacin, riboflavin, Ca and Mg content were not affected by the process method. From a consumer nutritional aspect, the compositional differences between the two types of processed meat were very small and may be of little concern; even the largest difference, observed in P content, constituted only about 1.6% US RDA per 100g tissue.

The data on ash content and on the moisture-to-protein ratio, when further refined, may be used as criteria to verify whether broiler meat has been hot-deboned or conventionally chill-processed, if such need arises in the future.

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Spaghetti Stickiness: Some Factors Influencing Stickiness and Relationship to Other Cooking Quality Characteristics

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ABSTRACT

Spaghetti was processed in a semi-commercial scale laboratory press from a range of raw materials, dried by a low temperature (LT) and a high temperature (HT) drying cycle, and assessed for stickiness and other important cooking quality attributes in cooking waters of varying hardness. Cooked HT spaghetti was generally less sticky, more resilient, firmer, and exhibited lower cooking loss than corresponding LT spaghetti. As cooking water hardness increased spaghetti became stickier and cooking loss increased. Stickiness was influenced by cultivar, wheat class, raw material granulation and protein content, but was not related to sprout damage. Stickiness was significantly correlated to cooking loss, cooked weight, degree of swelling, compressibility, recovery, and firmness. However, even when all these factors were included in a step-up regression less than 50% of the variance in stickiness could be predicted.

INTRODUCTION

TO PROPERLY ASSESS spaghetti cooking quality a number of factors must be considered. According to Manser (1981) the decisive criteria are firmness, elasticity, surface stickiness, cooking tolerance, water absorption, degree of swelling, and solids lost to the cooking water.

Of all the major cooking quality factors, surface stickiness, or gumminess, of cooked spaghetti has received the least attention by researchers. Some workers have demonstrated that as cooking water becomes harder ($CaCO_3$ increases) cooked spaghetti becomes stickier (Alary et al., 1979; D'Egidio et al., 1981; Menger, 1980). Others have reported that processing spaghetti under high temperature drying conditions may partly overcome cooked spaghetti stickiness (Dexter et al., 1981b; Manser, 1981). The relationship between stickiness and other important cooking quality factors has not been investigated.

The main reason spaghetti stickiness has received so little attention is that it is difficult to quantify. Taste panels have been used to estimate cooked spaghetti stickiness with some success. However, evaluation by taste panels is time consuming and requires large sample size. These disadvantages limit the scope of samples which can be studied. Further, the apparent stickiness of a given sample is strongly influenced by the amount of unabsorbed water associated with the cooked spaghetti following drainage, the length of time between drainage and testing, and relative humidity of the testing area.

Recently we adopted the general concept of Voisey et al. (1978) to develop a fast, reliable procedure for instrumental measurement of cooked spaghetti stickiness which requires less than 10g per analysis (Dexter et al., 1983). This has permitted us to extend previous investigations into factors associated with cooked spaghetti stickiness.

In the current study we compare the stickiness of spaghetti processed from a wide range of raw materials, dried by both a conventional low temperature and a high temperature process, and cooked in waters of varying hardness. The relationship between stickiness and other important cooking quality factors is also examined.

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MATERIALS & METHODS

Wheat

High protein and low protein samples of five North American amber durum wheat cultivars were prepared from residues remaining from fertilizer trials conducted at Saskatoon, Saskatchewan in 1980 (Dexter et al., 1982b). Each high and low protein pair represented composites of the nine highest protein replicates and the nine lowest protein replicates. All composites graded No. 1 CWAD (Canada Western Amber Durum).

All other wheats were composites from the 1981 Canadian crop. They included a No. 5 CWAD degraded because of sprout damage, a sound No. 3 CWAD and a No. 2 CWRS (Canada Western Red Spring).

Milling

The pure cultivar composites were milled (3 kg samples) into granulars (mean milling yield 69.9%) in a three-stand Allis-Chalmers laboratory mill in conjunction with a laboratory purifier (Matsuo and Dexter, 1980).

The No. 5 CWAD, the No. 3 CWAD and the No. 2 CWRS were milled in 30 kg lots by a modified Bühler laboratory milling procedure (Dexter et al., 1982a). Semolina yields for the No. 5 CWAD and the No. 3 CWAD were 59% and 65% respectively. The No. 2 CWRS wheat gave a farina yield of 48%. A portion of the No. 3 CWAD semolina and a portion of the No. 2 CWRS farina were reduced to flour by gradual reduction on smooth rolls until all stock passed through a 10 XX sieve.

Analytical tests

Protein content (% N x 5.7) was determined by the Kjeldahl method as modified by Williams (1973). Starch damage was estimated by the method of Farrand (1964). Amylograph peak viscosity was measured as described previously (Dexter et al., 1981b).

Spaghetti processing

Spaghetti was processed in a Demaco S-25 laboratory-scale continuous extrusion press (De Francisci Machine Corporation, Brooklyn, NY) under previously described conditions (Matsuo et al., 1978). Spaghetti was dried by a conventional low temperature (LT) drying cycle (39°C for 28 hr). Where quantity permitted a portion of the spaghetti was also dried by a high temperature (HT) drying cycle which featured a 1 hr conventional temperature predrier followed by 12 hr at 68–70°C. Details of both drying cycles have been published previously (Dexter et al., 1981b).

Cooking water

Spaghetti samples were assessed for cooking quality in well water (from Rossburn, MB), laboratory tap water, and deionized water prepared from tap water. All waters were collected and stored prior to experimentation to ensure uniformity of water properties. The deionized water was prepared by distillation, deionized by reverse osmosis, and passed through a series of columns (Milipore Corp., Belford, MS) including a prefiltration, charcoal, ion exchange and a final filtration column.

A portion of each water was forwarded to the W.M. Ward Technical Services Laboratory (Winnipeg, MB) for analysis by standard methods (American Public Health Association, 1981).

Cooking tests

Ten grams of spaghetti were added to 250 mL of rapidly boiling water. Spaghetti was cooked to optimum cooking time, defined as the time required for the white core in the strands to disappear (about 12 min). Time of disappearance of the core had been established in a preliminary cooking by removing strands from the cooking water at intervals and crushing them between two glass plates.

The instrumental test procedure for determining cooked spaghetti stickiness has been described in detail previously (Dexter et al., 1983). The basic principle of the test procedure is to compress cooked spaghetti strands under a plunger, and upon lifting the plunger, to measure the force of adhesion of the plunger to the spaghetti.

Spaghetti cooked weight and cooking loss were determined as described previously (Dexter and Matsuo, 1979b). Swelling index was measured from the ratio of water displacement of cooked spaghetti divided by water displacement of an equivalent amount of uncooked spaghetti.

Tenderness index, a measure of shear rate under increasing force was determined by the method of Matsuo and Irvine (1969). The firmer the sample the lower the tenderness index. Compressibility, a measure of deformation under constant force, and recovery, a measure of resilience, were determined as described by Matsuo and Irvine (1971).

Each of the above cooking tests was performed in triplicate on all spaghetti in deionized water and tap water. Well water quantity was limited. Accordingly, cooked weight and swelling index were not determined in well water, and the other tests were performed singly on a few selected samples.

Statistical analysis

The significance of the effects of various factors on each cooking quality characteristic was determined by analysis of variance (ANOVA) using factorial design (Snedecor and Cochran, 1967).

Cooking data in both tap and deionized water for all spaghetti samples were pooled and simple correlations among cooking quality characteristics were calculated. The extent that spaghetti stickiness could be predicted by combinations of the other cooking quality characteristics was investigated by step up multiple regression (Snedecor and Cochran, 1967).

Table 1—Cooking water properties

Property	Cooking water		
	Well	Tap	Deionized
Conductivity (μ mho/cm)	3444	180	1.29
pH (units)	8.00	7.80	6.05
Alkalinity total ($CaCO_3$) (mg/L)	482	78.0	2.0
Alkalinity (HCO_3) (mg/L)	588	95.2	2.44
Ca (mg/L)	80.2	26.1	<0.5
Mg (mg/L)	30.1	7.2	<0.5
Hardness ($CaCO_3$) (mg/L)	324	94.0	1.65
Mn (mg/L)	0.226	<0.02	<0.02
Fe (mg/L)	1.54	0.074	<0.02

RESULTS & DISCUSSION

Cooking water composition

In selecting cooking waters for the study we considered previous reports that cooking water composition, particularly hardness, influences spaghetti cooking properties (Alary et al., 1979; D'Egidio et al., 1981; Menger, 1980). Cooking water analyses (Table 1) confirmed that well water was very hard and contained considerable amounts of all minerals quantified. The tap water was intermediate in composition between the well water and the deionized water.

Durum wheat cultivars

Cooking quality data for spaghetti processed by low temperature drying (LT) from the five amber durum wheat cultivars at two protein levels are summarized in Table 2.

ANOVA showed that all of the cooking quality factors examined were significantly influenced by cultivar (Table 3). However, the presence of significant cultivar-protein and cultivar-water interactions for some of the cooking factors revealed that not all cultivars responded to cooking water and protein content in the same manner.

The various cooking quality factors were not all influenced by protein content to the same degree (Tables 2 and 3). Results were generally consistent with previous reports. Grzybowski and Donnelly (1979) showed that cooking loss was more strongly related to protein content than cooked weight, and in another study they concluded that degree of swelling during cooking was not related to protein content (Grzybowski and Donnelly, 1977). In the current study cooking loss exhibited a significant cultivar-protein interaction (Table 3) because Hercules was the only cultivar not to show a significant decrease in cooking loss at higher protein (Table 2).

The significant effect of protein content on cooked spaghetti resilience (compressibility and recovery) and firmness (tenderness index) observed in the current study has been demonstrated before on numerous occasions (e.g. Dexter and Matsuo, 1977; Dexter et al., 1980; 1982b; Grzybowski and Donnelly, 1979; Matsuo et al., 1982a; Matweef, 1966). Recovery and compressibility were especially strongly influenced by protein content (Tables 2 and

Table 2—Summary of cooking characteristics[a] of spaghetti (dried at 39°C) prepared from five amber durum wheat cultivars at two protein levels

Cultivar	Tap water								Deionized water						
	PR[b] (%)	ST[b] (N/m^2)	CL[b] (%)	SI[b]	CW[b] (g)	C[b] (%)	R[b] (%)	TI[b] (m/sec x 10^6)	ST[b] (N/m^2)	CL[b] (%)	SI[b]	CW[b] (%)	C[b] (%)	R[b] (%)	TI[b] (m/sec x 10^6)
Ward	13.2	607	5.4	3.23	29.4	77.0	32.0	45.0	487	5.1	3.10	28.4	77.3	27.3	45.7
	15.4	513	5.1	3.22	29.3	77.0	36.0	46.7	417	4.9	3.15	28.7	77.3	33.3	47.3
Wascana	11.5	687	6.1	3.17	29.1	76.0	30.7	45.7	563	5.5	3.14	28.9	74.7	30.3	46.0
	14.3	543	5.1	3.17	28.8	76.3	40.7	46.3	520	4.6	3.21	29.1	72.7	41.3	42.3
Wakooma	12.3	700	6.3	3.09	28.3	76.0	30.7	46.0	577	5.8	3.05	29.2	73.3	35.7	45.7
	15.4	677	5.4	3.07	28.0	72.0	44.3	39.7	527	4.7	3.02	27.7	67.7	48.3	38.3
Hercules	12.9	543	5.7	3.12	28.4	77.0	34.3	45.0	480	5.5	3.13	28.6	74.7	35.7	45.3
	15.3	510	6.1	3.07	28.2	74.7	39.0	45.3	497	5.4	3.11	28.3	73.7	39.0	44.3
Macoun	12.3	720	6.2	3.19	29.0	76.7	33.0	46.7	500	5.4	3.08	28.2	76.3	34.3	44.3
	14.3	683	5.6	3.10	28.6	73.3	43.0	44.3	587	4.8	3.09	28.6	73.3	40.3	44.3
Overall means	12.4	651	5.9	3.16	28.8	76.5	32.1	45.7	521	5.5	3.10	28.5	75.3	32.7	45.4
	14.9	585	5.5	3.13	28.6	74.7	40.6	44.5	510	4.9	3.12	28.5	72.9	40.4	43.3

[a] Mean of three replications.
[b] PR = semolina protein (14% moisture basis), ST = stickiness, CL = cooking loss, SI = swelling index, CW = cooked weight (10 g dry matter basis), C = compressibility, R = recovery, TI = tenderness index.

Table 5—Analysis of variance for cooking quality of Hercules and Macoun semolina processed by LT and by HT drying, and cooked in tap water and deionized water

Source of variation	Degrees of freedom	F-values						
		Stickiness	Cooking loss	Swelling index	Cooked weight	Compressibility	Recovery	Tenderness index
Reps	2	N.S.	N.S.	N.S.	N.S.	N.S.	4.7*	N.S.
Sample (S)	3	3.8*	14.4**	8.2**	5.3**	21.3**	36.4**	9.8**
Tamperature (T)	1	18.2**	N.S.	24.1**	6.8**	N.S.	32.0**	16.9**
Water (W)	1	31.7**	8.5**	17.9**	5.2*	N.S.	N.S.	N.S.
S*T	3	4.6**	N.S.	N.S.	N.S.	7.2**	15.9**	6.9**
S*W	3	N.S.	8.0**	N.S.	3.3*	N.S.	N.S.	N.S.
T*W	1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
S*T*W	3	N.S.	N.S.	N.S.	N.S.	3.5*	N.S.	N.S.
Error	30							
Total	47							

*Significant at $P < 0.05$
**Significant at $P < 0.01$

Table 6—Cooking characteristics[a] of Hercules and Macoun spaghetti when cooked in well water

Cultivar	PR[b] (%)	ST[b] (N/m^2)	CL[b] (%)	C[b] (%)	R[b] (%)	TI[b] (m/sec x 10^6)
				Dried at 39°C		
Hercules	12.9	1118	7.5	78	19	46
	15.3	1135	5.6	79	28	48
Macoun	12.3	1151	10.1	80	20	48
	14.3	1102	9.7	76	28	47
				Dried at 70°C		
Hercules	12.9	820	6.4	80	25	53
	15.3	1053	5.4	73	46	40
Macoun	12.3	1053	6.5	82	21	47
	14.3	950	5.2	76	34	44

[a] Single determinations
[b] PR = semolina protein (14% moisture basis), ST = stickiness, CL = cooking loss, C = compressibility, R = recovery, TI = tenderness index.

also cooked in well water (Table 6). Cooking quality of all spaghetti samples deteriorated markedly from that observed in tap water and deionized water (Tables 2 and 4). In well water all spaghetti samples became very sticky (Table 6). Cooking loss increased, particularly in the case of LT spaghetti, elasticity was reduced, and to a lesser degree, firmness was also reduced. The HT spaghetti generally cooked slightly better in well water than the LT spaghetti, but, except for a strong tendency for HT spaghetti to exhibit reduced cooking loss, the advantage of HT drying over LT drying was much reduced in well water compared to tap water.

Sprout damage

A badly sprouted semolina (amylograph peak viscosity less than 20 Brabender units) was processed under HT and LT drying conditions, and cooking quality was evaluated in tap water and deionized water (Table 7). The protein content of the sprouted semolina was 12.5%, similar to the average protein content of the low protein cultivar samples. A comparison of the cooking quality of the sprouted sample to the low protein cultivar samples (Tables 2 and 4) failed to demonstrate any detrimental effects of sprout damage to stickiness or any other cooking quality factor.

The effect of sprout damage on spaghetti stickiness has not been investigated previously. Dick et al. (1974) and Donnelly (1980) under LT conditions concluded that spaghetti cooked weight, cooking loss and firmness were unrelated to sprout damage. In contrast, Maier (1980) stated that relatively low levels of sprout damage can adversely affect firmness.

Matsuo et al. (1982b) recently attributed the variability of the effect of sprout damage on spaghetti quality noted in past studies to variability in alpha-amylase retention during semolina milling. They found under LT processing conditions that firmness was adversely affected for some semolina samples with exceptionally high amylase activity. However, they pointed out that under HT conditions significant amylase denaturation occurs (Dexter et al., 1981a, b) making it probable that sprout damage effects under HT conditions would be much reduced. In fact, under HT conditions spaghetti processed from sprouted semolina contains maltose levels comparable to spaghetti processed from sound semolina (Dexter et al., 1981a, b). The very low amylograph peak viscosity of the sprouted sample used in the current study is consistent with very high alpha-amylase activity. Thus, the likelihood is that except in extreme cases sprout damage has little effect on spaghetti cooking quality.

Semolina granulation

The cooking quality of spaghetti processed by HT and LT from amber durum semolina and various blends of semolina and amber durum flour is summarized in Table 8. The general tendency was for cooking quality to deteriorate with increasing quantity of amber durum flour. Results of the current study appear to be generally consistent with the very limited information currently available on semolina granulation effects. For example, Seyam et al. (1974) found that under LT processing conditions semolina granulation did not influence cooked weight and had only a marginal effect on firmness. They observed an increase in cooking loss as granulation became finer; a result confirmed later by Breen et al. (1977).

The most consistent trend observed in the current study was a tendency for stickiness to increase as flour content increased (Table 8). ANOVA confirmed other significant trends between samples (Table 9), but aside from a tendency for increased cooking loss with increased flour content, most changes were not great enough to be of great practical concern (Table 8).

The influence of granulation on cooking loss was particularly interesting because of a strong interaction with drying temperature (Tables 8 and 9). In both tap water and deionized water the LT spaghetti exhibited a nearly linear relationship between cooking loss and flour content (Table 8). This was anticipated because of increasing starch damage with increasing flour content (23, 26, 40, and 73 Farrand units for semolina, 20% flour, 40% flour, and 100% flour, respectively). When processed under HT conditions, the effect of flour content on cooking loss was drastically reduced. The reason for the strong effect of HT drying

on cooking loss is unclear. Previously we showed that under HT conditions starch gelatinization properties were not modified compared to LT conditions (Dexter et al., 1981a, b). However, protein denaturation was much greater under HT conditions. There is the possibility that during HT drying the protein film on the surface of spaghetti (Dexter et al., 1978) is strengthened leading to increased resistance to strand disintegration. This possibility gains some support from the tendency of recovery, which is very strongly influenced by gluten strength (Matsuo et al., 1982a) to improve more under HT conditions than the other cooking quality factors (Tables 2, 4–9).

The effect of cooking water on cooking quality factors for the series of semolina-flour blends (Table 9) was generally consistent with the results for the five durum cultivars (Tables 3 and 5) and the sprouted sample (Table 7). Stickiness, degree of swelling and cooked weight all decreased significantly in deionized water relative to tap water, whereas compressibility, recovery and tenderness index were not influenced by cooking water.

Hard red spring wheat blends

Cooking quality data for hard red spring wheat farina, flour, and blends with the durum wheat semolina used for the granulation study (Table 8) are summarized in Table 10. There was an obvious tendency for cooking quality to deteriorate with increasing amounts of hard red spring wheat flour or farina. The very slight difference in protein content between the durum semolina (12.8%) and the hard red spring farina and flour (12.5%) would not account for the cooking quality trends. These results support previous reports that addition of common wheat farina or flour to durum wheat semolina results in decreased cooking quality (Dexter et al., 1981c; Manser, 1980; 1981). Recently Wyland and D'Appolonia (1982) reported an improvement in spaghetti cooking quality with the addition of hard red spring wheat farina to durum semolina. Their results can be explained on the basis of the poorer cooking quality of American durum, compared to Canadian durum, a factor which has led recently to an active program in North

Table 7—Cooking characteristics[a] of spaghetti prepared from sprouted amber durum semolina when cooked in tap water and deionized water

Cooking water	Drying temp (°C)	ST[b] (N/m^2)	CL[b] (%)	SI[b]	CW[b] (g)	C[b] (%)	R[b] (%)	TI[b] (m/sec x 10^6)
Tap	39	667	5.5	3.29	28.8	76.0	36.0	46.7
	70	447	5.0	3.24	28.9	73.0	42.3	44.7
Deionized	39	520	5.6	3.06	27.6	74.7	35.0	44.7
	70	447	4.9	3.04	27.6	74.7	45.7	45.7

[a] Mean of three replicates
[b] ST = stickiness, CL = cooking loss, SI = swelling index, CW = cooked weight, C = compressibility, R = recovery, TI = tenderness index.

Table 8—Summary of cooking characteristics[a] of spaghetti prepared from various blends of amber durum wheat semolina and flour

	Tap water							Deionized water						
Spaghetti	ST[b] (N/m^2)	CL[b] (%)	SI[b]	CW[b] (g)	C[b] (%)	R[b] (%)	TI[b] (m/sec x 10^6)	ST[b] (N/m^2)	CL[b] (%)	SI[b]	CW[b] (g)	C[b] (%)	R[b] (%)	TI[b] (m/sec x 10^6)
Dried at 39°C														
100% durum semolina	657	5.2	3.30	29.3	76.7	34.0	48.3	510	5.3	3.13	27.0	75.0	33.3	43.3
20% durum flour[c]	667	5.4	3.23	28.6	75.7	35.7	44.3	597	5.8	3.03	27.1	71.0	39.7	44.7
40% durum flour[c]	690	6.0	3.23	28.7	75.3	33.0	46.3	593	5.9	3.09	27.6	79.0	29.0	48.7
100% durum flour	780	6.4	3.18	28.7	77.0	25.7	47.3	663	6.3	3.01	27.5	77.3	29.3	47.0
Dried at 70°C														
100% durum semolina	593	5.0	3.26	28.7	75.7	40.0	40.3	447	5.1	3.05	27.2	74.7	41.0	42.3
20% durum flour[c]	503	5.2	3.22	28.3	73.7	45.7	40.3	430	5.1	3.03	27.5	73.3	48.0	42.3
40% durum flour[c]	503	4.9	3.16	28.1	72.7	43.7	42.0	480	5.3	3.05	27.5	74.0	45.0	45.0
100% durum flour	657	5.1	3.15	28.0	76.0	36.0	44.7	527	5.5	3.04	27.8	74.7	35.3	45.0

[a] Mean of three replications
[b] ST = stickiness, CL = cooking loss, SI = swelling index, CW = cooked weight, C = compressibility, R = recovery, TI = tenderness index.
[c] Blends with durum semolina

Table 9—Analysis of variance for cooking quality of spaghetti from blends of durum wehat semolina and flour

Source of variation	Degrees of freedom	F - values						
		Stickiness	Cooking loss	Swelling index	Cooked weight	Compressibility	Recovery	Tenderness index
Reps	2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sample (S)	3	12.0**	18.3**	5.7**	N.S.	5.4**	23.0**	6.0**
Temperature (T)	1	74.5**	85.7**	N.S.	N.S.	9.4**	106.0**	32.9**
Water (W)	1	46.5**	4.4*	110.2**	46.0**	N.S.	N.S.	N.S.
S*T	3	N.S.	7.3**	N.S.	N.S.	N.S.	N.S.	N.S.
S*W	3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
T*W	1	N.S.	N.S.	N.S.	5.5*	N.S.	N.S.	4.4*
S*T*W	3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Error	30							
Total	47							

*Significant at $P < 0.05$
**Significant at $P < 0.01$

Dakota to develop durum wheat cultivars with better cooking quality (Quick et al., 1979; 1980).

Addition of hard red spring wheat farina or flour resulted in very sticky spaghetti when processed under LT conditions and cooked in tap water (Table 10). This result confirms the experience of several commercial pasta manufacturers (private communications). When cooked in deionized water, stickiness of the LT spaghetti decreased (Table 10). Thus, LT spaghetti containing appreciable amounts of hard red spring wheat appears to be particularly sensitive to cooking water hardness. The stickiness of the hard red spring wheat blends could not be explained on the basis of increased strand disintegration. In fact, cooking losses for the hard red spring wheat blends (Table 10) were comparable to, or lower than the durum wheat semolina and flour samples discussed previously (Table 8).

As expected, HT drying brought about some improvement in cooking quality (Tables 10 and 11). As was the case for the durum wheat semolina-flour blends (Table 8), cooking loss differences under HT conditions attributable to starch damage differences (2, 9, 24, and 33 Farrand units for 100% farina, 50% farina, 50% flour, and 100% flour, respectively) were much less than under LT conditions (Table 10). Combined data from LT spaghetti processed from the durum and the hard red spring blends exhibited a correlation of 0.96** (n = 8) between cooking loss and starch damage. Under HT conditions cooking loss still correlated to starch damage (r = 0.84**, n = 8), but the slope of the HT regression line was only one-third of the slope of the LT regression line.

Consistent with results from the other samples in this study, HT drying brought about an improvement in spaghetti stickiness for all the hard red spring wheat blends (Tables 10, 11), particularly when cooked in tap water. However, we were unable to verify Manser's (1980) claim that soft wheat products undergo a much greater improvement in firmness and resilience under HT drying conditions compared to durum wheat products. Our data show that compressibility of the hard red spring blends was not affected by HT drying, and although recovery and tenderness index improved significantly (Table 11), the magnitude of the improvement was slight (Table 10).

The effect of cooking water on the various cooking quality factors for the hard red spring wheat blends (Tables 10 and 11) generally followed a similar pattern to the other samples included in the study. Major effects were decreased stickiness concomitant with reduced swelling and cooked weight (Tables 10 and 11). Compressibility, recovery and tenderness index once again proved to be essentially independent of cooking water properties (Tables 10 and 11).

Relationship between stickiness and other cooking factors

The literature contains no information concerning the relationship of spaghetti stickiness to other cooking factors. Therefore, we pooled all the cooking data in both tap and deionized water from the 32 spaghetti samples examined in the current study, and determined simple correlation coefficients between stickiness and the other cooking quality characteristics (Table 12). Stickiness correlated significantly

Table 10—Summary of cooking characteristics[a] of spaghetti prepared from hard red spring wheat flour, farina and blends with amber durum semolina

	Tap water							Deionized water						
Spaghetti	ST[b] (N/m^2)	CL[b] (%)	SI[b]	CW[b] (g)	C[b] (%)	R[b] (%)	TI[b] (m/sec x 10^6)	ST[b] (N/m^2)	CL[b] (%)	SI[b]	CW[b] (%)	C[b] (g)	R[b] (%)	TI[b] (m/sec x 10^6)
Dried at 39°C														
50% farina[c]	770	5.3	3.31	29.2	76.7	27.7	48.7	587	4.9	3.09	27.9	76.7	26.7	44.7
50% flour[c]	800	5.6	3.24	28.7	78.0	23.0	47.7	610	5.4	3.12	27.1	81.3	20.3	51.3
100% farina	950	4.9	3.21	29.1	81.3	17.3	50.3	583	4.4	3.15	29.0	84.0	16.3	54.3
100% flour	903	5.9	3.15	28.7	87.7	10.3	61.3	713	5.5	3.02	27.1	87.0	8.3	56.0
Dried at 70°C														
50% farina[c]	627	4.9	3.29	29.5	78.3	28.7	44.7	550	4.7	3.06	27.9	76.0	30.3	46.0
50% flour[c]	590	5.1	3.23	28.3	78.3	29.7	47.0	573	5.1	3.01	27.5	78.3	24.7	46.3
100% farina	717	4.7	3.28	29.0	81.0	21.3	50.0	583	4.6	3.04	27.1	84.7	12.7	49.3
100% flour	683	5.0	3.20	28.3	83.7	13.0	53.3	623	4.9	2.99	27.5	87.3	11.3	57.7

[a] Mean of three replications
[b] ST = stickiness, CL = cooking loss, SI = swelling index, CW = cooked weight, C = compressibility, R = recovery, TI = tenderness index.
[c] Blends with durum semolina (see Table 8)

Table 11—Analysis of variance for cooking quality of spaghetti from blends of hard red spring wheat flour, farina and blends with amber durum semolina

Source of variation	Degrees of freedom	F - value						
		Stickiness	Cooking loss	Swelling index	Cooked weight	Compressibility	Recovery	Tenderness index
Reps	2	N.S.	N.S.	4.8*	N.S.	3.7*	7.9**	N.S.
Sample (S)	4	28.2**	15.3**	10.8**	5.0**	65.3**	132.3**	48.6**
Temperature (T)	1	70.1**	24.6**	8.0**	N.S.	N.S.	19.6**	18.4**
Water (W)	1	133.0**	5.8*	294.8**	81.9**	N.S.	6.6*	N.S.
S*T	4	N.S.	3.5*	N.S.	N.S.	N.S.	N.S.	N.S.
S*W	4	3.8*	N.S.	N.S.	N.S.	2.9*	N.S.	N.S.
T*W	1	24.0*	N.S.	14.3*	N.S.	N.S.	N.S.	N.S.
S*T*W	4	N.S.	N.S.	N.S.	2.9*	N.S.	N.S.	4.8*
Error	38							
Total	59							

*Significant at $P < 0.05$
**Significant at $P < 0.01$

Table 12—Simple correlation coefficients between cooking quality variables for 32 laboratory spaghetti samples cooked in two different cooking waters (n = 64)

	ST	TI	R	C	CW	SI	CL
Cooking loss (CL)	0.29*	NS	NS	NS	NS	NS	1.00
Swelling index (SI)	0.35**	NS	NS	NS	0.79**	1.00	
Cooked weight (CW)	0.31**	NS	NS	NS	1.00		
Compressibility (C)	0.47**	0.91**	−0.91**	1.00			
Recovery (R)	−0.56**	−0.87**	1.00				
Tenderness index (TI)	0.53**	1.00					
Stickiness (ST)	1.00						

*Significant at $P < 0.05$
**Significant at $P < 0.01$

Table 13—Step-up multiple regression for the prediction of cooked spaghetti stickiness by other cooking quality variables

Variable	r^2
Recovery	0.32
Cooked weight	0.40
Cooking loss	0.44
Swelling index	0.45
Compressibility	0.46
Tenderness index	0.48

to every cooking quality factor. Interestingly, aside from the strong relationships between compressibility, recovery and tenderness index, and a strong relationship between degree of swelling and cooked weight, the other cooking quality factors were largely unrelated.

The possibility that it might be possible to predict spaghetti stickiness by some combination of the other cooking quality characteristics was investigated by step-up multiple regression (Table 13). Despite the significant relationship of stickiness to all other cooking quality characteristics, and the fairly high degree of independence of many of the cooking characteristics, the inclusion of all the cooking characteristics still was unable to account for even 50% of the variability in spaghetti stickiness (Table 13). Clearly stickiness is affected by some factor or factors in addition to those influencing the other cooking quality characteristics.

It is well established that differences in cooked spaghetti firmness and resilience between durum wheat cultivars can be largely related to gluten strength (Dexter et al., 1980; Matsuo et al., 1982b; Quick et al., 1979; 1980). The possibility exists that gluten strength may be associated with cooked spaghetti stickiness as well. The significant differences between cultivars in the current study (Tables 2–4) merits further study. We are currently investigating the relationship between gluten properties and stickiness using lines from the Canadian durum wheat breeding program.

Another possible explanation for differences in stickiness between samples not considered in the current study has been put forward by D'Egidio and co-workers (1976; 1978; 1981; 1982). They claim that the amount of material that can be exhaustively rinsed from drained cooked spaghetti correlates better to stickiness than the traditional measurement of cooking loss. Currently we are attempting to quantitatively prepare and characterize material rinsed from the surface of a series of spaghetti samples ranging widely in stickiness.

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A Research Note

Pacific Cod (*Gadus macrocephalus*): Change in Sensory and Chemical Properties When Held in Ice and in CO_2 Modified Refrigerated Seawater

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ABSTRACT

Pacific cod, *Gadus macorcephalus*, were held in the round in ice and in CO_2 modified refrigerated seawater (MRSW), and examined for chemical changes as well as changes in palatability of steamed portions of blocks of fillets. Sensory evaluation of raw and cooked flesh indicated that the quality of cod was acceptable to 6 days in ice and to 9 days in MRSW. Absorption of salt may present a problem for holding cod in MRSW. Trimethylamine and total volatile acid content was significantly correlated with flavor scores.

INTRODUCTION

MANY REPORTS on factors that affect the food quality of Atlantic cod, *Gadus morhua*, have appeared, but comparatively few are available for Pacific cod, *Gadus macrocephalus*. Kramer et al. (1977) dealt with cod iced in the round, while Tomlinson et al. (1978) compared iced eviscerated cod with cod held in the round in refrigerated seawater (RSW), half-strength RSW and half-strength RSW containing 0.2% potassium sorbate. There are several reports (Lemon and Regier, 1977; Reppond et al., 1979; Collins et al., 1980) in which RSW or CO_2 modified refrigerated seawater (MRSW) has proven superior to ice as a holding medium for several species of fish. Domestic processing of Pacific cod caught in Alaskan waters has recently started and processors and fishermen need information on their fresh holding characteristics. The purpose of this study was to determine the chemical and sensory properties of Pacific cod when held in the round in ice and in MRSW.

MATERIALS & METHODS

THE COD were caught on September 22, 1978 by a commercial fishing boat using trawl gear in waters near Kodiak, Alaska. The fish were placed in metal baskets and held on deck until landed. The majority of the fish were still in rigor when landed. About 9 hr after capture, the fish were transferred into the ice and MRSW holding systems. A few hours later, a block of fillets was prepared and shall be referred to as the control or zero holding time sample. Approximately 150 kg of cod were placed in layers with an equal weight of ice in an insulated box. Fresh ice was added as needed to replace melted ice. The MRSW tank was loaded with 100 kg of cod and 200 kg of chilled brine (3.5% NaCl) saturated with CO_2 (pH = 4.3). The pH increased to 6.2 after 8 days and could not be reduced even with constant addition of CO_2. The temperature was maintained at −1.1°C. At regular intervals, cod were removed from the holding systems and filleted by hand. Fillets for sensory evaluation were frozen as blocks at −34°C. Fillets for chemical analysis were ground using the coarse plate of an Oster food grinder then frozen at −34°C.

Sensory evaluation

The blocks were sawed into portions measuring 80 x 50 x 20 mm. The control sample and samples from fish held in ice were lightly salted to minimize differences in salt content with samples from fish held in MRSW. The portions were thawed and placed in individual aluminum pans which were sealed with aluminum foil and steamed for 20 min. Drip was drained before serving. Each serving consisted of coded samples held an equal length of time either in ice or in MRSW plus a coded control (zero holding time). Judges (12) were asked to evaluate flavor and texture using a 5 point hedonic scale and to note if samples were too salty. Panelists were trained using fish of varying quality and were familiar with the scoring system. Sensory analysis was completed within 2 months of frozen storage at −34°C.

Chemical analysis

The frozen samples were tempered overnight in a refrigerator at 3°C and ground twice using the fine plate of an Oster food grinder. Analyses were carried out for total solids, chloride (AOAC, 1975), total volatile acid (TVA, Friedemann and Brook, 1938), and trimethylamine (TMA, Bullard and Collins, 1979). Chemical analyses were completed within a month of frozen storage at −34°C.

RESULTS & DISCUSSION

AT 3 DAYS, fish held in ice and fish held in MRSW were of high and equal quality based on an examination of round fish and their fillets. At 6 days, the quality of cod from MRSW appeared to be better than cod from ice where some spoilage was evident. Both samples were acceptable, however. Iced fish were judged not acceptable at 9 days because of the odor of the raw fillet, but odor of the cod held in MRSW was less intense and the fillets were still acceptable. At 12 days, all samples appeared to be spoiled.

The results from the sensory panel essentially agreed with results of the observation of the raw fish, i.e., cod in MRSW remained fresh longer than cod in ice (Table 1). The flavor of cod held in ice was good on the third day, acceptable on the sixth day, borderline on the ninth day, and unacceptable on the twelfth day. The flavor of cod held in MRSW decreased only slightly during the experiment and none were statistically ($p < 0.05$) different from the control sample. However, the 12-day sample was of unacceptable quality based on the smell of the raw fillets. Comments by the panel members indicated that low flavor scores were caused by the increased intensity of a "fishy" flavor. The texture scores slowly decreased with time of holding cod in ice, but only the control and the 12-day samples were

Table 1—Mean sensory scores, NaCl and total solids content of Pacific cod held in ice and in modified refrigerated seawater (MRSW)

Medium	Time of holding, (Days)	Flavor	Texture	NaCl[e] (%)	Solids (%)
I C E	0	3.8^{ab}	3.9^{ab}	0.15	16.9
	3	3.5^{ab}	3.6^{abc}	0.16	16.7
	6	2.9^{bc}	3.3^{bc}	0.19	17.2
	9	2.6^{cd}	3.3^{bc}	0.18	16.8
	12	1.8^{d}	3.1^{c}	0.19	16.2
M R S W	0	3.8^{ab}	3.9^{ab}	0.15	16.9
	3	3.9^{a}	4.3^{a}	0.48	17.9
	6	3.8^{ab}	4.1^{a}	0.69	18.2
	9	3.6^{ab}	3.8^{abc}	0.97	17.3
	12	3.0^{bc}	3.8^{abc}	0.96	18.3

[abcd] Mean sensory scores in the same column followed by a common superscript are not different ($p < 0.05$) by Least Significant Difference Test.
[e] Calculated from analysis for chloride.

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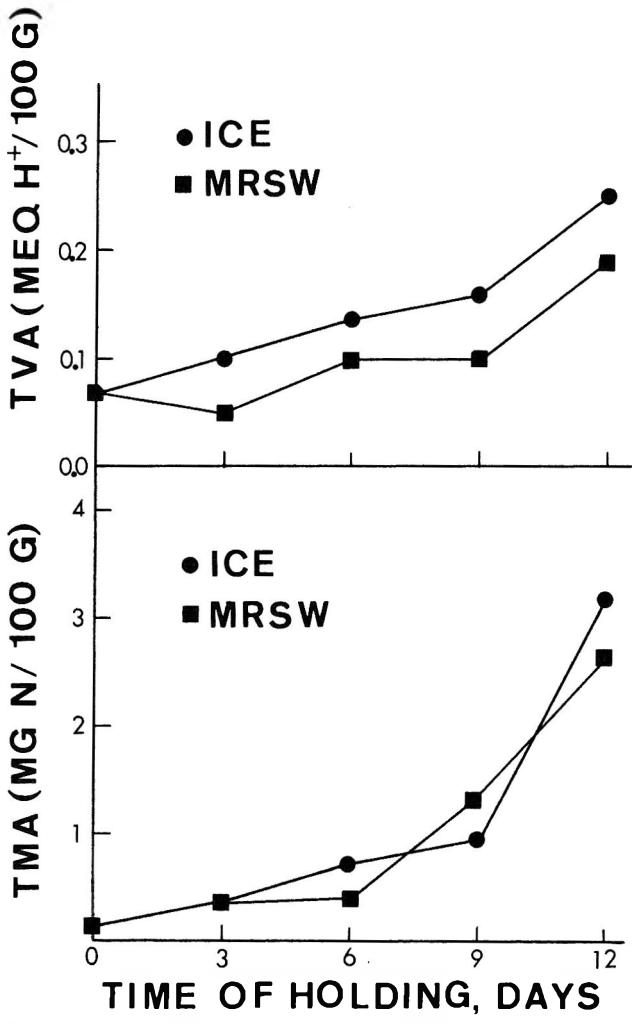

Fig. 1—Regression of trimethylamine (TMA) and total volatile acid (TVA) content on time of holding Pacific cod in the round in ice and CO_2 modified refrigerated seawater (MRSW).

statistically ($p < 0.05$) different. Comments from the judges indicated that low texture scores were due to softening of the flesh. For cod held in MRSW, no significant change in texture scores occurred with time of holding and the scores were higher (flesh was firmer), than those of the corresponding samples from fish held in ice.

Cod held in MRSW readily absorbed salt (Table 1) and at 9 days, 58% of the panelist thought the samples tasted too salty. If the ratio of fish to brine were higher, the absorption of salt should not be as much a problem. Total solids content decreased with time of holding in ice, but increased for fish in MRSW due to absorption of salt (Table 1). The TMA content increased for fish held in either media, and generally fish held in MRSW had a TMA content slightly less than that of fish held in ice (Fig. 1). Flavor scores and TMA values were correlated both for fish in ice ($r = -0.933$, $p < 0.05$) and in MRSW ($r = -0.969$, $p < 0.01$). Use of TMA content for an index of spoilage for cod held in MRSW may prove difficult because a sample (12 days) with an acceptable flavor score had a substantial TMA content and an unpleasant odor in the raw state. Changes in TVA content (Fig. 1) paralled those in TMA content and were significantly correlated with flavor scores: (Ice, $r = -0.993$, $p < 0.001$; MRSW, $r = -0.946$, $p < 0.05$).

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Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA, to the exclusion of other products that may be suitable.

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A Research Note

A Plating Technique for the Selective Isolation of Yeast Utilizing Water Immiscible Carbon

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ABSTRACT

An emulsion overlay plating technique was developed for the rapid and selective isolation of yeast that assimilate water immiscible substrates. The plating medium contains a defined enrichment broth and an Aniline blue agar base. Growth is easily detected by colony dye concentration, decolorization zones around colonies and fluorescence of colonies under long wave UV light.

INTRODUCTION

SEVERAL AGAR PLATE METHODS (Alford, 1976; Atlas and Bartha, 1973; Horowitz et al., 1975; Reisfeld et al., 1972; Rosenberg, 1981; Seki, 1973; Starr, 1941; Walker and Colwell, 1976; Zajic and Supplisson, 1972.) and general shake flask culture enrichment methods (Atlas, 1978; Horowitz et al., 1975; Iguchi et al., 1969; Jobson et al., 1972; Reisfeld et al., 1972; Zajic et al., 1977, 1974) have been described for the detection of microorganisms that utilize petroleum or edible vegetable oils as carbon sources. In addition, a polystyrene replica plate method (Rosenberg, 1981) has been devised for screening bacterial hydrophobilicity and provides a means by which bacteria, showing an affinity for hydrocarbons, could be selectively isolated. Although these procedures serve the purpose of their design, chiefly as primary isolation media, they lack the specificity, selectivity and/or ease of application to rapidly screen a large number of microbial samples for water immiscible substrate assimilating ability. We have developed an emulsion overlay agar technique which permits the rapid and selective isolation of yeast with the ability to utilize water immiscible substrates as the primary carbon source. The medium contains a defined enrichment broth and an Aniline blue agar base.

MATERIALS & METHODS

CANDIDA PARAPSILOSIS (ATCC 20246) *C. petrophilum* (ATCC 20226), *C. utilis* (ATCC 20248), *C. tropicalis* (ATCC 32113) and *Saccharomycopsis lipolytica* (ATCC 8662) were obtained from the American Type Culture Collection. The candida strains are cited as petroleum degrading yeasts (American Type Culture Collection, 1978; Asahi Kogyo Kabushiki Kaisha, 1971; Azarowicz, 1975) and *S. lipolytica* is cited as a fish oil utilizing yeast (American Type Culture Collection, 1978). All cultures were maintained on yeast mold agar (Difco) slants and cultured weekly in yeast mold broth (Difco).

Aniline Blue Agar (ABA) medium consists of an ABA base (0.0023% Aniline Blue (Sigma Chemical Co.) and 1.8% agar) supplemented with a chemically defined enrichment broth (0.67% Difco Yeast Nitrogen Base and 0.005% glucose) The medium was prepared in the following manner. Twenty grams of agar were dissolved with heating in 1L of distilled water. To this solution, 10 ml of a filtered (0.45μ Millipore membrane filter) 0.25% stock solution of Aniline blue was added to make the ABA base. The ABA base was distributed in 90 ml volumes in screw cap milk dilution bottles (Corning), sterilized by autoclaving (121°C, 20 min), and tempered (55°C) until ready for use. The enrichment broth was formulated in 10X strength, just prior to use, by dissolving 6.7g of Difco Yeast Nitrogen Base and 0.05g of glucose in 100 ml of distilled water. The broth concentrate was filter sterilized using a 0.45μ Millipore membrane filter. Ten ml of the 10X strength enrichment broth was added to 90 ml volumes of the ABA base just prior to pouring the plates. Approximately 15 ml of the tempered ABA medium was added to petri dishes (15 x 100 mm) and allowed to solidify. The final pH of the ABA medium is about 5.2 at 25°C. Pure edible vegetable oils (T.J. Lipton) with no added preservatives, were detoxified (removal of free fatty acids) as previously described (Alford, 1976). Hexadecane (Eastman) and paraffin oil (Baker) were used directly without further purification. Five ml of the filter sterilized (0.45μ Millipore membrane) water immiscible substrate to be tested was added to 90 ml of the ABA medium, emulsified by vigorous hand shaking, and ten ml portions immediately overlaid on the ABA plates. An opaque dispersion of oil droplets is observed in the agar overlay after solidification. Broth cultures of yeast were prepared 72 hr in advance of the test plating. Appropriate dilutions were made in Butterfield Buffer with 0.1% peptone (Scott Laboratories) to deliver 100–300 colony forming units per plate. ABA plates containing each of the water immiscible substrates were spread plated with the test cultures in duplicate, and incubated 5 days at 27°C'

Spirit Blue Agar was prepared with 5% of each water immiscible substrate emulsified with Tween 80 as previously described (Difco laboratories, 1972). Spirit Blue Agar paltes were spread plated in duplicate with 100–300 colony forming units of each culture and incubated 5 days at 27°C.

The ability of yeast to utilize water immiscible substrates was confirmed by shake flask culture (Zajic and Supplisson, 1972). Ninety ml volumes of distilled water were sterilized in 250 ml Wheaton shake flasks at 121°C for 20 min. Filter sterilized 10X strength enrichment broth (10 ml) was added to each flask. Five ml of the filter sterilized water immiscible substrate was added to the liquid medium. Duplicate shake flasks were inoculated with each yeast culture to give a final concentration of apporiximately 10^3 colony forming units per ml. Cultures were incubated for 5 days at 27°C on a rotary shaker at 250 rpm. Growth in shake flasks was confirmed by plate counts on yeast mold agar.

RESULTS & DISCUSSION

YEAST STRAINS demonstrating the ability to assimilate water immiscible substrates on ABA plates produced colonies that concentrated the Aniline blue dye. During the early stages of colony development the dye concentrating effect was most easily observed by viewing the underside of the plates. As colony growth became more dense, zones of decolorization were produced with a darkening of the surrounding medium. In addition, the colonies fluoresced yellow-green when illuminated with long wave UV light. Growth on ABA media was dependent on the ability of the yeast to utilize the water immiscible substrate as the primary carbon source. ABA media without water immiscible substrate did not support growth, indicating that the small amount of glucose (0.005%) in the media was not adequate to effect colony formation. In the absence of glucose, several yeast strains with the ability to assimilate water immiscible substrates did so much slower rates.

Spirit Blue Agar (Difco Laboratories 1972; Starr, 1941) is commonly used as a selection media for microorganisms that utilize edible oils. All five yeast strains showed positive

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Table 1—Growth of yeast on selective media after 5 days at 27°C

Yeast	Hexadecane			Paraffin oil			Soybean oil			Cottonseed oil		
	SBA[a]	ABA	SF[b]	SBA	ABA	SF	SBA	ABA	SF	SBA	ABA	SF
C. parapsilosis	+	–	–	+	–	–	+	+	+	+	+	+
C. petrophilum	+	+	+	+	+	+	+	+	+	+	+	+
C. tropicalis	+	–	–	+	–	–	+	–	+	+	+	–
C. utilis	+	–	–	+	–	–	+	+	+	+	+	+
S. lipolytica	+	+	+	+	–	–	+	+	+	+	+	+

[a] Spirit Blue Agar
[b] Shake Flask

grwoth responses on each of the substrates tested using Spirit Blue Agar (Table 1). However, using ABA, negative growth responses were shown by three strains for hexadecane, four strains for paraffin oil, and one strain for soybean oil (Table 1). Results from shake flask cultures were in agreement with the ABA growth responses with the exception of the response of *C. tropicalis* on soybean oil and cottonseed oil (Table 1). The presence of emulsifiers, which may be metabolized as carbon substrates (Alford, 1976), and other undefined substances in Spirit Blue Agar may account for the growth of the yeast strains on Spirit Blue Agar but not on ABA.

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A Research Note

Acetaldehyde Determination in Fermented Food Products by Direct 2,4-Dinitrophenylhydrazine Derivatization, Extraction and High Performance Liquid Chromatography

R. H. SCHMIDT, S. M. DAVIDSON, and R. P. BATES

ABSTRACT

Data obtained for acetaldehyde analysis of yogurt and wine by direct 2,4-dinitrophenylhydrazine (DNPH) derivatization and extraction are compared to those obtained by distillation and DNPH derivatization. Separation and analysis of acetaldehyde 2,4-dinitrophenylhydrazone (DNP) was by reverse phase high performance liquid chromatography (HPLC). Higher precision was obtained with the direct derivatization extraction technique than for the distillation derivatization technique. Data obtained by the direct derivatization extraction technique for acetaldehyde in commercial yogurt samples had less viriability than those for wine.

INTRODUCTION

THE IMPORTANCE of acetaldehyde to the flavor of fermented products such as yogurt and wine has been clearly demonstrated (Amerine, 1954; Bottazzi and Vescovo, 1969; Keenan and Bills, 1968; Law, 1981; Pette and Loklema, 1950). Reported data for yogurt acetaldehyde have recently been summarized (Tamime and Deeth, 1980). These data are generally characterized by a wide range and variability depending upon starter culture, method of manufacture, and other factors including analytical method. Typically, acetaldehyde analysis in fermented products or in microbial cultures involve distillation or micro-diffusion to isolate acetaldehyde from background followed by gas chromatography or spectrophotometric analysis of hydrazone or semicarbazide reaction products (Lees and Jago, 1969). Enzymatic procedures involving alcohol dehydrogenase have also been described for acetaldehyde analysis of yogurt, butter distillates (Veringa and Schijver-Davelaar, 1970) and wine samples (McCloskey and Mahaney, 1981).

Because of the extreme reactivity and volatility of acetaldehyde (bp 21°C), care must be taken in any analytical procedure. Thus, techniques involving chemical derivatization with 2,4-dinitrophenylhydrazine (DNPH) or related reagents may be advantageous over other techniques since the derivatives are less volatile. Results obtained using high performance liquid chromatography (HPLC) for acetaldehyde analysis in *Streptococcus lactis* culture distillates as its 2,4-dinitrophenylhydrazone (DNP) derivative (Schmidt et al., 1983) compared favorably with those using a spectrophotometric method (Lindsay and Day, 1965). For routine analysis of fermented products, it may be desirable to eliminate the distillation step in the DNPH-HPLC procedure. In addition to time savings, a direct derivatization extraction would also minimize possible artifact formation from heating during distillation. The objective of the present investigation was, therefore, to evaluate the application of an HPLC method involving direct DNPH derivatization and extraction for acetaldehyde analysis in yogurt and wine samples.

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MATERIALS & METHODS

Food samples

Commercial plain yogurt samples, purchased from local markets, were designated yogurt A (Dannon's; Dannon, Inc., Long Island, NY), B (Dairyfresh; Publix Super Markets Inc., Lakeland, FL), C (Superbrand; Borden's Inc., Tampa, FL), D (Breakstone; Kraft, Inc., So. Edmeston, NY), and E (Breyers; Kraft, Inc., Philadelphia, PA). To assess variability between production lots, samples of yogurt A, B and C were obtained from different markets at 2-wk intervals. Analysis of each yogurt sample was done in triplicate.

Wine samples analyzed included wines prepared from Florida grape varieties (red wines #289, 309 and 339; white wine #308; Bates et al., 1980) and commercial wines (Pink Chablis; Gallo Vineyards, Modesto, CA; and Cabernet Sauvignon; Sebastiani Vineyards, Sonoma, CA). Analysis of each wine sample was done in triplicate.

Acetaldehyde analysis

For the distillation/derivatization method, a 5.0 ml sample of diluted yogurt (1:1 with 20 mM sodium phosphate buffer at pH 7.0) or a 2.0 ml sample of wine (undiluted) was mixed with 25 ml water and steam distilled into the DNPH-celite reagent mixture and extracted with carbonyl free hexane (Schmidt et al., 1983). For the direct derivatization/extraction method, 5.0 ml diluted yogurt (1:1 with phosphate buffer) or wine (2:5 with phosphate buffer) was mixed directly with 200 mg of DNPH-celite reagent mixture and reacted at room temperature for 30 min. Carbonyl-free hexane (5.0 ml) was added with mixing and the derivatizing mixture was transferred onto a Clinelut™ extraction column and the carbonyl DNP's were eluted with 100 ml carbonyl free hexane.

Separation of acetaldehyde by HPLC was as previously described (Schmidt et al., 1983). Data are reported as μg acetaldehyde/g yogurt or μg acetaldehyde/ml wine.

RESULTS & DISCUSSION

Comparison of distillation/derivatization and direct derivatization/extraction techniques

For comparison of the two DNP derivatizing techniques, eight replicate samples of yogurt B or wine #339 were analyzed. These data are summarized in Table 1. The direct derivatization/extraction procedure had better precision for both product systems than did the distillation/derivatization procedure. Relative standard deviation (RSD) for the direct derivatization procedure applied to yogurt and wine were 3.3% and 5.5%, respectively. Mean acetaldehyde data for yogurt by the two procedures were not different ($p > 0.05$) while mean acetaldehyde levels in wine by direct derivatization were lower ($p < 0.05$) than for distillation derivatization techniques. While this may be due to dissociation of bound acetaldehyde during distillation (McClosky and Mahaney, 1981), further investigation would be necessary to elucidate reasons for the differences detected.

Acetaldehyde levels in fermented products

Data for acetaldehyde levels in yogurt samples are presented in Table 2. [RSD values for all yogurt samples evaluated ranged from 2.5–13.5%.] Highest acetaldehyde levels were observed for yogurt A. The direct DNPH derivatization HPLC procedure appears to be advantageous over

Table 1—Comparison of distillation/2,4-dinitrophenylhydrazine (DNPH) derivatization and direct DNPH derivatization/extraction techniques for acetaldehyde analysis in yogurt and wine

Sample	Derivatization procedure[a]	Acetaldehyde Mean[b]	Acetaldehyde R.S.D.[c]
Yogurt B	Distillation/derivatization	20.03	7.4
	Derivatization/extraction	21.36	3.3
Wine #339	Distillation/derivatization	37.25	6.0
	Derivatization/extraction	31.23	5.5

[a] Distillation/derivatization: Sample steam distilled into DNPH reagent and extracted. Derivatization/extraction: Sample mixed directly with DNPH reagent and extracted.
[b] Mean (μg acetaldehyde/g yogurt or μg acetaldehyde/ml wine) of eight replicate analyses.
[c] R.S.D. = relative standard deviation.

the HPLC technique involving distillation and DNPH derivatization with considerable time and savings and better data precision.

For wine samples analyzed (Table 2), higher data variability was observed than with yogurt samples. Further investigation may be needed using different sample size and altered extraction conditions for improved application of the technique to wine.

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Table 2—Acetaldehyde data for yogurt and wine samples obtained by direct 2,4-dinitrophenylhydrazine (DNPH) derivatization, extraction and high performance liquid chromatography (HPLC)

Sample	Acetaldehyde[a]
Yogurt:	
A (1)[b]	34.53 ± 3.02
(2)	23.24 ± 3.14
(3)	26.89 ± 1.65
B (1)	22.06 ± 0.75
(2)	22.82 ± 2.68
(3)	21.15 ± 1.66
C (1)	19.80 ± 0.93
(2)	22.89 ± 1.17
(3)	18.05 ± 0.89
D	23.08 ± 1.05
E	22.42 ± 0.56
Florida red wines:	
#289	18.07 ± 3.47
#305	17.20 ± 0.64
#339	31.24 ± 1.71
Florida white wine:	
#308	26.05 ± 4.25
Commercial wines:	
Pink chablis	2.17 ± 0.08
Cabernet Sauvignon	18.85 ± 1.86

[a] Mean (μg acetaldehyde/g yogurt; μg acetaldehyde/ml wine) of triplicate analyses ± standard deviation.
[b] Numbers in parentheses represent replicate yogurt samples obtained from local markets at 2-wk intervals.

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A Research Note

Polyethylene Film Packaging of Citrus Fruit: Containment of Decaying Fruit

C. R. BARMORE, A. C. PURVIS, and P. J. FELLERS

ABSTRACT

Decaying grapefruit (*Citrus paradisi* Macf.) stored under nonventilated conditions caused a substantial reduction in the oxygen and an increase in the carbon dioxide concentrations in the atmosphere. A comparable increase in the internal carbon dioxide concentration of sound fruit in the same container was also observed. Low oxygen caused an increase in the ethanol concentration in the juice of the sound fruit and the development of an undesirable stale flavor. Containment of the decaying fruit in polyethylene film limited the oxygen depletion. Thus, flavor quality of sound fruit from the containers with PE film-sealed decaying fruit was comparable to that of sound fruit stored in the absence of decaying fruit.

INTRODUCTION

INDIVIDUAL SEALING of citrus fruit with heat-shrinkable polyethylene (PE) film beneficially affects the postharvest quality of the fruit by reducing moisture loss and subsequent fruit deformation and preventing soilage of sound fruit by decayed fruit within the same container. Although, diffusion of oxygen and carbon dioxide is reduced by the PE film, fruit respiration and quality are not adversely affected during storage (Ben Yehoshua, 1977; Ben Yehoshua et al., 1979; Hale et al., 1982; Purvis, 1983).

Storage of citrus fruit under low oxygen levels can adversely affect their flavor (Chace et al., 1967). Since decaying fruit consume oxygen faster than sound fruit (Schiffmann-Nadel, 1977), they could contribute to low oxygen levels within containers especially under conditions of inadequate ventilation. The objective of this study was to examine the effect of containment of decaying citrus fruit in PE film on oxygen and carbon dioxide concentrations in containers and the flavor of the accompanying sound fruit.

MATERIALS & METHODS

GRAPEFRUIT (*Citrus paradisi* Macf. cv 'Marsh') were washed, treated with 600 ppm benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate), and waxed with Flavorseal. Fruit of a second lot, washed only, were inoculated by injection with a water suspension of *Penicillium digitatum* spores. One group of inoculated fruit was individually shrink-sealed in a low density PE film (27.4 μm) and one group was not sealed. Oxygen transmission of the film was 3477 ml $O_2/m^2/24$ hr at 23°C and atm. pressure. Commercial corrugated fiberboard trays were filled with 9 or 12 fruit comprising the following treatments: (1) uninoculated (sound) fruit; (2) sound and individually PE film-sealed inoculated fruit, and (3) sound and nonsealed inoculated fruit. Sound fruit in each treatment were not individually sealed in PE film. Each tray was then sealed with 27.4 μm PE film and placed into a nonventilated commercial fiberboard container and stored at 23°C for 14 days. Each treatment was replicated three times.

Oxygen and carbon dioxide concentrations within the trays were determined with a paramagnetic and an infrared analyzer, respectively. Ethylene was determined with a gas chromatograph. Internal atmosphere of the fruit was sampled by submerging the fruit in water and removing a volume of gas with a syringe from the center cavity (Wheaton et al. 1977). Juice from the sound fruit within each treatment was extracted with a Sunkist hand reamer and combined into a composite sample for ethanol and flavor analyses. The procedure of Davis and Chace (1969) was used for ethanol determination. Flavor was evaluated by an experienced taste panel providing 27 and 48 judgments on the 7 and 14 day stored fruit samples, respectively. Members scored the freshly extracted juices on a 9-point hedonic scale where 9 was liked extremely and 1 was disliked extremely. Samples were served at 23°C and presented to the panelist in standard taste booths under red light. Data were subjected to an analysis of variance.

RESULTS & DISCUSSION

DECAYING FRUIT caused a substantial decrease in the oxygen concentration and a concomitant increase in the carbon dioxide concentration after 14 days storage in nonventilated contaners (Table 1). Sealing the decaying fruit in PE film limited both the decrease in the oxygen and the increase in carbon dioxide concentrations. Rates of decay development of the sealed and nonsealed inoculated fruit were comparable. Increasing the number of decaying fruit from 4 to 8 per tray did not increase the reduction in oxygen during a 7-day storage period if the decaying fruit were sealed in the PE film. However, if the decaying fruit were not sealed in the PE film, increased numbers did cause further reduction in oxygen concentration. Summation of the oxygen and carbon dioxide concentrations averaged about 22% for each treatment. Internal carbon dioxide concentration in the sound fruit reflected the level of carbon dioxide within the trays of each treatment.

Ethylene concentrations within trays in nonventilated containers containing no decay, 4 sealed decaying, and 4 nonsealed decaying fruit were 0.16, 1.32, and 6.45 ppm, respectively.

Most of the decrease in the oxygen concentration can be attributed to the utilization of oxygen by the decaying fruit. However, ethylene produced by *Penicillium digitatum* (Schiffmann-Nadel, 1977) would cause an increase in the respiration rate of sound fruit in the tray (Biale, 1960). The high internal carbon dioxide concentration in the sound fruit stored in the presence of decaying fruit suggests that the respiration of these fruit was increased.

Table 1—Oxygen and carbon dioxide concentrations in nonventilated containers with decaying (Penicillium digitatum Sacc.) and/or sound grapefruit stored at 23°C

Treatment[a]	Storage (days)	Oxygen[b] (%)	Carbon dioxide[b] (%)
No decaying fruit	7	18.7 ± 0.9	2.2 ± 0.8
	14	19.1 ± 0.8	2.4 ± 1.0
Decaying fruit sealed in PE film	7	13.8 ± 2.0	8.6 ± 2.0
	14	13.9 ± 0.9	8.3 ± 0.5
Decaying fruit nonsealed	7	11.2 ± 1.9	11.1 ± 2.0
	14	7.5 ± 3.7	13.8 ± 3.5

[a] Each treatment consisted of 3 replicates of 9 fruit each including 0 or 3 decaying fruit
[b] Mean with standard deviation

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Table 2—Flavor score and ethanol concentration of juice from sound grapefruit stored in the presence of decaying fruit (Penicillium digitatum Sacc.) in nonventilated containers at 23°C

Treatment[a]	Storage (days)	Flavor[b]	Ethanol[c] (mg/100 ml)
No decaying fruit	7	6.1	5.3 ± 0.7
	14	5.8	10.2 ± 1.9
Decaying fruit sealed in PE film	7	6.1	8.8 ± 1.4
	14	5.6	16.2 ± 1.5
Decaying fruit nonsealed	7	5.6	15.7 ± 2.5
	14	4.9[d]	59.0 ± 14.9

[a] Each treatment consisted of 3 replicates each with 12 fruit including 0 or 4 decaying fruit
[b] Flavor scored on a 9-point hedonic scale where 9 was liked extremely and 1 disliked extremely
[c] Mean with standard deviation
[d] Significant within days stored at the 5% level using the Duncan Multiple Range Test

Taste evaluation by an experienced taste panel of the freshly extracted juices from the sound fruit within each treatment indicated that after 7 days storage there were no significant differences among the flavor scores of the 3 treatments, although the mean flavor score for the juice of the sound fruit with nonsealed decaying fruit was lower than the other 2 treatments (Table 2). The juice from sound fruit stored for 14 days with nonsealed decaying fruit did, however, develop an undesirable stale flavor. The mean flavor score of this juice was significantly lower than the flavor scores of the other 2 treatments.

Storage studies have shown that an oxygen concentration below 13% can lead to undesirable off-flavor development (Chace et al., 1967). The oxygen concentration in the trays with nonsealed decaying fruit was 7.5% after 14 days storage, thus, the undesirable flavor may have resulted from the production of anaerobic metabolites. Ethanol concentration in the juice of sound fruit from containers with nonsealed decaying fruit was approximately 6-fold higher than in the juice of sound fruit stored under about 19 or 14% oxygen. Ethanol has been reported by others to accumulate in fruit stored under conditions of low oxygen (Chace et al., 1967).

Sealing the inoculated fruit in the PE film limited the rate of oxygen utilization during the decay process. The PE film, being a partial barrier to oxygen diffusion, provided for an equilibrium between the rate of oxygen utilization by the decaying fruit and the diffusion of oxygen into the nonventilated container which was not completely impermeable to oxygen and carbon dioxide. Interestingly, increasing the number of film-sealed decaying fruit from 4 to 8 did not substantially lower the oxygen level. Although, containing the decaying fruit in PE film delayed the development of off flavors in the juice of sound fruit, the use of PE film for individually sealing of citrus fruit does not eliminate the need for ensuring adequate air circulation in and around containers during storage.

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A Research Note

A Simple Nondestructive Method for Determination of Air Content in Polymeric Packages

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ABSTRACT

A new nondestructive method of estimating the residual air volume in food packages was developed and compared with the standard destructive technique. The nondestructive method involved enclosing the test package in a nonflexing, plexiglass container and placing this container into a vacuum chamber. Monitoring changes in volume at two vacuum levels and using Boyle's Law, the air content of more than fifty polymeric packages was computed. The difference between the destructive and nondestructive volumes was found to be statistically insignificant.

INTRODUCTION

NO LONGER are glass or rigid metal containers utilized exclusively for packaging and processing of foods. The retort pouch, a flexible package, is gaining popularity as a substitute for the traditional containers. The increased use of these packages has led to extensive studies of the package's characteristics, including the determination of the residual air content. This information is vital to the food manufacturer because the residual air content affects the quality of the food, the rate of heat penetration, and the integrity of the package's seal (Ghosh and Rizvi, 1982). Since it is the absolute quantity of air (oxygen), not the vacuum level, in a food package that affects the quality of food within, a simple nondestructive method of its evaluation will be helpful in product development and quality control work.

Various methods for destructively and nondestructively determining the residual air content have been reported (Lampi, 1977). Using the principle of neutral buoyancy, the nondestructive method was found to give consistently lower values than those obtained by the destructive technique (Shappee and Workowski, 1972). A correction factor that increases the correlation between the two methodologies has been established (Ghosh and Rizvi, 1982). However, the application of this method is limited to packages that do not float in water under atmospheric pressure and become buoyant under reduced pressure. This study was designed to investigate the possibility of a nondestructive method that is not limited to nonfloating packages but determines the residual air contents of all different types of flexible packages.

MATERIALS & METHODS

FORTY-FIVE of the polymeric packages used in this experiment were made of polyethylene material. The pouches ranged in size from 7 cm x 2.5 cm to 7 cm x 12 cm. In addition, five (12 cm x 15 cm size) retort pouches constructed of polyester, aluminum foil, and polyolefin laminates were used. The test packages were filled with various quantities (ranging from 13–140g) of solid materials (lima beans, navy beans, split peas). The pouches were sealed at atmospheric pressure with an automatic bag sealer. All measurements were made at room temperature. The volume of residual air was first determined nondestructively and then destructively.

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Nondestructive method

The packages were placed in a nonflexing plexiglass container of approximately 1.6 liter capacity and consisting of a bottom and a top. The package was placed into the bottom portion of the container which was filled with water and to which 2 drops of octanol were added to suppress bubble formation. The top was attached with four clamps. Gaskets were used to seal the top and bottom to make them air tight. A burette was fixed with a union clamp to the top and make-up water was added through the burrette into the container until the water level became visible in the burette. Every effort was made to remove any entrapped air pockets; making the top convex prevented the entrapment of air bubbles. The filled container was placed in a vacuum chamber attached to a vacuum gauge and a vacuum pump (Fig. 1). Two different vacuum levels were applied. The vacuum reading and the volume of the water level in the burette were recorded. The volume of air in the packages (V_a) was determined using the following equation (see Nomenclature for symbol definition):

$$V_a = \frac{P_2 \cdot \Delta V_2}{(P_1 - P_2)} - \Delta V1 \qquad (1)$$

A detailed derivation of Eq. (1) is shown in the Appendix.

Destructive method

The pouches were cut open under water and the escaping gas collected in a graduated cylinder filled with water whose open end was held under water. Regression analysis and paired t-test were performed on the data (SAS, 1980).

RESULTS & DISCUSSION

THE VOLUMES of air in 50 packages tested nondestructively were calculated using Eq. (1) and were plotted against actual (destructive) volumes (Fig. 2). The relationship between destructive and nondestructive volume was essentially linear and the observations clustered around the 45 degree line drawn through the origin. At a level of significance of P = 0.6, the t-test indicated that the hypothesis of equal destructive and nondestructive volumes cannot be rejected. A linear regression analysis of nondestructive versus destructive volumes had a slope of 0.925 and y-intercept of

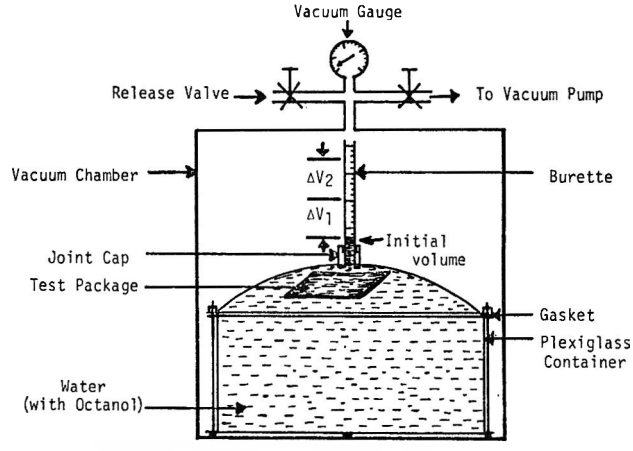

Fig. 1—Set-up for determining the nondestructive volume.

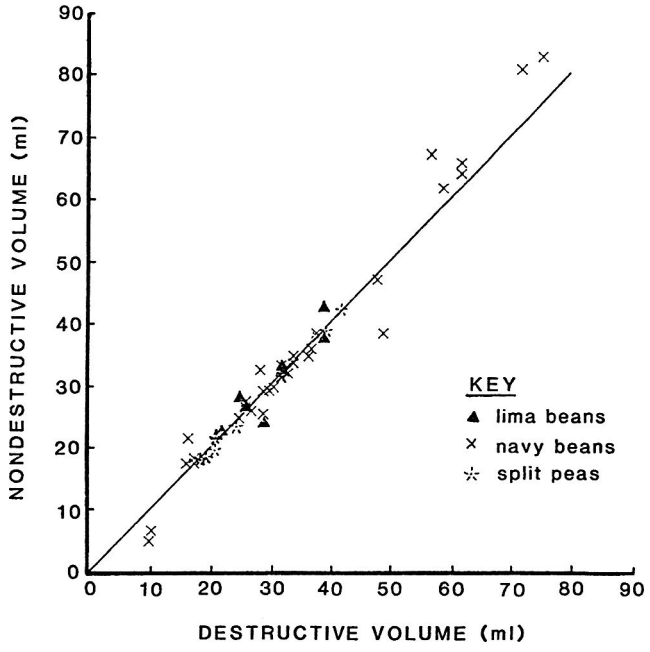

Fig. 2—Plot of nondestructive volume vs destructive volume.

3.8. The correlation coefficient was 0.98. The statistical tests and visual inspection indicated that, within experimental error, the two methods of determining volume were equivalent.

This linear relationship, however, appeared stronger within a certain residual air volume range. At high air volumes (greater than 50 ml) or at low air volumes (less than 15 ml), the correlation was not as precise as within the air volume range of 15–50 ml. At the upper and lower range of the tested volumes, experimental error became significant ($P < 0.05$) due to the limitations of the experimental setup. The height of the vacuum chamber limited the height of the burette used and thus the maximum volume change. The construction and material of the packages limited the minimum exertable pressure. Also at the low air volumes, experimental errors in determining the destructive volume were magnified.

Unlike previously reported techniques, this nondestructive method for determining the residual air volume of the pouch is not dependent on the weight and buoyancy characteristics of the package. This allows for wider application because less dense packages (higher air to solid ratio or utilization of lighter solids) may be used. This is an important consideration when attempting to determine the volume of air in a package by nondestructive methods. Since not all packaging materials are completely flexible, use of various materials gives different magnitudes of variability. Therefore, further testing to determine the application of this technique to a variety of other packaging materials is recommended.

CONCLUSIONS

THIS PAPER DESCRIBES a new technique for nondestructively determining the residual air content of food packages. By enclosing the pouch in a nonflexing plexiglass container, which is subsequently placed in a vacuum chamber, the volume of air in a package may be determined. This method gives good correlation with the destructive volume. Since the use of the latter involves the expenditure of the package's integrity, the described method is simple and adaptable for routine testing for residual air content of packages without any major investment.

NOMENCLATURE

P_G = Atmospheric pressure (760 Torr)
P_1 = Pressure at expanded volume V_1 (Torr)
= P_G – Vacuum Gauge Reading + Hydrostatic Pressure
P_2 = Pressure at expanded volume V_2 (Torr)
= P_G – Vacuum Gauge Reading + Hydrostatic Pressure
V_a = Volume of air inside sealed package at P_G (ml)
V_1 = Volume of air at P_1 (ml)
ΔV_1 = $V_1 - V_a$ (ml)
V_2 = Volume of air at P_2 (ml)
ΔV_2 = $V_2 - V_1$ (ml)

APPENDIX

Derivation of Eq. (1)

Assuming ideal behavior of air,

$$P_1 V_1 = P_2 V_2$$

But $V_2 = V_1 + \Delta V_2 \therefore P_1 V_1 = P_2(V_1 + \Delta V_2)$.

Or $V_1 = \frac{P_2 \cdot \Delta V_2}{(P_1 - P_2)}$. Also, $V_a = V_1 - \Delta V_1$. Substituting in above equation,

$$V_a = \frac{P_2 \Delta V_2}{(P_1 - P_2)} - \Delta V_1$$

P_1 and P_2 were corrected for hydrostatic pressure exerted on each package during testing. The hydrostatic pressure was computed by dividing the height of water above the test package by 13.69.

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A Research Note

Production and Textural Properties of Soy-Cheese Whey Curd

H. M. WU and A. C. PENG

ABSTRACT

Soy-cheese whey curd was prepared by mixing soymilk and cheese whey with the addition of glucono-delta-lactone (GDL) to coagulate the proteins. Three soymilk concentrations, 6:1, 8:1 and 10:1 (water:bean, v/w), and four cheese whey levels, 3%, 4.5%, 5.25% and 6% (w/v), were used. Heating the GDL treated soy-cheese whey milk in a hot water bath at 85°C for 25 min consistently produced a smooth and compact curd without syneresis. Textural properties of the curd were tested by an Instron machine. Five parameters—stiffness, bioyield point, firmness, relaxation and plasticity—were determined. Among these, only stiffness and firmness could be used as the textural parameters for the curd.

INTRODUCTION

SOYBEAN CURD, also known as tofu, has been used as a protein source in the Orient for many centuries. It is high in lysine and only moderately low in methionine. It is also an inexpensive protein source for human food as compared to animal sources such as pork and beef. Cheese whey has long been posing a waste problem of the dairy industry. Much effort has been extended to improve the utilization of whey for its high nutritional value, especially the sulfur-containing amino acids (Whitaker and Tannenbaum, 1977; Wingerd et al., 1970). Combination of soybean protein and cheese whey protein into a soy-cheese whey curd may upgrade the nutritional value of soybean curd, and promote the use of cheese whey in the food industry.

Yao and Peng (1976) have investigated the effects of coagulants on protein content and amino acid composition of soy-cheese whey curd. The purpose of this research was to develop a method which can consistently produce an attractive soy-cheese whey curd and to evaluate the textural properties of this product.

MATERIALS & METHODS

Materials

Soybeans of the Vickery cultivar were obtained from the Manchester Farm (Auglaize, OH). Sodium Protolac, a cheese whey protein concentrate was provided by the Industrial Food Products, Borden Inc. (Columbus, OH); and Glucono-delta-lactone (GDL) was purchased from Sigma Chemical Company (St. Louis, MO).

Soy-cheese whey curd preparation

After being washed and soaked overnight under refrigeration, the soybeans were blended with fresh tap water (pH 7.0) at water: bean ratios of 6:1, 8:1 and 10:1 (v/w) for 5 min in a Waring Blendor. The slurry was filtered, the filtrate was boiled for 15 min and then cooled to 20°C. Sodium Protolac at 3%, 4.5%, 5.25% and 6% (w/v) levels was dispersed into the cooled soymilk; the mixture deaerated for 1–1.5 hr, followed by mixing with 0.6% powdered GDL (w/v). The soy-cheese whey-GDL mixture was heated in a water bath at 85°C for 25 min, cooled under running cold tap water for 25 min to enhance the hardening of the gel, and refrigerated.

Analytical evaluation

Total solids (TS) content of soy-cheese whey milk. Five ml of soy-cheese whey milk in triplicate were dried in a Precision-Thelco recirculating oven at 95 ± 2°C for 18 hr. The weight difference (%) was used to calcualte the TS content of the mixture.

Moisture content of the soy-cheese whey curd. Triplicate samples of 1.00–5.00g were dried in a Precision-Thelco recirculating oven at 65 ± 2°C for 48 hr. The weight losses (%) were assumed to represent the moisture contents of the curd.

Protein content. The Micro Kjeldahl method was used (Croke and Simpson, 1971). The protein content was calculated by the equation:

$$\text{Protein content (\%)} = \frac{(\text{ml acid sample-ml acid blank}) \times 0.1 \times 14 \times 100}{50.0 \text{ mg of sample}} \times 6.25$$

Textural properties of the curd

An Instron Universal Testing Machine, Table Model TMM, CTM cell, was used to determine textural properties of the curd. Samples were tested in beakers, 6.80 cm inside diameter, filled to a depth of 3.20 cm. The flat plate plunger was 3.90 cm in diameter. during the descent of the plunger, the crosshead speed was set at 1.0 cm/min, chart speed was 20.0 cm/min, and full scale deflection was 2.0 kg. Plunger penetration length was stopped at 1.0 cm. The relaxation was obtained by continuing to record the decay of the force, after the plunger was stopped, until a certain period of time had elapsed. Samples were tested at 22.2–23.3°C. Three parameters: stiffness, bioyield point and firmness were determined in the descending test, and two additional parameters: relaxation and plasticity were obtained in the relaxation test.

RESULTS & DISCUSSION

Soy-cheese whey curd formation

All 102 samples prepared by the method reported in this experiment showed smooth and compact gels both in appearance and cut surface. No serum separation was observed. Curds were opaque and slightly yellowish in color. after 2 months of refrigeration, no syneresis was observed. During reheating of GDL treated soy-cheese whey milk (20°C) in a water bath to 85°C, heat gradually hydrolyzed GDL to form gluconic acid which decreased the pH of the protein solution to the isoelectric regions of soybean and whey proteins, thus forming a three-dimensional gel network. The excellent water-holding capacity of the cheese whey protein (McDonough et al., 1974) and GDL structure contributed to the nonsyneresis of the curd. Reheating also exerted a pasteurization effect on the curd.

Analytical evaluation

Effect of cheese whey concentrations. The results of analytical characteristics, such as total solids (TS) content, moisture content, and protein content of the curds prepared by mixing soymilk (6:1, water:bean, v/w) and sodium protolac at 3%, 4.5%, 5.25% and 6% (w/v) are summarized in Table 1. As indicated by the data, cheese whey concentration did affect the analytical characteristics. As it increased from 3% to 6%, TS content of cor-

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Table 1—Total solids (TS), moisture and protein content of soy-cheese whey milk and gelled products (%)

Analytical characteristics	Cheese-whey conc (w/v)[a]				Soymilk conc (H_2O:bean, v/w)[b]		
	3%	4.5%	5.25%	6%	6:1	8:1	10:1
TS of soy-cheese whey milk	11.0	12.3	12.9	13.5	14.7	13.0	11.6
Moisture of curd	87.8	86.6	85.8	85.4	84.3	86.1	87.8
Protein of curd	47.4	48.3	49.4	49.5	49.0	49.3	49.5

[a] Soymilk: 6:1 (H_2O:bean, v/w)
[b] Cheese whey: 6% (w/v)

Table 2—Textural parameters of soy-cheese whey curd as affected by cheese whey and soymilk concentration

Textural parameter	Cheese-whey conc (w/v)[a]				Soymilk conc (H_2O:bean, v/w)[b]		
	3%	4.5%	5.25%	6%	6:1	8:1	10:1
Stiffness (kg/cm)	3.3	3.4	3.6	4.0	4.3	3.7	2.9
Firmness (kg)	1.2	1.3	1.4	1.5	1.8	1.9	1.5
Bioyield point (kg)	0.7	0.8	0.8	0.9	1.0	0.9	0.8
Relaxation (min^{-1})	0.2	0.2	0.2	0.2	0.2	0.2	0.3
Plasticity	0.8	0.8	0.8	0.8	0.8	0.8	0.8

[a] Soymilk: 6:1 (H_2O:bean, v/w)
[b] Cheese whey: 6% (w/v)

responding soy-cheese whey milk increased, but the moisture content of the curd decreased. However, the protein content of the curd also increased.

Effects of soymilk concentrations. The results (Table 1) of TS, moisture and protein content of curd prepared at soymilk concentratons of 6:1, 8:1 and 10:1 (water:bean, v/w), and sodium protolac of 6% (w/v) showed that as water:bean ratio increased from 6:1 of 10:1, total solids of soy-cheese whey milk decreased, but moisture content of the curds increased. Protein content, however, was not affected by soymilk concentration.

Textural properties

Data in Table 2 specified that only stiffness and firmness were the two parameters affected by cheese whey concentrations. Curds prepared at 3% and 4% cheese whey levels had lower stiffness and firmness values than those of 6%. When curds were prepared at different soymilk concentrations, stiffness and firmness were also affected, both parameter readings were decreased as the water:bean ratio increased from 6:1 to 10:1. Other textural parameters were not altered. This leads us to believe that stiffness and firmness were the possible textural parameters for the product.

The data revealed that the textural properties of soy-cheese whey curds were more affected by total solids content than moisture or protein content of curds. However, the protein content in soymilk was almost at the same level in three concentrations, while in cheese-whey it increased from 47.4% to 49.5% as the cheese-whey concentration increased from 3% to 6%. This variation may disclose that different sources of protein may have different characteristics which may also affect the textural properties of the finished product. A group of factors, such as concentration of each individual protein, pH, ionic strength, heating time and temperature, and other components, e.g. lipid and lactose components, may contribute to the gel formation. Thus, more studies should be conducted to better understand the textural properties of this product.

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A Research Note

Nutritive Quality of Long-Distance Shipped Green Beans for Processing

L. M. MASSEY JR.

ABSTRACT

Vitamin A and C contents of bulk raw snap beans for canning from distant sources (up to 12 hr) were measured and compared to that from local sources. Statistically significant differences were found between vitamin contents of beans arriving from some locations for some seasons, but equally large season-to-season variations were also found. Much of this variation could be attributed to cultural conditions. Differences found were relatively minor, particularly in view of the large losses which are known to occur during processing. Our observations indicate that retention of these two vitamins following long distance shipping is at least as great as retention of other quality factors.

INTRODUCTION

OVER THE YEARS the food processing industry has shifted from numerous small plants to fewer but larger installations capable of sustained high-volume production. This and other converging trends have resulted in increasing use of long-distance transportation of bulk raw fruit and vegetable raw products for processing (Massey, 1972). Snap green beans in particular is an example of a highly mechanized crop which lends itself to such techniques. Although snap beans exhibit high respiratory activity and frequent loss in canning quality when improperly handled, bulk truckloads of this raw commodity are often made over shipping periods of 12 hr or more. These shipments are commonly made overnight in open trucks, frequently with air scoops and/or mesh sides to circulate ambient air through the commodity to control temperature rise and avoid anaerobiosis. Detrimental effects to quality are possible as pointed out by other investigators (Freeman and Sistrunk, 1978; Groeschel et al., 1966; Parker and Stuart, 1935). No study of the effect of such handling on nutritive contents has been reported. The purpose of this study was to gather information on vitamins A and C in beans following both long and short distance transport as they arrived at a large central New York bean canning plant.

MATERIALS & METHODS

THE PLANT SELECTED for this study had a packing capacity of about 2 million cases of 24-#303 cans per season utilizing in the order of 15,000 grade tons of commodity over the normal season of about 15 June through 15 September. Raw product for the first 5–6 wk of operation was drawn from New Jersey and the Eastern Shore region of Virginia. Samples for analysis were taken from bulk trucks during the unloading operation. Sample temperature (thermometer), location in the load, origin of the shipment, cultivar and grade were recorded. Samples were then removed to the laboratory, and the No. 4 and 5 sieve size beans combined for immediate moisture determination, or quickly frozen to −40°C for further analyses (usually less than 2 hr following arrival of the shipment). As required for analysis, subsamples were removed from the freezer and either analyzed immediately for Vitamin C [ascorbic acid (Robinson and Stotz, 1945)] or lyophylized for Vitamin A (beta carotene) determination (AOAC, 1975), as described by Lee et al. (1982). All results are expressed on a dry weight basis.

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RESULTS & DISCUSSION

EXPERIMENTS conducted during the 1980 season were principally of an exploratory nature. Emphasis was placed on examination of the commodity from long-haul trucks, but data were also taken on beans from local sources for purposes of comparison. Such variables as temperature upon arrival and sample location in the load relative to contents of vitamins A and C were examined. Beans from out-of-state were from the New Jersey area (ca 9 hr) and were of the Bush Blue Lake cultivar. Beans from local sources were of mixed cultivars. There was no significant difference in vitamin contents in beans taken from either the exterior or interior of the loads in shipments drawn from the New Jersey area, as indicated in Table 1. Commodity temperatures recorded upon arrival were usually found to be ambient or slightly below, averaging 18.3°C (range 13–23°C). There was no correlation between commodity temperature and vitamin contents in any of these samples. Beans from the local New York area likewise showed no difference in vitamin contents due to location in the load. Commodity temperatures recorded upon arrival were usually found to be ambient or slightly above, averaging 27.2°C (range 23–36°C). Some instances of local beans having been held overnight in presumably stationary trucks prior to morning delivery were noted. Here too, there was no correlation between commodity temperature and vitamin content. Comparison of the vitamin contents between out-of-state and local beans is shown in Table 2. The significant difference in carotene contents of beans from the two sources is probably not meaningful in view of the difference in cultivars being sampled.

These observations were repeated in more detail during the 1981 season in which shipments from New Jersey and Virginia (ca 12 hr) were compared. Although fewer shipments were involved, sampling from within various locations of the load was made in greater replication. Commodity temperatures were slightly lower in samples from New Jersey, averaging 18.6°C (range 16–21°C) than from Virginia, averaging 22.5°C (range 20–25°C). Again there was no correlation between commodity temperature and vitamin contents from either source. Also again, there was no significant difference between ascorbic acid and carotene content in beans from various locations in the load. How-

Table 1—Vitamin contents of snap bean samples taken from various locations from bulk loads following shipping[a]

Number of shipments	Location in load	Ascorbic acid (mg/100g)	Beta carotene (μg/g)
1980			
23	Exterior	89.5(±16.6)	28.0(±6.83)
23	Interior	89.7(±16.1)[NS b]	27.4(±7.82)[NS]
1981			
12	Exterior	115(±20.4)	30.8(±4.96)
12	Interior	120(±33.9)[NS]	31.8(±7.20)[NS]

[a] Dry weight basis. Vaues in parenthesis are standard deviations.
[b] Notation indicates no significant difference between compared values in columns per year.

Table 2—Vitamin contents of snap beans immediately following shipment from various geographic locations to a central New York canning plant.[a]

Vitamin	State of origin					
	Virginia		New Jersey		New York	
	Number of shipments	Mean	Number of shipments	Mean	Number of shipments	Mean
1980						
Ascorbic acid (mg/100g)	–	–	23	88.7(±10.8)[z]	18	85.3(±21.1)[z]
Beta carotene (μg/g)	–	–	23	30.5(±7.3)[z]	18	23.1(±3.2)[y]
1981						
Ascorbic acid (mg/100g)	4	138(±9.4)[z]	3	95.0(±13.0)[y]	–	–
Beta carotene (μg/g)	4	27.5(±3.0)[y]	3	36.3(±2.5)[z]	–	–
1982						
Ascorbic acid (mg/100g)	6	90.0(±16.2)[y]	17	79.1(±17.6)[y]	26	107(±27.4)[z]
Beta carotene (μg/g)	6	24.3(±1.60)[y]	17	32.5(±3.4)[z]	26	31.8(±7.14)[z]

[a] Dry weight basis. Values in parenthesis are standard deviations. Mean separation within rows each year by Duncan's multiple range test, 5% level.

ever, a significant difference between ascorbic acid and carotene content was noted in samples from the two areas (Table 2). As the cultivar in both cases was Bush Blue Lake and there was no notable difference between size grades utilized for determination, it is probable that the observed differences were due to cultural factors.

Experiments conducted in 1982 utilized beans from both relatively long distance (Virginia and New Jersey) and short distance (local New York). For these observations, samples were taken in duplicate from within each load. The results of these analyses are indicated in Table 2. This year, only samples from loads of the same cultivar (Bush Blue Lake) were utilized. Ascorbic acid contents of distant origin scored significantly lower than those of local origin. Carotene contents of beans from Virginia were significantly lower than those from either New Jersey or New York. In both cases, however, the differences between actual mean values were relatively small. Again, some evidence of local beans being held overnight in presumably stationary vehicles were observed. In two instances, loads from distant origin arrived after having been delayed en route an extra 24 hr. In both, the commodity was unfit for processing, showing high temperatures, and obvious anaerobiosis, and was dumped. Samples obtained from these loads were analyzed for both vitamins. Although it is not possible to apply statistical analysis to these data, it is of interest that in these cases both ascorbic acid and carotene values were somewhat above the yearly mean from that area (New Jersey).

We conclude from these studies that long-distance movement of bulk green beans can be accomplished successfully with only relatively minor differences in either ascorbic acid or beta carotene from those obtained from local sources. Although statistically significant differences can exist in these vitamins depending on both geographic location of the growing area and on season, in general these differences are relatively minor, particularly in view of the losses in both vitamins which occur during processing (Van Buren et al., 1982). Although other quality measurements of the suitability of raw product snap beans for processing were not made in these studies, it is our impression that the retention of these two vitamins following long distance shipping is at least as great if not greater than the retention of other quality factors.

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A Research Note

Use of Vegetable Oils in Pie Crusts

M. L. DREHER, P. T. BERGLUND, and C. J. DREHER

ABSTRACT

Limited research has been reported in the literature on the performance of vegetable oils in pastry. This study evaluated differences in quality between pie crusts prepared with four types of vegetable oils and those prepared with a standard hydrogenated vegetable shortening. Vegetable oils and shortening were evaluated for color, viscosity, free fatty acids, peroxide values and P/S ratio. Pie crusts were evaluated for color, shrinkage, flakiness, and sensory quality. Panelists detected no significant differences in color between vegetable oils or shortening pie crust. Percent shrinkage and flakiness of the crusts were directly related to the P/S ratio. Sensory texture and flavor scores showed no significant difference between vegetable oil crusts but the shortening crust was rated significantly lower in flavor ($P \leq 0.05$) than vegetable oil crusts.

INTRODUCTION

LIMITED RESEARCH has been reported in the literature on the performance of vegetable oils in pastry. Matthews and Dawson (1963) evaluated the performance of vegetable oils and shortenings in pastries and biscuits and found vegetable oils to be better shortening agents in pastry and vegetable shortening better in biscuits. They concluded that for good-quality pastry, even distribution and high specific gravity of the oil droplets are important.

Current dietary recommendations call for moderation in the consumption of dietary fat. Recommended dietary fat should be approximately 35% of the calories or less with a higher proportion of the total fat coming from polyunsaturated fatty acids contained in vegetable oils rather than from saturated fatty acid sources (Food & Nutrition Board, 1980). Potential beneficial health effects of polyunsaturated fatty acids have been noted (Food & Nutrition Board, 1980) and in response to this, consumers have increased their usage of polyunsaturated oils in areas where they traditionally used saturated fats. The purpose of this study was to determine any differences in quality between pie crusts prepared with vegetable oils and those prepared with a standard, hydrogenated shortening.

MATERIALS & METHODS

Samples

The four types of vegetable oils used were commercially available and included: safflower, sunflower, corn and peanut oils. Two different brands of each oil were evaluated. The control vegetable shortening was composed of partially hydrogenated soybean and palm oils with mono- and diglycerides added. Each oil and the shortening were incorporated into the following pie crust formulation: 43.0g oil/shortening, 130.0g sifted all purpose white flour and 33.4g cold water (20°C). The crust was rolled between sheets of plastic wrap using rod guides to obtain a uniform thickness (2 mm). The crusts were then baked at 220°C for 10 min.

Oil properties

The color and physicochemical properties of the shortening and oils were evaluated. Color was determined on a Gardner Tristimulus IX-23 Colorimeter using the L, a, b scale compared to a white standard (XL-23-246-D). Viscosities were determined with a Brookfield synchrolectric viscometer using spindle RV-1, 20 rpm and at ambient temperature. Free fatty acids and peroxide values were determined by official AOCS methods (1974). The P/S ratios were calculated from data included in Reeves and Weihrauch (1979).

Crust properties

The pie crusts were evaluated for color and consumer acceptance. Color was determined as described previously on the top and bottom of each sample. Shrinkage was determined by the following formula:

$$\% \text{ Shrinkage} = \frac{\text{Area before baking} - \text{Area after baking}}{\text{Area before baking}} \times 100$$

Flakiness was determined by measuring the height of each of two squares of baked pastry at the centerpoint of each of the four sides of the samples and then averaging the eight values obtained (McWilliams, 1977). Sensory quality attributes of color, texture, and flavor were evaluated in duplicate samples by seven trained panelists using a 9-point hedonic rating scale (1 = dislike extremely to 9 = like extremely) (Amerine et al., 1965; Johnston, 1979). The panel members consisted of faculty, staff and students. The test was performed in partitioned booths with fluorescent lighting. Samples were randomly coded and served individually with water. Each sample consisted of one 7.62 cm by 2.54 cm crust strip. Sensory data were statistically analyzed by analysis of variance and Duncan's multiple-range test. Shrinkage and flakiness were compared with P/S ratios by the correlation coefficient method.

RESULTS & DISCUSSION

DATA on the color and physicochemical properties of the vegetable oils and shortening are shown in Table 1. The L, a, b color values for all the vegetable oils and the melted shortening were similar but the solid shortening was lighter, less red and less yellow in color than the melted shortening. The safflower, sunflower and corn oils had similar viscosity values (65.0–85.0 cps) while peanut oil was higher (95.0–105.0 csp). This variation may be due to the degree of saturation of the oil. The free fatty acid content as oleic, ranged in decreasing order, was vegetable shortening, safflower oil, peanut oil, corn oil and sunflower oil. The peroxide values were highly variable (1.50–6.00 meq/kg). Oils selected for this study were representative samples of commercially available oils with P/S ratios of 0.5–1.9.

The pie crust characteristics are shown in Table 2. The Gardner color values displayed considerable variation in crust made from different brands of the same oil, especially the sunflower oil. However, the panelists did not perceive the Gardner color variability as a negative factor as the sensory scores were less variable and moderately highly rated. Within the vegetable oils, there were only slight variations between the top and bottom of the pie crust but the vegetable shortening showed a wide variation, especially for the L and a values. The panelists detected no significant ($F_{0.01,\ 8,\ 135} = 1.23$) color differences between the crust containing shortening and those containing vegetable oils. The percent shrinkage ($r = 0.87$) and flakiness ($r = 0.82$) of the crust were significantly related to the P/S ratio. The sensory texture ($F_{0.01,8,135} = 1.21$) and flavor ($F_{0.01,\ 8,\ 135}$

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Table 1—Vegetable oil characteristics

Oil	Color L	Color a	Color b	Viscosity[a] (CPS)	Free Fatty Acids as Oleic (%)	Peroxide Value (mEq/kg)	P/S Ratio
Safflower							
Brand A	11.4	−0.62	1.06	75.0	0.066 ± 0.001	2.75 ± 0.35	8.2
Brand B	10.0	−0.53	0.44	67.5	0.049 ± 0.001	4.45 ± 0.64	
Sunflower							
Brand A	12.0	−0.64	0.76	72.5	0.024 ± 0.002	6.00 ± 0.80	6.4
Brand B	10.1	−0.69	1.03	85.0	0.017 ± 0.003	2.75 ± 0.35	
Corn							
Brand A	9.8	−0.65	0.51	65.0	0.033 ± 0.003	1.50 ± 0.71	4.6
Brand B	12.1	−0.97	2.74	75.0	0.026 ± 0.002	5.50 ± 0.71	
Peanut							
Brand A	10.5	−0.74	1.16	105.0	0.034 ± 0.001	4.50 ± 0.00	1.9
Brand B	10.6	−0.69	0.94	95.0	0.032 ± 0.002	2.25 ± 0.35	
Vegetable Shortening							
Melted	8.00	−0.50	0.23	–	0.130 ± 0.014	0.50 ± 0.00	0.5
Solid	49.3	−3.21	−0.40	–			

[a] 25°C

Table 2—Pie crust characteristics

Crust		Color L	Color a	Color b	Shrinkage (%)	Flakiness (mm)	Sensory scores: Color	Texture	Flavor
Safflower Oil									
	top	60.7	4.44	20.4					
Brand A					20.8 ± 3.80	2.3 ± 0.46	6.25	6.25	6.46
	bottom	61.3	5.11	21.8					
	top	64.1	1.82	19.8					
Brand B					19.8 ± 5.30	2.4 ± 0.44	6.96	6.32	6.64
	bottom	65.1	2.69	21.8					
Sunflower Oil									
	top	61.5	5.14	21.8					
Brand A					15.2 ± 3.63	2.3 ± 0.46	6.93	6.82	6.71
	bottom	65.9	2.76	20.6					
	top	54.0	8.36	20.8					
Brand B					17.1 ± 4.05	2.1 ± 0.32	6.89	6.25	6.27
	bottom	52.5	10.3	21.6					
Corn Oil									
	top	53.4	8.15	20.2					
Brand A					14.4 ± 0.09	1.9 ± 0.32	6.33	6.46	6.53
	bottom	55.3	8.13	20.9					
	top	58.7	7.43	22.4					
Brand B					17.9 ± 2.35	2.1 ± 0.58	6.80	6.37	6.87
	bottom	63.3	5.11	21.9					
Peanut Oil									
	top	62.9	3.80	20.3					
Brand A					13.5 ± 1.08	1.8 ± 0.26	6.57	6.17	5.90
	bottom	62.2	3.84	21.2					
	top	68.5	0.88	19.5					
Brand B					14.7 ± 0.30	2.1 ± 0.42	6.93	6.70	6.87
	bottom	68.7	1.02	20.9					
	top	63.2	4.23	19.4					
Vegetable Shortening					12.0 ± 2.62	1.9 ± 0.39	6.28	5.83	5.46
	bottom	52.1	9.69	22.2					

= 2.01) scores showed no significant difference between the vegetable oil crusts but the shortening crust flavor was rated significantly ($P \leq 0.05$) lower and its texture score was lower (not significantly) than the vegetable oil crusts. The results of this study, using this specific pie crust formulation, showed vegetable oils to produce better flavor and flakiness than partially hydrogenated vegetable shortening. Additionally, the use of high polyunsaturated vegetable oils in pie crust may have a nutritional advantage due to the high P/S ratio. The benefits of high polyunsaturated vegetable oil in pastries needs to be further evaluated.

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A Research Note

Method of Preparation and Sensory Evaluation of Sweet Potato Patties

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ABSTRACT

Sweet potato patties were prepared from Jewel and Centennial cultivars which were freshly harvested or cured and stored. The patties were prepared with additions of sugar, starch and other minor ingredients to peeled, cooked sweet potatoes. The resulting mixture was comminuted, heated by steam injection, vacuum cooled and molded into patties. The patties were cooked in peanut oil and evaluated by a sensory panel. The evaluations indicated that patties of acceptable quality could be prepared from either freshly harvested sweet potatoes or from roots that had been cured and stored for up to 6 months.

INTRODUCTION

THERE IS A WIDE VARIANCE in the size of fresh sweet potato roots ranging from 4 cm (canners) to 15 cm (Jumbos) in diameter. In general, there has been a good market for the small roots for the canning trade and the no. 1 or medium size roots for the fresh market. However, it is often difficult to find a market for the large "jumbo" and misshapened roots except at a very reduced price. Thus, there is a need for better utilization of the large roots in order for the farmer to market them in a more economic manner.

Sweet potatoes are normally harvested and cured at about 30°C and 80–90% relative humidity for 5–8 days (Wilson et al., 1980). During curing, there is a rapid build-up of alpha-amylase (Deobald et al., 1969). This enzyme, in concert with endogenous beta-amylase, softens the tissue during cooking by starch hydrolysis (Walter et al., 1975). However, if the roots are rapidly heated, the amylolytic enzymes are inactivated before significant starch hydrolysis and accompanhing softening occurs (Hoover and Harmon, 1967). Rapid enzyme inactivation is necessary if processed sweet potato products such as French fries or patties are to be produced from cured and stored roots.

In the United States sweet potatoes are normally harvested from about August 15 to November 15. Good quality sweet potato patties have been produced commercially for several years from freshly harvested Centennial variety roots (Kimbrough and Kimbrough, 1961). However, due to the short harvest season, only a limited number of patties could be produced from freshly harvested roots. Up to now a good quality patty from cured roots has not been available.

The purpose of this study was to develop a method for producing a good quality, firmly textured patty from cured and stored sweet potatoes. This capability could extend the processing period from about 3 months to as much as 8 or 9 months. Among the factors investigated were the effects of curing, storage, variety, and varying amounts of added starch and sugar.

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MATERIALS & METHODS

CENTENNIAL AND JEWEL sweet potatoes were harvested and cured for 8 days at 30°C and 80–90% relative humidity. After curing, the roots were held at about 15°C (80% relative humidity) until processed. Roots were processed immediately after harvesting (uncured), immediately after curing and at 2-month intervals thereafter up to 6 months of cured storage.

Processing conditions

Roots were washed, peeled and cut into strips 3/4-inch wide and 1/4-inch thick. The strips were then cooked for 5 min in a continuous cooker at 100°C. After cooking, varying amounts of unmoidified cornstarch and sucrose were added. The starch added ranged from 6–15% by weight of the cooked potato. The sucrose added ranged from 0–15% by weight of the cooked potato. In addition, the following minor ingredients were added to each formulation based on the weight of cooked sweet potatoes: mono- and diglycerides, 1%; sodium chloride, 0.3%; sodium acid pyrophosphate, 0.05%; FD/C yellow no. 6 color, 0.015%. After the cooked strips and other ingredients were blended together in a Hobart mixer, the mass was ground through a 0.25 inch screen in a Fitz mill operated at medium speed, heated in a steam injector cooker to 104–116°C in order to finish the cook and gelatinize the added starch and then vacuum cooled at 0.5 atmosphere to about 60–71°C to better facilitate the forming of the patties. The warm puree was filled into a mold under pressure to give patties that were 2 inches in diameter by 1/2 inch thick. The patties were then frozen and stored in polyethylene bags at −26°C until evaluated.

Sensory evaluation

Immediately before sensory evaluation, the frozen patties were cooked in peanut oil (170°C for 2.5 min). The oil-cooked patties were presented to a 20-member, untrained sensory panel consisting of staff and graduate students from the Dept. of Food Science. Panelists were served coded samples on white plates in fluorescent lighted rooms and were asked to evaluate color, flavor and texture on a 5-point scale (5 = excellent, 1 = unacceptable). At each sitting the treatments from one variety were evaluated (3 to 6 patties). Each set of samples was evaluated at three sittings. The data were analyzed with the Statistical Analysis System's (SAS, 1979) analysis of variance (ANOVA) and general linear mode (GLM) procedures.

RESULTS & DISCUSSION

AFTER MUCH PRELIMINARY WORK, the procedure reported in this paper was selected as the one best suited for this investigation. A number of cooking methods were treid and eliminated for various reasons. These included cooking in water at varying temperatures, different cooking times and also grinding the raw product prior to steam cooking. Several starches were evaluated. Unmodified cornstarch provided the best combination of characteristics compatible with the desired sensory and physical properties for the product. Most of the modified starches evaluated either caused off-flavor when added at the desired level or affected the texture by forming a tough skin on the surface of the patty when it was cooked.

Serial addition of sucrose and starch to patties

The addition of various levels of sucrose ranging from 0–15% to patties containing 8% starch (group A) and varying amounts of starch ranging from 6–15% to patties con-

Table 1—Effect of varying the starch and sugar content on the color, flavor, and texture of sweet potato patties utilizing cured (4-month storage) Jewel variety roots

Group[a]	Sucrose (%)[b]	Starch (%)[b]	Color rating	Flavor rating	Texture rating
A	0	8	4.2	2.8[c]	3.3[c]
	3	8	4.1	3.2	3.5
	6	8	4.1	3.7	3.8
	9	8	4.2	3.9	3.6
	12	8	4.2	4.2	4.0
	15	8	4.2	4.2	3.9
B	10	6	4.2	4.3	3.6[d]
	10	9	4.2	4.3	4.2
	10	12	4.3	3.7	4.0
	10	15	3.9	3.4	3.4
C	12	8	4.3	4.0	3.6
	12	9	4.2	4.0	3.7
	12	10	4.2	3.9	3.6
D	10	9	4.0	3.9	3.6
	12	9	4.0	3.9	3.7
	14	9	3.9	3.7	3.5

[a] Groups: A—8% starch, variable sucrose; B—10% sucrose, variable starch; C—12% sucrose variable starch; D—9% starch, variable sucrose.
[b] Added as percent of weight of cooked sweet potatoes.
[c] Statistically significant ($P \leq 0.01$) linear trend within respective group.
[d] Statistically significant quadratic function ($P \leq 0.01$) indicating that texture score is highest at the two intermediate starch levels.

taining 10% added sucrose (group B) resulted in significant flavor and texture differences (Table 1). Statistical analysis of the sensory data for group A indicated that flavor and texture ratings increased linearly ($P \leq 0.01$) until between 9 and 12% sucrose had been added. In group B there was a linear decrease ($P \leq 0.01$) in the flavor rating of patties with more than 9% added starch and a plateau in texture rating was reached between 9 and 12% starch addition ($P \leq 0.01$). Patties made from cured roots to which less than 8% starch was added were too soft to mold well, and when deep fat fried, tended to disintegrate. There was no significant difference in the color rating that could be attributed to any of the treatments, probably because the added FD&C yellow no. 6 was present in sufficient amounts to mask any natural color differences. Moreover, there were no significant differences in the flavor and texture of samples in groups C or D. Since the data in this study indicated that the addition of 8–12% unmodified cornstarch and 10–12% sucrose provided the most acceptable patties, we chose a mixture containing 8% cornstarch and 10% sucrose for the study of curing and storage on patty acceptability.

Effect of curing and storage on patty acceptability

There was little variation in the color rating of Centennial patties that could be attributed to curing and storage treatments; however, the color rating of Jewel patties was higher for those prepared from roots stored for 2 and 4 months (Table 2). The color rating of Centennial patties was higher ($P \leq 0.05$) than Jewel only for roots stored 4 months. There was a significant decline in texture scores after 4 months of storage for Centennial patties, while Jewel patties showed no significant changes in either flavor or texture ratings that could be attributed to storage treatments. If ratings for the two varieties are considered on the basis of each storage date, there were no significant differences except that Jewel patties made from roots stored 6 months received a higher acceptance rating for texture than Centennial patties from similar storage. Our data indicate that high quality sweet potato patties can be prepared from both freshly harvested and cured stored roots from Centennial and Jewel cultivars.

Table 2—Effect of curing and storage on the color, flavor and texture of sweet potato patties[a]

Factor	Uncured	Freshly cured	Storage 2 months	4 months	6 months	LSD[b]
Centennial variety						
Color (mean)	4.20	4.35	4.44	4.25	4.26	0.29
Flavor (mean)	3.30	3.75	3.75	3.60	3.60	0.44
Texture (mean)	3.70	3.70	3.80	3.25	3.15	0.49
Jewel variety						
Color (mean)	4.25	4.15	4.70	4.90	4.30	0.29
Flavor (mean)	3.50	3.65	3.85	3.75	3.90	0.44
Texture (mean)	3.75	3.95	4.05	3.75	3.75	0.49

[a] Prepared from a mixture containing the following components based on the weight of cooked sweet potatoes: 10% sucrose, 8% cornstarch, 1% mono- and diglycerides, 0.3% NaCl, 0.05% sodium acid pyrophosphate, and 0.015% FD and C eyllow no. 6.
[b] Least significant different ($P \leq 0.05$).

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A Research Note

Synthesis and Aroma Properties of New Alkyloxazoles and Alkylthiazoles Identified in Cocoa Butter from Roasted Cocoa Beans

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ABSTRACT

Previous work provided evidence for the occurrence of new alkyloxazoles and alkylthiazoles in the volatiles of cocoa butter from roasted cocoa beans. The synthesis, mass spectral data and sensory properties of these compounds are reported.

INTRODUCTION

THE OCCURRENCE OF OXAZOLES in cocoa has been reported by Vitzthum et al. (1975). They identified 2,5-dimethyloxazole, 4,5-dimethyloxazole, 2,4,5-trimethyloxazole and 2-propyl-5-methyloxazole in the volatile basic fraction from cocoa liquor. Numerous thiazoles have been identified in a wide variety of food flavors (Ho et al., 1982). However, only 4-methyl-5-vinylthiazole (Stoll et al., 1967) and benzothiazole (Flament et al., 1967; van der Wal et al., 1968; 1971) have been reported in cocoa flavor.

The isolation and identification of the volatile flavor constituents from cocoa butter have been recently described (Carlin et al., 1982). A total of twelve compounds was identified in the acidic fractions, 218 in the neutral fractions and 83 in the basic fractions. The compounds identified in the basic fractions of cocoa butter sample included 62 pyrazines, 9 oxazoles, 4-thiazoles, 4 pyridines, and 4 miscellaneous compounds (Carlinet al., 1982). This paper reports the synthesis, mass spectral data and sensory properties of seven alkyloxazoles and two alkylthiazoles identified in cocoa butter from roasted cocoa beans.

MATERIALS & METHODS

Synthesis of α-bromoketones

1-Bromo-2-propanone, 3-bromo-2-pentanone, 2-bromo-3-pentanone, 3-bromo-2-heptanone, 4-bromo-3-heptanone and 4-bromo-3-octanone were synthesized using the method described by Catch et al. (1948) which involves essentially direct bromination of acetone, 2-pentanone, 3-pentanone, 2-heptanone, 3-heptanone and 3-octanone, respectively. With unsymmetrical ketones, two bromoketones are formed. In all case, the bromoketone or the mixture of two bromoketones was taken through the oxazole or thiazole synthesis.

Synthesis of α-bromoacetaldehyde

α-Bromoacetaldehyde was synthesized by the method of Bedoukian (1944), which involves conversion of acetaldehyde to its enol acetate followed by the addition of bromine and then conversion to the dimethylacetal and hydrolysis to α-bromo-acetaldehyde.

Synthesis of alkyloxazoles

The alkyloxazoles were synthesized using the method of Theilig (1953). One equivalent of α-bromoketone was allowed to react with two equivalents of amide. Yields were satisfactory at about 50%. The distilled products were purified by GC. Gas chromatography was performed on a Beckman GC-55 gas chromatograph, fitted with a 12 ft long x 1/8 in. o.d. stainless steel column packed with 10% SP-1000 on 80-100-mesh chromosorb W. The flow rate was 30 ml/min. The column temperature was programmed from 50 to 230°C at a rate of 5°C/min. The boiling points of the alkyloxazoles were: 2-methyl-4-ethyl-5-propyloxazole, bp. 82–87°C/19 mm Hg; 2,4-dimethyl-5-butyloxazole, bp. 95–101°C/19 mm Hg; 2-isopropyl-4-ethyl-5-methyloxazole, bp. 74–76°C/10 mm Hg; 2-methyl-4-ethyl-5-butyloxazole, bp. 94–98°C/19 mm Hg; 2-butyl-4-methyl-5-ethyloxazole, bp. 97–101°C/10 mm Hg; 2-butyl-4-ethyl-5-methyloxazole, bp. 90–92°C/10 mmHg; 2,5-dibutyl-4-methyloxazole, bp. 116–119°C/10 mm Hg.

Synthesis of alkylthiazoles

The alkylthiazoles were synthesized using the method of Kurkjy and Brown (1952). This involves addition of the α-bromoketone or α-bromoaldehyde to the preformed thioamide. Yields were satisfactory at about 50% and the distilled products were purified by GC. Gas chromatographic conditions used were the same as described for the purification of alkyloxazoles. The boiling points of the alkylthiazoles were: 2-isopropyl-4-methylthiazole, bp. 59–62°C/10 mm Hg; 2-pentylthiazole, dp. 98–102°C/20 mm Hg.

Mass spectrometry

Mass spectrometry was conducted using a Du Pont 21-490 mass spectrometer which was interfaced by a jet separator to a Varian Moduline 2700 gas chromatograph. The gas chromatograph was equipped with an FID detector and a 1/8 in. o.d. x 12 ft stainless steel column packed with 10% OV-101 on 80-100 mesh Chromosorb-W. The ionization voltage was 70 eV.

RESULTS & DISCUSSION

SEVEN ALKYLOXAZOLES and two alkylthiazoles tentatively identified in the volatiles of cocoa butter from roasted cocoa beans (Carlin, 1980) were synthesized by the reaction of corresponding α-bromoketones with amide (Theilig, 1953) or thiomide (Kurkjy and Brown, 1952). The mass spectral data of the synthesized alkyloxazoles and alkylthiazoles are tabulated in Table 1. The mass spectra of the isolated compounds are also listed in Table 1 and match well with the synthetic compounds.

The aroma qualities of the alkyloxazoles and alkylthiazoles synthesized were described by an expert flavorist and are also listed in Table 1. The alkyloxazoles are mainly associated with a green, sweet, earthy and vegetable-like aroma impression. It is generally agreed that alkylthiazoles are important constituents of food aromas (Ohloff and Flament, 1978). 2-Pentylthiazole has strong fatty, green and sweet notes and may be an important contributor to cocoa butter flavor.

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Table 1—Oxazoles and thiazoles identified in cocoa butter from roasted cocoa beans

	Compounds isolated from cocoa flavor; MS data[a], m/z (%)	Reference compounds	
		MS data[a], m/z (%)	Odor description[b]
2-methyl-4-ethyl-5-propyloxazole	27(30), 29(15), 41(25), 43(35), 55(37), 56(5), 69(6), 71(5), 82(7), 83(15), 96(3), 97(3), 110(2), 111(2), 124(100), 125(10), 138(3), 153(5), 154(2)	27(20), 29(12), 41(28), 43(36), 55(32), 56(5), 69(7), 71(4), 82(7), 83(13), 96(2), 97(3), 110(2), 111(1), 124(100), 125(9), 138(3), 153(6), 154(2); M = 153	green fatty, vegetable-like
2,5-dimethyl-4-butyloxazole	27(10), 28(4), 42(28), 43(85), 53(5), 55(15), 69(30), 70(20), 81(5), 82(2), 96(3), 97(5), 110(89), 111(100), 124(10), 125(2), 138(5), 153(30), 154(5)	27(11), 28(4), 42(28), 43(70), 53(3), 55(7), 69(33), 70(12), 81(2), 82(2), 96(3), 97(3), 110(77), 111(100), 124(6), 125(3), 138(5), 153(21), 154(5); M = 153	fresh acidic, green, pickle-like
2-isospropyl-4-ethyl-5-methyl-oxazole	27(55), 28(57), 41(50), 43(100), 55(17), 56(22), 69(40), 70(10), 83(2), 84(10), 96(39), 97(3), 110(35), 111(2), 124(2), 138(95), 139(15), 152(5), 153(25),	27(43), 28(60), 41(42), 43(100), 55(18), 56(21), 69(44), 70(10), 82(2), 84(8), 96(37), 97(3), 110(30), 111(3), 124(4), 138(84), 139(10), 152(5), 153(26); M = 153	sweet, fruity
2-methyl-4-ethyl-5-butyloxazole	27(30), 29(50), 41(45), 43(56), 55(48), 56(15), 68(4), 69(10), 82(9), 83(25), 96(4), 97(4), 110(15), 111(8), 124(100, 125(9), 138(5), 152(2), 167(19), 168(5)	27(36), 29(45), 41(48), 43(50), 55(46), 56(8), 68(4), 69(9), 82(7), 83 (17), 96(5), 97(4), 110(9), 111(7), 124(100), 125(9), 138(3), 152(1), 167(12), 168(2); M = 167	acidic, fatty, sweet, flowery
2-butyl-4-methyl-5-ethyloxazole	27(85), 29(73), 39(30), 41(71), 54(85), 55(35), 68(9), 69(10), 83(25), 84(14), 96(10), 97(10), 110(8), 111(4), 125(100), 126(15), 138(35), 139(27), 152(7), 153(2), 166(2), 167(10)	27(62), 29(63), 39(31), 41(68), 54(85), 55(33), 68(9), 69(9), 83(12), 84(7), 96(10), 97(11), 110(7), 111(2), 125(100), 126(10), 138(36), 139(24), 152(7), 153(1), 166(2), 167(7); M = 167	green, sweet
2-butyl-4-ethyl-5-methyloxazole	28(25), 29(10), 41(38), 43(59), 56(14), 59(19), 69(23), 72(4), 82(15), 84(15), 96(4), 100(5), 124(18), 125(100), 138(30), 139(18), 152(15), 153(2), 166(5), 167(21),	28(25), 29(16), 41(26), 43(58), 56(10), 59(18), 69(17), 72(3), 82(12), 84(15), 96(3), 100(6), 124(17), 125(100), 138(27), 139(10), 152(19), 153(2), 166(3), 167(15); M = 167	green, herbal, weak, acidic, slight buttery
2,5-dibutyl-4-methyloxazole	27(22), 29(25), 41(30), 43(22), 55(9), 57(15), 68(9), 69(7), 83(4), 85(22), 96(19), 97(4), 110(45), 111(10), 122(2), 124(3), 138(2), 139(2), 152(100), 153(99), 166(25), 167(10), 180(3), 195(20), 196(2)	27(12), 29(17), 41(31), 43(17), 55(11), 57(15), 68(8), 69(7), 83(4), 85(21), 96(17), 97(6), 110(38), 111(6), 122(2), 124(4), 138(2), 139(2), 152(100), 153(90), 166(21), 167(7), 180(3), 195(16), 196(3); M = 195	sweet, fruity
2-isopropyl-4-methylthiazole	27(30), 28(10), 39(41), 45(48), 54(10), 55(10), 71(57), 72(49), 82(2), 85(1), 96(15), 99(15), 112(2), 113(5), 126(100), 127(10), 140(18), 141(65)	27(24), 28(10), 39(37), 45(49), 54(10), 55(11), 71(55), 72(48), 82(1), 85(1), 96(13), 99(14), 112(1), 113)3), 126(100), 127(8), 140(15), 141(40); M = 141	strong, camphorous, nutty
2-pentylthiazole	27(15), 29(24), 39(21), 40(25), 58(37), 59(15), 68(2), 71(2), 85(1), 86(2), 98(10), 99(100), 112(39), 113(7), 126(15), 127(2), 138(1), 140(1), 154(2), 155(4)	27(22), 29(23), 39(12), 41(20), 58(39), 59(15), 68(1), 71(1), 85(1), 86(2), 98(8), 99(100), 112(28), 113(7), 126(10), 127(2), 138(1), 140(1), 154(1), 155(2); M = 155	strong green, fatty, sweet

[a] The two most intense ions every 14 mass units above 20 are listed.
[b] The compound was purified by GC collected in a small glass trap and evaluated in its pure form.

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A Research Note

Effects of Low Frequency Ultrasound on Properties of Restructured Beef Rolls

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ABSTRACT

The effects of low frequency ultrasound on exudate yield, breaking strength, cooking yield, water-holding capacity, color, and muscle microstructure were investigated by exposing pieces of lean muscle to slow tumbling and low frequency ultrasonic waves. Results indicated that beef rolls exposed to low frequency ultrasound without added salt were superior in breaking strength and cooking yield to those tumbled with neither ultrasound nor salt and were comparable in breaking strength, cooking yield, exudate yield and water-holding capacity to those tumbled with salt and no ultrasound exposure. Furthermore, the beef rolls exposed to ultrasound and no salt were superior in color to those to which salt was added. Low frequency ultrasound caused muscle fiber disruption and separation of up to approximately 1 cm in depth in muscle microstructure in the pieces of lean muscle.

INTRODUCTION

COLOR AND OXIDATIVE RANCIDITY problems in fresh restructured pork and beef products, with sodium chloride being the primary cause, have been reported (Huffman and Cordray, 1979; Booren et al., 1979.) Sodium chloride has been added routinely to enhance salt soluble protein extraction, releasing various sticky protein fractions termed exudates (Mandigo, 1975), and muscle sections have been tumbled or massaged to disrupt cellular integrity. An alternate approach to achieve adequate myofibrillar protein extraction might be use of ultrasound without sodium chloride. However, if sodium chloride is to be used in restructured meat products, technology must be developed that will prevent unacceptable color and oxidative rancidity problems.

The purposes of this study were to examine the effects of low frequency ultrasound on myofiber disruption, to enhance myofibrillar protein extraction for use in restructured meat products and to determine the water-holding capacity, cooking loss, color and histological characteristics of restructured beef rolls as influenced by ultrasound.

MATERIALS & METHODS

RESTRUCTURED BEEF ROLLS were made from the shanks and necks of U.S. Good grade beef carcasses. Sections, 3 x 3 x 3 cm, were tumbled at 20 rpm for 60 min (model stainless steel tumbler canister 13.5 cm diameter and 20 cm long) in 1 kg lots with and without NaCl (sprinkled over the meat within the first 5 min of tumbling) and with and without ultrasound (alternate 5 min stimulation and 10 min rest during a 60 min continuous tumbling period). A 1.9 cm hole halfway from the ends of the tumbler canister allowed for insertion of a Branson ultrasonic horn (catenoidal horn, 1.3 cm, 609-001-020), coupled to a Branson converter and 184 V power supply (15 kHz frequency). After tumbling, twenty 4 cm^2 areas of muscle were gently scraped twenty times with a scalpel (blade #21) and the exudate was weighed.

Water-holding capacity of 1g samples was determined by a modification of the press method of Grau and Hamm (1957), using Whatman #1 filter paper and 22.7 kg of force for 5 min. Areas of meat and moisture were measured with a planimeter. One kg of tumbled muscle sections were hand stuffed into 6.4 cm fibrous casings and compressed with a Rheem Tipper clipper.

Each beef roll was weighed and placed on an individual cooking pan, then cooked in a 165°C pre-heated conventional electric oven to an internal temperature of 70°C. Cooking yields were determined.

Binding strength was determined on strips, (0.5 x 6 x 3 cm) sliced with a sample sizer, using an Instron Universal Testing Machine (table model no. TM) fitted with a pull force instrument adjusted to a crosshead travel speed of 10 cm/min. Four slices (2 cm thick) of each beef roll were allowed to oxygenate 10 min prior to measurement of color at room temperature. L, a and b values were determined on a Hunter colorimeter (Model D25M/L-Z). One cm^3 of muscle was removed from each unsalted treatment, frozen in liquid nitrogen, secitioned and stained by a modification of the Harris hematoxylin and eosin staining procedure described by Theno et al. (1978). Least-squares analysis of variance of the data by treatment with differences between means using the student's T-test was conducted with the SAS computer program (Barr et al., 1979).

RESULTS & DISCUSSION

WATER-HOLDING CAPACITY was similar in restructured beef rolls with salt added and restructured beef rolls with no salt added but exposed to ultrasound. There was an additive effect of salt and ultrasound, which enhanced water-holding capacity (Table 1). The color L, a and b values are shown in Table 1. The no-ultrasound and ultrasound, no-salt treatment restructured beef rolls had higher L and a values indicating that there was less destruction of myoglobin in these treatments. Beef rolls with salt added were substantially darker in color than the no salt groups as indicated by the lower L values.

The ultrasound treatment with no salt was not different from the salt added treatment (Table 1). The ultrasound salt added treatment restructured beef rolls were superior in cooking yield and exudate yield. Reynolds et al. (1978) reported similar findings, with ultrasound increasing cooking yield of restructured cured ham rolls.

The no-ultrasound, no-salt treatment group demonstrated the lowest binding strength values. Furthermore, despite the slightly lower binding strength of the ultrasound, no-salt treatment group compared to the salt added, no-ultrasound and ultrasound exposed treatment groups, all treatment groups appeared visually to have adequate binding strength for acceptable restructured products.

The light micrographs in Fig. 1 show the effects of ultrasound and tumbling on muscle. Muscles in photomicrographs A and B were tumbled while those in C and D were tumbled and exposed to ultrasound. The micrographs on the bottom are cross sections at 0.5 cm from the surface of the muscle pieces and those on the top are cross sections at 1 cm from the surface. At 1 cm depth the tissues are relatively normal in appearance with little separation or misalignment. At 0.5 cm the tissues show a greater degree of separation and some fiber disruption, especially in the muscle exposed to ultrasound. There is a substantial difference in the gap or endomysial space of the ultrasound treatment muscle compared to that exposed only to tumbling.

Both tumbling and ultrasound create cellular disruption and enhance protein extraction. However, ultrasound seems

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Table 1—Effect of ultrasound on water-holding capacity, colorimeter assessment, cooking yield, exudate yield, and binding strength

Treatment	Water-holding capacity[a]	Color assessment[b] L	a	b	Cooking yield, %[c]	Exudate yield[d]	Binding strength[e]
No ultrasound, no salt	3.8[f]	37.0[h]	16.6[g]	11.8	78.1[f]	0.42[f]	270[f]
No ultrasound, salt	3.1[fg]	32.6[f]	6.7[f]	8.5	82.1[g]	0.76[fg]	805[h]
Ultrasound, no salt	3.2[fg]	35.1[g]	14.9[g]	11.3	81.5[g]	0.71[fg]	590[g]
Ultrasound, salt	2.6[g]	32.1[f]	7.0[f]	8.4	83.8[g]	0.87[g]	860[h]

[a] Press force of 22.7 kg for 5 min; the meat area (tissue ring) was divided into the moisture area (moisture ring) to give a ratio for expressed fluid or water-holding capacity.
[b] Color assessment by a Hunter Colorimeter in the L, a and b values.
[c] The cooked weight divided by the fresh weight x 100.
[d] Grams exudate scraped/20 meat sections in area of 4 cm^2.
[e] Binding strength between muscle sections in grams peak force required to pull apart the bonded muscle sections.
[fgh] Means in the same column with different superscripts are different ($P < 0.05$).

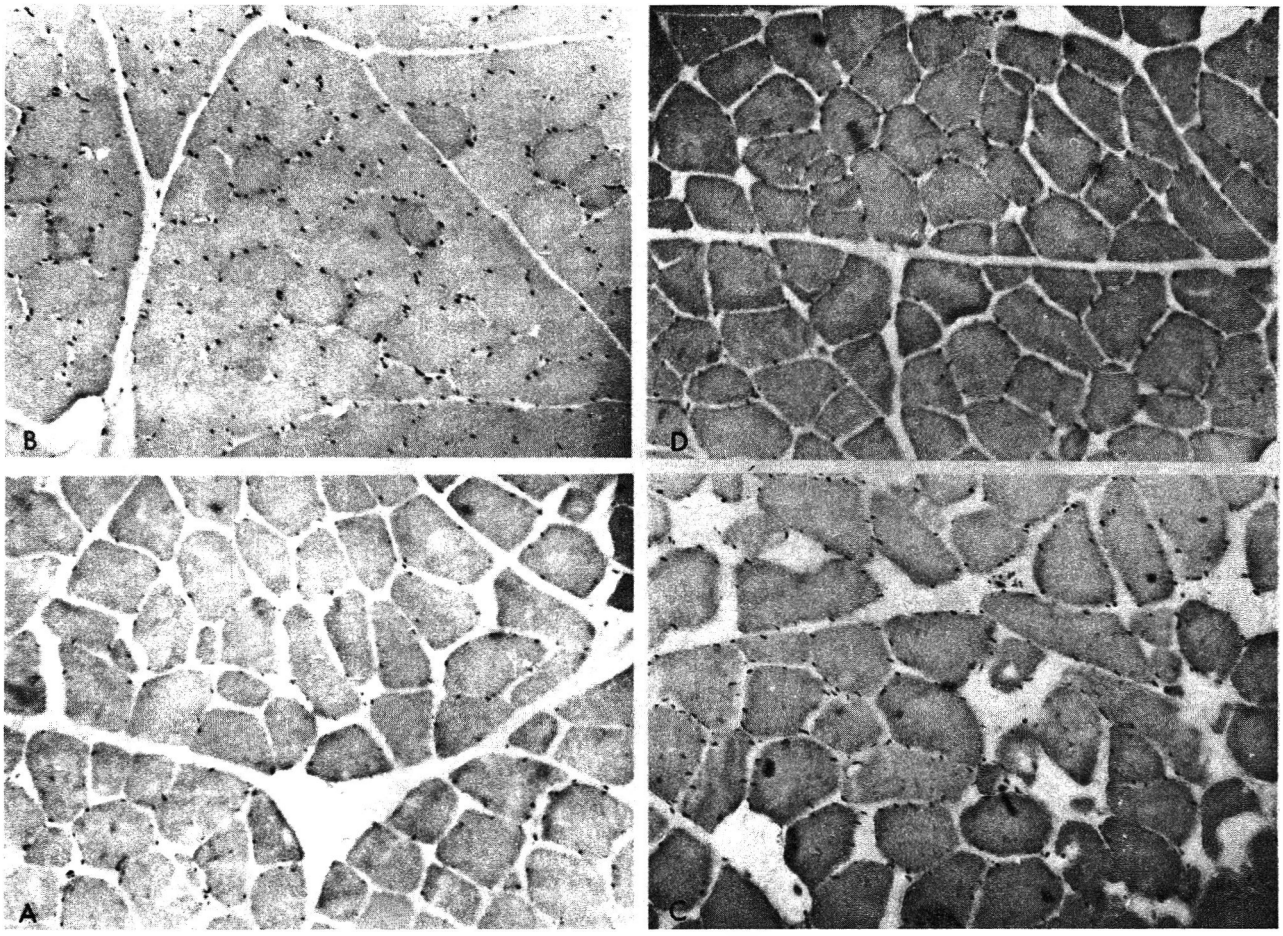

Fig. 1—Light micrographs of muscle fiber cross sections for controls at .5 cm (A) and 1 cm (B) and ultrasound treatments at .5 cm (C) and 1 cm (D). Magnification is X250.

to be most disruptive within the upper cm from the surface of pieces of meat. Although tumbling or massaging of muscle are currently the major ways in accomplishing effective salt-soluble protein extraction; ultrasound may offer an alternative to accomplish this objective without the use of sodium chloride or with the use of sodium chloride at very low levels.

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A Research Note

Antibotulinal Activity of Methyl and Ethyl Fumarates in Comminuted Nitrite-Free Bacon

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ABSTRACT

Mono- and dimethyl and ethyl esters of fumaric acid were evaluated for their antibotulinal efficacy in cans of comminuted nitrite-free bacon. At 0.125%, all were equal or superior to 120 ppm nitrite in preventing toxin formation in cans incubated at 30°C for 8 wk. No cans swelled or became toxic when mono- or dimethyl fumarate was added. With monoethyl fumarate, two cans out of twenty swelled but were nontoxic. The least effective ester was diethyl fumarate but its activity was equal to that of 120 ppm nitrite.

INTRODUCTION

THE NITRITE IN BACON is singularly suspect as a carcinogenic precursor, since it can nitrosate amines during frying (NAS, 1982). Nitrite however, acts as an inhibitor of *Clostridium botulinum* toxin formation in bacon. Decreasing or eliminating it could increase the risk of botulism from temperature-abused bacon (Hauschild, 1982) although decreasing the potential for nitrosamine formation. Although the risk of botulism from bacon is probably less than that from other cured meat products, a number of substitutes have been proposed. Among them are irradiation (Rowley et al., 1982), sorbate/sorbic acid (USDA, 1979; Ivey et al., 1978; Huhtanen and Feinberg, 1980), sodium hypophosphite (Pierson et al., 1982), and natural acidification by lactic acid bacteria (Tanaka et al., 1980).

Our laboratory has been involved in a program to find a replacement for nitrite for inhibiting *C. botulinum* in bacon. A number of alkynoic and alkenoic acids and esters were included in this program; some exhibited antibotulinal activity (unpublished results). The most promising compounds were esters of fumaric acid. This paper is a report of their activity against *C. botulinum* in a comminuted bacon system.

MATERIALS & METHODS

Comminuted bacon

Bacon was prepared in a commercial processing plant using a nitrite-free curing brine which contained 12.5% salt, 0.86% sodium tripolyphosphate, 0.39% erythorbate, and 0.11% liquid smoke. The target pump was 14% with processing back to 4% above green weight. After processing, the bacon was frozen and comminuted by grinding in a Hobart model 84-145 bowl cutter (3/16-in. plate). It was then mixed in a "Butcher Boy" model B52 food chopper. The bacon was distributed in 2-kg quantities, heat sealed in plastic pouches, and frozen at −23°C until use. Packages were thawed under running tap water. The meat was spread out in a thin layer (1–1.5 cm) and spores, esters, or $NaNO_2$ (H_2O solution) were added. These were spread out on the surface with gloved hands followed by hand mixing. Spore suspensions were added by pipetting 0.1 ml onto each 75g portion of meat. Water was added where necessary to maintain equivalent liquid additions. The bacon was distributed in 70–75g amounts into 208 x 107 aluminum tab cans which were sealed under vacuum in a Rooney canner. The cans were heated for 30 min at 68°C (center temperature) and rapidly cooled in tap water. Temperature abuse was at 30°C; cans were observed daily (once on weekends) and were removed when swollen or at predetermined intervals (1, 2, 4, and 8 wk).

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Spores

Heat-shocked spores of 20 strains (12 type A, 8 type B) of *C. botulinum* were used as previously reported by Huhtanen and Feinberg (1980), with the exception that strain 5 was omitted. Stock spore suspensions (3×10^5/ml) were kept frozen and were thawed when needed. The final concentration was 400 spores/gm, which was determined by serial dilution (in triplicate) in a fluid thioglycolate medium (Huhtanen and Feinberg, 1980).

Fumarate esters

The monomethyl and dimethyl esters of fumaric acid were purchased from Pfalz and Bauer (Stanford, CT); the monoethyl and diethyl esters were purchased from Aldrich Chemical Company (Milwaukee, WI). The purity of the first two esters is not given by the distributor, but the monethyl ester is listed as being 95% pure, while the diethyl is 99%. These were added at a concentration of 0.125% (2 oz/100 lb).

Extract preparation

Swollen cans (refrigerated after removal from the incubator) or those removed at predetermined time intervals, were opened while cold in a fume hood and approximately 15g of bacon were placed in a 40 ml polypropylene centrifuge tube. Two volumes of gelatin phosphate buffer (0.2% gelatin, 0.4% Na_2HPO_4, pH 6.2) were added and the samples mixed with wooden tongue depressors. The tubes were centrifuged at 3000g for 20 min at 5°C. The supernatants were placed in serum bottles which were sealed and kept frozen until tested.

Toxin testing

Two mice (15–20g) were each injected i.p. with 0.5 ml of each supernatant. If either mouse died, another pair of mice were injected with a heated sample (100°C, 10 min) and the sample was classified as positive if the latter injection failed to produce botulism symptoms (respiratory distress followed by death). If the original injection produced no symptoms in either mouse the sample was classified as negative. In practice, all samples producing symptoms of botulism were negative when the extracts were heated and the injections repeated.

RESULTS

A PRELIMINARY EXPERIMENT using monomethyl fumarate at concentrations of 0.200%, 0.175%, 0.150%, 0.125%, 0.100%, and 0.075% showed complete inhibition of *C. botulinum* spore germination and outgrowth (based on can swelling) at levels of 0.125% or above. At 0.100% and 0.075%, four out of five cans swelled before the termination of the experiment (60 days). The level of 0.125% was therefore chosen for the experiment whose results are shown in Table 1.

A comparison of the antibotulinal activity of the fumarate esters with that of nitrite is shown in Table 1. All 10 control cans swelled and were toxic in 7 days. In addition, one nonswollen can of bacon treated with 120 ppm nitrite was toxic at 7 days. There were, however, no additional toxic cans from this treatment until after 4 wk. Four of the nitrite cans swelled between 36 and 43 days, one at 56 days; all were toxic.

Cans containing bacon treated with 0.125% mono- or

Table 1—Comparison of fumarate esters and nitrite for antibotulinal activity in comminuted bacon

	Abuse period (days)[a]									
	7[b]		14		28		56		Cumulative	
	Cans[c]		Cans		Cans		Cans		Cans	
Addition	Swollen	Toxic	Swollen	Toxic	Swollen	Toxic	Swollen	Toxic	Swollen	Toxic
none	10	10	ND[f]	ND	ND	ND	ND	ND	10	10
120 ppm $NaNO_2$	0	1	0	0	0	0	5[d]	5	5	6
0.125% MMF[e]	0	0	0	0	0	0	0	0	0	0
0.125% DMF	0	0	0	0	0	0	0	0	0	0
0.125% MEF	0	0	0	0	0	0	2	0	2	0
0.125% DEF	0	0	1	0	0	0	4	5	5	5

[a] Abuse temperature 30°C.
[b] Ten cans per treatment interval; all the rest had five cans.
[c] No. of cans swollen or toxic between the abuse periods shown.
[d] The nitrite treated cans of meat swelled at 36, 39, 40, 43, and 56 days; the two MEF treated cans swelled at 49 days; the DEF treated cans swelled at 11, 33, 47, 47, and 49 days.
[e] MMF = monomethylfumarate; DMF = dimethylfumarate; MEF = monoethylfumarate; DEF = diethylfumarate.
[f] ND = not done.

dimethyl fumarate did not swell or become toxic, even after 8 wk of temperature abuse. Two cans of bacon treated with monoethyl fumarate swelled at 49 days but neither was toxic. With diethyl fumarate, there was one nontoxic, slightly swollen can at 11 days and four more toxic, swollen (these swelled between 33 and 49 days) cans before the termination of the experiment. There was one nonswollen toxic can at 8 wk in the group treated with diethyl fumarate.

DISCUSSION

THE RESULTS indicate that fumarate esters at 0.125% were at least as effective as 120 ppm nitrite in preventing toxin formation by *C. botulinum* in the comminuted bacon. To be useful for bacon produced under commercial conditions, however, it would have to be demonstrated that these compounds are safe and free of properties which may adversely affect the organoleptic properties of the bacon. Very little is known about the toxicity of these esters. According to the "Registry of Toxic Effects of Chemical Substances" (DHEW, 1975), the oral LD_{50} for rats of the fumaric acid diethyl ester is 1780 mg/kg; for the dimethyl ester it is 2240 mg/kg. By comparison, the LD_{50} of sodium nitrite is 85 mg/kg. More comprehensive studies of their acute and chronic toxicity would need to be done before their commercial use could be contemplated.

Several of these esters, particularly the dimethyl and diethyl fumarates, have a "perfumey" odor which, if carried through to the finished products, would make these compounds organoleptically undesirable.

In addition, to be commercially feasible, nitrite substitutes should be usable without major changes in processing conditions. Solubility in the curing brine is an important consideration in this respect. Of the four esters, the most soluble in water at 25°C was monomethyl fumarate (4%) followed by the monoethyl ester (1.8%). The dimethyl and diethyl esters were less than 1% soluble. Because of their inhibitory activity and favorable solubilities, the most promising esters for further studies would be the monomethyl and monoethyl fumarates.

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

A Research Note

Selected Nutrients in Ground and Mechanically Separated Veal

LOUIS L. YOUNG, G. K. SEARCY, L. C. BLANKENSHIP, J. SALINSKY, and DOUGLAS HAMM

ABSTRACT

Paired samples of ground veal (GV) and mechanically separated veal (MSV) were analyzed for moisture, protein, crude fat, ash, cholesterol, purine bases and nucleic acids. The MSV product was lower than the GV in protein and hypoxanthine and higher in crude fat, ash, cholesterol, adenine and guanine, DNA and total nucleic acids than the ground product. There were no differences between the products in moisture, xanthine, RNA or total purines. The data are limited but indicate that until more data are available, care should be exercised in using high levels of this product in formulations to be consumed by people with a tendency to hyperuricemia or hypercholesterolemia.

INTRODUCTION

MECHANICAL PROCESSING EQUIPMENT for recovering adherent tissue from scrap animal bones has been an important development in the meat industry. Field (1976) estimated that if all suitable bones were processed in this manner, the worldwide production of mechanically separated meat (MSM) would reach 2.1 million metric tons. Thus, this process offers an inviting opportunity to improve the edible yield from processing plants and increase the animal protein in our food supply.

MSM is not without controversy, however. Most reports indicate that MSM is lower in protein and higher in fat and cholesterol than conventional meats (Kunsman and Field, 1976, Kolbye et al., 1977; Arasu et al., 1981; Kunsman et al., 1981). Furthermore, there are indications that MSM may be higher in nucleic acids than ground muscle tissue (Arasu et al., 1981). There is concern that over consumption of MSM by persons prone to hyperuricemia or hypercholesterolemia might exacerbate their conditions. It was in part for these reasons that the USDA has recently decided to sharply limit the amount of MSM in processed foods (USDA, 1982).

Very little data are published on the composition of mechanically separated veal (MSV). Since beef and veal muscle tissues differ in their content of some important components (Watt and Merrill, 1963), it seems likely that the composition of MSV is different from that of mechanically separated beef (MSB). Thus, the current use regulations for MSM may be unsuitable for MSV. In this brief communication, we report our analyses of a limited sample of ground veal (GV) and mechanically separated veal (MSV).

MATERIALS & METHODS

SINGLE PAIRED SAMPLES of GV and MSV were obtained from a northeastern processing plant on four different days. This plant processed 40,000–50,000 pounds (18,144–22,600 kg) of product per month and at the time of this study was the only processor in this country preparing MSV under USDA inspection. An auger-type deboner was used to prepare the MSV. The samples were frozen and shipped to the USDA-FSIS Eastern Laboratory (Athens, GA), where they were thawed, mixed, sampled in duplicate for determination of nitrogen (macro-Kjeldahl), moisture (oven drying), crude fat (soxhlet extraction) and ash (incineration at 550°C). The samples were then refrozen. AOAC (1980) procedures were used for all proximate analyses. Within one week, each sample was analyzed in duplicate for cholesterol (AOAC, 1980) and for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) using the Schmidt-Thanhauser (1945) procedure as modified by Flick and Munro (1962). The levels of purine bases were analyzed using the procedure described by Young (1980). The data are reported as either percent or mg/100g of raw, wet tissue.

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RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION and cholesterol content of GV (Table 1) was similar to the data published by Watt and Merrill (1963) for thin veal carcasses without kidney or kidney fat. To our knowledge, no other data are published on the purine or nucleic acid content of GV. Arasu et al. (1981) reported RNA levels in beef muscle which are higher than ours. It is unknown if the difference between our data and theirs reflects a difference in laboratories or a difference between beef and veal.

Except for lower fat content, the proximate composition of MSV is similar to mechanically deboned chicken meat (Young, 1975), MSB (Kunsman and Field, 1976) and MSV (Field, 1976). The low fat content should not be surprising since the GV had less than 3% fat.

The cholesterol content of MSV was much higher than that of lean beef as reported by Kunsman et al. (1981). Watt and Merrill (1963) reported that veal contains somewhat more cholesterol than beef. Moreover, the proportion of lean to bone in veal may be lower than in beef. Since much of the cholesterol in MSB (and presumably MSV) comes from bone marrow and spinal cord (Kunsman et al., 1981), one might expect high cholesterol levels in MSV.

The adenine and guanine content of MSV were higher than those of GV, but the hypoxanthine was lower in MSV. These relationships are consistent with our previous results on mechanically deboned chicken meat (Young, 1980). There was no difference in total purines. There was more DNA, but the same RNA in MSV and GV. Similar results were reported by Arasu et al. (1981) using beef and MSB.

–Continued on page 1578

Table 1–Composition of selected nutrients in ground veal (GV) and mechanically separated veal (MSV)

Nutrient	GV	MSV
Protein (%)	20.4	14.3*
Total Lipid (%)	2.7	8.4*
Moisture (%)	76.6	75.9
Ash (%)	1.0	3.0*
Cholesterol (mg/100g)	103.6	214.3*
Adenine (mg/100g)	32.2	51.98*
Guanine (mg/100g)	35.1	53.6*
Hypoxanthine (mg/100g)	59.4	17.4*
Xanthine (mg/100g)	6.7	11.0
DNA (mg/g)	1.5	4.0*
RNA (mg/g)	1.7	1.8
Total purine (mg/100g)	133.5	135.0
Total nucleic acids (mg/100g)	3.3	5.8*

* MSV significantly different from GV ($P \leq 0.05$)

A Research Note

Shear Force Values for Steaks from the Semitendinosus Muscle of Pre-Rigor Leg-Twisted Beef Carcasses

S. O. ODUSANYA and A. O. OKUBANJO

ABSTRACT

The imposition of leg-twist treatment to intact beef carcass sides increased the mean, mode and median of the shear values of cooked semitendinosus muscle. Steaks from treated sides were uniformly tougher than steaks from nontreated sides. In both treated and nontreated sides, the anterior end of the muscle was tougher than the posterior end.

INTRODUCTION

VARIOUS STUDIES have identified some end-to-end and cross-sectional variation in the physical structure of certain muscles (Alsmeyer et al., 1965; Hansen, 1972; Hostetler et al., 1972; Buege and Stouffer, 1974). In some of these reports, it appears that postmortem treatments which increase or decrease the tenderness of some major muscles of the intact carcass make the shear values more uniform both within (Buege and Stouffer, 1974) and between muscles (Hostetler et al., 1972; Okubanjo, 1978a).

The present study evaluates the physical changes within the beef semitendinosus muscle as a result of the imposition of leg-twist treatment to the intact side.

MATERIALS & METHODS

FIVE GOOD GRADE White Fulani steers of approximately B$^-$ maturity, and with small amount of marbling, were used in this study. The previous nutritional background of the animals was unknown except that they were grazed for 2 wk prior to slaughter on a grass-legume (*Cynodon nlemfuensis; Centrosema pubescens*) pasture.

Dressing, splitting of the carcass and the application of the conventional and leg-twist methods of carcass suspension were as described by Okubanjo (1978a, b). Essentially, one hind leg selected at random was left intact for leg-twisting purposes. The hoofs on that leg were removed by peeling after immersion in boiling water for 5 min. Leg-twisting was effected by inserting one of the third phalanges into a slit made at the lower anterior tip of the *Rectus abdominis* muscle. The thin part of the flank thus formed a sling tucked between the two sets of phalanges. Both the conventional and leg-twisted sides were chilled at 1°C.

After 48 hr of chilling, the semitendinosus muscles from both treatments were excised at their origin and insertion. Each muscle was tightly wrapped in a polythene bag, straightened out, blast-frozen and subsequently stored at −18°C until needed. The muscles were equilibrated for 16 hr at 1°C prior to use. Five 3.75 cm thick steaks were cut from each muscle and were numbered sequentially from the anterior to the posterior ends. Each steak was broiled to an internal endpoint temperature of 72 ± 2°C, as measured with a stainless steel skewer thermometer and then cooled to room temperature. The maximum number of good 1.3 cm diameter cores was taken from each steak; this number varied from 3 to 5. Individual cores were sheared three times on a Warner-Bratzler shear machine.

As a result of differences in the number of cores per slice of steak, the range of shear values was reduced to relative frequency distributions after grouping at 1 kg intervals. Other statistics were obtained as necessary. The data were subjected to analysis of variance (Snedecor and Cochran, 1973) and the multiple range test (Duncan, 1955).

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RESULTS & DISCUSSION

SHEAR VALUES are presented in Fig. 1 with the bars of the histograms representing the percentage of total shear values that were of a certain magnitude. Other statistics from these data are shown in Table 1. Shear values decreased from the anterior to the posterior ends of the semitendinosus although only steak 1 was significantly different ($P < 0.05$) from steaks 3, 4 and 5 for both control and treated steaks.

The leg-twist treatment induced an upward shift in the mean, mode and median values in all steaks although significant difference ($P < 0.05$) was noted only for means for steaks 2, 3 and 4. Leg-twisting also narrowed the range

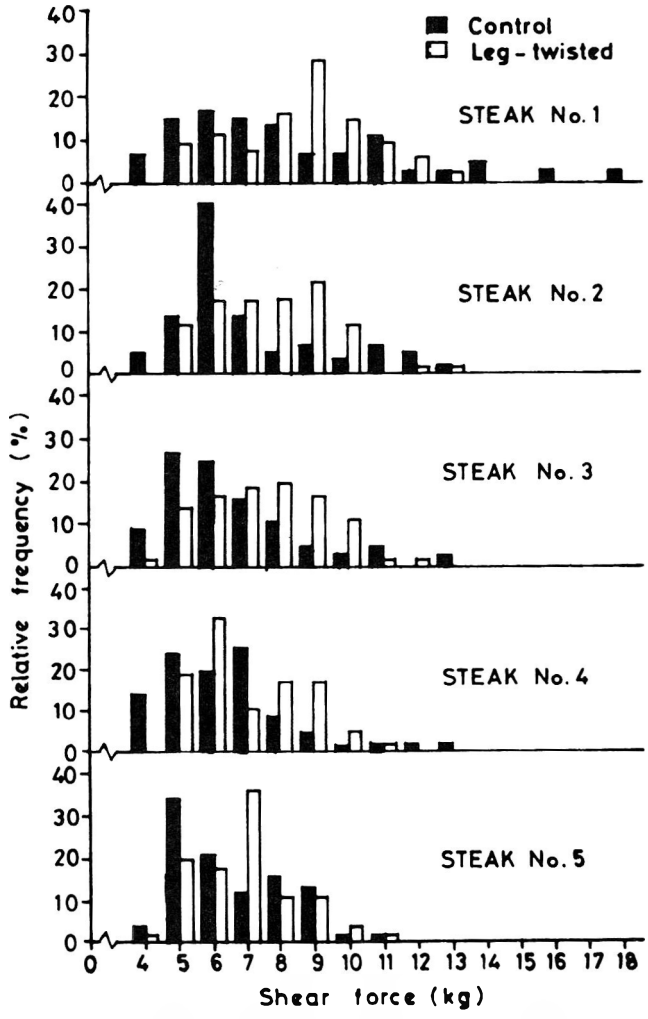

Fig. 1—Effect of leg-twisting on end-to-end variation in shear values of beef semitendinosus muscle.

Table 1—Effect of leg-twisting on shear force (kg) in five steaks of beef semitendinosus muscle

		Mean	Standard error	Mode	Median	Shear force Minimum	Shear force Maximum	Kurtosis	Skewness
Control steak	No. 1	7.15	0.46	6	6.36	4	18	0.98	1.09
	2	6.08	0.29	6	5.29	4	13	0.29	1.09
	3	5.59	0.25	5	5.12	4	13	1.58	1.29
	4	5.33	0.22	7	5.14	4	13	2.34	1.25
	5	5.56	0.21	5	5.15	4	11	−0.61	0.60
Treatment steak	No. 1	7.58	0.26	9	7.78	5	13	−0.45	−0.11
	2	6.71	0.21	9	6.71	5	13	−0.03	0.36
	3	6.50	0.21	8	6.50	4	12	−0.63	0.16
	4	6.03	0.21	6	5.50	5	11	−0.88	0.46
	5	5.88	0.20	7	5.80	4	11	0.05	0.51

of shear values from between 7 and 14 units in the control steaks to between 6 and 8 units in the treated steaks. Some individual shear values in the control steaks were greater than those obtained in the treated steaks; such shear values were masked when only mean values were reported for both groups.

In the treated group, the mode was approximately midway between the minimum and maximum shear values. In the control group, the mode was within one to three units of the minimum value but was 6 to 12 units lower than the maximum value. With one exception (steak 5), there was a general shift from positive kurtosis in the shear values for control steaks to negative kurtosis in the shear values for treated steaks. For both control and treated steaks, distributions were positively skewed but with a marked decrease in the magnitude of skewness in the treatment group.

In conclusion, data from the present study indicate that, irrespective of the treatment, the anterior end of the semitendinosus was tougher than the posterior end. The leg-twist treatment, while increasing the toughness of cooked meat, induced more uniform shear values, the majority of which were below the modal class. It is probable that treatments which improve tenderness may also behave in this manner, as was suggested for various muscles of leg-twisted mutton carcasses by Okubanjo (1978a).

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The significance of the high nucleic acid and purine content of MSV is that dietary nucleic acids and two of the purines (adenine and hypoxanthine) have been shown to exacerbate hyperuricemia and gout in pateints prone to such a condition (Clifford et al., 1976). Considering the high cholesterol, adenine and DNA content of MSV, it seems appropriate to include these items in the diets of people with hypercholesterolemia or hyperuricemia only after careful consideration. Further and more extensive studies might show that such caution is unwarranted, but until such data are available, caution seems the prudent approach.

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