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BASIC RESEARCH

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APPLIED SCIENCE AND ENGINEERING

RESEARCH NOTES

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Volume 44: Number 5

Letters

EXPERIMENTAL DESIGN/RESULTS CRITICIZED

I wish to comment on the paper "Metmyoglobin reduction in beef systems as affected by aerobic, anaerobic and carbon monoxide-containing environments by T.C. Lanier, J.A. Carpenter, R.T. Toledo and J.O. Reagan. [*J. Food Sci.* 43: 1788 (1978)].

I feel that the paper reflects a disturbing lack of awareness and rigor in the experimental design and execution, and, it follows, in the interpretation of results. Certain of the conclusions could not possibly be supported by their associated experiments. The following example should serve to illustrate this.

To test whether ferrimyoglobin reduction in beef muscle extracts is enzymic or nonenzymic, the authors start with obviously well 'aged' meat (extract pH 5.5–5.6, the usual ultimate post-rigor pH) of unknown history (i.e., obtained at "a local market"). For this and other experiments involving reducing capacity, converted to a rather crude aqueous extract, a portion of which is partially exhausted of its ferrimyoglobin reducing capacity by repeated ferricyanide treatment. Some of the latter is set aside (in air at 2°C), while the remainder is completely exhausted of its reducing capacity by further ferricyanide treatment, followed by dialysis against untreated extract (5 hr at 2°C with stirring). The "pigment composition" of the oxidized-dialyzed extract is then compared spectrophotometrically with that of the partially exhausted, nondialyzed aliquot. The latter exhibited around half the "%Mb^+ reduction" of the fully exhausted–dialyzed sample. The conclusion is that this indicates "a nonenzymatic electron transfer *to* Mb^+ from the unoxidized extract to the oxidized dialyzate." In explaining the occurrence of significant Mb^+ reduction in aerobic extracts in the presence of ferricyanide, in the abstract the statement is made that "a nonenzymatic electron transfer was demonstrated in extracts under these conditions."

Even if the execution of the experiment was flawless (which it undoubtedly was not; for example, there is no indication of a pre-dialysis set of measurements, merely a measurement of percent oxidized myoglobin at the end), it could not possibly answer the question being addressed because any enzymic component would have existed on both sides of the dialysis membrane. About the only relevant things the central dialysis step could have accomplished are (a) removal of ferricyanide from the fully exhausted aliquot (elsewhere the authors admit to the use of excess ferricyanide for pigment oxidation) and (b) replenishing the treated-dialyzed aliquot with low MW, dialyzable sources of, and carriers of reducing equivalents. Surely any passage of reducing equivalents through the wall of the membrane would in all probability be via a passive diffusion mechanism; but nothing definitive can be said from this experiment about subsequent transfer of reducing equivalents to ferrimyoglobin within the dialysis bag.

This example is, unfortunately, typical of nebulous experiments and confused reasoning elsewhere in the paper. For example, for reducing activity experiments with intact meat the authors appear to have selected for naturally exhausted samples to begin with. Further, from the results of an experiment employing arsenate as an uncoupler of oxidative phosporylation in mitochondria of intact meat and slurries, the 'blanket' statement is made that "it would appear that reversal of electron transport likely does not contribute to MB^+ reduction." However, the inference could only be valid if it were known with certainty that the arsenate was influencing *energy-linked* reversal in tightly coupled structurally and functionally intact mitochondria. Besides there being no way of knowing if this were the case in the systems employed, it is virtually certain that mitochondria in the aged, pH 5.5–5.6 meat, had long since lost any *energy-linked* reversal capacity. In an uncited review (Giddings, G.G. 1977. The basis of color in muscle foods, CRC Crit. Revs. in Food Science & Nut. 9: 81), I reiterated and further developed the relevant point in the cited review that reversal-connected reduction in aged beef (i.e., pH 5.5–5.6) would surely involve submitochondrial particles and be nonenergy linked.

Finally, the authors appear to be rediscovering textbook properties of carbon monoxide (and inferring possible disproval of Warburg regarding photosensitivity in the process), and, confusing properties of cyanides that are associated with their ability to ligand to ferrimyoglobin with suggested analogous properties of CO which does not.

–*George G. Giddings,* Manager Marine Resources, Fundación Chile, P.O. Box 773, Santiago, Chile

We appreciate Dr. Gidding's comments concerning this paper; however, we feel some clarification on our part would indicate that there was no 'lack of rigor' associated with the research. This work arose from previous studies concerning the stability of color in hanging beef during transport. As a result, we were primarily concerned with what processes contributed–or might be induced to contribute–to the maintenance of the meat pigment in the reduced or pinkish-red state. Thus, the samples chosen for this study were representative of beef normally encountered in trade.

The essential finding reported in this paper is the enhancement of Mb^+ reduction in beef in the presence of atmospheres containing even small percentages of carbon monoxide. This enhancement was demonstrated to occur in the presence or absence of ferricyanide, thus precluding the objection to use of excess ferricyanide as an oxidant, although an increased rate of reduction was observed when ferricyanide was present, as noted in the paper. Adequate controls were present in each experiment involving Mb^+ reduction, including predialysis measurements in the particular experiment mentioned, as evidenced by the results being reported as "% Mb^+ reduction." Therefore, while the results of particular experiments designed to elucidate the mechanism for this enhancement phenomenon may be subject to different interpretations, we are unaware of any "textbook properties" of carbon monoxide which would have predicted such enhancement of Mb^+ reduction at such low concentrations. Furthermore, we believe that a better understanding of this enhancement effect might enable the development of feasible methods for directing the Mb^+ reducing capability of red meats toward the prolongment of their color shelf-life.

–*T.C. Lanier,* Dept. of Food Science, North Carolina State Univ., P.O. Box 5992, Raleigh 27650
–*J.A. Carpenter, R.T. Toledo* and *J.O. Reagan,* Dept. of Food Science, Univ. of Georgia, Athens, GA 30601

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A relatively simple and efficient method has been developed and is described for the preparation of lysozyme from food or biological tissues prior to quantitation. Deaminated chitin has a high specificity and capacity for the enzyme (squid chitin more so than crab chitin), along with good stability and fast flow rates. The enzyme-substrate reaction is effective at pH 7.0-8.0; all other proteins pass through the column. Elution is carried out under acid conditions. Studies have shown the method to be excellent for tissues and foods.

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(Continued from p. 5)

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NITRITE-LIPID REACTION IN AQUEOUS SYSTEM: INHIBITORY EFFECTS ON N-NITROSAMINE FORMATION

TSUTAO KURECHI and KIYOMI KIKUGAWA

ABSTRACT

The chemical interaction of lipids and lipid-containing foods with nitrite in a mild acidic aqueous system was investigated. Methyl linoleate-coated silica gel, Intralipid, cow's milk, mayonnaise, yolk and *miso* reduced a considerable amount of nitrite. Methyl linoleate-coated silica gel and cow's milk extensively prevented the formation of carcinogenic N-nitrosamines. It seemed likely that the unsaturated fatty acid residues were responsible for the interaction of lipids with nitrite. Methyl linoleate was changed into two or more unidentified products, neither of which was the hydroperoxides of the ester.

INTRODUCTION

NITRITE is used in many countries as a deliberate food additive, serving formation of the characteristic color of cured meat products and inhibition of *Clostridium botulinum* outgrowth (Duncan and Foster, 1968; Cho and Bratzler, 1970). Nitrate, which is widely distributed in vegetables such as spinach, beets, celery, lettuce and Chinese cabbage (Yanagihara et al., 1963), undergoes bacterial reduction (Ayanaba and Alexander, 1973) and salivary reduction (Ishiwata et al., 1975) to produce nitrite. Nitrite readily produces N-nitrosamines by reaction with secondary amines under mild acidic conditions (Mirvish, 1970), which have been elucidated to be potential carcinogens in laboratory animals (Druckrey et al., 1967). They may be produced in food itself during processing (Wasserman et al., 1972) or in the human stomach by acid catalyzed reaction between secondary amines and nitrite (Sander and Seif, 1969).

Certain compounds such as ascorbate (Mirvish et al., 1972), tannic acid derivatives (Gray and Dugan, 1975; Yamada et al., 1978) and sorbic acid (Tanada et al., 1978), which are endogeneous to foodstuffs or may be added to foods for preservative purposes, have been shown to inhibit the formation of N-nitrosamines. Ascorbate (Mirvish et al., 1972) and sorbic acid (Tanaka et al., 1978) have been shown to react with nitrite to reduce the available nitrite in the nitrosation.

In the course of our investigation on lipid oxidation, it was found that lipids readily reacted with nitrite in aqueous model system. Frouin et al. (1975) and Goutefongea et al. (1977) have demonstrated the interaction of nitrite with unsaturated fatty acid in cured meat products. This paper deals with the reaction of nitrite with lipids and lipid-containing foods under gastric conditions, and subsequent inhibition of the formation of carcinogenic N-nitrosamines. The lipids and lipid-containing foods dealt with here were methyl linoleate-coated silica gel, Intralipid, cow's milk, mayonnaise, yolk and *miso*, one of the flavors used in Japan.

Authors Kurechi and Kukugawa are affiliated with the Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-30, Japan.

0022-1147/79/0005-1263$02.25/0

EXPERIMENTAL

Materials

Commercially available methyl linoleate (1.0g, Tokyo Kasei Kogyo Company, Ltd.) was purified by passing through a silica gel column (10g, 100 mesh, Kanto Chemical Co., Inc.) by elution with n-hexane-benzene (2:1). Methyl linoleate-coated silica gel was prepared according to the method of Wu et al. (1977). Silica gel (20g, 100 mesh), which had been dehydrated at 105°C for 2 hr, was stirred for 1 hr in 400 ml of n-hexane containing 5.62g of the purified methyl linoleate. The precipitated silica gel was collected and dried in vacuo, which adsorbed 0.212g of methyl linoleate/g of silica gel when estimated according to Wu et al. (1977). Methyl stearate-coated silica gel (0.217 g/g) was prepared similarly by mixing 10g of silica gel and 200 ml of n-hexane containing 2.8g of methyl stearate (Tokyo Kasei Kogyo Company, Ltd.). These lipid-coated silica gels were used as soon as possible after preparation.

The fat emulsion for intravenous injection was Intralipid 10%® (product of Green Cross Corp.) and contained 10% purified soybean oil, 1.2% purified yolk lecithin, and 2.5% glycerin. The diameter of the particles of the emulsion was less than 1 μm. Several brands of sterilized cow's milk in Japan were used, constituents of which were 3.3% lipid, 2.9% protein, 4.5% lactose, 0.7% minerals and vitamins, and 88.6% water. Lactose and starch potato were obtained from Wako Pure Chemical Industries, Ltd. Skim milk (Snow Brand Milk Products Company, Ltd.) contained 1.0% lipid, 35% protein, 52.0% sugar, 8.0% minerals, and 4.0% water and vitamins. Mayonnaise (Q.P. Corp.) made from vegetable oil, yolk, acetic acid and NaCl, contained 72% lipid, 2.8% protein, 0.3% sugar, 2.4% minerals, 22% water, and no food additives other than chemical flavors. *Miso* prepared from soybean, rice, NaCl and alcohol, contained 4.3% lipid, 10% protein, 14.3% sugar, 12.4% minerals, 3.1% fibers and so on.

N-Nitrosodimethylamine (standard for gas chromatography) showing $\lambda_{max}^{CH_2Cl_2}$ 351 nm (ϵ : 110) and N-nitrosodiethylamine showing $\lambda_{max}^{CH_2Cl_2}$ 355 nm (ϵ : 102) were the products of Wako Pure Chemical Industries, Ltd.

Apparatus

Ultraviolet absorption spectrum was measured with a Shimadzu Double Beam Spectrophotometer, UV-200S. A Yanaco Gas Chromatograph Model G80 equipped with a hydrogen flame ionization detector and a glass column (3 mm i.d. × 3m) of polyethylene glycol 6000 on 80–100 mesh Chromosorb W AW was used to determine N-nitrosamines. The chromatograph was operated isothermally at 130°C (column temperature) and 150°C (injection temperature) with carrier nitrogen gas flow of 20 ml/min. The chromatograph was run with retention time of 5.5 min of the internal standard (ethyl caproate), 7.5 min for N-nitrosodimethylamine and 11.0 min for N-nitrosodiethylamine. The amount of the N-nitrosamine was determined by comparing the peak area of the samples with that of each authentic standard solution (0.40 mg/ml). A Shimadzu LC-2 Liquid Chromatograph equipped with a Shimadzu Spectrophotometric Detector SPD-1 and a stainless steel column (7.9 mm i.d. × 0.5m) of Permaphase® ODS was used for methyl linoleate analysis. The chromatograph was operated at 20°C with mobile phase (60% methanol) at 0.5 ml/min.

Determination of nitrite

Sample suspensions (methyl linoleate, silica gel, methyl linoleate-coated silica gel, Intralipid, cow's milk, starch potato, skim milk, mayonnaise, yolk, egg white and *miso*), sample solutions (glycerin, lactose and ascorbate) and the control solution, all containing 0.05M sodium citrate and 0.2–0.4 mM $NaNO_2$, were adjusted to an indicated pH with concentrated hydrochloric acid. Each mixture (100 ml) placed in a stoppered flask (150 ml-content) was incubated at 37°C. Suspensions of methyl linoleate, silica gel and methyl linoleate-coated silica gel were continuously stirred during

the incubation. After incubation, the nitrite concentration in the aqueous phase was estimated by the sulfamine-naphthylethylenediamine method. The suspensions of cow's milk, starch potato, skim milk, egg white and *miso* were filtered, and the filtrates were subjected to nitrite determination. The emulsions of Intralipid, mayonnaise and yolk were treated as follows before nitrite determination. To 10 ml of each mixture were added 1.0 ml of 5N NaOH, 2.0g of NaCl and 20 ml of ethyl acetate, and the mixtures were vigorously shaken to facilitate separation of the aqueous layer (13.0 ml) from the organic layer.

Determination of N-nitrosamines

To 0.1M sodium citrate (100 ml) or cow's milk (100 ml) containing 2.94g of sodium citrate dihydrate (0.1M), was added 345 mg of $NaNO_2$ (0.05M). The mixtures were adjusted to pH 3.0 or 4.0 with perchloric acid, and added with either dimethylamine hydrochloride (800 mg, 0.1M) or diethylamine hydrochloride (1.10g, 0.1M). They were incubated at 37°C for 5.5 hr in stoppered flasks (150 ml-content). A 10 ml-aliquot of each control sodium citrate solution was diluted to 50 ml with water and extracted with 40 ml of CH_2Cl_2 in the presence of 5 ml of 5N NaOH and 10g of NaCl. A 20 ml-aliquot of each milk suspension was diluted to 100 ml, filtered through a filter paper, and a 50 ml-portion of the filtrate was extracted with 40 ml of CH_2Cl_2 in the presence of the alkali and the salt. The CH_2Cl_2-extracts were subjected to ultraviolet and gas chromatographic analysis of the N-nitrosamines.

Recoveries estimated by gas chromatography were 93% for the control and 89% for the milk when the samples were incubated with the known concentration of the authentic N-nitrosodimethylamine at pH 3.0.

To 0.1M sodium citrate (50 ml) containing 0.05M $NaNO_2$ and 0.1M dimethylamine was added 5.0g of silica gel, methyl linoleate-coated silica gel or methyl stearate-coated silica gel. Each suspension was adjusted to pH 3.0 and stirred at 37°C for 5.5 hr in stoppered flasks (100 ml-content). Ten milliliters of the filtrate from each mixture were diluted to 50 ml and extracted with 40 ml of CH_2Cl_2 in the presence of the alkali and the salt. Recoveries of the N-nitrosamine were 92% for silica gel and 93% for methyl linoleate-coated silica gel, when the authentic N-nitrosodimethylamine was incubated under the same conditions.

Reaction of methyl linoleate with $NaNO_2$

Methyl linoleate-coated silica gel (5.0g) was suspended in 50 ml of 0.1M sodium citrate (pH 3.0) containing 0.05M $NaNO_2$. The mixture was stirred at 37°C for 5.5 hr. The suspension was settled to obtain the yellow-colored lipid layer on the surface of the buffer, which was collected for analysis by high performance liquid chromatography. A control experiment without $NaNO_2$ was performed similarly.

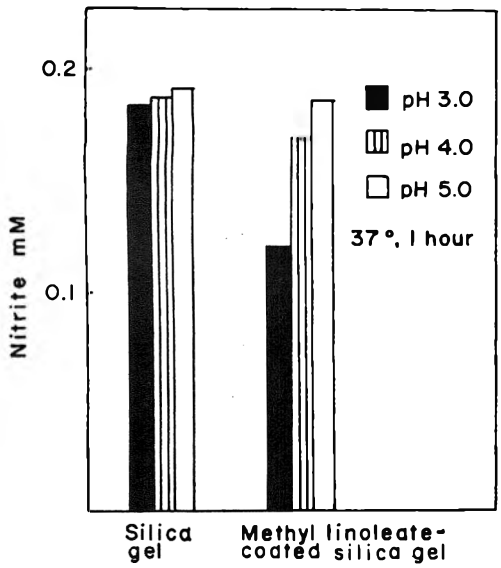

Fig. 1—Reduction of nitrite by incubation with silica gel (1.0%) and methyl linoleate-coated silica gel (1.0%).

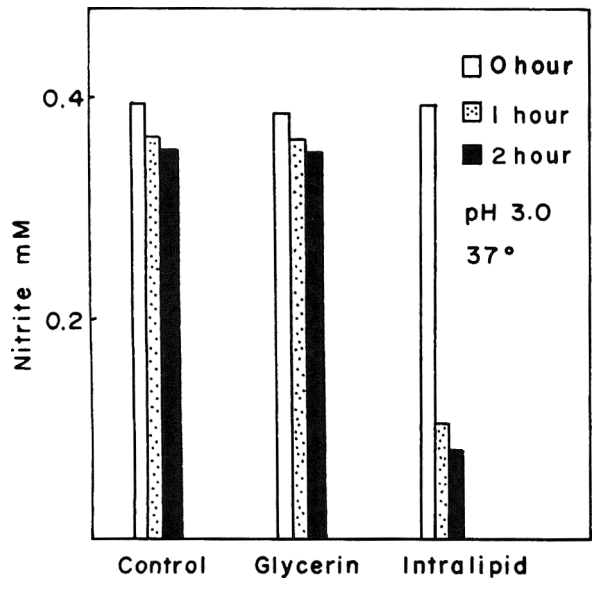

Fig. 2—Reduction of nitrite by incubation with Intralipid. Control: 0.05M sodium citrate; Glycerin: 1.0% solution in citrate; Intralipid: Intralipid 10% suspended in 2 volumes of citrate.

RESULTS & DISCUSSION

A METHYL LINOLEATE-coated silica gel suspension (1.0%) in sodium citrate solution containing 0.2 mM $NaNO_2$ was stirred at 37°C for 1 hr. At pH 3.0 the nitrite concentration in the aqueous phase decreased to 66% when compared to that of the control, silica gel alone, although it decreased little at pH 4.0 and 5.0 (Fig. 1). The nitrite in 0.2 mM $NaNO_2$-sodium citrate solution at pH 3.0 was, however, scarcely lost by incubation with methyl linoleate (1.0%) at 37°C for 1 hr. A large surface area of methyl linoleate coated on silica gel seemed to be a requisite for the interaction with nitrite in an acidic aqueous medium.

A fat emulsion used for infusion therapy, Intralipid, is a pure lipid emulsion of large surface area composed of soybean oil, yolk lecithin and glycerin. The emulsion in 0.4 mM $NaNO_2$-sodium citrate (pH 3.0) was incubated at 37°C for 2 hr. The nitrite concentration in the aqueous phase

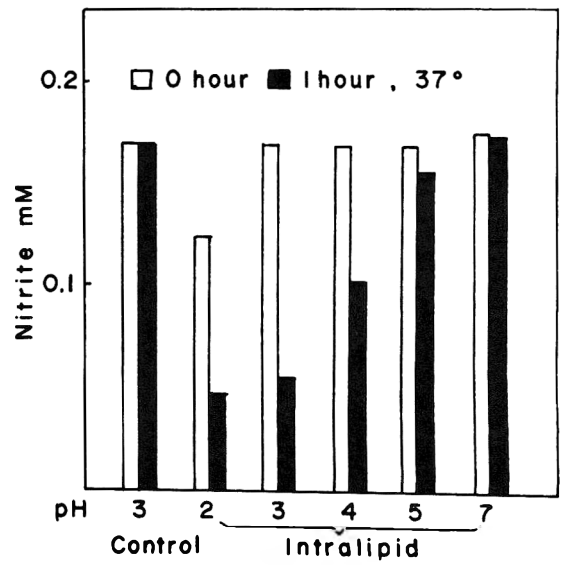

Fig. 3—Effect of pH on nitrite reduction by Intralipid. Control: 0.05M sodium citrate; Intralipid: Intralipid 10% suspended in 2 volumes of citrate.

decreased to 29% and 21% after 1 and 2 hr, respectively, when compared to those of the control solution. Nitrite in glycerin solution was little retarded (Fig. 2). Figure 3 shows the effect of pH on the nitrite reduction by Intralipid. The rate of loss was maximum at pH 2–3 (70–73%) and decreased with increasing pH. The results also revealed that the lipid emulsion having a small particle size or a large surface area was effective to reduction of nitrite in acidic media.

Interaction of nitrite with lipid-containing foods was then investigated. Table 1 shows the nitrite reduction when several foods were incubated in 0.4 mM $NaNO_2$ at pH 3.0. Cow's milk containing 3.3% of lipid reduced the nitrite concentration 95% after 1 hr incubation. Nitrite was little retarded by incubation with lactose, one of the major constituents of the milk, or starch potato. Skim milk suspension containing a large amount of proteins and sugars and a lesser amount of lipids lost less nitrite than cow's milk. Mayonnaise suspension containing 2.2% lipid reduced the nitrite concentration 85%. Egg white, consisting mainly of protein, reduced little nitrite (17%), whereas yolk, consisting mainly of lecithin, reduced much nitrite (57%). *Miso* suspension, containing 0.2% lipids, also reduced nitrite considerably (68%).

The results shown in Table 1 indicate that the extent of reduction of nitrite by foods was dependent largely upon their lipid content and little on the protein content. Among the lipid-containing foods tested, cow's milk was the most potential. Identical data were obtained with different brands of milk sold in Japan. The effects of the milk were comparable to those of 2 mM ascorbate, which reduced nitrite 94% after 1-hr incubation at pH 3.0. The effect of pH on nitrite reduction by cow's milk (Fig. 4) was similar to those by methyl linoleate-coated silica gel (Fig. 1) and Intralipid (Fig. 3).

Formation of N-nitrosamines by reaction between nitrite and secondary amines under mild acidic conditions was prevented by cow's milk, when mixtures of 0.1M dimethyl (or diethyl) amine and 0.05M $NaNO_2$ in the milk were incubated at 37°C for 5.5 hr (Table 2). Formation of N-nitrosodimethylamine was inhibited 66% (estimated by UV) or 68% (by GC) at pH 3.0, and 46% (by UV) or 46% (by GC) at pH 4.0. Formation of N-nitrosodiethylamine was inhibited 49% (by UV) or 52% (by GC) at pH 3.0, and 12% (by UV) or 22% (by GC) at pH 4.0. Methyl linoleate-coated silica gel also inhibited the formation of N-nitrosodimethylamine (Table 3). Silica gel alone and methyl stearate-coated silica gel, however, only slightly inhibited the formation of N-nitrosamine. Therefore, the unsaturated fatty acids seemed responsible for the inhibition of N-nitrosamine formation.

A methyl linoleate-coated silica gel suspension was treated with $NaNO_2$ at 37°C for 5.5 hr. The yellow-colored

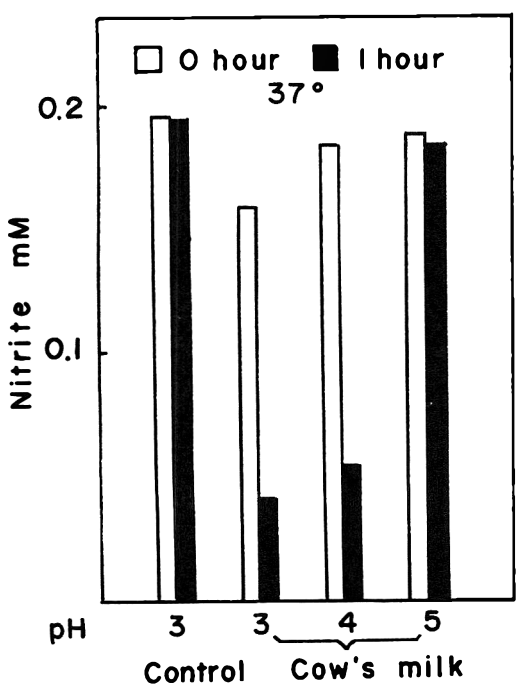

Fig. 4—Effect of pH on nitrite reduction by cow's milk. Control: 0.05M sodium citrate; Cow's milk: cow's milk containing 1.47g of sodium citrate dihydrate/100 ml.

Table 1—Reduction of nitrite by lipid-containing foods

Solution or suspenion[a]		Lipid (%)	Protein (%)	%Reduction of nitrite
Control		0	0	8
Cow's milk	100%*	3.3	2.9	95
Lactose	3%	0	0	9
Starch potato	3%*	0	0	19
Skim milk	10%*	0.1	3.5	63
Mayonnaise	3%*	2.2	0.1	85
Yolk	3%*	(rich)	(poor)	57
Egg white	3%*	(poor)	(rich)	17
Miso	5%*	0.2	0.5	68

[a] Each suspension (*) or solution in 0.4 mM $NaNO_2$—0.05M citrate (pH 3.0) was incubated at 37°C for 1 hr.

Table 2—Effects of cow's milk on the N-nitrosamine formation

			N-Nitrosodimethylamine[b]		N-Nitrosodiethylamine[b]	
pH		Detm[a] by	mg/ml	%Inhibition	mg/ml	%Inhibition
3.0	Control	UV	0.780	0	0.275	0
		GC	0.748	0	0.276	0
	Milk	UV	0.269	65.5	0.140	49.0
		GC	0.239	68.0	0.134	51.5
4.0	Control	UV	0.345	0	0.125	0
		GC	0.315	0	0.116	0
	Milk	UV	0.188	45.5	0.110	12.0
		GC	0.170	46.0	0.090	22.0

[a] UV = ultraviolet absorption spectrophotometry; GC = gas chromatography.

[b] The mixtures of 0.1M dimethylamine (or diethylamine) and 0.05M $NaNO_2$ in the citrate were incubated at 37°C for 5.5 hr in the presence and absence of the milk.

Table 3—Effects of methyl linoleate-coated silica gel on the formation of N-nitrosodimethylamine

	N-Nitrosodimethylamine[a]	
	mg/ml	%Inhibition
Control	1.07	0
Silica gel (10%)	0.95	11.0
Methyl linoleate-coated silica gel (10%)	0.61	43.0
Methyl stearate-coated silica gel (10%)	0.93	13.0

[a] The mixtures of 0.1M dimethylamine and 0.05M $NaNO_2$ were incubated at pH 3.0 and 37°C for 5.5 hr in the presence and absence of lipid-coated silica gel. N-Nitrosodimethylamine was determined by GC.

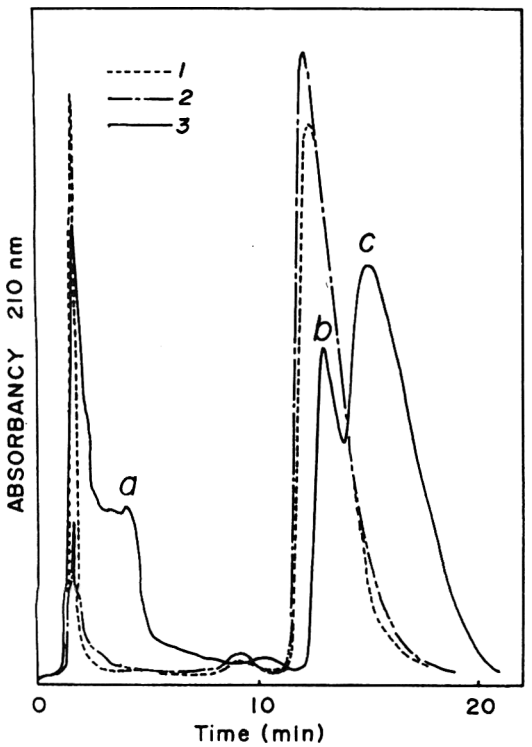

Fig. 5—High performance liquid chromatogram of methyl linoleate. (1) Authentic methyl linoleate; (2) Methyl linoleate recovered from the treatment of the coated silica gel at pH 3.0 and 37° C for 5.5 hr; (3) Methyl linoleate recovered from the treatment of the coated silica gel with $NaNO_2$ at pH 3.0 and 37° C for 5.5 hr.

oil separated from the silica gel was collected and subjected to high performance liquid chromatography. The chromatogram revealed a characteristic profile [Fig. 5 (3)], while the chromatogram of colorless oil recovered from the incubation without $NaNO_2$ showed an almost identical profile [Fig. 5 (2)] to that of the authentic methyl linoleate [Fig. 5 (1)]. Two new peaks (a and c) indicated the formation of two or more products, and both the peaks, showing end absorption in the ultraviolet absorption region, did not correspond to the hydroperoxide form of the ester which might show characteristic absorption maximum at 234 nm. Trials to isolate these reaction products were not performed.

Interest has been growing concerning the formation of carcinogenic N-nitrosamines by reaction between secondary amines and nitrite. Several compounds related to foodstuffs and food additives have been investigated as inhibitors of N-nitrosamine formation. Ascorbate reacts with nitrite to form dehydroascorbic acid and nitric oxide (Dahn et al., 1960) and reduces available nitrite in the nitrosation reaction (Mirvish et al., 1972). Sorbic acid also reacts with nitrite and prevents the nitrosation (Tanaka et al., 1978). Tannic acid derivatives are also shown to influence the nitrosation (Gray and Dugan, 1975; Yamada et al., 1978).

This time, it was found that lipid and lipid-containing foods readily reacted with nitrite at gastric pH in aqueous system to reduce the available nitrite and subsequently inhibited the formation of the carcinogenic N-nitrosamines. Comparison of the effect of methyl linoleate suspension with that of the monolayer of methyl linoleate coated on silica gel suggested that a large surface area of lipids seemed to be a requisite for effective interaction with nitrite. Unsaturated fatty acid residue, such as linoleic acid, would be responsible for the interaction, since saturated fatty acid residue such as stearic acid interacted little with nitrite.

Intralipid, cow's milk, mayonnaise, yolk and *miso* reduced a considerable amount of nitrite, among which cow's milk was the most efficient and effectively inhibited the formation of N-nitrosamines. The results were consistent with the previous observation described by Fan and Tannenbaum (1973) who demonstrated that reconstituted dehydrated milk retarded the nitrosation of morpholine. They did not, however, explain why nitrosation was retarded in a milk system. The reaction of nitrite with protein has been well-established in the modifications of the enzymes (Kurosky and Hofmann, 1972), in gastric model systems (Knowles et al., 1974) and in cured meat model system (Woolford et al., 1976). The results shown in Table 1 indicated that the reduction of nitrite by foods seemed dependent upon the lipid content rather than protein content, although the loss of nitrite by protein or by nitric oxide gas evolution described by Walters and Taylor (1964) could not be neglected.

Thomas et al. (1968) and Felmeister et al. (1968) demonstrated the interaction of nitrogen dioxide gas with rat lung lipids or unsaturated lecithin monolayer films to induce lipid peroxidation. Frouin et al. (1975) and Goutefongea et al. (1977) have reported that ^{15}N from $Na^{15}NO_2$ binds to unsaturated fatty acids in adipose tissue when the conditions were similar to those for meat curing. Our results relating to the nitrite-lipid interaction under gastric conditions confirmed these observations, and suggested the role of lipid in the fate of nitrite in the digestion process.

Although the previous observation by Mottram et al. (1977) demonstrated that lipids provide an environment conductive to nitrosamine formation in fried bacon, our lipid-containing system was inhibitory against nitrosamine formation. Discrepancies might be explained by the difference of the temperature of the treatments.

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EFFECTS OF SODIUM NITRITE ON *Clostridium botulinum* TOXIN PRODUCTION IN FRANKFURTER EMULSIONS FORMULATED WITH MEAT AND SOY PROTEINS

J. N. SOFOS, F. F. BUSTA and C. E. ALLEN

ABSTRACT

Botulinal toxin production, residual nitrite depletion, and total microbial growth were measured in meat and/or soy-containing frankfurter formulations during 27°C temperature abuse. Botulinal toxin production in formulations containing soy proteins was equal to or less rapid than in all-meat samples, both in the absence and presence of nitrite. Of the nine soy proteins examined, nitrite did not delay *C. botulinum* toxin production in products made from the isolate form, whereas it was an effective control in textured, flours and concentrates. In all-meat formulations the effectiveness of nitrite decreased with increasing spore load. Residual nitrite depletion in meat was more rapid than in soy-containing treatments. Among the soy proteins, nitrite depletion was more rapid in the isolate than in the textured form tested. Total microbial growth was similar in both meat and soy-containing samples. Gas production was an inadequate index of toxin development.

INTRODUCTION

PREVIOUS STUDIES in this laboratory have shown that residual nitrite levels in frankfurter formulations containing soy proteins were higher than in all-meat treatments 5 days after processing (Sofos and Allen, 1977; Sofos et al., 1977). That observation suggested a different reaction of nitrite in meat and soy proteins. Since nitrite is known to affect *Clostridium botulinum* toxin production in cured meat products, it seemed appropriate to study the botulinal safety of such products formulated with increased levels of different soy proteins.

A variety of forms of soy protein products exist in the market. Such products may differ in the type of their manufacture and processing, in composition, and in functionality related to the state of their proteins. All these products are recommended by their manufacturers as suitable for human consumption and are already being incorporated into food products of one type or another. It was pertinent to study a few of these products in order to determine their effect on *C. botulinum* toxin production both in the presence and absence of nitrite. The major objectives were to determine the effects of sodium nitrite on *C. botulinum* toxin production in frankfurter emulsions formulated with meat and/or different soy proteins, to measure the rate of nitrite depletion in such products during temperature abuse and to determine the relationship between botulinal spore concentration and nitrite effectiveness in these products.

MATERIALS & METHODS

THE STUDY consisted of a total of nine trials. Two replicate trials (Fig. 1) were formulated to determine the effects of nitrite (156 μg/g) on *C. botulinum* toxin production in all-meat, meat-soy, and all-soy frankfurter-type mixtures. Two more trials (Fig. 2) were conducted to study botulinal toxin production in mixtures formulated with nine different soy protein forms. In one of these trials nitrite was omitted while in the second, 156 μg of nitrite per gram of product were added. The influence of spore inoculum size and its relationship to nitrite botulinal inhibition were examined in a fifth all-meat trial (Fig. 3).

Residual nitrite depletion and total microbial growth for the all-meat, meat-soy, and all-soy treatments detailed in Figure 1 were measured in a sixth uninoculated trial (Fig. 4 and 6). Residual nitrite depletion for each one of the two soy forms used in the soy-containing treatments detailed in Figures 1, 4 and 6 was measured in a seventh trial (Fig. 5). Finally, two uninoculated, no added nitrite trials were used to determine possible raw ingredient and processing botulinal contamination. Heat-shocked (80°C, 15 min) anaerobic five-tube most probable number (MPN) determinations of all inoculated treatments (except Fig. 3) after cooking indicated the presence of an average of 975 spores per gram of product.

Table 1—Proximate composition and protein dispersibility index (PDI) of soy products

Soy product type	Product code	Moisture (%)	Fat (%)	Protein (%)	PDI
Textured	A	8.5	0.38	51.2	17
	B	7.1	1.00	51.4	20
Flours	C	5.9	0.60	51.5	85
	D	7.0	1.60	51.8	28
Concentrates	E	4.1	0.20	67.8	49
	F	4.6	0.50	67.9	2
	G	5.1	0.60	68.1	7
Isolates	H	6.8	0.26	86.5	88
	I	7.0	0.47	86.7	91

Lean meat

Beef from the chuck was trimmed, ground twice, and frozen until used. Three different lots of ground beef were used to formulate the treatments containing meat. The treatments of replicate Y (Fig. 1) were formulated with the first lot of ground beef containing 59.4% moisture; 21.0% fat; and 17.9% protein as determined by standard AOAC procedures. The meat-containing treatments of trial Z (Fig. 1), those presented in Figure 3 and the uninoculated, no added nitrite meat treatments (data not shown) were based on ground beef from a second lot. The proximate composition of this lot of ground beef was 70.7% moisture; 6.8% fat; and 20.7% protein. Finally, a third lot of ground beef containing 61.8% moisture; 20.4% fat; and 17.8% protein was used to formulate the uninoculated treatments found in Figures 4 and 6.

Pork backfat

Ground pork backfat (7.5% moisture; 90.0% fat; and 2.0% protein) was used to adjust the total fat content of all treatments in all trials to the maximum level allowed in frankfurter-type products (30%).

Soy protein products

Nine unfortified soy protein products of different type and from different manufacturers were used in the studies. The data in Table 1 present the type, proximate composition and protein dispersibility index (PDI) of the soy forms used. Of the nine soy products tested, two were of the textured form; two were flours; three were concentrates; and two were isolates. A mixture of the first textured soy protein (PDI 17, A, Table 1) and the first isolate (PDI 88, H, Table 1) was used in the appropriate treatments presented in Figures 1, 4 and 6. The same soy products were tested separately in the treatments presented in Figure 5.

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Product formulation

Batches of 1,000–1,500g were formulated depending on the trial. Common ingredients and their concentrations in all treatments were: salt, 2.5%; sugar (sucrose), 1.0%; white pepper, 0.25%; nutmeg, 0.07%; ascorbic acid (Mallinckrodt, Inc., St. Louis, MO), 0.04%; and liquid smoke (Charoil, Red Arrow Products Co., Manitowoc, WI), 0.5%. Twenty percent water was added to each treatment in order to introduce the spore inoculum and ingredients such as nitrite, and to account for losses during cooking. All treatments were formulated to contain similar moisture (53–54%) contents.

The meat treatments (Fig. 1, 3, 4 and 6) contained 45.6% lean and no soy protein. In the meat-soy formulations (Fig. 1, 4 and 6) half of the above was lean and the other half a mixture of 17.8% hydrated (1:2, wt./wt., dry soy/water) textured soy protein (PDI 17) and 5.0% hydrated (1:4) soy protein isolate (PDI 88). Hydrated soy protein replaced lean meat on wt./wt. basis. The all-soy mixtures (Fig. 1, 2, 4, 5, and 6) contained 3.0% lean contributed from the pork backfat; 35.6% hydrated (1:2) textured soy protein (PDI 17) and 5.0% hydrated (1:4) soy protein isolate (PDI 88). The hydration ratio of the nine soy protein products (Fig. 2 and 5) when tested individually ranged from 2.27–2.43 depending on their fat and moisture contents (Table 1). Based on the total weight of each batch (treatment), sodium nitrite (Mallinckrodt Inc., St. Louis, MO) was added when appropriate at a level of 156 μg per g of product.

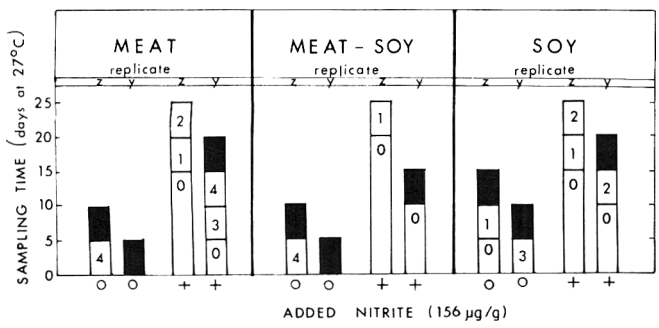

Fig. 1–C. botulinum toxin production in all-meat, meat-soy, and all-soy frankfurter-type mixtures formulated with and without nitrite (156 μg/g) during temperature abuse. Numbers indicate toxic samples out of five tested and dark bars demonstrate that all five samples were toxic.

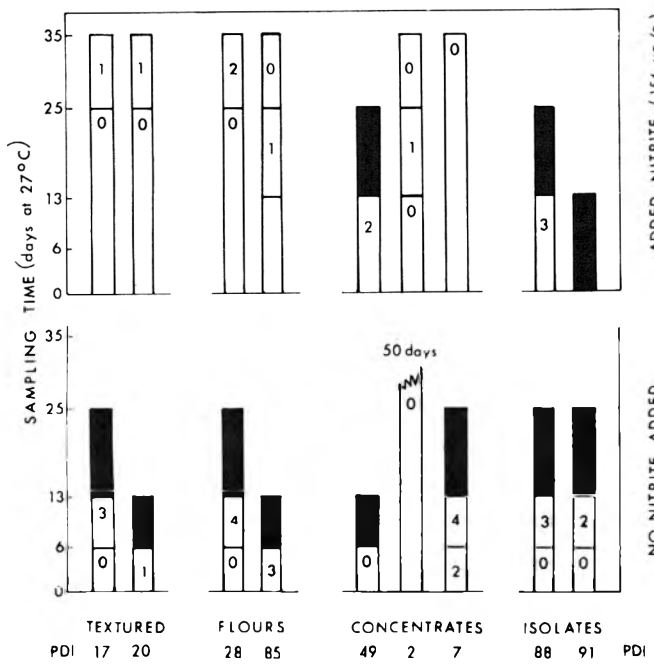

Fig. 2–C. botulinum toxin production in frankfurter-type mixtures from different forms of soy proteins formulated with and without nitrite (156 μg/g) during temperature abuse. Numbers indicate toxic samples out of five tested and dark bars demonstrate that all five samples tested were toxic. PDI, protein dispersibility index.

Product formation, inoculation and analyses

The meat and/or soy formulations were inoculated with a composite of five type A and five type B strains of *C. botulinum* spores, emulsified, processed, and incubated at an abuse storage of 27°C for various times prior to sampling for toxicity. Except for the replacement of mechanically deboned chicken meat with soy and/or meat ingredients, methods for product formation, inoculation, incubation, spoilage assessment, sampling, residual nitrite and toxicity testing have been detailed by Sofos et al. (1979a). Microbiological procedures were described by Sofos et al. (1979b,c).

RESULTS & DISCUSSION

Nitrite effects in meat and/or soy mixtures

The data presented in Figure 1 indicate that *C. botulinum* toxin production was delayed by the inclusion of nitrite (156 μg/g) in the formulation. This delay in toxin production by nitrite was recorded in all treatments and replicates tested, including all-meat, meat-soy and all-soy (plus pork backfat) mixtures. In the absence of nitrite, all-meat and meat-soy samples were toxic in 5 days of 27°C incubation. All-soy samples were toxic after five or ten days of temperature abuse, depending on the replicate (Fig. 1). When nitrite was present, toxin was produced in the all-meat treatments between 5 and 10 or between 15 and 20 days of incubation; in the meat-soy formulations between 10 and 15 or between 20 and 25 days; and in the all-soy mixtures between 10 and 15 or between 15 and 20 days.

The effectiveness of appropriate nitrite concentrations in delaying botulinal toxin production is well established and has been reported for different cured meat products including comminuted pork, bacon and wieners (Christiansen et al., 1973; 1974; Hustad et al., 1973). The results included in Figure 1 confirm the effectiveness of nitrite to control *C. botulinum* and also indicate that the nitrite concentration tested (156 μg/g) is capable of delaying *C. botulinum* toxin production during temperature abuse of emulsified meat products. The results also extend these generalizations to emulsified products containing soy proteins. Botulinal toxin production was somewhat slower in soy-containing treatments (Fig. 1). Obviously, replacement of part or, if practical, all the lean meat by soy proteins would give products at least as safe as all-meat formulations, both in the presence or absence of nitrite. Figure 1 also shows reproducibility of the effects observed in these types of treatments. Samples of all treatments of replicate Y became toxic sooner than those of replicate Z, which was conducted at a different time and formulated with a different lot of ground beef. However, the nitrite (156 μg/g) effectiveness for delaying toxin production was observed in both instances. Variations in the rate of botulinal toxin production and in the effect of nitrite are common among laboratories, lots of meat, types of meat, and even in the same laboratory and the same product formulated at the same or different time (Tompkin et al., 1978b; 1977; Rhodes and Jarvis, 1976).

Nitrite effects in different soy proteins

Samples of one of the concentrates (PDI 2) were not toxic for up to 50 days of incubation (Fig. 2). The reason for this presumably was the low pH (4.64) of the product formulated with this (PDI 2) soy concentrate. The pH values of products formulated with the textured, flours, and remaining concentrates were in the range 5.76–5.97 while mixtures containing the soy isolates had higher pH values (6.38 and 6.55).

Generally, the results shown in the lower portion of Figure 2 indicate that in formulations without nitrite, botulinal toxin was produced in all soy forms studied (except the low pH concentrate mentioned above) after 13 days of incubation. However, at the first time of sampling (6 days), toxin was detected in only one of the textured, one of the flours, and only one of the concentrates tested. Generally, no major differences were observed among soy proteins tested in the absence of nitrite. The low pH concentrate was an exception and its practical significance as a component in frankfurter-type products is questionable due to potential emulsion stability problems associated with low pH. The results in the lower portion of Figure 2, when compared to those for the all-meat-no nitrite formulations of Figure 1, indicate that in the absence of nitrite the soy forms individually tested may be less supportive of *C. botulinum* toxin production than all-meat formulations. The same conclusion was reached when a combination of two soy forms was tested (Fig. 1).

Addition of nitrite (156 μg/g) appeared to delay botulinal toxin production in all textured, flours, and concentrates tested, but did not in the isolates (Fig. 2, upper portion). However, this nitrite effectiveness was not evident in one of the concentrates. A skip in toxin production was recorded for one of the flours and one of the concentrates tested. One toxic sample was detected in each one of those samples after 25 days but none after 35 days. The concentrate sample showing the skip was the low pH soy concentrate. In this trial the pH (5.20) of the concentrate with nitrite was higher than that of the no-nitrite formulation (4.64). The increase in pH from 4.64 to 5.20 could be due to ingredient differences, such as pork backfat, used to formulate the two treatments.

Both textured products and the remaining flour were free of toxin at 25 days and showed 1 or 2 toxic samples after 35 days of temperature abuse. The third concentrate was found nontoxic at the last sampling time (35 days). Since no sampling was performed at 6 days, it is difficult to conclude that toxin production in the isolates was faster in the presence of nitrite. However, it can be concluded from the data in the lower and upper portions of Figure 2, that nitrite (156 μg/g) did not have any effect on botulinal toxin production in products formulated with soy isolates. As one of the controls used here, all uninoculated, no added nitrite samples were found toxin-free after 25 days at 27°C (data not shown), indicating botulism-free raw materials and good sanitary practices in the laboratory.

Spore concentration effects

Data shown in Figure 3 indicate that in the absence of nitrite a spore load difference of about one order of magnitude (130 vs 1090 spores per gram) made very little, if any, difference in the rate of *C. botulinum* toxin production.

In product formulated with nitrite (156 μg/g), there was a direct relationship between development of detectable toxin and number of spores in the formulation. With 140 or 580 spores per gram of cooked product, toxic samples were not detected after 25 days of temperature abuse. On the other hand when the spore load was increased to 940/g, a toxic sample was found after 20 days of 27°C incubation. Differences in spore concentration such as the above or of higher magnitude could be responsible for some of the differences in nitrite responses found in the literature. The fact that increases in the spore inoculum level can override the inhibitory effect of nitrite and allow toxin production is often found in the literature (Pivnick et al., 1969; 1970; Christiansen et al., 1973; 1974; Hustad et al., 1973; Bowen et al., 1974). As stated by Ingram (1974), systems found safe with 1–10 spores/g failed when challenged with inoculum sizes 100–1000 times greater. A relationship between spore inoculum level and nitrite concentration necessary to

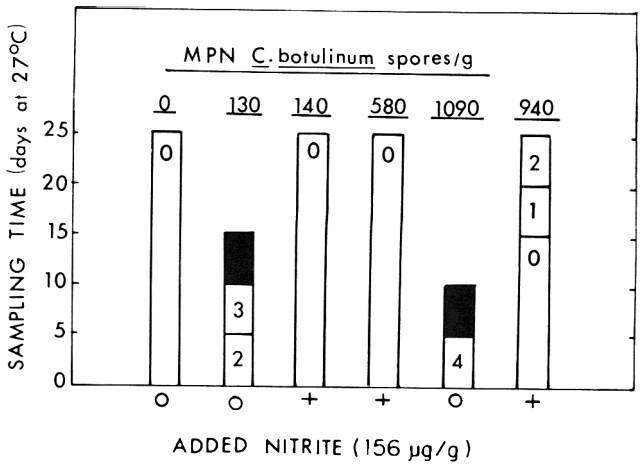

Fig. 3—Relationship of C. botulinum spore concentration and nitrite to toxin production in all-meat frankfurter-type mixtures during temperature abuse. Numbers indicate toxic samples out of five tested and dark bars demonstrate that all five samples were toxic.

inhibit toxin production was also demonstrated by Christiansen et al. (1973). The results reported here agree and confirm the above findings and demonstrate that nitrite effectiveness may depend on the spore load present.

Before any inoculation studies are conducted, the above and other relevant factors must be considered. In order for any such studies to be of practical significance, the average natural botulinal spore contamination of meat should be taken under consideration. The average natural botulinal contamination of meat was estimated to be about 1 spore per 1–7 lb of meat (Lechowich et al., 1978). Considering the above, we used an inoculum level in the range 200–700

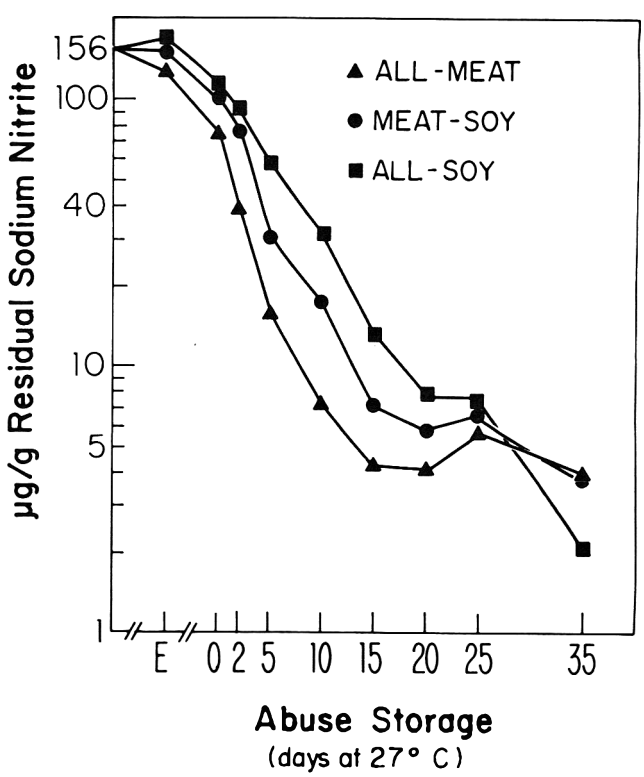

Fig. 4—Sodium nitrite depletion in all-meat, meat-soy, and all-soy frankfurter-type mixtures during processing and temperature abuse. ▲, all-meat; ●, meat-soy; ■, all-soy; E, uncooked emulsion.

Table 2—Gas and botulinal toxin production relationship in meat and/or soy-containing frankfurter emulsions formulated with 156 μg/g nitrite

Sampling time (Days at 27° C)	Treatments					
	Meat		Meat-soy[d]		Soy[e]	
	Y[a]	Z	Y	Z	Y	Z
5	0/0[b]	0/0	0/0	0/0	5/0	0/0
10	2/3	2/0	5/0	0/0	5/0	0/0
15	4/4	4/0	5/5	3/0	5/2	3/0
20	5/5	4/1	–	5/0	5/5	5/1
25	–[c]	5/2	–	5/1	–	5/2

[a] Replicates
[b] Numerator represents gassy and denominator toxic samples out of five tested at each particular sampling time
[c] Incubation was discontinued after all five samples were toxic.
[d] Lean (beef, pork backfat), 22.8%; fat (beef, pork backfat), 30.0%; textured soy protein (A, PDI 17, hydrated 1:2, wt/wt.), 17.8%; soy protein isolate (H, PDI, 88, hydrated 1:4, wt/wt.), 5.0%.
[e] Lean (pork backfat, 3.0%; fat (pork backfat), 30.0%; textured soy protein (A, PDI 17, hydrated 1:2, wt./wt.), 35.6%; soy protein isolate (H, PDI 88, hydrated 1:4, wt/wt.), 5.0%.

spores per gram of product in subsequent studies (Sofos et al., 1979; Sofos et al., 1979b,c,d).

Residual nitrite

Figure 4 shows residual nitrite depletion during processing and storage in all meat, meat-soy and all-soy treatments formulated with 156 μg/g nitrite. The results confirm previous findings (Sofos and Allen, 1977; Sofos et al., 1977) and further demonstrate that nitrite depletion during processing and storage was slower as the soy content of the formulation increased or as the meat content decreased. However, after 25 days of temperature abuse all the treatments reached similar levels of residual nitrite. The reasons for differences in nitrite depletion rates are not known but the following speculation can be made. General compositional differences as well as differences in the type, form, and state of proteins present in meat and soy products could be responsible for the different rates of nitrite depletion recorded. Also the possibility of different rates of pH decrease during 27°C storage between meat and soy-containing treatments due to growth of different microorganisms might have influenced the rates of nitrite depletion.

The soy-containing treatments presented in Figures 1 and 4 consisted of a mixture of a textured soy and a soy isolate. Each was tested separately for its effect on residual nitrite depletion (Fig. 5). The results show that nitrite was depleted faster in the mixture formulated with the isolate than with the textured soy. This would indicate that the slower rate of nitrite depletion observed in the soy-containing treatments of Figure 4 was due mostly to the presence of textured soy protein.

Textured soy proteins in general are highly heat treated during manufacture. Increased heat treatment denatures proteins and changes their state. In contrast, soy isolates are generally chemically processed and their proteins are less damaged. Differences among textured and isolates in the state of the proteins and possibly other components such as minerals may be affecting the reactions of nitrite and thus influence the rate of nitrite depletion.

Based on the theory developed by Tompkin et al. (1978a,b,c) that residual nitrite, or some compound associated with it, is active in botulinal control, some speculations can be made to explain the observed toxicity results. The decreased rate of toxin production associated with the texture-based formulations (Fig. 2) and the soy-containing formulations in general (Fig. 1) could be due to the slower rate of nitrite depletion in those treatments. In addition, the ineffectiveness of nitrite to delay toxin production in

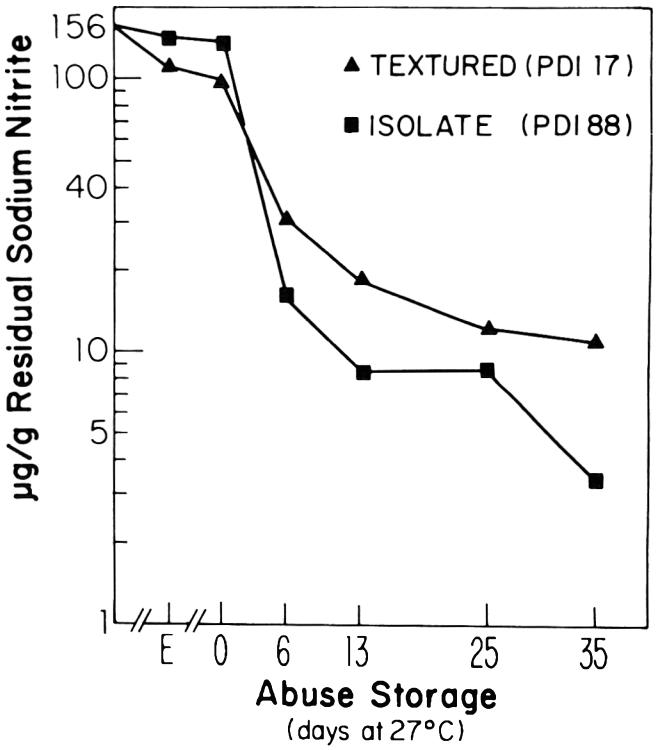

Fig. 5—Sodium nitrite depletion in a textured and an isolate soy protein frankfurter-type mixture during processing and temperature abuse. ▲, textured; ■, isolate, PDI, protein dispersibility index; E, uncooked emulsion.

isolate-based formulations (Fig. 2) might be the result of a rapid rate of nitrite depletion in such treatments (Fig. 5) or due to a possible increased iron concentration in isolates. Also at the higher pH levels (6.38–6.55) of such formulations, nitrite might have lost its effectiveness.

Total microbial growth and gas production

The effect of soy protein on the growth of mesophilic microorganisms that can grow anaerobically is shown in Figure 6. The results indicate no major differences in the rate and extent of total growth among all-meat, meat-soy and all-soy treatments formulated with 156 μg/g nitrite. The data of Table 2 indicate that gas formation is not al-

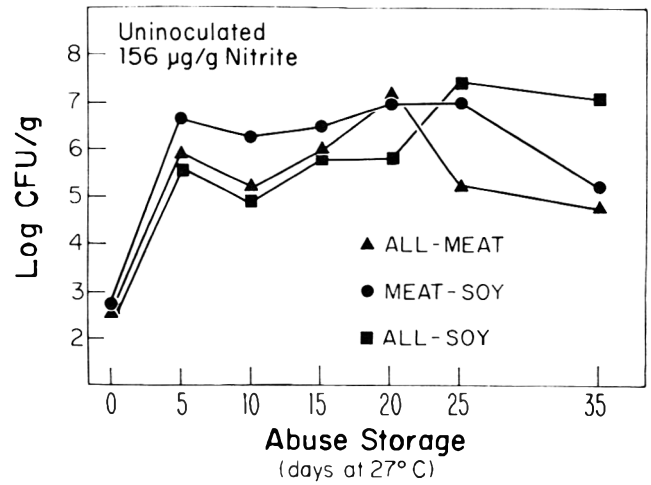

Fig. 6—Total aerobic plate counts (CFU/g) in all-meat, meat-soy and all-soy frankfurter-type mixtures during temperature abuse. ▲, all-meat; ●, meat-soy; ■, all-soy.

ways a good index of toxin production. In meat formulations, toxin was found in the absence of gas, and in all formulations not all gassy samples were toxic. Regular sampling times and toxin analyses should be the sole determinants for sample selection.

SUMMARY

ADDITION of sodium nitrite (156 μg/g) delayed botulinal toxin production in beef-pork backfat frankfurter products as well as in similar mixtures formulated with textured soy proteins, soy flours, and soy concentrates. In formulations consisting of soy isolates and pork backfat, nitrite was found ineffective.

In beef-soy and in all-soy mixtures, botulinal toxin production was equal or less rapid than in all-beef products in the absence or presence of nitrite (156 μg/g).

The ineffectiveness of nitrite in controling *C. botulinum* toxin production in all-soy isolate mixtures is not of concern since such (all-isolate) formulations would be impractical. However, this does demonstrate that nitrite does not effectively control *C. botulinum* toxin production in all protein systems.

The effectiveness of a given nitrite concentration to delay *C. botulinum* toxin production may disappear if the spore load in the formulation is increased extensively.

Residual nitrite is lost at a slower rate in soy-containing formulations as compared to all-meat products. Such slower nitrite depletion appears to be due mostly to textured soy protein rather than soy protein isolate.

Total microbial growth was not affected by the presence of soy proteins in the formulation. Gas production is not an adequate index of toxin, since gas production preceded toxin in soy and/or meat-containing samples and toxin was also detected in the absence of detectable gas in all-meat samples.

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MICROSTRUCTURE OF ISOLATED SOY PROTEIN IN COMBINATION HAM

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ABSTRACT

Combination hams were prepared by injecting excised porcine muscles with a brine containing solubilized isolated soy protein. The microstructure of the muscle in the combination ham was compared to that of a water added ham to determine the location of the isolated soy protein. The effect of massaging on the location of the isolated soy protein was also studied. The results showed that the isolated soy protein occupied primarily perimysial spaces and the massaging acts to incorporate these proteins into the endomysial spaces and mix them with extracted myofibrillar proteins. The isolated soy protein appeared to enhance myofibrillar protein extraction by binding water, thus increasing the effective concentration of salt and phosphate. Scanning electron microscopy showed that the gel formed by the isolated soy protein possessed a denser structure than the gel formed by a crude myosin preparation. The gel formed by a mixture of myosin and isolated soy protein possessed a structure more comparable to the structure of the crude myosin gel.

INTRODUCTION

SECTIONING MUSCLE into smaller pieces and then forming these pieces into a continuous body of meat has become a very important process in the meat industry. Sectioned and formed hams are produced in this manner and comprise 18.7% of all the ham sold in the United States (Anonymous, 1977). Theno et al. (1977) have reviewed the actions of massaging and tumbling as the most common processes utilized to produce this product. When used in conjunction with salt and phosphate these mechanical treatments act to extract the salt-soluble muscle proteins, the binding agents, to surfaces of the muscle chunks. Studies on the binding of meat pieces in poultry rolls (Schnell et al., 1970; Vadehra and Baker, 1970; Maesso et al., 1970; Acton, 1972a, b; Meydav et al., 1978) and pork or beef rolls (Rahelic et al., 1974; Pepper and Schmidt, 1975; Moore et al., 1976; Rejt et al., 1978; Siegel et al., 1978a, b) have shown that those physical and chemical treatments which enhance extraction of the myofibrillar proteins, and/or the water-binding capacity of the muscle, also enhance the meat-to-meat binding in roll type products.

Changes in the microstructure of muscle due to various chemical and physical treatments have proved to be very useful in determining the mechanism of action behind these treatments. Studies on the microstructure of sectioned and formed ham have been conducted by Theno et al., (1978a, b, c). By examining the exudate with light microscopy (Theno et al., 1978b) and the ultrastructural changes in the muscle with scanning electron microscopy (Theno et al., 1978c), massaging was shown to increase myofibrillar protein extraction by breaking muscle fibers loose from muscle surfaces as well as in the interiors of muscles.

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The use of isolated soy protein in sectioned and formed meats offers improvements in binding qualities and yields of these products while at the same time reducing costs. The method for introducing this protein into cured meats involves the solubilization of the isolated soy protein in the curing brines injected into the meat during the production of these items. Massaging is then commonly employed to uniformly distribute this brine within the musculature. Recent research in this laboratory on ham rolls prepared in this manner has examined the effects of isolated soy protein and the level of injection on binding strengths, cooking yields, and taste panel scores (Siegel et al., 1979). The results showed that isolated soy protein functions to improve both binding strengths and cooking yields due to the higher levels of water. The level of injection was also found to have an effect on panel scores for flavor, uniformity, textural appeal and overall acceptability. Improved panel scores for juiciness and tenderness were found when increased levels of injection were used. Other research has shown improved panel scores for flavor, tenderness, texture and acceptability in turkey rolls containing this isolated soy protein (Kardouche et al., 1978). Microscopic examination of the isolated soy protein within the musculature would provide a basis for determining the mechanism of its functional contributions.

The objectives of this study were to determine the location of isolated soy protein after being solubilized and injected into ham muscles, and to determine the mechanism of the functional contributions offered by isolated soy protein in this combination ham. The objectives were accomplished by examining the ultrastructure of the gelled isolated soy protein in the meat system as well as in a model system using light microscopy and scanning electron microscopy. Since myosin is the most functional of the muscle proteins in meat systems, the ultrastructure of the gelled isolated soy protein was then compared with the ultrastructure of a crude myosin gel. This comparison provides the basis for understanding the mechanism behind the functionality of solubilized isolated soy protein in meat systems.

MATERIALS & METHODS

Ham preparation

The hams were prepared according to Hawley and Tuley (1976, 1977) as described by Siegel et al. (1979). Briefly, excised ham muscles were pumped to either 130%, 145%, or 160% of their green weight with brines containing solubilized isolated soy protein. After pumping, the muscles were massaged, stuffed and cooked.

Crude myosin and soy isolate gel preparation

Crude myosin was prepared similar to the method of Macfarlane et al. (1977). Briefly, three volumes of Guba-Straub solution (0.3M KCl, 0.15M potassium phosphate, pH 6.4) were homogenized with 1 volume of prerigor ground bovine neck muscles and allowed to extract for 15 min. The suspension was centrifuged at 1000 × G for 1 hr. The crude myosin was precipitated out of the supernatant by diluting to an ionic strength of 0.04 with deionized distilled water. After settling the crude myosin was retrieved by centrifuging at 12,000 × G for 1 hr. Protein was measured using the Kjeldahl method and then adjusted to 5%. Salt and phosphate were adjusted to 6% and 2%, respectively.

A solution of soy isolate containing 10% Supro 620 (Ralston Purina Co., St. Louis, MO) was prepared according to Siegel et al.

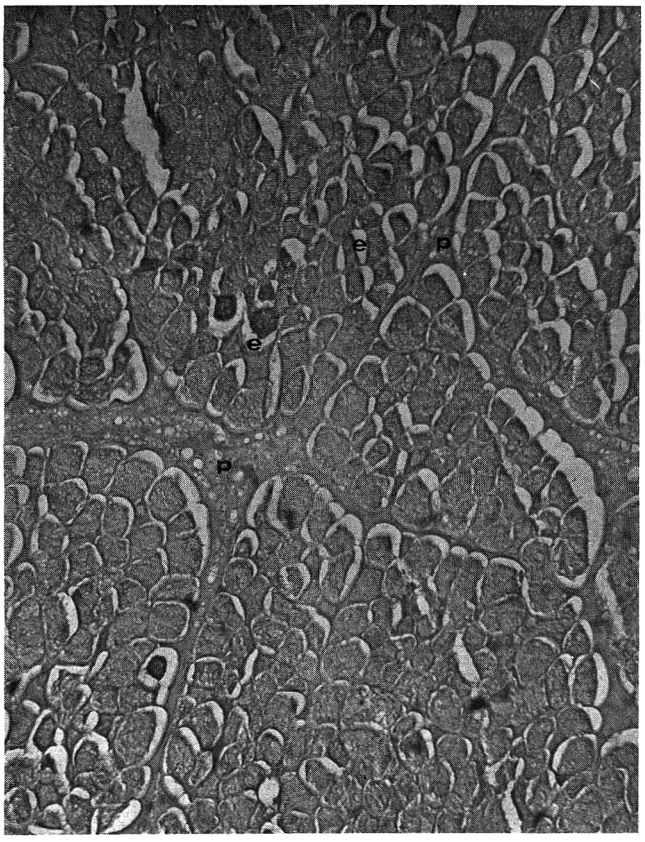

Fig. 1—Light micrograph of a cross-section of the cooked massaged muscle from the water added ham receiving a 160% pump showing coagulated myofibrillar protein in the endomysial (e) and perimysial (p) spaces. (Magnification = 60X)

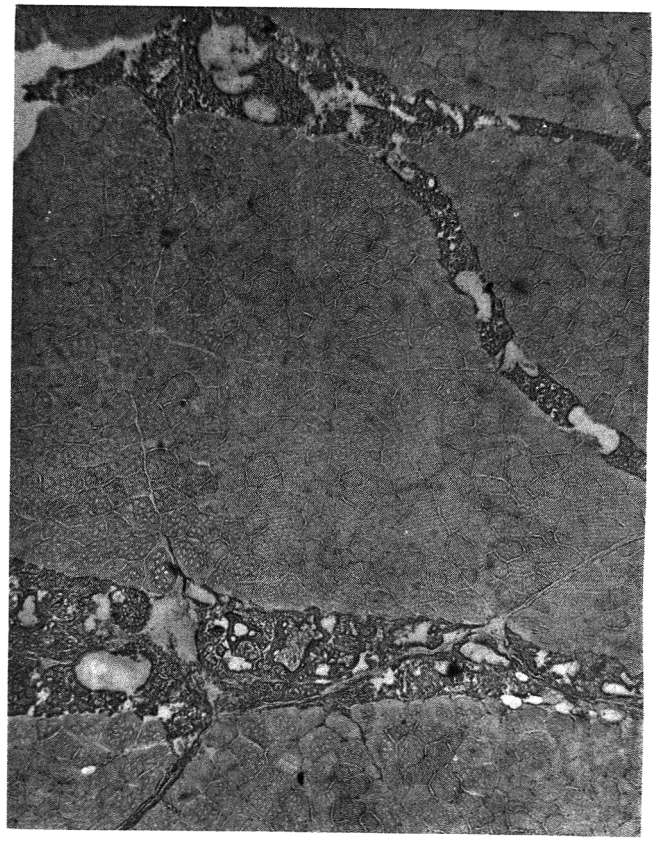

Fig. 2—Light micrograph of a cross-section of the raw unmassaged muscle injected 145% with hydrated Supro 620 showing the location of the Supro 620 within the musculature. (Magnificantion = 60X)

(1979). The composition of the solution was comparable in salt and phosphate to the brine used for the 145% pump treatment. These levels were 8 and 1.3%, respectively. A mixture of crude myosin with the isolated soy protein also was prepared simply by mixing one part of the crude myosin preparation with one part of the solution of soy isolate.

Gels of the crude myosin, the soy isolate and the mixture of crude myosin with the soy isolate were formed by heating these solutions in 90°C water bath for 15 min. After cooling in an ice bath, samples having a volume of approximately 1 cm^3 were prepared for examination under the scanning electron microscope.

Preparation of samples for light microscopy and scanning electron microscopy

Before cooking and within 72 hr after cooking, two cubes of muscle approximately 1 cm^3 were removed from the interior of muscles comprising each ham roll. One of the meat cubes was examined with light microscopy and the other was examined using scanning electron microscopy according to the procedures described by Theno et al. (1978a and 1978b, c, respectively).

RESULTS & DISCUSSION

Light microscopy

The effect of massaging on muscle microstructure was presented in a previous study (Theno et al., 1978a). It was shown that massaging acts to extract myofibrillar protein into extracellular spaces by rupturing endomysial and perimysial sheaths. The areas occupied by these extracted proteins were found to increase when brines were injected at high levels. After cooking these areas possessed a gel-like appearance (Fig. 1).

The locations of isolated soy protein in these products were easily observed using light microscopy by recognizing areas of abnormal morphology. Immediately after injection the isolated soy protein was found to occupy primarily perimysial spaces (Fig. 2). However, after massaging, the isolated soy protein penetrated endomysial spaces. The structure of isolated soy protein in the massaged product appeared to be finer than its structure in the unmassaged product. Since the solution of isolated soy protein also contained salt and phosphate, the myofibrillar proteins were probably extracted into these areas and the mixing of these proteins with the isolated soy proteins incurred by massaging may help explain the finer structure of these areas in the massaged product. This mixing of extracted myofibrillar proteins with the solubilized soy proteins illustrates one of the most important effects massaging has on these products.

In the cooked combination ham (Fig. 3) the pockets of isolated soy protein exhibited a finer structure than in the raw products as recognized by the loss of granularity. This may be attributed to the gelation of the myofibrillar plus soy protein mixture. Distinct soy particles (arrows) were visible in these soy pockets where they appear to have been embedded in a gelled protein matrix. The veins of isolated soy protein also contained many large vacuoles which may be attributed to air introduced into the isolated soy protein during its hydration.

Scanning electron microscopy

Examination of the ultrastructure of the massaged muscle showed fiber shredding and disrupted sarcolemma and connective tissue sheaths. Previous work in this laboratory corroborates these observations and gives a detailed description of the ultrastructural changes incurred by massaging (Theno et al., 1978c).

Figure 4 is a scanning electron micrograph of a cross-sec-

tional view of a typical fiber isolated in the cooked product receiving 160% pump without isolated soy protein. This micrograph shows that although the fiber itself appears to be intact, its internal structural integrity is lost. It appears as though the myofibrils in this fiber have been solubilized. The coagulation of these solubilized proteins due to heating has generated a gel-type structure. This occurrence is comparable to the granulation that Hearne et al. (1978) described in their phase contrast micrographs of cooked bovine semitendinosus muscle. It was recognized that a finer texture was observed toward the periphery of these fibers. This may be attributed to either increased solubilization due to an ionic gradient effect or to the extraction of some of the protein from this area. Closer examination of this area showed that it possessed a porous structure typical of the classical gel structure as described by Paul and Palmer (1972) and Anglemier and Montgomery (1976).

Since the location of the isolated soy protein was described using light microscopy and since it is so difficult to confidently recognize these areas using scanning electron microscopy, no micrographs of the isolated soy protein in the musculature are presented. However, it was considered appropriate that an examination of the ultra structure of a pure gel formed by the isolated soy protein would relate to its properties in a meat system. The structure of this gel is shown in Figure 5. The gel was found to be composed of small hollow holes where water was trapped by the solid matrix. Upon examining the solid component of this gel at a higher magnification, a solid and very compact texture was revealed. It was not found to possess the porous nature typical of the classical gel structure. When the ultrastructure of the crude myosin gel was examined, a very typical gel structure was revealed (Fig. 6). The three dimensional network of coagulated myosin molecules or filaments is

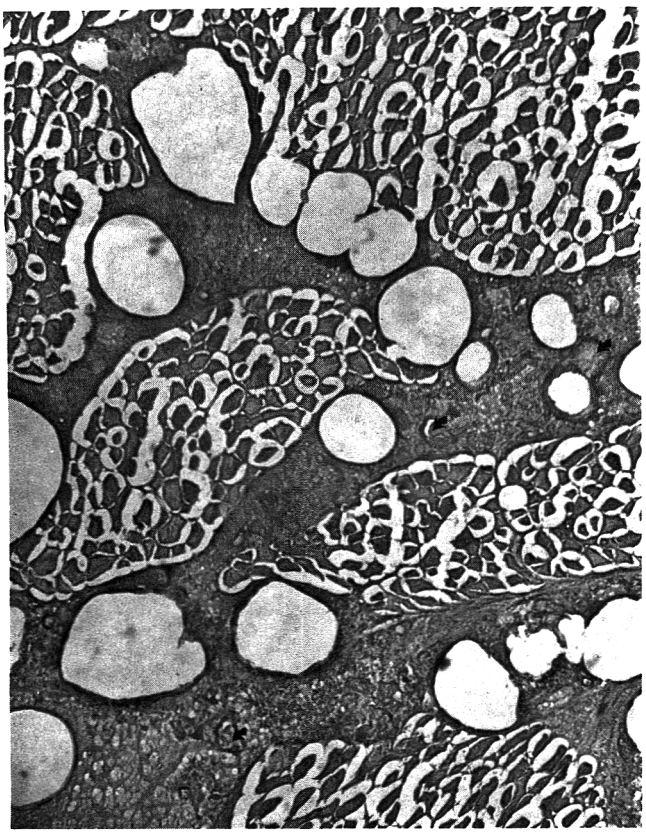

Fig. 3—Light micrograph of a cross-section of the cooked massaged muscle injected 160% with hydrated Supro 620 showing the texture of the isolated soy protein. (Magnification = 60X)

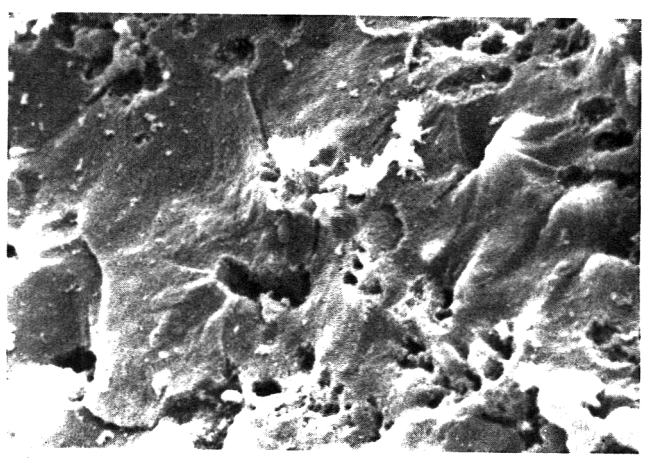

Fig. 5—Scanning electron micrograph showing the structure of the gel formed by the brine containing isolated soy protein that was used for the 145% level of injection. (Magnification = 2200X)

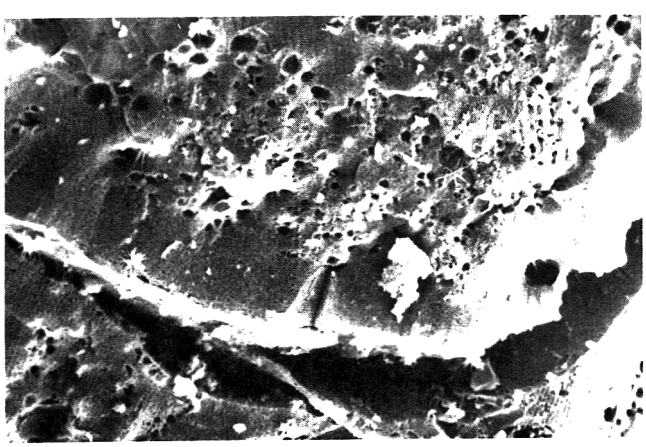

Fig. 4—Scanning electron micrograph of a cross sectional view of a cooked fiber from combination ham receiving the 160% level of injection. (Magnification = 715X)

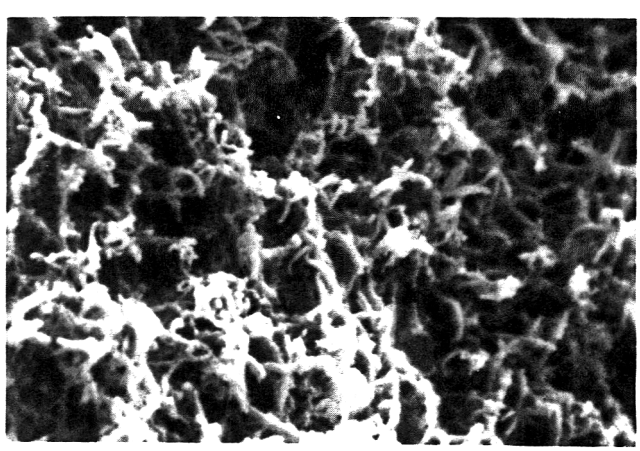

Fig. 6—Scanning electron micrograph showing the structure of the gel formed by a crude myosin preparation. (Magnification = 3300X)

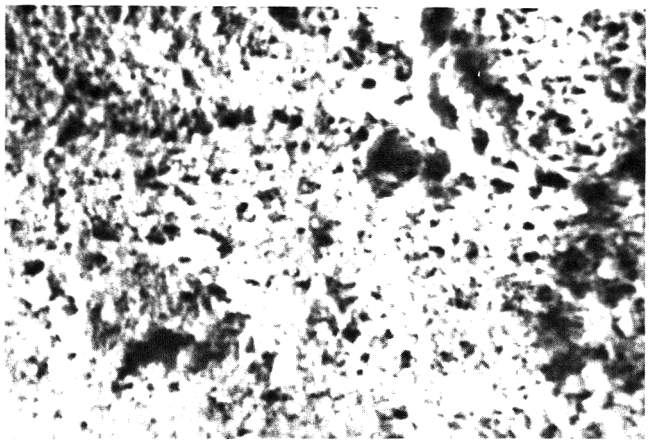

Fig. 7—Scanning electron micrograph showing the structure of the gel formed by a 1:1 mixture of crude myosin and the brine containing isolated soy protein that was used for the 145% level of injection. (Magnification = 2750X)

clearly evident. This stabilized lattice formed by the gelation of crude myosin provides the basis for its large water binding capacity and its functionality in processed meats.

Since light micrographs of the combination ham suggest that the mixing of extracted myofibrillar proteins with the added isolated soy protein enhances the functionality of the soy isolate, the ultrastructure of a 1:1 mixture of the isolated soy protein with crude myosin was examined (Fig. 7). The texture of this gel possessed the porous structure typical of normal gel structure. This texture was not as fine and fibrous as the texture of the crude myosin gel. However, it also did not possess the compacted texture found in the soy isolate gel. From these observations it appears as though the properties of isolated soy protein in this meat system are improved by its subsequent mixture with extracted myofibrillar proteins. This mixing of the soy isolate with the extracted myofibrillar proteins does not appear to hinder the binding ability of the extracted myofibrillar proteins.

CONCLUSIONS

EXAMINATION of microstructure of muscle in the combination ham revealed the most important effects of massaging as the distribution of the solubilized isolated soy protein within the musculature, and the mixing of this protein with extracted myofibrillar proteins. Pockets of the isolated soy protein possessed a finer structure after cooking which was attributed to its gelation. The gel formed by isolated soy protein possessed an atypical structure, while the gel formed by crude myosin possessed a typical structure. Therefore, it appears different types of molecular interactions between proteins are responsible for the gelation of crude myosin and isolated soy protein. By examining gel ultrastructure, the potential functionality of nonmeat proteins can be studied.

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GEL STRUCTURE OF NONMEAT PROTEINS AS RELATED TO THEIR ABILITY TO BIND MEAT PIECES

D. G. SIEGEL, K. E. CHURCH and G. R. SCHMIDT

ABSTRACT

Investigations on the binding abilities of some nonmeat proteins showed that those studied were inferior to the binding ability previously reported for myosin. Their binding abilities in the presence of 8% salt and 2% sodium tripolyphosphate were ranked from highest to lowest as wheat gluten, egg white, corn gluten, calcium reduced dried skim milk, bovine blood plasma, isolated soy protein and sodium caseinate. Structures of gels formed by these proteins and mixtures of them with crude myosin showed that a three dimensional network of protein fibers is not indicative of good binding ability. The types of molecular interactions stabilizing their gel structures are thought to be more important.

INTRODUCTION

"BINDING" is used by meat scientists to express several different meanings. It is used to refer to water-holding capacity, emulsification capacity or the sticking together of meat pieces. The present study is concerned with sectioned and formed types of meat. Therefore, the term "binding" will be used to refer to the sticking together of meat pieces.

The binding properties of chunk-type products (Macfarlane et al., 1977; Ford et al., 1978; Siegel et al., 1978; Siegel and Schmidt, 1979a, b) as well as comminuted or emulsified sausages (Fukazawa et al., 1961a, b, c; Samejima et al., 1969; Nakayama and Sato, 1971a, b, c) have been shown to be dependent on the presence of myosin. Many nonmeat proteins are capable of offering meat systems improved fat and water-binding properties (Lancaster, 1975; Lauck, 1975); however, their ability to bind meat pieces has not been fully investigated. The objective of this study was to measure the binding abilities of some nonmeat proteins and to study how the ultrastructure of gels formed by these proteins relates to their ability to bind meat pieces.

MATERIALS & METHODS

Preparation of proteins

The isolated soy protein was Supro 620 which is commercially produced by Ralston Purina Company, St. Louis, MO.

The wheat gluten was Vital Wheat Gluten which is commercially produced by General Mills Inc., Minneapolis, MN.

The sodium caseinate was Erie Ultra Supreme which is commercially produced by Erie Casein Company, Erie, IL.

The calcium reduced dried skim milk was Savortex which is commercially produced by Western Dairy Products, San Francisco, CA.

The corn gluten used was obtained from A.E. Staley Company at Decatur, IL.

These proteins were hydrated with cold distilled water in a 2°C cold room for 15 min with a Waring Blendor set as low speed. Enough water was used to make a protein concentration of 13%. After hydration the protein solutions were centrifuged at 500 × G for 10 sec to remove air incorporated during the hydration process.

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Table 1—Binding abilities (B.A.)[a] of various nonmeat proteins in the presence and absence of salt and phosphate

Protein	B.A. (g) 8% salt, 2% phosphate	Protein	B.A. (g) 0% salt, 0% phosphate
Wheat gluten	175.4a	Bovine blood plasma	24.1e
Egg white	120.3b	Wheat gluten	23.5e
Control	107.0bc	Isolated soy protein	8.7e
Calcium reduced dried skim milk	74.5cd	Control	0e
Bovine blood plasma	71.9d	Corn gluten	0e
Isolated soy protein	66.7d	Calcium reduced dried skim milk	0e
Sodium caseinate	0e	Sodium caseinate	0e

[a] Values are the mean of these replications. Those not followed by the same letter are different at the 0.05 level of confidence. $LSD_{0.05} = 34.9$

Blood plasma was separated from fresh bovine blood after adding 1 mg/ml EDTA by centrifuging at 3500 × G for 30 min. Kjeldahl analysis showed that it was composed of 7% protein.

Egg white was separated from hen eggs. Kjeldahl analysis showed that it contained 10% protein.

Each protein solution was measured for its ability to bind meat pieces in the presence and absence of 8% salt and 2% sodium tripolyphosphate using the method of Siegel and Schmidt (1979a). Briefly, meat slices (1.5 in. thick) were removed from the semitendinosus muscle by cutting perpendicular to the longitudinal axis and then trimmed to a square shape (4 cm × 4 cm). Each protein solution was spread on the surfaces of two of these slices at the rate of 0.1 g/cm^2. The slices were sandwiched together and roasted until an internal temperature of 75°C was reached. Uniform sections, 0.5 cm thick by 1 cm wide, were removed from the sandwiches by cutting parallel to the longitudinal axis of the muscle fibers. The peak force required to separate the meat pieces at their binding junction was measured with an Instron Universal Testing Machine equipped with pneumatically powered jaws. The average of five of these peak forces was recorded as the binding ability. Binding abilities were measured in triplicate for each protein and then compared to that of a control consisting of 90% water, 8% NaCl and 2% sodium tripolyphosphate.

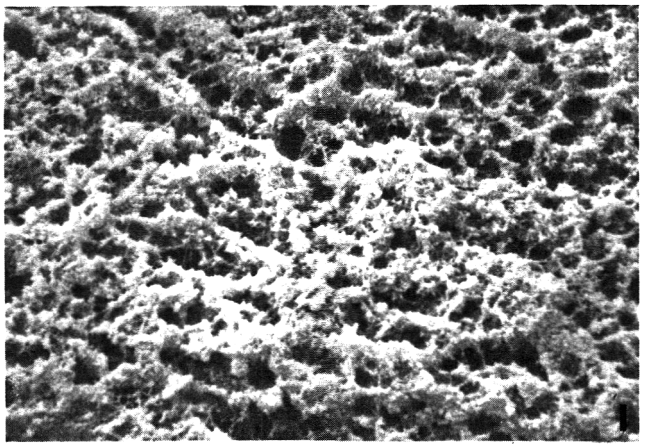
The gel structure of each protein was examined using scanning electron microscopy according to the procedure of Theno et al. (1978). The protein solutions were gelled by immersing a test tube containing 3 ml in a boiling water bath for 5 min. After cooling, the ability of each protein to form a heat initiated gel was tested by inverting the test tube. If the protein solution retained its shape in the bottom of the test tube, its ability to gel was recorded as positive. Since all of the proteins do not form heat initiated gels, the gel structures of 1:1 mixtures of a 7% crude myosin preparation with each of these proteins were also examined. The crude myosin was prepared from prerigor beef with the Guba-Straub solution as described by Perry (1955).

Statistical analysis

The binding abilities of the proteins were compared using an analysis of variance with Fischer's Least Significant Difference (Steel and Torrie, 1960).

RESULTS & DISCUSSION

RESULTS of binding abilities showed that in the presence of salt and phosphate wheat gulten was the only protein having a significantly higher binding ability ($P < 0.05$) than the nonprotein control (Table 1). Egg white and corn glu-



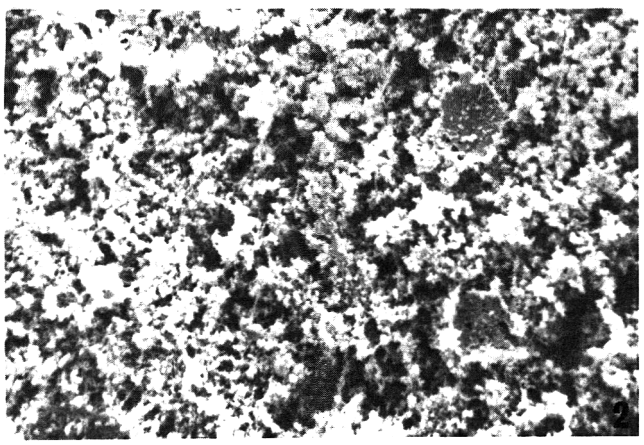
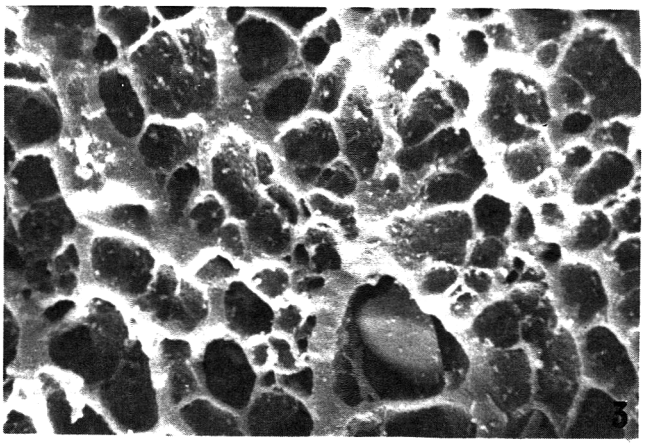


Fig. 1–2–Scanning electron micrographs showing the ultrastructure of gels formed by egg white (10% protein) in the absence (Fig. 1) and presence (Fig. 2) of 8% NaCl and 2% sodium tripolyphosphate. Magnification = 1200X.



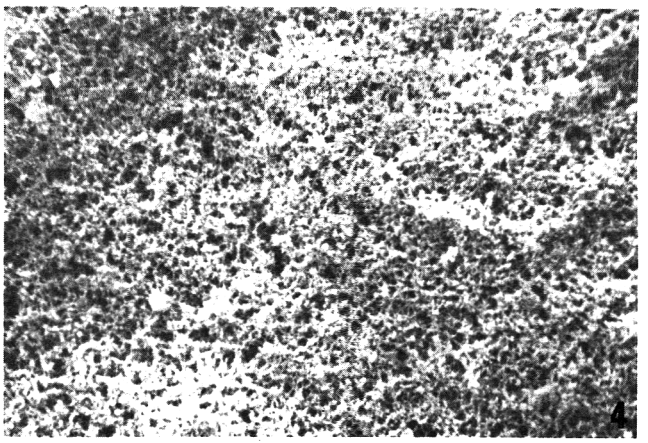


Fig. 3–4–Scanning electron micrographs showing the ultrastructure of gels formed by bovine blood plasma (7% protein) in the absence (Fig. 3) and presence (Fig. 4) of 8% NaCl and 2% sodium tripolyphosphate. Magnification = 1100X.

ten had comparable binding abilities to that of the control while calcium reduced dried skim milk, bovine blood plasma and isolated soy protein were less able to bind two pieces of meat, and sodium caseinate was unable to bind at all. It should be mentioned that the bovine blood plasma and egg white used in this study were in their natural form. Therefore, their concentration of protein was lower than that of the other proteins. Comparisons with these proteins would be invalid and little emphasis should be placed on their relative binding abilities. This is especially true for the blood plasma as its binding ability was lower than expected.

It was shown in a previous study (Siegel and Schmidt, 1979a) that a 5% solution of crude myosin has a much better binding ability than any of the proteins studied here as well as the nonprotein control. The high binding ability found for the nonprotein control may be attributed to the extraction of myosin from the surfaces of the meat pieces being bound. This extracted myosin then functions as the binder upon cooking. It appears as though those proteins which offer binding properties comparable to that of the nonprotein control (egg white and corn gluten) do so by not hindering the extraction of myosin. Perhaps those proteins which offer poorer binding properties than that of the nonprotein control (calcium reduced dried skim milk, bovine blood plasma and isolated soy protein) either hinder the extraction of myosin, by reducing the effective concentration of salt and phosphate, or interfere with the binding ability of the extracted myosin.

Scanning electron microscopy

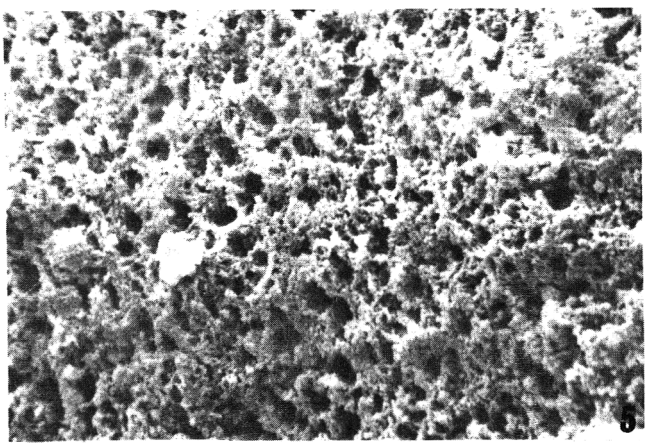
Although the proteins examined here possess good fat and water binding properties (Lancaster, 1975), their poor ability to bind meat pieces suggests that these properties are unrelated. An examination of the ultrastructure of gels formed by these proteins would help explain their poor ability to bind meat pieces. Since all of these proteins do not form heat initiated gels, the gels formed by mixtures of each of these proteins with crude myosin were examined to determine if their poor binding abilities may be attributed to a negative effect on the gel structure of crude myosin.

Table 2–Ability of various nonmeat proteins to gel upon heating in the presence and absence of salt and phosphate

Protein	Ability to gel 0% salt, 0% phosphate	Ability to gel 8% salt, 2% phosphate
Egg white	+	+
Bovine blood plasma	+	+
Wheat gluten	+	–
Isolated soy protein	+	–
Calcium reduced dried skim milk	–	–
Corn gluten	–	–
Sodium caseinate	–	–

The ability of the various proteins to form a heat-stabilized gel in the presence and absence of salt and phosphate is shown in Table 2. Since the proteins capable of gelling are egg white, plasma, wheat gluten and isolated soy protein, their gel structure was also examined without mixing them with crude myosin.

Egg white formed a three dimensional network of coagu-



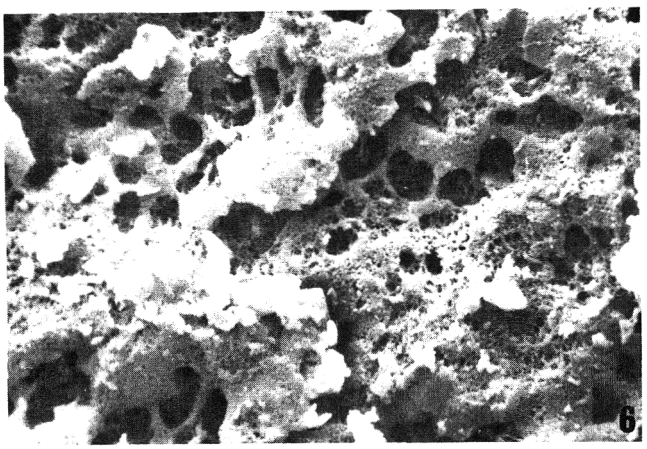


Fig. 5–6–Scanning electron micrographs showing the ultrastructure of gels formed by a mixture of salted egg white and crude myosin (Fig. 5) and a mixture of salted blood plasma and crude myosin (Fig. 6). Magnification = 1200X.

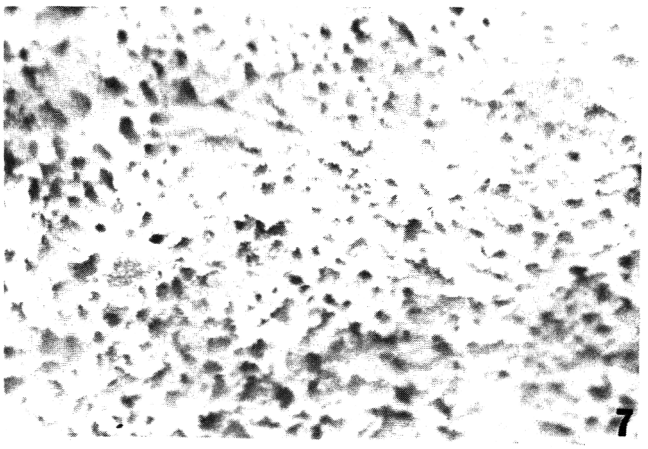


Fig. 7–8–Scanning electron micrographs showing the ultrastructure of gels formed by isolated soy protein (Fig. 7) and wheat gluten (Fig. 8) containing 13% protein. Magnification = 1200X.

lated protein in the absence of salt and phosphate (Fig. 1) and it formed a more porous network in the presence of salt and phosphate (Fig. 2). Both of these structures are well suited to bind water; however, only the salted egg white was capable of binding meat pieces. Salt and phosphate had more of an effect on the ultrastructure of the gel formed by plasma. In the absence of salt and phosphate (Fig. 3) plasma coagulated into a structure possessing very large holes where water is most likely harbored. However, in the presence of salt and phosphate (Fig. 4) its structure was composed of a very fine three dimensional network of interweaving fibers. This structure is better suited to entrap water and is very comparable to the gel structure of crude myosin shown in Siegel and Schmidt (1979b). The structures of both egg white and plasma gels are typical of protein gels as described by Paul and Palmer (1972) and Fennema (1976).

The gel structure of the mixture of crude myosin with egg white (Fig. 5) has a network comparable to that of crude myosin. This suggests that egg white does not interfere with interactions between myosin molecules, and the types of molecular interactions stabilizing the egg white gel are most likely of the same nature as those stabilizing the myosin gel.

The gel structure of the mixture of crude myosin with plasma (Fig. 6) shows that it possesses a more irregular network than those of either salted plasma or crude myosin. From this observation and plasma's low binding ability, the presence of plasma interferes with interactions between myosin molecules and the types of molecular interactions stabilizing the plasma gel probably are not of the same nature as those stabilizing the myosin gel.

The structure of the isolated soy protein gel (Fig. 7) was porous with a dense solid character and that of the wheat gluten (Fig. 8) was less porous and possessed more of the dense solid character. These differences may be attributed to the syneresis that occurred upon heating wheat gluten. Therefore, the wheat gluten gel contained a higher protein concentration.

Examination of the mixture of crude myosin with isolated soy protein (Fig. 9) shows an irregular network with the dense solid character. These observations and the low binding ability of isolated soy protein suggests that it interferes with the interactions between myosin molecules, and the molecular interactions stabilizing it are of a different nature than those stabilizing myosin.

Examination of the mixture of crude myosin with wheat gluten (Fig. 10) reveals a more uniform and more fibrous structure than is seen in the isolated soy protein and crude myosin mixture. A comparison of the gel structure of wheat gluten with that of the mixture of crude myosin with wheat gluten, and the high binding ability of wheat gluten, suggests that it does not interfere with interactions between myosin molecules. The binding ability of wheat gluten, being even higher than the control, suggests that its gel stabilizing molecular interactions are similar to those of myosin and it perhaps enhances these interactions.

Gel structures formed by mixtures of crude myosin with either calcium reduced dried skim milk (Fig. 11), corn gluten (Fig. 12) or sodium caseinate (Fig. 13) showed net-

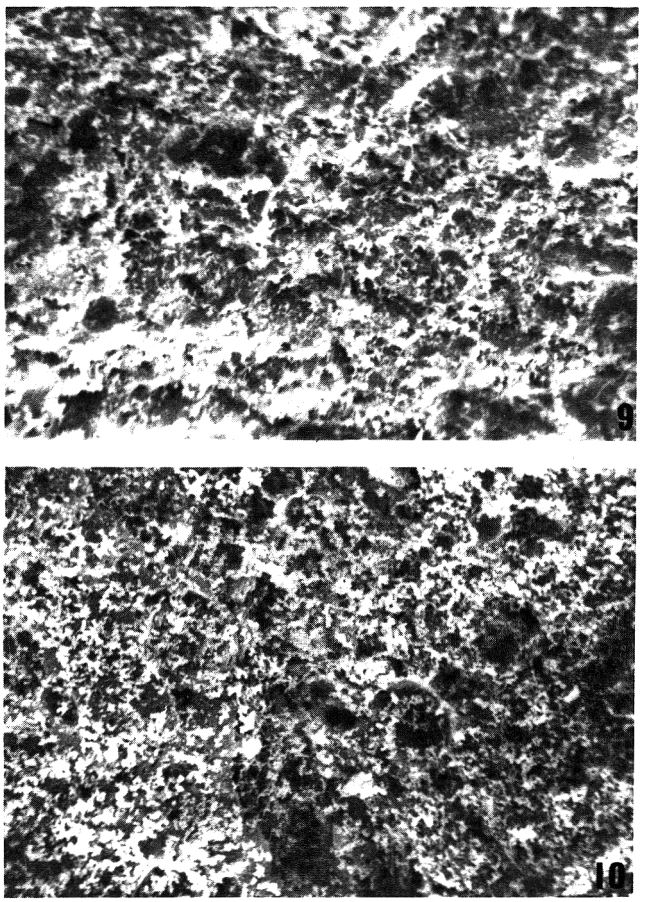


Fig. 9–10–Scanning electron micrographs showing the ultrastructure of gels fromed by a mixture of wheat gluten and crude myosin (Fig. 9) and a mixture of isolated soy protein and crude myosin (Fig. 10). Magnification = 1200X.

works which are typical of protein gels. The mixture with calcium reduced dried skim milk possessed a very fibrous character. However, its low binding ability suggests that its molecular interactions are weaker than those of myosin. The mixture with corn gluten showed the presence of large vacuoles surrounded by a denser area that was very fibrous. Its intermediate binding quality suggests that this gel structure is not beneficial to binding. The mixture with sodium caseinate produced a structure possessing a globular character in addition to a fibrous character. Although this structure appears to be well suited to the retention of water, the absence of binding ability by soidum caseinate suggests that it inhibits the interactions between myosin molecules.

CONCLUSIONS

THE BINDING ABILITIES of the nonmeat proteins investigated here may be ranked from highest to lowest as wheat gluten, egg white, corn gluten, calcium reduced dried skim milk, bovine blood plasma, isolated soy protein and sodium caseinate. The ability of wheat gluten to bind meat pieces may be attributed to its ability to interact with myosin while the inability of sodium caseinate to bind meat pieces may be attributed to its inhibitory effect on interactions between myosin molecules. The ability of these proteins to bind fat or water is not related to their ability to bind meat pieces.

The ability of proteins to form heat initiated gels and the ultrastructure of these gels are not indicative of a protein's ability to bind meat pieces. Although a three-dimensional network of protein fibers is beneficial to binding ability, the types of molecular interactions stabilizing this structure are more important. They must be of the same nature as those stabilizing the gel formed by myosin so that interactions between molecules of the nonmeat protein with those of myosin are possible.

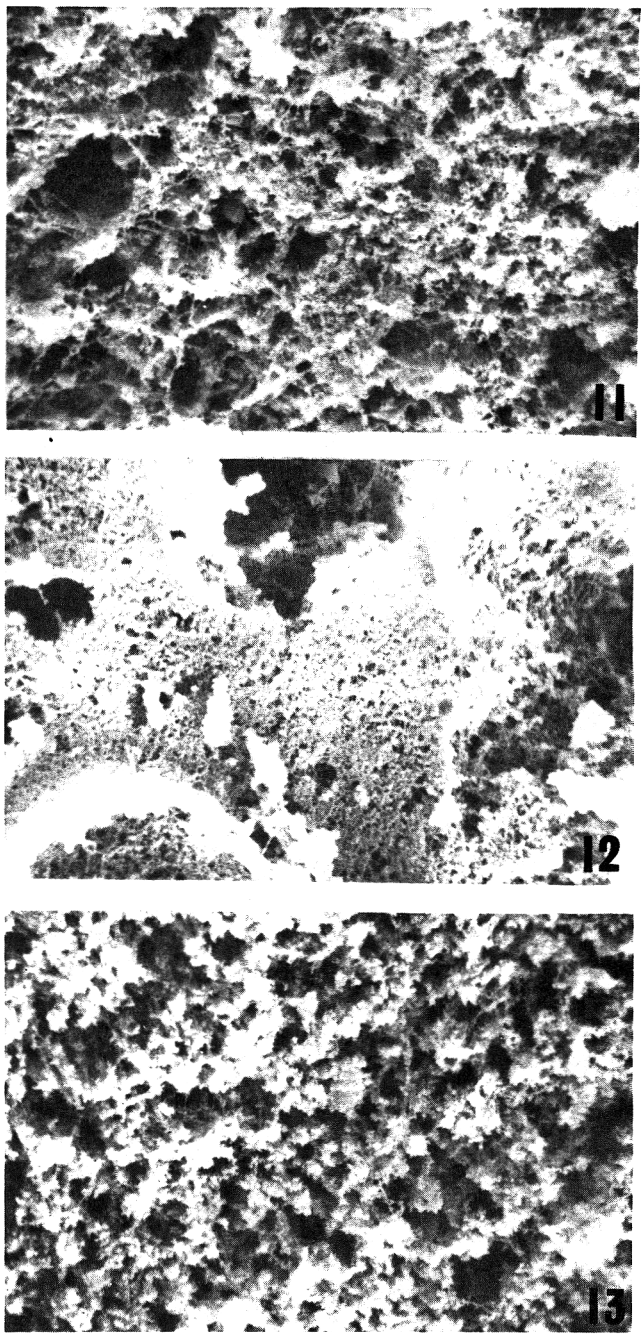


Fig. 11–13–Scanning electron micrographs showing the ultrastructure of gels formed by a mixture of calcium reduced dried skim milk and crude myosin (Fig. 11), a mixture of corn gluten and crude myosin (Fig. 12), and a mixture of sodium caseinate and crude myosin (Fig. 13). Magnification - 1100X.

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HEAT-INDUCED GELATION OF MYOSIN: FACTORS OF pH AND SALT CONCENTRATIONS

MAKOTO ISHIOROSHI, KUNIHIKO SAMEJIMA and TSUTOMU YASUI

ABSTRACT

The heat-induced gelation of myosin was optimally developed at temperatures between 60° and 70°C and at pH 6.0 as studied quantitatively by measuring shear modulus of myosin in 0.6M KCl. No difference was observed in the gelation profile, when KCl was replaced by NaCl. The shear modulus of the gel formed at 65°C and pH 6.0 increased proportionally to the 1.8 power of myosin concentration. Although the heat gelling ability of myosin as measured by the shear modulus did not increase with increasing salt concentration from 0.4–1.0M, irrespective of storage time at 0°C of the stock myosin solution (0.6M KCl) or suspension (0.1M KCl), it varied drastically with time of storage at salt concentrations between 0.1 and 0.3. This variation in shear modulus at 0.1–0.3M KCl was apparent due to the storage conditions. These changes in the salt concentration dependence of the heat gelation of myosin corresponded well with the changes in solubility of myosin during storage. Studies on selected physico-chemical properties of the original stock myosin during storage indicated gradual increase in viscosity with little inactivation of ATPase as well as very slight decrease in sulfhydryl content, suggesting the spontaneous transformation (aggregation) of myosin molecules to a less soluble state.

INTRODUCTION

THE DECISIVE ROLE played by myosin in the development of binding properties of meat products such as sausages was first demonstrated by Fukazawa et al. (1961a, b, c). They showed that myofibrils from which myosin had been selectively removed lost their ability to produce an aggregate without liberation of water. Since the term "Binding" refers to the "Bonding together of pieces of meat to produce a unit system, where adhesion of pieces of meat to each other is initiated by the mechanical formation of a protein exudate, followed by protein gelation during thermal processing" (Macfarlane et al., 1977), it seems to be of great importance to study the mechanism of heat-induced gelation of myosin which as yet has not been clearly elucidated.

From the practical point of view some information is available as to how various treatments affect the knitting of pieces of poultry meat (Schnell et al., 1970; Vadhra and Baker, 1970; Acton, 1972a, b; Wardlaw et al., 1973), beef (Pepper and Schmidt, 1975; Moore et al., 1976) and pork (Gillet et al., 1978; Siegel et al., 1978a, b). The heat gelling and binding qualities of purified proteins have been studied in gel systems (Samejima et al., 1969; Sato and Nakayama, 1970; Nakayama and Sato, 1971a, b, c; Grabowska and Sikorski, 1976), and Macfarlane et al. (1977) actually measured the binding strength between adjacent meat pieces using myosin, actomyosin and sarcoplasmic proteins as binding agents. However, in these earlier investigations, no attempt was made to measure the gelation of the purified proteins at elevated temperatures.

The present study was designed to investigate the factors which affect the heat-induced gelation of myosin, by measuring changes in the shear modulus of myosin under various conditions. Conditions and factors for this study were selected according to the dictates of practical meat processing operations.

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MATERIALS & METHODS

Myosin preparation

Myosin was prepared according to the method described previously (Yasui et al., 1979) from M. longissimus thoracis of rabbit. The pH value of the stock myosin solution (in 0.6M KCl) or suspension (in 0.1M KCl) was about 6.5. The stock myosin (about 25 mg/ml) was stored in ice at 0°C. When stored for more than 7 days, sodium azide (final concentration, 1 mM) was added to inhibit bacterial growth. The protein concentration was determined by the Biurel method (Gornall et al., 1949), and when used, the desired quantity of myosin was diluted to appropriate concentrations by the addition of 3M KCl and 0.2M phosphate buffer (pH 6.0) to final concentrations of 0.6M and 20 mM, respectively.

Gelation

To monitor the occurrence of heat-induced gelation of myosin, we selected the change in the shear modulus of the protein sample under various conditions, which was measured by means of the shear modulus tester reported previously (Yasui et al., 1979). A sample solution or suspension in a cuvette (1 cm) was placed in the thermostatically controlled cell holder and incubated at the desired temperature. After a 20–30 min incubation at each temperature the shear modulus was measured. The value of shear modulus remained constant after 15–30 min at each fixed temperature.

Solubility

Since freshly prepared myosin is known to precipitate quantitatively at ionic strengths below 0.1, changes in solubility were investigated by centrifuging myosin at various salt concentrations at 10,000 × G for 30 min. The solubility of myosin hereafter is expressed as protein concentration of the supernatant/protein concentration of the sample before centrifugation × 100 (%).

Scanning electron microscopy

Scanning electron microscopic observations were made on the heat-induced gel of myosin (Yasui et al., 1979) using a Hitachi HHS-2R scanning electron microscope.

ATPase assay

The inorganic phosphate liberated during the hydrolysis of ATP was measured by the method of Fiske and SubbaRow (1925). The reaction mixture, which had the following final concentrations: ATP, 1 mM; KCl, 0.6M; EDTA, 1 mM; Tris-HCl buffer, 20 mM (pH 7.8); myosin, 0.2 mg/ml, was maintained at 25°C, and the reaction was terminated by the addition of 15% trichloroacetic acid. The use of EDTA as a modifier was discussed in the previous paper (Yasui et al., 1975).

Determination of sulfhydryl groups

For the determination of sulfhydryl groups in myosin, the Ellman procedure (1959) was found most suitable (Buttkus, 1971; Kawakami et al., 1971). The assay with 5,5'-dithio-bis-(2-nitro benzoic acid) (DTNB) was carried out in saturated urea and borate (0.2M, pH 8.0). The optical density readings were measured against appropriate blanks at 412 nm and calculations were carried out using the extinction coefficient of 1.36×10^4 (for the thiophenol anion of DTNB) as given by Ellman (1959).

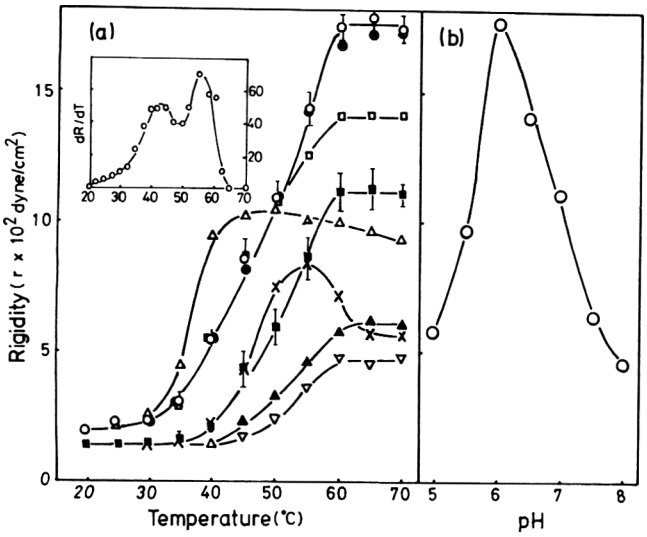

Fig. 1—Changes in the shear modulus of thermally treated myosin: (a) Myosin (4.5 mg/ml) dissolved in 0.6M KCl or NaCl and 20 mM citrate (pH 5.0–5.5), phosphate (pH 6.0–7.0) or tris-HCl (pH 7.5–8.0) buffer was incubated for 25 min at temperatures from 20–70°C. Shear modulus of each system was measured at the temperature indicated. (X) KCl at pH 5.0, (Δ) KCl at pH 5.5, (○) KCl and (●) NaCl at pH 6.0, (□) KCl at pH 6.5, (■) KCl at pH 7.0, (▲) KCl at pH 7.5 and (▽) KCl at pH 8.0. Bars for pH 6 and 7 indicate standard errors of 10 experiments on 6 myosins from different animals. Other points were the mean of measurements on 2–3 samples. (Inset): Derivative plot as a function of temperature. Differential shear modulus (ΔR/ΔT) of the system at pH 6.0 (KCl) was plotted against temperature. (b) pH dependence of heat-induced gellation of myosin (4.5 mg/ml) in 0.6M KCl at 65°C.

Viscosity

Viscosity measurements were made with Ostwald-type viscometers at 20°C and pH 7.0. The flow-time for water was 45 sec.

RESULTS

Effects of temperature and pH on gelation

As presented in Figure 1(a), the heating of myosin solutions at pH 6.0 and temperatures from 20–30°C for 25 min resulted in only minor increases in the shear modulus of the system. Similar heating at temperatures from 35–55°C brought about a dramatic increase of the shear modulus, nearly as high as that produced by heating at temperatures from 60–70°C. The temperature profile of gel formation of myosin was not affected by the replacement of KCl with NaCl in the system [Fig. 1(a)]. Since gels formed at 60–70°C exhibited the highest shear modulus value, a heating temperature of 65°C was used in subsequent experiments.

Figure 1(a) insert illustrates the differential shear modulus (ΔR/ΔT) plotted against temperature, showing two transition temperatures (Tm_1 and Tm_2) at 43° and 55°C.

The effect of pH on heat-induced gelation of myosin is shown in Figure 1(a) and (b). While some firm gels remained in the cell when it was inverted and showed shear modulus values higher than 1,000 dyne/cm² were formed by heating myosin over the pH range of 5.0–8.0, distinct quantitative differences in the shear modulus were noted as an effect of changes in pH. At 65°C, gels formed at higher or lower pH values were considerably lower in measured shear modulus than were gels formed at 6.0. As would be expected, gels formed at lower pH exhibited more synerisis and those at higher pH were more translucent in appearance than the white gels formed at 6.0. It is evident from the results in Figure 1 that the optimal pH and temperature for the heat-induced gelation of myosin are pH 6.0 and 60–70°C, respectively.

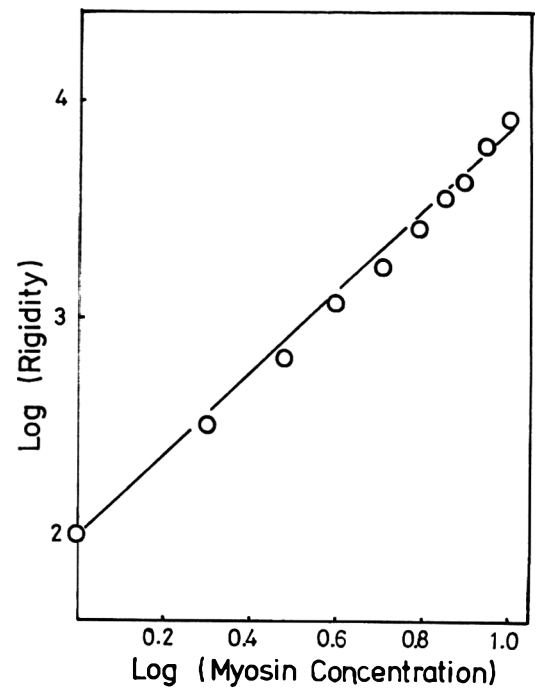

Fig. 2—Protein concentration dependence of heat-induced gellation of myosin at 65°C and pH 6.0.

Effect of protein concentration on gelation

The effect of varying concentration from 0.1–1.0% on quantitative gel formability of myosin is summarized in Figure 2. The value calculated from the slope was 1.8. Therefore, it may be concluded that the shear modulus of the heat-induced myosin gel increases proportionally to the square of myosin concentration under these experimental conditions.

Effects of salt concentration and storage time on gelation

Dependency of heat-induced gelation on salt concentration of myosin used within 24 hr after preparation was characterized by an extremely high shear modulus at 0.1–0.2M KCl [Fig. 3(a)]. The shear modulus then decreased drastically at salt concentration up to 0.4M. The peak position and the extent of shear modulus shifted towards higher salt concentration and decreased, respectively, with storage time of the myosin in 0.6M KCl at 0°C. The increase in shear modulus due to ionic strength on the 15 day storage occurred between 0.2 and 0.3M KCl and after 0.3M KCl the shear modulus stayed steady.

In the stock myosin suspensions stored (1–10 day) at 0.1M KCl, the shear modulus of their heat-induced gelation were extremely high at 0.1–0.2M KCl and decreased drastically with increasing salt concentration up to 0.4M KCl [Fig. 3(b)]. The 15 days storage, on the other hand, had a peak of rigidity at 0.3M KCl though an extremely high shear modulus could not be seen at 0.1–0.2M KCl. The same trend was found with the stock myosin suspension stored in 0.1M KCl [Fig. 3(b)] as in 0.6M KCl [Fig. 3(a)] between 0.4 and 0.6M KCl on the shear modulus. There were no significant differences in shear modulus between 0.6 and 1.0M KCl although those results were not included in this figure. It is to be noted that in both cases [Fig. 3(a) and (b)], salt concentration dependence at 0.4–1.0M of heat-induced gelation of myosin was shown to be independent of storage time.

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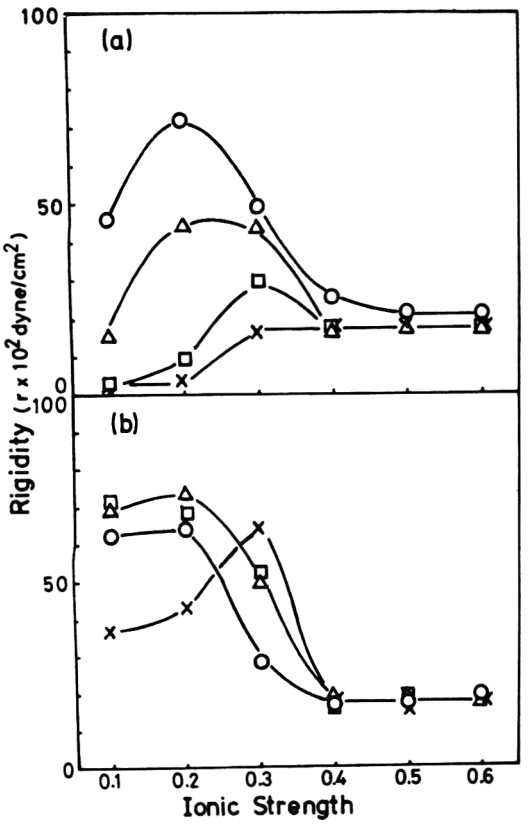

Fig. 3—Effects of salt concentration and storage time on changes in the shear modulus of myosin treated for 25 min at 65°C and pH 6.0. The stock myosin was stored at 0°C in 0.6M (a) and 0.1M (b) KCl. For shear modulus measurements myosin (4.5 mg/ml) in 20 mM phosphate buffer and various concentrations of KCl indicated was used. Symbols for (a) and (b): (○) 1 day-storage, (△) 5 day-storage, (□) 10 day storage and (X) 15 day-storage.

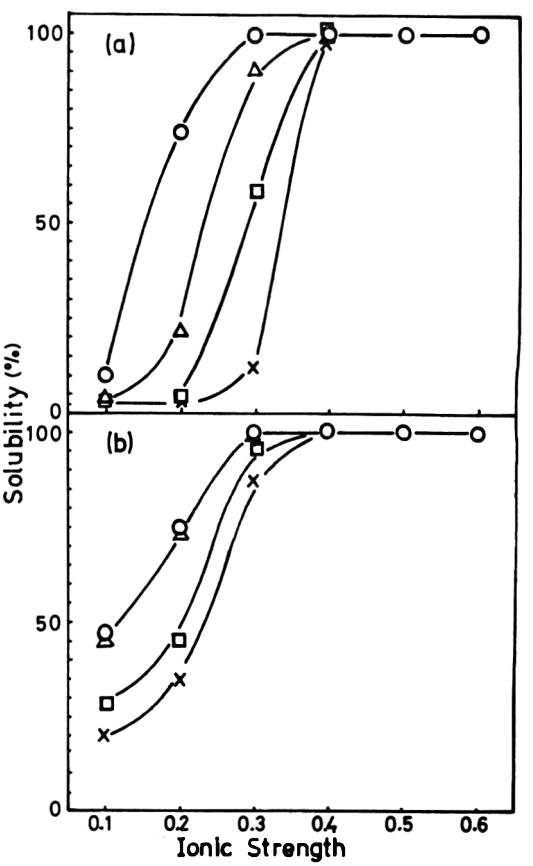

Fig. 4—Changes in solubility of myosin as a function of salt concentration during storage at 0°C. (a) storage in 0.6M KCl and (b) storage in 0.1M KCl. Conditions and symbols were the same as in Figure 3.

Figure 4(a) and (b) demonstrate, respectively, changes in solubility of myosin stored in 0.6M (a) and 0.1M (b) KCl under the same conditions as in Figure 3. That solubility of fresh myosin in 0.1–0.3M KCl was decreased with storage time suggests increasing formation of aggregates that do not dissociate at low ionic strengths.

Changes in selected physicochemical properties of myosin during storage were studied under the experimental conditions described above. While viscosity increased gradually with storage time, ATPase activity exhibited little change and sulfhydryl content decreased slightly (Table 1). This indicates aggregation of myosin molecules without loss of their enzymatic activity during storage.

Scanning electron microscopic observations on gels of fresh myosin at 0.2M and 0.6M KCl are shown in Figure 5. Finer network structure was observed with the gel at 0.2M KCl, as compared to the ultramicrostructure at 0.6M KCl. Formation of the finer protein matrix appears to cause higher shear modulus of the system.

DISCUSSION

THE SALT used commonly in the commercial production of reformed meats is sodium chloride at a concentration range between 2.0 and 3.0%. Molar concentrations calculated from those % give 0.47–0.68M, assuming that lean meat (skeletal muscle) contains 75% water. Since substitution of NaCl for KCl did not reveal any difference in the heat-induced gelation profile of myosin (Fig. 1), the results obtained in this study using KCl as a salt may be compared with those reported previously, in which NaCl was used as a salt (Fukazawa et al., 1961a, b, c; Samejima et al., 1969; Nakayama and Sato, 1971a, b, c; Macfarlane et al., 1977; Gillet et al., 1978; Siegel et al., 1978a, b). At this salt concentration range, the shear modulus of myosin has been found to be almost independent of salt concentration and storage time (Fig. 3). The optimum temperature and pH for development of gelation are at 60–70°C and pH 6.0, respectively. The optimum pH observed here is in agreement with that reported by Trautman (1966) who studied the effect of pH on the gelling properties of salt soluble proteins from ham muscle using a semiquantitative technique.

The existence of two transition temperatures (Tm_1 and

Table 1—Changes in ATPase activity, viscosity and sulfhydryl content of myosin during storage at 0°C[a]

KCl conc (M)	Storage time (Day)	ATPase activity (moles Pi/min/ mg of protein)	Viscosity (dl/g)	SH content (moles/10^5 g protein)
0.1	1	0.84	2.04	8.51
0.1	5	0.85	2.82	8.22
0.1	10	0.84	2.87	8.09
0.1	15	0.83	2.94	8.05
0.6	1	0.84	2.15	8.51
0.6	5	0.83	3.40	8.34
0.6	10	0.83	3.75	8.26
0.6	15	0.82	3.78	7.85

[a] Myosin (25 mg/ml) in 0.6M or 0.1M KCl was stored in ice at 0°C. pH of these stock solutions or suspensions was maintained at about 6.5.

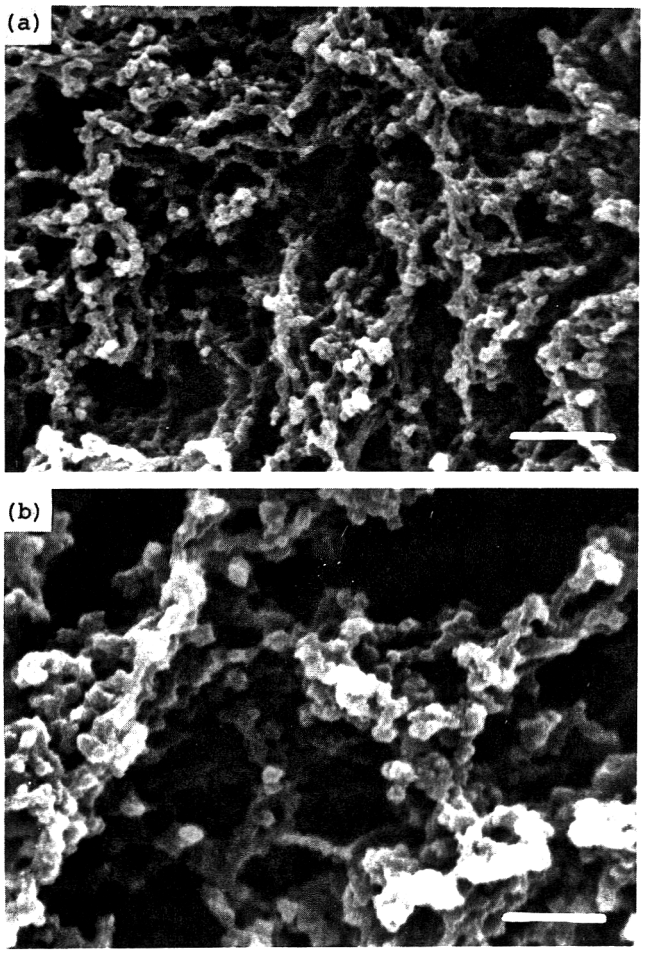

Fig. 5—Scanning electron micrographs of myosin (4.5 mg/ml) gels formed at 60°C and pH 6.0. (a) 0.2M KCl and (b) 0.6M KCl. Bar length is 1.0μ.

Tm_2) in the heat-induced gelation of myosin (Fig. 1) is thought to be important. This implies that in the gelling reaction myosin undergoes at least two conformational changes of the molecule each with a different Tm. Tm_1 and Tm_2 obtained in this report are 43° and 55°C, respectively. These values correspond to those for the helix-coil transition of the myosin rod (Burke et al., 1973; Goodno and Swenson, 1975; Samejima et al., 1976), 43–47.5°C for Tm_1 and 55°C for Tm_2. The coincidence of the transition temperatures for myosin gelation with those for thermal unfolding of the helical structure of the myosin rod suggest that the unfolding of the helical tail portion of the myosin molecule may play a role in the heat-induced gelation of myosin.

At pH 6.0 heat-induced gelation of myosin has been found to begin at temperature of 35°C (Fig. 1). Denaturation studies on myosin reported previously (Yasui et al., 1966; Kawakami et al., 1971) indicate that the aggregation of myosin molecules was due to fusion of the head portion of the molecules by heat, which took place at such relatively low temperatures as 35°C. The lower transition temperature of the two might involve this thermal aggregation of myosin heads in addition to the unfolding of the tail portion of the molecule.

Time of storage for stock myosin at 0°C does not seem to affect the heat-induced gelation of myosin at ionic strengths between 0.4–1.0, but time exerts a remarkable influence over gelation at ionic strengths at between 0.1–0.3 (Fig. 3). The shorter the storage time after preparation, the greater was the extent of shear modulus (maximally about 5–6 times higher than the value at high ionic strength) attained and lower was the optimum ionic strength (at about 0.1–0.2). This extremely high gel-forming ability gradually decreases, shifts towards higher ionic strengths (at 0.2–0.3) with storage time, and eventually disappears (Fig. 3). The myosin stored for more than 15 days was no longer able to exhibit gel-formation of desirable shear modulus unless the ionic strength of the system is brought to 0.4–1.0.

Solubility studies (Fig. 4) performed under the same conditions, indicate loss of myosin solubility at low ionic strengths with storage time. The ionic strength range at which the solubility of myosin changed has been found to be between 0.1–0.3. The apparent coincidence of the changes in gel-forming ability at low ionic strengths of myosin, with the changes in solubility can be explained by the well known filament formation of myosin monomers at low ionic strengths (Huxley, 1963; Kaminor, 1966). That is, the finer network structure shown in Figure 5(a) is formed from myosin filaments at low ionic strengths. In fact, Yasui et al. (1966) demonstrated the loss of filament formability of myosin at very early stage of denaturation, where the ATPase activity of myosin is still quite high. Unlike the uniform length of filaments formed by native myosin, huge amorphous aggregates are formed at physiological ionic strength (Yasui et al., 1966), which must affect the solubility of myosin.

The results in Table 1 may confirm that aggregate formation is occurring as the myosin solution age. Although changes in ATPase activity and sulfhydryl content of myosin are small, a gradual increase in viscosity from 2.04 to 2.94 dl/g is observed, suggesting the spontaneous aggregation of myosin molecules, which seems to be a seed of irregular mass (aggregate) at low ionic strength. Furthermore, a slight decrease in sulfhydryl groups in myosin indicates a possible contribution of this reaction to the formation of the myosin aggregates through oxidation of SH groups followed by intermolecular SH- S-S exchange reaction during storage, as has been suggested by Buttkus (1971). The spontaneous transformation of myosin to a less soluble state has also been reported by Connel (1960), Johnson and Rowe (1961) and Mackie (1966).

It is to be noted that the rate of exchange in gel-formability as well as solubility of myosin with storage time is considerably reduced, if the myosin is stored at KCl concentration of 0.1M. This may be explained by the hindering effect of organized myosin filaments on the collision of molecules due to micro-Brownian motion in solution. The effect would be expected to be enhanced, if myosin filaments are immobilized in the built-in architecture of myofibrils, because the myosin in muscle fiber bundles has been reported to show neither aggregation nor inactivation during storage at 3°C and pH 5.5 (Yasui et al., 1973).

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ROLE OF PHOSPHOLIPIDS AND TRIGLYCERIDES IN WARMED-OVER FLAVOR DEVELOPMENT IN MEAT MODEL SYSTEMS

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ABSTRACT

The effects of triglycerides and phospholipids on development of warmed-over flavor (WOF) in cooked meat was studied using model systems from beef and from chicken dark and light meat. Triglycerides, total lipids, total phospholipids, phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were added back to the lipid extracted muscle fibers in each system and WOF development was followed by the TBA test and taste panel scores after heating to 70°C and holding at 4°C for 48 hr. Total phospholipids, especially PE, were shown to be the major contributors to development of WOF in cooked meat. The triglycerides enhanced development of WOF only when combined with the phospholipids (as total lipids). Phosphatidyl choline (PC) did not influence WOF in the model system. Changes in the PUFAs of the phospholipids were shown to be related to development of WOF in cooked meat. Addition of 156 ppm of nitrite significantly ($P < 0.01$) reduced TBA numbers and prevented development of WOF.

INTRODUCTION

CONTROL of lipid oxidation in meat and meat products has increased in importance with greater consumption of precooked meat items for both institutional and home use. Warmed-over flavor (WOF) development occurs in a matter of hours as compared to months for normal oxidative rancidity, hence control is much more critical (Tims and Watts, 1958; Love and Pearson, 1971; Pearson et al., 1977). Although phospholipids have been implicated as being major contributors to WOF (Younathan and Watts, 1960; Zipser and Watts, 1961; Keskinel et al., 1964; Jacobson and Koehler, 1970; Wilson et al., 1976), the relative contributions of total and individual phospholipids to WOF and of total lipids and triglycerides have not been studied in model systems.

The present investigation was undertaken to explore the role of total lipids, phospholipids (total and individual), and triglycerides to lipid oxidation and off-flavor development in cooked model systems of beef and of chicken dark and white meat. The cooked meat model systems offer a unique and useful approach for determining the contributions of the different lipid components to WOF. The role and function of nitrite in controlling lipid oxidation in the cooked meat model systems were also investigated.

MATERIALS & METHODS

Source of meat

The beef, chicken dark and white meat used in this study were obtained from the Michigan State University Meat and Poultry Processing Laboratories. Portions of longissimus dorsi (LD) muscle were excised from beef carcasses at 24 hr postmortem. Thigh (dark meat) and breast (white meat) meat were also removed from chicken carcasses at 24 hr postmortem.

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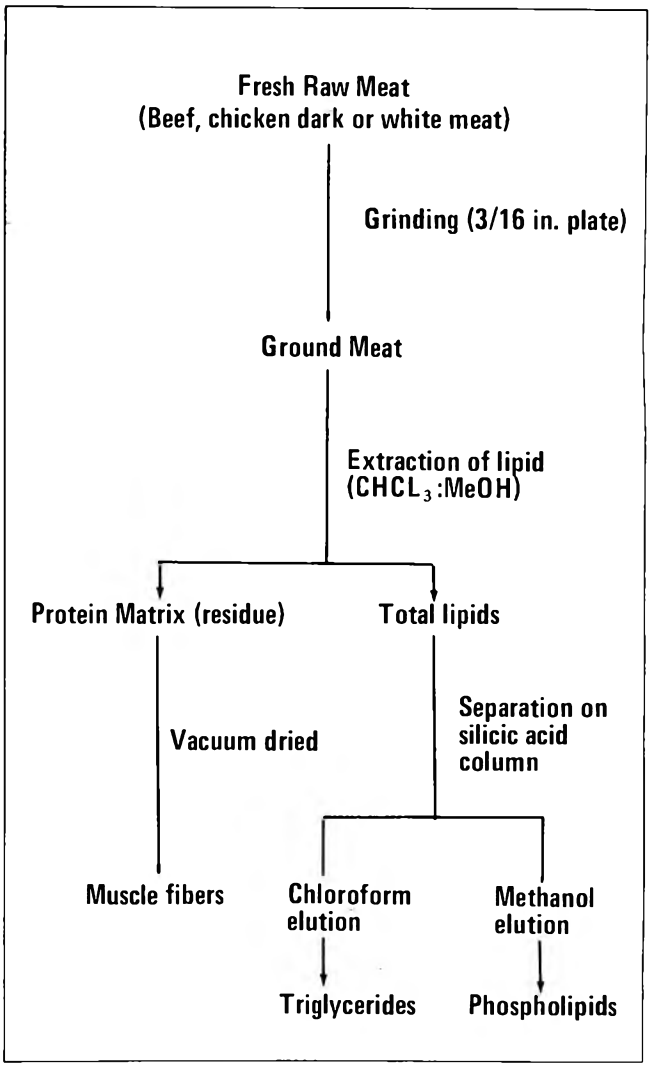

Fig. 1—Preparation of model meat system.

Preparation of model meat system

Lipid-extracted muscle fibers were used as the matrix for the model system. Fresh raw beef, chicken dark and white meat were each ground once through a 3/16-in. plate using a Hobart meat grinder. Total lipids were extracted from the ground meat by the method of Folch et al. (1957). The phospholipids were separated from total lipids using the procedure of Choudhury et al. (1960). The solvent was removed from the residue (protein matrix) by drying under vacuum and later in a stream of nitrogen gas at room temperature. Following removal of the solvent, the model systems were used immediately. The preparation of the model system is outlined schematically in Figure 1.

Design of model meat experimental treatments

The amount of total lipids, triglycerides and phospholipids added back to the model systems closely corresponded to that removed during the extraction process. Total phospholipids were used in the beef and chicken meat model systems at levels of 0.8 and

Table 1—Design of model meat experimental treatments[a]

Meat source	Code no.	Composition of sample systems
Beef	B_1	0.8% beef phospholipids in beef muscle fibers
	B_2	9.2% beef triglycerides in beef muscle fibers
	B_3	10% beef total lipids in beef muscle fibers
	B_4	Beef muscle fibers only (control)
Chicken dark meat[b]	D_1	0.7% dark meat phospholipids in dark meat muscle fibers
	D_2	4.3% dark meat triglycerides in dark meat muscle fibers
	D_3	5.0% dark meat total lipids in dark meat muscle fibers
	D_4	Dark meat muscle fibers only (control)
Chicken white meat[b]	W_1	0.7% white meat phospholipids in white meat muscle fibers
	W_2	4.3% white meat triglycerides in white meat muscle fibers
	W_3	5.0% white meat total lipids in white meat muscle fibers
	W_4	White meat muscle fibers only (control)

[a] Muscle fibers without added lipids served as controls. Each experiment was replicated 4 times.

[b] The same amount of lipids were applied to chicken dark meat and white meat fibers in order to eliminate variations due to lipid levels.

0.7%, respectively. Total lipids and triglycerides were added to the beef model system at levels of 10.0 and 9.2%, respectively. Total lipids and triglycerides were added to both the chicken dark meat and chicken white meat model systems at concentrations of 5.0 and 4.3%, respectively. The design of the experimental treatments is shown in Table 1.

A second experiment was designed (Table 2) to study the relative contributions of PC, PE and total serum phospholipids on development of WOF using the model system. Pure bovine, PC, PE and a serum lipid mixture containing specified amounts of various phospholipid components (Supelco Inc., Bellefonte, PA) were added back to the model system at a level of 0.0375%, either with or without 156 ppm of nitrite. Each of the 8 experimental treatments was prepared using 50 ml deionized water. There was no evidence of WOF or other objectionable odors in the freshly prepared uncooked samples.

Cooking

The samples to be cooked were packed in 6 1/4 × 8 1/2 inch retortable pouches (Continental Diversified Industries, Chicago, IL) and heat sealed under vacuum with a Multi-Vac sealing machine (Busch, W. Germany). The bags containing the samples were cooked to an internal temperature of 70°C in boiling water. Following cooking, the bags were opened, and the contents thoroughly mixed. The meat was then stored at 4°C for 48 hr. TBA values, taste panel scores and the fatty acid composition of the different model systems were then determined.

TBA test

The distillation method of Tarladgis et al. (1960) was utilized to measure the development of oxidative rancidity by the TBA test, except for the samples containing nitrite. Since Hougham and Watts (1958), Younathan and Watts (1959) and Zipser and Watts (1962) have shown that nitrite interferes with the distillation step by nitrosation of malonaldehyde, the modified TBA test of Zipser and Watts (1962), in which sulfanilamide is added to the sample, was utilized for all preparations containing nitrite. TBA numbers were expressed as mg malonaldehyde/kg meat.

Taste panel evaluation

Sensory evaluation was carried out for WOF by 12 trained panelists after 48 hr storage of the cooked meat at 4°C. At each setting, all panelists were presented with four or eight coded samples. A control sample consisting of freshly cooked meat was also presented along with the treated samples. All experimental samples were reheated and served while hot. The panel scoring system was as follows: 1 = very pronounced WOF; 2 = pronounced WOF; 3 = moderate WOF; 4 = slight WOF; and 5 = no WOF. Thus, panel scores concentrated solely on the intensity of WOF.

Table 2—Design of experiment to determine the influence of PC, PE, total serum phospholipid and nitrite on development of WOF using beef model system[a]

Treatment	Composition of sample systems
1	Model system only (control)
2	Model system + nitrite
3	Model system + PC
4	Model system + PC + nitrite
5	Model system + PE
6	Model system + PE + nitrite
7	Model system + serum phospholipids
8	Model system + serum phospholipid + nitrite

[a] Phospholipids were added to the model system at a level of 0.375% and nitrite at 156 ppm: PC = Phosphatidyl choline; PE = Phosphatidyl ethanolamine; Serum phospholipids (lysophosphatidyl choline, 8%; sphingomyelin, 24%; PC, 53%; phosphatidyl serine, 1%; phosphatidyl inositol, 4%; PE, 6%; phosphatidyl glycerol, 2%; cardiolipin, 1%; and phosphatidic acid, 1%).

Lipid analyses

Phospholipids were separated into their classes by one-dimensional thin-layer chromatography (Rouser et al., 1966), using 20 × 20 cm precoated silica Gel G (500 μm thickness) glass plates (Fisher Scientific Co., St. Louis, MO). Exactly 50 mg/ml of total phospholipids (equivalent to 0.457 mg phospholipid/spot) were spotted on each plate under a stream of nitrogen gas. The spots were identified by using a standard mixture of authentic phospholipids (Supelco, Inc.) which was simultaneously spotted on the left hand side of the plate. The separation was accomplished by using a solvent system of chloroform:methanol:water (65:25:4, v/v). For determination of phosphorus, the spots were made visible by spraying with potassium dichromate/sulfuric acid followed by charring and were analyzed as described by Rouser et al. (1966). The values for phosphorus were expressed as mg phosphorus/g phospholipid. Plates for the determination of fatty acids in PC and PE were prepared and sprayed with iodine vapors. The spots containing PC and PE were immediately recovered and eluted with chloroform-methanol (4:1, v/v), evaporated to dryness under nitrogen and redissolved in chloroform, and further rechecked for purity by TLC.

The standard triglycerides, total phospholipids, PC and PE used for fatty acid analyses were converted to methyl esters by the borontrifluoride/methanol procedure as described by Morrison and Smith (1964). The analysis of all fatty acid methyl esters was performed using a Perkin-Elmer model 900 gas chromatography equipped with a hydrogen flame ionization detector. The column, 6 ft × 2 mm (i.d.) stainless steel, was packed with 10% (w/w) diethylene glycol succinate (DEGS) on Supelcoport (Supelco, Inc.). The column was set at 185°C, the injection port at 220°C and the detector at 250°C. the carrier gas was helium and the flow rate was maintained at 30–40 ml/min while hydrogen gas and air were adjusted to 30 and 285 ml/min, respectively. Quantitative identification of the emerging peaks was done using retention times of standard mixtures of known fatty acid methyl esters (Applied Science Lab., Inc., State College, PA; Supelco, Inc.). Peak areas were calculated quantitatively as the product of peak height and width at half height and results were expressed as percentage of the total area.

Statistical methods

Analysis of variance for TBA values and taste panel was calculated using a Michigan State University computer package program identified as MSU Stat System and was run on a Control Data Corporation (CDC) 6500 computer. Correlation coefficients were also calculated and the significance between treatments was determined using Tukey's test for multiple comparisons.

RESULTS & DISCUSSION

Changes in TBA values and in taste panel scores

Mean TBA values and their corresponding mean sensory scores are presented in Table 3. Results showed that the model systems containing added total lipids gave the highest TBA values, followed by samples containing total phospholipids, triglycerides, and the lipid-free muscle fibers

Table 3—TBA numbers and sensory scores for cooked beef, chicken dark meat and white meat model systems[a,b]

Meat type	Composition of treatments	Mean TBA no.	Mean sensory scores[c]
Beef	B_1=0.8% phospholipids	5.76b	2.69a
	B_2=9.2% triglycerides	1.88a	3.84b
	B_3=10% total lipids	6.81b	2.64a
	B_4=control	1.16a	3.75b
Chicken dark meat	D_1=0.74% phospholipids	8.48b	2.97a
	D_2=4.3% triglycerides	6.30a,b	3.65a,b
	D_3=5% total lipids	11.74c	3.25a
	D_4=control	5.78a	3.88a,b
Chicken white meat	W_1=0.7% phospholipids	5.03b	2.80a
	W_2=4.3% triglycerides	2.99a	3.35a
	W_3=5% total lipids	5.53b	3.09a
	W_4=control	2.18a	4.02b

[a] There were four replicates for each treatment.
[b] Taste panel score were from 1–5, where 1=very pronounced WOF and 5=No WOF.
[c] All numbers in same column within a meat type bearing the same letter are not significant at the 5% level.

Table 4—TBA numbers and sensory scores for cooked meat model systems containing added PC, PE, serum phospholipids or nitrite[a,b]

Experimental treatment		Mean TBA number	Mean sensory score
1.	Model system only	0.36 ± 0.07b	3.33 ± 0.42a,b
2.	Model system + nitrite	0.26 ± 0.06a	4.11 ± 0.44b,c
3.	Model system + PC	0.34 ± 0.03a,b	3.36 ± 0.51a,b
4.	Model system + PC + nitrite	0.29 ± 0.07a	4.52 ± 0.27c
5.	Model system + PE	0.81 ± 0.13d	2.64 ± 0.58a
6.	Model system + PE + nitrite	0.36 ± 0.07b	3.61 ± 0.23b
7.	Model system + TP	0.62 ± 0.12c	3.29 ± 0.49a,b
8.	Model system + TP + nitrite	0.34 ± 0.05a,b	4.04 ± 0.43b,c

[a] Each treatment was replicated four times.
[b] A significant ($P < 0.01$) "r" value of −0.62 was found between TBA numbers and sensory score. Numbers in the same column bearing same letter are not significant at 5% level.

(control), respectively. Although the samples containing total lipids exhibited the highest TBA values, they did not consistently show the lowest flavor ratings. This is consistent with the report by Love and Pearson (1971) suggesting that neutral lipids in association with polar lipids may trap volatile decomposition products of polar lipids and thus reduce their effect upon flavor.

Analysis of variance for TBA values and sensory scores revealed that the experimental treatments were significantly ($P < 0.001$) different. Total lipids and total phospholipids significantly ($P < 0.01$) increased TBA values while significantly ($P < 0.05$) reducing flavor scores. Thus, total lipids and total phospholipids were shown to be major contributors to development of WOF in the cooked meat model systems.

Results showed that neither the model system (control) nor the triglycerides alone significantly influenced development of WOF in cooked meat. This is in agreement with the report of Acosta et al. (1966), who pointed out that the phospholipid fraction of total lipids rather than the triglycerides is responsible for oxidative deterioration in cooked turkey meat. Younathan and Watts (1960) have demonstrated that the TBA reactive material in cooked pork was largely associated with the proteolipid fraction. Thus, the evidence seems clear for the involvement of total phospholipids in development of WOF in cooked meat. The present study confirms the reports of a number of investigators (Younathan and Watts, 1960; Keskinal et al., 1964; Jacobson and Koehler, 1970; Wilson et al., 1976), who have suggested that total phospholipids are responsible for development of WOF in cooked meat. Although triglycerides alone did not significantly influence the development of WOF in cooked meat, they did have an additive effect when combined with phospholipids.

Statistically significant ($P < 0.05$) correlation coefficients of $r = -0.57$ and $r = -0.51$ were found between TBA values and sensory scores for beef and chicken white meat model systems, respectively. Results confirm the relationship between WOF and panel scores, although the degree of association indicates that changes in TBA values account for only 25 and 32% of the variation in panel scores for the chicken white meat and beef model systems, respectively. The data are in agreement with the results of Zipser et al. (1964), who reported a good relationship between TBA values and development of oxidized flavor in cooked meat.

In the case of chicken dark meat, however, the correlation between TBA values and sensory scores was not statistically significant. The low correlation coefficient may be due to the retention of lipids by the extracted muscle fibers. Chicken dark meat fibers (control) had a higher mean TBA value of 5.78 in comparison to values of 1.16 and 2.18 for beef and chicken white meat, respectively, indicating that the chicken dark meat model system probably contained some unextracted lipids. The unextracted lipids in combination with heme pigments have been reported to accelerate autoxidation of cooked mullet by Zipser and Watts (1961), suggesting that the heme pigments may help contribute to the high TBA values in the residual dark meat system of the current investigation. Any residual lipids would cause high TBA values of the extracted muscle fibers and decrease the relationship with sensory scores. There is, however, no apparent influence on sensory scores, which suggests the major effect is upon TBA values. However, the relatively high TBA value for the control would decrease the relationship between TBA values and panel scores.

It should be pointed out that TBA values are not specific for WOF but are indicative only of oxidation (Sato and Hegarty, 1971; Sato et al., 1973; Pearson et al., 1977). Use of a trained taste panel is probably the best way to measure WOF, but even then it is not clearly established that a panel can differentiate between WOF and other types of autoxidative changes. Nevertheless, TBA values have been widely used to follow WOF development (Tims and Watts, 1958; Sato and Hegarty, 1971; Sato et al., 1973; Love and Pearson, 1974).

As mentioned earlier herein, Zipser and Watts (1961) suggested that heme pigments may catalyze tissue oxidation and account for the increase in TBA numbers and development of rancidity in cooked meat. Support for this concept is found in the work of Keskinel et al. (1964) who reported higher TBA values in cooked turkey dark meat than in light meat. The catalytic effect of heme pigments has been espoused by a number of investigators (Lew and Tappel, 1956; Younathan and Watts, 1959; Liu, 1970; Liu and Watts, 1970). More recently, several studies (Sato and Hegarty, 1971; Sato et al., 1973; Love and Pearson, 1974) with model systems have demonstrated that myoglobin does not catalyze WOF, whereas, ferrous iron accelerated autoxidation. Recently, Igene et al. (1979) clarified the role of myoglobin and ferrous iron in development of WOF by demonstrating that cooking releases the iron from myoglobin and thereby indirectly accelerates development of autoxidation. Thus, myoglobin per se is not the catalyst for oxidation but cooking cleaves the heme pigments to release

free non-heme iron, which then serves as the direct catalyst for autoxidation.

Effect of PC, PE and serum phospholipids on development of WOF in cooked meat

Since total phospholipids were major contributors to development of WOF in cooked meat, it was considered imperative to evaluate the contributions of PC and PE (which made up over 75% of total phospholipids) and nitrite in development of WOF in cooked meat. Results of this experiment are presented in Table 4. Differences between experimental treatments were statistically significant ($P < 0.001$) for both TBA numbers and sensory scores. Addition of PE and serum phospholipids significantly ($P < 0.01$) increased TBA values while decreasing sensory scores. The addition of PC did not significantly influence either TBA numbers or sensory scores. Addition of 156 ppm of nitrite significantly ($P < 0.01$) reduced TBA numbers and improved sensory scores for all samples.

Sato and Hegarty (1971) showed that nitrite will completely eliminate WOF at 220 ppm and will inhibit development at 50 ppm. Recently, Fooladi (1977) showed that 156 ppm of nitrite effectively prevents WOF in cooked meat and poultry. Zipser et al. (1964) proposed that nitrite forms a stable complex with iron porphyrins in heat denatured meat, thereby inhibiting the development of WOF. The inhibition of lipid oxidation in model systems containing only residual pigments suggests that nitrite converted the pigments to catalytically inactive forms and may have also directly reacted with unsaturated fatty acids. Thus, the results are in agreement with the report by Cassens et al. (1976) indicating that nitric oxide reacts with unsaturated fatty acids.

Results of this study have demonstrated that PE is the most important phospholipid component contributing to development of WOF. However, other phospholipid components also have an additive effect. Corliss and Dugan (1970) and Tsai and Smith (1971) reported that PE exerted a much greater pro-oxidant effect than PC. Hence, this study confirms the involvement of PE in development of off-flavors, and especially in WOF in cooked meat and meat products.

Changes in phospholipid components due to cooking and storage at 4°C

Quantitative composition of phospholipid components was determined before and after cooking the samples using the phosphorus assay procedure of Rouser et al. (1966). The compositional changes in the phospholipid components as a result of cooking are presented in Table 5. Results indicated that cooking caused significant ($P < 0.01$) decreases in total phospholipids. PC and PE did not consistently behave in the same manner during cooking. While a significant decrease in PE was not observed in beef, PC was found to decrease by 45.0% after cooking. Changes in PE and PC in chicken dark meat were opposite to those for beef, i.e., PE significantly decreased while PC was stable to cooking. In chicken white meat, highly significant ($P < 0.01$) losses occurred in both PE and PC during cooking and

Table 5—Mean changes in phospholipids due to cooking and storage at 4°C[a,b,c]

Classes of phospholipids	Beef model system		Chicken dark meat model system		Chicken white meat model system	
	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked
LPC[d]	2.23	0.97	2.55	1.44	1.86a	1.15a
PE[e]	3.57a	2.78a	5.81	2.55	7.91	4.00
PC[f]	10.57	5.73	15.48a	13.48a	18.81	10.86
Others[g]	3.0	1.16	7.67	10.24	4.58	7.97
Tp[h]	19.34	10.64	31.51	28.14	33.16	23.98

[a] Values given are mg phosphorus/g phospholipid.
[b] There were four replicates for each component.
[c] All numbers in same column within a given meat type bearing the same letter are not significantly different at $P < 0.05$.
[d] LPC = Lysophosphatidyl choline.
[e] PE = phosphatidyl ethanolamine.
[f] PC = Phosphatidyl choline.
[g] Other phospholipids were calculated by difference.
[h] Total phospholipids.

Table 6—Mean fatty acid composition of triglycerides in model meat systems[a]

Fatty acid	Beef		Chicken dark meat		Chicken white meat	
	Prior to cooking	After cooking	Prior to cooking	After cooking	Prior to cooking	After cooking
14:0	3.15	1.86	0.54	0.58	0.84	0.63
14:1	–	0.80	–	–	–	–
15:0	–	–	–	–	0.80	–
16:0	25.26	32.92	22.46	19.94	25.14	23.08
16:1	3.62	4.20	3.70	4.08	2.77	3.85
17:0	1.02	0.53	–	0.69	–	–
18:0	22.64	14.16	7.57	7.23	8.30	7.30
18:1	42.19	44.29	39.07	41.27	34.32	39.49
18:2	1.27	1.24	25.03	24.48	24.93	23.94
18:3ω6	–	–	–	–	–	1.20
18:eω3	0.85	–	1.63	1.73	2.90	0.51
% Sat.	52.07	49.47	30.57	28.44	35.08	31.01
% Mono.	45.81	49.29	42.77	45.35	37.09	43.34
% Dienoic	1.27	1.24	25.03	24.48	24.93	23.94
% Polyenoic	0.85	–	1.63	1.73	2.90	1.71
Total Unsat.	47.93	50.53	69.43	71.56	64.92	68.99

[a] Percent of total fatty acids

Table 7—Changes in the fatty acid composition of total phospholipids of meat model systems due to cooking and storage at 4°C[a]

Fatty acid	Beef		Chicken dark meat		Chicken white meat	
	Prior to cooking	After cooking	Prior to cooking	After cooking	Prior to cooking	After cooking
14:0	1.46	0.72	0.14	0.17	1.54	0.87
14:1	0.52	–	–	–	–	–
15:0	1.16	1.86	0.37	0.57	1.94	0.69
16:0	18.49	18.73	15.60	16.52	21.94	18.98
16:1	3.61	3.67	1.05	1.26	1.49	2.76
16:2	0.74	0.25	–	–	–	–
17:0	0.92	0.84	–	–	–	1.07
18:0	12.95	12.83	18.16	21.03	10.25	10.74
18:1	33.44	34.81	20.43	19.74	25.62	33.87
18:2	10.52	12.66	21.51	19.22	20.32	16.65
18:3ω6	0.37	–	0.37	0.23	–	–
18:3ω3	1.29	0.69	0.41	0.43	0.57	0.85
20:2	0.69	0.21	–	–	0.61	0.56
20:3	2.77	1.92	0.53	0.52	1.20	0.62
20:4	8.51	9.32	17.41	18.88	11.26	6.90
20:5	0.76	–	–	–	0.43	1.62
22:4	0.88	0.42	1.23	0.57	–	0.36
22:5ω6	–	–	–	–	2.22	2.15
22:5ω3	0.92	1.08	–	–	0.60	1.30
22:6	–	–	2.79	0.86	–	–
% Sat.	34.98	34.98	34.27	38.29	35.67	32.35
% Mono.	37.57	38.48	21.48	21.00	27.11	36.63
% Dienoic	11.95	13.12	21.51	19.22	20.93	16.65
% Polyenoic	15.50	13.42	22.74	20.83	16.28	14.37
Total Unsat.	65.02	65.02	65.73	61.05	64.32	67.65

[a] Percent of total fatty acids

storage. Decreases in the component phospholipids may be due to either autoxidation, hydrolytic decomposition, lipid browning *reactions* or lipid-protein co-polymerization as outlined by Lea (1957). Products of lipid autoxidative degradation have been associated with development of off-flavors, principally with WOF (Ruenger et al., 1978).

Changes in fatty acid composition due to cooking

Triglycerides. The fatty acid profiles of the samples containing added triglycerides were determined prior to cooking and again following cooking and storage, and are presented in Table 6. In beef samples, the saturated fatty acids decreased from 52.07 to 49.47%, while the monoenes increased from 45.81 to 49.29%. Altogether this resulted in a net increase of 2.60% in total unsaturation (Table 6). In the chicken samples, total unsaturation increased from 69.42 to 71.56% in dark meat, and from 64.92 to 68.99% in the white meat. These results are consistent with reports showing that only minor changes take place in the fatty acid content of the triglycerides due to cooking (Chang and Watts, 1952; Giam and Dugan, 1965; Campbell and Turkki, 1967). Thus, the stability of the triglycerides is consistent with the low TBA values (Table 3).

Total phospholipids. Compositional changes in the fatty acid profiles in total phospholipids of meat model systems due to cooking and storage are shown in Table 7. In beef, the levels of saturated fatty acids were unchanged. The monoenes increased slightly, the dienes from 11.95 to 13.12%, while the polyenes decreased from 15.50 to 13.42%; a loss of 13.42% in unsaturation from the original value. In chicken dark and white meat, the concentration of $C_{18:2}$ fatty acid decreased during cooking. Arachidonic acid ($C_{20:4}$) increased from 17.41 to 18.88% in chicken dark meat but decreased from 11.26 to 6.90% in chicken white meat. The level of polyenoic acids decreased from 22.74 to 20.83% and from 16.28 to 14.37% in chicken dark meat and white meat, respectively.

Although total unsaturation was not drastically affected by cooking and storage, nevertheless, the moderate changes that occurred in both the dienoic and the polyenoic fatty acids may be important in development of WOF. Several workers (Lea, 1957; Younathan and Watts, 1960; Keller and Kinsella, 1973) have reported that PUFAs may be involved in the heat induced degradation of lipids which results in off-flavors. Recently, Ruenger et al. (1978) have reported that WOF is a result of autoxidation of tissue lipids. Thus, the present study demonstrates that decreases in total phospholipids, as well as in PUFAs, may be related to the development of WOF in cooked meat.

The fatty acid profiles for PC and PE in beef phospholipids are presented in Table 8. Following cooking, total unsaturation in PC increased from 53.36 to 65.44%, mainly at the expense of the saturated fatty acids. $C_{18:2}$, $C_{20:4}$ and $C_{22:4}$ fatty acids increased from the original value by 106.0, 34.4 and 59.1%, respectively. In PE, however, total unsaturation decreased from 67.30 to 64.67%. $C_{20:2}$ and $C_{22:4}$ fatty acids of PE decreased by 74.5 and 55.1% from the original value, respectively.

The fatty acid profiles of PC and PE for chicken dark meat phospholipids are presented in Table 9. Total unsaturation in PC increased from 58.90 to 64.99% following cooking and storage. A slight increase in total unsaturation of PE also occurred. Unlike beef, in chicken dark meat the $C_{18:2}$ fatty acid decreased in both PC and PE. Arachidonic acid increased from 17.84 to 21.10% in PC but decreased from 18.68 to 13.95% in PE.

The fatty acid profiles recorded for PC and PE in chicken white meat are presented in Table 10. In PC, $C_{18:2}$, $C_{20:4}$, $C_{20:5}$ and $C_{22:4}$ fatty acids decreased by 61.30, 53.60, 27.75 and 23.52% from the original value, respectively. For PE, $C_{18:2}$ decreased from 11.08 to 8.92%, $C_{20:2}$ from 5.97 to 1.22%, and $C_{20:4}$ decreased from 17.52 to 3.36% during cooking and storage. The significant loss of $C_{20:4}$ underlines its vulnerability to cooking, especially in PE.

Holman and Elmer (1947) attributed the tendency of

Table 8—Changes in fatty acid composition of phosphatidyl choline and phosphatidyl ethanolamine of beef model system[a]

	Phosphatidyl choline (PC)		Phosphatidyl ethanolamine (PE)	
Fatty acid	Prior to cooking	After cooking	Prior to cooking	After cooking
12:0	6.05	0.38	0.48	0.95
14:0	5.14	0.44	1.55	1.04
14:1	–	–	–	1.08
15:0	1.00	0.32	0.33	2.50
16:0	21.97	14.81	13.96	10.42
16:1	0.73	0.95	1.65	–
17:0	1.94	0.76	1.49	2.78
18:0	10.53	17.85	14.89	16.79
18:1	23.73	20.25	19.50	11.56
18:2	4.48	9.23	4.80	9.75
18:3ω6	–	–	–	0.33
18:3ω3	0.27	–	0.37	0.28
20:1	–	–	–	–
20:2	0.78	2.47	1.06	0.27
20:3	–	0.38	0.37	0.78
20:4	20.34	27.34	32.75	37.35
20:5	–	–	–	0.76
22:4	3.03	4.82	6.80	3.05
22:5	–	–	–	0.31
% Saturated	46.63	34.56	32.70	34.48
% Monoencic	24.46	21.20	21.15	12.64
% Dienoic	5.26	9.23	5.86	10.02
% Polyenoic	23.64	35.01	40.29	42.56
Total Unsaturation	53.56	65.44	67.30	64.67

[a] Percent of total fatty acids

Table 9—Changes in fatty acid composition of phosphatidyl choline and phosphatidyl ethanolamine of dark meat model system[a]

	Phosphatidyl choline (PC)		Phosphatidyl ethanolamine (PE)	
Fatty acid	Prior to cooking	After cooking	Prior to cooking	After cooking
14:0	–	–	0.25	0.22
14:1ω6	–	0.53	0.33	–
15:0	0.93	0.92	1.60	1.53
16:0	19.70	15.56	15.73	16.70
16:1ω7	–	–	–	–
16:2	–	–	–	–
17:0	3.35	1.98	2.70	2.74
18:0	17.14	15.75	23.23	20.95
18:1ω9	15.45	14.56	16.39	23.00
18:2ω6	10.78	8.39	11.84	6.41
18:3ω6	0.42	0.53	0.33	0.60
18:3ω3	0.35	0.27	0.28	1.70
20:2ω6	2.76	3.94	1.06	0.75
20:3ω6	0.24	0.24	0.23	0.26
20:4ω6	17.84	21.10	18.68	13.95
20:5ω3	0.28	2.32	–	4.43
22:3ω6	–	–	0.35	0.75
22:4ω6	10.78	13.91	5.94	5.45
22:5ω6	–	–	0.57	0.56
22:6ω3	–	–	0.49	–
% Saturated	41.12	34.21	43.51	41.92
% Monoenoic	15.45	15.09	16.72	23.22
% Dienoic	13.54	12.33	12.90	7.16
% Polyenoic	29.94	37.57	26.87	27.70
Total Unsaturation	58.90	64.99	56.49	58.08

[a] Percent of total fatty acids

Table 10—Changes in fatty acid composition of phosphatidyl choline and phosphatidyl ethanolamine of white meat model system[a]

Fatty acid	Phosphatidyl choline (PC)		Phosphatidyl ethanolamine (PE)	
	Prior to cooking	After cooking	Prior to cooking	After cooking
14:0	0.34	–	1.80	–
14:1	1.38	–	1.56	–
15:0	1.55	0.97	4.14	0.47
16:0	17.84	16.54	13.85	15.42
16:1	–	1.72	–	3.39
16:2	–	–	–	–
17:0	1.72	4.47	2.25	3.34
18:0	12.07	9.45	13.64	14.02
18:1	14.94	20.79	15.92	23.13
18:2	10.11	3.91	11.08	8.92
18:3ω6	0.52	–	0.28	–
18:3ω3	0.40	6.44	0.33	6.68
20:2	5.52	9.71	5.97	1.22
20:3	–	4.12	0.28	3.36
20:4	16.66	7.73	17.52	3.36
20:5	11.89	8.59	7.58	9.11
22:3	–	–	–	–
22:4	5.06	3.87	3.79	7.24
22:5ω6	–	–	–	0.35
22:5ω3	–	1.68	–	–
22:6	–	–	–	–
% Saturated	33.52	31.43	35.68	33.25
% Monoenoic	16.32	22.51	17.48	26.52
% Dienoic	15.63	13.62	17.05	10.14
% Polyenoic	34.53	32.43	29.78	30.10
Total Unsat.	66.48	68.56	64.31	66.76

[a] Percent of total fatty acids

the phospholipids to oxidize to their high content of $C_{20:4}$, $C_{22:3}$, $C_{22:4}$, $C_{22:5}$, and $C_{22:6}$ fatty acids. Thus, the changes in some specific PUFAs, which occurred in total phospholipids (Table 7) and PC and PE (Tables 8, 9 and 10), may be of great significance to development of WOF. Greater losses in PUFAs occurred in PE than in PC. Most of the losses were observed in the $C_{18:2}$, $C_{20:4}$ and $C_{22:4}$ fatty acids associated with chicken dark and white meat. Love and Pearson (1971) and Keller and Kinsella (1973) have indicated that loss of $C_{20:4}$ fatty acid was consistent with its greater propensity to undergo autoxidation, especially when associated with PE. Thus, results of this study confirm that PUFAs associated with PE tend to be more labile to heat than those of PC. Hence, PE appears to be more significant in the development of WOF than PC.

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PROTEIN EFFICIENCY RATIO AND AMOUNTS OF SELECTED NUTRIENTS IN MECHANICALLY DEBONED TURKEY MEAT

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ABSTRACT

Three types of mechanically deboned turkey meat (MDTM) were obtained from a Pennsylvania poultry processing plant and studied for nutrient composition. Samples of MDTM included meat from turkey breast cages (protein 15.4%, fat 10.2%, moisture 71.3%), turkey racks (protein 13.4%, fat 17.0%, moisture 67.9%), and turkey backs (protein 13.0%, fat 21.7%, moisture 65.9%). Protein efficiency ratios (PER) were determined on each type, and with and without an antioxidant. Material without an antioxidant resulted in gross errors. Adjusted PER values of MDTM treated with an antioxidant were significantly higher than the 2.50 value for the standard casein diet. Amino acids, fatty acids, caloric content and selected minerals were also measured.

INTRODUCTION

MECHANICALLY DEBONED poultry meat (MDPM) or specific class separations of this material, mechanically deboned turkey meat (MDTM) and mechanically deboned spent layers (MDSL) are currently being used in a variety of food products. The major use is in sausage-type products such as frankfurters and bologna.

Blackshear et al. (1966) were among the earliest reporters on the possible uses of mechanically deboned poultry meat. Later studies dealt more specifically with the use of this material in frankfurter emulsions (Saffle, 1968; Froning et al. 1971). Schnell et al. (1973) demonstrated that MDPM can be used at the 100% level for chicken frankfurters. Compositional studies by Grunden et al. (1972) provided additional information on the quality and functional properties of MDPM. Microbiological studies of MDPM were conducted by Ostovar et al. (1971), Maxey et al. (1973), Mast and MacNeil (1975) and Mulder and Dorresteijn (1975).

The concern of consumer groups and federal regulatory agencies has created a need for information on the nutritional characteristics of MDPM. Amino acid profiles of MDPM were first reported by Essary and Ritchey (1968). Brinkman and MacNeil (1976) reported unadjusted protein efficiency ratio (PER) values of 3.60 for hand deboned broiler neck meat and 3.70 for mechanically deboned neck meat. Later MacNeil et al. (1978) reported an adjusted PER value of 2.65 for mechanically deboned broiler meat.

While some nutritional information of MDPM is beginning to appear in the literature, additional data for MDTM are required on the various types now available. This study was undertaken to provide this additional information.

EXPERIMENTAL

THREE TYPES of MDTM were obtained from a commercial processing plant in Pennsylvania and were selected to reflect types available commercially. Samples consisted of deboned meat from turkey breast cages (BR), whole turkey racks or frames (RK) and turkey backs (BK). All turkey parts were from 21-wk old Broad Breasted Bronze males. A small portion of back skin remained with RK and BK samples. Three 22.7 kg boxes of each type were obtained at approximately 10 minute intervals from a Yieldmaster deboning machine set to draw between 20–25 amps. Approximately 30 min were allowed to elapse between different types to make sure that any material from the previous testing had been ejected from the machine. The nine boxes of MDTM, cooled to 5°C were immediately transported to the poultry products laboratory at the University and placed in a −37°C air blast freezer. Additional samples (3 boxes of each type) were obtained in which BHA plus citric acid was added at 0.02% of the fat content. These samples were to be freeze-dried for rat feeding trials. Prior to freezing a pooled sample was obtained by removing at random 4.1 kg from each sample box. The combined material was blended in a commercial bakery mixer for 2 min. The three pooled samples were frozen at −37°C.

Official methods of the AOAC (1975) were used to determine fat (petroleum ether extractables), moisture, ash, fatty acids and protein by Kjeldahl nitrogen determination (N × 6.25 for meat protein). Iron and zinc were measured on the dry-ashed samples by atomic adsorption spectrophotometry (Perkin-Elmer Co., Norwalk, CT), calcium content by a Corning Calcium Analyzer (Corning Scientific Inst. Medford, MA). Calorie content was measured by using approximately 1g of dried meat subjected to analysis according to the procedure outlined for the Parr Automatic Adiabatic Calorimeter (Parr Instrument Co., Moline, IL). Total amino acids were determined using procedures for the Beckman Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA), and oxidative stability changes were monitored by the 2-thiobarbituric acid (TBA) test (Tarladgis et al., 1960). All analyses, with the exception of amino acids, fatty acids and PER, were performed in duplicate on each sample by making a total of six determinations per MDTM type.

PER values were determined on the pooled samples of MDTM. Frozen blocks of MDTM were cut into 5.1 cm cubes using a meat band saw, packed in plastic bags, and freeze dried by a commercial freeze-drying plant. After freeze drying the material was ground in a Hobart Meat grinder using a 0.32 cm plate opening. This material was placed in sealed containers and held at 21°C until incorporated into the experimental diets. Proximate analysis, amino acids and fatty acids were determined on freeze-dried material as well as fresh MDTM to detect possible changes during freeze-drying.

AOAC (1975) procedures for PER were followed except that fat contents of the diets were adjusted to compliment the higher fat content of the test material. For example, inclusion of turkey backs in the diets in amounts to give the desired 9% protein level resulted in a 14.5% dietary fat content which was in excess of the 8% recommended in the test procedure. Thus, all other diets were adjusted to 14.5% fat with turkey fat containing BHA + citric acid in order to minimize variation in dietary fat content. This procedure is similar to that suggested by Hurt et al. (1974). The casein containing reference diets were fed at both the 8 and 14.5% fat contents. Each test diet was fed to ten male weanling rats (Wistar strain) which were housed in individual cages. Feed and water were supplied ad libitum.

The PER values obtained were adjusted with the 14.5% fat reference diet being given the value of 2.5. Analyses of Variance and Duncan's Multiple Range (Duncan, 1955) test were performed on the actual PER values before adjustment to 2.5.

Sensory analyses of the three types of MDTM were conducted by a trained taste panel using test procedures as outlined by Larmond (1977). Tests were performed on samples stored at −22°C for 1 month. At this same time additional samples were removed from the freezer, held at room temperature for 2 hr then placed in a refrigerator (3°C) for 24 hr. Samples were then cooked in electric skillets until all the pink color had disappeared (approximately 20

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min). Cooked material was then transferred into small paper cups, placed in coded cup holders, and presented to the panel member. Red lights were used to mask any color differences. All tests were conducted in the Sensory Evaluation Laboratory of the Food Science Department.

RESULTS & DISCUSSION

MECHANICALLY DEBONED turkey meat from BR (Table 1) contained 15.4% protein while MDTM from RK and BK was slightly lower at 13.4 and 13.0%, respectively. Fat content was highest (21.7%) for BK and lowest for BR (10.2%) with meat from RK containing 17.0%. The fat content reported here for BK is somewhat lower than that reported earlier by MacNeil et al. (1978) for broiler backs; i.e. 24.1%. It still reflects the fact that when skin is processed through a deboner, fat is expressed from the skin and included with the meat. This was first reported by Froning et al. (1973) and later confirmed by MacNeil et al. (1977).

Moisture content varied with source of MDTM and indicated that breast cages had the highest moisture content and reflect the ability of these materials to retain or absorb more moisture than the back portion, which also contains some skin.

Selected nutrient contents for the three types of MDTM showed that zinc content was significantly ($P < 0.01$) higher for MDTM from BK while calcium was significantly ($P < 0.01$) higher in MDTM from BR. As expected kilocalories were significantly ($P < 0.01$) different for each type and increased as fat content increased.

In a first experiment MDTM was used in a determination of PER values in which the test material was not treated with an antioxidant. It was obvious very early in the test that the rats did not readily eat the test diets containing MDTM. On closer inspection the odor of these diets indicated that the diet materials had become rancid. TBA values obtained initially and on the test diets at 21 days are included along with the test data (Table 2) for the 28 day feeding period. It can be seen that turkey RK and BK have relatively high TBA values before the feeding trial had started with the lower fat turkey BR showing good storage

Table 1—Proximate analysis and selected nutrient content of mechanically deboned turkey meat (wet weight basis)

Characteristic	BR	RK	BK
Protein (%)	15.4a[a]	13.4b	13.0b
Fat (%)	10.2a	17.0b	21.7c
Moisture (%)	71.3a	67.9b	65.9c
Ash (%)	1.4a	1.1b	0.9c
Calcium (%)	0.235a	0.145b	0.095c
Iron (mg/100g)	1.7a	1.6a	1.7a
Zinc (mg/100g)	2.7a	2.9b	3.1c
Kilocalories/100g	184a	233b	261c

[a] Means followed by the same letter are not significantly different ($P > 0.01$) from each other. Each mean represents six determinations.

Table 2—Growth and protein efficiency ratios (PER) for rats fed mechanically deboned turkey meat without an added antioxidant

Diet	Total wt gained (g)	Total feed consumed (g)	Total protein consumed (g)	PER	TBA values[a]	TBA values[b]
Casein (8% fat)	111.7	401.7	36.2	3.09		.37
Casein (14.5% fat)	111.9	381.9	34.4	3.25		.33
Turkey BR	61.6	302.5	27.2	2.26	1.93	7.92
Turkey RK	9.56	155.2	14.0	0.68	5.88	8.58
Turkey BK	1.37	158.8	14.3	0.09	5.51	8.97

[a] Values are obtained on MDTM prior to freeze-drying and is reported as mg of malonaldehyde per 1000g of sample.
[b] Values obtained at 21 days on test diets.

Table 3—Growth and protein efficiency ratios (PER) for rats fed mechanically deboned turkey meat.

Diet	Total wt gained (g)	Total feed consumed (g)	Total protein consumed (g)	Adjusted PER
Casein (8% fat)	94.7b[a]	435.8b	39.2b	2.42b
Casein (14.5% fat)	95.7b	432.4b	38.6b	2.50b
Trukey BR	137.8a	526.9a	47.4a	2.92a
Turkey RK	139.8a	505.0a	45.5a	3.09a
Turkey BK	134.1a	491.5a	44.2a	3.05a
Broiler necks (skinless)	139.5	451.2	40.6	2.65

[a] Means within columns followed by the same letter are not significantly different ($P > 0.01$) from each other.

Table 4—TBA values of mechanically deboned turkey meat used in PER studies

	MDTM after freeze-drying	Diets containing MDTM after 35 days
Turkey BR	2.32[a]	2.32
Turkey RK	2.32	3.16
Turkey BK	3.10	5.50

[a] Each value represents the mean of two determinations.

Table 5—Amino acid analysis of freeze-dried deboned turkey meat (grams) of amino acid residue per 100g of total amino acid residues)

Amino acid	MDTM source BR	RK	BK
	Essential Amino Acids[a]		
Histidine	2.79	3.11	2.29
Lysine	7.64	7.56	7.44
Threonine	5.12	5.11	5.20
1/2 Cystine	0.86a[c]	0.78a	0.51b
Valine	4.57ab[c]	4.45b	4.64a
Methionine	2.22	2.41	2.46
Isoleucine	3.83	3.66	3.68
Leucine	8.09a[c]	7.91b	8.08a
Phenylalanine	3.15	3.19	3.19
Tryptophan	ND[b]	ND	ND
Total	38.26	38.18	37.49
	Nonessential Amino Acids		
Arginine	4.89	4.76	4.77
Aspartic acid	9.90	9.73	9.77
Serine	5.64	5.62	5.75
Glutamic acid	13.78b[c]	14.51a	14.35a
Proline	5.47	5.40	5.58
Glycine	9.89	9.93	10.06
Alanine	9.53	9.34	9.57
Tyrosine	2.56	2.51	2.58
Total	61.66	61.79	62.43

[a] Each value represents the mean of 4 determinations on the pooled sample.
[b] ND—Not determined.
[c] Means followed by the same letter are not significantly different ($P > 0.01$) from each other

Table 6—Fatty acid composition in machanically deboned turkey meat

	Fatty acids							
	Lauric	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
Treatment	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
	Percent of total fatty acids							
Turkey BR	0.8[a]	1.8	24.0	1.5	10.3	32.2	27.2	1.8
Turkey RK	0.9	1.8	22.0	1.7	11.1	32.2	28.2	2.2
Turkey BK	0.6	1.5	21.4	2.0	11.9	32.9	27.9	1.8
Broiler SNBK[b]	Trace	0.8	24.9	4.8	7.1	46.8	14.3	1.5

[a] Each value represents the mean of 4 determinations on the pooled sample.
[b] Skinless necks and backs from broilers (MacNeil et al., 1978).

stability. At the 21-day test period TBA values for the three test diets did not show any drastic differences which is not consistent with the data on feed consumption and resultant PER value. Granted the turkey BR material had a slightly lower TBA value when compared to turkey RK or turkey BK but that would hardly account for the reduced feed intake of 147.3g for RK or 143.7g for BK. These data serve to illustrate the care that must be taken in determining PER values for food materials subject to lipid oxidation. Caution should also be exercised in using TBA values as an index of lipid oxidation under similar circumstances as that presented in this paper. A second test was conducted in which the test material had been treated with an antioxidant as outlined in the procedure.

Results of the PER test are shown in Table 3. In each case the MDTM from BR, RK and BK had a significantly ($P < 0.01$) higher PER value than either of the two standard casein diets. There were no significant differences in PER values between the three types of MDTM studied. Included in this table is a PER value calculated for broiler skinless necks (MacNeil et al., 1978). PER values reported here are higher than the 2.55 (adjusted to 10% casein diet) reputed by Yang et al. (1959) on hand deboned meat from turkey carcasses. It is interesting to note the effect of the added antioxidant in improving the stability of the three test materials. Table 4 shows the TBA values obtained on the freeze-dried meat and on the test diets one week after the completion of the 28-day feeding test.

Amino acid profiles of the three types of MDTM are shown in Table 5 and are grouped into essential and nonessential amino acids. Procedures used in this study did not include a determination of tryptophan. Histidine and cystine are included as essential amino acids because histidine is essential to the rat and cystine can replace part of the methionine. There was very little variation in the amino acid profile of each type. Analysis of variance on the essential amino acid grouping showed that 1/2 cystine, valine and leucine were significantly ($P < 0.01$) different when the three types were compared. These values for essential amino acids are only slightly lower than those reported by Happich et al. (1975) for lean ground beef. Studies by Lee et al. (1978) on various blends of ground beef showed several essential amino acid totals that were comparable to those found in this study.

Fatty acid composition (Table 6) shows only slight differences between the three types tested. Of particular interest is the high level of linoleic acid when compared to that found in mechanically deboned broiler meat. It is also lower in oleic acid when compared to broiler materials.

Sensory analysis of all three types of MDTM are summarized in Table 7. Panelists had no difficulty in distinguishing between all three types indicating that there were identifiable flavor or texture differences between the three types. When either BR X BK or BR X RK was tested for

Table 7—Sensory evaluation[a] of three types of MDTM

Pair tested		No. of panelists	No. of panelists identifying correct pair	No. of panelists preferring		
				BR	RK	BK
BK	BR	35	34**	30**		4**
BK	RK	35	29**		19	10
RK	BR	35	32**	28**	4**	

[a] Values represent data from three different days of testing.
** Values are significant ($P < 0.01$).

preferences there was a significant ($P < 0.01$) preference for the BR over either the BK or RK MDTM. When RK X BK was tested there was a significant ($P < 0.01$) preference for the RK material. It should also be pointed out that TBA values for this same material are presented in Table 2 and show much higher TBA values for RK and BK. It appears that turkey BR had better storage stability than the other two samples tested.

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STABILITY OF STERILE BEEF AND BEEF EXTRACT TO PROTEASE AND LIPASE FROM Pseudomonas fragi

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ABSTRACT

Extracellular protease and lipase from *Pseudomonas fragi* significantly ($P < 0.05$) decreased color stability of sterile beef at 4°C and sterile beef extract at 4°C and 21°C. Concentrations of free fatty acids (FFA) in enzyme-treated sterile beef samples were significantly ($P < 0.05$) higher than in sterile controls held at 4°C indicating hydrolysis of glycerides by lipase from *P. fragi*. Concentrations of nonprotein nitrogen (NPN) and free amino acids in sterile beef extract treated with extracellular enzyme and held at 4°C and 21°C were significantly ($P < 0.05$) higher than in controls indicating proteolysis by protease from *P. fragi*. This was confirmed by SDS-polyacrylamide gel electrophoresis of enzyme-treated beef extract stored at 4°C for 6 days. Sephadex G-100 gel filtration and polyacrylamide disc electrophoresis indicated that the molecular size and electrophoretic mobility of the protease and lipase were similar.

INTRODUCTION

Pseudomonas fragi has been reported to cause discoloration in beef (Bala et al., 1977). *P. fragi* produces extracellular proteases (Hasegawa et al., 1970; Dutson et al., 1971; Tarrant et al., 1973) and lipases (Mencher and Alford, 1967). This investigation reports the effects of protease and lipase from *P. fragi* on color and lipid stability of sterile beef and on color and protein stability of beef extract.

MATERIALS & METHODS

Preparation of "Crude" Extracellular Enzymes

Freeze-dried cells of *P. fragi* (ATCC 4973) were suspended in Brain Heart Infusion (BHI) broth (BBL, Cockeysville, MD) and incubated at 21°C for 48 hr. BHI broth was inoculated (1%) with this culture and incubated at 21°C for 72 hr. Bacteria were separated by centrifuging at 16000 × G for 20 min. The supernate was sterilized by filtration (0.45 μm membrane filter) before ammonium sulfate (55% of saturation) was added slowly while stirring. Precipitate that formed after 24 hr at 4°C was collected by centrifugation. Precipitate from 1L of filtrate was dissolved in 10 ml of 0.002M phosphate buffer (pH 7.3) and dialyzed (24 hr) against six changes of cold deionized water to remove salts. It was then dialyzed against 0.002M phosphate buffer for 12 hr at 4°C.

These "crude" extracellular enzyme preparations were concentrated by ultrafiltration. The apparatus was equipped with a membrane (PM10) designed to retain molecules with greater than 10,000 molecular weight.

Isolation of protease and lipase

Concentrated crude enzyme was placed on a Sephadex G-100 column (2.5 × 100 cm) previously equilibrated with 0.002M phosphate (pH 7.3) buffer. The column, maintained at 4°C, was eluted with the same buffer at flow rate of 72 ml/hr. Five ml fractions were collected with an ISCO fraction collector (Model 327) and volumeter (Model 400). Elution was monitored at 280 nm with an ISCO Model UA-5 Absorbance Monitor.

Fractions with protease and lipase activities were identified by saturating filter paper discs and placing them on casein digest agar (protease) and spirit blue agar plates (lipase). Fractions having protease and lipase activity were pooled and concentrated. Homogeneity of the isolated enzymes was checked by polyacrylamide disc gel electrophoresis as outlined by Davis (1964).

Quantitation of protease activity

Protease activity was determined by the method of Hull (1947) using the Folin-Ciocalteau reagent. In this test the quantities of free tyrosine and tryptophan were measured spectrophotometrically after incubation of the enzyme with a 2.5% solution of casein in phosphate buffer (0.02M, pH 7.4) for 24 hr at 21°C. A standard curve for tyrosine was used to convert absorbance to tyrosine equivalent.

Quantitation of lipase activity

Lipase activity of enzyme preparations was assayed by titration of fatty acids released from emulsified tributyrin (Mencher and Alford, 1967).

Preparation of sterile beef samples

Sterile samples were prepared from USDA Choice grade beef short loins (Bala et al., 1977). Forty-four samples, approximately 1.5 cm thick, were aseptically cut from a pair of cores; face-to-face samples were designated as (1) sterile control and (2) sterile enzyme-treated. Odd numbered samples were sterile controls and even numbered samples were treated with protease and lipase. Sterile controls were dipped for 5 min in cold sterile deionized water and enzyme-treated samples were dipped for the same time in isolated (chromatographed) sterile enzyme solution. (The enzyme solution contained 4.6 mg protein per ml. One ml of enzyme released 400 μg of tyrosine from 5 ml of 2.5% casein solution and 0.324 mM of butyric acid from 2 ml of 5% tributyrin emulsion.) Both control and enzyme-treated samples were aseptically packaged.

Preparation of sterile beef extract

Sterile beef extract was prepared by the procedure of Bala and Naumann (1977). Enzyme-treated sterile beef extract samples were prepared by mixing 0.34 mg of freeze-dried isolated enzyme per ml of extract. The specific activity of protease in the enzyme mixture was 150.

Storage

Samples of beef and beef extract were stored at 4°C for 0, 2, 4 and 6 days and samples of beef extract were also stored at 21°C for 0, 12 and 24 hr. Beef samples were randomly assigned to treatments (time). Following each storage time, beef samples were analyzed for color and titratable free fatty acids (FFA). Beef extract samples were analyzed for color, nonprotein nitrogen (NPN) and free aromatic amino acids. Protein degradation in samples of beef extract was determined by SDS-polyacrylamide gel electrophoresis after 6 days of storage at 4°C.

Color measurement of beef

Color of beef samples was measured by the method of Snyder (1964). Values of "L" (measure of total light reflected), "a" (measure of redness) and "b" (measure of yellowness) were determined with a Color/Difference Meter (Hunter Model D25D2) which was standardized against Hunter Standard Tile No. C2-3395 with a clear glass optical flat ("L" = 67.6, "a" = 22.7, "b" = 9.8, Y = 45.7, × = 53.4 and Z = 42.8). A clear glass optical flat was placed between the meat sample and the source (over the sample port) to provide a flat meat surface for the measurements. The ratio a/b is the degree of discoloration; the lower the value, the higher is the degree of discoloration. Color differences (ΔE) of beef samples were determined as follows: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$.

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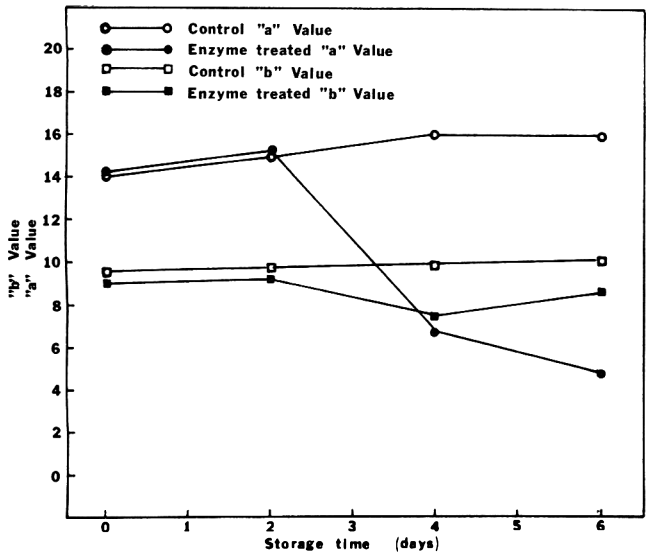

Fig. 1—Effects of protease and lipase from P. fragi on Hunter Color /Difference Meter "a" and "b" values of sterile beef stored at 4° C.

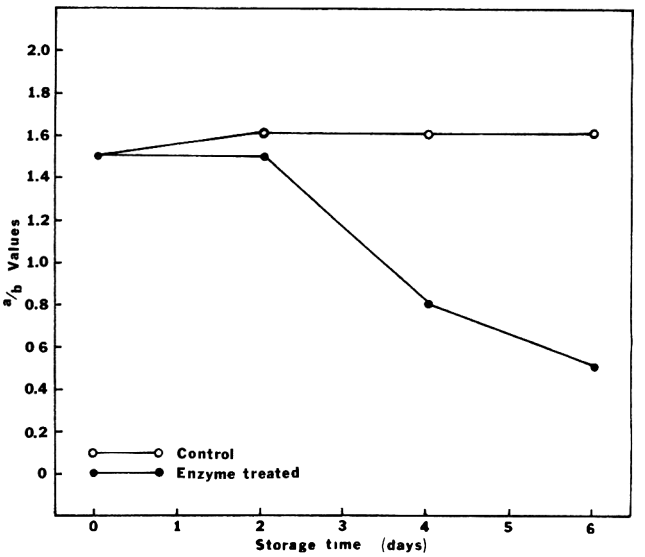

Fig. 2—Effect of protease and lipase from P. fragi on Hunter Color/ Difference Meter a/b values of sterile beef stored at 4° C.

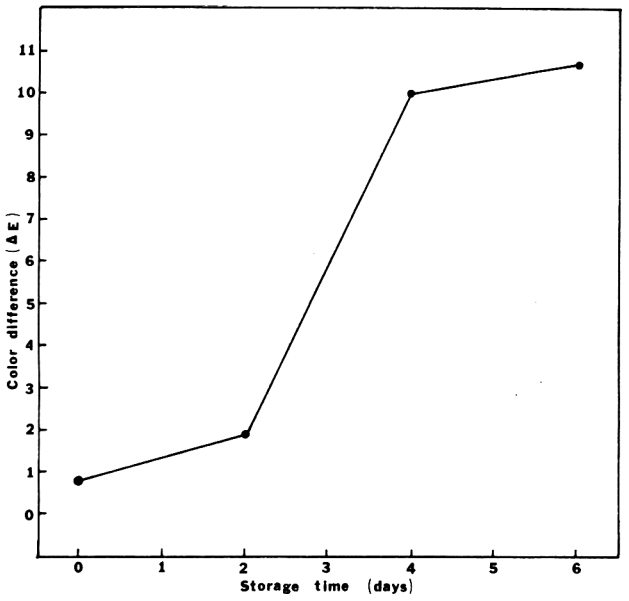

Fig. 3—Effects of protease and lipase from P. fragi on color difference values (ΔE) of sterile beef stored at 4° C.

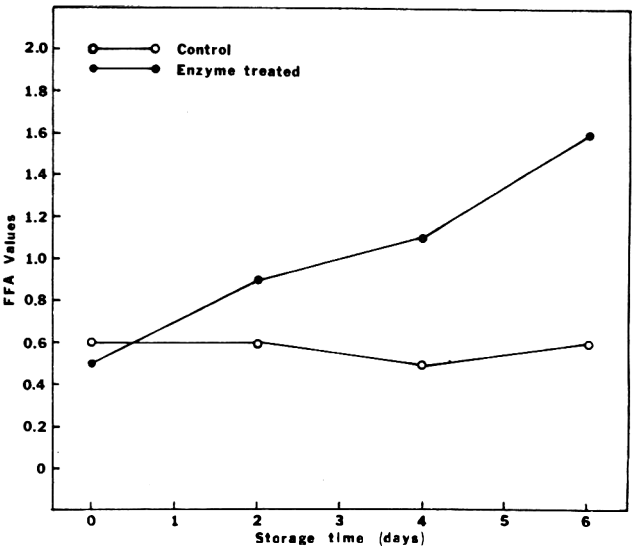

Fig. 4—Effects of lipase from P. fragi on FFA values of sterile beef stored at 4° C.

Measurement of free fatty acids (FFA)

Quantities of FFA in the top (approx 5 mm) surfaces of beef samples were determined by the procedure of Govindarajan et al. (1977).

Color measurement of beef extract

Absorbancy of beef extracts was determined at 473, 507, 573 and 597 nm using a Model 24 Beckman Spectrophotometer. From values obtained the relative concentrations of myoglobin (Mb), oxymyoglobin (O_2Mb) and metmyoglobin (MMb) were calculated by the absorbancy ratio method of Broumand et al. (1958).

Nonprotein nitrogen (NPN)

Nonprotein nitrogenous compounds in beef extract were measured by the Biuret method (Gornall and Bardawill, 1948) which is based on the principle that substances containing two or more peptide bonds form a purple complex with copper salts in alkaline solutions. Protein was precipitated by addition of 4 ml of 10% TCA to 4 ml of beef extract. The mixture was cooled at 4°C for 2 hr and filtered through Whatman No. 1 filter paper. Three ml of filtrate were transferred to a clean test tube, mixed with 3 ml Biuret reagent and 0.02 ml carbonate free 1:1 NaOH (to neutralize the acid). Absorbance was measured at 540 nm after 20 min. Nonprotein was determined from a standard curve for bovine albumin.

Free amino acid analysis by a spectrophotometric method

Concentrations of the aromatic amino acids tyrosine, phenylalamine and tryptophan, were measured by absorbance at 280 nm. Solutions obtained as described under NPN analysis were tested in a Model 24 Beckman Spectrophotometer.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis

Electrophoresis was conducted according to procedures described by Weber and Osborn (1969).

Statistical analyses

Analyses of variance, least significant differences and correlation coefficients were used to statistically analyze the data (Snedecor and Cochran, 1967).

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Table 1—Analysis of variance for the effects of protease and lipase from P. fragi, on "L", "a", a/b and FFA values of beef stored at 4° C

Source	DF	Mean Squares "L"	"a"	"b"	a/b	FFA
Replication (A)	2	4.2^{ns}	2.2^{ns}	0.05^{ns}	0.05^{ns}	0.10^{ns}
Storage time (B)	3	5.9^{ns}	25.5**	0.80^{ns}	0.20*	0.30**
Enzyme treatment (C)	1	0.4^{ns}	127.9**	5.10**	1.00**	1.30**
B X C	3	6.7^{ns}	49.5**	4.00**	0.30**	0.30**
Error	14	4.3	1.2	0.60	0.04	0.03

** Significant ($P < 0.01$)
* Significant ($P < 0.05$)
^{ns} Not significant ($P > 0.05$)

Table 3—Analyses of variance for the effects of P. fragi protease and lipase on %Mb, %O_2Mb, %MMb, nonprotein nitrogen (NPN) and absorbance at 280 nm of beef extract stored at 4° C

Source	DF	Mean squares %Mb	%O_2Mb	%MMb	NPN	Absorbance at 280 nm
Replication (A)	2	6.5^{ns}	130.7*	151.2*	0.000^{ns}	0.06*
Storage time (B)	3	103.4*	5981.7**	4598.7**	0.100**	0.30**
Enzyme treatment (C)	1	294.0**	8893.5**	5953.5**	0.400**	0.50**
B X C	3	103.4*	2278.5**	1457.9**	0.100**	0.09**
Error	10	36.0	25.4	19.0	0.005	0.01

** Significant ($P < 0.01$)
* Significant ($P < 0.05$)
^{ns} Not significant ($P > 0.05$)

RESULTS

CONTROL and enzyme-treated beef samples had no detectable microbial growth throughout storage at 4°C for 6 days. Protease and lipase from *P. fragi* had a significant ($P < 0.01$) effect on color ("a", "b", a/b) and FFA values of the beef samples (Table 1). There was a significant interaction between storage time and treatment (Table 1).

Redness and discoloration of beef

Sterile control samples remained bright red in color (Fig. 1, "a" value) and there was no discoloration (Fig. 2, a/b value) during storage. Protease- and lipase-treated samples had a significantly ($P < 0.05$) lower degree of redness (Fig. 1, "a" value) and significantly ($P < 0.05$) more discoloration (Fig. 2) than control samples. Redness decreased significantly (LSD = 0.33) by day 4 (Fig. 1). Color differences (ΔE) between the control and enzyme-treated beef samples increased significantly during storage (Fig. 3).

Effects of enzyme treatment on lipid stability

Enzyme-treated samples had ($P < 0.05$) higher FFA values than control samples (Fig. 4). Differences were significant (LSD = 0.31) from day 2 through day 6.

The correlation coefficient of 0.93 between degree of redness ("a") and discoloration (a/b) (Table 2) indicated that in protease- and lipase-treated samples discoloration increased as the bright red color decreased. As the FFA values increased, "a" values decreased (r = 0.97). This suggests that hydrolysis of lipids may have influenced color stability of beef at 4°C.

Table 2—Correlation coefficients of "L", "a", "b", a/b and FFA values for sterile beef treated with protease and lipase at 4° C[a]

Parameters	"a"	"b"	a/b	FFA
"L"	−0.16^{ns}	−0.09^{ns}	−0.20^{ns}	0.37*
"a"		0.76*	0.93*	−0.79*
"b"			0.53*	−0.42*
a/b				−0.77*

[a] Number of comparisons = 24
* Significant ($P < 0.05$)
^{ns} Not significant ($P > 0.05$)

Table 4—Effects of P. fragi protease and lipase on %Mb, %O_2Mb, NPN and absorbance at 280 nm of beef extract at 21° C[a]

Parameters studied	Storage time (hr)	Treatment Control	Enzyme treated
Mean %Mb[b]	0	0.0a	0.0a
	12	0.0a	6.7a
	24	1.7a	8.3a
Mean %O_2Mb[c]	0	97.3d	100.0d
	12	68.3d	20.0b
	24	49.3c	0.0a
Mean %MMb[d]	0	2.7a	0.0a
	12	31.7b	73.3d
	24	49.0c	91.7e
Mean NPN[e]	0	0.5a	0.5a
	12	0.5a	0.7b
	24	0.5a	0.7b
Mean absorbance at 280 nm[f]	0	0.5a	0.6a
	12	0.7a	1.2b
	24	1.0b	1.6c

[a] Means within individual parameters tested bearing a common letter do not differ ($P < 0.05$).
[b] LSD ($P < 0.05$) = 8.8.
[c] LSD ($P < 0.05$) = 10.4.
[d] LSD ($P < 0.05$) = 10.8.
[e] LSD ($P < 0.05$) = 0.16.
[f] LSD ($P < 0.05$) = 0.28.

Color and protein stability of beef extract

Protease and lipase-treatment had a significant ($P < 0.01$) effect on color (% O_2Mb and %MMb) and protein stability (NPN and absorbance at 280 nm) of beef extract at 4°C (Table 3) and at 21°C. (Mean squares in the analysis of variance for experiments at 21°C were much like those in Table 3; therefore, they are not included.) There was also a significant ($P < 0.05$) interaction between enzyme treatment and storage time at 4°C and 21°C. Enzyme-treated samples had significantly ($P < 0.05$) more discoloration than controls at 21°C (Table 4) and 4°C (Fig. 5). Enzyme treatment caused significant ($P < 0.05$) protein degradation in beef extract as determined by the increase in NPN and increase in absorbance at 280 nm (Table 4, Fig. 6).

Decreases in %O_2Mb were strongly and inversely correlated with %MMb (r = −0.99). There were also significant ($P < 0.01$) negative correlations between %O_2Mb and NPN and between %O_2Mb and absorbance at 280 nm at 4°C (Table 5). Correlation coefficients were similar but slightly lower in the same experiment performed at 21°C. The significant ($P < 0.01$) positive correlation between NPN and absorbance at 280 nm (Table 5) resulted from the increase of both peptides and free amino acids by protease. The SDS-polyacrylamide gel electrophorogram (Fig. 7) illustrates proteolysis by proteases of *P. fragi* in beef extract.

DISCUSSION

PROTEASE and lipase of *P. fragi* had significant detrimental effects on the color and lipid stability of beef samples at 4°C and on the color and protein stability of beef extract at 4°C and 21°C. Protease was not separated from lipase by Sephadex G-100. As illustrated in the polyacrylamide gel disc electrophorogram (Fig. 8), protease and lipase of *P. fragi* had the same electrophoretic mobility and appeared as a single band. Thus, Sephadex gel filtration and polyacrylamide gel disc electrophoresis indicated that the molecular size and electrophoretic mobilities of protease and lipase from *P. fragi* were similar. This is not meant to

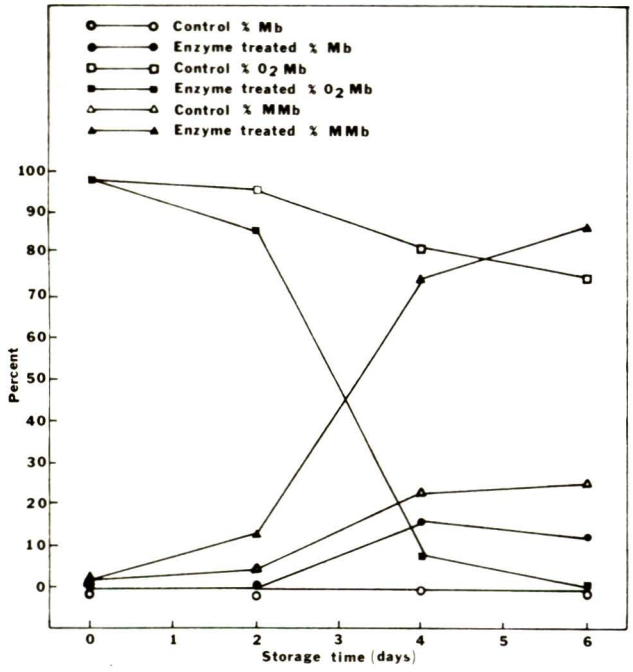

Fig. 5—Effects of protease and lipase from P. fragi on Mg, O_2Mb and MMb concentration of sterile beef extract at 4° C.

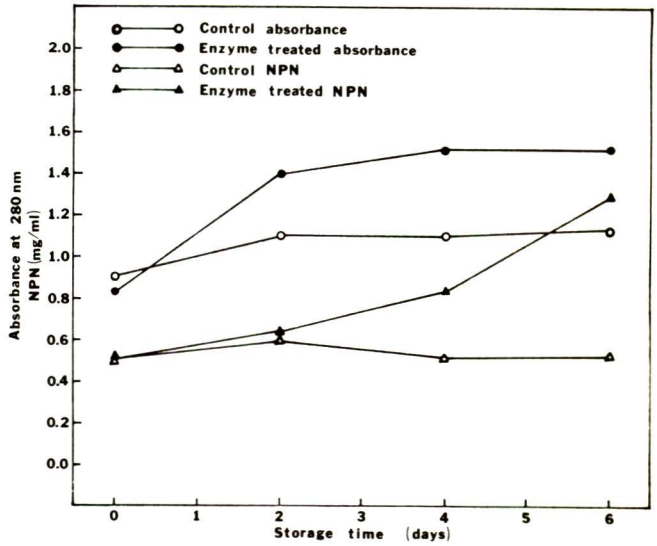

Fig. 6—Effects of protease on nonprotein nitrogen and free amino acid values of beef extract at 4° C.

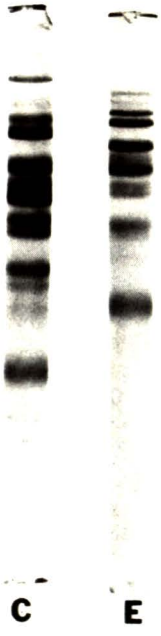

Fig. 7—SDS polyacrylamide gel electrophoretogram of aqueous beef extract. Electrophoresis was performed at a constant current of 5mA/tube for 7 hr. Protein conc: Control beef extract (C) = 45 μg /tube; Enzyme-treated beef extract (E) = 45 μg/tube.

Fig. 8—Polyacrylamide disc electrophoretogram of extracellular enzymes, protease and lipase from P. fragi. Protein conc: 15 μg.

Table 5—Correlation coefficients of %Mb, $\%O_2$Mb, %MMb, nonprotein nitrogen (NPN) and absorbance at 280 nm of beef extract treated with P. fragi protease and lipase and stored at 4° C[a]

Parameters	$\%O_2$ Mb	%MMb	NPN	Absorbance at 280 nm
%Mb	−0.79**	0.68**	0.61**	0.55**
$\%O_2$ Mb		−0.99**	−0.86**	−0.72**
%MMb			0.86**	0.72**
NPN				0.68**

[a] Number of comparisons = 24
** Significant ($P < 0.01$)

imply that a single enzyme had both activities. Our findings suggest that *P. fragi* lipase caused discoloration in beef accompanied by release of free fatty acids from the lipid muscle fraction. This agrees with the findings of Govindarajan et al. (1977). They also reported that lipase treatment had an accelerating effect on lipid oxidation. It is likely that unsaturated fatty acids in the lipid molecule are protected by steric hindrance against oxidation. However, when the glyceride bonds are hydrolyzed by lipase, the fatty acid moieties set free are more readily oxidized because of their availability to react. In meat, oxidation of fat and oxidation of heme pigments are closely related and each reaction can accelerate the other (Watts, 1954).

In aqueous beef extract the discoloration must have been associated with protease since there was no substrate for lipase. The SDS-PAGE electrophorogram (Fig. 7) illustrates that the protease of *P. fragi* caused degradation of water-soluble proteins in beef extract stored for 6 days at 4°C. Protease activity is important in discoloration, since the globin part of the myoglobin molecule is susceptible to attack by protease (Fox, 1968). It is also possible that protease caused discoloration by degradation of the peptide chain in the myoglobin molecule.

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A SIMPLE METHOD FOR THE DETERMINATION OF CHOLESTEROL AND SOME PLANT STEROLS IN FISHERY-BASED FOOD PRODUCTS

M. I. P. KOVACS, W. E. ANDERSON and R. G. ACKMAN

ABSTRACT

An efficient method has been developed for the microdetermination of cholesterol and some plant sterols such as brassicasterol, campesterol, stigmasterol, and β-sitosterol. The method is a greatly simplified analytical procedure in which samples are directly saponified, the unsaponifiable substances extracted, and the sterols estimated by gas liquid chromatography without further processing. The sterol contents from the new method are at least as high, and generally higher, than those from the official method, indicating superior recovery. The analysis has been found to be simple, sensitive, economical of time and particularly of solvents. It is probably adaptable to a wide variety of food ingredients or products.

INTRODUCTION

THE DETERMINATION of the sterol components, particularly cholesterol, among the unsaponifiable lipids of food is of growing importance to the food industry. The qualitative and quantitative evaluation of sterols is of interest to the consumer because of an apparent relationship between increased incidence of coronary heart disease and elevated serum cholesterol (National Diet-Heart Study Research Group, 1968; Brown, 1968).

The Liebermann-Burchard methods and that using ferric chloride/sulfuric acid reagent have been evaluated and their limitations are well understood (Sweeney and Weihrauch, 1976; Tonks, 1967; Vanzetti, 1964). Cholesterol determination by gas liquid chromatography (GLC) is usually more accurate than colorimetric procedures (Vanzetti, 1964), and because of its greater specificity the GLC analysis of steroids has become the preferred method in recent years (Blomhoff, 1973; Ferrell et al., 1977). This is especially true when samples contain both cholesterol and the closely related phytosterols such as brassicasterol, campesterol, stigmasterol, β-sitosterol, etc. common in vegetable oils.

There are numerous GLC methods available for sterol determination which, while often rigorous, are generally cumbersome and time-consuming (Punwar, 1975; Sheppard et al., 1977; AOCS, 1972). At the same time, there is little uniformity among these methods. Even the currently accepted standard methods differ not only in details, but also in significant points such as the respective extractions of total fat and of unsaponifiables from total fat, the most critical steps in the analysis (Hubbard et al., 1977). In fact all the standard methods require more or less complicated sample preparations and extraction procedures, using highly toxic and flammable chemicals in large quantities.

Derivatization of steroids for GLC is still a common practice in the standard analyses. However, recent improvements in thermostable polysiloxanes for liquid phases, more inert supports, and other improvements in instrumentation now make it possible to analyze sterol fractions in the free-OH form (Seher and Vogel, 1976). Derivatization of sterols is not only time consuming but contributes to increased noise and decreased linearity due to silicon deposits on the FID originating from the trimethylsilyl (TMS) derivatives (Tamura et al., 1976).

The aim of the present study was to develop and then evaluate a simple method for the determination of cholesterol in composite food products, preferably while minimizing utilization of expensive, dangerous and environmentally objectionable solvents and reagents.

METHODS

General procedure

Sample preparation. Food products of tuna (canned in vegetable oil) and fish cakes, purchased from local retail stores, consisted of 12 packages or cans having total net weights of about 1.5 kg. Fresh cod was obtained from the local fish market. Samples were homogenized in a Hobart Silent Cutter.

GLC determination. The unsaponifiables were analyzed either as TMS derivatives or as free sterols with a Perkin-Elmer 3920B gas chromatograph equipped with flame ionization detector and glass column (80 cm × 2 mm i.d.), packed with GAS-CHROM Q, 80–100 mesh, coated with 3% OV-17. The column was operated at 230°C with helium as carrier gas at 40 ml/min. Injector and detector temperatures were 235°C and 240°C, respectively. For quantitation 5α-cholestane was used as the internal standard and all sterols were identified by comparing their retention times with authentic standards.

Extraction

Method A. Lipids were extracted from a 100–150g aliquot of homogenate by the method of Bligh and Dyer (1959). About 5 g of the lipid extract was saponified according to the official method of the American Oil Chemists' Society (AOCS, 1972). The unsaponifiables were analyzed for free sterols using GLC.

Method B. About 50 mg of lipid extract was saponified in a tightly capped (Teflon lined) 15 ml centrifuge tube containing 0.5 ml 50% KOH and 2 ml 95% ethanol. The tube contents were boiled on a hot plate for 1 hr while being continuously stirred with a magnetic bar. After cooling 1.5 ml of distilled water was added and unsaponifiables were extracted 4 times with hexane (2.5 ml each). The combined extracts were concentrated and analyzed for free sterols using GLC.

Method C. Approximately 0.5g of well-homogenized sample was saponified as described in Method B using 30 ml centrifuge tubes containing 1 ml 50% KOH and 4 ml 95% ethanol. It is important that all the homogenate be suspended and not adhere to the tube wall above the saponifying solution. At the end of the saponification the tubes were cooled to room temperature and 2.5 ml of H_2O was added. The unsaponifiables were extracted with four portions of hexane (5 ml each). The combined extracts were concentrated and analyzed for free sterols using GLC.

Recovery studies

Recovery of cholesterol and its esters was evaluated by adding

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cholesterol and cholesteryl palmitate to the samples prior to homogenization.

Tissue residue after fat extraction. After extraction of lipids (Method A) the tissue residue was transferred into a 500 ml round-bottom flask containing 20 ml 50% KOH and 80 ml 95% ethanol. The contents were then boiled under a reflux condenser for 1 hr while continuously stirred with a magnetic bar. Then the flask was cooled and after adding 60 ml water the unsaponifiables were extracted four times with hexane (100 ml each). The extracts were combined then concentrated and analyzed for sterols by GLC.

Methanol-water layer. The methanol-water layer from Method A was quantitatively evaporated to dryness and the residue was saponified and analyzed as described in Method C.

Direct saponification of tissue. After adding cholesterol or cholesteryl palmitate to the samples, it was homogenized and analyzed as in Method C.

Table 1—Cholesterol content in cod muscle and canned tuna[a], determined by three different methods

		Cod muscle			Canned tuna[a]	
		Method			Method	
Homogenate	Detn[b]	A	B	C	A	C
1	1	36.3	40.3	46.6	30.1	34.3
	2	37.5	39.7	47.4	31.1	34.7
2	1	37.4	40.5	50.5	33.9	36.3
	2	36.1	42.1	48.8	33.4	35.7
3	1	39.3	41.2	49.8	32.1	34.0
	2	39.4	41.2	51.2	30.2	37.2
4	1	40.3	43.7	48.1	30.4	37.8
	2	40.4	42.5	46.2	28.7	35.2
5	1	40.7	40.9	48.7	28.6	36.3
	2	40.3	40.2	50.4	30.5	37.1
6	1	39.2	43.3	50.3	25.0	34.7
	2	38.6	44.1	50.4	24.4	33.7
7	1	41.4	42.5	51.2	29.3	33.3
	2	40.8	43.0	50.2	30.2	34.8
8	1	40.1	41.1	50.6	28.8	36.9
	2	40.5	42.4	49.5	29.0	35.5
9	1	38.7	42.7	48.8	23.3	35.2
	2	38.6	44.1	50.3	22.2	35.9
10	1	39.8	41.1	47.2	32.1	33.7
	2	39.6	39.1	49.4	32.9	34.2
Mean		39.3	41.8	49.3	29.3	35.3
Std. dev.		1.48	1.47	1.5	3.2	1.3
Coeff. of var. %		3.8	3.5	3.0	10.9	3.7

[a] Canned in vegetable oil

[b] Duplicate analysis of the same sample. Results in mg/100g sample.

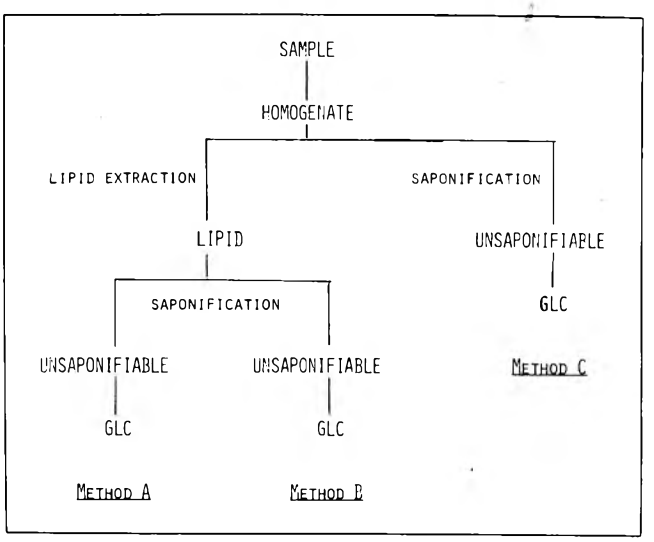

Fig. 1—Flow diagram of the methods: (A) AOCS (1972); (B) simplified saponification of extracted lipids; (C) direct saponification of the tissue homogenate.

Table 2—Cholesterol, brassicasterol, campesterol and β-sitosterol content of fish cakes, determined by three different methods

		Cholesterol			Brassicasterol			Campesterol			β-Sitosterol		
		Method			Method			Method			Method		
Homogenate	Detm[a]	A	B	C	A	B	C	A	B	C	A	B	C
1	1	18.2	18.5	19.1	2.5	1.7	1.6	8.7	8.3	8.9	16.1	15.8	18.0
	2	18.2	18.5	20.3	2.9	1.7	2.6	8.7	8.8	9.7	16.0	16.5	18.7
2	1	15.5	20.0	19.9	2.4	2.3	2.0	7.3	9.7	9.6	13.1	18.0	18.9
	2	17.8	19.4	19.3	3.1	2.5	1.8	8.7	10.1	9.1	15.5	19.1	17.8
3	1	17.6	19.9	20.1	2.0	2.3	2.3	8.7	9.1	9.8	16.0	17.5	18.9
	2	17.6	19.6	19.7	2.2	2.2	2.2	8.6	9.0	9.3	16.4	17.2	18.1
4	1	17.6	18.1	19.3	2.2	1.8	1.9	8.8	8.8	8.2	15.7	16.8	16.4
	2	17.3	19.6	19.2	2.0	1.7	2.3	8.2	9.0	9.8	15.5	17.3	19.5
5	1	18.0	19.6	19.5	2.3	1.9	1.9	8.6	9.0	8.9	15.7	19.5	17.0
	2	17.8	19.2	20.8	2.2	2.0	2.4	8.5	9.7	9.9	16.5	18.4	19.4
6	1	17.2	19.2	20.7	2.0	1.9	1.9	8.3	9.4	10.2	15.8	18.2	20.1
	2	17.5	17.5	20.4	2.2	1.9	1.8	8.8	9.6	9.8	16.5	19.2	19.6
7	1	18.7	18.2	20.8	2.3	2.0	2.4	9.0	8.9	10.3	17.4	17.1	20.0
	2	18.9	18.8	21.3	2.3	2.4	2.7	9.3	9.4	10.9	17.7	17.7	20.5
8	1	18.3	19.7	19.8	2.2	2.0	2.4	9.0	9.7	9.7	17.1	18.8	19.1
	2	18.7	17.8	20.3	2.5	2.3	2.5	9.3	10.3	9.9	17.5	19.6	19.4
9	1	18.8	18.4	20.3	2.2	2.1	2.5	9.0	9.3	10.1	17.4	17.3	19.0
	2	19.0	17.9	20.0	2.1	2.1	2.3	8.9	9.2	10.0	17.1	16.8	19.6
10	1	17.7	19.7	20.1	2.0	1.7	2.4	8.5	10.2	9.1	16.5	19.5	18.3
	2	17.7	17.9	21.8	2.1	1.6	2.8	8.4	8.9	10.3	16.2	17.0	20.1
Mean		17.9	18.9	20.1	2.3	2.0	2.2	8.7	9.3	9.7	16.3	17.9	18.9
Std. Dev.		0.79	0.80	0.71	0.29	0.26	0.33	0.43	0.52	0.61	1.02	1.12	1.1
Coeff. of var. %		4.4	4.2	3.5	12.6	13.0	15.0	4.9	5.6	6.3	6.3	6.3	5.8

[a] Duplicate analysis of the same sample. Results in mg/100g sample.

Table 3—Recovery studies of cholesterol from cod muscle[a]

Method A	Sample wt (g)	Cholesterol added (mg)	Cholesterol recovered: Chloroform (mg)	Chloroform (%)	Tissue Residue (mg)	Tissue Residue (%)	Methanol-Water (mg)	Methanol-Water (%)	Total (mg)	Total (%)
L	100	–	44.4	96.9	0.6	1.3	0.8	1.8	45.8	100
L + Ch	100	50	91.9	95.5	1.2	1.3	3.2	3.4	96.3	101
L + ChP	100	62[a]	98.9	94.0	2.0	1.9	5.4	5.1	105.3	98

Method C	Sample wt (g)	Cholesterol added (mg)	Cholesterol recovered (mg)	Cholesterol recovered (%)
T	1	–	0.485	100
T + Ch	1	0.50	1.02	103.5
T + ChP	1	0.62	1.09	104.0

[a] Values are the means of duplicate analyses: Ch = Cholesterol; ChP = Cholesteryl palmitate; L = lipid; T = tissue.

RESULTS & DISCUSSION

A SIMPLIFIED FLOW diagram comparing the steps in the sterol analysis is shown in Figure 1. Analysis via A represents the official method of AOCS (AOCS, 1972). Silylation did not result in significant differences in sterol recovery (data not included), consequently it was omitted from the procedure. Methods B and C were developed in our laboratory respectively for extracted fat and/or homogenized tissue.

Due to the specificity and sensitivity of gas-liquid chromatography, large sample sizes are unnecessary. For this reason the saponification in Methods B and C is simplified by using a single closed tube, eliminating the commonly used reflux systems of conventional methods. Consequently it was possible to saponify up to twelve samples at the same time on a single stirring hot plate.

Saponification of extracted fat in a closed tube (Method B, Tables 1 and 2) gave results comparable to those of the official Methods A (AOCS, 1972).

The saponification of the whole tissue gave significantly higher cholesterol estimates than saponification of the fat extracted with solvents (Table 1). It has to be noted that the sterol of cod fillets is almost exclusively cholesterol; however, tuna canned in vegetable oil also contains plant sterols (data for the latter not given in table). Similar results were obtained when the cholesterol was determined in the presence of plant sterols in fish cakes (Table 2). With the exception of brassicasterol, all the sterols determined gave higher results using Method C (direct sample saponification). The differences in the results from the other methods probably arise from the structural involvement of cholesterol in the cell. Cholesterol forms complexes with other biological molecules, primarily phospholipids and proteins (Sabine, 1977) which change their total physical properties, resulting in inefficient extraction and this explains the poor recovery of cholesterol during what is considered as one of the most critical steps in lipid analysis (Hubbard et al., 1977), the fat extraction (Table 3).

Figure 2 shows typical GLC separations of cholestane, cholesterol, brassicasterol, campesterol and stigmasterol. It can be seen that samples prepared after direct saponification (Fig. 2) do not contain materials which interfere with either the separation or quantitation.

The life time of the relatively short GC column (80 cm) was about 2–3 months. Moreover, it was found that injection of a relatively high amount of cholesterol was necessary to resaturate the absorption sites each morning to obtain stable response ratios. Longer columns (2 ml) were found necessary to improve separations in samples containing complex mixtures of sterols, e.g. shellfish (unpublished data). However, in our evaluation of methods the short column gave satisfactory separations with reduced analysis time.

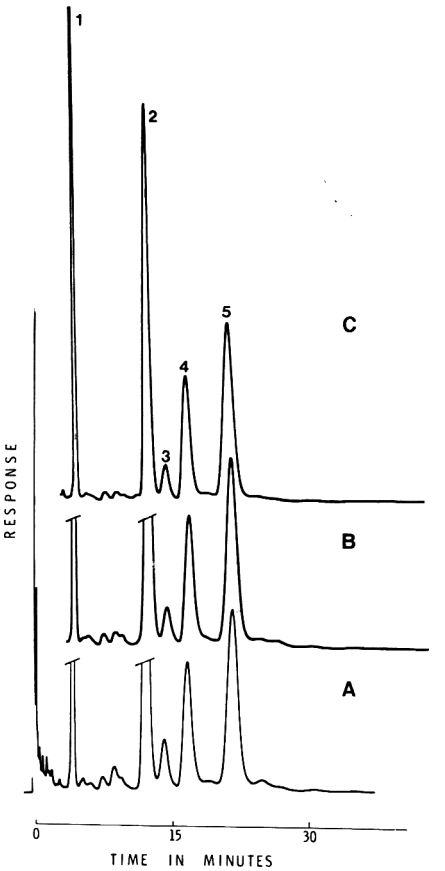

Fig. 2—Gas chromatogram of cholestane (1), cholesterol (2), brassicasterol (3), campesterol (4) and sitosterol (5) obtained by methods A, B and C.

The official methods recommended by the AOAC and AOCS require a minimum of 400 or 600 ml organic solvents per sample respectively, while our economical direct saponification method requires at most only 24 ml. Furthermore, about twenty times as many samples can be analyzed during a given time by our method as by the conventional methods.

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NUTRIFICATION OF DRY BEAN (Phaseolus vulgaris, L.) BY METHIONINE INFUSION

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ABSTRACT

The technological conditions for the nutrification of beans by methionine infusion are described. The best conditions seem to be infusion for 1 hr with a 5% methionine solution at 50°C. By this procedure the methionine was raised from 1.2 to 24 g/100g of bean protein and the absorbed water was only 40% of the beans weight. The beans infused under these conditions, after drying, could be mixed 1:7 (w/w) with original beans and the mixture after cooking in the presence of the soaking water still contained 3% methionine on the protein basis. During soaking (prior to cooking) about one-third of the infused methionine was diffused out in the remaining water. Therefore, elimination of the soaking water before cooking should be discouraged. Infusion with methionine raised the bean PER from 0.9 to 2.6 and the efficiency of a 10% bean protein diet from 9.4 to 26.6%. There was no difference in preference between the original and infused beans when the Directional Paired Comparison test was applied. However, when the Non Structured Scale test was used for the odor and taste of cooked beans there was no preference for the original beans when seasoning was used, whereas there was a significant difference at 5% probability level in favor of the original beans when the samples were cooked and tasted without seasoning.

INTRODUCTION

DRY BEAN (*Phaseolus vulgaris*) is an important source of protein in the human diets of many countries. The protein contents of different varieties may range from 20–35% on a dry basis. Genetic as well as environmental factors play an important role in determining the protein contents of the seeds (Kelly, 1971; Lantz et al., 1958; Pant and Tulsiani, 1969; Silva and Iachan, 1975).

The main problem in relying on beans as the main source of dietary proteins is the deficiency of sulfur-containing amino acids in their molecules (Jaffé, 1949; Tandon et al., 1957; Bressani et al., 1961; Jaffé and Brucher, 1974). In addition to the limiting total concentration of methionine and cysteine in the bean proteins, their biological availability is very low (Kakade et al., 1969; Evans et al., 1974; Sgarbieri et al., 1979). Another important nutritional implication related to the availability of the sulfur-containing amino acids from beans is the recent finding (Antunes and Sgarbieri, 1979) that methionine and cysteine availability may drop quite rapidly and significantly with storage time, particularly under conditions of a relatively high temperature and moisture content.

Several approaches have been used in an attempt to increase methionine content of dry bean based diets. The genetic aspect has been studied all over the world (Milner, 1973; Kelly, 1971); complementation of bean protein with other foods richer in methionine (Bressani, 1973; Sgarbieri et al., 1978; Antunes and Markakis, 1977) and, addition of synthetic methionine in some food items ready to consume have also been applied.

In this paper an infusion method to enrich dry bean kernels with methionine is described. This seems to be practicable and of nutritional interest in areas of the world where beans are consumed together with other food items which could not compensate for the methionine deficiency.

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EXPERIMENTAL

Bean variety

The bean used in this study was the commercial variety "Rosinha G2" obtained from the Agronomic Institute of Campinas, S.P., Brazil.

Infusion procedures

Infusion was accomplished by soaking the beans in aqueous methionine solutions of different concentrations and at different temperatures, i.e. (a) 3% methionine at 25°C, and (b) 1–5% methionine at 50°C.

After the soaking period the beans were quickly washed under running tap water and then dried in a circulating hot air oven, at 55°C for 20 hr. The final moisture content of the dried beans was 9.8%.

Analytical procedures

Crude protein, crude fiber, ash and lipid content of the beans were determined by the procedures described in the AOAC (1975). Carbohydrate was calculated by difference.

Total methionine in the infused and dried beans as well as in the original beans was determined by the colorimetric procedure of Lunder (1973).

Biological assays

Protein efficiency ratios (PER) were determined for the diets containing the original beans and the ones infused with methionine under different conditions. Before use, beans were cooked for 45 min in a pressure cooker (120°C, 15 psi). For each diet, six male weanling rats of the Wistar strain were used. The procedure followed was that described in the AOAC (1975).

Methionine availability from the original and the methionine infused beans was determined in a balance study using rats of approximately 40g each. For the first 5 days the animals were maintained on the experimental diets for adaptation followed by another 5 days in which the food intake was recorded and feces were collected and analyzed for methionine content. A protein-free group was used to estimate the endogenous methionine excretion.

Procedures for sensory evaluation

The preference between the original and the infused beans was determined in the sensory evaluation laboratory employing two different methods: (1) Directional Paired Comparison (Amerine et al., 1965; Garruti, 1976); (2) Non Structured Scale method (Amerine et al., 1965; Raffensperger et al., 1956).

The infused beans (1% and 5% methionine solutions) as well as the original beans were soaked in distilled water for 12 hr and then cooked in a pressure cooker for 45 min (120°C, 15 psi) using the same soaking water.

For the Directional Paired Comparison tests the products (10g) were offered to the panel members (5 women and 5 men, 20–40 years old) in 50 ml beckers maintained at 45°C by a temperature controlled heater. The beans infused with 5% methionine solution (50°C) were mixed 1:7 (w/w) with the original beans prior to cooking. The taster received, in each test, three pairs of samples and was asked to indicate the preferred one in a specially designed sheet.

For the Non Structured Scale tests the samples were prepared and offered to the panel under the same conditions used in the previous method. For these tests were used only original beans and the ones infused with 5% methionine solution. The infused beans

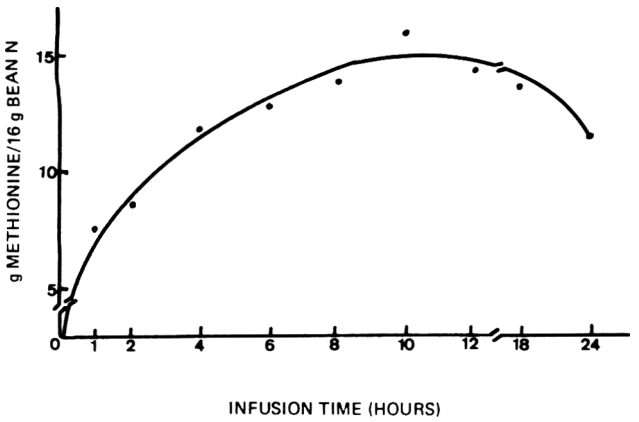

Fig. 1—Time dependent methionine absorption curve of a dry bean (Phaseolus vulgaris) infused with a 3% methionine solution at 25° C.

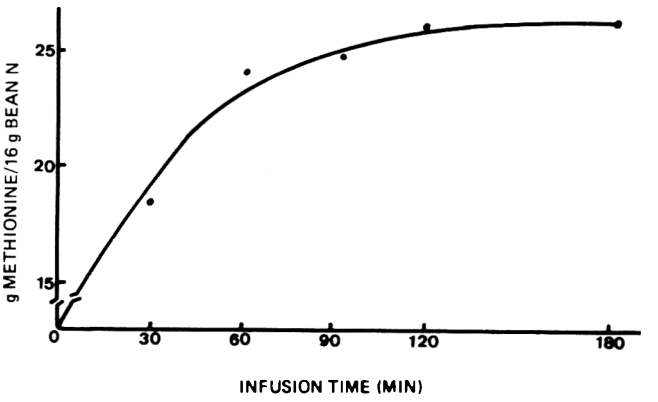

Fig. 2—Time dependent methionine absorption curve of a dry bean (Phaseolus vulgaris) infused with a 5% methionine solution at 50° C.

were mixed in proportions of 1:4 and 1:7 (w/w) with the original beans before cooking. For each sample the panel members were asked to express their judgment about different parameters related to taste and odor of cooked beans.

Statistical analyses

The results of the sensory evaluation were submitted to statistical analyses according to the following procedures: (1) data from the Directional Paired Comparison tests were analyzed using the Table of significance for two-tailed tests (Roessler et al., 1956); (2) for the data of the Non Structured Scale tests the Analysis of Variance (Cochran and Cox, 1957) and the Dunnett test for the significance between mean values were used.

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION of the beans used in all experiments is shown in Table 1. In the formulation of the rat diets the beans were used as the only source of protein. Carbohydrate and lipid contents of the beans were discounted from the added components.

The absorption rate of methionine by infusion with a 3% methionine solution (25°C) is shown in Figure 1. Maximum absorption was observed only after 10 hr of infusion when the beans had absorbed the equivalent of their own weight in water and about 16g methionine/100g of bean protein. With a longer period of time the methionine started diffusing out into the water solution. The main inconvenience of the infusion under these conditions is that the evaporation of the absorbed water, if the infused beans are to be stored dry, is very expensive. In addition, the appearance of the beans after evaporation of the water was considerably inferior to that of the original (noninfused).

In Figure 2 the rate of methionine absorption by the beans is shown for the infusion with a 5% methionine solution at 50°C. The rate of absorption is greatly accelerated under these conditions. Thus after 1 hr of infusion the absorbed methionine was 24g/100g of bean protein, where-

Table 1—Proximate percent composition of a dry bean (Phaseolus vulgaris) variety Rosinha G2.

Component	Percentage
Protein	23.7
Lipid (P.E. extract)	1.8
Ash	2.9
Crude fiber	4.6
Water	9.0
Carbohydrate (difference)	58.0

Table 2—Methionine content of cooked dry bean (Phaseolus vulgaris) before and after infusion with methionine for 1 hr at 50° C

Description of samples[a]	Methionine contents (g/16gN)
Original beans cooked without soaking water	0.85 (F)[b]
Infused with 1% methionine solution, cooked without soaking water	2.13 (C)
Infused with 1% methionine solution, cooked with soaking water	3.24 (D)
Infused with 5% methionine solution, mixed (1:7 w/w) with otiginal beans, cooked without soaking water	1.98 (B)
Infused with 5% methionine solution, mixed with original beans (1:7 w/w), cooked with soaking water	3.02 (E)
Casein used as standard	3.15 (A)

[a] Soaked for 12 hr in distilled water (25°C) before cooking. Cooked for 45 min in a pressure cooker (120°C, 15 psi).

[b] Letters in parenthesis refer to corresponding growth curves of Fig. 4.

Table 3—Body weight gain, diet consumption, PER and efficiency of diets prepared with noninfused beans and beans infused with methionine, cooked with and without soaking water[a]

Description of samples used as protein source	Body wt gain (g/rat/day)	Diet consumption (g/rat/day)	Diet efficiency (%)	PER ± SD
Original bean cooked without soaking water	0.6	6.0	9.4	0.9 ± 0.24
Infused with 1% met. solution, cooked without soaking water	1.2	6.7	18.4	1.6 ± 0.29
Infused with 1% met. solution, cooked with soaking water	2.1	7.9	26.6	2.6 ± 0.23
Infused with 5% met. solution, mixed (1:7 w/w) with original beans, cooked without soaking water	1.0	7.0	14.6	1.3 ± 0.22
Infused with 5% met. solution, mixed (1:7 w/w) with original beans, cooked with soaking water	2.1	8.8	24.5	2.4 ± 0.26
Casein used as standard	2.5	7.9	31.6	3.4 ± 0.27

[a] All samples were soaked for 12 hr in distilled water (25°C) before cooking.

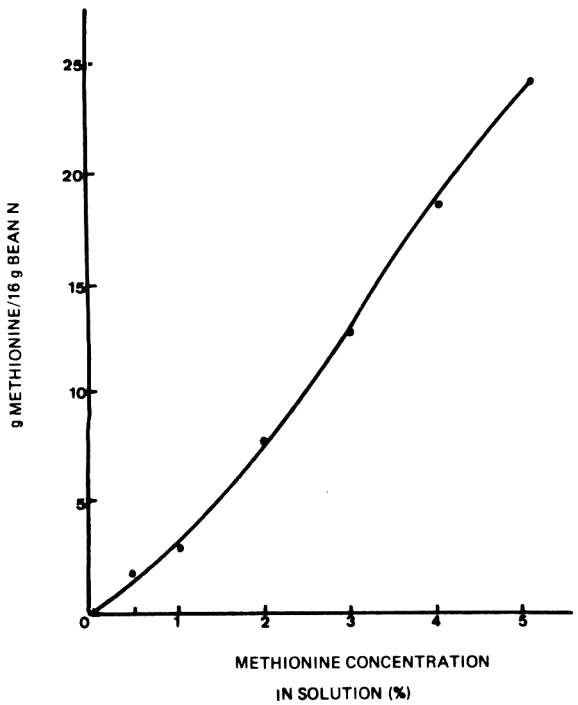

Fig. 3—Concentration dependent curve for the absorption of methionine by a dry bean (Phaseolus vulgaris) during 1 hr infusion at 50° C.

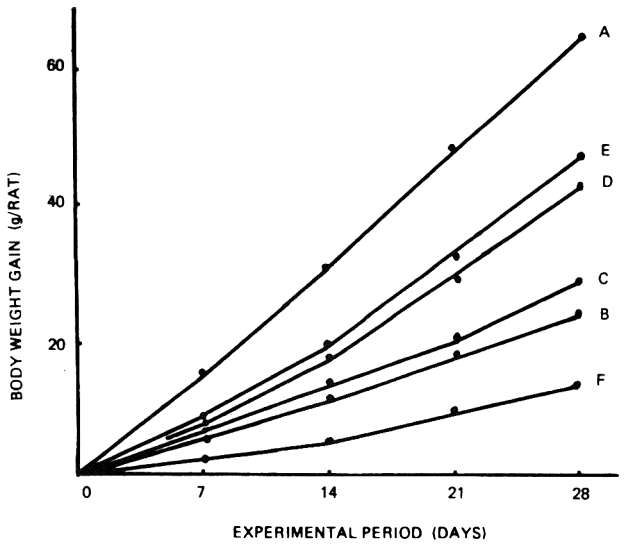

Fig. 4—Growth rate curve for weanling rats on 10% protein diets. F, original beans cooked without soaking water; D and C, beans infused with 1% methionine solution and cooked with and without soaking water, respectively; E and B, beans infused with a 5% methionine solution mixed 1:7 (w/w) with original beans, and cooked with and without soaking water, respectively; A, casein standard diet.

as the absorbed water was only 40% of the bean weight. When dry, the beans infused under these conditions (1 hr at 50°C) exhibited a much better appearance being practically undistinguishable from the original ones. The kernels infused under these conditions contained sufficient methionine to be used as a fortification material for the noninfused beans at a proportion of 1:7. The methionine content of the protein in this mixture was approximately 3%, very similar to casein (Table 2). Infusion at 50°C using a 5% methionine solution would represent economy in the drying process following the infusion, besides the fact that the dried kernels exhibited a better appearance.

The influence of methionine concentration in the rate of infusion at 50°C is shown in Figure 3. The time allowed for infusion was 60 min for all concentrations. The absorption rate was almost a linear function of methionine concentration.

The concentration of methionine in the integral flour of this variety was 1.2g/16g of nitrogen. The methionine contents of the cooked original beans and of beans infused with methionine under different conditions as well as of mixtures (1:7 w/w) of the original and infused beans are shown in Table 2. It should be realized that when the infused beans were soaked before cooking, part of the infused methionine diffused out into the soaking water. Therefore, elimination of the soaking water prior to cooking resulted in a lower concentration of methionine in the product. The materials analyzed for total methionine (Table 2) were the same utilized for PER determination.

The PER values and the efficiency of the diets prepared with 10% protein furnished by the original cooked beans as well as by beans infused with methionine and cooked under various different conditions are shown in Table 3. Examination of the data of Table 3 shows clearly that the PER values and the efficiency of the diets are directly proportional to the methionine added to the beans by infusion. The increased content of methionine of the infused beans greatly improved the efficiency of the diets and the growth rate of the rats without affecting substantially the consumption of the diets. Bean diet with methionine content equivalent to casein, as percent of the protein, was not as efficient as the milk protein diet.

The growth rate of the rats on 10% protein diets from the original and infused beans was compared with a casein standard and appears in Figure 4.

The biological availability of methionine as determined by the metabolic balance technique was 55% for the original cooked beans and increased to 83% for the mixture 1:7 (w/w) of original beans and beans infused with a 5% methionine solution. The methionine added by infusion was 100% available.

The results of the sensory evaluation for preference of cooked original and infused beans are shown on Tables 4 and 5. Table 4 compares the judgments of the original beans (A) with those infused with 1% methionine solution

Table 4—Preference test (Directional Paired Comparison) for beans (Phaseolus vulgaris), original and infused with methionine, expressed as frequency (N = 40)[a]

	Comparisons between samples[b]			
Repetition	A × B		A × C	
I	9*	1	6	4^{ns}
II	5	5^{ns}	4	6^{ns}
III	5	5^{ns}	5	5^{ns}
IV	5	5^{ns}	7	3^{ns}
Totals	24	16^{ns}	22	18^{ns}

[a] Significance of the paired test (Roessler et al., 1956). ns = Nonsignificant; * significant (p = 0.05).

[b] A = original cooked beans (Reference sample); B = beans infused with 1% methionine solution; C = mixture 1:7 (w/w) of original beans and beans infused with 5% methionine solution.

Table 5—Preference (Non Structured Scale Method) for odor and taste of beans, original and infused with methionine

Samples[b] (Treatments)	Bean odor[a]		Bean taste[a]	
	Seasoned	Without seasoning	Seasoned	Without seasoning
A	7.83[ns]	7.84*	7.44[ns]	6.68*
B	7.39	7.09	7.08	6.09
C	7.28	7.22	7.19	6.07

[a] Mean values of 60 replications; ns = nonsignificant; * Significant (p = 0.05).

[b] A, original cooked beans (reference sample); B, 1:4 (w/w) mixture of original beans and beans infused with a 5% methionine solution; C, 1:7 (w/w) mixture of original beans and beans infused with a 5% methionine solution.

(B) and, (A) with the 1:7 (w/w) mixture of the original beans and the ones infused with a 5% methionine solution (C), by the Directional Paired Comparison test. No statistically significant difference between treatments was found. Table 5 shows the results of the sensory evaluation of the noninfused original beans (A) compared with beans infused with a 5% methionine solution and mixed in proportion of 1:4 (B) and 1:7 (C) with the original beans, respectively, using the Non Structured Scale method. In these experiments the beans were cooked and served both with and without seasoning and evaluated for bean taste and odor. The statistical analysis (Dunnett test for means) revealed a significant preference (p = 0.05) for the original beans (A) as compared with the B and C mixtures when the beans were cooked and served without seasoning. However, no difference in the mean values between treatments was detected when the beans were cooked and served seasoned as they are traditionally prepared in Brazil. Sulfur odor was slightly perceived by only one person in the laboratory during cooking.

The results reported in this paper seem to indicate a good possibility of applying the infusion method to enrich common beans and other legume seeds with sulfur-containing amino acids, particularly methionine. This can be done in areas where the staple food including beans does not supply enough methionine to compensate for the deficiency of this amino acid in the seed proteins.

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NUTRITIONAL EVALUATION OF FOUR VARIETIES OF DRY BEANS (Phaseolus vulgaris, L.)

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ABSTRACT

Proximate composition, globulin, albumin, nonprotein nitrogen and amino acid contents were determined in four varieties of dry bean. Protein and nonprotein nitrogen contents were similar in all four varieties. The lysine was higher in the varieties Rico 23 and Rosinha -G2. The sulfur-containing amino acids were higher in Rico 23. PER, apparent biological value, nitrogen retention and methionine availability were highest in Rico 23 and lowest in the variety Carioca. For these two varieties PER values ranged from 1.32–0.75, apparent biological value from 58.9–39.4%, nitrogen retention from 36.1–21.0 mg N retained/rat/day and methionine availability from 40.6–29.3%, respectively. Apparent digestibility was lowest for Rico 23 and highest for Piratã-1. Piratã-1 ranked second in all biological parameters, except for methionine availability. Cooking the raw bean did not raise digestibility beyond 66%. Availability of iron was in the range 4–5% for all varieties.

INTRODUCTION

LEGUME SEEDS constitute an excellent source of protein and energy to improve diets based on cereals and roots in low income countries (Bressani, 1973; Souza and Dutra de Oliveira, 1959; Sgarbieri et al., 1978).

In several Latin American countries, particularly in Brazil, common bean is by far the most important legume seed consumed directly as human food. It contains over 20% proteins and about 60–70% carbohydrates in addition to several essential minerals and vitamins.

Because of its high lysine content the bean proteins show a beneficial complementary effect when consumed with cereal proteins which are low in lysine. On the other hand the cereal proteins are complementary to the bean proteins by contributing methionine and cysteine which are limiting amino acids in the bean proteins. Rice and bean constitute the staple in Brazil and the mixture of both is of good nutritive value (Souza and Dutra de Oliveira, 1959).

The nutritive values of seeds from different bean varieties and of the various protein fractions extracted from them have not been studied adequately. Common bean is generally considered a good source of dietary iron. However, there are indications that the iron of bean could be largely unavailable to the organisms (Layrisse et al., 1969).

In this paper some compositional and nutritive properties of four bean varieties largely cultivated and consumed in Brazil are described.

EXPERIMENTAL

Varieties

The bean varieties were obtained locally from the Agronomic Institute of Campinas, São Paulo, Brazil. The variety Rico 23 is black skinned Rosinha-G2 is pink whereas the other two are of light brown color. All four varieties are commercially available and consumed by all sectors of the population.

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Analytical procedures

Prior to analyses and biological assays the beans were soaked in tap water overnight, the soaking water was discarded and the kernels were cooked for 2 hr in an open kettle. This is the normal way of cooking beans in the rural areas of Brazil. After cooking the samples were frozen freeze-dried and ground in a hammer mill to pass a 70-mesh screen.

Crude protein, petroleum ether extractable material, crude fiber, ash and moisture contents were determined by the procedures described in the AOAC (1975). Carbohydrate was calculated by difference.

The amino acid composition was determined by the method of Spackman et al. (1958) using the Beckman 120C Amino Acid Analyzer and the procedure described by the manufacturer.

Methionine and cysteine (1/2 cystine) were determined in the hydrolysate of the sample previously oxidized with performic acid and hydrogen peroxide at 4°C for 15 hr, as methionine sulfone and cysteic acid, according to the method of Jamalian and Pellet (1968).

Tryptophan was determined colorimetrically in the enzyme hydrolysates following the procedure of Spies (1967).

For extraction, 50g of ground seeds were suspended in 500 ml of 2% sodium chloride and stirred at 25°C for 2 hr. The soluble material was separated from the insoluble residue by centrifugation (10,000 × G, 20 min), in a Sorvall refrigerated centrifuge.

Nonprotein nitrogen was determined in an aliquot of the extract by the method of Becker et al. (1940). The remainder of the extract was dialyzed against distilled water at 4°C for 48 hr with at least six changes of water. The globulin precipitated during dialysis and the albumin remained in solution. The two fractions were separated by centrifugation (10,000 × G, 20 min). The relative amount of globulin and of albumin was established by determining total nitrogen (Kjeldahl) in the dialysed suspension before centrifugation and in the supernatant after centrifugation (albumin fraction). The globulin content was calculated by difference. The sum of globulin, albumin and nonprotein compounds, subtracted from crude protein gave the amount of unrecovered nitrogenous compound. The globulin to albumin ratios were calculated to indicate the predominance of globulin in the 2% NaCl extract.

The contents of iron in the bean flours, in the prepared diets for the rats and in the rat's blood and feces were determined by atomic absorption spectrophotometry following the method described in the AOAC (1975).

Procedures employed for nutritional evaluation

Protein efficiency ratio (PER) was determined essentially by the method of Osborne et al. (1919). Weanling male rats of the Wistar strain (average initial weights = 40g) were caged individually and fed a 10% protein diet (N × 6.25) and water ad libitum for 28 days. Six rats were used in each experimental group.

Apparent biological value, apparent digestibility and nitrogen retention were determined essentially by the method of Mitchell (1923). Male rats of the Wistar strain (average weight = 60g) were held individually in metabolic cages while fed a 10% protein diet (N × 6.25) and water ad libitum for 10 days. Five rats were used in each experiment. An initial 5-day adaptation period to the diet was allowed. From the sixth to the tenth days, feces and urine were collected and the food intake was recorded for estimation of the protein intake.

Digestibility was also determined in vitro for the raw and cooked (121°C, 15 min) beans employing the method of Akeson and Stahmann (1964).

The percentage of available methionine was determined biologically using rat assay and the addition procedure for calculating availability. For each assay 20 male weanling rats of the Wistar strain were divided into four groups of approximately the same body weight. The animals received a basal diet which contained 10% protein exclusively from bean. All the other nutrients were added as necessary to make the diet adequate for the rat. One of the groups received only the basal diet and to the diets of the other three

Table 1—Proximate composition of four varieties of beans (Phaseolus vulgaris, L.)

Component (%) Dry basis	Varieties			
	Rico 23	Rosinha-G2	Carioca	Piratã-1
Protein (% N X 6.25)	25.53	25.77	23.37	23.62
Lipid (P.E. extract)	2.12	1.85	1.45	1.25
Ash	4.20	3.79	4.18	3.58
Crude fiber	5.67	4.57	3.82	4.13
Carbohydrate	62.48	64.02	67.18	67.42

Table 2—Distribution of nitrogenous compounds in four varieties of beans (Phaseolus vulgaris, L.) in different fractions (grams per 100g sample)

Determination[a]	Varieties			
	Rico 23	Rosinha-G2	Carioca	Piratã-1
Crude protein (%)	25.53	25.77	23.37	23.62
Globulin (%)	8.89	8.82	9.10	10.97
Albumin (%)	2.39	3.97	4.08	4.34
Globulin/Albumin	3.72	2.22	2.23	2.53
Nonprotein (%)	4.88	3.75	3.99	3.38
Unrecovered material (%)	9.37	9.23	6.20	4.93

[a] Calculated by multiplying % N by 6.25.

groups methionine was added at 0.3, 0.6 and 0.9% of the protein, respectively. Cysteine was also added to all four diets at 1% of the protein to spare the available methionine. The growth response to the added methionine, which is 100% available made possible the calculation of the availability of the methionine from the bean in the basal diet.

The biological availability of the iron from the bean was evaluated by two different procedures. The availability relative to ferrous sulfate was determined by the slope-ratio assay of Amine et al, (1972). The rats were put initially in an iron-free diet, containing 15% casein as the only source of protein, until a reduction of 50% in the total blood iron was measured. Subsequently they were divided into groups of four rats and each group received for 10 days a different level of iron in the diet, derived either from ferrous sulfate or from one of the four bean varieties studied. When the iron was supplied by the bean, which contains also protein, an equivalent amount of casein was withdrawn from the diet to keep the protein level constant. The bean protein was also balanced with added methionine and cysteine to values equivalent to those of casein. The slope of the response line of iron hemoglobin due to added bean to the iron-free diet was compared with the slope of the response line due to addition of ferrous sulfate taken as 100 and expressed as percentage of the response to ferrous sulfate.

The absolute iron availability from ferrous sulfate was determined in an iron balance experiment (iron absorption) using four groups of six anemic rats, which were fed for 7 days. The first 2 days were allowed for the animals to adapt themselves to the diet, and used for the 5 remaining days, collecting all the feces and recording food intake. The iron ingested minus the iron excreted in the feces gave the proportion which was absorbed. The availability of iron from the beans was recalculated on the basis of the absolute availability of iron from ferrous sulfate.

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION for the bean varieties appear in Table 1. The differences in gross composition between varieties are minimal and do not suggest any significant nutritional difference between varieties.

The fractionation of the bean proteins by extraction, dialysis and centrifugation furnished the results shown in Table 2. It should be noticed that globulin was the predominant type of protein in the 2% NaCl extract. The ratio of globulin to albumin ranged from 2.2–3.7. The lowest values were encountered for the varieties Rosinha-G2 and Carioca whereas the highest value was for Rico 23. The nonprotein nitrogen ranged from 3.4–4.0%. The highest value was found again for the variety Rico 23. The unrecovered nitrogenous material represented 36.7, 35.8, 26.5 and 20.8% of the total crude protein for the varieties Rico 23, Rosinha-G2, Carioca and Piratã-1, respectively. Most of this material remained unextracted in the insoluble residue. It is likely that a small fraction, which was not determined as nonprotein nitrogen, was lost in the material eliminated by dialysis.

Table 3—Amino acid composition of four varieties of beans (Phaseolus vulgaris, L.)

Amino acid (g/16gN)	Varieties			
	Rico 23	Rosinha-G2	Carioca	Piratã-1
Lysine	8.77	8.80	6.84	6.54
Histidine	2.27	2.33	2.53	2.94
Amonea	1.73	2.18	1.85	1.56
Arginine	4.97	6.40	6.22	5.14
Tryptophan[a]	1.02	1.03	1.17	1.32
Aspartic acid	13.80	14.51	12.47	14.12
Threonine	4.70	5.17	4.21	4.53
Serine	6.63	6.62	5.97	5.92
Glutamic acid	20.74	19.53	18.32	19.41
Proline	4.05	3.86	3.34	3.67
Glycine	4.40	4.35	3.65	3.99
Alanine	4.81	4.52	3.86	4.23
1/2 Cystine[b]	1.51	1.11	1.12	1.31
Valine	5.57	6.15	4.06	4.35
Methionine[b]	1.42	1.30	1.13	1.22
Isoleucine	4.75	4.62	3.26	3.50
Leucine	9.94	9.70	7.27	7.75
Tyrosine	2.47	2.56	2.37	2.60
Phenylalanine	5.52	5.86	5.00	5.62

[a] Determined in the enzyme hydrolysate by the p-dimethylamino-benzal-dehyde reaction.
[b] Determined as cysteic acid and methionine sulfone, respectively.

The higher ratio of globulin to albumin in the variety Rico 23 could be of nutritional importance since it was demonstrated in this laboratory (unpublished data) that the isolated globulin from Rosinha-G2 is of better nutritional quality than the albumin.

The amino acid composition of all four varieties is shown in Table 3. No great differences are noticeable from one variety to another except that Rico 23 and Rosinha-G2 showed a higher lysine content than Carioca and Piratã-1 and that the variety of Rico 23 was a little higher in sulfur-containing amino acids (methionine plus cysteine) than the other three varieties. Tryptophan was slightly higher for Piratã-1 than for the other varieties studied.

The nutritional qualities of the proteins of the four bean varieties were evaluated by several biological parameters. The results are summarized in Table 4. Except for digestibility the variety Rico 23 was superior in all parameters studied. The variety Piratã-1 ranked second, except for available methionine. Rosinha-G2 and Carioca were comparatively of inferior nutritional value.

The most undesirable nutritional properties in the bean proteins, in general, seem to be the low digestibility and the very low availability of methionine, which is the limiting essential amino acid. The digestibility (52–69%) for these varieties is quite low compared with other legume seeds in which digestibility ranged from 80–90% (Sgarbieri, 1978). The explanation for the low digestibility of the common bean proteins is still not available. The low digestibility in

Table 4—Nutritional evaluation of the proteins from four varieties of beans (Phaseolus vulgaris, L.)

Determination	Varieties			
	Rico 23	Rosinha-G2	Carioca	Piratã-1
Protein efficiency ratio (PER)[a]	1.32	0.85	0.75	1.12
Apparent biological value (%)	58.9	38.3	39.4	49.7
Apparent Digestibility (%)	52.0	58.0	64.5	69.5
Nitrogen balance (Retention, mg)[b]	36.1	24.0	21.0	29.2
Available Metionine (%)	40.6	36.6	29.3	34.7

[a] PER for casein control 3.4
[b] Average value per rat per day

Table 5—Digestibility in vitro of the proteins of four varieties of beans (Phaseolus vulgaris, L.) before and after cooking

Variety	Digestibility	
	Raw	Cooked[a]
Rico 23	39.2	60.5
Rosinha-G2	45.2	66.2
Carioca	43.9	62.8
Piratã-1	45.0	65.5

[a] Cooked in autoclave (15 min, 121°C)

Table 6—Content and biological availability of iron of four varieties of beans (Phaseolus vulgaris, L.)

Variety	Iron content (mg/100g)	Iron availability ($FeSo_4$ = 100%)	Iron availability ($FeSO_4$ = 30%)
Rico 23	9.93	16.25	4.88
Rosinha-G2	7.93	15.38	4.61
Carioca	8.70	13.75	4.05
Piratã-1	5.79	17.50	5.25

the uncooked (raw) bean is attributed to the activity of protease inhibitors which act in diminishing the activity of the digestive enzymes. Heat treatment of the bean in the cooking process blocks the activity of the protease inhibitors thus exerting a beneficial effect on the digestibility as shown in Table 5. However, even after cooking, the digestibility of the bean proteins is not improved beyond 70% (Table 4).

Seidl et al. (1969) reported on a globulin fraction from kidney bean which showed protease inhibiting activity even after relatively severe heat-treatment and was thus resistant to the action of the proteolytic enzymes. Since this fraction accounted for a significant part of the total bean proteins, the above authors suggested that this particular globulin should be held responsible for the low digestibility of total protein in the ground integral seeds.

Contrary to this concept, at least for the variety Rosinha-G2, the isolated total globulin exhibited the highest digestibility (80–90%) which was considerably higher than the digestibility of the total protein in the integral seed (unpublished data). The low digestibility of the bean protein in the whole seed could be explained by one or both of the following: (a) the proteins which remain in the insoluble residue (unextracted in NaCl) which accounts for 20–30% of the total proteins is very indigestible thus diminishing the digestibility of the crude protein in the whole grain; (b) the proteins in the ground seeds react with other tissue cell components forming less digestible complexes.

The highest availability of methionine was 41% in the variety Rico 23 and the lowest was 29% in the variety Carioca. There was a good apparent correlation between either PER and available methionine or nitrogen retention and available methionine. The percentages of available methionine observed for the four varieties of *Phaseolus vulgaris* studied were considerably lower than the 50% availability reported for Navy beans (Evans and Bauer, 1978).

The results of determination of iron content and biological availability are shown in Table 6. The iron contents were 9.93, 7.93, 8.70 and 5.79 mg/100g of bean flour for the varieties Rico 23, Rosinha-G2, Carioca and Piratã-1, respectively.

The biological availability of iron was first determined using ferrous sulfate as reference. Percent availability was calculated considering ferrous sulfate iron as 100% available. By this criterion the bioavailability of the bean iron ranged from 13.7% for Carioca to 17.5% for Piratã-1.

The absolute availability of the ferrous sulfate iron was determined by an iron absorption experiment performed with a casein diet containing ferrous sulfate as the only source of iron. From this experiment, the availability of iron from ferrous sulfate was observed to be only 30%.

Recalculation of iron available from the beans based on 30% availability of this element from ferrous sulfate gave results ranging from 4.05–5.25% as shown in Table 6. These results are within the range of iron absorption expected for vegetable foods.

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FUNCTIONAL ROLE OF THE ARABINOGALACTAN OF BLACK GRAM (*Phaseolus mungo*) IN THE TEXTURE OF LEAVENED FOODS (STEAMED PUDDINGS)

N. S. SUSHEELAMMA and M. V. L. RAO

ABSTRACT

The arabinogalactan of black gram (Phaseolus mungo) exerts high viscosity in aqueous medium and thus stabilizes the network of foam formed by the surface active protein both at room and at higher (culinary) temperatures. This is essential for the raising of the rice-black gram batters (autofermented or chemically leavened) and the porosity of the steamed puddings (*Idli*). Proportions of black gram flour and rice semolina in the batters of 1:1 to 1:2 are optimal to achieve a balance between surface activity and viscosity to obtain products with low bulk density and satisfactory texture.

INTRODUCTION

THE PRESENCE of a mucilagenous principle has long been recognized to be a distinctive feature of black gram (*Phaseolus mungo / Vigna mungo*) as contrasted with other common grain legumes. By virtue of this it has found traditional application in some of the most popular and typically Indian food preparations such as the *Idli*—a steamed pudding made out of finely ground (100 mesh) black gram flour and coarsely ground (30 mesh) rice semolina batters after fermentation, and *Dosai*—a pan cake made out of black gram and rice flour mix from a thinner batter after fermentation. Soft and spongy texture and low bulk density are their major and much desired textural attributes.

In early experiments carried out to identify the factors responsible for the texture, emphasis was placed on the mucilagenous principle of black gram (Kadkol et al. 1961). Subsequent investigations (Susheelamma and Rao, 1974) more clearly indicated that the texture principles in black gram could be resolved into (1) a highly surface active (foam forming) protein (globulin) responsible for the soft and porous texture of leavened foods and (2) an arabinogalactan type of polysaccharide which stabilizes this texture.

The surface active proteins have been purified and characterized (Susheelamma and Rao, 1978a). The polysaccharide—an arabino-galactan shown to have a high molecular weight ca 144000 daltons (Susheelamma and Rao, 1978b)—is highly viscogenic and swells and forms rather weak gels in water. These hydrocolloidal properties showed interesting correlations with the changes in volumes of autofermented or chemically leavened batters, bulk densities of the steamed puddings as well as the stabilization of the spongy texture. These findings helped to elucidate the manner in which the arabinogalactan in association with the surface active protein influences the texture of leavened foods. The results are discussed in this paper.

MATERIALS & METHODS

Materials

Finely ground (100 mesh) flours of black gram, bengal gram, green gram, red gram, coarsely ground (30 mesh) rice semolina and the saline soluble globulin (SE) and the trichloro-acetic acid extractable polysaccharide (TCA-polysaccharide or P) from black gram and other pulses were prepared as described earlier (Susheelamma and Rao, 1974).

The viscosity of aqueous dispersions of pulse flours (0–40%) and their batters containing rice semolina with different proportions of pulse flours (20–70%) was determined in a Brookfield viscometer with LVT model spindles.

The volumes of rice-black gram flour batters (prepared in graduated boiling tubes) were noted before and after steaming in the autofermented and chemically leavened samples. Test *Idli* preparations from these batters and also with isolated protein and polysaccharide either alone or in combination with different *proportions* of the polysaccharide were prepared and their bulk densities determined as described earlier (Susheelamma and Rao, 1974).

Foam stabilization

TCA polysaccharide from black gram and other pulses, guar gum (Dealca P/225) and gelatinized soluble starch were added to the solution of surface active protein (SE) of black gram in varying amounts with $NaHCO_3$ and citric acid as the in situ source of CO_2. The contents were mixed well, allowed to stand at room temperature (25°C) for 10 min and heated in a water bath at 95°C. The foam volumes were noted before and after acidification and after heating. Photograph of the foam columns was taken after 3–4 min standing at 95°C.

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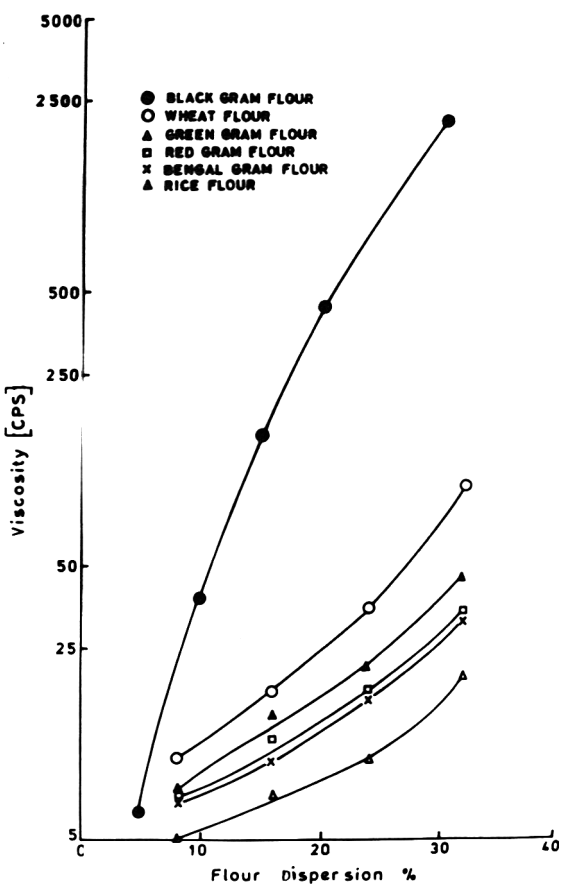

Fig. 1—Viscosities of pulse flour dispersions.

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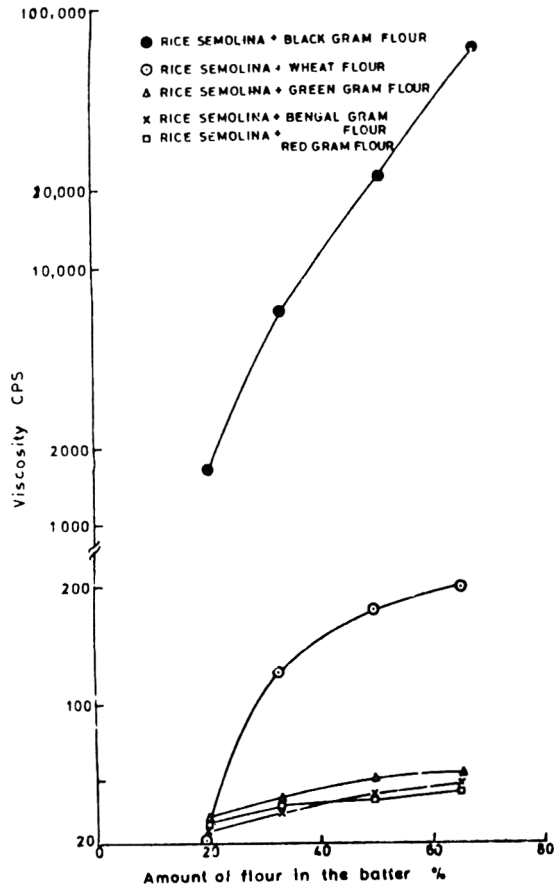


Fig. 2—Viscosities of pulse flour batters.

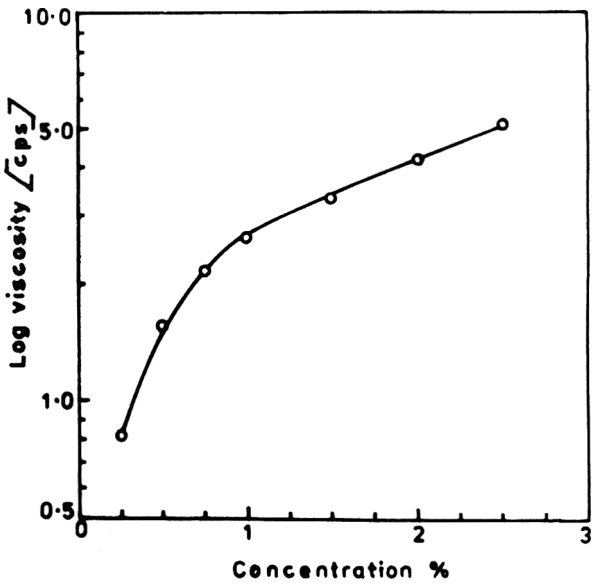


Fig. 3—Viscosity of arabinogalactan dispersions. Successive points in the graph represent viscosity of 6.5, 37.5, 150, 450, 2250, 16200 and 130000 cps units.

RESULTS & DISCUSSION

Viscosities of flour dispersions and batters

The viscosities of aqueous dispersions of black gram flour at various concentrations were one to two orders higher (Fig. 1) than those of other common legume/pulse flour dispersions of corresponding concentrations. Those of rice flour dispersions were very low and of wheat flour dispersions intermediate between black gram and other pulse flour dispersions. The viscosities of unfermented pulse flour batters showed a similar trend (Fig. 2). The viscosity of black gram flour dispersions were nearly the same as those of equivalent concentrations of the isolated arabinogalactan as could be seen from Figure 3, and hence this polysaccharide is the major contributor to the viscosity of black gram flour dispersions or batters. As this polysaccharide swells and weakly gels in water the viscosity of batters with rice semolina (which binds the water) is much higher (by one to two orders) compared to that of flour dispersions (Fig. 1 and 2) and on *a priori* considerations viscosity was the first to be implicated in its functional role.

Batter volumes

It could be seen from Figure 4(A) that the surface activity (which is responsible for dough raising/porosity) of the batters increased linearly. Volumes before and after steaming differed in the autofermented and chemically leavened samples. The initial volumes were the same in both cases. At lower levels of black gram in the mix the volumes were very high in the chemically leavened and steamed samples, moderate in fermented and low in fermented and steamed samples. This is due to the slower dough raising during autofermentation wherein the air cells are smaller and uniform while leavening with chemicals liberates the gas (CO_2) more quickly and the air cells are considerably bigger. The trend of the changes was the same in both cases—volumes decreasing between 30–50% and remaining fairly constant above 60% of flour in the mix.

Bulk density

The bulk densities of steamed puddings which bear an inverse relationship to the volume decreased at first [Fig. 4(B)], remained constant between 30–50% of flour in the mix and then increased sharply disproportionate to the volume changes, while viscosity which stabilizes the batter volume/dough raising increased logarithmically with increasing proportions of black gram in the mix. Bulk densities could not be determined between 10 and 20% of pulse flour in the mix.

Texture

The bulk density curve [Fig. 4(B)] could be divided into four segments A, B, C and D corresponding to regions with significant differences in the texture of *Idli*. Rice semolina with very low proportions of black gram flour [Fig. 4(B) A] gave products which were very hard. Between 10 and 20% of flour in the mix [Fig. 4(B) B] there was excessive expansion of the matrix and the structure was floury and unstable. Hence bulk densities could not be determined. Between 30–50% of black gram flour in the mix was optimal [Fig. 4(B) C] for producing the soft and spongy texture with uniform porosity and physical stability. At levels exceeding the optimal (> 50%) range [Fig. 4(B) D] the gas diffusion was retarded considerably eventuating in irregular porosity or foam cells and congealing of such cells to form large gas pockets randomly distributed and the products were sticky with unsatisfactory texture.

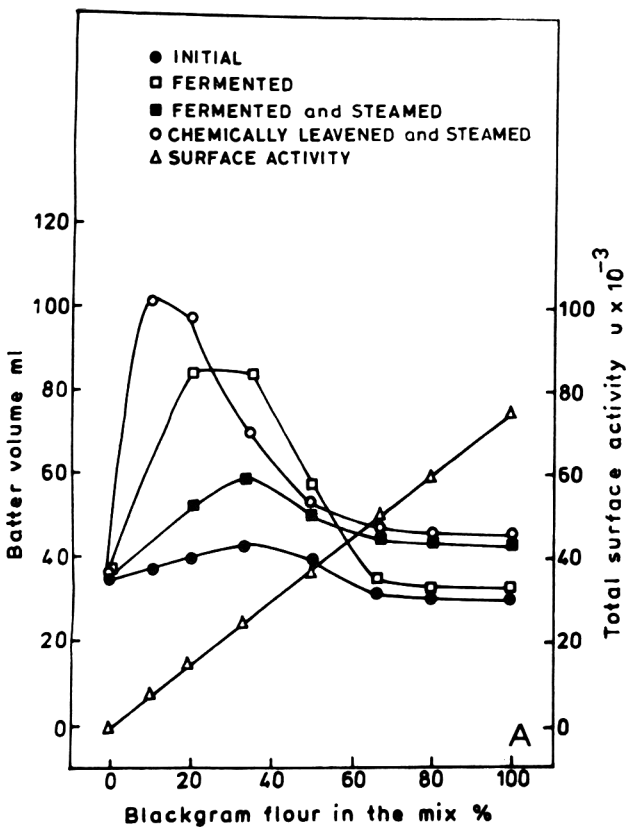


Fig. 4(A)—Changes in batter volumes after autofermentation or chemical leavening and steaming. The total weight of solids was 15g in each experiment. 1.8 parts of water was added and mixed. 25 mg of D-glucono-δ-lactone and 12.5 mg of $NaHCO_3$ per g of the solid (Susheelamma and Rao, 1974) was added for chemical leavening. The contents were mixed well before fermentation or steaming.

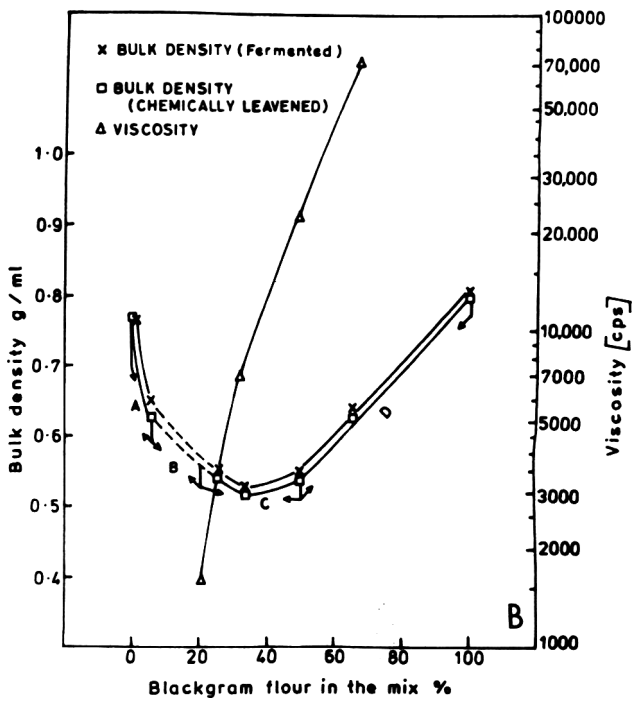


Fig. 4(B)—Bulk density of Idli preparations and the viscosities of batters containing different proportions of black gram flour and rice semolina. A, B, C and D represent regions with different textures: A—hard; B—floury and unstable; C—optimal porosity and satisfactory; D—Sticky and unsatisfactory.

These results indicate that viscosity is an important parameter which determines the volume of batters both in the cold and after steaming and hence the bulk density of the final products. A balance between factors contributing to dough raising/porosity (surface activity) and those which impart adhesion/binding, but prevent excessive expansion (viscosity) has to be achieved for getting *Idlis* with low bulk density and satisfactory texture, and this evidently obtained at 30–50% of black gram flour in the mix.

Table 1—Bulk density of steamed puddings containing rice semolina and isolated protein and polysaccharide from black gram

Components[a](g)				Bulk density g/ml
RS	BLG	SE	P	
5	–	–	–	0.79
5	2.5	–	–	0.55
5	–	0.25	–	0.63
5	–	–	0.125	0.75
5	–	–	0.250	0.69
5	–	0.25	0.065	0.64
5	–	0.25	0.125	0.56
5	–	0.25	0.185	0.61
5	–	0.25	0.250	0.66
5	–	0.25	0.310	0.71

[a] Rice semolina (RS), black gram flour (BLG), dialyzed and freeze-dried saline extract (SE) and TCA-polysaccharide (P) from black gram. The mean deviation was found to be ±0.03 in three independent experiments.

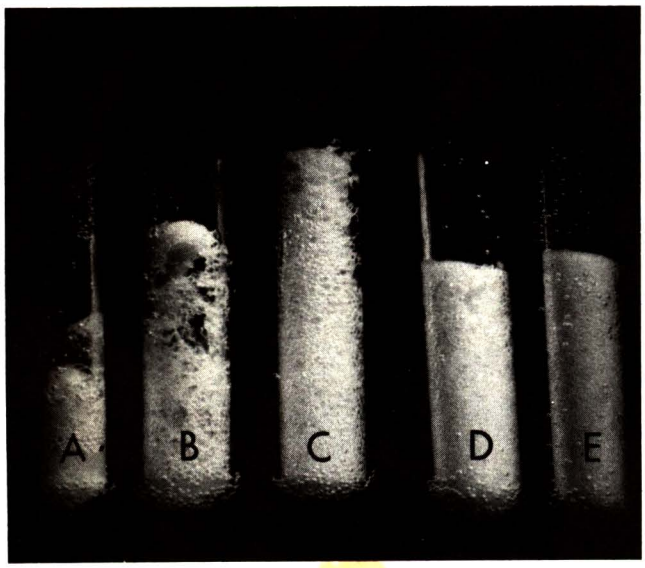
Studies with isolated protein and polysaccharide

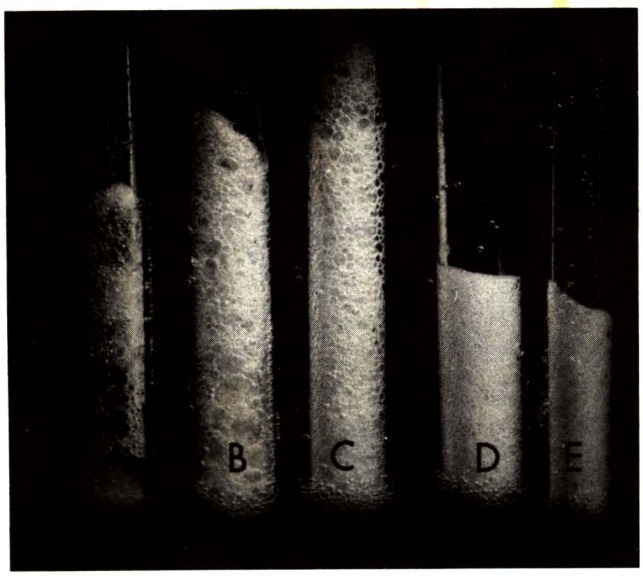
Experiments carried out with isolated surface active protein fraction (SE) and TCA-polysaccharide (P) of black gram confirmed the above findings. It is seen from Table 1 that combination of SE and P at a proportion equivalent to 30% of black gram flour along with rice semolina gave products with lowest bulk density and good texture comparable to that of rice with 33% of black gram flour in the mix. Addition of polysaccharide above this concentration (proportions corresponding to 60–75% levels of black gram flour in the mix) increased the bulk density of the product and the texture was adversely affected.

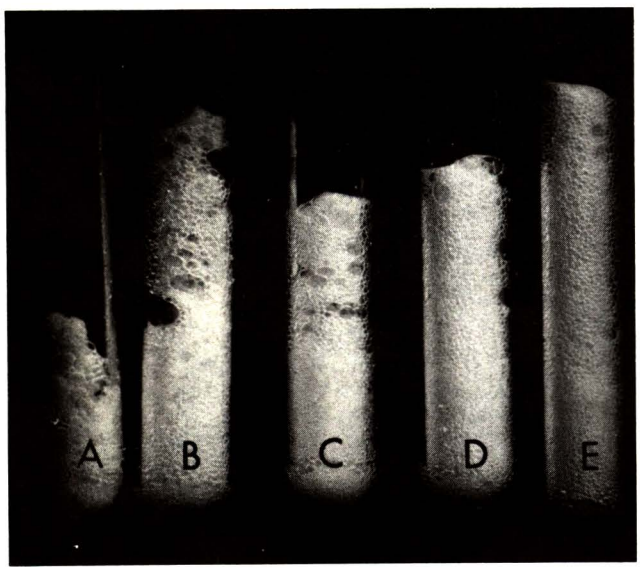
Foam stabilization

The protective action of the polysaccharide against thermal disruption of the foam formed by the surface active protein fraction has been demonstrated by the results shown in Table 2. In presence of an in situ source of CO_2, the saline extract gave rise to a foam column, which increased slightly after heating, but was unstable to heat. Addition of TCA-polysaccharide from black gram increased the foam volumes after heating and also stabilized this foam. TCA-polysaccharide from other pulses failed to stabilize the foam at higher temperature.

Figure 5 shows the foam columns after standing for 3 min at 95°C. It is clearly seen [Fig. 5(top) B, C] that maximum volume expansion with good cellular structure of







foam was attained at 1–1.5% of the polysaccharide corresponding to 30–40% of legume flour in the mix. The concentrations at which guar gum (Dealca P/225) [Fig. 5(center) B, C] and gelatinized soluble starch [Fig. 5(bottom) D, E] were effective are 0.5–1% and 2.5–3.2% respectively, which correspond to the isoviscous levels of these gums with the black gram polysaccharide. The decrease in foam volume [Fig. 5(top) and (center) D, E] at higher levels and the disruption of the foam at lower levels [Fig. 5(top–bottom) A] are also clearly demonstrated in the picture. Foams formed by synthetic detergents were however, not thus stabilized at the higher temperature by the polysaccharide.

Mode of action

The above findings taken in toto lead to the postulation of the following mode of action of the arabinogalactan of black gram and other hydro colloids-guar gum and gelatinized soluble starch.

The surface active proteins form copious foams by virtue of their ability to hold the gas and thus give rise to products with good porosity. But the foam cells tend to get frayed or break up after long time at room temperature or immediately after heating. The surface active proteins in presence of low concentrations of the polysaccharide (low viscosity) form large foam cells which expand greatly on heating and lead to the formation of a structure, which is unstable and becomes floury. The presence of protein with sufficient amounts of polysaccharide gives rise to stable foams with optimal viscosity, which prevents the back flow of liquids both at room and at higher (culinary) temperatures from the films of foam. Presumably the hydrocolloids through

–Continued on page 1316

Table 2–Stabilization of foam by TCA-polysaccharide

Components[a] (mg)					Vol. after acidification	Vol. after 10 min of acidification	Vol. at 95°C
	P						
SE	BG	GG	RG	BLG			
25	–	–	–	–	3.0	2.0	3.5
50	–	–	–	–	3.0	2.5	5.0
25	25	–	–	–	3.0	2.0	5.0
25	–	25	–	–	3.5	2.5	5.0
25	–	–	25	–	3.5	1.5	6.0
25	–	–	–	10	4.0	3.0	8.5[b]
25	–	–	–	25	3.5	2.8	10.5[b]

[a] TCA-polysaccharide (P), dialyzed and lyophilized saline extract of black gram (SE), bengal gram (BG), green gram (GG), red gram (RG) and black gram (BLG). Protein was dispersed in 1 ml of water. 0.25 ml of $NaHCO_3$ (5%) was added, mixed well. Volume before acidification was 1.5 ml in all samples. 0.25 ml of citric acid (5%) was added to these samples. The values represent averages of three independent experiments.

[b] After 3 min at 95°C the foam columns were stable only in these two and collapsed in other samples.

Fig. 5–Stabilization of the foam formed by surface active protein fraction SE (dialyzed and freeze-dried saline extract of black gram) against thermal disruption. The protein solution (1 ml of 2% solution) was tested in presence of $NaHCO_3$ (0.5 ml of 5% solution) and citric acid (0.5 ml of 5% solution) as in situ source of CO_2 along with; (top) (A) 0, (B) 0.5, (C) 1.0, (D) 1.5 and (E) 2.5% of TCA-polysaccharide from black gram; (center) (A) 0, (B) 0.5, (C) 1.0, (D) 1.5 and (E) 2.5% of guar gum (Dealca P/225); (bottom) (A) 0, (B) 0.8, (C) 1.6, (D) 2.4 and (E) 3.2% of gelatinized soluble starch.

CONDENSED TANNINS OF RAPESEED MEAL

J. LEUNG, T. W. FENTON, M. M. MUELLER and D. R. CLANDININ

ABSTRACT

The study was undertaken to determine the amount and nature of condensed tannins in rapeseed meal. No condensed tannins were found in rapeseed meats (endosperm plus germ). The hulls, however, yielded 0.1% condensed tannins by 70% aqueous acetone extraction, the bulk of the condensed tannins in the hulls being unextractable by the extractants used. The basic unit of the isolated polymeric flavanols appeared to be leucocyanidin.

INTRODUCTION

TANNINS are usually classified into hydrolyzable and condensed catagories on the basis of their structural differences and diverse hydrolytic reactivities (Ribéreau-Gayon, 1972; Haslem, 1966). Tannins have been extensively investigated because of their adverse effects on growth of animals (Glick and Joslyn, 1970) and birds (Chang and Fuller, 1964; Vohra et al., 1966; Marquardt et al., 1977), protein utilization (Bornstein et al., 1965; Vohra et al., 1966) and metabolizable energy value of feeds (Yapar and Clandinin, 1972). It has been reported that rapeseed meal (RSM) contains approximately 3% of tannins (Clandinin and Heard, 1968) as determined by the method for tannins in cloves and allspice (AOAC, 1965). However, this value has been shown (Fenwick and Hoggan, 1976) to include sinapine. This choline ester of sinapic acid constitutes about 1.5% of RSM (Mueller et al., 1978). Hence, it would appear that RSM contains only about 1.5% of tannins. Relatively little is known about the chemical nature of tannins in RSM. The presence of condensed tannins in rapeseed hulls (RSH) was first reported in 1959 by Bate-Smith and Ribéreau-Gayon and verified by Durkee (1971) who identified cyanidin, pelargonidin and an artifact, the n-butyl derivative of cyanidin in the hydrolytic products of RSH. To date, the condensed tannins of RSH have not been isolated and their physical and chemical properties have not been extensively studied. The present work was undertaken to isolate, purify and characterize these polymeric phenolic compounds in the hope that their contribution to the total tannin content of RSM could be evaluated. The hull part of therapeseed was chosen for the study on condensed tannins since their presence could not be detected in the meat (endosperm plus germ) fraction in our laboratory, confirming the report by Durkee (1971). In light of recent successes with polydextran LH-20 in alcoholic media as adsorbant for the isolation of condensed tannins from sorghum grain (Strumeyer and Malin, 1975), faba bean hulls (Marquardt et al., 1977) and pasture legume species (Jones et al., 1976), a similar procedure was adopted in the present investigation.

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MATERIALS & METHODS

Materials

The RSH (*Brassica napus*, cv. Tower) used in this investigation were kindly supplied by Dr. J.D. Jones, Food Research Institute, Ottawa, Canada. The brown-coated sorghum grain was provided by Dr. K.G. Briggs of the Plant Science Department, University of Alberta, Edmonton, Canada, and the faba beans (*Vicia faba minor* cv. Diana) were obtained through a commercial source. These plant materials were initially defatted by soxhlet extraction with hexane, finely ground in a micromill and further defatted by soxhlet extraction with hexane. Condensed tannins of sorghum grain and faba bean hulls were isolated according to the method of Strumeyer and Malin (1975). Pelargonidin hydrochloride was purchased from K & K Laboratories Inc., Plainfield, NY. Catechin and epicatechin were obtained from Sigma Chemical Company, St. Louis, MO. U.S.A..

Absorption and mass spectra

Absorption spectra in the visible and ultra-violet region were obtained using a Unicam spectrophotometer, model SP1800, and 1 cm cuvets. Mass spectra and microanalysis were run by the Mass Spectroscopy Laboratory and Spectral Services Laboratory, respectively, of the Chemistry Department, University of Alberta, Edmonton, Canada.

Chromatography

Sephadex G-50 (medium) and LH-20 gels obtained from Pharmacia (Canada) Ltd., Montreal, were equilibrated in appropriate solvents overnight before use. Column flow rates were maintained constant by a Mariotte flask and the samples were collected in various sizes by a fraction collector. All solvent systems were deaerated briefly and gased with nitrogen for column chromatographic and extraction purposes. The void volume of Sephadex G-50 columns were calibrated in 50% aqueous acetone by dextran blue.

Thin-layer chromatography was carried out on silica gel G-25 UV-254 plates (Macherey-Nagel) at room temperature. Paper chromatography was performed in a descending manner using Whatman No. 3MM paper. Solvents used for thin-layer chromatography were: (a) chloroform-2-methoxyethanol-88% formic acid-acetic acid (CMFA) 60:20:8:12; (b) benzene-ether-ethanol-acetic acid (BEEA) 52:45:2:0.2; (c) n-butanol-acetic acid-water (BAW 612) 6:1:2; (d) n-butanol-acetic acid-water (BAW 411) 4:1:1 and (e) 2% aqueous acetic acid. The developing solvents employed for paper chromatography were: (a) acetic acid-HCl-water (Forestal) 30:3:10; (b) n-butanol-acetic acid-water (BAW 415) 4:1:5, upper phase, and (c) 88% formic acid-HCl-water (FHW) 5:2:3. All the thin layer and paper chromatograms were viewed by ultra violet light (UV) (360 nm and 254 nm) before treatment with specific sprays.

Visualization reagents employed in chromatography

Most of the phenolic compounds in the RSH extract on the TLC plates could readily be visualized because of their fluorescent properties when viewed under UV light before and after exposure to ammonia. The reagent sprays used for visualization were as follows: (a) 5% H_2SO_4 followed by charring for organic compounds; (b) 1% aqueous $FeCl_3$ and $K_3Fe(CN)_6$ for phenolics (Ribéreau-Gayon, 1972); (c) diazotized p-nitroaniline for phenolics (Ribéreau-Gayon, 1972); (d) 10% vanillin in conc HCl and 3% p-toluenesulphonic acid in ethanol (Roux and Maihs, 1960) for flavanols; (e) ninhydrin for amines and amino acids (Clark, 1964) and (f) anisaldehyde-H_2SO_4 (Stahl, 1969) for sugars.

Extraction of condensed tannins from rapeseed hulls

In order to remove chlorophyll and some of the low molecular weight phenolics present, 0.5g of defatted RSH was extracted thrice with 5 ml of 95% ethanol by homogenization with a polytron (Brinkmann) set at the maximum speed for 1 min each time, a cold water bath being used to prevent evaporation of the solvent during the extraction. The extract was separated from the hulls by centrifu-

gation. The alcoholic supernatants were combined and components were determined by TLC. The alcohol treated RSH was then extracted 3 times with 5 ml of 70% aqueous acetone by homogenization as described above. The combined supernatants were evaporated under reduced pressure to almost dryness. The yellowish residue was redissolved in a minimum amount of 50% aqueous acetone and was used immediately for the isolation of condensed tannins by column chromatography. Additionally, the constituents of the extract were investigated by TLC. The residual RSH was subjected to further extraction and acid hydrolysis to determine the effectiveness of the extraction method for removal of the condensed tannins from RSH.

Isolation of condensed tannins

The procedure undertaken was primarily adapted from the work of Jones et al. (1976) and could be summarized as follows. The sample extract was applied to a Sephadex G-50 column which was previously equilibrated with the same solvent. The size of the sample was about 2% of the bed volume of the column. The column was eluted with 50% aqueous acetone at a flow rate of 12 ml/hr and 1-ml fractions were collected. The fractions were assayed for condensed tannins by TLC with BAW (612) as developing solvent. UV light and 10% vanillin-conc HCl spray were used for visualization. Condensed tannins were detected at the void volume of the column. The fractions which contained condensed tannins were combined and evaporated to almost dryness under reduced pressure. 50% aqueous methanol was added to redissolve the residue for further purification by Sephadex LH-20 in the same solvent. The flow rate was kept at 20 ml/hr and 2 ml fractions were collected. 500 ml of solvent was used to remove the remaining phenolic contaminants. The fractions were assayed for condensed tannins by the method mentioned above. The top region of the column which showed a pale brown coloration due to the presence of condensed tannins was removed and repacked into a small column (approx 2.2 × 4 cm) and the condensed tannins were eluted with 75 ml of 70% aqueous acetone. The gel remaining in the original column was eluted with 150 ml of the same solvent. Acetone of both eluates was evaporated under reduced pressure and the remaining aqueous solutions were freeze-dried. Both portions were examined by TLC for their content and purity in regard to condensed tannins.

Acid hydrolysis of the condensed tannins isolated from rapeseed hulls

Condensed tannins were refluxed in 5% conc HCl in n-butanol for 30 min. The pale yellowish brown solution turned pinkish within minutes. The resulting solution was concentrated under reduced pressure, applied onto a sheet of Whatman No. 3 MM paper and developed in Forestal solvent. In order to identify the anthocyanidins formed, pelargonidin hydrochloride and the hydrolytic products of condensed tannins of sorghum grain and which had been reported to contain cyanidin (Strumeyer and Malin, 1975) were chromatographed simultaneously for reference purposes. It was observed that the hydrolytic products of condensed tannins of faba bean hulls contained both cyanidin and delphinidin so this was also used for reference. The pink bands with rf values in the region of 0.5 given by the three hydrolyzed samples were cut out and eluted separately with 0.1% HCl in methanol. The solutions were evaporated to almost dryness and rechromatographed with the same developing solvent. The pink compounds were again isolated as before. The dry residues were dissolved in 0.1% HCl in ethanol for spectroscopic characterization.

For further confirmation of the identification of the anthocyanidins formed, the hydrolytic products of the three types of condensed tannins were subjected to paper chromatography developed in BAW (415) and FHW (523) systems. The chromatograms were sprayed with 4% aqueous ammonium molybdate (Mullick, 1969) and 1% lead acetate in 75% aqueous ethanol (Fuleki and Francis, 1967) to determine the hydroxy substitution pattern in the aromatic 'B' ring of the anthocyanidins.

Mild acid hydrolysis of the condensed tannins isolated from rapeseed hulls

Condensed tannins in 0.1N HCl (2 mg/ml) were heated for 10 min in a boiling water bath. The pale tan-colored reaction mixture was analyzed for its contents immediately by TLC in BAW (612), BAW (411), CMFA and BEEA systems. Authentic samples of glucose, galactose, arabinose, xylose, catechin, epicatechin and gallic acid were used as reference standards. The chromatograms were treated with vanillin-conc HCl, anisaldehyde-H_2SO_4 and 1% aqueous $FeCl_3$ and $K_3Fe(CN)_6$.

RESULTS & DISCUSSION

Isolation of condensed tannins from rapeseed hulls

Various solvent systems which included methanol, ethanol, water, acidic methanol and aqueous solutions of methanol, ethanol and acetone were investigated for their extraction efficiency of condensed tannins under different conditions such as soxhlet extraction, shaking at various temperatures for different lengths of time and homogenization using a polytron. It was concluded that polytron homogenization in 70% acetone was most effective.

Using systems BEEA, CMFA and BAW (612) as developing solvents, TLC chromatograms of the ethanolic extract of the hulls did not indicate the presence of simple phenolic acids such as ferulic, coumaric, caffeic, sinapic, and p-hydroxybenzoic acid, these findings being in line with the report of Durkee and Thivierge (1975) who suggested that these phenolics occurred mainly in bound condition in RSH. Condensed tannins which were found only in traces in the ethanolic extract occurred in significant amounts in the aqueous acetone extract. They remained at the origin of the chromatograms as revealed by vanillin-conc HCl spray. Most of the noncondensed-tannin compounds present in the aqueous acetone extract were identified as sinapic acid, derivatives of sinapic acid and low molecular weight carbohydrates.

Utilizing Sephadex G-50 in 50% aqueous acetone as a fractionation aid, condensed tannins came out in the void volume of the column along with a small amount of non-tannin compounds. Sephadex LH-20 in 50% aqueous MeOH was found to be an effective, although not excellent, tool for further purification of the crude condensed tannins. It was observed that almost all of the noncondensed tannin compounds could be removed by exhaustive washing of the column with 50% aqueous MeOH. However, condensed tannins were also detected in these washings, the amount being approximately 4–5% of that of the condensed tannins isolated in the pure state as determined by TLC. Compounds which exhibited condensed tannin properties remained as a defuse pale brownish band at the top of the column. They could be effectively released by 70% aqueous acetone. To minimize the risk of oxidation on prolonged elution and because it was found that traces of a sugar ester of sinapic acid remained tightly bound to the gel in the lower portion of the column in spite of exhaustive washing by aqueous methanol, the upper region was removed and the condensed tannins were released separately rather than down the column. After freeze-drying, 0.58 mg of condensed tannins representing 0.12% of the defatted RSH used was isolated as a white, amorphous compound with a light brown tint which could be due to oxidation during the experimental procedure. This procedure was repeated several times and the amount of condensed tannins isolated was found to be consistently about 0.1% of the defatted RSH. The purity of the isolated condensed tannins was tested by 2-dimensional TLC in BAW (612) and 2% aqueous acetic acid. Only one spot with some tailing was detected when the chromatograms were visualized by: (a) UV (short and long wavelength); (b) vanillin-conc HCl and p-toluenesulphonic acid in ethanol (flavanols); (c) 5% H_2SO_4 followed by charring (organic compounds); (d) 1% aqueous $FeCl_3$ and $K_3Fe(CN)_6$ (phenolics) and (e) diazotized p-nitroaniline (phenolics). Also no amino acids or amines (ninhydrin) and carbohydrate compounds (anisaldehyde-H_2SO_4) were detected indicating the isolated condensed tannins were pure. A series of tests were also performed on the isolated compound for its characterization (Table 1).

The compounds which were released by 70% aqueous acetone from the remaining gel in the original column was found to contain a sugar ester of sinapic acid with rf=0.72

on the chromatogram developed in CMFA, and traces of another compound (rf=0.09 in BAW (612)) which gave a positive reaction to vanillin-conc HCl. It was evident that the compound with the low rf value was flavanolic in nature and could be condensed tannins with a lower degree of polymerization. Based on comparison by TLC, the content of condensed tannins present in this preparation was found to be minute and estimated to be approximately 1% of the pure condensed tannins isolated from the upper portion of the column.

When the RSH residue was subjected to further extraction, it was observed that the majority of the condensed tannins susceptible to the employed procedure had been removed. However, on acid hydrolysis, intense red-colored anthocyanidins continued to be produced even when the degradation was repeated 6 times. This finding showed that most of the condensed tannins remained tightly bound in the hulls.

Acid hydrolysis of the condensed tannins isolated from rapeseed hulls

In order to obtain an insight into the basic units of the polymeric condensed tannins isolated, the hydrolytic products were examined by paper chromatography and visible absorption spectroscopy. The results are shown in Table 2. The paper chromatograms of the reaction mixture of RSH showed the presence of two distinctive pink bands. The compound in the lower region bore the same rf value as that of the cyanidin produced from the condensed tannins of FBH and sorghum grain in the developing solvents. used. These pigments turned blue on exposure to NH_3. This color reaction due to the change in pH is typical of anthocyandins. Furthermore, the color changes displayed by these pigments towards lead acetate and ammonium molybdate were similar to those reported by Durkee (1971) and Mullick (1969). Hence, it can be concluded that the aromatic 'B' ring of the anthocyanidin present had adjacent hydroxy substituents as in cyanidin. The other pink compound with greater rf value was probably the butyl derivative of cyanidin as suggested by the same author and Mathew (1969). These observations indicated that leucocyanidin was the only elementary unit of the condensed tannins isolated from RSH.

Based on his work on the hulls of *Brassica campestris* cv. Echo, Durkee (1971) reported that cyanidin and perlargonidin were obtained when RSH were degraded by hot acidic butanol. According to the findings in our laboratory, *no* perlargonidin was detected when RSH (*Brassica napus* cv. Tower and *Brassica campestris* cv. Echo from four different regions across Western Canada) were subjected to the same treatment.

Table 1—Properties of condensed tannins isolated from rapeseed hulls

Solubility	(water, aq acetone, aq MeOH)> (EtOH, MeOH)
Elements	Absence of sulphur (mass spectrum) and nitrogen (ninhydrin and microanalysis)
1% gelatin	White ppt
Neutral lead acetate	White ppt
Ferric chloride	Dark ppt
Color of aq solution	Pale yellow at neutral pH
Diazotized p-nitroaniline	Yellow with orange tint
Absorption spectrum (H_2O)	203 nm and 280 nm
(0.1N NaOH)	222 nm
UV	Nonfluorescent

Mild acid hydrolysis of the condensed tannins isolated from rapeseed hulls

It was reported (Forsyth and Roberts, 1960; Ito and Joslyn, 1965; Mathew et al., 1969) that condensed tannins with flavan-3-ols and flavan-3,4-diols as sub units, upon dilute acid hydrolysis, gave catechin and epicatechin rather than anthocyanidins due to depolymerization. The TLC of the hydrolytic mixture of the condensed tannins of RSH did not indicate the presence of epicatechin or catechin. The phenolics which show a strong vanillin positive reaction characteristic of flavanols were found at the origin with some tailing, a similar pattern exhibited by the starting material of the reaction. Simple phenolic acids such as gallic acid and sinapic acid, and sugars were not observed. This further substantiated that the condensed tannins of RSH solely existed as polymers of leucocyanidin and the isolated polymeric compound was free of hydrolysable tannins.

Concurrently, an effort was made to detect the presence of gallotannins and ellagitannins by dilute acid hydrolysis

Table 2—Characteristics of cyanidin obtained from the condensed tannins of rapeseed hulls, faba bean hulls and sorghum grain

	Rf values		Colors				
	Forestal	FHW (523)	Visible	UV	Ammonium molybdate[a]	Lead acetate[b]	Max nm
Cyanidin (rapeseed hulls)	0.49	0.22	pink	red	violet	blue	544
N-butyl-cyanidin (rapeseed hulls	0.78	0.46	pink with lt orange tint	red	bluish	blue	539
Cyanidin (faba bean hulls)	0.49	0.22	pink	red	violet	blue	544
Cyanidin (sorghum grain)	0.50	0.23	pink	red	violet	blue	544
Pelargonidin hydrochloride	0.71	0.43	orange	orange red	no change	no change	532
Cyanidin[c] (literature)	0.49	0.22	–	–	–	–	–
N-butyl-cyanidin[d] (literature)	0.82	0.46	–	–	–	–	–

[a] Observations under UV (360nm) right after spraying: cyanidins and n-butyl-cyanidin = dark spots and pelargonidin = fluorescent red.
[b] Observations under UV (360nm) right after spraying: cyanidins = dull red; n-butyl-cyanidin = dark bluish grey and pelargonidin = fluorescent orange.
[c] Harborne (1959).
[d] Mathew (1969).

on both RSM and RSH. No gallic acid was detected by TLC indicating the amount of these hydrolysable tannins must be, if present, below our detection level (1 μg of gallic acid applied at a 5 μl spot and viewed under short wave UV).

CONCLUSIONS

BASED on the present experimental findings, we conclude that the major portion of the condensed tannins of RSH can not be extracted by common solvents. Condensed tannins isolated by the employed procedure were estimated to be 0.1% of the hulls by weight. Cyanidin was detected as the principal degradation product of the isolated polymeric flavanols which implies that condensed tannins exist solely as the polymers of leucocyanidins. In view of the low extractability of condensed tannins from RSH, absorption of these polymeric compounds by the digestive system of the birds and animals would probably be insignificant.

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their affinity for water act as protective colloids, preventing the coalescence of protein micelles and their gross precipitation which would disrupt the physical stability of the matrix or network of foam. At very high concentration of flour or polysaccharide, although there is a corresponding increase in the content of surface active protein, the formation and expansion of foam is retarded or prevented due to a very high increase in viscosity (as it increases logarithmically with concentration). Hence the bulk density grossly increases and the texture is adversely affected. At high concentrations of the gum greater water retention by the swollen hydrocolloid and to some extent also decreased loss of water due to unsatisfactory foam formation and consequent decrease in the evaporative surface contribute to the sharp increases in bulk density which appears to be noncommensurate with the volume changes which vary only within a narrow range.

CONCLUSIONS

THE ABOVE MODE of functioning of the viscogenic hydrocolloids is consistent with all the experimental observations and serves as a working hypothesis for understanding how polysaccharides as diverse as guar gum, gelatinized soluble starch and black gram polysaccharide, which differ in chemical composition but commonly share certain hydrocolloidal characters function similarly. It will also help in further quests for possible alternates for the special functional constituents of black gram.

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OLIGOSACCHARIDES OF 13 AMERICAN CULTIVARS OF COWPEAS (Vigna sinensis)

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ABSTRACT

Mature dry seeds of 13 American cowpea (*Vigna sinensis*) cultivars were subjected to proximate analysis with the following results: moisture 9.6%; crude protein 23.9%; crude fat 1.6%; crude fiber 4.1%; ash 3.3%; and nonfiber carbohydrates by difference 57.7%. The sugars of these cultivars were extracted with 70% ethanol and separated by paper chromatography. On the basis of R_G and R_f values, and several confirming tests, sucrose, raffinose, stachyose and verbascose were identified in these extracts. On a dry basis the average content of the cowpeas in these sugars was: sucrose 2.2%; raffinose 1.2%; stachyose 3.4%; and verbascose 0.9%.

INTRODUCTION

Oligosaccharides have been found in a number of legume seeds (Nigan and Giri, 1961; Korytnyk and Metzler, 1962; Gould and Greenshields; 1964; Kim et al., 1973; Cerning et al., 1975). The oligosaccharides which contain α-galactosidic bonds, such as raffinose, stachyose and verbascose, have been implicated in the incidence of flatulence (Steggerda, 1961; Hellendorn, 1969; Rackis et al., 1970). Flatus generation has been related to the absence of α-galactosidase activity in the upper intestinal tract. Apparently, these nondigestible sugars pass into the large intestine, where they are fermented by microorganisms with the formation of gas. Incidentally, intolerance to lactose, a β-galactoside, is similarly attributed to lack of β-galactosidase in susceptible individuals.

There are few data (Nigam and Hiri, 1961; Lee et al., 1970) dealing with the oligosaccharide composition of cowpeas, also known as black-eye peas (*Vigna sinensis*). The purpose of this study was to provide additional information on the oligosaccharides of a number of cowpea cultivars.

MATERIALS & METHODS

MATURE DRY SEEDS from 13 cultivars of cowpeas grown in the southern United States were analyzed. The samples were subjected to proximate analysis before they were analyzed for oligosaccharides. Moisture, ash, crude protein (N × 6.25), and crude fat were determined according to AOAC (1975) procedures. Crude fiber was determined by the method of Whitehouse et al. (1945). Carbohydrate content was estimated by difference.

The oligosaccharides were determined as follows: a 10-g sample of seeds was ground to 60-mesh size in a Wiley mill, covered with 100 ml 70% ethanol (v/v) and agitated for 12 hr. The mixture was then filtered, the residue was washed with another 50 ml of 70% ethanol and the combined extracts were concentrated under reduced pressure at 40°C. The syrup obtained was made up to 25 ml with water and subjected to paper chromatography.

For qualitative work, the cowpea extracts were spotted along the short edge of a 46 × 57 cm Whatman No. 1 filter paper, next to spots of authentic sugars. Since no authentic verbascose was available for direct chromatographic comparison, the section of a paper chromatogram which presumably contained this sugar was cut off, eluted with water, the eluate was hydrolyzed (1N H_2SO_4, 70°C, 1 hr) and the hydrolysis products were determined qualitatively and quantitatively. For quantitative work, 50 μl of cowpea extract was streaked to a length of 30 cm with the authentic sugars spotted next to the streak.

Among several solvent systems tried the following two resulted in the best separation of the cowpea oligosaccharides: (a) 1-butanol, ethanol, water (5:3:2 by vol) (BEW); and (b) 1-butanol, pyridine, water (6:4:3 by vol) (BPW). By using descending irrigation and stopping the solvent front just before it reached the far edge of the paper it was possible to obtain R_f values which were necessary for the use of special formulae connecting papergram mobility to structure (French and Wild, 1953). When greater separation was desired, the solvent was allowed to run over the far edge which was serrated for more even flow; the mobility in this case was expressed in R_G values.

The benzidine reagent (Smith, 1958) was used as a general chromogenic spray for all sugars. The triphenyl tetrazolium chloride (TTC) reagent (Mattson and Jensen, 1950) served as an indicator of reducing sugars. The α-naphthol-phosphate (NP) reagent (Albon and Gross, 1950) was employed to detect bound fructose. Raybin's test (Raybin, 1937) was used to demonstrate a sucrose unit in an oligosaccharide. Invertase hydrolysis and acid hydrolysis (1N H_2SO_4, 70°C, 1 hr) were used in confirming the identity of the sugars.

In quantifying the cowpea oligosaccharides, the phenol-sulfuric acid colorimetric method of Dubois et al. (1956) was employed. The paper chromatogram segments corresponding to the standard sugar spots were cut off, eluted with water, the eluates were reacted with the phenol reagent and the resulting color was measured in a Beckman DU spectrophotometer at 490 nm. The absorbance was converted to concentration by means of reference curves. Because authentic verbascose was not available for the preparation of a reference curve, the eluate containing this sugar was hydrolyzed with invertase and the freed fruxtose was determined by Roe's method (1934). The fructose content was converted to verbascose content assuming one fructose residue per verbascose molecule.

RESULTS & DISCUSSION

THE RESULTS of the proximate analysis are shown in Table 1. The averages of the 13 cultivars analyzed are close to the values appearing in Agriculture Handbook No. 8 (USDA). Worth noting is the wide range in protein content among cultivars: 21.0–26.3%.

The chromatographic pattern of the sugars for all 13 cultivars were similar: four spots appeared. The R_G values and R_f values of three of these spots (Table 2) indicated that the probable identities of the corresponding sugars were sucrose, raffinose and stachyose. Confirmation for this identification was derived from the following observations, which also helped identify the fourth sugar.

(a) The TTC test was negative for all four sugars indicating that none of them is reducing.

(b) The NP test was positive for all four sugars indicating the presence of fructose in all of them.

(c) Raybin's test was also positive indicating that all four sugars contain sucrose residues in them.

(d) Treatment with invertase resulted in releasing fructose from all four cowpea sugars. In addition, glucose was found in the invertase hydrolysate of the sugar which was presumed to be sucrose; melibiose was present in the hydrolysate of presumed raffinose; from the invertase hydrolysate of the third cowpea sugar a spot corresponding to a trisaccharide was obtained and from the hydrolysate of the

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Table 1—Proximate composition of cowpeas from 13 cultivars (g/100g)[a]

	Cultivars													
	California No. 5	Cabbage Cowpea	Auburn 70.3	Pinkeye Purple Hull	Mississippi Silver Skin	Magnolia Blackeye	Alabama 963.8 G1-5	Alabama 562 3-1-2	Knuckle Purple Hull	Big Boy Cowpea	Sa Dandy Cowpea	Tenn. White-crowder	Giant Blackeye	AVERAGE
Moisture	7.9	9.1	8.4	10.1	10.1	9.9	9.0	10.9	10.6	8.3	10.1	11.7	8.9	9.6
Protein (N X 6.25)	26.3	26.3	24.2	24.5	23.6	21.0	22.8	22.8	24.5	24.5	23.5	22.7	24.5	23.9
Fiber	2.2	2.4	4.4	3.4	4.6	5.2	5.1	3.3	3.6	4.1	5.9	2.9	5.7	4.1
Ash	3.0	3.4	3.2	3.5	3.3	3.5	3.3	3.2	3.1	3.4	3.5	3.3	3.7	3.3
Lipids	1.3	1.3	1.8	1.9	1.7	1.7	1.5	1.3	1.8	1.8	1.5	2.0	1.3	1.6
Carbohydrates (by difference)	59.3	57.5	58.0	56.6	56.7	58.7	58.3	58.5	56.4	57.9	55.5	57.4	59.9	57.7

[a] Average of two determinations differing by less than 4%.

fourth cowpea sugar a spot indicating a tetrasaccharide.

(e) Complete acid hydrolysis yielded fructose, glucose and galactose from three of the cowpea oligogassacharides and fructose plus glucose from one of them.

Table 2—R_G and R_f values of authentic and cowpea sugars

	R_G values		R_f values			
	BEW[a]		BEW		BPW[b]	
	Authentic	Cowpea	Authentic	Cowpea	Authentic	Cowpea
Sucrose	0.69	0.68	0.24	0.24	0.23	0.24
Raffinose	0.30	0.28	0.11	0.11	0.11	0.11
Stachyose	0.13	0.12	0.05	0.04	0.04	0.04
Verbascose	–	0.06	–	0.02	–	0.02

[a] BEW 1-butanol, ethanol, water, 5:3:2 by volume.
[b] BPW 1-butanol, pyridine, water, 6:4:3 by volume.

Table 3—Oligosaccharide content of cowpeas from 13 cultivars (g/100g dry basis)[a]

	Sucrose	Raffinose	Stachyose	Verbascose
California No. 5	2.4 ± 0.02	1.1 ± 0.08	3.6 ± 0.18	0.9 ± 0.05
Cabbage Cowpea	2.1 ± 0.04	1.2 ± 0.03	3.4 ± 0.11	0.9 ± 0.06
Aub. 70.3	1.8 ± 0.03	1.1 ± 0.05	3.1 ± 0.06	0.9 ± 0.01
Pinkeye Purple Hull	2.1 ± 0.01	1.3 ± 0.01	3.6 ± 0.03	0.9 ± 0.06
Mississippi Silver Skin	1.9 ± 0.05	1.1 ± 0.01	2.9 ± 0.09	1.0 ± 0.05
Magnolia Blackeye	2.4 ± 0.02	1.2 ± 0.06	3.4 ± 0.01	1.0 ± 0.01
Alabama 963.8 G1-5	1.8 ± 0.07	1.1 ± 0.05	4.1 ± 0.14	0.6 ± 0.01
Alabama 562 3-1-2	1.9 ± 0.04	1.3 ± 0.04	3.5 ± 0.09	0.6 ± 0.03
Knuckle Purple Hull	1.8 ± 0.04	1.1 ± 0.08	3.4 ± 0.13	1.0 ± 0.03
Big Boy Cowpea	3.0 ± 0.06	1.1 ± 0.06	3.6 ± 0.01	1.0 ± 0.06
Sa Dandy Cowpea	2.0 ± 0.14	1.2 ± 0.02	3.3 ± 0.01	0.9 ± 0.06
Tenn. White-crowder	3.1 ± 0.05	1.1 ± 0.12	2.9 ± 0.21	0.8 ± 0.04
Giant Blackeye	2.6 ± 0.10	1.2 ± 0.19	3.8 ± 0.03	0.9 ± 0.02
Average	2.2 ± 0.06	1.2 ± 0.06	3.4 ± 0.08	0.9 ± 0.04

[a] Mean of four determinations ± one standard deviation

These tests leave no doubt that three of the cowpea sugars are sucrose, raffinose and stachyose, and indicate that the fourth sugar is probably verbascose.

This indication regarding the identity of the fourth sugar is strengthened by the fact that the R_f values for that sugar fit the linear relationship that exists between log (R_f/(1 – R_f)) versus number of hexose units in a sugar (French and Wild, 1953). When the R_f values obtained with either BEW or BPW were plotted as log (R_f/(1 – R_f) against number of hexose units, the fourth sugar was shown to correspond to a pentasaccharide. Again, when the R_f values of the glucose terminal residues left after invertase hydrolysis were similarily plotted, the fourth-sugar-minus-fructose fragment was found to correspond to a tetrasaccharide. Finally, when the fourth sugar was completely acid hydrolyzed, the three sugars of the hydrolysate were found to be present in the following quantitative relationship: fructose 1.0; glucose 1.1; galactose 2.9, which is not very different from the theoretical 1:1:3 relationship for verbascose.

The quantitative results of the sugar analysis applied to 13 cowpea cultivars are shown in Table 3. On the average, stachyose was present in the highest proportion (3.4% on dry weight), followed by sucrose (2.2%), raffinose (1.2%) and verbascose (0.9%). Sucrose content (1.8–3.1% on dry weight) showed the greatest variability among cultivars. The nondigestible sugars of cowpeas (raffinose, stachyose and verbascose) represent 5.5% of the total solids, and 9.5% of the total carbohydrates (excluding fiber) in this legume.

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REMOVAL OF TANNINS AND IMPROVEMENT OF IN VITRO PROTEIN DIGESTIBILITY OF SORGHUM SEEDS BY SOAKING IN ALKALI

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ABSTRACT

Soaking of sorghum seeds at 30°C in 0.05M sodium hydroxide and potassium hydroxide removed 75–85% of the tannins in 24 hr. Treatment at 100°C increased the rate of extraction and reduced the time required to leach 75–85% tannins from 24 hr to 20 min. Under similar conditions, 0.05M sodium carbonate removed 77% whereas soaking in distilled water removed only 30%. Dry matter loss due to the treatments with alkaline reagents ranged from 1.2–2.3%. Extraction of tannins in 0.05M alkaline solution at 100°C for 20 min improved in vitro protein digestibility of high tannin seeds 2.5 fold as compared to untreated seeds.

INTRODUCTION

THE PRESENCE of tannins in sorghum seeds is well documented (Salunkhe et al., 1977). Tannins affect the availability of amino acids (Rostango, 1972) and utilization of protein (Eggum and Christensen, 1975). Growth retardation has been observed in chicks fed on diets containing high tannin sorghum (Chang and Fuller, 1964).

Tannins in sorghum seeds are located in the pericarp and its removal significantly reduces the tannin content (Jambunathan and Mertz, 1973). However, this treatment causes a loss of dry matter. It was observed that the extraction of tannins from high tannin sorghum results in increased weight gain and feed efficiency in the experimental animals. Our earlier report (Chavan et al., 1978) has shown that during the process of commercial milling, tannins are in the flour and cannot be removed by screening. Hence, studies have been conducted to develop a suitable method to remove tannins from seeds. The effectiveness of such treatments was tested by electrophoretic technique and in vitro protein digestibility of the treated seeds. The present communication reports the results of this study.

MATERIALS & METHODS

Sorghum seeds

Two low tannin, IS 4129 (0.46% tannin) and IS 3421 (0.40% tannin) and two high tannin, IS 2825 (3.44% tannin) and IS 8202 (3.60% tannin) cultivars were obtained as pure inbred lines from ICRISAT (International Crop Research Institute for Semi Arid and Tropics), Hyderabad.

Tannin determination

Tannin content in control (untreated) and treated seeds was determined by the method of AOAC (1965) as modified by Christensen (1974). Each sample was measured four times and to these samples, different amounts of tannic acid were added as an internal standard.

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Table 1—Effects of water treatments on the removal of tannins from sorghum seeds, cultivar – IS 2825

Extraction time	Tannin content of treated seeds (%)	Removal of tannins (%)
Hours	Soaking at 30°C	
0	3.44	–
1	2.99	13
3	2.94	15
6	2.67	22
12	2.42	30
24	2.37	31
Minutes	Soaking at 100°C	
5	2.94	15
10	2.56	26
20	2.44	29

Dehulling of seeds

The seeds of high tannin cultivar (IS 2825) were dehulled by 20% hot alkali (Blessin et al., 1971).

Chemicals

To remove tannins, sodium hydroxide and potassium hydroxide (0.005, 0.01, 0.025, and 0.05M), sodium carbonate (0.025, 0.05, and 0.1M), and distilled water were used.

Chemical treatments

One gram seeds were soaked in 25 ml of various solutions at either 30°C or 100°C for different lengths of time as indicated and subsequently washed thoroughly with distilled water several times. All washings were collected and the volume was made to 50 ml. Tannins were determined from this extract.

Electrophoresis of soluble proteins

The soluble proteins were extracted from the seeds in 0.1M Tris-HCl buffer, pH 7.2. The proteins were resolved by acrylamide gel electrophoresis using 7.5% gel according to the method of Davis (1964).

In vitro protein digestibility (IVPD)

The IVPD of proteins of various samples of seeds was obtained by using digestive enzymes, pepsin and trypsin, by the method of Fasold and Gundlach (1964). The rate of digestion was expressed in terms of μmoles of amino acids (L-leucine) released by the digestive enzymes per gram of fat free powdered sample in 4 hr when corrected to zero time control. The percent IVPD was also determined by pepsin hydrolysis (AOAC, 1965). The IVPD was calculated from the difference in N content of sample before and after pepsin hydrolysis.

RESULTS & DISCUSSION

THE SOAKING of seeds in distilled water at 30°C removed 31% tannins in 24 hr (Table 1). When soaking was done at 100°C, it removed about the same amount of tannins in 20 min. Sodium hydroxide at 0.05M concentration extracted 84% tannins when soaking was conducted at 30°C for 24 hr (Table 2). When the extraction was made at 100°C, the time required to remove the same amount of tannin was reduced to 20 min (Table 3). Thus, the extraction at high temperature enhanced the rate of removal of tannins and reduced the time. Increase in concentration of sodium

hydroxide from 0.005 to 0.05M improved the efficiency of removal from 36–84% (Table 2). When 0.05M potassium hydroxide was used, it removed 84% tannins in 24 hr at 30°C (Table 2). When the extraction was made at 100°C, about 87% tannins were removed in 20 min (Table 3). Sodium carbonate also yielded similar results (Table 4). Acetic acid, hydrochloric acid, and ethyl alcohol were all ineffective.

Strumeyer and Malin (1975) isolated and characterized tannins present in sorghum as condensed tannins. The active groups on tannins are the phenolic hydroxyl groups. Phenols dissolve readily in dilute sodium hydroxide and form sodium phenate (Brewster and McEwen, 1971). A similar reaction might be involved in dissolution of tannins in alkaline reagent (Wah et al., 1977).

The distribution pattern of soluble proteins of the low tannin cultivar, IS 4149 showed 17 protein components in polyacrylamide gel (Fig. 1–A). However, not a single band was detected in high tannin seeds of IS 2825 (Fig. 1–B). In this case proteins remained at the top of the running gel. When these high tannin seeds were dehulled, before extraction of proteins, 10 bands were detected (Fig. 1–C). These results indicate that the tannins interfere with the entry of proteins into the gel in the high tannin seeds. Tannins form hydrogen bonds with free carbonyl groups of protein and form complexes with proteins in sorghum seeds (Schaffert et al., 1974). The tannin from high tannin seeds was extracted by alkaline reagents and the proteins of such treated seeds were resolved by electrophoresis (Fig. 1–D to F). After such treatments the electrophoretic pattern of treated seed and dehulled seed proteins exhibited similarities.

Table 2–Effects of soaking at 30°C in sodium hydroxide and potassium hydroxide on the removal of tannins from sorghum seeds, cultivar – IS 2825

		Sodium hydroxide		Potassium hydroxide	
Extraction time (hr)	Conc (M)	Tannin content of treated seeds (%)	Removal of tannins (%)	Tannin content of treated seeds (%)	Removal of tannins (%)
0	–	3.44	–	3.44	–
1	0.005	2.84	17	2.99	13
3	0.005	2.74	20	2.89	16
6	0.005	2.54	26	2.29	33
12	0.005	2.34	32	2.24	35
24	0.005	2.19	36	1.94	44
1	0.01	2.74	20	2.89	16
3	0.01	2.49	28	2.74	20
6	0.01	2.44	29	1.94	44
12	0.01	2.24	35	1.94	44
24	0.01	2.14	38	1.89	45
1	0.025	2.24	35	2.59	25
3	0.025	1.84	47	2.14	38
6	0.025	1.74	49	1.35	61
12	0.025	1.69	51	1.14	67
24	0.025	1.64	52	1.14	67
1	0.05	1.84	47	2.05	41
3	0.05	1.34	61	1.49	57
6	0.05	0.94	73	1.04	70
12	0.05	0.64	81	0.64	81
24	0.05	0.54	84	0.64	81

Table 3–Effects of heating at 100°C in sodium hydroxide and potassium hydroxide on the removal of tannins from sorghum seeds, cultivar – IS 2825

		Sodium hydroxide		Potassium hydroxide	
Extraction time (min)	Conc (M)	Tannin content of treated seeds (%)	Removal of tannins (%)	Tannin content of treated seeds (%)	Removal of tannins (%)
0	–	3.44	–	3.44	–
5	0.005	2.84	17	2.86	17
10	0.005	2.64	23	2.74	20
20	0.005	2.04	41	2.04	41
5	0.01	2.64	23	2.74	20
10	0.01	2.14	38	2.54	26
20	0.01	1.74	49	1.49	57
5	0.025	2.19	36	2.34	31
10	0.025	0.94	73	1.84	47
20	0.025	0.74	79	1.24	64
5	0.05	1.74	49	2.19	36
10	0.05	0.64	81	0.94	73
20	0.05	0.49	86	0.44	87

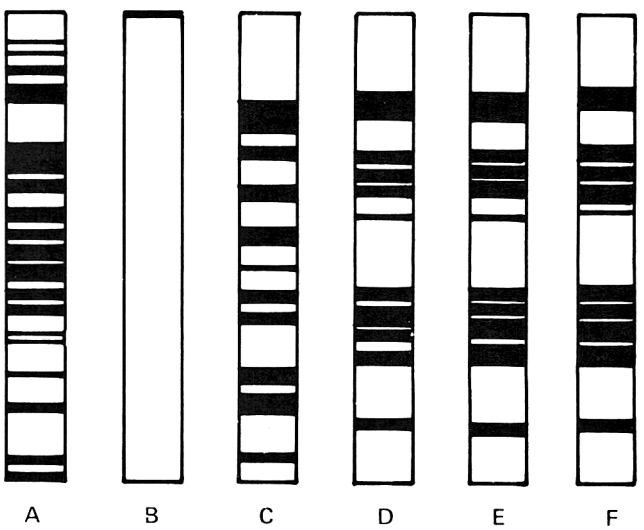

Fig. 1–Effects of chemical treatments on the electrophoretic pattern of soluble proteins of high tannin cultivar, IS 2825. The seeds were soaked at 100°C for 20 min in various chemical solutions. The soluble proteins were extracted in Tris-HCl buffer (0.1M, pH 7.2) and electrophoresed as described under Materials and Methods. (A) Low tannin, IS 4129; (B) High tannin, IS 2825; (C) Dehulled high tannin, IS 2825; (D) Soaking in sodium hydroxide; (E) Soaking in potassium hydroxide; and (F) Soaking in sodium carbonate.

Table 4–Effects of heating at 100°C in sodium carbonate on the removal of tannins from grain sorghum seeds, cultivar – IS 2825

Extraction time (min)	Conc (M)	Tannin content of treated seeds (%)	Removal of tannins (%)
0	–	3.44	–
5	0.025	2.84	17
10	0.025	2.24	35
20	0.025	2.14	38
5	0.05	2.64	23
10	0.05	2.04	41
20	0.05	0.79	77
5	0.10	2.59	25
10	0.10	1.44	58
20	0.10	0.59	83

Table 5—Effects of alkali treatments on in vitro protein digestibility of sorghum seeds

Sorghum seeds samples	In Vitro protein digestibility (μmoles of leucine formed per g per 4 hr) Pepsin	Trypsin	IVPD %
Low tannin (untreated)			
IS 4129	130	148	77
IS 3421	150	180	73
High tannin (untreated)			
IS 8202	55	53	51
IS 2825	53	40	48
Dehulled high tannin (IS 2825)[a]	155	158	79
Alkali extracted high tannin (IS 2825)			
Sodium hydroxide	122	133	69
Potassium hydroxide	120	133	72
Sodium carbonate	120	125	69

[a] The high tannin seeds (IS 2825) were soaked in 0.05M solutions of the various alkaline chemicals at 100°C 20 min, washed thoroughly several times and dried at 60°C. The dried grains were ground to 100 mesh, defatted and used for in vitro protein digestibility studies.

There were no differences in the distribution pattern of proteins among the treatments.

The IVPD of two low tannin, two high tannin, dehulled high tannin and the alkali treated high tannin cultivar are shown in Table 5. The low tannin cultivars showed higher IVPD values than high tannin cultivars. In sorghum, most of the tannins are present in the pericarp layer. Dehulling of high tannin seeds had significant positive effect on IVPD. The gain in IVPD on dehulling was much higher to that of the untreated seeds. The alkali treated seeds exhibited significant increase in IVPD as compared to untreated seeds. The tannin content in alkali treated seeds (0.5–0.6%) was very close to values of tannin observed in dehulled seeds (0.45–0.47%). This suggests that very little tannin goes to the endosperm due to alkali treatment at 100°C. It is possible that the effects of tannin on IVPD is expressed only when the tannin content exceeds a certain threshold value (Fuller et al., 1966).

Tannins inhibit the activities of digestive enzymes (Tamir and Alumot, 1969). The condensed tannins present in seed coat of high tannin sorghum may have inhibited the activities of proteolytic enzymes like pepsin and trypsin as indicated by significantly lower values of IVPD in high tannin untreated seeds. The inhibitory effects of tannins were markedly reduced when high tannin seeds were extracted in alkaline solutions.

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CHEMICAL AND PHYSICOCHEMICAL CHANGES IN FIELD BEAN AND SOYBEAN PROTEINS TEXTURIZED BY EXTRUSION

J. JEUNINK and J. C. CHEFTEL

ABSTRACT

The solubility of extruded field bean and soybean protein in a pH = 6.9 buffer was less than 22%. Extensive solubilization could be obtained in buffers containing sodium dodecyl sulfate (SDS) and SDS plus dithiothreitol (DTT). The cysteine + cystine content decreased by 8.5% after extrusion of field bean protein, but remained constant with soybean protein. The content of all other amino acids was not changed; 1.4% (soybean) to 3.2% (field bean) of the lysine residues became chemically unavailable after extrusion. There was no significant formation of lysinoalanine or lanthionine. Electrophoretic patterns of initial and extruded proteins (SDS plus DTT solubilized) were very similar except for the appearance of a low mobility aggregate in extruded field bean protein.

INTRODUCTION

IN ORDER TO increase the consumer's acceptability of vegetable proteins, various texturization processes have been developed. Since ancient times, texturized vegetable proteins have been produced in Japan. For example, tofu is prepared by calcium or acid coagulation of soybean milk, yuba is a heat induced soy milk "skin" and aburage a heat expanded tofu gel. Newer types of texturized vegetable proteins used as meat analogs are produced by fiber spinning, thermoplastic extrusion or other techniques (Kinsella, 1978).

The mechanism of protein texturization during gelation, spinning and extrusion is still poorly understood. However, the tofu gel appears to be formed through disulfide bonds, calcium bridges and noncovalent interactions with the contribution of disulfide bonds being different with the 7S or the 11S protein (Saio and Watanabe, 1978). Disulfide bonds together with noncovalent interactions seem to be responsible for the formation of spun soy fibers (Kelley and Pressey, 1966).

The mechanism of texturization by thermoplastic extrusion has received little attention. Process parameters and feed composition are reported to influence textural characteristics (Taranto et al., 1975; Aguilera and Kosikowski, 1976; Kinsella, 1978; Maurice and Stanley, 1978). Both structural and chemical studies can lead to a better understanding of the texturization process. Using electron microscopy, fiber-like structures consisting of proteins (Cumming et al., 1972) or of proteins and insoluble carbohydrates (Taranto et al., 1978) were observed in the extruded material. The chemical basis of extrusion texturization is believed to be the formation of covalent bonds between polypeptide chains or between these chains and other constituents. Cumming et al. (1973) have shown by electrophoresis that extrusion causes a dissociation followed by an aggregation of the water-soluble soy proteins. Since sulfhydryl-disulfide interchange reaction is reported to be involved in the insolubilization of the 11S soy protein upon heating (Wolf and Tamura, 1969), disulfide bonding may be responsible for the texturization of soy proteins by extrusion. However, in solubility experiments, Burgess and Stanley (1976) did not confirm such a hypothesis, and suggested instead that "isopeptide" crosslinks may play a role. Another possibility is the formation of lysinoalanine (LAL) and lanthionine (LAT) crosslinks. These unusual amino acids are known to occur both in alkali-treated and heat-treated proteins (Sternberg et al., 1975; Aymard et al., 1978).

The present study concerns chemical and physicochemical changes occurring in field bean and soybean proteins as a result of texturization by thermoplastic extrusion. Initial and extruded protein concentrates are compared on the basis of (1) protein solubility in the presence of agents known to disrupt noncovalent interactions or to reduce disulfide bonds; (2) general amino acid composition and formation of some crosslinking amino acids; and (3) electrophoretic patterns.

MATERIALS & METHODS

Vegetable protein concentrates and extrusion conditions

A protein concentrate from field beans (*Vicia faba* var *minor*) was produced by air classification (Grandes Minoteries à Fèves de France, Marseille). It contained 60% protein (N × 6.25, d.b.) and 9% moisture. It was extruded by C.T.U. (Nogent s/Marne, France) in a Creusot-Loire (Firminy, France) double screw extruder, Model BC 45 (de la Guérivière et al., 1978). Four different extruded samples A, B, C and D were obtained. Operating conditions included a feed rate of 400 g/min, 10% water added (except for sample D: 15%), a screw rotation of 50 rpm (except for sample C: 100 rpm) and a maximum temperature in the barrel of 245°C (except for sample A: 265°C). Separate experiments with full fat soybean have shown that the maximum product temperature in the final section of the same extruder is 20–40°C less than in the barrel. The moisture content of the four extruded samples was 7–10%. Only sample A kept its structure after rehydration in boiling water.

The defatted soybean protein concentrate (Unilever, Zwijndrecht, Netherlands) contained 62% protein (N × 5.7, d.b.) and 9% moisture. It was extruded by Unilever (Duiven, Netherlands) in a Troester (Hannover, Germany) single screw extruder, Model UP 30-D15 (clearance between screw and barrel: 0.3mm), with a feed rate of 75 g/min (including 32% added water), a screw rotation of 40 rpm, a product temperature of 145°C at the die and a mean residence time of 32 sec. The extruded product contained 19% moisture and was subsequently dried to 5%.

For the determination of the water absorption capacity the extruded products were heated in distilled water at 100°C for 10 min and then drained on a nylon sieve (ϕ = 0.1 mm) for 10 min.

Determination of protein solubility

Samples of initial or extruded protein concentrates were extracted with 0.1M phosphate buffer solutions (pH = 6.9) containing various concentrations of sodium dodecyl sulfate (SDS) (an agent known to disrupt noncovalent interactions), and of dithiothreitol (DTT) (an agent known to reduce disulfide bonds). One-gram samples (initial protein concentrate or finely ground extruded concentrate) were extracted for 2 hr at 37°C under moderate stirring with 12.5 ml of the buffer solution. After centrifugation (30000 × G, 20 min, 4°C), the nitrogen content of the supernatant (field bean) or of the paper-filtered supernatant (soybean) was determined, using the

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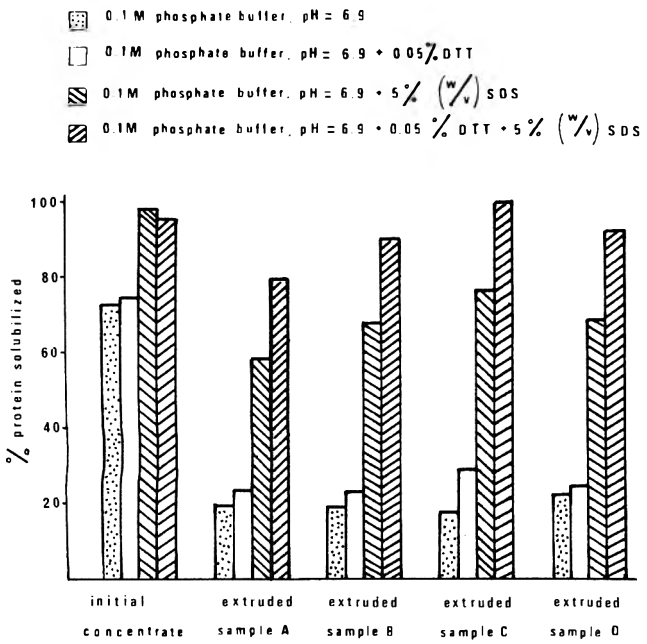

Fig. 1—Protein solubility of initial and extruded field bean protein concentrates in different extraction solutions (conditions of extraction: 37°C, 2 hr, ml extraction solution/g sample = 12.5). Results for the initial concentrate and extruded sample A are the mean of 2 determinations (variation in % protein solubilized less than 8%).

micro-Kjeldahl method. The protein content was calculated with a conversion factor of 6.25 (field bean) or 5.7 (soybean).

Amino acid analysis

Protein hydrolysis was carried out with 6 mg protein in 2 ml 6N HCl at 110°C for 24 hr under nitrogen. Amino acid separation was done with a NC 1 Technicon autoanalyzer at 60°C on a 75 × 0.6 cm ion-exchange column consisting of a Chromobeands C_2 resin. The elution gradient was as described by Provansal et al. (1975). Norleucine was used as an internal standard.

Determination of cysteine + cystine and methionine

100 mg protein were heated at 60°C under reflux for 1 hr in 7.5 ml of 98% (v/v) formic acid; 22.5 ml of a solution of performic acid, prepared as indicated by Hirs (1956), were then added and the mixture was kept at 0°C for 3 hr. After hydrolysis in 150 ml 6N HCl at 110°C, cysteic acid, methionine sulfone and methionine sulfoxide (when present) were separated and determined on a 140 × 0.6 cm ion-exchange column according to Cuq et al. (1973). Compensation for experimental protein losses was calculated on the basis of valine recovery.

Determination of lysinoalanine (LAL)

LAL (Miles-Yeda, Rehovot, Israel) was determined at 60°C on a 25 × 0.6 cm Chromobeads C_2 column equilibrated with 0.05M sodium citrate-HCl buffer, 0.2N Na^+, pH = 3.8, and eluted with a similar buffer, 0.8N Na^+, pH = 5.0. The detection limit of LAL was close to 250 μg/g protein. Protein hydrolysis prior to LAL determination was carried out with 20 mg protein in 2 ml 6N HCl at 110°C for 24 hr under nitrogen. Compensation for experimental protein losses was calculated on the basis of lysine recovery.

Determination of lanthionine (LAT)

One of the two isomers of LAT (Koch-Light Laboratories, Colnbrook, Bucks, England) was separated from glycine (the other was still eluted under glycine) on a 75 × 0.6 cm Chromobeads C_2 column through a slight modification of the elution gradient used for amino acid analysis. Chamber 2 was filled with 12 ml of the 0.05M sodium citrate-HCl buffer, 0.2N Na^+, pH = 2.75 and 28 ml of a similar buffer, 0.2N Na^+, pH = 3.1. Chamber 3 contained 40 ml of a similar buffer, 0.2N Na^+, pH = 3.8. Since thermal or alkaline treatments of proteins give a racemic mixture of LAT, the total LAT content was calculated as the double of the content of one isomer. The detection limit of LAT was close to 600 μg/g protein. Protein hydrolysis was carried out as described for LAL determination. Compensation for experimental protein losses was calculated on the basis of leucine recovery.

The LAT content in protein hydrolysates was also determined by thin layer chromatography (TLC) according to Aymard et al. (1978).

Determination of unavailable lysine

The reaction between protein and 1-fluoro-2,4-dinitrobenzene (FDNB) was carried out according to the "Silcock" method (Milner and Westgarth, 1973). Residual lysine was determined, after protein hydrolysis, on a 25 × 0.6 cm Chromobeads C_2 column, following the procedure described for the determination of LAL. Compensation for experimental protein losses was calculated on the basis of phenylalanine recovery.

Polyacrylamide gel electrophoresis (PAGE)

Sodium dodecyl sulfate-polyacrylamide gels (0.5 × 7.8 cm) were prepared according to Weber and Osborn (1969). The final concentration in the gel was 5% acrylamide. Soluble extracts from field bean protein concentrates in phosphate buffer pH = 6.9 (containing 5% (w/v) SDS and 0.05% DTT) and soluble extracts from soybean protein concentrates in phosphate buffer pH = 6.9 (containing 5% (w/v) SDS and 0.2% DTT) were used for electrophoresis. *Before* application onto the gel, each soluble extract (75 μl) was incubated with glycerol (25 μl) and excess DTT (10 mg) for 20 min at 25°C. Five μl of the resulting solution, containing 80–110 μg protein, were applied to the gel. Electrophoresis was carried out at 5 mA per gel for 6 hr. The gels were then stained for 1 hr with a 0.05% Coomassie blue solution in an acetic acid-methanol-water mixture (1:5:5), destained by electrophoresis, and stored in a 7.5% (v/v) acetic acid solution.

Molecular weights of protein subunits were calculated from the plot of log molecular weight vs the distance traveled. References were RNA polymerase subunits (167000, 155000 and 39000), B.S.A. (68000) and trypsin inhibitor (21500).

Densitometry of the various peaks was carried out with a Vernon photometer.

RESULTS & DISCUSSION

Protein solubility

The percent protein solubilized from the field bean protein concentrates by four different extraction solutions is given in Figure 1. In the plain phosphate buffer (pH = 6.9) 72% of the total protein of the initial field bean concentrate is soluble, but only 18–22% after extrusion (extruded samples A to D). In the phosphate buffer containing 5% SDS, and in the buffer containing 5% SDS plus 0.05% DTT, 58 and 80% respectively of the total protein of extruded sample A can be solubilized. Practically, 100% of the total protein of the initial field bean concentrate is soluble in the SDS or SDS plus DTT-containing buffers. It therefore appears that extrusion of the field bean protein concentrate causes a marked decrease in the solubility of the protein constituents; a major part of this reduced solubility is probably due to: (1) noncovalent interactions between polypeptide chains, or between polypeptide chains and other constituents, since more protein is soluble in the SDS-containing buffer than in the plain buffer; (2) the formation of new disulfide bonds (either by interchange reactions or by additional cystine formation from cysteine), since more protein is soluble in the SDS plus DTT-buffer than in the SDS-buffer. It can be noted however that the disulfide breaking agent has no solubilizing effect when it is used without SDS.

The possible formation of other covalent bonds cannot be excluded, especially in the proteins remaining insoluble in the SDS plus DTT-containing buffer.

Preliminary data appear to show that texturization is not directly related to insolubilization. Whereas the extruded field bean sample A absorbs 300% water when placed in boiling water, the extruded samples B, C and D disintegrate totally in these conditions. The four extruded samples show no significant differences in protein solubility (Fig. 1). This would indicate that marked differences in the texture of

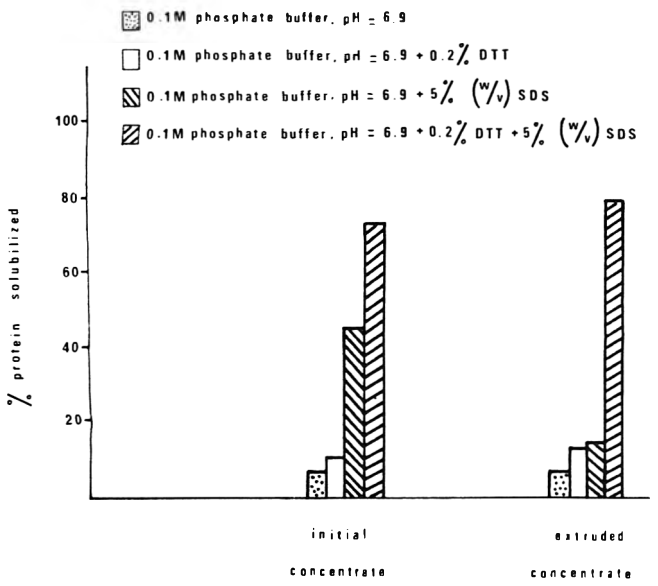

Fig. 2—Protein solubility of initial and extruded soybean protein concentrates in different extraction solutions (conditions of extraction: 37°C, 2 hr, ml extraction solution/g sample = 12.5). Each result is the mean of 2 determinations (variation in % protein solubilized less than 8%).

extruded samples are not reflected by differences in the extent of protein solubilization by reagents disrupting disulfide bonds and/or noncovalent interactions. However, it is still possible that disulfide bonds and/or noncovalent interactions play a role in protein texturization only when they are properly positioned.

As much as 93% of the proteins from the initial soybean concentrate is insoluble in the plain phosphate buffer (Fig. 2). This is probably due to the prior delipidation and extraction processes. The corresponding extruded concentrate, which has a water absorption capacity of 160%, presents an unchanged proportion of insoluble protein. Extraction with the SDS-containing buffer solubilizes 45 and 14% of the proteins of the initial and the extruded concentrates respectively, while extraction with the SDS plus DTT-containing buffer solubilizes 73 and 79%. These data indicate that noncovalent interactions and disulfide bonds are probably responsible for the poor protein solubility of the initial concentrate. The disruption of disulfide bonds has a stronger solubilizing effect on the extruded than on the initial soybean proteins. This finding can be compared to the observation of Wolf and Tamura (1969) that sulfhydryl-disulfide interchange reactions play a role in the aggregation of soybean proteins through heating. In similar solubility experiments Burgess and Stanley (1976), however, discount the importance of disulfide bond formation in extruded soybean proteins. A knowledge of the cysteine/cystine ratio in the various protein samples would be useful. However, a suitable method for the determination of thiol groups, and eventually of disulfide bonds, in highly insoluble proteins is not readily available.

Amino acid analyses

The amino acid compositions of the field bean and soybean concentrates both before and after extrusion are summarized in Table 1.

The amino acid content of the field bean concentrate is not modified by extrusion except for the cysteine + cystine content, which is reduced from 1.16 to 1.06g per 100g protein (8.5% loss). This is in agreement with the well-known thermal lability of cysteine (Cheftel, 1977). There is

Table 1—Amino acid composition[a] of field bean and soybean protein concentrates

	Field bean[b]		Soybean[c]	
Amino acid	Initial conc	Extruded sample A	Initial conc	Extruded conc
Asp	12.5	12.5	13.5	13.0
Thr	3.6	3.8	4.5	4.5
Ser	5.3	5.3	5.9	5.9
Glu	17.0	18.0	21.0	20.5
Pro	4.2	4.3	5.8	5.7
Gly	4.2	4.3	4.8	4.7
Ala	4.3	4.3	5.1	5.0
Val	4.8	4.9	5.2	5.2
Ileu	4.5	4.4	5.2	5.1
Leu	8.1	7.9	9.1	8.9
Tyr	3.2	3.0	3.7	4.0
Phe	4.5	4.5	5.7	5.6
Lys	6.8	6.7	7.1	7.1
Unavailable Lys[d]	0.40	0.62	0.41	0.51
His	2.5	2.5	3.1	3.0
Arg	10.0	10.0	8.5	8.1
Cys[e,f]	1.16 ± 0.02	1.06 ± 0.02	1.67	1.70 ± 0.05
Met[f,g]	0.80 ± 0.02	0.84 ± 0.02	1.54 ± 0.01	1.45 ± 0.10

[a] g/100g protein
[b] Mean values of 4 assays unless otherwise indicated
[c] Mean values of 2 assays unless otherwise indicated
[d] Mean values of 2 assays
[e] Determined as cysteic acid
[f] Mean value and standard error of the mean from 5 or 6 assays (field bean) and 2 or 3 assays (soybean). Loss of cysteine + cystine in field bean protein due to extrusion is significant at P = 0.01
[g] Determined as methionine sulfone

however no loss of cysteine + cystine during extrusion of the soybean protein concentrate, probably because of the milder time-temperature extrusion processing.

The lysine content remains constant after extrusion both with field beans and with soybeans, with 3.2% (field bean) and 1.4% (soybean) of the lysine residues becoming chemically unavailable after extrusion. This small increase in unavailable lysine may be due to the formation of "isopeptide" crosslinks, i.e. ε-(γ-glutamyl)-lysine or ε-(β-aspartyl)-lysine (Hurrell et al., 1976), or less likely to Maillard condensation with reducing carbohydrates. It is therefore not excluded that "isopeptide" crosslinks are involved in protein texturization.

LAL can be detected in the initial field bean concentrate and in the extruded sample A (Table 2). The content of this crosslinking amino acid corresponds to 0.25% (mole basis) of the total lysine content, and is therefore negligible.

Lanthionine (LAT), another crosslinking amino acid, cannot be detected in the initial and extruded field bean concentrates by ion-exchange chromatography. Using TLC,

Table 2—LAL and LAT content[a] of field bean and soybean protein concentrates

Sample	LAL[b]	LAT[b]	LAT[c]
Field bean			
Initial conc	270 ± 25	< 600	200
Extruded sample A	260 ± 35	< 600	200
Soybean			
Initial conc	< 250	2350 ± 10	1 500
Extruded conc	< 250	2700 ± 250	2 000

[a] μg/g protein; mean values of 2 assays
[b] Analysis by ion-exchange chromatography
[c] Analysis by TLC

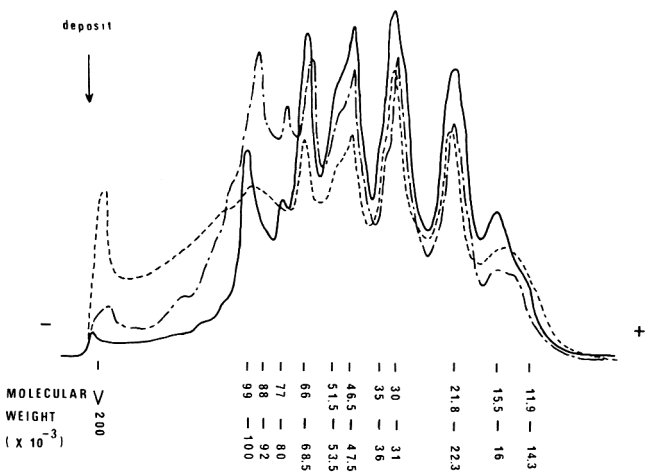

Fig. 3—Densitogram of PAGE of soluble extracts of field bean protein concentrates (extraction with 0.1M phosphate buffer, pH = 6.9 + 5% (w/v) SDS + 0.05% DTT):—Initial conc;-- Extruded Sample A; —- Extruded Sample C.

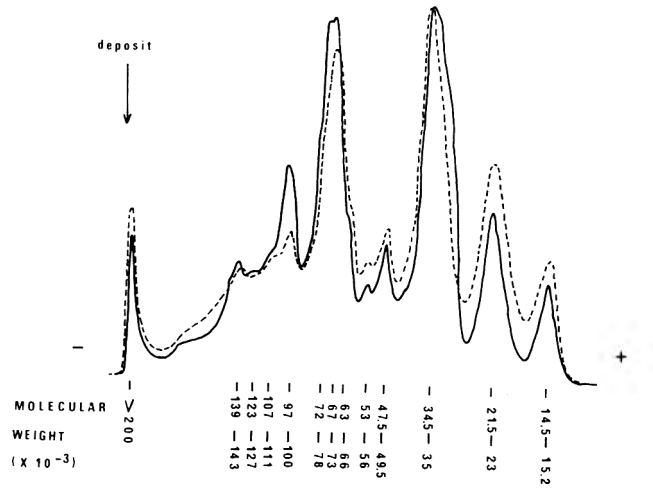

Fig. 4—Densitogram of PAGE of soluble extracts of soybean protein concentrates (extraction with 0.1M phosphate buffer, pH = 6.9 + 5% (w/v) SDS + 0.2% DTT):—Initial conc; --Extruded conc.

in which both isomers of LAT are eluted in one spot, LAT can just be determined in both concentrates (Table 2). No information could be obtained from the processor concerning the temperature during grinding, but the average particle size of the initial field bean concentrate is close to 1 μm. The presence of LAL, LAT and unavailable lysine in the initial field bean concentrate may be due to the grinding treatment.

LAL cannot be detected in the initial and extruded soybean concentrates. Using ion-exchange chromatography, LAT content is found to be 2350 μg/g protein in the initial soybean concentrate and 2700 μg/g protein in the corresponding extruded concentrate. This corresponds to 8 and 9% (mole basis) respectively of the total cysteine + cystine content. It therefore appears that the LAT content does not increase significantly as a result of extrusion. The presence of LAT and unavailable lysine in the initial soybean concentrate is probably due to prior processing.

Thus, it appears that extrusion of field bean or soybean proteins in the conditions used in this study does not cause a significant formation of lysinoalanine or lanthionine crosslinks.

Polyacrylamide gel electrophoresis

The soluble extract (in SDS plus DTT buffer) from the initial field bean concentrate, representing 96% of the total protein of the concentrate, has been submitted to PAGE in the presence of SDS plus DTT. The densitometric pattern of this electrophoresis is shown in Figure 3. At least 11 distinct peaks and shoulders can be detected, and correspond to subunit molecular weights from 11900 to 100000. The pattern of the soluble extract from the extruded field bean sample A, representing 80% of the total protein, shows the same 11 subunits. An additional peak appears at the origin, together with a wide band between the origin and the first peak. Consequently, in the extruded field bean concentrate, the relative importance of the peak area representing the low molecular weight subunits (Mol wt < 53500) is decreased relative to that in the initial concentrate (from 74% to 55%). This is also the case but less so in the "badly texturized" extruded field bean sample C. It therefore appears that extrusion of the field bean concentrate causes some protein aggregation. Since both extraction and electrophoresis are carried out in the presence of SDS plus DTT, it is likely that aggregation is due to covalent crosslinks other than disulfide bonds. However, extraction is performed in the presence of 5% SDS, while the electrophoresis buffer contains 0.1% SDS, thus noncovalent aggregation during electrophoresis is not totally excluded.

Figure 4 shows the densitometric patterns of the SDS plus DTT soluble extracts of both the initial soybean concentrate and the corresponding extruded concentrate. These extracts represent 73 and 79% of the total protein. At least 13 peaks and shoulders can be detected corresponding to subunits with molecular weights from 14500 to 143000. The pattern is almost the same before and after extrusion, and there is no formation of aggregates due to extrusion.

In conclusion, a high degree of protein insolubility is found after extrusion. This low solubility appears to be due mainly to noncovalent interactions and to disulfide bonds. Extrusion causes no significant formation of LAL and LAT, in spite of a decrease in the cysteine + cystine content of field bean protein. The possible formation of "isopeptide" crosslinks is at present under investigation.

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A SIMPLE AND RAPID METHOD FOR ESTIMATION OF CONCENTRATION OF TEXTURED SOYBEAN PROTEIN IN RAW BEEF-SOY BLENDS

S. D. CUNNINGHAM, P. J. DENMAN and W. L. SANDERS

ABSTRACT

Two batches of raw ground beef were prepared to yield four levels of fat (18, 22, 26 and 30%) and levels of hydrated textured soy protein (TSP) within each of the fat levels of 0, 5, 10, 15, 20, 25 and 30%. The 28 different sample combinations were thoroughly blended and then frozen. 25-g portions of each sample were then blended with water, filtered and analyzed for soluble carbohydrate content with a spectrophotometer to determine the relationship between absorbance at 490 μm and the actual content of hydrated TSP present in the ground beef. The four fat levels were used to test the potential effect of the fat content on the spectrophotometer readings. It was determined that absorbance values were linearly dependent on the amount of hydrated TSP in the beef-soy blend. Fat level in the ground beef did not significantly affect spectrophotometer readings. Using the 95% confidence limits, it was possible to estimate soy content in raw beef-soy blends to ± 3.3%.

INTRODUCTION

UTILIZATION of Textured Soybean Proteins (TSP) as extenders in various meat products has increased markedly over the past decade. By 1980, TSP is expected to share as much as 10–20% of the total beef market and as much as 25–50% of all ground or processed meat products (Wilding, 1974). TSP has already been a tremendous asset to the School Lunch Program (SLP), primarily due to the significant reduction in meal cost afforded by use of TSP which cost less than ground beef (McCloud, 1974). Ground beef used in the SLP may contain up to 30% hydrated TSP (Bird, 1974), but at the present time, no practical method exists to estimate TSP content in beef-soy blends. Many institutions are aware of the potential savings offered by the use of TSP as a meat extender and analytical methods for TSP quantitation are needed for quality control and to assure the purchaser of obtaining the proper proportion of ingredients as agreed upon by contract with the supplier.

Various methods have been proposed to detect the presence or amount of TSP in ground beef. Electrophoretic techniques have been proposed to identify (Frouin et al., 1973; Persson and Appelquist, 1977; Guy and Willcox, 1977; Hofmann, 1977) and quantitate (Lee et al., 1975; Parsons and Lawrie, 1972, 1977) the TSP concentration in meat products. Methods based on isoelectric focusing (Llewellyn and Flaherty, 1976), selective staining of carbohydrates (Coomaraswamy and Flint, 1973; Parisi et al., 1974; Flint and Lewin, 1976) flourescence (Holmes, 1976), and enzyme resistant peptide analysis (Bailey, 1976; Llewellyn et al., 1978) have been reported. Immunochemical detection systems (Hauser et al., 1974; Bystra, 1975; Baudner, 1977) have also been attempted, with some success. Clearly, numerous analytical systems have been investigated for use in the detection and quantitation of added TSP in meat products but most are time consuming, require considerable technical training and are rarely applicable to cooked samples.

In this study, an adaptation of a colormetric method for carbohydrate analysis (Dubois et al., 1956) has been used in an attempt to develop a simple and rapid procedure to estimate TSP content in beef-soy blends. Variation due to different beef fat levels and commercial TSP sources have been considered.

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MATERIALS & METHODS

TWO 28-LB BATCHES of ground beef representing a wide variety of animal grades and breeds were obtained from the Meats Laboratory at the University of Tennessee. TSP used in preparation of the beef blend was Mira-Tex 110-F supplied by Staley Manuf. Co., Decatur, IL. Variation in absorbance values also was determined for TSP's from the following manufacturers: Vita Pro 555, Lauhoff Grain Co., Danville, IL; Ultra Soy minced, Ultra Soy QR 800N and Ultra soy minced (F), Far-Mar-Co., Inc. Hutchinson, Kansas; ADM 165118, Archer Daniels Midland Co., Decatur, IL; Centex 300 and Centex 300 SL4135 Central Soya, Fort Wayne, IN; Griffith Structured Soy flour, Griffith Laboratories, Inc. Chicago, IL; Mira-Tex 405-F, Mira-Tex 210 and Mira-Tex 210-F, A.E. Staley Co., Decatur, IL.

Preparation of soy-fat-beef

The percent of fat in a 28-lb lot of ground beef was determined in triplicate by a modified Babcock analysis (Smith et al., 1975). The meat then was divided into four portions and ground fat was added to yield four different fat levels: 18, 22, 26 and 30%. The fat was carefully mixed with the meat and then the sample was ground twice through a plate with 3 mm holes in a grinding attachment on a Hobart Kitchen Aide (Mixer Model 5A) to insure a homogenous mixture. The TSP was hydrated by mixing with hot tap water in the ratio of 1 part TSP to 2 parts water and allowed to hydrate for 30 min. Within each fat level, hydrated TSP was added to produce seven levels of TSP: 0, 5, 10, 15, 20, 25 and 30%. Each of these 28 different soy-fat combinations was thoroughly mixed then ground twice. Each soy-fat-beef combination (1 lb) then was formed into patties for easy handling. Samples were stored at −18°C. This procedure was repeated to yield two replications.

Carbohydrate analysis

The procedure used was a modification of the method of Dubois et al. (1956). Each of the soy-fat-beef combinations was sampled three times in triplicate, yielding 252 observations per replication.

Portions of each combination were allowed to thaw at room temperature and a 25-g sample was taken. The sample then was mixed with 1 liter distilled H_2O and blended in a Waring Blendor for 3 min at maximum speed. The blend then was filtered through glass wool, and the filtrate was shaken immediately prior to pipetting to eliminate any variation due to settling. Triplicates of 0.4 ml of filtrate were pipetted into test tubes and 1.6 ml distilled H_2O added to each test tube to bring the volume to 2 ml. One ml 5% phenol was added to each test tube before mixing on a Vortex mixer. Then, 5 ml conc H_2SO_4 was rapidly added and the test tubes were mixed again and placed in a water bath at 25°C for 15 min. The tubes were removed and allowed to stand until the A^{490} was recorded in a double beam Perkin-Elmer (model 124) spectrophotometer using a reagent blank.

Comparison of commercial TSP sources

Samples of several commercial TSP's were each ground with a mortar and pestle. Five-tenths gram of each sample was transferred

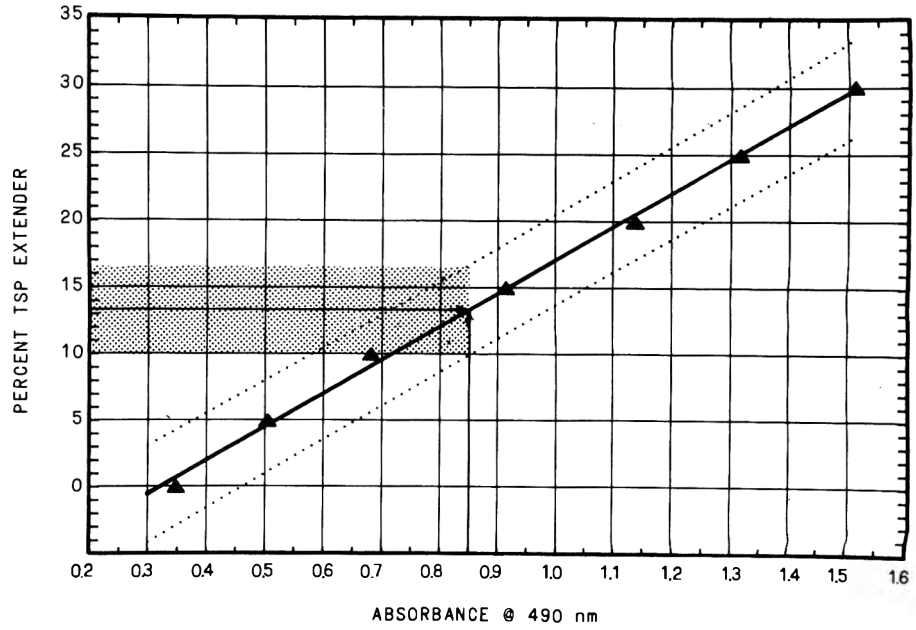

Fig. 1—Relationship between absorance values and percent TSP extender in raw beef-soy blends.

into a blender canister and was blended for 3 min in 1 liter of distilled water and then was filtered, through glass wool. Two ml of each sample was transferred to a test tube and was treated with phenol and sulfuric acid (see above) before determination of A^{490} against a reagent blank. Each commercial TSP was sampled three times.

Statistical analysis

The soy-fat combinations were arranged in randomized complete blocks. Each soy-fat combination within each replication was subsampled. Each subsample was evaluated in triplicate. Data were analyzed by analysis of variance techniques and the means for soy levels across fat levels were separated by orthogonal polynomials.

Data from different TSP sources also were analyzed by analysis of variance to determine if there were significant differences among sources.

RESULTS

AN OVERALL ANALYSIS of variance of carbohydrate absorbance values is presented in Table 1. Clearly, the relationship between TSP content in ground beef and carbohydrate absorbance values is linear as evidenced by the fact that the nonlinear terms (quadratic and lack of fit) were not significantly different from zero. Concern for possible interference by fat content of the ground beef on absorbance values motivated inclusion of various fat levels in the experiment. However, neither fat nor the soy × fat interaction was significant which suggests that the relationship between absorbance values and soy level in the meat blend is independent of meat fat level.

The relationship between soy level and absorbance values in the meat blends as well as a 95% confidence interval are presented in Figure 1. The independent variable (% TSP) is plotted on the ordinate while the dependent variable (absorbance) is plotted on the abscissa. This format was chosen since in most applications TSP is to be predicted from measured absorbance. The confidence interval was calculated based on methods presented by Snedecor and Cochran (1967) for linear calibration.

The soy content of an unknown beef-soy blend can be estimated from absorbance values of carbohydrate extracted from the sample. For example, if the mean absorbance value of an unknown sample were 0.85 then the estimated hydrated TSP content is about 13.5% ± 3.3%. However, a broadening of the confidence interval would be expected in applications where sampling plans were less intense.

Table 2 contains absorbance values (A^{490}) for 11 different commercial forms of TSP. No significant differences in absorbance values were found among the various TSP sources ($P < 0.05$). These results suggest the possible application of this rapid and simple measurement of soluble car-

Table 1—Analysis of variance for carbohydrate absorbance values of meat blends containing various levels of TSP and fat

Source of variation	d.f.	M.S.
Soy	6	13.3073
linear	1	79.6391**
quadratic	1	0.0252NS
lack of fat	4	0.0449NS
Fat	3	0.1218
Soy X fat	18	0.0321
Rep	1	0.8764
Error[a]	27	0.0742
Samples/(soy, fat, Rep)	112	0.0211
Det/(samples, soy, fat rep)	336	0.0027

[a] Soy, Fat and Soy X Fat were tested with Error. **$P < 0.001$

Table 2—Absorbance values and means for various commercial TSP sources

		Sample			
	TSP	(1)	(2)	(3)	Xi
1.	Ultra Soy:minced	1.22	1.32	1.44	1.33
2.	Ultra Soy QR 800N	1.25	1.26	1.32	1.28
3.	Ultra Soy minced (F)	1.24	1.23	1.32	1.26
4.	Centex 300 SL 4135	1.30	1.37	1.33	1.33
5.	Centex 300	1.39	1.33	1.36	1.36
6.	Mira-Tex 405-F	1.30	1.28	1.12	1.23
7.	Mira-Tex 210	1.20	1.30	1.38	1.29
8.	Mira-Tex 210-F	1.29	1.34	1.32	1.32
9.	Vita Pro 210-F	1.31	1.10	1.32	1.24
10.	Griffith Structured soy flour	1.16	1.12	1.16	1.15
11.	ADM 165118	1.25	1.32	1.44	1.24

Overall Mean = 1.28

S = 0.0601

S^2 = 0.0033

bohydrates as a quality control procedure for estimation of the amount of textured or structured soy flour used as an ingredient in ground beef-soy blends.

DISCUSSION

DEVELOPMENT of analytical systems for detection and/or quantitation of TSP concentration in meat blends involves some very fundamental questions. The problem more practically stated is "Can animal and plant tissues be accurately and quantitatively differentiated in meat food products?" Most existing analytical systems have been based upon differentiation of the protein component of food products but these systems (electrophoretic and immunochemical) are time and labor intensive and require a high degree of skill on the part of the technician. In this study we have attempted to develop a method, based on the lack of appreciable soluble carbohydrate in ground beef, to quantitate TSP content in beef-TSP blends. By extraction and analysis of the soluble carbohydrates in a meat-TSP blend the difficulties involved in determination of the origin of the various protein components have been avoided and a linear relationship between absorbance readings and TSP content of ground beef has been demonstrated.

The method is simple, straight-forward and requires no special equipment. Approximately 40 meat samples (in triplicate) may be analyzed in a 8-hr work day by a single technician. The procedure is not applicable to meat blends extended with soy protein concentrates or isolates and may have only limited value in more complex meat blends. The procedure is applicable to meat blends extended with soybean flour or its structured derivitives.

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EFFECT OF SOAKING TEMPERATURE ON COOKING AND NUTRITIONAL QUALITY OF BEANS

SAMUEL KON

ABSTRACT

California small white beans were soaked at different temperatures, in 10°C increments, from 20° C to 90°C, until maximum imbibition was achieved. Losses of total solids, N compounds, total sugars, oligosaccharides, Ca, Mg, and three water-soluble vitamins (thiamin, riboflavin and niacin) were measured and found to be very small at soaking temperatures up to 50°C. An increase in those losses of from three- to fourfold was found when the soaking temperature was raised to 60°C or above. Cooking rates followed a bell-shaped pattern with the peak, longest cooking time, being for beans soaked at 70°C and shortest cooking time for beans soaked at 90°C. Cooking rates corresponded closely to the amount of organic phosphate left in the beans after soaking at the different temperatures.

INTRODUCTION

WHEN BEANS are prepared for consumption they are usually soaked before cooking. This is done to facilitate quicker cooking. This is particularly true when dry beans are used for canning in commercial production. Soaking, however, is by itself a long process and numerous ways to shorten it were devised. Some of those methods have to do with mechanically increasing the rate of water imbibition by applying a vacuum infiltration technique, but more commonly increasing the temperature of the soaking water is used to increase the imbibition rates. This latter technique is quite effective and soaking time can be reduced from 16 hr at room temperature to less than an hour at 90°C. Other changes might and do occur during this shortened soaking at elevated temperatures. The purpose of this report is to investigate the effects these changes, caused by soaking at elevated temperatures, have on the cooking characteristics of dry beans as well as on their nutritional qualities.

MATERIALS & METHODS

CALIFORNIA small white beans were used in all the experiments reported here. They were obtained from the growing area around Salinas, CA.

Soaking was done in baskets submerged in a water bath kept at the desired temperatures ± 1°C. The rate of water imbibition was determined by weighing the basket periodically and noting the increase in weight. Before weighing, the baskets were allowed to drain for a specified time. Soaking continued until two consecutive weighings were the same. The soaking water was freeze-dried and the solids redissolved in a measured amount of water.

Cooking was done on the experimental cooker as described by Burr et al. (1968) and the cooking rates were followed.

The concentration of thiamin, riboflavin, and niacin was determined as described by Miller et al. (1973).

Total sugars were determined by the anthrone colorimetric method while the values for oligossacharides were determined by gas-liquid chromatography of their trimethylsilyl derivatives as described by Becker et al. (1974).

Total and organic phosphate was determined by the colorimetric method of Allen (1940). Ca and Mg were determined by atomic absorption and N by Kjeldahl.

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Table 1—Composition of California small white beans (dry wt)

Kjeldahl N	3.55%
Total sugars	7.74% (anthrone)
Oligosaccharides[a]	6.26% (gas chromatography)
Total P	0.46%
Inorganic P	0.05%
Organic P	0.41%
Total Ca	0.18%
Total Mg	0.20%
Thiamin	0.56 mg/100g
Riboflavin	0.19 mg/100g
Niacin	1.29 mg/100g

[a] Including sucrose, raffinose and stachyose.

Table 2—Soaking time for maximum imbibition at the different temperatures and cooking time for 95% cooked product

Soaking temp (°C)	Soaking time (min)	or (hr)	Cooking time (min)
RT[a] (20)	960	16	28
40	300	5	25
50	240	4	27
60	90	1.5	33
70	70	1.2	37
80	60	1.0	33
90	50	0.8	21

[a] Room temperature

RESULTS

IN TABLE 1 are the results of compositional analysis of the beans used in these experiments. The results in all the other tables and figures are reported as percent of the values in Table 1. The value for oligosaccharides reported here included sucrose, raffinose, and stachyose. Those three oligosaccharides account for 81% of beans total sugars. Of the three oligosaccharides found in beans, sucrose was the biggest fraction (3.03%) followed very closely by stachyose (2.94%) and then raffinose which was found in much smaller quantities (0.29%), only about 10% of stachyose.

As can be seen from Table 2 and Figure 1, and as was expected, soaking at elevated temperatures increased the rate of water imbibition and decreased the time required for maximum water absorption. As can be seen from Figure 1, the values for final weight of the beans soaked at the higher temperatures, 60°C and above, was lower than for beans soaked at the lower temperatures. This was due to the large increase in the extraction of bean soluble material at those temperatures as shown in Table 3. It was interesting to note that there seem to be two groups with similar imbibition rates; one for the beans soaked at temperatures of 50°C and below and one for beans soaked at 60°C and above. This grouping was very apparent also in the results shown in Table 2, where a jump in the imbibition rate between 50°C and 60°C soaking temperature was demonstrated. The same jump was shown also, as will be discussed later, for the extraction of soluble material as seen in Table 3.

The results shown in Table 2 and particularly in Figure 2 indicated that soaking temperature had an important effect

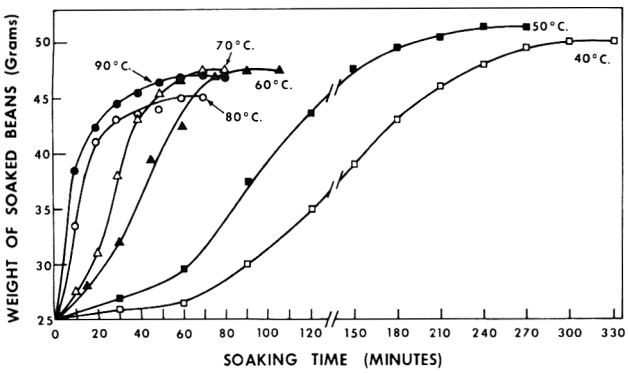

Fig. 1—Imbibition rates at different soak water temperatures.

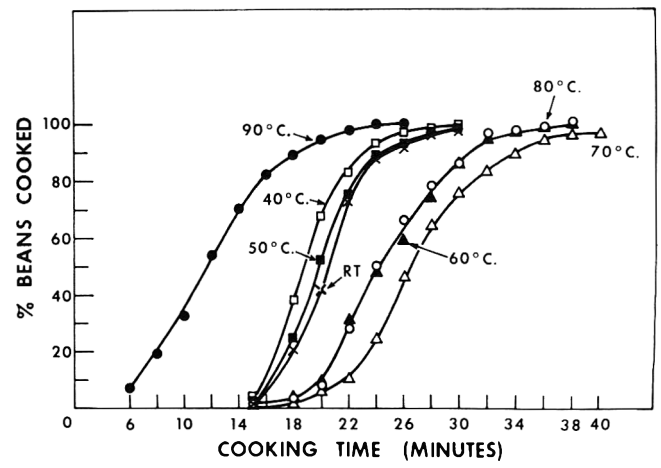

Fig. 2—Cooking rates of beans soaked at different temperatures.

on the cooking rate of beans. The cooking rates as seen in this figure were bunched in three groups. The slowest cooking rate was for beans soaked at 70°C followed by the ones soaked at 60°C and 80°C with very similar cooking rates. The second group was composed of beans soaked at RT (room temperature about 20°C), 40°C and 50°C, with the one soaked at 40°C having the shortest cooking time in this group. The last group had only one member, those beans soaked at 90°C; these beans cooked at considerably shorter time than the rest of the samples.

In Tables 3 and 4 the concentrations of the different components that were followed were reported for the amount extracted (Table 3) and for the amount left in beans (Table 4). In general, more solids were extracted at the higher soaking temperatures. An exception to this general trend was found when comparing extraction at RT with extraction at 40°C. Here, less material was extracted at 40°C than at RT even though the temperature was higher. However, in this particular case, there was a large reduction in soaking time from 16 hr to 5 hr. As seen in the two tables, it seems that at 60°C there was somewhat more extraction than at 70°C and 80°C, this difference if it is real, might be explained by a faster inactivation at the higher temperatures of catabolic enzymes which play an important role in the extraction rate.

Extraction of N-compounds, total sugars, oligosaccharides, total P, Ca and Mg all followed the same pattern as extraction of total solids. As mentioned before, there seemed to have been a breaking point between soaking at 50°C and 60°C. In all cases, soaking at 60°C or above increased extraction by about fourfold compared to extraction while soaking below 60°C. The values for oligosaccharides were reported for all three rather than individually because interconversion from the longer ones to the shorter ones due to the action of bean α-galactosidase during the soaking period made it very difficult to assess them individually. However, soaking at 60°C and above removes 50–57% of stachyose and raffinose from beans, while soaking below 60°C removes very small fractions of those two oligosaccharides (about 5% at RT and about 15% at 50°C).

During soaking, organic phosphate compounds were

Table 3—Percent[a] of individual components in soak water at the different soaking temperatures

Soaking temp (°C)	Total solids	N	Total sugars	Oligo-saccharides	Total P	Organic P	Inorganic P	Ca	Mg
RT (20)	3	1	5	6	1	1	3	2	3
40	2	1	3	3	1	Trace	5	1	2
50	4	3	12	16	4	2	22	2	5
60	12	9	46	44	20	10	98	9	31
70	11	7	34	44	15	8	77	6	26
80	11	7	35	45	14	5	88	6	32
90	12	9	36	47	17	7	99	7	30

[a] Results are reported as percent of original composition.

Table 4—Percent[a] of individual components left in beans after soaking at different soaking temperatures

Soaking temp (°C)	Total solids	N	Total sugars	Oligo-saccharides	Total P	Organic P	Inorganic P	Ca	Mg
RT (20)	96	99	92	91	99	69	352	94	76
40	97	95	95	96	89	58	344	93	70
50	94	93	73	81	87	57	336	91	64
60	83	81	61	60	74	50	272	91	52
70	85	83	55	59	74	47	208	93	52
80	85	84	55	59	75	55	152	96	52
90	82	87	55	61	82	76	136	91	52

[a] The results are reported as percent of original composition.

Table 5—Distribution of thiamin after soaking at different temperatures

Soaking temp (°C)	Residue in beans (%)	Present in soak water (%)	Total recovered (%)
RT (20)	98	1	99
40	98	1	99
50	92	9	101
60	69	32	101
70	63	39	102
80	63	34	97
90	63	40	103

Table 6—Distribution of riboflavin after soaking at different temperatures

Soaking temp (°C)	Residue in beans (%)	Present in soak water (%)	Total recovered (%)
RT (20)	99	1	100
40	94	2	96
50	90	9	99
60	80	24	104
70	70	29	99
80	70	30	100
90	53	35	88

being continuously hydrolyzed to inorganic phosphates by the action of bean phytase enzyme. It seems, from the two tables, that more organic phosphates were hydrolyzed at lower temperatures but that more were leached into the soak water at the higher temperatures. This is in agreement with the results of Becker et al. (1974) who found that the optimum temperature for phytase activity in beans is around 40°C. As can be seen in Table 4 the content of organic phosphate in the beans is not dependent only on the leaching of soluble compounds but also on the activity of the enzyme phytase, so that the presence of organic phosphorous material in the residue of soaked beans is not directly related to the soaked water temperature.

In the case of Ca and Mg again there was at least a fourfold jump in the amount extracted at 60°C and above as compared to the amounts extracted at lower temperatures. About four times as much Mg as Ca was present in the soak water, especially at the higher temperatures (60°C and above), which will imply that Mg is present *in the beans* in a more extractable form and is in general agreement with results reported by Meiners et al. (1976).

A large jump in the extraction of soluble materials when the soaking water temperature was raised from 50°C to 60°C was seen again in Tables 5, 6, and 7 pertaining to three water-soluble vitamins (thiamin, riboflavin, and niacin). In this case at least a threefold increase in extractability was observed. As in the case of total sugars and oligosaccharides (Tables 3 and 4) there was also a small jump in

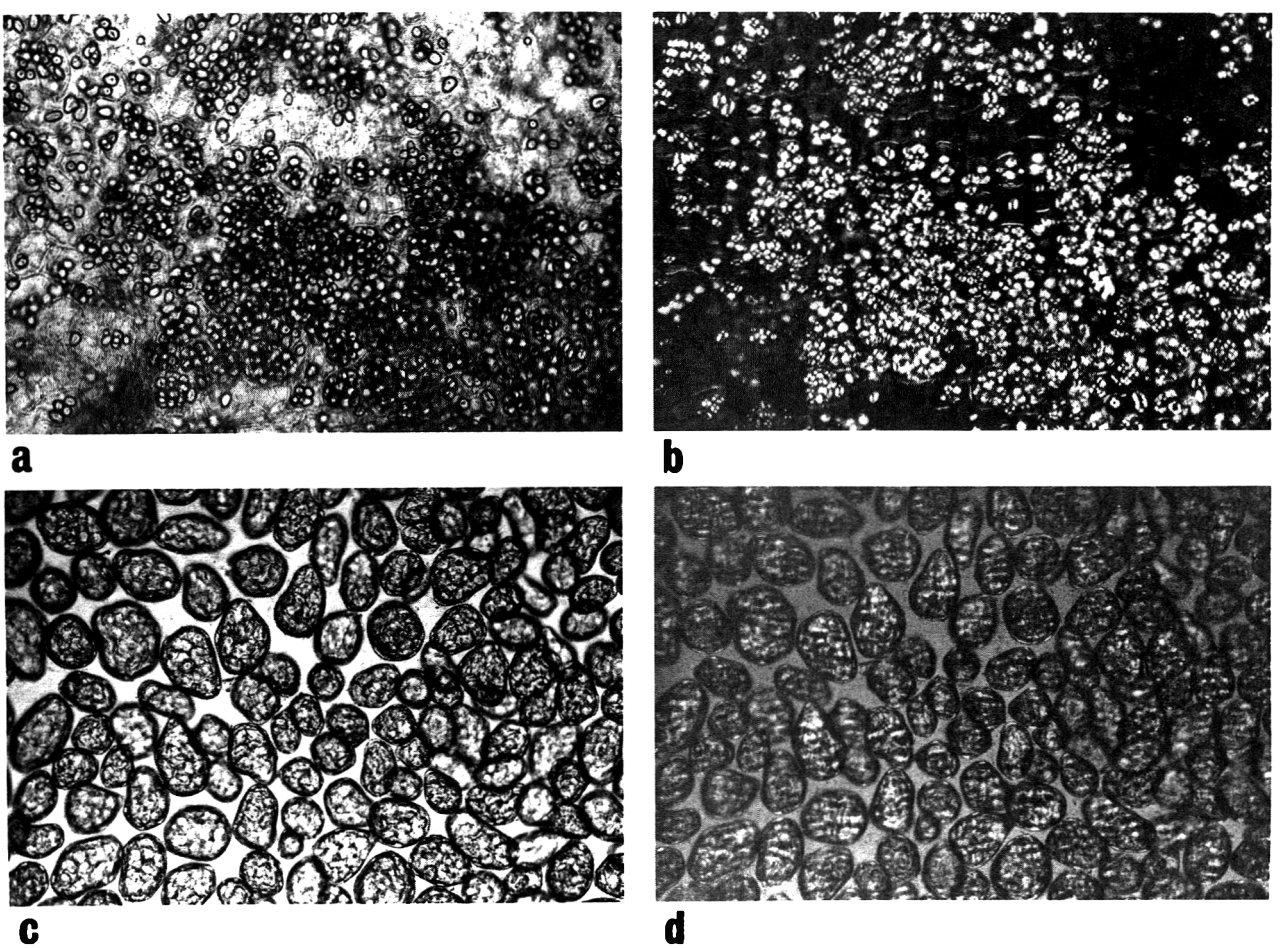

Fig. 3—Photomicrographs of beans soaked at 40°C: (a) regular; (b) polarized light. The same beans after cooking (c) regular; (d) polarized light.

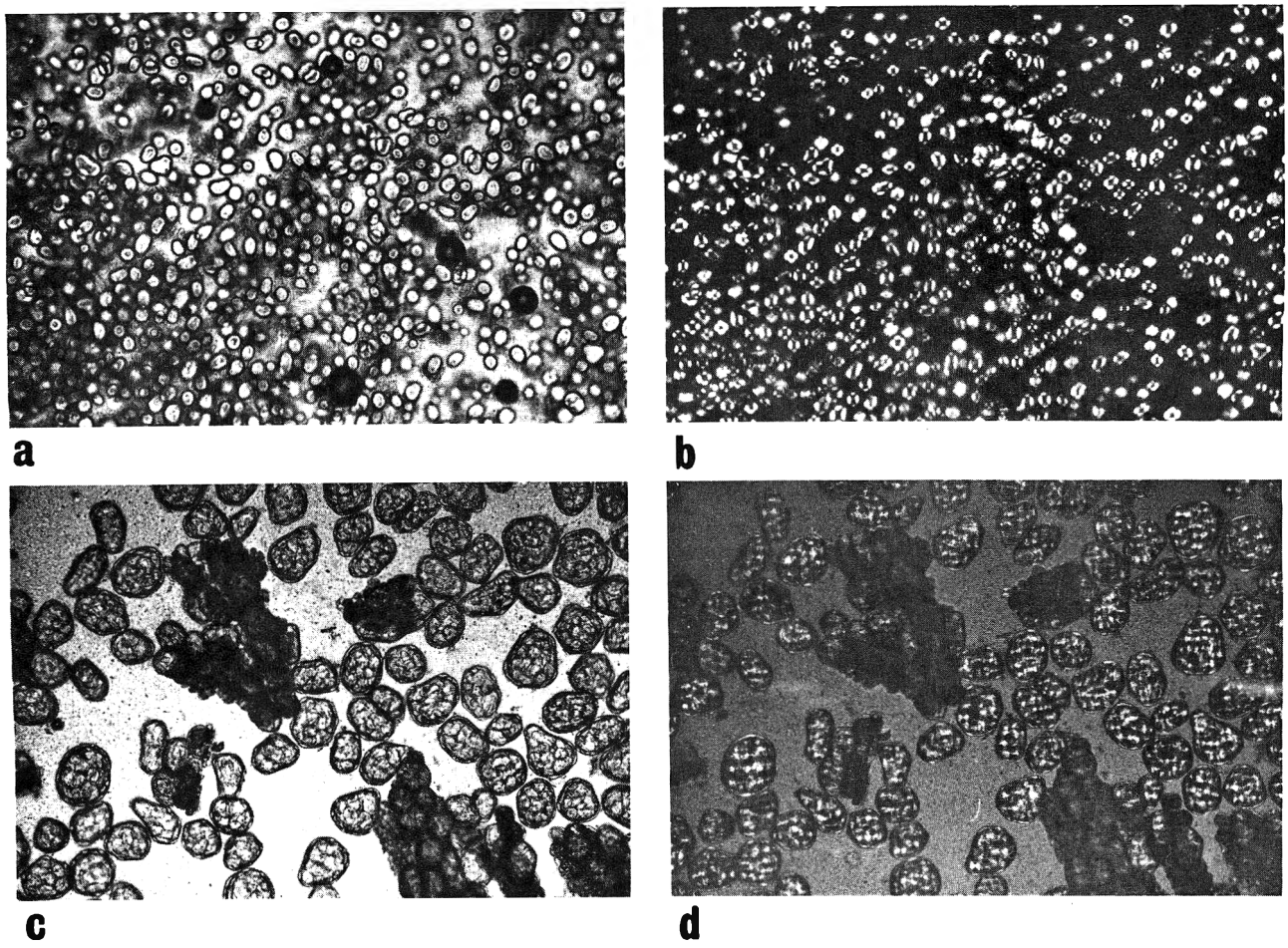

Fig. 4—Photomicrographs of beans soaked at 80°C: (a) regular; (b) polarized light. The same beans after cooking (c) regular; (d) polarized light.

the extractable vitamins when the soaking temperature was increased to 50°C from either room temperature or 40°C.

As discussed in some of our previous papers (Kon et al. 1970, 1971, 1974) cells of raw dry beans tend to break very easily when pressure is applied on them, releasing their contents to the surrounding media; this is true also when the beans are fully hydrated. However, after cooking this does not happen, and when pressure is applied the cells tend to separate rather than break. This can be seen in Figure 3 for beans soaked at 40°C. In Figure 3a and b it is quite clear that the cell walls are broken and that the cell content was released to the surrounding media. The starch granules do not seem to be very swollen and under polarized light they are birefringent and exhibit the characteristic cross which disappears on gelatinization. It is possible to see in the background (Fig. 3a and b) the outline of the empty cells. After cooking, Figures 3c and d, the cell separated whole rather than broke when pressure was applied. The starch inside the cells seems to be mostly gelatinized as no birefringence is seen in Figure 3d. When looking at results reported in Figure 4 the same seems to be true for beans soaked at 80°C only that the soaked starch granules (Fig. 4a and 4b) seem to be more swollen than the one soaked at 40°C (Fig. 3). Soaking at 90°C seems to cause a change in the characteristic pattern described above. As seen in Figure 5, even soaking for a short time (20 min) caused some of the bean cells to separate rather than break, and soaking for 50 min, for complete hydration, made all the cells separate rather than break under those stress conditions. The starch, however, was not gelatinized under those conditions as seen in Figure 5d. Cooking the beans that were soaked at 90°C did not gelatinize the starch, as seen by residual birefringence present in Figure 6b. This can be partially explained by the short cooking time (21 min) required after soaking at this high temperature, and partially by the fact, observed by us previously, that gelatinization rate of starch held at high temperatures prior to gelatinization decreases markedly. When those beans were cooked for 40 min the starch seemed to be gelatinized (Fig. 6d).

DISCUSSION

FROM THE RESULTS presented here it seems that very little material is extracted from beans soaked at 50°C and

Table 7—Distribution of niacin after soaking at different temperatures

Soaking temp (°C)	Residue in beans (%)	Present in soak water (%)	Total recovered (%)
RT (20)	93	4	97
40	92	3	95
50	87	16	103
60	58	53	111
70	55	61	116
80	52	50	102
90	51	55	106

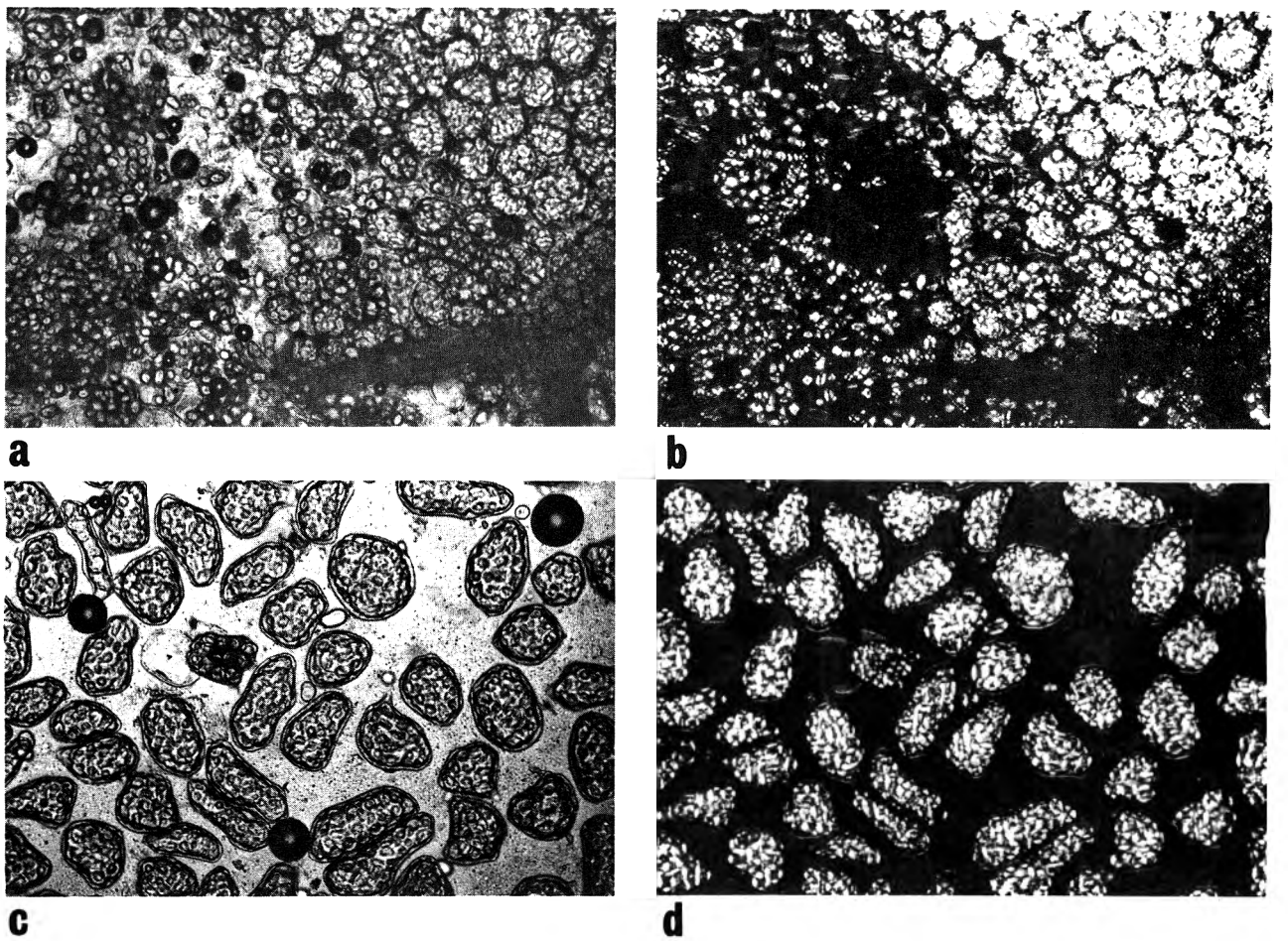

Fig. 5—Photomicrographs of beans soaked at 90° C: (a) for 20 min, regular light; (b) same under polarized light; (c) for 50 min, regular light; (d) same under polarized light.

below. The least extraction of any of the nutrients in beans occurs when the soaking is done at 40°C. This, as mentioned before, is due to the fact that considerably less time is required for complete hydration at 40°C than at room temperature (5 hr compared to 16 hr). There is a marked increase in nutrients lost on soaking at 60°C and above. This would seem to indicate that at this temperature (60°C) the permeability of the cell membrane is affected and therefore more materials can diffuse out (Schwimmer, 1972). Also because of this destruction of inter- and intracellular membranes an intermixing of catabolic enzymes and substrates occurs under those conditions (Schwimmer, 1969) which will then facilitate the diffusion of the resulting smaller molecules.

It appears from analyzing the diffused materials that only the smaller compounds of the cell diffuse out under any of the conditions observed, so that only nonprotein N components of the cell are found in the soak water (California small white beans have 12% of their N as nonprotein compounds). It is possible that at the higher soaking temperatures when one would expect some protein material to be diffused, due to membrane destruction, the protein is denatured by the high temperature, becomes insoluble, and thus does not diffuse out.

Among the materials that are extracted at higher temperatures of soaking are the oligosaccharides of beans. When beans are soaked at temperatures above 60°C about 50% of the oligosaccharides are extracted into the soaking water. In addition to simple diffusion, those compounds are also hydrolyzed under those conditions as the activation temperature for bean α-galactosidase was found to be around 55°C (Kon et al., 1973; Becker et al., 1974; Kon and Wagner, 1977). In contrast to the other nutrients that are lost while soaking at higher temperatures, this loss might be quite advantageous because two of the bean oligosaccharides, raffinose and stachyose, have been implicated as contributing significantly to flatulence (Cristofaro et al., 1970; Becker et al., 1974; Wagner et al., 1976); loss of about 50% of those oligosaccharides might reduce appreciably the discomfort associated with flatulence.

As shown in the results section, starch gelatinization is affected by the soaking temperature. At 90°C soaking temperature, some changes occur in the starch granule that cause it to maintain its birefringence under polarized light for a longer time than without this initial heating. Persistent birefringence is indicative of slower gelatinization and might have an effect on digestibility because in in vitro experiments, nongelatinized starch is much less susceptible to enzymatic digestion than gelatinized starch (Kon et al., 1971). From our discussion so far, and from the results presented here, it is quite possible that beans that were soaked at 90°C and then cooked for the relatively short time required, as indicated by softness of the beans, will have much of their starch in nongelatinized form and thus not as available nutritionally as when it is fully gelatinized.

The effect that soaking at different temperatures has on the extractability of phosphate is different from the effect on the extractability of other constituents tested. This is because two different reactions affect phosphate extraction; one is the conversion of organic phosphates to inor-

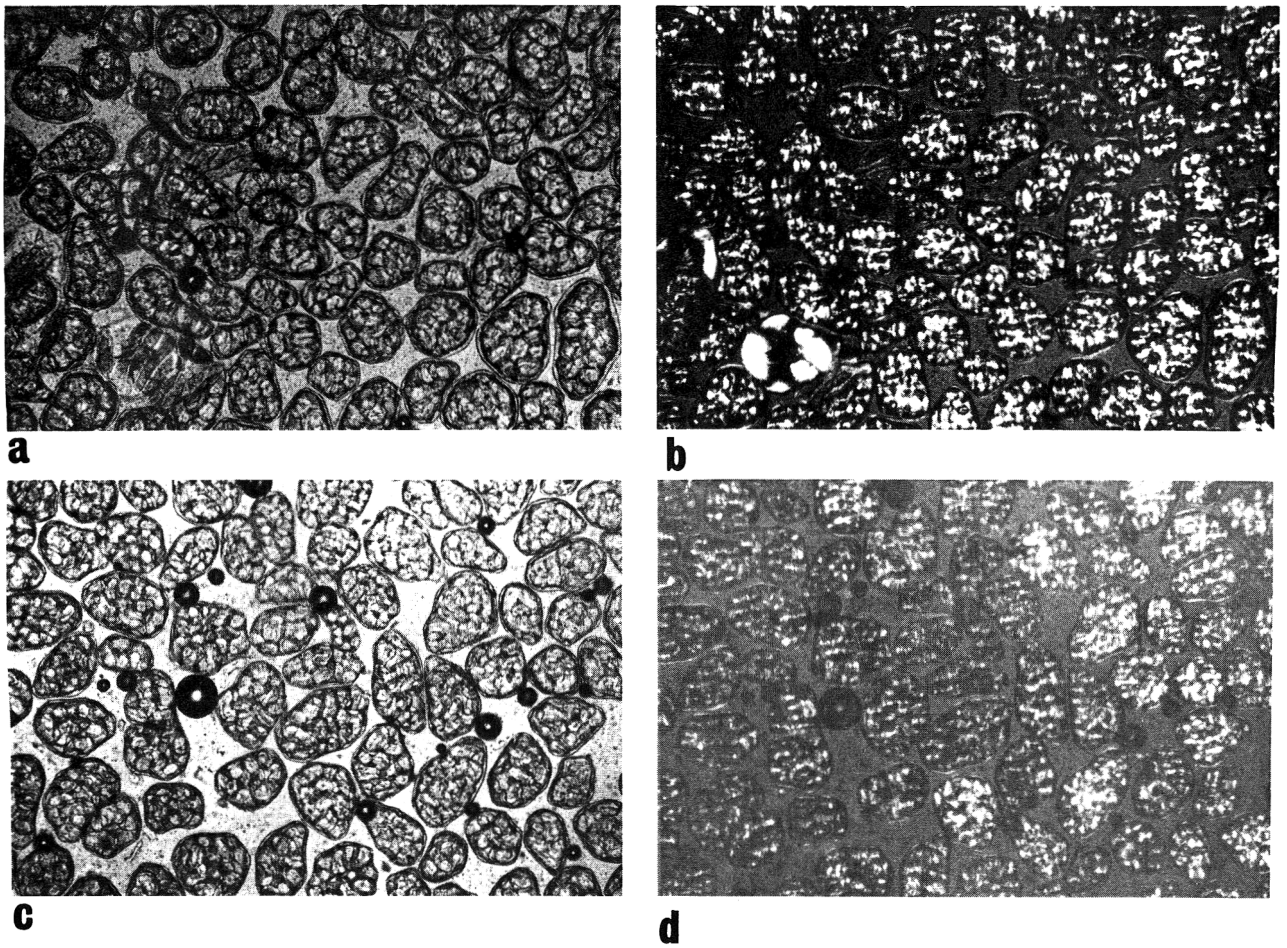

Fig. 6—Photomicrographs of beans soaked at 90° C for 50 min and cooked: (a) for 21 min, regular light; (b) same under polarized light; (c) for 40 min, regular light; (d) same under polarized light.

ganic phosphates which is an enzyme dependent reaction (phytase), and the second is the diffusion of inorganic (and some organic) phosphate compounds through the membrane. The two reactions do not respond the same way to changes in soak water temperature. More organic phosphate was hydrolyzed to inorganic phosphate at the lower temperatures, which is in agreement with the results of Becker et al. (1974), but more phosphate containing material was leached at the higher temperatures. As a consequence of those two independent actions, the soaked beans with the least organic phosphate left in them after soaking were the ones soaked at 70°C, which corresponded also to the beans with the longest cooking time.

A correlation between phytic acid content and cooking rate of legumes was discussed by Mattson (1946) and Muller (1967). Some years ago we proposed a model for the softening of legumes during cooking which involved an exchange of Ca from the mostly insoluble Ca pectate present in legumes (Kon, 1968) with Na and K from the mostly soluble Na and K phytate. (Chang et al., 1977). This model is supported by the results reported by Kumar et al. (1978) who showed that germination of cowpeas and greengram, while reducing phytate content, increases cooking time, by Molina et al. (1975) who showed that cooking of beans prior to storage reduces the number of hard cooking beans that develop; and, now by our correlation between cooking time and amount of organic phosphate left in beans after soaking at different temperatures, as shown in Figure 7. Soaking at higher temperatures that allowed hydrolysis of phytate and diffusion out of phosphate caused the beans to require longer cooking time. Soaking at 90°C caused rapid inactivation of legume phytase allowing only 25% of the phytate to be hydrolyzed and made the beans cook rather rapidly as compared to the others tested.

From the preceding discussion it appears that soaking at temperatures between 60°C and 80°C might have a significantly adverse effect on the nutritional qualities of the

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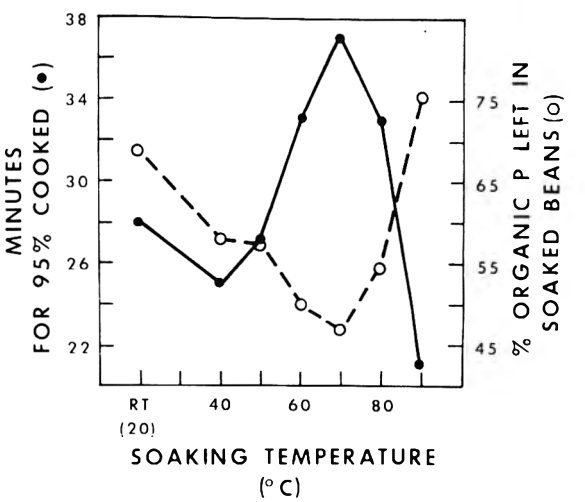

Fig. 7—Correlation of cooking time with organic P content of beans soaked at different temperatures.

RAPID EVALUATION OF PROTEIN QUALITY OF FOODS USING Clostridium perfringens

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ABSTRACT

A 1-day protein quality evaluation procedure was developed utilizing the anaerobic microorganism, *C. perfringens* strain 3624. The food sample required no pretreatment except for particle size reduction. Culture development was measured manometrically. Contaminating microorganisms were inhibited by cultural conditions which included anaerobiosis, 46°C incubation and a large initial inoculum. Culture response was expressed as Relative Slope Ratio (RSR) which compared the response slopes of the test nitrogen source with that of a standardized mixture of synthetic amino acids. A preliminary study comparing RSR values with Protein Efficiency Ratios (PER) for 25 foods showed a linear correlation coefficient of 0.74. A study comparing RSR and PER values for 15 foods representing a wide variety of protein sources indicated that the RSR method cannot be substituted for the PER method, but there was an indication that foods may be grouped for improved analysis. The RSR assay was sensitive to thermal treatment variations and responded to sulfur containing amino acid supplementation of heated products. Further evaluation of the technique for rapid assessment of protein quality changes resulting from processing variables seems warranted.

INTRODUCTION

A RAPID and inexpensive method for determining the quality of a protein is highly desirable to aid in the development of new protein sources, to meet the requirements established by the Food and Drug Administration concerning nutritional labeling and to assist in the assessment and improvement of nutritional status of humans.

Biological evaluation is the preferred method for protein quality determination since the ultimate value of the protein is measured by its ability to support growth and maintenance in the form of cellular synthesis. Included in the biological methods presently used for assessing the nutritive value of protein are the biological value (BV) (Thomas, 1909), net protein ratio (NPR) (Bender and Doell, 1957), net protein utilization (NPU) (Miller and Bender, 1955), slope ratio assay (Hegsted and Neff, 1970) and the protein efficiency ratio (PER) (Osborne et al., 1919). The PER method is currently recognized as the official AOAC method for protein quality evaluation, primarily because of its long-standing use. All of these biological methods involve time consuming, costly and cumbersome animal feeding studies. Simplified techniques proposed for the biological evaluation of protein quality include the chemical score method, (Block and Mitchell, 1946) blood amino acid profile methods, (Swendseid et al., 1963) and microbiological methods (Fernell and Rosen, 1956; Ford, 1960). The objection to the chemical score is that it is an in vitro analysis of the amino acid profile of a protein and is not truly indicative of the growth or maintenance potential of the protein source (Hackler, 1977). The blood amino acid method is also not clearly related to the quality of protein and tends to provide extremely variable results (Bodwell, 1975).

Microbiological methods, which have been reported in the literature, appear to have some potential for protein quality evaluation. Two of the microbiological assays that have been successful in correlating with the rat assay values involved the use of the protozoan flagellate *Tetrahymena pyriformis* (Fernell and Rosen, 1956; Rosen and Fernell, 1956; Stott et al., 1962; Frank et al., 1976; Evancho et al., 1977) and the bacterium *Streptococcus zymogenes* (Ford, 1960; Waterworth, 1964; Ferrando et al., 1968). A major advantage of microbial methods over other biological methods for protein quality evaluation is their relatively brief assay times. The method using *T. pyriformis* takes 4 days to complete and that using *S. zymogenes* takes only 2 days. The other advantage of microbiological assays is their relatively low cost. Microbiological assays for protein quality in foods, as described above, may be challenged on the basis of several factors. First, the need to reduce the indigenous microflora of the test food material by a thermal treatment (Frank et al., 1976) which could potentially alter the protein of the food. Second, the need for pretest enzymic proteolysis of the food protein (Frank et al., 1976) to make it available to the microorganism, thereby potentially confusing the food protein with the enzyme protein. Third, the need for growth evaluation by destructive alteration of the growth system thus yielding a single point measurement (Frank et al., 1976) which renders the response time dependent rather than dynamic.

The potential for using *C. perfringens* as a protein quality assay tester organism seemed high because: (1) the amino acid requirements of *C. perfringens* (Boyd et al., 1948; Fuchs and Bonde, 1957; Murata et al., 1965; Riha and Solberg, 1971) include most of those required by humans and rats; (2) it would eliminate the need for thermal pretreatment of the test sample by severely limiting the growth of the natural microflora through the use of anaerobic conditions, 46°C temperature of incubation, large pure culture inoculum and rapid rate of growth; (3) the organism produces proteolytic enzymes; (4) the organism produces large quantities of gas thus permitting the evaluation of growth response indirectly via gas production so that culture turbidity is no problem and there is no need to weigh cellular matter, to make direct microscopic counts, to cultivate colonies or to titrate; and (5) the growth response is a dynamic one which gives a complete growth curve, offering numerous possibilities for evaluation, such as adjustment phase duration, slope and time to reach the stationary phase.

The objectives of this study were to develop a method for the evaluation of protein quality using the organism *C. perfringens* strain 3624 and to evaluate the method using a wide variety of food products and several process variables.

EXPERIMENTAL

Test organism

C. perfringens strain 3624 was obtained from the culture collection of Rutgers University, Food Science Dept. New Brunswick, NJ. The culture was maintained in Cooked Meat Medium (CMM) (BBL,

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Difco). The organism was transferred on a monthly basis by two consecutive transfers in Fluid Thioglycollate Medium and incubated at 37°C. Final transfer of the cultures was in CMM, incubated at 37°C and then stored at 4°C until needed. The stored cultures were the stock cultures for subsequent experimentation.

R&S medium preparation

R&S medium was prepared as described by Riha and Solberg (1971) on a daily basis except that the medium was adjusted to pH 7.0 before use and filter sterilization was performed under a stream of oxygen-free nitrogen (Ades and Pierson, 1973).

R&S base was prepared similarly except that no amino acids were included and no heat was necessary.

Inoculation of cuvettes

Two 20-ml portions of sterile R&S medium were transferred under nitrogen to two sterile Coleman cuvettes (25 × 105 mm). The cuvettes and flasks containing filtered media were sealed and placed at 4°C until ready for further use within 18 hr.

Inoculation was accomplished by adding 0.5 ml from the stock culture, which was first thoroughly shaken, to the pretempered cuvettes (37°C) containing 20 ml of sterile R&S medium. The inoculated cuvettes were then incubated at 37°C for 9–12 hr. Without agitation of the cuvette, the absorbance at 600 nm was then measured in the Coleman Jr. Spectrophotometer. The absorbance should be 1.7–1.8.

Washed cell inoculum

Eight Coleman cuvettes were filled with 18 ml of R&S medium under a stream of oxygen-free nitrogen after which they were pretempered in a 46°C water bath. Seven of these cuvettes were each inoculated with 2 ml of the 9–12 hr culture. The uninoculated cuvette served as a spectrophotometric control. Culture development was monitored spectrophotometrically at 600 nm without agitation until an absorbance of 0.6–0.7 was reached.

The seven cuvettes were then transferred into three 46°C pretempered stainless steel 50 ml centrifuge tubes (Dupont-Sorvall) and centrifuged in a Sorvall Super Speed Centrifuge type SS-1 for 5 min at 14,350 × G. The supernatant was poured off and 15 ml of pretempered (46°C) R&S base were added to each centrifuge tube. After vigorous shaking, the contents of the three tubes were combined into one centrifuge tube and centrifuged at 14,350 × G for 5 min. The cells were then resuspended in 15 ml of 46°C R&S base and recentrifuged two more times, after which the cells were resuspended in 15 ml of 46°C R&S base and transferred to a pretempered (46°C) Coleman cuvette. The absorbance at 600 nm was adjusted to 1.4 by adding pretempered (46°C) R&S base. This was the inoculum and represents approximately 2×10^9 colony forming units (CFU)/ml.

Plate counts

The inoculum was serially diluted in 0.1% peptone water and plates were poured in duplicate in Tryptose Sulfite Cycloserine (TSC) Agar (Harmon et al., 1971), without cycloserine or egg yolk. The plates were incubated in anaerobic glass jars for 18–24 hr at 37°C before CFU's were counted. The CFU's were counted with a Quebec colony counter to confirm proper inoculum viability.

Gas evolution measurement

U-tube glass manometers (40 cm high) (Fisher) were used for measurement of gas evolution of the microorganisms in the reaction flasks. The manometers were approximately half-filled with red colored kerosene (sp gr 0.826). A manometer unit was equivalent to 0.29 cm^3 of gas.

Reaction vessels

The principal reaction vessel was a side arm flask of 300 ml capacity manufactured by Bellco Glass Inc., (stock number 2574-50002 made from blueprint F 1630). The top of the flask was closed with a one-hole number 6 rubber stopper fitted with a 1.5 inch length of glass tubing. Attached to the glass tubing was a 24 inch length of latex rubber tubing (3/16 inch i.d.). The side arm opening was fitted with a rubber serum bottle stopper. Uniform lengths of rubber tubing lead from one side of the manometer to a three-way aquarium type needle point valve.

A second reaction vessel consisted of a 25 × 105 mm test tube fitted with a one-hole rubber stopper through which was inserted a 1.5 inch length of glass tubing attached to latex rubber tubing as described above.

Preparation of the test system

Eight sterilized 300 ml side-arm reaction flasks were filled with 150 ml of R&S medium. Another eight reaction flasks were filled with 150 ml R&S base medium. One drop of Dow Corning antifoam was added to each of the 16 flasks to reduce foaming during nitrogen flushing.

The eight R&S base containing flasks were pretempered to 46°C after which the test protein was added (see section: Preparation of food suspension). The pH of all 16 flasks was adjusted to 7.0 and then each flask was sealed with modeling clay around the rubber stopper. Nitrogen flushing was accomplished by insertion of a 6 inch, 18 gauge, stainless steel syringe needle, through the serum bottle stopper into the flask and submerged below the liquid level. The needles were connected to the nitrogen supply by 30-inch lengths of rubber tubing (3/16 inch i.d.), joined by y-tube connectors to a common inlet. After 30 min of nitrogen flushing, the needles were removed and the valves were closed simultaneously leaving a slight positive pressure in the flask-manometer system.

1.5 ml of prepared inoculum was introduced into each of 15 flasks with a 3 cc disposable syringe through the rubber serum bottle stopper. The 16th flask was injected with 1.5 ml of R&S base medium and served as a control to correct all manometer readings for changes in temperature and barometric pressure during the experiment. After inoculations were completed, the three-way valves were opened just long enough to allow the manometer fluid to come to zero and then closed. Manometer readings were recorded at 10-min intervals, until 300 units of gas were produced in the system. As each manometer reached 15 units, the three-way valve was opened to the atmosphere for an instant to reset the manometer to its zero point. Accumulation of 300 units of gas required 4–5 hr. The values from 10–100 units were used to calculate the slope of the growth response curve for each flask. The mean log slope for the seven R&S medium flasks was divided into each of the test flask slopes to obtain the Relative Slope Ratio (RSR). Relative Slope Ratio (RSR) was calculated thus:

$$RSR = \frac{\text{mean log slope for the test medium}}{\text{mean log slope for R\&S medium.}}$$

The test tube reaction vessels were filled with 20 ml of R&S medium or R&S base. Nitrogen flushing was accomplished by direct immersion of syringe needles through the open test tube. Inoculation was accomplished by adding 0.5 ml of the inoculum preparation into each test tube reaction vessel. The test tube reaction vessel procedure was described in detail by Blaschek and Solberg (1978).

Nitrogen determination of food samples

The determination of the percent nitrogen present in the test food samples was accomplished by one of two methods.

1. Use of Coleman Nitrogen Analyzer (Model 25A, Coleman Ind. Inc., Maywood, IL).
2. Semi-micro modification of the AOAC Kjeldahl method as described by Henwood and Garey (1971).

Preparation of food suspension

Samples of foods to be tested were analyzed for their nitrogen content. The test food was added to 150 ml of R&S base on an isonitrogenous basis compared to R&S medium. This amounts to 2.6 mg of nitrogen per ml of medium or 2.44g of protein per reaction flask. The test foods were prepared in powder or mashed forms by mechanical methods which included grinding, pulverizing or blending with liquid nitrogen used to freeze samples. The flasks containing 150 ml of sterile, pretempered (46°C) R&S base were placed on magnetic stirrers. While stirring, the food samples were added to each flask. The stirring continued for 20 min. The pH of each flask was adjusted to pH 7.0 and then each flask was stoppered and sealed with modeling clay around the top opening.

When tubes were used as the reaction vessel, 0.33g of protein were added to each tube which contained 20 ml of R&S base and dispersion was accomplished by vortex mixer agitation for 10 min. Other procedures were the same as those described previously for reaction flasks.

Protein efficiency ratio (PER) determination

PER values were determined using methods described in the AOAC (1975).

Animal room was maintained at 25 ± 1°C with 12 hr light and 12 hr dark cycles. Food and water were provided ad libitum with

water changes on alternate days and food changes or additions as required. All glass feeders with feed followers were used. Moist diets were protected with 0.05% sodium benzoate and were changed daily except on weekends. A low protein control diet (6.4%) was used for instant potato test, a high fat control diet (468 cal/100g) was used for Isomil and Similac tests and a low calorie (250 cal/100g) and high fiber (6g/100g) control diet was used for the high moisture containing canned pork shoulder test thus maintaining isocaloric conditions without free liquid in the control diet. All other diets contained 8.9–10.4% protein and were compared with control diets containing 8.9–9.2% protein. The canned peas, precooked breaded fish, beef stew, frozen peas, chicken-a-la-king and vegetarian baked beans were freeze dried prior to evaluation, to concentrate the protein for diet preparation purposes.

Statistical analyses

Data were subjected to a completely randomized design analysis of variance with F tests carried out at the 0.01 level to determine the presence of differences. The data were then subjected to a modified multiple range test with 95% confidence limits as described by Woolf (1968).

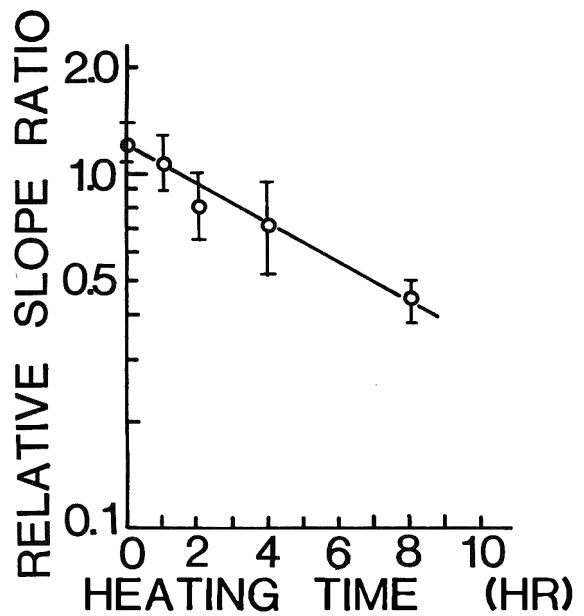

Fig. 1—Relative Slope Ratios determined for a series of frankfurter samples heated for various time periods at 105° C. Each point is the mean of 10 trials except for the 8-hr value which represents 4 trials. The extensions above and below each point represent the standard deviation.

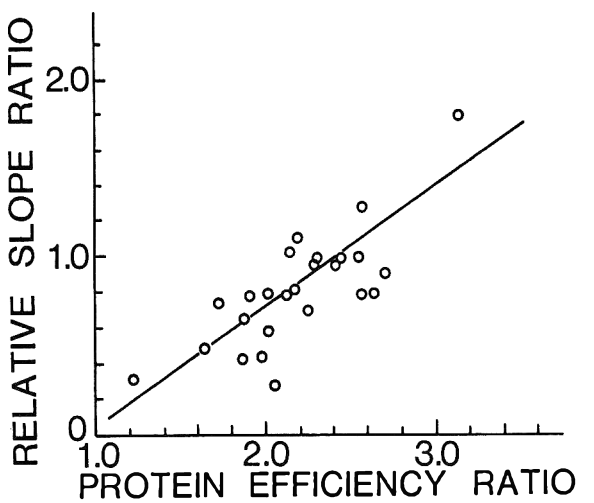

Fig. 2—Comparison of microbially determined Relative Slope Ratio with rat assay Protein Efficiency Ratio for a variety of food items.

RESULTS & DISCUSSION

Assay development

Storage of cultures at 4°C in CMM resulted in uniform recoveries of CFU's over a period from 1 to 25 days.

Cultures did not develop during 4-hr incubation periods in the absence of carbohydrate (unpublished data) or in the absence of amino acid or protein nitrogen (Blaschek and Solberg, 1978).

Culture development in R&S medium with individual amino acids removed, yielded reduced RSR values for all amino acids other then serine, glycine, isoleucine, aspartic acid, proline and hydroxyproline. Of the essential amino acids required by rats and humans, only isoleucine failed to give a significant depression in metabolic activity when omitted from the medium.

Alanine, tyrosine, glutamic acid and cystine, which are essentially ubiquitous in foods (FAO, 1970) are the nonessential amino acids required by the test organism. The addi-

Table 1—Comparison of relative slope ratio with protein efficiency ratio for a variety of foods

Product	Number of RSR replicates	RSR	Std. dev	PER
1. Hot dog	7	1.80	0.01	3.1
2. Hot dog dried	5	0.35	0.13	1.2
3. Oven dried dough	6	0.73	0.03	1.7
4. Flour	4	0.79	0.05	2.0
5. Soy flour	4	0.64	0.04	1.9
6. Texturized soy	4	1.10	0.09	2.2
7. Texturized vegetable protein + wheat flour - type 1	2	0.58	0.02[a]	2.0
8. Milk powder - type 1	3	1.00	0.00	2.3
9. Milk powder - type 2	2	0.81	0.20[a]	2.2
10. Rice + vegetable protein - type 1	1	0.26	–	2.1
11. Milk based powder - type 3	2	0.79	0.10[a]	2.1
12. Milk based powder - type 4	2	1.18	0.16[a]	2.4
13. Vegetable protein + rice	3	1.02	0.03	2.1
14. Vegetable protein - type 1	3	1.00	0.11	2.4
15. Vegetable protein flakes	5	0.96	0.04	2.3
16. Texturized vegetable protein + rice - type 1	2	0.69	0.03[a]	2.2
17. Texturized vegetable protein + rice - type 2	2	0.92	0.02[a]	2.7
18. Texturized vegetable protein + rice - type 3	4	0.79	0.02	2.6
19. Texturized vegetable protein + rice - type 4	1	0.78	–	2.6
20. Wheat gluten - type 1	1	0.48	–	1.7
21. Soy flour - type 1	3	0.78	0.01	1.9
22. Wheat gluten - type 2	1	0.43	–	2.0
23. Texturized vegetable protein + wheat flour - type 2	3	1.00	0.04	2.6
24. Soy flour - type 2	3	0.22	0.06	1.9
25. Texturized vegetable protein + rice - type 5	3	1.27	0.05	2.6
26. Cracker	1	1.75	–	2.7
27. Cookie	1	0.46	–	0.1
28. Cookie + Lactalbumin - type 1	1	0.37	–	0.6
29. Cookie + lactalbumin - type 2	1	0.52	–	0.7
30. Biscuit	1	0.31	–	0.0
31. Biscuit + milk protein - type 1	1	0.41	–	0.0
32. Biscuit + milk protein - type 2	1	0.46	–	0.6
33. Biscuit + milk protein - type 3	1	0.46	–	0.9
34. Full fat soy	2	1.21	0.03[a]	1.7
35. Triticale	6	1.74	0.08	1.5
36. Rice + vegetable protein - type 2	7	0.92	0.06	2.6
37. Vegetable protein - type 2	8	0.91	0.08	1.6
38. 85% soy + 15% egg	14	1.36	0.09	2.0
39. 75% soy + 25% egg	2	1.50	0.16[a]	2.6

[a] Number represents the range since only two samples were evaluated.

tion of the required "nonessential" amino acids to the base medium in the amounts present in R&S medium (Riha and Solberg, 1971) had no effect upon culture development in wheat gluten, two types of vegetable protein, triticale and two types of frankfurter systems and caused depressed culture development in a soy flour system, possibly due to the creation of a highly unbalanced nitrogen source with resulting toxicity.

Casein was inhibitory to culture development. The RSR value for casein was 0.31 with a standard deviation of 0.06. Supplementation of the casein with cystine and tryptophan improved culture development but not to the level of the defined medium. The RSR values for amino acid supplemented casein were, 0.51 (σ = 0.07) for cystine, 0.39 (σ = 0.06) for tryptophan and 0.58 (σ = 0.13) for cystine and tryptophan combined. Casein was reported as an inhibitor of *Bacillus stearothermophilus* by Ashton et al. (1968). The effect could be through the ability of casein to chelate metal ions in the medium or the inability of the microorganism to hydrolyze the casein into usable peptides or amino acids. Studies of these hypotheses are in progress.

Assay sensitivity to process variables

Heating of ground frankfurters in a convection oven at 105°C for 1, 2, 4 and 8 hr resulted in a gradual decrease in RSR values suggesting a change in protein quality. The results may be seen in Figure 1. It is reasonable to assume that the protein is involved in the altered growth response, although there is the possibility that an inhibitor could be formed. Heated protein type foods yield lysinoalanine (Sternberg et al., 1975). The removal of lysine, cystine and serine could inhibit the growth of *C. perfringens* 3624, which demonstrates a strong dependence upon both lysine and the sulfur containing amino acids.

Another processing sensitivity study compared a frankfurter-like product before and after drying. In this case the RSR values changed from 1.80 (σ = 0.10) to 0.35 (σ = 0.13). Comparison of these data with PER values showed a concomitant drop from 3.1 to 1.2. These results show that both the microorganism and the rat detected the detrimental effects of the drying process upon their respective growth responses.

A similarly comparable response was observed in soy flour converted to texturized soy. The process raised the PER from 1.9 to 2.2. The RSR values were raised from 0.64 (σ = 0.04) to 1.10 (σ = 0.09). The increase in apparent protein quality in soy flour may derive from a gentle heat treatment which inactivates inhibitors and through a heat induced unfolding or denaturing of protein molecules which renders them more available to microbial attack and utilization as suggested by Anglemier and Montgomery (1976), Liener (1977) and Cheftel (1977).

Food evaluations and comparison to Protein Efficiency Ratio

A variety of 25 food items (listed as items 1 through 25 in Table 1) were tested for their RSR values. These food items were assayed for PER for purposes other than the development of the RSR method. The ingredients of the products and the methods of manufacture were not available. The number of replicates in this study varied from as few as one in four cases to as many as seven in one case with most samples being run in duplicate or triplicate due to the small quantity of material available. Figure 2 shows the RSR values plotted against the PER values as determined by rat feeding studies. A regression analysis was carried out to determine the best fitting linear response. The equation for the straight line is Y = 0.65 X − 0.60. The linear coefficient of the curve presented is 0.74 and the standard error of the correlation coefficient is 0.02. While this correlation coefficient is statistically significant by F test at a probability of 0.01, it is not good enough for general use. It was believed that much of the problem in these preliminary studies may have been caused by insufficient replication of both the test materials and the R&S controls.

Table 2—Comparison of relative slope ratio (RSR) with protein efficiency ratio (PER) for a variety of foods using complete food preblending to provide comparable samples for both RSR and PER determinations

Group	Product	Number of RSR Replicates	RSR	Std. dev	PER
1	Frozen peas	8	2.05	0.12	1.1
	Canned beef stew	23	2.29	0.39	1.7
	Canned peas	15	0.67	0.06	0.7
	Vegetarian baked beans	7	0.85	0.11	0.7
	Special K breakfast cereal	15	0.36	0.07	<0.1
2	Potato granules	7	0.81	0.10	1.6
	Similac baby formula	7	0.88	0.16	1.5
	American cheese	6	1.43	0.31	2.3
	Morning Star breakfast strips	6	1.79	0.14	2.2
	Chicken a la king frozen	16	1.78	0.35	2.3
3	Isomil baby formula	7	0.33	0.05	1.6
	Powdered dry milk	8	0.45	0.07	1.9
	Oatmeal	12	0.49	0.05	1.8
	Precooked breaded fish frozen	8	1.17	0.07	2.5
4	Canned pork shoulder	8	0.51	0.04	2.7

The foods presented as items 26 through 33 in Table 1 were available in such small quantities that each sample could be evaluated only once. These foods were obtained from various sources where PER tests had been carried out and PER values were known. These data demonstrate that the RSR value is incapable of dealing with foods which have PER values less than 1.0 due to the base line effect of the RSR which is between 0.3–0.4. Items 34, 37, 38 and 39 in Table 1 were all soy products and fall above the range of the curve in Figure 2 but in order with respect to the PER values. Item 36 in Table 1 is a rice and vegetable protein mixture which fits the curve described in Figure 2, many of the points on which were derived from similar mixtures. Item 35 in Table 1 is a Triticale sample which the microorganisms found highly suitable but was much less so for the rats.

A second study was carried out using 15 different foods. These foods were purchased in quantities great enough for both rat studies and microbial studies. The foods were ground, mixed and blended so that each represented a uniform sample.

A comparison of the PER values with the RSR values for the 15 foods is presented in Table 2. The correlation coefficient for the comparisons is 0.77 with a standard error of 0.18 but the F test shows that there is no significance to this correlation value. The increased replication design did not yield a highly significant correlation between PER and RSR. It is clear that the wide variety of food was beyond the limits of the RSR method; therefore, the RSR method, as used in this study cannot be substituted directly for the PER test in the evaluation of protein quality. Figure 3, which is a comparison of the log RSR vs PER, indicates that the wide variety of foods tested may fall into approximately four groups. Three of the groups yield curves of similar slopes displaced from one another, while the fourth group consists of a single item. The foods in group 3 of Table 2 match up with the curve developed from the food

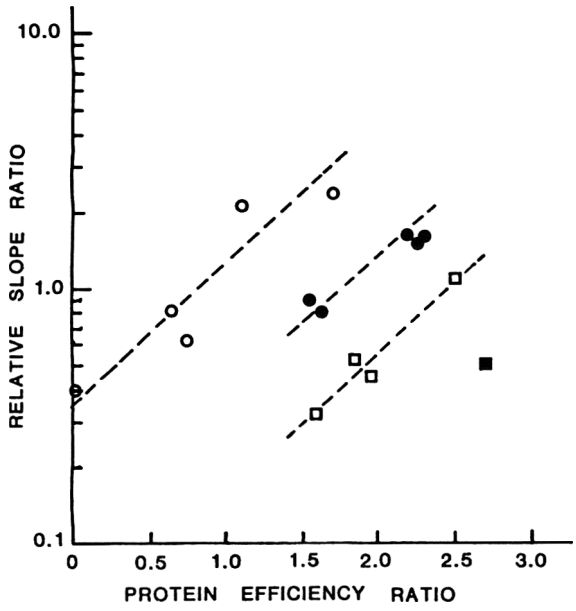

Fig. 3—Comparison of $\log_{10}$ Relative Slope Ratio to the Protein Efficiency Ratio for 15 food items preblended to assure sample equivalency: Group 1 (○---○); Group 2 (●---●); Group 3 (□---□); Group 4 (■).

products listed as 1 to 25 in Table 1. Although there are some relationships within the 4 groups, there are no completely clear distinctions.

Process variable assays with supplementation

The frozen peas (RSR-2.13) and the canned peas (RSR-0.63) seemed to indicate the effect of process variables, therefore additional studies were carried out. Frozen storage and autoclave heating of moistened freeze-dried peas for various time periods showed reductions in RSR values as may be seen in Table 3. The addition of 0.40 mg/ml cystine and 0.35 mg/ml methionine to the R&S base medium containing the various pea samples brought the stored and heated peas back to the original RSR value (α = 0.01). The addition of either lysine or trypthophan had no effect upon the RSR values. A similar set of results was observed when a soy-meat analog was subjected to dry heat at 105°C for periods up to 24 hr. The addition of 0.40 mg/ml cystine and 0.35 mg/ml methionine resulted in total RSR recovery in the sample heated for 12 hr but only partial recovery of those samples heated for 24 hr. These data are also presented in Table 3. The partial recovery may be attributable to losses other than the sulfur containing amino acids becoming significant due to the extreme stress placed upon the system.

Comparison of reaction vessels

A comparison of the test tube reaction vessel and the flask reaction vessel was carried out. The results using nine of the foods listed in Table 2 showed that the two methods were equivalent with a mean difference in Relative Slope Ratio of 0.07 units and a maximum difference of 0.13 units. These results permit the test to be carried out on much smaller samples (0.33g protein vs 2.44g protein) and require the preparation of a smaller quantity of inoculum (0.5 ml/tube vs 1.5 ml/flask).

Additional research required

Several additional problems were encountered during the studies. RSR values for both casein and egg are far below those which would be expected. This may be due to insufficient proteolytic activity by the selected *C. perfringens* strain, the presence of inhibitors, the chelation of minerals by the protein, or some other factors.

Value of the assay

The sensitivity of the RSR technique to storage, heating and dehydration alterations of protein coupled with the rapidity, simplicity, and low cost of the assay indicate the potential value of this new protein quality evaluation procedure.

Additional studies may provide means to overcome some of the weaknesses observed thus leading to a rapid, simple and inexpensive general assay for protein quality.

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Table 3—Relative Slope Ratios before and after the addition of amino acids for frozen peas subjected to freeze drying followed by frozen storage, moistening and heating and for a frozen analog bacon-like product subjected to frozen storage and heating

Product	Storage time (mos)	Heat treatment		Relative[a] Slope Ratio	Relative Slope Ratio after adding[b]		
		Temp (°C)	Time (hr)		CYS + MET	LYS	TRY
Peas	3	–	–	2.08 (0.13)a	–	–	–
	6	–	–	1.79 (0.10)b	–	–	–
	6	121	0.08	1.60 (0.21)bca'	2.01 (0.04)a'	–	–
	6	121	0.25	1.49 (0.12)cdb'	2.51 (0.45)c'	–	–
	6	121	0.50	1.45 (0.08)cdd'	2.18 (0.24)e'	1.54 (0.04)d'	1.36 (0.06)d'
	6	121	1.00	1.25 (0.29)d	–	–	–
Analog	3	–	–	1.79 (0.13)e	–	–	–
	6	–	–	1.71 (0.13)ef	–	–	–
	6	105	1	1.85 (0.15)e	–	–	–
	6	105	2	1.83 (0.16)e	–	–	–
	6	105	4	1.68 (0.12)ef	–	–	–
	6	105	8	1.57 (0.18)f	–	–	–
	6	105	12	–	1.67 (0.16)f'	1.05 (0.18)g'	1.19 (0.34)f'
	6	105	24	0.91 (0.07)gh'	1.25 (0.12)i'	0.78 (0.02)h'j'	0.68 (0.04)j'

[a] The RSR values are the means of at least six samples. The numbers in parentheses are the standard deviations of the means. Those values followed by the same letter are from sample means which are not significantly different at the 0.05 level.

[b] The RSR values are the means of three samples at 5 min and 15 min of heating, two samples at 30 min and 24 hr of heating and four samples at 12 hr of heating. The numbers in parentheses represent the range when only two samples were run and the standard deviation in all other cases. Those values followed by the same primed letter designation are not statistically different at the 0.05 level.

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EFFECT OF SOAKING TEMPERATURE ON BEANS . . . From page 1334

beans, and most certainly will have an adverse effect on the cooking time required for obtaining soft edible beans. Because of the extraction of about 50% of bean oligosaccharides under those soaking conditions, the resultant beans might be less flatulent.

It seems that the fastest processing time will be achieved when beans are soaked at 90°C; however, under those conditions some nutrients are lost and the total nutritional value of the beans might also be affected due to the possible effect on the availability of starch.

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Reference to a company and/ or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

DEGRADATION OF THE O-GLYCOSIDICALLY LINKED CARBOHYDRATE UNITS OF OVOMUCIN DURING EGG WHITE THINNING

AKIO KATO, KIMINORI OGINO, YUKIKO KURAMOTO and KUNIHIKO KOBAYASHI

ABSTRACT

β-Elimination reaction of the O-glycosidically linked carbohydrate units of ovomucin was followed by determining the increase of unsaturated amino acid and chromogen of glycopeptide I in alkaline solution. The Arrhenius plot for β-elimination reaction gave an activation energy of 23 kcal/mol. During egg white thinning the O-glycosidically linked carbohydrate units were gradually liberated from serine or threonine residues in ovomucin. It was proposed that the liberation of O-glycosidically linked carbohydrate units might cause the deterioration of ovomucin gel structure during storage of egg.

INTRODUCTION

THE THICK WHITE of chicken egg is known to be held by the swollen rigid gel structure mainly composed of ovomucin. As the fresh egg is stored at room temperature, the gel structure of thick white is destroyed gradually, that is, undergoes egg white thinning. It has been proposed during egg white thinning that either (1) ovomucin is depolymerized by the reduction or alkaline hydrolysis of disulfide bonds (MacDonnell et al., 1951; Donovan et al., 1972; Tominatsu and Donovan, 1972; Beveridge and Nakai, 1975), or (2) ovomucin-lysozyme interaction is responsible for the rigidity of the gel structure of thick white and the interaction decreases gradually (Cotterill and Winter, 1955; Brooks and Hale, 1961; Robinson and Monsey, 1972a). However, little is hitherto known about the chemical changes which ovomucin undergoes during egg white thinning. In addition, although ovomucin is glycoprotein, little is also known about changes in the properties of glycoprotein during egg white thinning.

In the previous papers (Kato et al., 1971; Kato and Sato, 1971, 1972), we showed that ovomucin consisted of the carbohydrate rich component (noted as the F-component) and the carbohydrate poor component (noted as the S-component). During egg white thinning the former component was gradually solubilized from ovomucin gel of thick white, while the latter component was not despite containing more disulfide bond than the former component (Kato et al., 1971; Kato and Sato, 1972). Robinson and Monsey (1971, 1972b) also obtained similar results on the behavior of ovomucin during egg white thinning, and they designated the carbohydrate poor component as α-ovomucin and the carbohydrate rich component as β-ovomucin. Thus, the macromolecular changes in ovomucin during egg white thinning have been elucidated. However, there is no elucidation on the reason why the specific solubilization of carbohydrate rich ovomucin occurs during egg white thinning.

It is well known that the pH of egg white increases to about 9.5 during storage. It has been suggested by some workers (Donovan et al., 1972; Tomimatsu and Donovan, 1972; Kato et al., 1972) that the increase of pH is the cause of egg white thinning. In alkaline solution, the protein usually tends to undergo some destruction of cystine, while the O-glycosidic glycoprotein β-elimination of O-glycosidically linked carbohydrate unit. Donovan et al. (1972) suggested that the alkaline hydrolysis of disulfide bonds in ovomucin could occur in alkaline solution. In the previous papers (Kato et al., 1978a, b), we suggested that the O-glycosidically linked carbohydrate units of ovomucin were liberated from serine or threonine residues by alkali treatment and that the structure of its main carbohydrate unit was *N*-acetylneuraminyl-(2→3)-galactosyl-(1→3)-N-acetylgalactoamine-6-sulfate. It would be interesting to see whether alkaline degradation of ovomucin occurs in fact or not in the mild alkaline conditions arising during storage of chicken eggs. This paper describes the natural degradation of ovomucin, especially β-elimination of O-glycosidically linked carbohydrate units, during egg white thinning.

MATERIALS & METHODS

Preparation of ovomucin

Ovomucin was prepared by the methods described in the previous paper (Kato et al., 1971). The fresh thick white was separated into gel and liquid parts by ultracentrifugation for 60 min at 59,000 × G. The gel part was washed directly with 2% KCl solution without diluting with water until the washing was free from protein. The gel-like precipitate was finally washed with water until the washing contained no chloride.

Preparation of glycopeptide I

Glycopeptide I was prepared by the method described in the previous paper (Kato et al., 1978b). Ovomucin was throughly digested with pronase and subsequently papain. The proteolytic digests were fractionated on a column (1.8 × 120 cm) of Bio-Gel P-100. The flow-through fraction was pooled, and purified on a column (1.4 × 17 cm) of DEAE-Sephadex A-25, with a linear gradient of 0.1–1.0M pyridine-acetate buffer, pH 5.0.

Alkali β-elimination of glycopeptide I

Glycopeptide I was treated with 0.1N NaOH at different temperature. Aliquots were taken at a given interval for determination of unsaturated amino acid and chromogen formed by β-elimination with alkali. The quantity of unsaturated amino acid was determined by the increase of absorbance at 241 nm (Nashef et al., 1977). The quantity of chromogen formed by the N-acetylgalactosamine eliminating from serine or threonine residues was determined by the method of Reissig et al. (1955). Five-tenth ml of sample was added to 0.2 ml of 5% borate solution, pH 8.9, and the mixtures were cooled at 0°C and then added to 3 ml of p-dimethylaminobenzaldehyde solution (10g/100 ml acetate containing 12.5% 10N HCl). After the mixtures were allowed to stand for 20 min at 36–38°C, the absorbance at 544 nm was determined.

Determination of chromogen in stored egg white and ovomucin solution

The O-glycosidically linked carbohydrate units liberated in stored egg white were collected and determined by the following method. Egg white was aseptically stored at 30°C for a given time, as described in the previous paper (Kato et al., 1971). Fifty ml of stored egg white was added to 50 ml of ethanol to precipitate the egg white proteins. The filtrate was dialyzed against 0.1M pyridine-acetate buffer, pH 6.0, for 3 days. The outer dialyzate was concentrated on evaporation. Taking into account the possibility that the reducing end N-acetylgalactosamine liberated from serine and threonine residues can not form chromogen in stored egg white, the quantity of chromogen was determined by the method of Ressig et

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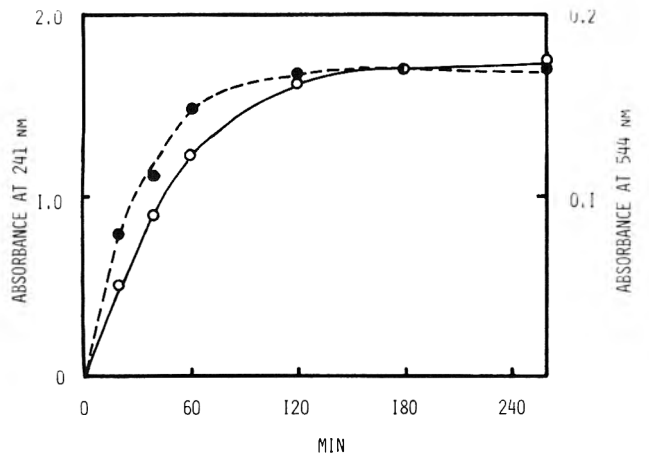

Fig. 1—Changes in absorbance at 241 nm and 544 nm of 0.1% glycopeptide I in 0.1N NaOH at 60°C: ○——○ 241 nm; ●---● 544 nm.

al. (1955) after causing the complete formation of chromogen in the concentrate. One ml of the concentrate was added to 0.2 ml of 5% borate solution, pH 8.9, and the mixture was heated at 100°C for 3 min. After cooling, the mixtures were added to 3ml p-dimethylaminobenzaldehyde solution (10g/100 ml acetate containing 12.5% 10N HCl). After the mixtures were allowed to stand for 20 min at 36–38°C, the absorbance at 544 nm was measured. Taking into account the other possibility that chromogen is formed from the original free N-acetylhexosamine in egg white, the amounts of chromogen produced during storage were expressed as values subtracting chromogen from the original free N-acetylhexosamine.

The O-glycosidically linked carbohydrate units in stored ovomucin solution were collected and determined by the following method: 0.2g ovomucin in 100 ml 0.05M carbonate buffer (pH 9.5–11.5) was stored at 30°C for a given time, with addition of 0.005M KCN to protect from microorganisms. Two ml of ovomucin solution was dialyzed against 0.1M pyridine-acetate buffer, pH 6.0, for 3 days. The outer dialyzate was concentrated on evaporation. The quantity of chromogen of the concentrate was determined in a similar method as above.

The complete β-elimination of egg white and ovomucin solution was carried out in 0.1N NaOH at 60°C for 2 hr to determine total amounts of O-glycosidically linked carbohydrate units. And then their chromogen was determined by the method stated above.

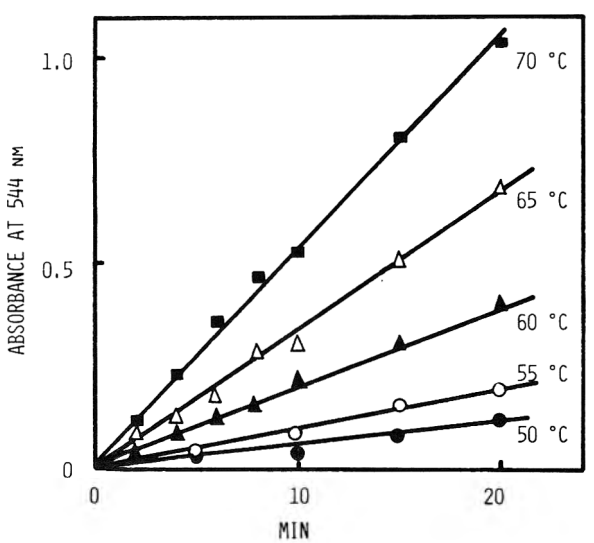

Fig. 3—Increase in absorbance at 544 nm as a function of temperature of 0.5% glycopeptide I in 0.1N NaOH.

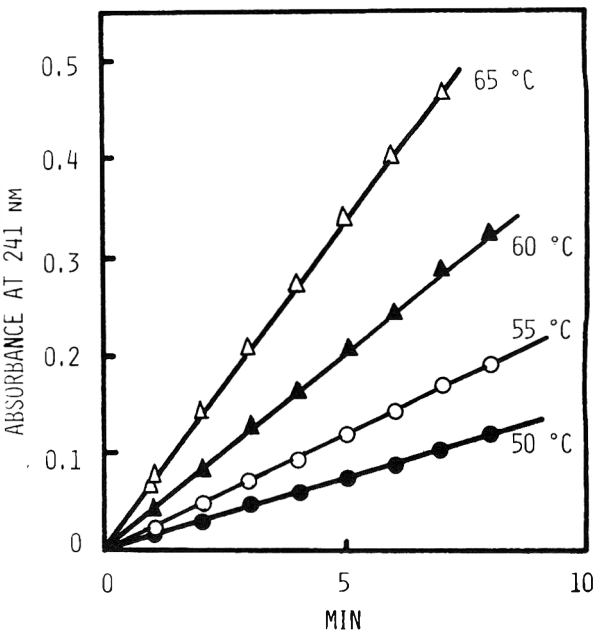

Fig. 2—Increase in absorbance at 241 nm as a function of temperature of 0.2% glycopeptide I in 0.1N NaOH.

Determination of SH- and SS-groups in stored egg white

All chicken eggs were collected within 24 hr after laying, and the shell was sterilized with 70% ethanol. The thick white was separated from the thin white and stored aseptically in the sterilized flasks at 30°C for a given time. Thoroughly blended egg white (1.5g) was diluted to 10 ml with Tris-glycine buffer (10.4g Tris, 6.9g glycine and 1.2g EDTA per liter, pH 8.0) containing 1% NaCl. The quantity of SH- and SS-groups in diluted egg white was determined by the method of Beveridge et al. (1974).

RESULTS & DISCUSSION

Alkali β-elimination reaction of glycopeptide I

As shown in the previous paper (Kato et al., 1978b), all of the O-glycosidically linked carbohydrate units of ovomucin are contained in glycopeptide I and no disulfide bonds are contained in glycopeptide I. Therefore, the glycopep-

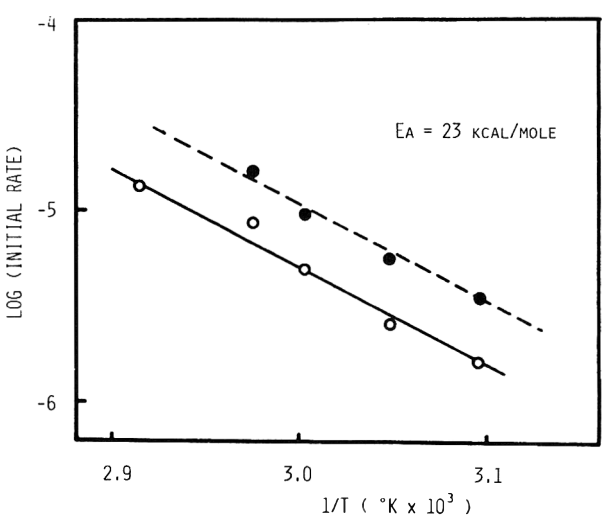

Fig. 4—Arrhenius plot for initial rates of β-elimination of glycopeptide I. Rates were calculated from Fig. 2 and 3: ●---● from Fig. 2; ○——○ from Fig. 3.

tide I was chosen as a model for the β-elimination of the O-glycosidically linked carbohydrate units of ovomucin in alkaline solution. The isolation and identification of β-elimination reaction products of glycopeptide I were already reported in the previous paper (Kato et al., 1978a, b), and the N-acetylgalactosamine at the reducing end of carbohydrate units was shown to be linked to serine and threonine residues of ovomucin. When 0.1% glycopeptide I was treated with 0.1N NaOH at 60°C for 4 hr, an increase of the absorbance at 241 nm was observed (Fig. 1). Its increase is known to be caused by producing the unsaturated amino acid residues from O-substituted serine and threonine by β-elimination with alkali (Gottschalk, 1972). On the other hand, the N-acetylgalactosamine at the reducing end of the O-glycosidically linked carbohydrate units liberated from serine and threonine residues is also known to result in the formation of chromogen (3-acetoamido-5-dihydroxyethylfuran) by its rapid degradation in alkaline solution (Kochetkov et al., 1970). Since chromogen gives the Ehlich reaction, its formation was followed by determining the absorbance at 544 nm after the Ehlich reaction (Reissig et al., 1955). When glycopeptide I was treated with 0.1N NaOH at 60°C for 4 hr, the absorbance at 544 nm increased correspondingly at a rate depending on an increase of the absorbance at 241 nm (Fig. 1). This result indicates that the alkali β-elimination reaction of ovomucin can be followed by determining not only the increase of unsaturated amino acid but also the increase of chromogen. As shown in Figure 2 and 3, the rate of the β-elimination of glycopeptide I was a function of temperature. The Arrhenius plot for the β-elimination reaction of glycopeptide I gave an activation energy of 23 kcal/mol (Fig. 4), by calculation from either Figure 2 or 3. This value is almost similar to that for β-elimination of disulfide (Nashef et al., 1977). Lee et al. (1977) showed that an activation energy for β-elimination reaction of the O-glycosidically linked carbohydrate units in antifreeze glycoprotein was 9.6 kcal/mol. The structure of the O-glycosidically linked carbohydrate unit of antifreeze glycoprotein (Vandenheede et al., 1972) is galactosyl-(1→3)-N-acetylgalactosamine, whereas that of ovomucin (Kato et al., 1978a) is N-acetylneuraminyl-(2→3)-galactosyl-(1→3)-N-acetylgalactosamine-6-sulfate. It seems likely that the difference in the structure of O-glycosidically linked carbohydrate unit reflects that of activation energy for β-elimination.

Vandenheede et al. (1972) suggested that absorbance at 241 nm was interfered by chromogen formation during β-elimination reaction. In addition, the unsaturated amino acid is known to be reactive with internal protein nucleophilic groups and external nucleophiles. Therefore, the increase of chromogen was followed to evaluate the β-elimination reaction of ovomucin during egg white thinning.

β-Elimination of O-glycosidically linked carbohydrate units of egg white protein during the thinning

The O-glycosidically linked carbohydrate units of ovomucin are contained in F-ovomucin (β-ovomucin) which plays an important role in holding the swollen rigid gel structure of ovomucin fiber (Kato et al., 1971). Therefore, it is very significant to check whether the carbohydrate units, sensitive to mild alkali, are liberated from serine and threonine residues in ovomucin or not during storage of egg. Thus, the extent of the β-elimination of the O-glycosidically linked carbohydrate units was evaluated. Egg white was stored at 30°C and its pH was 9.5 after 2 days of storage. Figure 5 shows the gradual increase in β-elimination in stored egg white. The extent of the β-elimination in stored egg white is shown as a percentage of the total amounts of the O-glycosidically linked carbohydrate units in egg white. This result seems to be caused by β-elimination of the O-glycosidically linked carbohydrate units of ovomucin, because most of the O-glycosidic glycoproteins in egg white are ovomucin. About 12% of the β-elimination of O-glycosidically linked carbohydrate units occurred in egg white after 30 days of storage.

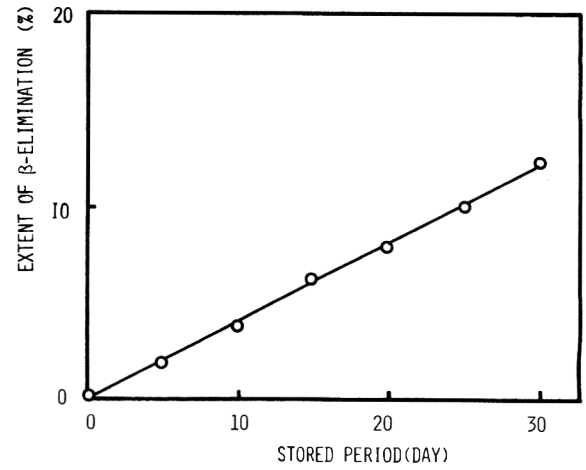

Fig. 5—Increase in β-elimination in stored egg white at 30°C.

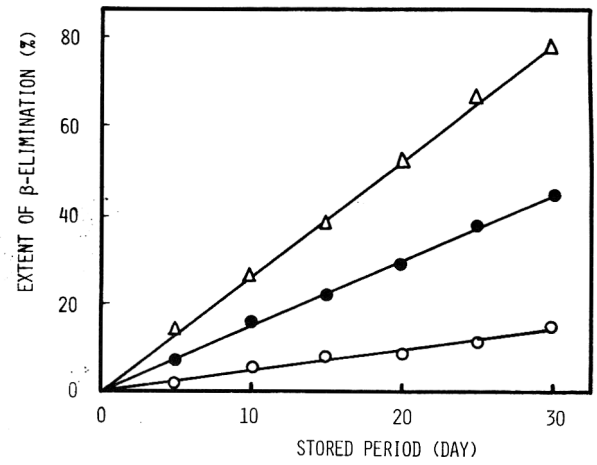

Fig. 6—Increase in β-elimination in ovomucin solution at various pH: ○—○ pH 9.5; ●—● pH 10.5; △—△ pH 11.5.

β-Elimination of O-glycosidically linked carbohydrate units of ovomucin in mild alkaine solution

The β-elimination of the O-glycosidically linked carbohydrate units of ovomucin during egg white thinning was also confirmed by storing ovomucin in mild alkaline solution, near the pH value found in stored egg white. Figure 6 shows the pH dependence of β-elimination in ovomucin solution. The extent of β-elimination of ovomucin is shown as a percentage of the total β-elimination of O-glycosidically linked carbohydrate units of ovomucin. This result indicates that the gradual β-elimination of the O-glycosidically linked carbohydrate units occurs in stored ovomucin solution, even at pH 9.5, which is near the pH in stored egg white. About 10% of the total β-elimination was shown to occur in ovomucin solution stored in pH 9.5 at 30°C for 30 days.

Thus, it was confirmed that the O-glycosidically linked carbohydrate units of ovomucin were gradually liberated by alkali β-elimination reaction during egg white thinning. Glycoproteins with O-glycosidic linkages are contained in common in highly viscous epithelial glands. It was suggested by Gottschalk and Thomas (1961) that N-acetylneuraminic acid in the O-glycosidically linked carbohydrate units might play an important role on viscous properties of mucin in

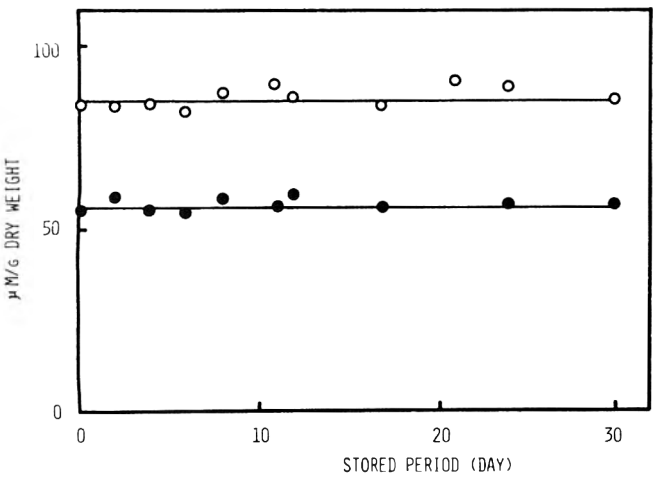

Fig. 7—Changes in SH- and SS-groups in stored egg white at 30°C: ○—○ SS-group; ●—● SH-group.

epithelial glands. In addition, N-acetylneuraminic acid is known to interact with lysozyme (Robinson and Monsey, 1972a; Kato et al., 1975) and its interaction may be responsible for the rigidity of gel structure of egg white. Therefore, the gradual liberation of the O-glycosidically linked carbohydrate units of ovomucin may possibly affect partially the gel structure of egg white.

Changes in SH- and SS-groups in stored egg white

Despite the similar activation energy for β-elimination reaction of O-glycosidically linked carbohydrate units, the degradation of disulfide bonds was not observed in stored egg white, as shown in Figure 7. This result suggests that the hydrolysis of disulfide bonds of ovomucin do not also occur in stored egg white. However, since ovomucin is only a few percent of egg white proteins and contains only 3% cystine, the hydrolysis of disulfide bonds in ovomucin might not be detectable under these experimental conditions. The experiment should be carried out with purified ovomucin, but it is diffiuclt to follow exactly the changes in SH- and SS-groups during storage, because of the insolubility of ovomucin. Even if this is taken into consideration, the observation that there were no significant changes during storage of egg white for a month is suggestive of the possibility that the hydrolysis of disulfide bonds of ovomucin do not occur in stored egg white. The question has been raised whether the reduction or alkaline hydrolysis of disulfide bonds in ovomucin is responsible for egg white thinning.

We proposed previously (Kato et al., 1971; Kato and Sato, 1972) on a model of egg white thinning. According to the model, the swollen rigid gel structure of ovomucin which is supported both by the S-ovomucin (α-ovomucin) and F-ovomucin (β-ovomucin) changes into the shrunken gel structure with the dissociation of F-ovomucin during storage of egg. The O-glycosidically linked carbohydrate units of ovomucin are contained in F-ovomucin which plays an important role in holding the swollen rigid gel structure of ovomucin fiber (Kato and Sato, 1972). The gradual liberation of O-glycosidically linked carbohydrate units of ovomucin may affect the gel structure of ovomucin to cause the dissociation of F-ovomucin during egg white thinning.

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CHROMATOGRAPHY AND ELECTROPHORESIS OF NATIVE AND SPRAY-DRIED EGG WHITE

R. D. GALYEAN and O. J. COTTERILL

ABSTRACT

Effects of the spray-drying process on egg white proteins were studied by diethylaminoethyl cellulose ion-exchange chromatography and polyacrylamide gel electrophoresis. Major chromatographic peaks of native egg white were characterized by electrophoresis. Chromatograms and electrophoretographs of native egg white were compared to those of egg white which was adjusted to various pH levels, then spray-dried. Changes in spray-dried egg white protein patterns were minor, even at pH levels where conalbumin is heat sensitive. Egg white globulin proteins appear most sensitive to the spray-drying process. Large scale denaturation of egg white protein does not occur during spray-drying, but may initiate changes in the protein which affect the functional properties of rehydrated products. Globulin proteins require further characterization in order to properly describe their electrophoretic and chromatographic behavior.

INTRODUCTION

Changes in functional properties of egg albumen due to processing treatments are a major concern of the egg product manufacturing industry. In order to minimize problems caused by processing, a great number of experiments have been conducted on the alteration of egg white due to various treatments. Baldwin et al. (1967), Zabik and Brown (1969), Hill et al. (1965), and others, using functional property testing methods, concluded that one or more egg proteins were altered during processing. Fractional denaturation of albumen proteins has been hypothesized as the major cause of changes in egg white functional properties. Other workers utilized chemical and physical properties of albumen proteins to evaluate changes in egg white. Evolution of ammonia and sulfhydryl activity of albumen (Cotterill et al., 1967), ion-binding properties of conalbumin (Seideman et al., 1963; Cunningham and Lineweaver, 1965), and viscosimetric data (Payawal et al., 1946) were used to measure changes in protein, usually in verification of changes observed in functional properties.

Some investigators have related heat-induced coagulation of egg albumen to alteration of specific egg white proteins. Heat sensitivity of conalbumin at neutral pH and lysozyme at high pH was demonstrated by paper electrophoresis (Seideman et al., 1963). Pasteurization damage to ovomacroglobulin was shown by Chang et al. (1970), and Cunningham (1974). Additionally, Cunningham and Lineweaver (1967) noted the formation of an insoluble lysozyme-ovalbumin complex when egg white was heated to 60°C at pH 9.0. A decrease in the size of chromatographic patterns of ovalbumin was shown by Meehan et al. (1962) to parrallel the decreased angel cake performance of albumen obtained from stored eggs.

Chromatographic methods have also been utilized in other studies of eggs and egg products. Cunningham and Cotterill (1971) observed changes in chromatographic patterns due to yolk contamination of egg white. Seideman and Cotterill (1969) and Seideman et al. (1969) successfully fractionated natural and processed egg yolk on carboxymethyl (CM) cellulose. Parkinson (1967; *1968a, b*; 1970) utilized CM cellulose and diethylaminoethyl (DEAE) cellulose chromatography to study the effects of pasteurization on whole egg. McBee (1974) used DEAE and CM cellulose chromatography to separate and identify yolk fractions.

This study was initiated to investigate changes in egg white caused by processing. Specific objectives were to investigate the DEAE ion-exchange chromatograhpic and disc gel electrophoretic properties of untreated native egg white, and compare those properties to the corresponding chromatographic and electrophoretic properties of spray-dried egg white.

MATERIALS & METHODS

Egg albumen

Native egg white (NEW) was prepared from unwashed, refrigerated, 1- or 2-day old eggs produced by Single-Comb White Leghorns. Care was taken to assure that this egg white was yolk-free. Where indicated, desugared egg white pH was adjusted with 1M NaOH or 1M HCl to pH 4.5, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. Liquid egg product was stirred constantly with a magnetic stirrer during all pH adjustments. Before electrophoresis or chromatography, NEW was dialyzed at 4°C against distilled water for 16 hr; albumen was measured into cellulosic tubing, placed in a dialysis chamber and stirred constantly until completion of the procedure.

Desugarization of albumen

Naturally-occurring reducing sugars were removed from egg albumen before spray-drying. Desugarization was accomplished by bacterial fermentation with an inoculum of *Enterobacter aerogenes*. Fermentation was conducted at 36.7°C and was allowed to continue until the egg white gave a negative reaction to glucose-sensitive test paper. After completion of fermentation, precipitated ovomucin was removed by straining egg white through two layers of cheesecloth. Fermented albumen was stored at 4°C until required for further treatment.

Spray-drying

Dehydration of egg white was performed in a pilot plant spray-dryer equipped with a pneumatic atomization system. Liquid egg white was spray-dried with an inlet temperature regulated at 137°C and exhaust temperature 65–68°C, at a rate of 250 ml/min. The spray-dried egg white (SDEW) was collected and sealed in glass containers for permanent sample storage at 12°C until required for analysis. Dehydrated egg product was rehydrated by transferring 6.50g of powder to 25 ml of distilled water which was continuously stirred by a magnetic stirrer. The albumen was further stirred constantly for 15 min. Any unrehydrated egg white adhering to the sides of the container was washed back into the solution with an additional 25 ml of distilled water. After completion of the rehydration period, egg white was immediately centrifuged at 1500 × G for 20 min to remove insoluble material, and then dialyzed exhaustively against distilled water. An estimated 25–30 mg of sample protein was placed on DEAE cellulose, and 200 μg of protein was used for each electrophoresis gel.

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Protein analysis

Protein was determined by the micro-Kjeldahl procedure (AOAC, 1970). Where indicated, other protein determinations were performed by Biuret (Henry, 1968).

Chromatography

Ion-exchange chromatography was performed by a modification of Mandeles' (1960) procedure. DEAE cellulose, (0.75 meq/g exchange capacity) was washed with 1000 ml each of the following reagents: 0.1M HCl–0.1M NaCl, distilled water, 1M $NaHCO_3$, distilled water, 1M Na_2CO_3, distilled water, 0.1M NaOH, distilled water, ethanol (95%), and distilled water. After the last wash, the DEAE cellulose was mixed with distilled water and allowed to settle overnight. Any unsettled particles were then removed by aspiration. Excess water was removed by filtration, and the DEAE cellulose suspended in 0.1M NaOH. Washed DEAE cellulose was packed in a 2.0 × 30.0 cm column (Glenco Scientific, Inc., 2802 White Oak Drive, Houston, TX). The column was packed under a 2.0 ml/min flow rate of 0.1M NaOH. After packing, the DEAE cellulose was washed with 500 ml of distilled water, then immediately equilibrated with 0.02M glycine pumped at a flow rate of 2.0 ml/min.

Chromatographic separation of egg white protein was initiated with a continuous elution gradient at a flow rate of 2 ml/min. The second buffer in the reservoir was allowed to flow into the closed mixing flask of starting buffer at the same rate as the starting buffer emptied. When the reservoir volume was depleted, it was refilled with succeeding buffers. The elution schedule used in chromatography was as follows:

A. Starting buffer (pH 5.8) – 175 ml; 0.02M glycine.
B. Second buffer (pH 6.8) – 200 ml; 0.02M glycine, 0.02M K_2HPO_4, 0.02M KH_2PO_4.
C. Third buffer (pH 4.4) – 200 ml; 0.02M glycine, 0.10M KH_2PO_4, 0.10M NaCl.
D. Fourth buffer (pH 2.5) – 200 ml; 0.02M glycine, 0.10M KH_2PO_4, 0.10M NaCl, 0.03M HCl.

DEAE cellulose was removed from the column after each chromatographic separation, and regenerated by suspension in 500 ml of 0.1M NaOH. The suspension was constantly stirred for 5 min, the DEAE allowed to settle, and the supernatant removed by aspiration This procedure was repeated three times. The DEAE cellulose was then resuspended in 0.1M NaOH and repacked into the chromatographic column.

Flow rate of chromatographic separation was controlled with an ISCO Model 312 syringe type metering pump (Instrument Specialties Co., 4700 Superior, Lincoln, NE). Chromatographic elution of protein was monitored at 280 nm with a dual beam ISCO Model UA-2 ultraviolet analyzer so that elution buffers were monitored before and after flowing through the column. Absorbance signals were recorded on a strip chart recorder. Chromatographic fractions were obtained by collection of 20 ml increments of eluant with an ISCO Model A fraction collector. Each fraction was dialyzed, freeze-dried, then rehydrated to the appropriate concentration for analyses.

Electrophoresis

Electrophoresis was conducted on polyacrylamide gels using a 12-tube Canalco Model 1200 unit (Canalco, 5635 Fisher Lane, Rockville, MD). The procedure utilized a discontinuous three-gel system consisting of: (1) sample gel (2.5% polyacrylamide in pH 8.9 Tris-HCl buffer); (2) stacking gel (2.5% polyacrylamide in pH 8.9 Tris-HCl buffer); and (3) separating gel (7% polyacrylamide in pH 9.5 Tris-HCl buffer). Preliminary experiments indicated optimal sample size was 200 μg protein. Electrophoresis of protein was accomplished by applying a constant current for four milliamps per gel for a period of 30–45 min. Bromphenol blue tracking dye (0.005%) was used to monitor the progress of protein separation. After completion of electrophoresis, gels were placed in 12% trichloroacetic acid for 30 min, rinsed with distilled water, then immersed 1.5 hr in stain solution (0.025% Coomassie Blue in 7% acetic acid). The gels were destained overnight in 7% acetic acid.

RESULTS & DISCUSSION

Chromatographic analysis of NEW

Preliminary results indicated that Mandeles' (1960) chromatographic methodology could be abbreviated with little loss in resolution by decreasing sample size and eluant volumes, thus shortening the time required for completion. Flow rate of the elution buffers remained the same. A typical chromatogram is shown in Figure 1 with the ionic strength and pH Profiles of eluting buffers.

Electrophoretic analysis of NEW and chromatographic fractions

Figure 2 shows the electrophoretic separation of NEW. Individual protein bands were examined for electrophoretic mobility and compared to the mobility of egg white proteins previously reported in the literature (Chang et al., 1970; Cunningham, 1974; Longsworth et al., 1940). Two bands of conalbumin (CON), 3 bands of globulin (G_2), 5 bands of globulin (G_3), 3 bands of ovalbumin (A_1, A_2, A_3), and 1 band of ovomacroglobulin (OMG) and flavoprotein (F) were observed. Globulin proteins of egg white are not well characterized, but were labeled according to available literature. The G_3 globulins were consistent enough in electrophoretic behavior to merit further distinction, and were therefore subscripted with letters A, B and S. The globulin subscript lettering system was a modification of the heterozygous globulin protein nomenclature of Baker and Manwell (1962).

Ten discernible chromatographic peaks were selected for further investigation by electrophoresis. Peak A was identified as lysozyme (globulin G_1) by its enzymatic activity and its anodic electrophoretic migration. Peaks B and C remain unidentified. Although cathodic and anodic electrophoresis were performed on these peaks, no protein stain was observed on the electrophoretic gels. Lysozyme activity determinations on these peaks indicated no apparent enzyme activity present. A previous report (Cunningham and Cotterill, 1971), utilizing Mandeles' (1960) technique, indicated that the equivalent peaks B and C contained lysozyme activity, and this dissimilarity may be a genetically-determined variant of egg white lysozyme. Electrophoretograms of the remaining fractions are shown in Figure 2. Peak D was composed mainly of the conalbumin heterozygote forms and globulins G_2 and G_{3A}. The major portion of conalbumin was found in fractions 14–16, but was present in small concentrations through fraction 24. Peaks E and F (fractions 15 and 16) contained combinations of conalbumin, globulin G_2 and globulin G_{3A}. Globulin G_{3A} was completely eluted in fractions 14, 15 and 16. The major components of peaks G (fraction 18), H (fraction 21) and I (fractions 23, 24 and 25) were identified as ovalbumins A_1, A_2 and A_3. Globulin G_{3B} and ovomacroglobulin were also components of peaks G and H. The three ovalbumins were relatively well separated during chromatography. Fraction 23 contained ovalbumins A_2 and A_3; fraction 24 contained A_1 and A_2 with traces of A_3; and fraction 25 was almost entirely A_1 with bands of globulin G_{3S}. Peak J was identified as a riboflavin-containing flavoprotein and its conjugate apoprotein. The identification was based on the yellow appearance of the fraction as it was eluted from the column, and its high electrophoretic mobility.

Generally, the identification of chromatographic peaks in this study confirm the earlier observations of Mandeles (1960). Even though they contained a major protein, most peaks were contaminated with other proteins. However, lysozyme contained no contaminants and was considered a pure protein fraction after one chromatographic separation.

The ion-exchange separation of egg white proteins was related to protein isoelectric point. Ovalbumin, with an isoelectric point of 4.5, was chromatographically separated with ovomacroglobulin, with an isoelectric point estimated at 4.5–4.7. However, the G_{3B} and G_{3S} globulins were also eluted with ovalbumin. The high isoelectric point reported for G_3 globulins (pH 5.8) by Baker (1968) would suggest that chromatographic elution should be closer to that of

conalbumin. In this study, the combined electrophoretic and chromatographic evidence showed that G_{3B} and G_{3S} globulins may be more acidic in nature than reported by Baker. Egg white globulin proteins are not well characterized, and this study indicates that further research in globulin characterization is required to properly describe their chromatographic and electrophoretic behavior.

Chromatography of SDEW

Results of chromatographic separation of egg white spray-dried at different pH levels are shown in Figure 3. A chromatogram of NEW is included for comparison. Generally, the chromatograms of SDEW were less resolved than those of NEW. The peaks were also eluted at lower pH and higher ionic strength. Except for egg white dried at pH 4.5, the spray-drying process did not disrupt the overall integrity of the chromatograms of egg white proteins. It is concluded, therefore, that the spray-drying process at pH levels other than 4.5 is a relatively gentle process with respect to the egg white protein ion-exchange characteristics. These results are in agreement with Bergquists's (1978) conclusions that egg white proteins are protected by the cooling effects of evaporating water when atomized droplets of egg white are exposed to a stream of heated air. The protective mechanisms are highly effective as judged by the conalbumin fraction. Conalbumin is very sensitive to heat treatment at pH 7.0 (Seideman et al., 1963). Yet when spray-dried at this pH, no major difference was observed between egg white conalbumin (Peak D) and that of egg white dried at other pH levels. The protein also appeared relatively stable to spray-drying at pH 5.0 and 6.0. These results agree with the conclusions of Hill et al. (1965), who reported no damage to ovalbumin, conalbumin, or lysozyme in egg white spray-dried below pH 6.0.

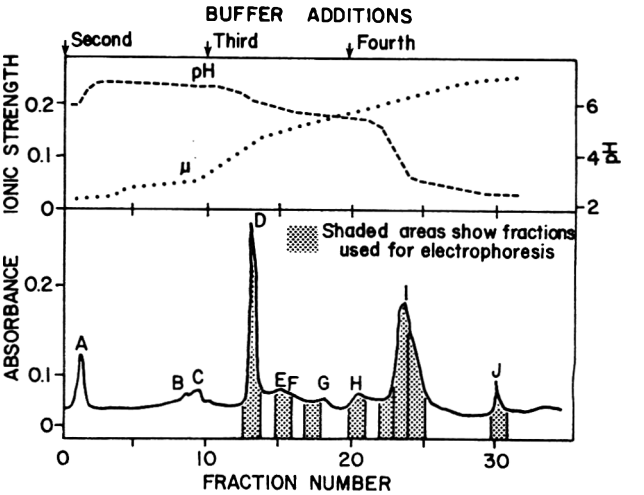

Fig. 1—Chromatographic separation of native egg white on DEAE cellulose. Ionic strength and pH conditions leading to elution of proteins are as indicated. Identification of major protein contained in peaks: A = lysozyme; B, C = unidentified; D = conalbumin; E, F = conalbumin and globulins; G, H, I = ovalbumins; and J = flavoprotein. Fraction size = 20 ml, flow rate = 2.0 ml/min.

It is probable that there is some generalized protein damage during the spray-drying process. Peak sharpness was de-

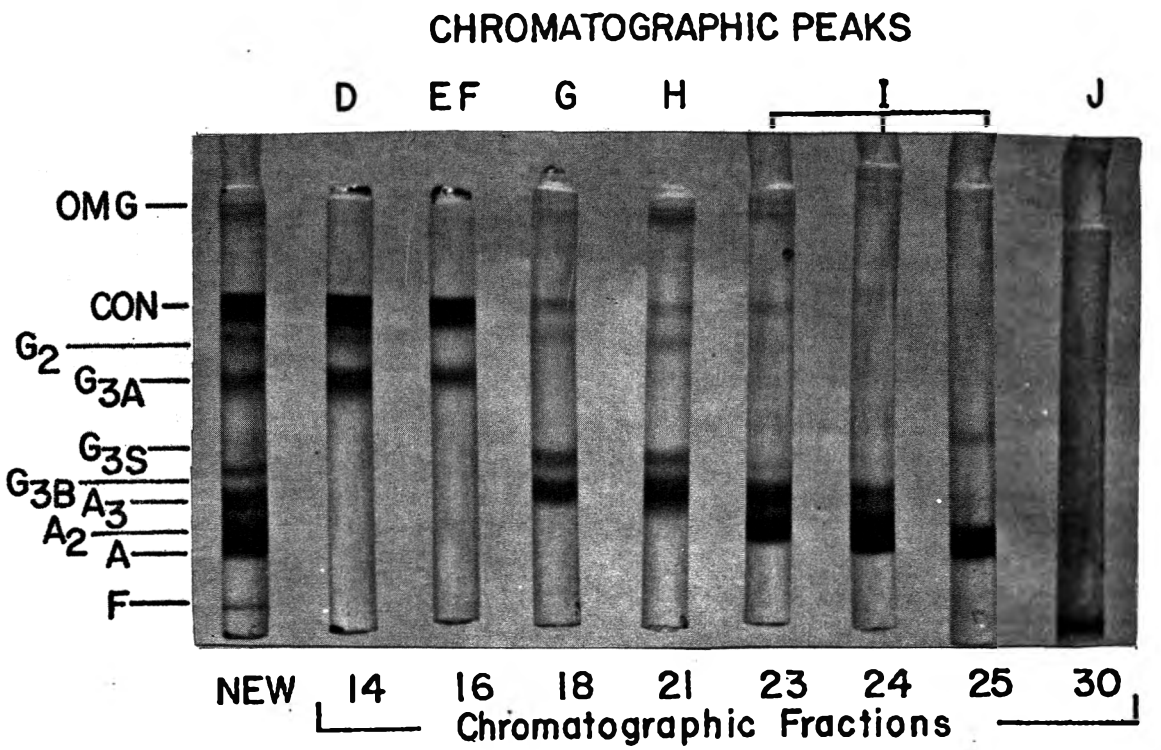

Fig. 2—Electrophoretograph of native egg white chromatographic fractions. See Figure 1 for protein peak identification. Abbreviations as follows: OMG = ovomacroglobulin; Con = conalbumin = G_2, G_{3A}, G_{3B}, G_{3S} = globulins; A_1, A_2, A_3 = ovalbumins; F = flavoprotein. Identification of fractions: Fraction 14 = peak D (conalbumin and globulin G_2); Fraction 16 = peak E, F (conalbumin and globulin G_2); Fraction 18 = peak G (globulin G_{3B}, ovalbumin A_3); Fraction 21 = peak H (globulin G_{3B}, ovalbumin A_3); Fraction 23 = peak I (ovalbumin A_3, A_2); Fraction 24 = peak I (ovalbumin A_3, A_2, A_1); Fraction 25 = peak I (ovalbumin A_1, globulin G_{3S}); Fraction 30 = peak J (flavoprotein).

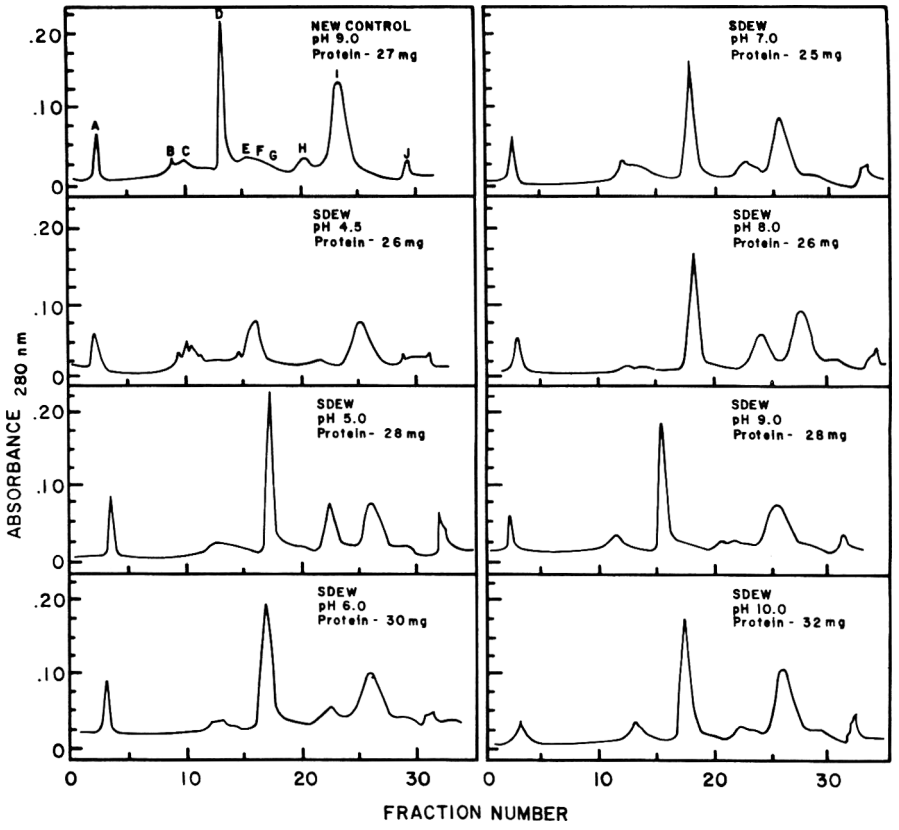

Fig. 3—Chromatographic separations of native egg white and pH-treated spray-dried egg white on DEAE cellulose. See Figure 1 for definition of letter designations on native egg white chromatograph. Fraction size = 20 ml, flow rate = 2.0 ml/min.

creased in comparison to NEW in all spray-dried samples. For example, as shown in Figure 3, conalbumin (Peak D) was eluted at fraction 14 in the native product and in fraction 16–19 in SDEW. Similarly, the main ovalbumin peak (Peak I) was recorded at fraction 23–25 in NEW, but at fraction 25–30 in the spray-dried product. In an ion-exchange gradient elution scheme, these results suggest slight changes in the ionic character of the egg white proteins which lead to chromatographic peak broadening and a decrease in the resolution of the protein chromatograms. Slight alterations in the secondary or tertiary structure of proteins could account for this result. A change in ovalbumin (Peak I) was also evident in SDEW due to the consistent appearance of a shoulder on the trailing side of Peak I. This trailing peak was never in evidence in chromatograms of NEW.

Since ovalbumin is susceptible to surface denaturation (Bull and Neurath, 1937), it is concluded that ovalbumin is partially denatured by exposure to new surface areas during the atomization of spray-drying. The data suggest that spray-drying of egg white results in slight alterations without simultaneously causing large-scale denaturation of the egg white protein. Hill et al. (1965) observed that conalbumin and lysozyme are more sensitive to heat treatment in rehydrated egg white which was spray-dried below pH 6.0. The results in this study indicate that small changes in the SDEW at pH levels below 7.0 may initiate changes in protein structure which cause higher heat sensitivity of the protein subsequent to rehydration.

Peaks E, F and G, the globulin portion of egg white, were the only constituents drastically reduced in the SDEW chromatograms. In most chromatograms of the SDEW, these peaks were absent. It is concluded that the globulin proteins were denatured during the spray-drying process and were insoluble in the rehydration of the spray-dried product. The damage to globulin proteins at all pH values is probably due to surface denaturation by foaming (MacDonnell et al., 1955). Since egg white is exposed to shear forces during the atomization of the liquid (Bergquist and Stewart, 1952), the shear forces are likely to cause mechanical denaturation of globulins during spray-drying.

Electrophoretic analysis of SDEW

The electrophoretic separations of experimental SDEW are compared to NEW in Figure 4. Many protein bands of each SDEW sample were diffused, particularly the globulin bands. Egg white dried at pH 4.5 and pH 7.0 showed the most change. Samples spray-dried at pH 5.0 and pH 6.0 most closely duplicated the electrophoretic characteristics of the NEW control.

The electrophoretic results generally indicate that conalbumin, ovalbumin and flavoprotein were not seriously damaged by the drying process. However, conalbumin bands were slightly less intense at pH 7.0. Spray-drying damage was apparent in the globulin fractions. The globulin proteins most affected by spray-drying treatment were globulins G_{3A} and G_{3S}. Globulin G_{3A} appeared normal only in egg white spray-dried at pH 5.0. Globulin G_{3S} appeared normal only in egg white samples spray-dried at pH 5.0 and pH 6.0.

The results of electrophoretic analysis of egg white adjusted to various pH levels before spray-drying tend to confirm observations made during chromatographic analysis. Conalbumin was not visibly altered to any great extent in electrophoretic properties. Globulin proteins were diffused or absent in electrophoretic patterns. Except for globulins, large scale denaturation of egg white protein does not occur, and the disruption of globulin proteins may be related to impaired functional properties caused by spray-drying.

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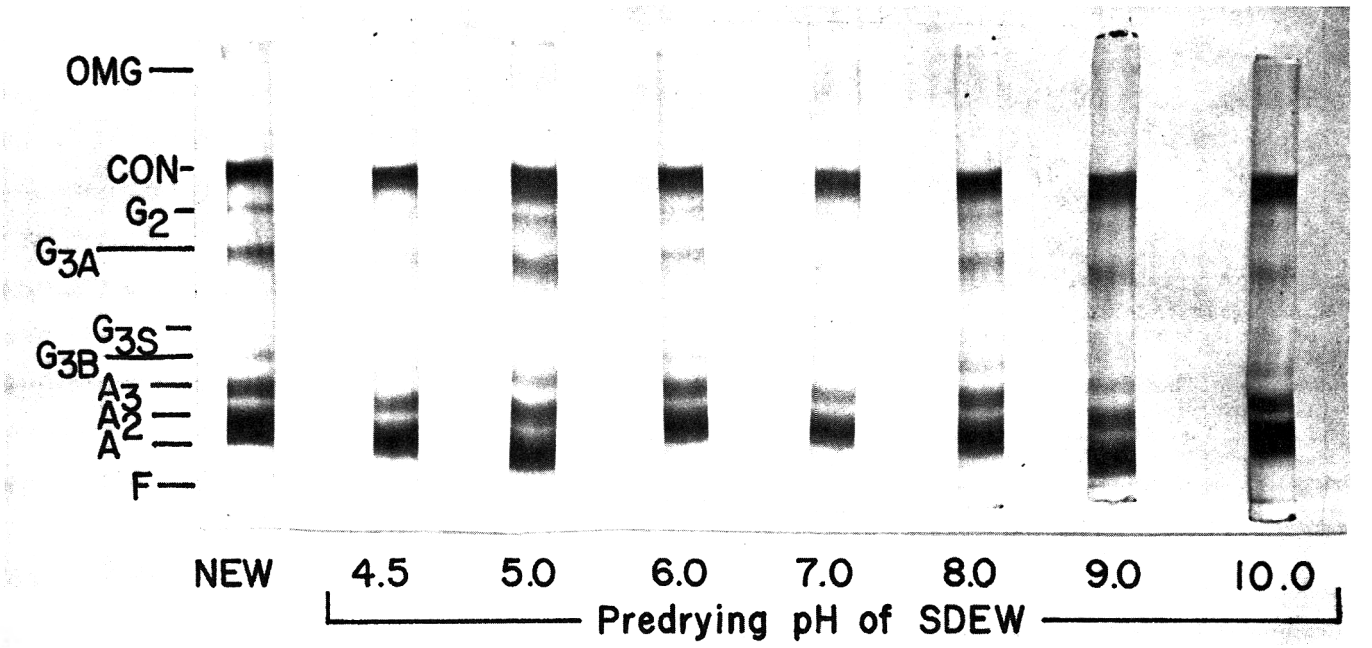

Fig. 4—Electrophoretograph of rehydrated spray-dried egg white adjusted to various pH values before spray-drying. See Figure 2 for definitions of protein band abbreviations.

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INTRACELLULAR PROTEINASE FROM Streptococcus cremoris

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ABSTRACT

An intracellular proteinase from *Streptococcus cremoris* was isolated, partially purified and characterized. A 40-fold purification was obtained with retention of 73% of the original activity. The proteinase exhibited normal reaction kinetics with respect to enzyme concentration and reaction rate. Proteinase activity was optimal at pH 7.0 and 37°C. The enzyme showed maximum activity on casein, the alpha casein being preferentially degraded. Effect of metal ions, some inhibitors and reducing agents were studied.

INTRODUCTION

PROTEOLYTIC ACTIVITY of lactic acid bacteria plays an important role in the development of the flavor and texture in cheese and in other fermented milk products (Reiter et al., 1967; Ohimiya and Sato, 1970a, b, 1972). The purpose of this study was to isolate, purify and partially characterize the interacellular proteinase from *Streptococcus cremoris* (H).

Williamson et al. (1964), Grutter and Zimmerman (1955), Sasaki and Nakae (1959) detected extracellular proteinase in lactic acid bacterial cultures. Baribo and Foster (1952), Vanderzant and Nelson (1953), Cowman et al. (1967), Sato and Nakashima (1965), Krishna and Dutta (1974), and Ohmiya and Sato (1975) detected intracellular proteolytic activity in cell-free extracts of lactic acid bacteria.

The importance of intracellular proteinase activity was demonstrated by Ohmiya and Sato (1970a, b) in aseptic rennet curd containing cell pellets of *S. cremoris* and *Lactobacillus helveticus*. Although *S. cremoris* is one of the most important starter organism used in the Dairy Industry, information about the proteolytic enzymes of this organism is very limited (Extertake, 1975).

EXPERIMENTAL

CASEIN was prepared from fresh skim milk by precipitation at the isoelectric point. α-, β- and κ-fractions of casein were isolated by the method of Singhal and Ganguli (1972) and stored lyophilized at −10°C.

Sephadex G-100 (Pharmacia Fine Chemicals, Sweden), diisopropylfluorophosphate (DFP), 1, 10-phenanthroline, idoacetamide and p-chloromercuribenzoic acid were products of Koch-light Laboratories Ltd., England. All the salts were reagent grade.

Organism

S. cremoris (H) was obtained from the culture collection of National Dairy Research Institute, Karnal.

Growth condition of S. cremoris

The organism was grown in Cowman and Speck's (1967) medium with a casein concentration of 0.2%. The organism was activated by repeated subculture.

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Table 1—Purification of intracellular proteinase from S. cremoris (H)

Procedure	Total enzyme units	Total protein (mg)	Specific activity	Purification (-fold)	Yield (%)
Cell-free extract	2500	80.00	31.25	1	100
Dialysis	4000	79.70	50.19	1.61	160
Ammonium sulfate fractionation (30–50% saturation)	3040	7.20	422.22	13.51	121.60
Sephadex G-100 gel filtration	1825	1.45	1258.62	40.28	73.00

Table 2—Action of intracellular proteinase on different substrates

Substrate[a]	Enzyme activity (units)
Whole casein	28
α_s-Casein	35
β-Casein	10
κ-Casein	30
Bovine serum albumin	12
Hemoglobin	3

[a] 1% solution in 0.05M phosphate buffer (pH 7.0).

Determination of proteolytic activity

The reaction mixture containing 1 ml of 1% casein solution, 1 ml of 0.05M phosphate buffer (pH 7.0) and 1 ml of enzyme solution was incubated at 37°C with a layer of toluene as a preservative. After the 10-hr incubation, an equal volume of 12% TCA was added; then the mixture was filtered through Whatman No. 1 filter paper. The amount of tyrosine liberated from casein in the deproteinized filtrate was determined from color intensity at 660 nm according to Hull (1947). A unit of proteinase activity was defined as the quantity of enzyme which produced TCA soluble fragments giving blue color equivalent to 1 μg tyrosine under the assay condition.

Protein determination

Protein was determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Preparation of the cell mass

Ten liters of casein medium were inoculated with 1% culture of *S. cremoris* and incubated at 30°C for 18–24 hr. The cells were harvested by continuous centrifugation of the broth culture in a Sharples centrifuge at 22,000 rpm and the cell mass was washed twice with 0.05M phosphate buffer (pH 7.0) and suspended in the buffer to a final volume of 100 ml.

Preparation of the cell-free extract

Twenty-milliliter portions of the washed cells were subjected to ultrasonic vibrations using Branson's Sonifier (Model B-12) at maximum amplitude of 60 watts, until the cells were disrupted. The temperature during the treatment was kept below 5°C by keeping the cuvette containing cell suspension in a freezing mixture of ice and common salt. Subsequently the cell debris was removed by centrifugation at 12,000 rpm at 5°C for 30 min. The precipitate was discarded. The cell-free extract thus obtained was stored at −10°C and used in further investigations.

Purification of the enzyme

The cell-free extract from *S. cremoris* (H) was dialyzed against

0.05M phosphate buffer (pH 7.0) for 24 hr at 5°C and then treated with ammonium sulfate at various levels of saturation. The precipitated fractions were centrifuged at 15,000 rpm at 5°C for 30 min and again dialyzed to remove ammonium sulfate residue. The active enzyme fraction (30–50% saturation of $(NH_4)_2SO_4$) obtained was passed through Sephadex G-100 column (2.5 × 50 cm). The enzyme was eluted with 0.05M phosphate buffer (pH 7.0) at a flow rate of 24 ml/hr, and 5 ml fractions were collected, and assayed for proteinase activity and protein content.

RESULTS & DISCUSSION

THE PROTEINASE from *S. cremoris* (H) was purified approximately 40-fold with 73% recovery of the original activity by the sequence of procedures reported in Table 1. The increase in activity (160%) after dialysis may be due to the removal of small molecular weight inhibitors present in the crude preparation. Maximum enzyme activity was observed in the fractions from 23–30 after gel filtration (Fig. 1).

Properties of the purified intracellular proteinase

Effect of temperature on enzymatic activity and stability. The enzyme activity was determined at pH 7.0 varying the temperature. The highest activity was observed at 37°C (Fig. 2A). The enzyme solution in 0.05M phosphate buffer (pH 7.0) without any substrate was incubated at various temperatures for 30 min and the residual activity was assayed at 37°C. Thermostability of the enzyme is depicted in Fig. 2B. On the contrary, Sato and Nakashima (1965) and Ohmiya and Sato (1975) found maximum proteinase activity of *S. lactis, S. cremoris* and *S. cremoris* H-61 between 20 and 30°C. McDonald (1964), Carini (1969), Wallace and Harmon (1970), Krishna and Dutta (1974) and Desnazeaud and Zevaco (1976) observed increased activity of these enzymes with increasing temperature up to 45°C.

Effect of pH on enzyme activity and stability. With casein as the substrate the optimum activity of the enzyme was noticed at pH 7.0 and 37°C when incubated for 10 hr (Fig. 3A). The enzyme was also more stable at pH 7.0 (Fig. 3B). The relative activity of this enzyme on casein at pH values encountered in cheese (pH 5.3–5.8; mean value, pH 5.5) was about 70% (Fig. 3A). The near neutral pH optimum observed for proteinase in the present study is similar to the eariler reports of several workers for proteinases of other lactic acid bacteria (Vadehra and Boyd, 1963; Sato and Nakashima, 1965; Krishna and Dutta, 1974; Ohmiya and Sato, 1975).

Effect of enzyme concentration. The enzymatic activity was proportional to enzyme concentration within the range of 3 μg to 30 μg/ml. The initial reaction rate was proportionate to the time of incubation period (Fig. 4). The results obtained in the present study are in good agreement with the findings of Wallace and Harmon (1970) who observed a linear relationship between enzyme concentration and its activity.

Other proteins as substrates. The results on the action of intracellular proteinase on casein and its fractions, bovine serum albumin and hemoglobin are presented in Table 2. The maximum activity of the enzyme was observed with casein as the substrate. The enzyme preferentially degraded α_s-casein moiety of casein.

The preferential degradation of casein as compared to other proteins is in conformity with the findings of McDonald (1964). A wide difference of opinion, however, exists as regards to the effect of these proteinases on casein and its fractions. Libudzisz and Metodieva (1970) reported that these enzymes digested casein more rapidly than its fractions. On the other hand, Ohmiya and Sato (1967, 1968 and 1969) observed α_s- or crude κ-casein to be degraded more than β- or whole casein which is comparable to the results in the present study.

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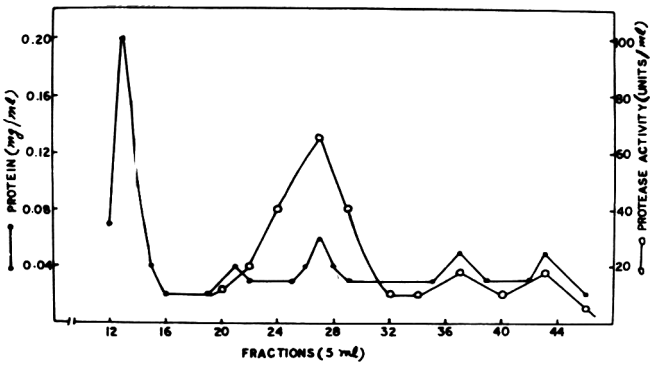

Fig. 1—Gel filtration of intracellular proteinase from S. cremoris (H) on Sephadex G-100 column (2.5 X 50 cm). Eluant was 0.05M phosphate buffer (pH 7.0), 7.20 mg sample load, 24 ml/hr flow rate.

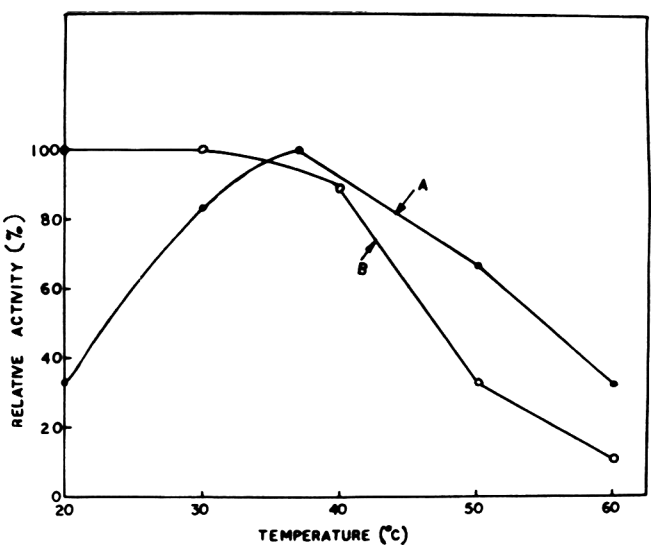

Fig. 2—Effect of temperature on the proteolytic activity (A) and stability (B) of the intracellular proteinase from S. cremoris (H). Stability was presented by the activity remaining in the enzyme, which was incubated in 0.05M phosphate buffer solution (pH 7.0) for 30 min at a given temperature.

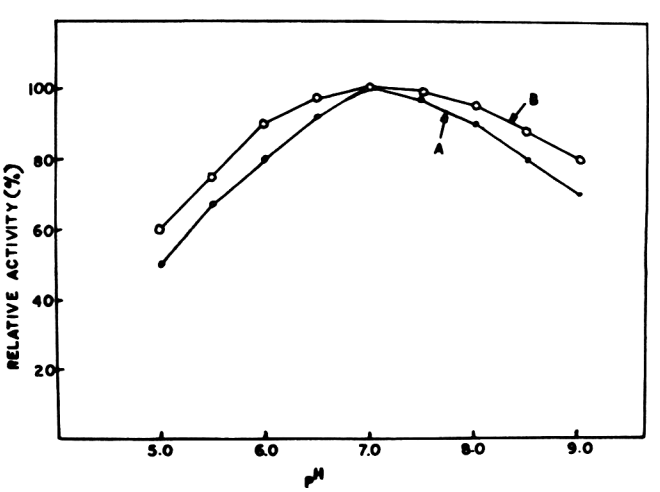

Fig. 3—Effect of pH on the activity of intracellular proteinase from S. cremoris (H) towards casein (A) and its stability to pH (B). Enzyme solution adjusted to various pH values was incubated for 30 min at 37°C before assaying the proteinase activity.

Effect of various metal ions and salts. The effect of various metal ions and salts on the intracellular proteinase activity of *S. cremoris* (H) was investigated by adding them at a concentration of 10^{-5} M to the reaction mixture. The results are recorded in Table 3. The metal ions Zn^{++}, Cu^{++}, Ni^{++}, Al^{+++} and Li^{+} caused 60–70% inhibition, while Co^{++}, Na^{+} and Pb^{++} did not show any effect on the activity. Manganese caused 266% activation (Table 3). These results are comparable to the reports by Vanderzant and Nelson (1953), Baribo and Foster (1952), Krishna and Dutta (1974) and Ohmiya and Sato (1975).

Table 3–Effect of various metal ions on the intracellular proteinase activity

Metal salts[a]	Relative activity (%)
None	100
$ZnSO_4$	30
$CuSO_4$	33
$CoCl_2$	100
$HgCl_2$	70
$FeSO_4$	66
$MgSO_4$	34
$Al_2(SO_4)_3$	32
Na_2SO_4	100
$MnCl_2$	266
$Zn(CH_3COO)_2$	66
$Pb(NG_3)_2$	100
$NiSO_4$	28
Li_2SO_4	40

[a] Final concentration of metal ions in the reaction mixture was 10^{-5} M.

Table 4–Effect of various chemical reagents on intracellular proteinase activity

Reagents[a]	Relative activity (%)
None	100
Ethylenediaminetetraacetic acid	100
Diisopropylfluorophosphate	0
1,10-phenanthroline	0
$K_4Fe(CN)_6$	0
Sodium diethyldithiocarbomate	40
Glutathion	180
Ascorbic acid	160
Cysteine	100
Iodoacetamide	100
P-Chloromercuribenzoic acid	88

[a] The concentration of various chemical reagents in the reaction mixture was 10^{-5} M

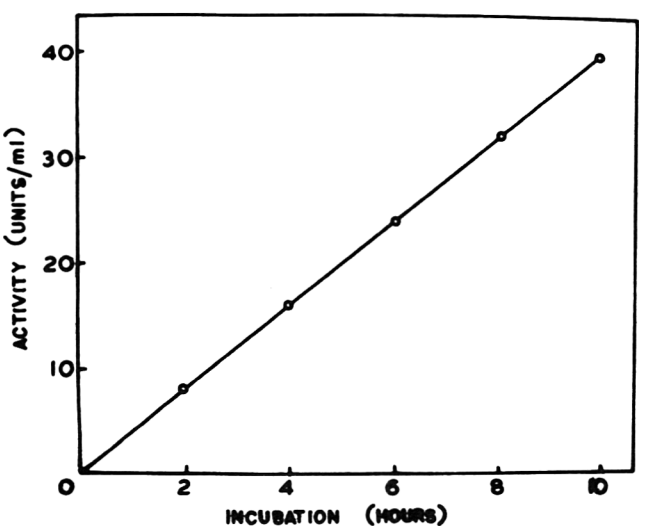

Fig. 4–Effect of incubation period on the activity of intracellular proteinase from S. cremoris (H).

Effect of various chemical reagents. The effect of selected chemical reagents at 10^{-5} M concentration on the intracellular proteinase activity of *S. cremoris* (H) was investigated. It may be seen from the results presented in Table 4 that diisopropylfluorophosphate, 1, 10-phenanthroline and $K_4Fe(CN)_6$ inactivated the enzyme completely. An increase in activity of the enzyme was observed in the presence of glutathion and ascorbic acid (Table 4). A metal chelating agent, ethylene diaminetetraacetic acid (EDTA) was not inhibitory to the proteinase activity of *S. cremoris* (H). The lack of influence of iodoacetamide, p-chloromercuribenzoic acid and cysteine on the activity of intracellular proteinase of *S. cremoris* (H) suggests that a thio- or disulfide group does not seem to be essential for enzymatic activity. This characteristic is in conformity with that of an intracellular proteinase of *S. cremoris* H-61 and from a mutant of *S. lactis* as reported by Ohmiya and Sato (1975) and Westhoff et al. (1971).

SUMMARY & CONCLUSIONS

AN INTRACELLULAR PROTEINASE from *S. cremoris* was purified 40-fold using dialysis, ammonium sulfate fractionation and Sephadex G-100 gell filtration. The proteinase exhibited normal reaction kinetics with respect to enzyme concentration and reaction rate. The enzyme activity was optimal at pH 7.0 and 37°C. The proteinase showed maximum activity on casein, the alpha-casein being preferentially degraded. Divalent Zn, Cu, Ni and trivalent Al ions caused 60–70% enzyme inhibition, while Mn^{++} caused activation up to 266%. The proteinase was activated by glutathion and ascorbic acid and completely inhibited by DFP, 1, 10-phenanthroline and $K_4Fe(CN)_6$. A metal chelating agent (EDTA) was not inhibitory to the proteinase activity. Variations observed in the properties of *S. cremoris* (H) as compared to earlier reports on *S. cremoris* can be due to strain differences.

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DETECTION AND QUANTIFICATION OF WHEY INGREDIENTS IN MILK CHOCOLATE USING SDS-GEL ELECTROPHORESIS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

P. F. HEMMATI and P. G. KEENEY

ABSTRACT

Sodium dodecylsulfate (SDS)-polyacrylamide disc gel electrophoresis and high performance liquid chromatography (HPLC) were utilized for identification and quantitative determination of whey powder replaced for whole milk powder in chocolate. SDS-phosphate buffer allowed complete solubilization of casein and whey proteins in chocolate. Therefore, isolation and fractionation of protein components of chocolate prior to electrophoresis were not required. Localization of protein bands was made possible by staining with Coomassie Brilliant Blue R-250 after separation. In all chocolate products containing whey powder as a partial replacement for whole milk powder, casein and β-lactoglobulin (β-Lg) were completely resolved and yielded sizable peak areas upon densitometric measurement. Therefore, casein as the major milk protein and β-Lg as the principal protein in the whey protein fraction were chosen for quantitative purposes. Peak area ratios (casein/β-Lg) for these proteins decreased as the percentage of whole milk powder replaced by whey powder increased, and the relationship was essentially linear. The ratio (casein/β-Lg) was similar to that of the whole milk powder ingredient only in milk chocolate which did not contain any whey. Lactose, determined by HPLC, increased linearly in chocolate products with increasing percentage of whole milk powder replacement by whey powder.

Using casein as a measure of whole milk solids content, the amount of β-Lg and lactose not belonging to whole milk solids can be used to estimate the type and amount of whey ingredient present in milk chocolate of unknown composition.

INTRODUCTION

CHEESE WHEY in its various forms has become an important component of many confectionery products. Whey's attractiveness centers on functional properties imparted relative to the development and control of flavor, color, and texture, and its efficacy as an inexpensive bulking agent. Although widely used in nonstandardized confections, whey solids are not permitted in milk chocolate as defined under federal standards of identity. Whether geared to the enforcement of the current standard or an amended version which allows whey, methods of analysis for the detection and measurement of whey solids in milk chocolate need development.

The standard of identity for milk chocolate specifies minima of 10% chocolate (chocolate liquor) and 12% whole milk solids. Essentially all of the remainder is sucrose and cocoa butter, the latter being used to raise the fat content to the level needed for optimum melting and viscosity characteristics. Depending upon flavor and texture properties desired, most commercial milk chocolate products will have quantities of chocolate liquor and whole milk solids which are considerably higher than the minima listed in the standards.

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Several complications must be considered when analyzing for a whey ingredient in milk chocolate. Any measurable component of whey, used as an index of whey solids amount, will also be present in the whole milk solids portion, the latter itself being a formula variable of milk chocolate. Also to be contended with are differences in composition among whey powders, and among other whey ingredients which might be used, such as modified wheys and whey protein concentrates. Recognizing that a single method may not suffice and that several types of analytical data might be needed to cover all eventualities, the investigation reported herein concentrated on the measurement of casein, β-lactoglobulin, and lactose in determining the amount of whey ingredient in milk chocolate. Sodium dodecyl sulfate, (SDS) polyacrylamide gel electrophoresis was employed for the milk protein components and lactose quantification was by high-performance liquid chromatography (HPLC).

Other approaches to whey solids quantification, such as amino acid analyses and nitrogen distribution determinations, were studied, but they are not covered in detail in this report. Although they do provide useful information, the electrophoresis and HPLC techniques are of greatest value. Interrelationships among the various methods are discussed.

MATERIALS & METHODS

Reagents

Acrylamide, bisacrylamide, N,N,N′,N′-tetramethylethylenediamine (TMED), sodium dodecyl sulfate (SDS), Coomassie Brilliant Blue R-250, ammonium persulfate and ion-exchange resin AG 501-X8 were from BIO-RAD Laboratories (Richmond, CA). Other analytical grade chemicals were anhydrous methanol, acetic acid, hydrochloric acid, bromophenol blue, sulfosalicylic acid, sucrose, and mono- and dibasic sodium phosphate.

Standard proteins

Standard α_{s1}-casein, β-lactoglobulin (β-Lg), α-lactalbumin (α-La) and bovine serum albumin (BSA) were supplied by M.P. Thompson (ERRC, USDA, Philadelphia, PA).

Preparation of milk chocolate confections

The standard milk chocolate formula used in the trials was 14% chocolate liquor, 20% whole milk solids (whole milk powder), 17% cocoa butter, and 49% sucrose. The principal variable was sweet whey powder as a replacement of whole milk powder (0–100% substitution in 10% increments). Trials were also conducted using whey powder as a supplement to the 20% whole milk level in the standard formula. Samples containing whey protein concentrate (28% protein), rather than whey powder, were also prepared.

Each milk chocolate sample was prepared in an identical manner, mix ingredients being pulverized together in a water-cooled Micro-Mill (Chemical Rubber Co., Cleveland, OH) to reduce particle size and obtain a homogeneous mass. After pulverizing and blending, the chocolate was placed in screw-cap jars for storage at ambient temperature.

Kjeldahl nitrogen determination

Kjeldahl nitrogen values in triplicate were determined on milk chocolates and chocolate ingredients (AOAC, 1975). Protein nitrogen was obtained using N × 6.38 for milk and whey powder and N × 6.25 for milk chocolate and chocolate liquor (RIC, 1972).

SDS-Disc Gel Electrophoresis

Electrophoresis was primarily as described by Weber and Osborn

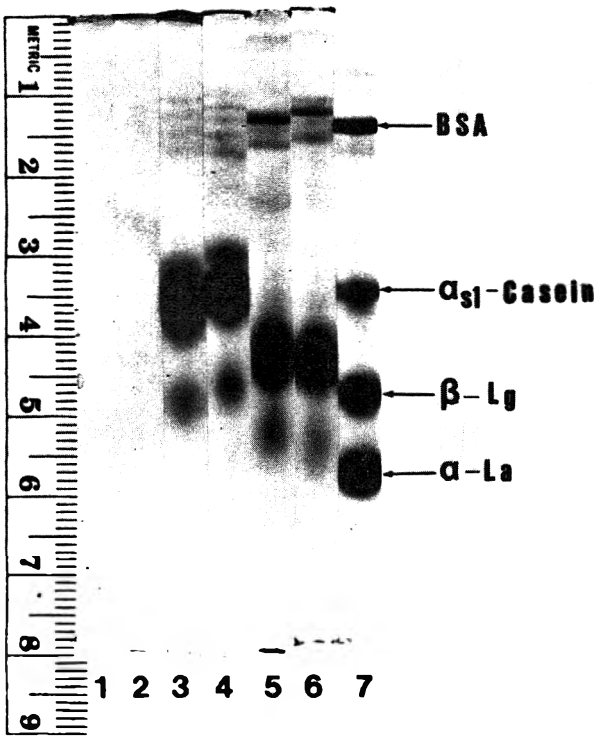

Fig. 1—SDS-polyacrylamide disc electrophoretic patterns of chocolate ingredients and mixture of standard proteins on a 10% gel stained with Coomassie Blue: (1,2) chocolate liquor; (3,4) whole milk powder; (5,6) whey powder; (7) mixture of standard proteins (2 μg BSA, 10 μg α_{s1}-casein, 5 μg β-Lg, and 5 μg α-La). Gels 1–6 were loaded with 20 μg protein each.

(1969) with modification. The standard SDS-phosphate gel system was chosen for protein separation and reagents were prepared accordingly.

Preparation of protein solution. Milk chocolates and individual ingredients were dispersed in sample buffer (0.01M sodium phosphate, pH 7.2, containing 1% SDS and 1% 2-mercaptoethanol). For complete denaturation of protein and reduction of disulfide bonds in polypeptide chains, samples were soaked in buffer at room temperature for 2 hr. Using protein values obtained by macro-Kjeldahl (AOAC, 1975; RIC, 1972), final protein concentration was adjusted to 1 μg/μl) for each sample. Suspended particles were then removed by centrifugation (Sorvall RC-5 Superspeed) at 48,000 ×G for 20 min. The supernatant solution was collected and kept frozen until used.

Standard α_{s1}-casein, β-lactoglobulin, and α-lactalbumin were dissolved in sample buffer (1 μg/μl) without further centrifugation and kept frozen until used. BSA was similarly treated, but its concentration was 0.25 μg/μl. In all cases the weight ratio of SDS (in buffer) to protein was at least 10:1.

Preparation of gels. For a 10% acrylamide concentration, the gel mixture was prepared by mixing 13.5 ml acrylamide solution (22.2g acrylamide, 0.6g methylene bis acrylamide, and distilled water to 100 ml), 15.4 ml gel buffer (7.8g $NaH_2PO_4 \cdot H_2O$, 38.6g $Na_2HPO_4 \cdot 12H_2O$, 2g SDS, distilled water to 1 liter, pH adjusted to 7.2 by concentrated NaOH), and 0.04 ml TEMD. The glass gel tubes (9 cm × 5.5 mm i.d.), cleaned by the procedure suggested by Webber and Osborn (1969), were filled with unpolymerized gel mixture up to the required length (7.5 cm) and overlayered with distilled water. After polymerization, the gel tubes were placed in an 18-tube capacity electrophoresis cell (Buchler Instrument, Fort Lee, NJ) equipped with a Buchler power supply. Reservoir buffer (1 part gel buffer and 1 part distilled water) was added to both the lower and upper compartments (400 ml each).

Preparation of electrophoresis samples. Frozen protein solutions were thawed and 20 μl (containing 20 μg protein) were mixed in a small vial (2 ml) with 5 μl 2-mercaptoethanol, 5 μl bromophenol blue (0.05% in 0.01M phosphate buffer, pH 7.2) and several crystals of sucrose. Sucrose crystals were dissolved in the sample mixture to retard diffusion of samples into the upper buffer chamber.

Electrophoresis separation. The electrophoresis sample mixture was layered on top of the gel tube through the reservoir buffer using a 50 μl syringe. The sample vial was then rinsed twice with 10 μl of sample buffer, which was also added to the gel. In each case, protein load on the gel was 20 μg with a total sample volume of 50 μl. In every trial one of the gels was loaded with a mixture of standard casein (10 μg) and whey proteins (2 μg BSA, 5 μg β-Lg, and 5 μg α-La). Separation was carried out at room temperature with a constant current of 8 mA per gel.

Upon completion of electrophoresis (4 hr) the gels were removed from the glass tubes using a plunger while the gel tubes were submerged under distilled water. The gels were then fixed in a solution of 20% sulfosalicylic acid in distilled water in 10 × 1.4 cm test tubes. After 16 hr the sulfosalicylic acid solution was discarded and the gels were stained in the same tubes for 3 hr using 0 25% Coomassie Brilliant Blue R-250 (1.25g Coomassie Blue, 227 ml methanol, 46 ml acetic acid and distilled water to 500 ml). At the end of the staining period, the staining solution was discarded and excess dye was removed by washing the gels with distilled water. Gels were then subjected to destaining for 1.5 hr in a solution of 5% methanol and 7.5% acetic acid with three changes of destaining solution.

The gels (in 16 × 125 mm screw-cap culture tubes) were further destained with 10–12 ml of destaining solution in the presence of 2g of ion exchange resin AG 501-X8. The culture tubes were capped and put into a plastic container (23.5 × 12.5 × 6.5 cm), which was then placed in a Dubnoff shaking incubator (Precision Scientific, Chicago, IL). The shaker was operated at half speed at 45°C, the internal temperature of the culture tubes being 37–40°C. Protein bands were visible after a few hours and clear background was obtained after 48 hr. Destained gels were stored in stoppered tubes (16 × 125 mm) containing 7.5% acetic acid until subjection to densitometric scanning.

Densitometric Scanning. The gels, supported in 10-cm glass tubing containing 7.5% acetic acid, were scanned at 600 nm using a Gilford spectrophorometer 2400-S equipped with a Gilford Model 2410-S linear transport attachment (Gilford Instrument Laboratory, Oberlin, OH). A 0.5-mm vertical slit was interposed between the glass tube and the photometer. Scan rate was adjusted to 1 cm/min, and the absorbance pattern was recorded as a series of peaks with chart speed at 1 min/inch.

To obtain a permanent record, the gels were photographed using a 4 × 5 View Camera, Kodak Tri-X film and a K2 filter. Light was passed through the gels by placing them on a glow-box Model 12-120-2 (Instrument for Research and Industry, Cheltenham, PA).

High-performance liquid chromatography

Sample preparation. Milk chocolate (1g) was defatted by shaking with 30 ml of ethyl ether in a 50-ml centrifuge tube followed by centrifugation and decanting of the ether layer. This treatment was repeated, after which the fat-free residue was dispersed in 10 ml distilled water and held 2 hr at room temperature to assure maximum extraction of sugars from the residue. The extract was then centrifuged 20 min at 48000 × G to remove suspended solids, after which the supernatant was passed through a Millipore filter (Millipore Corporation, Bedford, MA). The filtrate was frozen until analyzed. The same procedure was followed in preparing sugar extracts of individual chocolate ingredients (chocolate liquor, whole milk powder, and whey powder).

A Model ALC/GPC 201 liquid chromatograph (Waters Associates, Milford, MA) equipped with a differential refractometer having a sensitivity of 1 × 10^7 refractive units, a Model 6000A pump, and a U6K universal injector was used to separate and quantify lactose in the samples. Sugar extract (15 μl) was injected onto a 30 cm × 4 mm ID stainless steel "μ BONDAPAK Carbohydrate" column (Waters Associates). To filter out colloidal material which would deteriorate the column, a 60 mm × 2 mm stainless steel precolumn with 5-μ end fittings was connected in series. The precolumn was packed with 37-50μ Corasil I (Waters Associates). Acetonitrile (Burdick and Jackson, UV grade) was mixed with distilled water (3/1, v/v) and used as the mobile phase during chromatography, after passing the mixture through a 0.45μ filter. Solvent flow was adjusted to 2 ml per min. Lactose peaks were recorded on a Beckman 10-inch recorder at a speed of 0.2 inch/min.

Quantification was accomplished by comparing peak area (cm^2) of the sample to peak area of a 5% standard solution of lactose.

Standard solution was prepared by dissolving analytical grade lactose in distilled water.

RESULTS & DISCUSSION

SDS-gel electrophoresis

Protein extraction and electrophoresis procedure. Proteins denatured with SDS, were separated by electrophoresis in polyacrylamide gel-supporting media according to their molecular weights. SDS-phosphate buffer allowed complete solubilization of casein and whey proteins in chocolate. Therefore, isolation and fractionation of the proteins of chocolate prior to electrophoresis were not required. Visualization of individual proteins as distinct bands was made possible by the Coomassie Brilliant Blue R-250 staining and destaining techniques employed.

Quantitative studies with standard proteins showed that the upper limits beyond which densitometric response was no longer linear were: BSA, 3 μg; α_{s1}-casein, 21 μg; β-Lg, 22 μg; and α-La, 10 μg. Through calculations based on these values, a 20-μg load of protein onto gels was adopted for chocolate samples having whole milk powder replaced by whey powder at varying levels. With this quantity of total protein, the amount of each milk protein in the samples under study was within the concentration range over which it yielded a linear densitometer response.

Ingredients. Typical SDS gel polyacrylamide gel electrophoresis patterns for chocolate liquor, whole milk powder, whey powder, and standard milk proteins are presented in Figure 1. Even though chocolate liquor was 13.3% protein, no protein bands appeared on gels for this extracted ingredient (gels 1 and 2, Fig. 1). Extraction of milk proteins, but not cocoa proteins, into the sample buffer was fortuitous. With only milk protein bands appearing on developed gels, quantification by densitometric scanning was facilitated.

Separation patterns obtained for casein and whey proteins were comparable to those reported in the literature (Melachouris, 1969; Josephson, 1972; Badui and Josephson, 1974; and Lee et al., 1974). Whole milk powder (gels 3 and 4 Fig. 1) showed two major protein bands (casein and β-Lg) and four minor bands with smaller electrophoretic mobilities. Comparison with standard proteins indicated that the minor bands, being in the region of 60,000 daltons and higher, were BSA and immunoglobulins. Casein itself resolved into two bands, a slower moving lightly stained band immediately followed by an intense wide band. The former had an electrophoretic mobility characteristic of β-casein on SDS gel (Lee et al., 1974), while the dark band in the 34,000 region corresponded to α_{s1}-casein.

A band for BSA, but not α-La, was evident in the gel for whole milk powder. This occurred even though the contribution of α-La to the total milk protein fraction, 2–5%, is more than twice that of BSA, 0.7–1.3% (Whitney et al., 1976). The appearance of BSA, but not α-La, is probably due to the former's greater dye-binding capacity and the fact that it moved as a sharp and very narrow band, which would favor high color intensity upon staining.

Upon densitometric scanning of the gels, whole milk powder yielded two measurable peaks corresponding to α_{s1}-casein and β-Lg (Fig. 2). The lightly stained β-casein band was recorded as a shoulder at the front edge of the α_{s1}-casein peak. Alpha-lactalbumin and BSA were barely detectable by scanning. Although casein and β-Lg both produced sizable peaks, only casein, the major milk protein, was used for quantification of whole milk powder in subsequent milk chocolate trials.

Whey powder (gel 5 and 6, Fig. 1) exhibited two fast-moving bands having mobilities characteristic of β-Lg and α-La, and several slow moving bands corresponding to BSA and immunoglobulins. Since only β-Lg yielded a measureable peak when gels were scanned (Fig. 3), it was selected for quantitative purposes relative to whey ingredient concentration in milk chocolate.

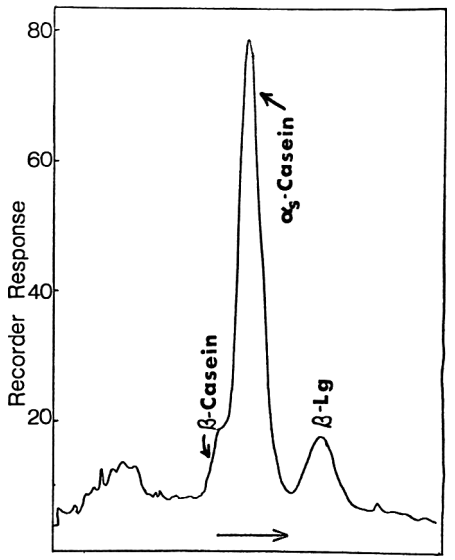

Fig. 2—Typical densitometric trace of Coomassie Blue-stained gel which had been loaded with whole milk powder. λ = 600, Slit = 0.5 mm, Units of absorbance = 2.0, gel length = 8.0 cm, and protein load = 20 μg.

Whey powder in chocolate. Each electrophoresis trial involved 15 disc gels. Eleven gels were loaded sequentially with 20 μg of total protein extracted from chocolate samples in which whole milk powder was replaced by whey powder at levels of 100% to 0% in 10% increments (gels 1–11 Fig. 4). In 20-μg quantities, gel 12 was loaded with protein extracted from whole milk powder and gel 13 with protein from whey powder. Mixtures of standard proteins (α_{s1}-casein, BSA, β-Lg, and α-La) in known amounts were applied to the remaining gels. Comparison with the patterns for standard casein and whey proteins indicate a high degree of reproducibility for SDS gel electrophoresis.

In Figure 4, immunoglobulins, BSA, casein and β-Lg are evident in all samples. An α-La band was less apparent, particularly in chocolate having reduced levels of whey substitution. Obviously, α-La concentration in the samples was

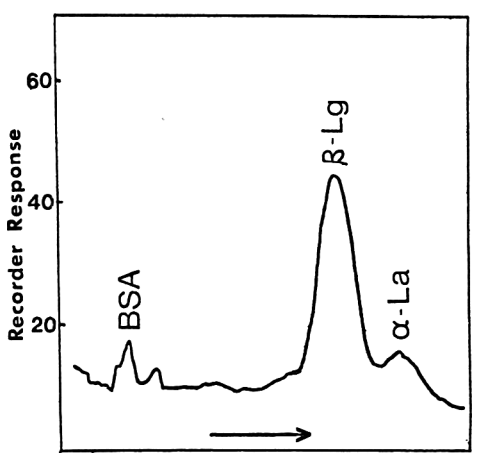

Fig. 3—Densitometric trace of Coomassie Blue stained gel loaded with 20 μg protein extract from whey powder. λ = 600, slit = 0.5 mm, Units of absorbance = 2.0, and gel length = 8.0 cm.

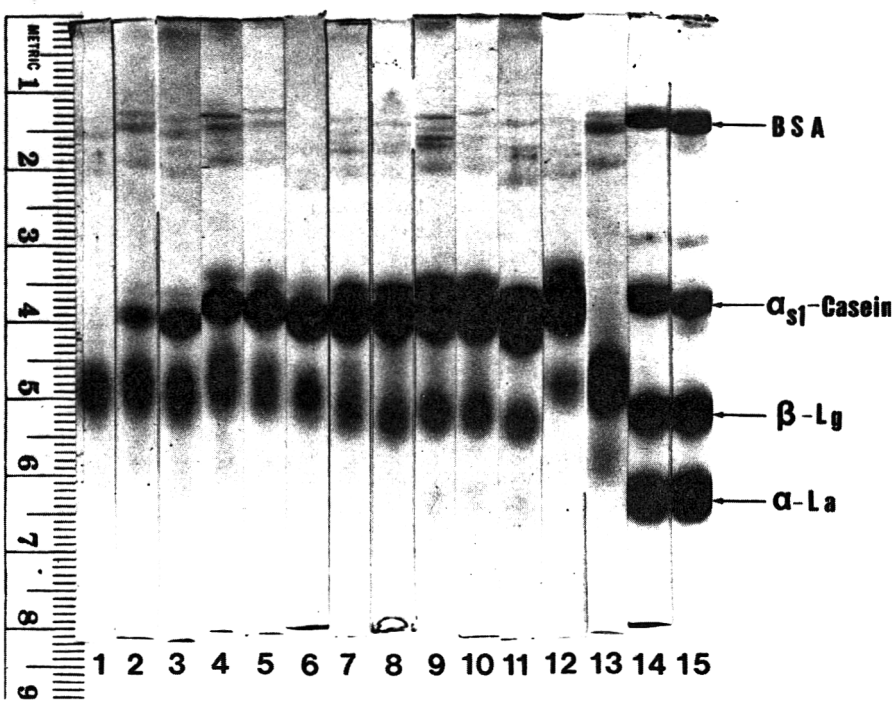

Fig. 4—SDS-polyacrylamide disc electrophoretic patterns of: (1—11) chocolate with varying levels (from 100 to 0%) of whole milk powder replaced by whey powder; (12) whole milk powder; (13) whey powder; (14—15) mixture of standard proteins (2 μg BSA, 10 μg α_{s1}-casein, 5 μg β-Lg, and 5 μg α-La), all on a 10% gel stained with Coomassie Blue. Gels 1—13 were loaded with 20 μg protein each.

near or below that needed for detection under the conditions selected for this study.

No casein was detected in gel 1 (Fig. 4), which had been loaded with extract from chocolate having only whey powder as a source of milk protein. As the percentage of whey powder substitution for whole milk powder decreased, the intensity of the casein band became progressively more pronounced, while that for β-Lg diminished (gels 1-11). Upon densitometric scanning, these changes could be expressed quantitatively, as presented in Figure 5.

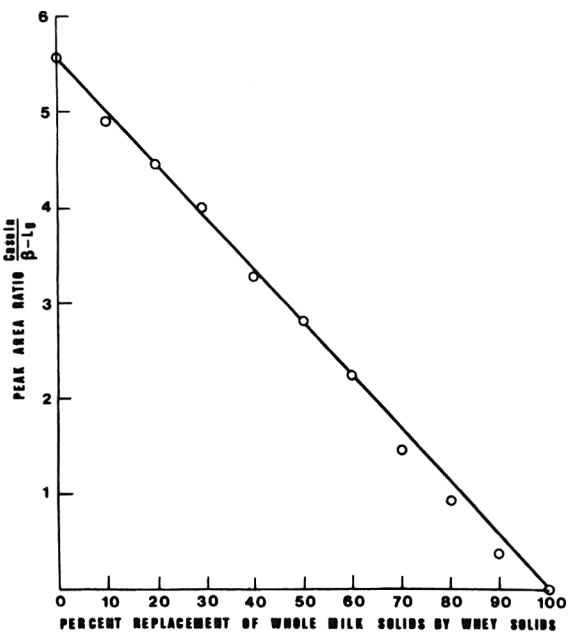

Fig. 5—Changes in casein/β-Lg peak area ratio due to percentage increase of whole milk powder (whole milk solids) replacement by whey powder (whey solids) in chocolate. Each point represents the mean of four determinations. Protein loaded = 20 μg.

The casein component in chocolate resolved into two bands on gels, one lightly stained followed immediately by a dark band, which upon scanning produced one dominant peak with a shoulder at the front edge similar to whole milk powder (Fig. 2). For the quantitative measurement of casein, the area of densitometric trace included the lightly stained band (shoulder).

For the determination of the amount of whey powder in chocolate, the casein peak, as the dominant milk protein in milk powder, and β-Lg, as the major whey protein, were chosen, since both yielded large, well resolved peaks by densitometer scanning. Areas under these peaks (recorded at units of absorbance = 2.0) were measured, ratios were calculated and then plotted against the percent of whole milk powder replaced by whey powder in chocolate (Fig. 5). The peak area ratio of casein/β-Lg decreased linearly as substitution by whey increased in chocolate.

Based on a large number of trials carried out, it was concluded that the protein load could be as high as 50 μg without changing the relationship illustrated in Figure 5. However, beyond a load of 30 μg protein, spectrophotometric scanning of stained bands had to be performed above two absorbance units which resulted in a greater inaccuracy of the absorbance measurements.

Lactose measurement by HPLC

Lactose content of ingredients and the various milk chocolate samples were determined quite easily and with accuracy by the HPLC method adopted for this investigation. Quantification was arrived at by comparing the peak areas (cm^2) for lactose in the sample with a standard solution of 5% lactose.

HPLC determinations revealed 41%, 73%, and 0% lactose in whole milk powder, whey powder, and chocolate liquor, respectively. These pronounced differences could be expected to have a measureable effect on the lactose content of milk chocolate in which the ratio of whole milk powder to whey powder was varied. For the series of milk chocolate samples prepared in this investigation, lactose content

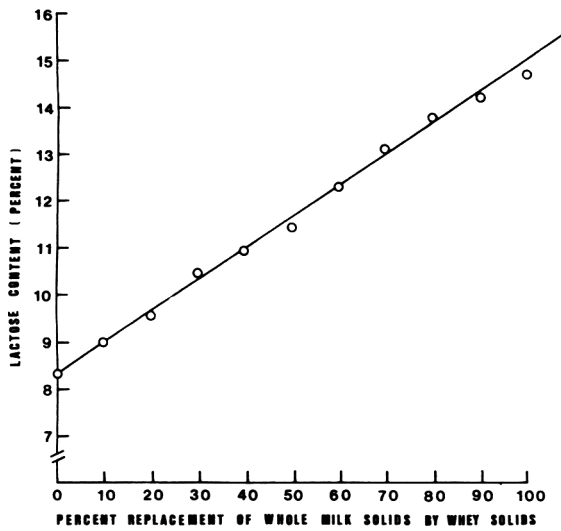

Fig. 6—Changes in lactose content of chocolate due to the percentage increase of whole milk powder (whole milk solids) replacement by whey powder (whey solids). Each point represents the mean of 8 determinations.

increased from 8.36% to 14.8% as replacement of whole milk powder by whey powder increased from 0% to 100%. As shown in Figure 6, the relationship was essentially linear. Thus, lactose measurements would play a key role in the detection and quantification of whey powder substitution for whole milk powder in milk chocolate.

Other studies

Although only trials involving whey powder as a substitute for whole milk powder have been detailed, mention of other chocolate models investigated seems appropriate relative to the analytical methods employed. Besides whey powder, a whey protein concentrate (28% protein) was used as a substitute for whole milk powder over a range of concentrations. In addition, trials were carried out with whey powder and whey protein concentrate as supplements to rather than replacements for milk powder. Based on the known compositions of these models, values obtained by analysis for casein, β-Lg, and lactose could be used to estimate the amounts of milk-based ingredients in chocolate. Agreements with known compositions were equal to those realized in the whey powder substitution trials.

In the study of whey ingredient determination in milk chocolate, approaches other than electrophoresis and HPLC were investigated. Included were nitrogen measurements (protein and nonprotein) and amino acid analyses. Nonprotein nitrogen was of no value, because most of it was contributed by the alkaloids, primarily theobromine, present in the chocolate liquor fraction. For the models analyzed, protein nitrogen was predictive of whey ingredient content when combined with lactose determinations, and differences in amino acid profiles were demonstrated, especially for the sulfur amino acids which are more prominent in whey protein than in whole milk protein. However, chocolate liquor makes significant contributions to both protein nitrogen and sulfur amino acid contents (Zak and Keeney, 1976). Since the chocolate liquor content of commercial milk chocolate is a variable, and the protein and sulfur amino acid levels in liquor are influenced by cocoa bean variety and post harvest treatments, those types of analyses can only be used as supplements to gel electrophoresis and HPLC measurements of whey ingredients in milk chocolate.

CONCLUSIONS

IN DETERMINING the whey solids content in milk chocolate of unknown composition a combination of analyses may be necessary, with lactose, casein, and β-lactoglobulin being most important. Casein and β-Lg can be quantitated through polyacrylamide gel electrophoresis. Knowing the casein content, the amount of whole milk solids in milk chocolate can be estimated, since whey does not contribute any casein. Using an average casein/β-Lg ratio for milk, β-Lg contributed by the whey ingredient can be calculated. Similarly, knowing the amount of whole milk solids (based on casein), its share of total lactose is arrived at. The difference is lactose contributed by the whey ingredient. Combining whey lactose and whey β-Lg, judgment can be made relative to the type and amount of whey ingredient present in milk chocolate.

Not covered in this investigation is the compositional variability of milk and whey, especially β-Lg, nor have the effects of both milk and chocolate processing practices been assessed. These will have to be dealt with before final conclusions can be drawn. Relative to the described methodology, the availability of instruments, laboratory facilities, and trained personnel have to be considered in determining if analyses can be performed routinely.

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NARINGIN BITTERNESS OF GRAPEFRUIT JUICE DEBITTERED WITH NARINGINASE IMMOBILIZED IN A HOLLOW FIBER

ALFRED C. OLSON, GREGORY M. GRAY and DANTE G. GUADAGNI

ABSTRACT

Naringinase from *Aspergillus niger* was immobilized in a hollow fiber reactor and used to hydrolyze naringin in grapefruit juice. Naringinase activity in the reactor was 0.04 μmoles naringin hydrolyzed/min/mg protein at 25°C compared to 0.06 μmoles/min/mg protein for the enzyme in solution using grapefruit juice reconstituted from a frozen concentrate as the substrate. Unclarified juice containing particulate matter could be pumped through the reactor with no adverse effects. A high correlation was obtained between sensory perception of bitterness in grapefruit juice and different lowered naringin levels in the juice produced by the hollow fiber/naringinase reactor.

INTRODUCTION

THE PROCESSING of grapefruit to yield grapefruit juice is complicated by several factors which affect the bitterness of the final product (Burdick, 1961; Hagen et al., 1966; Maier, 1969). It would be advantageous for the processor to have the capability of controlling the final product bitterness without waste or adverse effect on other desirable properties of the juice. Excessive bitterness in grapefruit products and how to control it has been the subject of a number of papers and reviews (Chandler and Nicol, 1975; Dunlap et al., 1962; Goldstein et al., 1971; Ono et al., 1977; Maier et al., 1977). Naringin is present in some juices in excess of 700–800 ppm, amounts definitely shown to be responsible for making the juice too bitter (Horowitz, 1977).

Naringin, or 4′,5,7-trihydroxyflavanone-7-rhamnoglucoside, can be hydrolyzed by an α-rhamnosidase to rhamnose and prunin, which is 1/3 as bitter as naringin (Horowitz, 1964), and then by a β-glucosidase to glucose and the aglycone naringenin, which is not bitter.

$$\text{Naringin} \xrightarrow{\alpha\text{-rhamnosidase}} \text{Prunin} + \text{Rhamnose}$$

$$\text{Prunin} \xrightarrow{\beta\text{-glucosidase}} \text{Naringenin} + \text{Glucose}$$

Both enzymes are present in commercially available enzyme preparations labeled naringinase.

Hollow fiber (HF) immobilized enzymes have been shown to be excellent for application to food processing systems compared to packed columns or other chemically immobilized enzyme systems (Olson and Korus, 1977). The HF reactor has a high surface area:volume ratio, permits high flow rates, is insensitive to solids of small particle size and can be rapidly cleaned out and reloaded. Pectinases, which could cause loss of juice cloud (Chandler and Nicol, 1975), would not be a problem in this system since the high molecular weight pectins could not diffuse through the hollow fiber membrane to come in contact with the enzyme. The objective in this work was to evaluate the use of naringinase in a HF reactor to control the amount of naringin, and thus, the final bitterness in grapefruit juice as measured by naringin content and sensory evaluation of the product.

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EXPERIMENTAL

Hollow fiber

The HF cartridge used in this study was a Romicon HF1.1-43-PM10 having 45 anisotropic polysulfone hollow fibers with an inside diameter of 1.1 mm (0.043″) potted (epoxied) at each end into a 63.5 × 2.5 cm clear plastic shell. The inner membrane surface of this bundle of fibers was 990 cm^2 (1.1 sq ft) in area and had a nominal molecular weight cutoff of 10,000. Total fiber hold-up volume was 27 ml. A Romicon HF2.5 manifold was used at each end of the cartridge with food-grade nylon ("Fast & Tite," Parker-Hannifin) fittings that accepted both semi-rigid (Eastman, foodgrade EVA-66) and flexible (food-grade Tygon B-44-44, or food-grade silicone, Cole-Parmer #6411) tubing. Valving was accomplished by pinch-clamping the flexible tubing. The fluid pump used was a Cole-Parmer "Ultra Master Flex" peristaltic tubing pump with a #7015 pump head using silicone tubing.

Enzyme loading and hollow fiber cleaning

Cleaning of the HF cartridge was accomplished by pumping solutions of commercial dairy cleaners (Monarch Spin and Monarch C.I.P., H.B. Fuller Company, 15g and 15 ml per 4.0 liters respectively) through the cartridge in both the ultrafiltration and backflush modes (Breslau and Kilcullen, 1978) to force the cleaning solutions to permeate the membrane and spongy regions and wash out the shell. After the cleaners, a solution of 200 ppm NaOCl (diluted Chlorox) in glass distilled water was used to sterilize the system, and a final rinse of 4–6 liters of glass distilled water used to remove the NaOCl before use again. Periodically the fittings and tubing connections were disassembled and cleaned to preclude particle or chemical build-up.

The naringinase (Sigma Chem. Co., #N-1753) used in the work was a preparation from *Aspergillus niger* which contained two enzymes: an α-rhamnosidase, EC 3.2.1.40, and a β-glucosidase, EC 3.2.1.21. It contained an inert material which was removed by slurrying the solid in glass distilled water and filtering through a double layer of glass fiber filters (Reeve Angel 934 AH). The nearly clear preparation which contained all of the naringinase activity was then diluted to approximately 1 mg protein per ml. The clean sterile hollow fiber was loaded by very slowly pumping the appropriate amount of enzyme solution through 0.45 μm pore membrane filters (Millex, Millipore Corp.) into the shell of the reactor in the backflush mode. With the second shell port closed and the lumen ports open, the enzyme was ultrafiltered into the sponge region of the hollow fibers and retained by the membrane with the excess liquid passing across the membrane and being removed through the lumen. After all the enzyme solution had been pumped into the shell, the reactor was tilted several times to distribute the enzyme evenly. The remainder of the liquid was removed by slowly pumping filtered air into the shell using a syringe and fresh dry filter. Thus loaded, the enzyme was located in the sponge and the shell was devoid of any free liquid. The shell was left slightly pressurized after the excess liquid was pumped out. Enzyme protein content was measured by Kjeldahl nitrogen analysis × 6.25.

Substrate

The grapefruit juice used as the enzyme substrate was obtained from the Florida Citrus Commission as a frozen concentrate. It was reconstituted 1:5, w:w with glass distilled water. This gave a juice with 285 ± 10 μg/ml naringin, 9.7° Brix, pH 3.2.

Analysis

The high pressure liquid chromatographic (HPLC) analysis of grapefruit juice for naringin content was performed using the procedure of Fisher and Wheaton, (1976) on similar equipment. A 2 mm

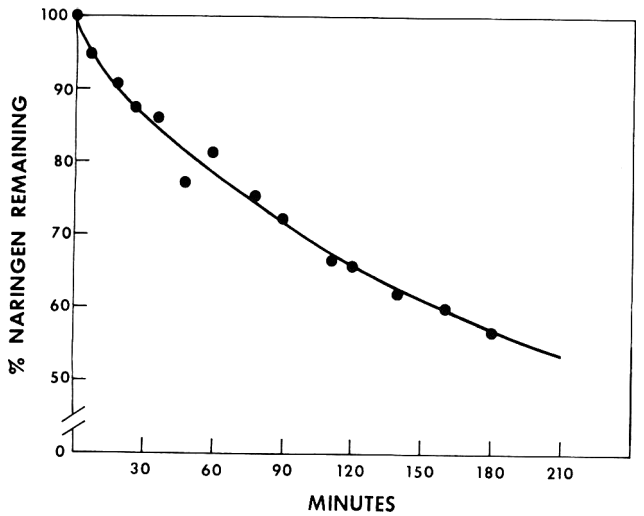

Fig. 1—Naringin hydrolysis curve: 1000 ml grapefruit juice (285 μg/ml naringin), recycled at 300 ml/min and 25°C through HF/naringinase reactor containing 112 mg (protein) immobilized enzyme.

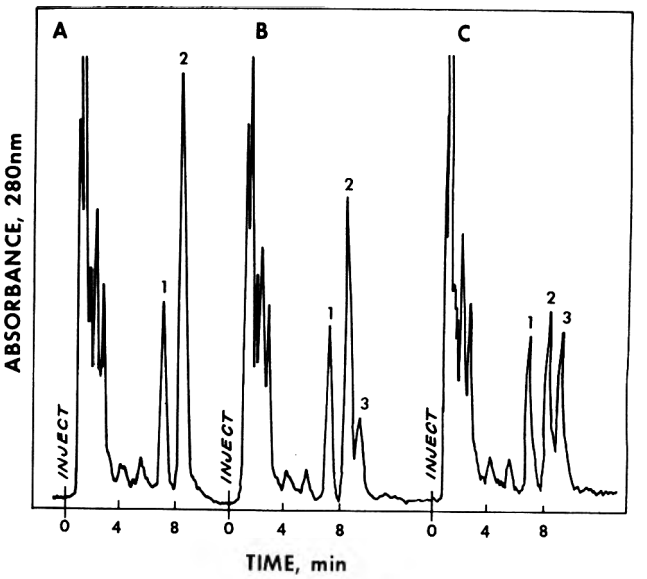

Fig. 2—Typical chromatograms of grapefruit juice: A, untreated grapefruit juice, 285 μg/ml naringin; B, juice treated for 1.5 hr in HF/naringinase reactor, naringin 28% hydrolyzed; C, juice treated for 4.0 hr, naringin 66% hydrolyzed. Peak #1, naringenin 7-β-rutinoside; #2, naringin; #3, prunin.

i.d. by 75 mm long guard-column of Bondapak C_{18}/Porasil B (Waters Associates) was employed to protect the μBondapak C_{18} analytical column. With a flow rate of 2.0 ml/min the average chromatograph of a grapefruit juice sample took 12 min.

Naringin standard solutions were made from naringin (Sigma Chem. Co., #N-1376, Grade II) that was recrystallized from ethanol/water after hot filtration. Melting point (172°C) and moisture content results indicated the resulting naringin crystals were dihydrates. The standard solutions were filtered through 0.22 μm pore membrane filters (Millex, Millipore Corp.). The grapefruit juice samples were filtered through 0.45 μm Millex filters prior to injection into the HPLC. Usually 10 μl of either sample or standard was injected. The standard curve was linear within the 50–500 μg/ml range used. Prunin and naringenin 7-β-rutinoside samples for identification purposes were provided by R. Horowitz (USDA, Pasadena, CA). Naringenin was obtained from Sigma Chem. Co., #N-1251.

Enzyme activities in the soluble enzyme system were measured by incubating grapefruit juice and enzyme solution at 25°C in a shaker bath. Naringin concentrations were monitored by removing small aliquots at appropriate intervals, boiling for 2 min, and analyzing by HPLC. In the HF immobilized enzyme system batches of from 500–3000 ml of grapefruit juice were continuously recycled through the reactor from the room temperature (23–26°C) stirred reservoir. Small aliquots were withdrawn from the reservoir, filtered, and analyzed by HPLC. Initial rate activities were measured by drawing tangents to the curves of decreasing naringin and calculated on the basis of μmoles naringin hydrolyzed/mg protein.

Sensory evaluation

Samples for the taste panel were prepared in small batches by recycling the grapefruit juice through the enzyme HF reactor until a desired concentration of naringin was obtained. The batches for a particular panel sample were then mixed to give the final desired naringin concentration to be tested. Each sample was then frozen until it was used.

The enzyme treated juices were evaluated by a 20-member panel experienced in grapefruit juice tasting. Each sample was replicated 4 times. The juices were rated on a structured bitterness scale in which 1 = no bitterness, 2 = just perceptible, 3 = definitely perceptible, 4 = moderately intense, 5 = very intense, and 6 = extremely intense. Bitterness threshold of the treated juices was also estimated by a slight modification of the procedure described by Gregson (1962). The threshold values for bitterness represent the percentage of juice in pure water that gives a perceptible bitter taste.

RESULTS & DISCUSSION

THE IMMOBILIZATION and use of naringinase in the PM10 hollow fiber was successful. The enzyme was not inactivated by contact with the fiber materials nor were there problems with enzyme leakage (Korus and Olson, 1977). No naringinase activity was detected in the ultrafiltrates from the enzyme loading procedures. The commercial enzyme preparation contained 28% protein. The initial rate activity of the enzyme in soluble form, as prepared to load the HF reactor, was 0.06 μmoles naringin hydrolyzed/min/mg protein using grapefruit juice with 285 μg/ml naringin at 25°C as substrate. Immobilized in the HF reactor the enzyme had an activity of 0.04 μmoles naringin hydrolyzed/min/mg protein with the same substrate (Fig. 1).

There are several methods (Soderquist, 1978) of following the enzymatic hydrolysis of naringin. Measuring the glucose and rhamnose increase by colorimetric or chromatographic procedures (high pressure liquid chromatography, gas liquid chromatography) is complicated by the native sugar concentrations in the grapefruit juice. Monitoring the naringenin concentration and calculating the naringin decrease is complicated by the presence of two substrates (Versteeg et al., 1977) in grapefruit juice that yield prunin and naringenin. They are naringin and naringenin 7-β-rutinoside, a non-bitter isomer of naringin (Horowitz, 1964).

In this work, the isocratic procedure of Fisher and Wheaton was used to follow the hydrolysis and only naringin was quantitated. As shown in the chromatographs, Figure 2, naringin and naringenin 7-β-rutinoside are clearly resolved. Since both compounds are substrates for naringinase, as the hydrolysis proceeded prunin accumulated and is seen as the peak immediately following naringin. Naringenin 7-β-rutinoside, naringin, prunin, and naringenin were identified from retention times of known samples. Results using peak heights or peak areas for quantitation were the same. Since peak height measurements were significantly more rapid, they were used in this work.

Hollow fiber reactor

A microscopic examination of the pellet from centrifuged grapefruit juice indicated that the maximum particle

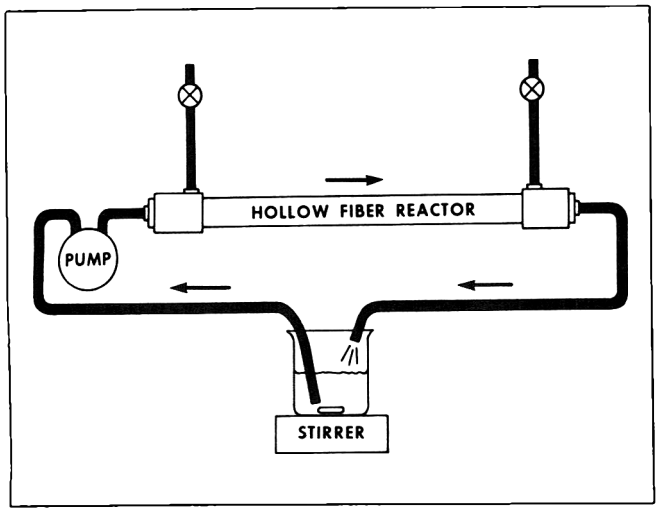

Fig. 3—Single HF system, recycle mode.

dimension was about 200 μm, and that the large particles are almost entirely juice sac membrane and were extremely flexible. When the juice was pumped at flow rates of 100 ml/min or more, no indications of plugging or deposition of the particles was noted. This feature of being able to receive juice feeds with insoluble solids is a great advantage over packed columns or reactors and would give the juice processor more freedom in the placement of the HF reactor in the processing stream for maximum control of debittering.

In typical operation, the substrate was pumped through the reactor at flow rates from 100–1000 ml/min in a recycle mode, Figure 3, with a substrate volume of 500–3000 ml held in a stirred room temperature reservoir. With proper precautions, including only filtered (0.45 μm) solutions in the shell, closed or covered containers, and a sterile water washout after the day's use with overnight storage at 1–2°C, the reactor could be run for 8 hr per day at room temperature for three consecutive days with no change in activity and no detectable formation of off flavors or loss of cloud.

To determine to what extent substrate recycle flow rates affected the apparent reactor activity, a large batch of grapefruit juice was prepared; 500 ml aliquots were recycled at varying flow rates from 100–1000 ml/min for 1 hr each. The results indicated a small flow rate dependence.

To determine how enzyme loading affected the reactor activity, a large volume of enzyme solution was prepared. Increasing amounts of enzyme were applied to the hollow fiber, and aliquots of the juice were recycled for 1 hour at each enzyme level. The results (Fig. 4) indicate that for up to about 60 mg protein applied, hydrolysis is proportional to total enzyme loaded. Above this amount of enzyme the hydrolysis curve flattens considerably which is probably due to inhibition of the β-glucosidase by the native sugars present in the juice (Thomas et al., 1958; Ting, 1958; Versteeg et al., 1977). This should lead to an increase in prunin concentration. Chromatographs of samples with 50% or more decrease in naringin showed a large prunin peak.

The enzyme-substrate contact in the above system should be governed by trans-membrane diffusion. To determine whether this was the case, an alternative mode of operation which should yield more control over this aspect of the contact process was considered. Grapefruit juice was pumped through a primary HF in the ultrafiltration mode to yield a clarified juice. The clarified permeate from the shell of this primary HF was passed into the shell of a secondary HF which contained the enzyme (see Fig. 5). This secondary HF was used in the backflush mode (the membranes containing the enzyme) and passage of the clarified substrate into the secondary HF shell and out the lumen ports should have prevented trans-membrane diffusion from being a limiting factor in enzyme-substrate contact. An experiment using this alternative system gave the same conversion rates as the use of a single HF in recycle operation for the same time period. These results indicate the trans-membrane diffusion in the single HF system did not significantly limit enzyme-substrate contact or conversion rates.

Sensory evaluation

The data (Table 1) shows an excellent correlation between naringin content of the treated juices and bitterness threshold and bitterness rating. The juice used in the sensory evaluation was prepared following procedures outlined for single hollow fiber recycle mode. Bitterness ratings were reduced from moderately intense to definitely perceptible, and threshold values were increased about four-fold for juices ranging from 285 to 95 μg/ml naringin. The increasing threshold values are directly proportional to the de-

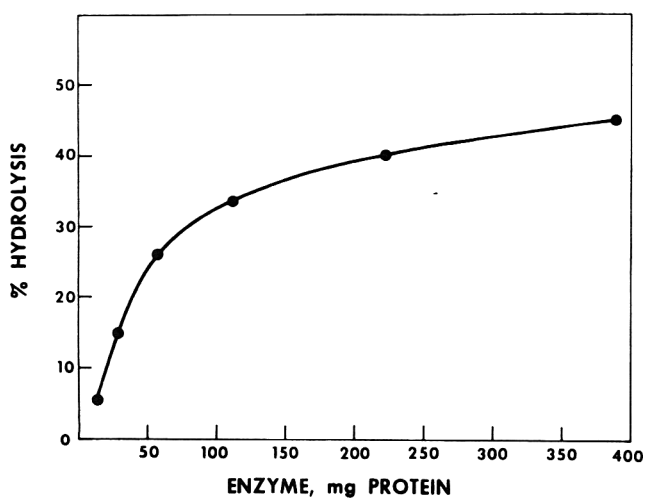

Fig. 4—Effect of enzyme load in HF reactor; 500 ml batches of grapefruit juice recycled for 1 hr at 25°C at 500 ml/min flow rate at each enzyme load level.

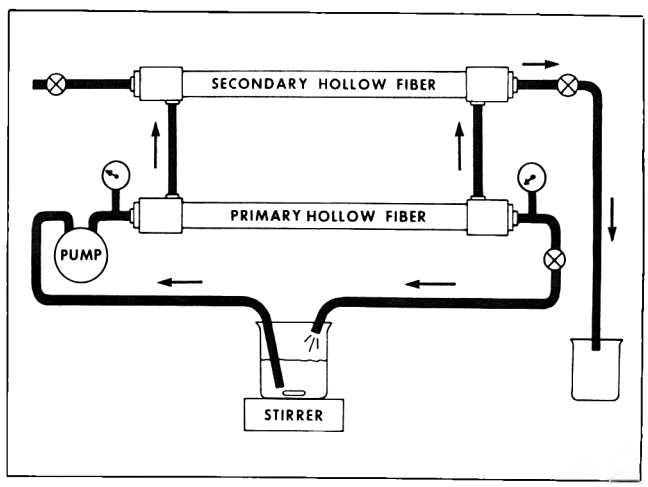

Fig. 5—Double HF system: primary HF producing permeate; secondary HF immobilizing enzyme.

Table 1—Effect of enzyme treatment on naringin content and bitterness perception in grapefruit juice

Sample[a]	Naringin content μg/ml	Bitterness threshold[b]	Bitterness rating[c]
Control	285	8	3.8
1	240	14	3.6
2	195	20	3.3
3	122	29	3.2
4	95	34	2.9

correlation coefficients

Naringin content vs bitterness threshold	−0.999
Naringin content vs bitterness rating	0.971
Bitterness threshold vs bitterness rating	−0.979

[a] Samples as follows: Control-untreated reconstituted grapefruit juice concentrate; sample 1—prepared in two batches, 1500 ml each, recycled through HF reactor for 100 min and 30 min respectively, then blended; sample 2—two batches, 1500 ml each, 180 min and 145 min, blended; sample 3—six batches, 500 ml each, 120 min each, blended; sample 4—six batches, 500 ml each, 125—150 min each, blended.

[b] Percentage of grapefruit juice in water to cause just perceptible bitterness.

[c] Ratings: See text, sensory evaluation.

crease in naringin content and bitterness. This demonstrates that any desired level of naringin bitterness could be achieved in grapefruit juice by properly manipulating the factors affecting naringin hydrolysis.

The basic scheme of debittering unclarified (but finished) grapefruit juice with immobilized naringinase has been demonstrated. Additional research is needed to determine pilot plant operational parameters including reactor half-life, potential microbial contamination problems, and optimum reactor placement in the processing stream. The hollow fiber method of immobilization requires no chemical agents or other procedures that may be detrimental to food processing operations, and, in addition, is easily cleaned and recharged with commercially available enzyme. The sensory results indicated that this method of debittering could improve the acceptability of juices with excessively high naringin levels.

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Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

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AN ENZYMATIC METHOD FOR YEAST AUTOLYSIS

D. KNORR, K. J. SHETTY, L. F. HOOD and J. E. KINSELLA

ABSTRACT

Treatment of intact yeast cells with lytic enzymes, i.e. zymolase and lysozyme, markedly increased the release of nitrogen and proteins during incubation. The addition of pancreatin or pronase during incubation of cells with lytic enzymes caused the concurrent hydrolysis of the yeast proteins. The precipitable yeast protein at pH 4.5 decreased from 73 to 21% within 60 min. This procedure may be used for the commercial production of yeast autolysate.

INTRODUCTION

AUTOLYSED YEAST EXTRACT which is composed of the protein, low molecular weight polypeptides and other intracellular material has long been used as an additive to food products such as soups, sauces, gravies, meat products, meat flavors, cocktail snacks, seasonings, ready-made dishes, cheese products, spreads, canned vegetable and TVP products (Hough and Maddox, 1970; Dogman, 1977). Yeast extracts are prepared by autolysis, plasmolysis and hydrolysis (Johnson, 1977). The disadvantages of conventional methods are long reaction time (up to 48 hr at temperatures between 30 and 70°C), relatively low yield, and risk of deterioration due to microbial contamination during processing, which requires aseptic procedures (Tannenbaum, 1968; Vosti and Joslyn, 1954; Johnson, 1977). In the common commercial process, nonpolar organic solvents (such as toluol, chloroform, ethyl acetate or amyl acetate) or inorganic salts such as sodium chloride are often used to accelerate autolysis. The addition of solvents is undesirable when yeast autolysate is intended for food products. Sodium chloride, while inducing rapid autolysis, does not always prevent contamination (Johnson, 1977) and high concentrations are undesirable.. Consequently there is interest in shortening the process without using undesirable additives, especially when autolysates are intended for human consumption.

Yeast cells contain a full complement of intracellular proteolytic enzymes which have been extensively studied (Rostum et al., 1971; Pringle, 1975; Diezel et al., 1972; Hata et al., 1967). These enzymes are liberated and activated after cells are disintegrated either by autolysis (Hough and Maddox, 1970; Shetty and Kinsella, 1978) or by mechanical disruption (Follows et al., 1971; Lindblom, 1977).

The use of proteolytic enzymes to disrupt yeast cells and aid in proteolytic breakdown during autolysis has been reported (Corteel, 1972; Reed and Peppler, 1973).

Enzymatic lysis of yeast cell walls using exogenous enzymes is very effective (Knorr et al., 1979). A coupled zymolase/lysozyme treatment followed by extraction at pH 9.0 yielded more than 80% of the total nitrogen of the yeast cell. Because this treatment activated the endogenous proteases, we studied the potential of coupling lytic enzymes with exogenous proteolytic enzymes for the rapid preparation of yeast autolysates.

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EXPERIMENTAL

Brewer's yeast (*Saccharomyces carlsbergensis*) in the resting phase was washed three times with distilled water, freeze-dried and stored at 3–5°C.

Enzyme treatments of yeast cells

Lytic enzymes. The coupled zymolase/lysozyme treatment was carried out as described earlier (Knorr et al., 1979). Dried, intact yeast cells were suspended in phosphate buffer of pH 7.5 (2.5%; w/v) then zymolase (Zymolase-5000, The Research Laboratories, Kirin Brewery Co., Ltd., Takaksaki, Japan) and lysozyme (Sigma Chemical Co., St. Louis, MO) were added and incubated for different intervals of time. After incubation for 30, 60 and 90 min, the pH of the suspension was increased to 9.0 and stirred for 45 min at 25°C. Samples were then centrifuged and the nitrogen and protein content of the supernatant were determined. For comparison intact yeast cells and cells disrupted in a Braun mechanical cell homogenizer (Braun Melsung, W. Germany) were treated under identical conditions without the addition of the lytic enzymes.

Proteolytic enzymes. To test the effects of enzyme combinations for preparation of autolyzates, proteolytic enzymes were added after 30 min of zymolase/lysozyme treatment. Pancreatin (Grade VI, Sigma Chemical Co., St. Louis, MO), at concentrations of 4 and 8 mg/g yeast, or pronase (B grade, Calbiochem, San Diego, CA) at 1 and 4 mg/g yeast, were added and incubated as indicated in the results.

Effect of lytic enzymes on the integrity of yeast cell wall. The effect of lytic enzymes on the integrity of yeast cell wall was examined by electron microscopy. Washed yeast was treated with the lytic enzymes and fixed in 2% $KMnO_4$ in water for 30–60 min at 22–24°C. The yeast preparations without enzyme treatments also were fixed. After washing four times in 5°C water, specimens were dehydrated at 5°C through an aqueous acetone series. Subsequent dehydration and infiltration was at 22–24°C. Specimens were infiltrated with propylene oxide and then Spurr resin. Specimens were allowed to settle through freshly prepared Spurr resin in Beem capsules and the resin polymerized at 60°C for 24 hr followed by 70°C for 24 hr. Thin sections (approximately 1000 Å) were cut on a Sorvall MT2 ultramicrotome, stained with 2% aqueous uranyl acetate and with lead citrate (Reynolds, 1963) and examined in a Philips 300 electron microscope at 80kv.

Total nitrogen and protein

Total nitrogen was determined by micro-Kjeldahl method (AOAC, 1975) and protein was assayed by the method of Lowry et al. (1951). The amount of nitrogen and protein was calculated as percent of dry yeast.

Determination of the extent of proteolysis during incubation

The amount of protein precipitable at pH 4.5 (isoelectric point of yeast protein) was used to determine the rate of proteolysis during the incubation of yeast cells with lytic and proteolytic enzymes. An aliquot (25 ml) of the supernatant from the extraction at pH 9 was adjusted to pH 4.5. The protein precipitated was separated by centrifugation, dissolved in 0.1N NaOH and both nitrogen and protein content were determined.

RNA hydrolysis

The effect of incubation of yeast cells with zymolase and proteolytic enzymes on the RNA hydrolysis was also monitored by determining the released ribose using the orcinol method described by Herbert et al. (1971). Ribose was used for the construction of the standard graph. Two ml of the enzyme treated yeast suspension

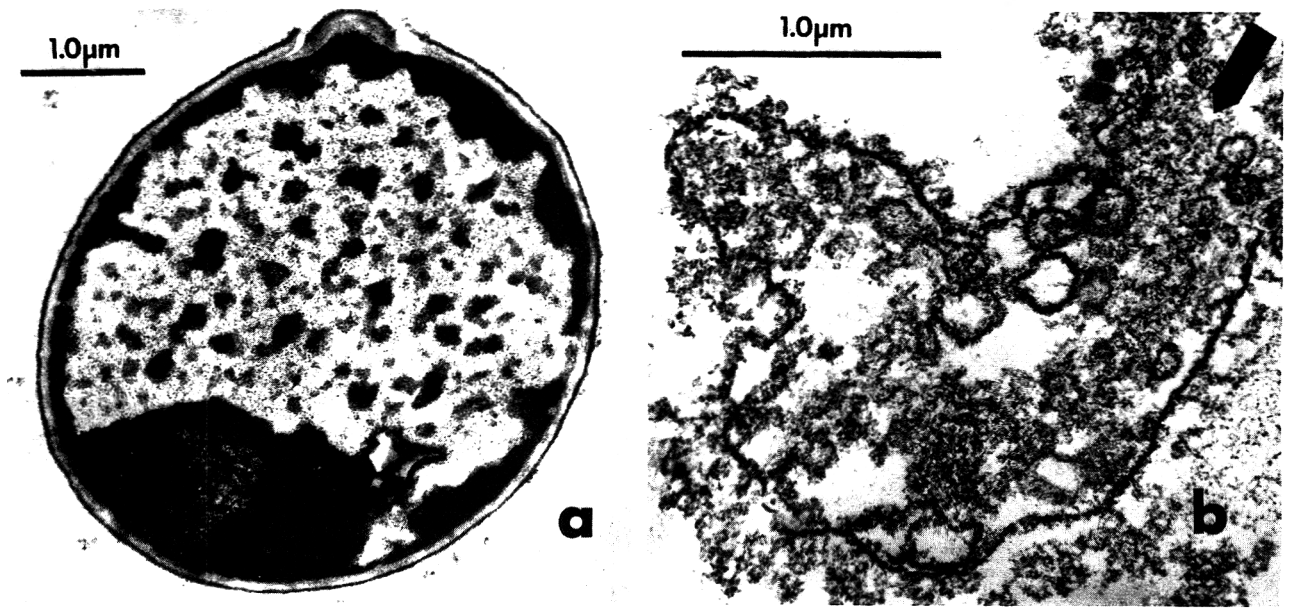

Fig. 1—Electron micrographs of untreated (a) and enzyme-treated yeast cells (b). Lytic enzymes lysed the cell walls and facilitated the release of cytoplasm (arrow).

were mixed with two ml of perchloric acid (1M), cooled and centrifuged. Ribose was estimated in the supernatant and expressed as mg/g of yeast.

Amino acid estimation.

The extent of proteolysis during incubation of yeast cells with the addition of lytic enzymes and exogeneous proteolytic enzymes was also determined by estimating the released free amino groups according to the method described by Paik and Kim (1972). In a typical experiment, to 2 ml of the incubated sample, 2 ml of 10%

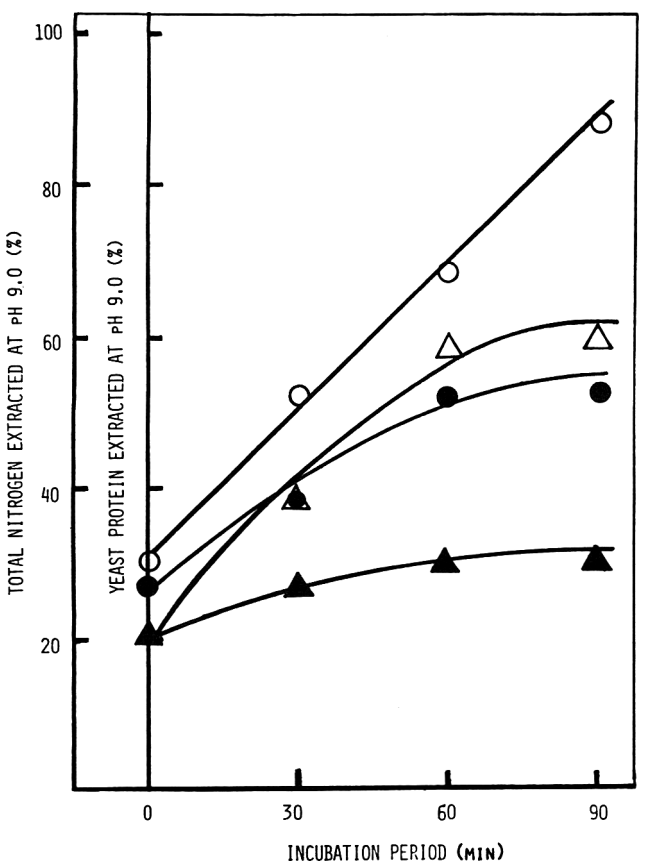

Fig. 2—The release of nitrogen and protein during incubation of intact and enzyme treated (zymolase) yeast cells: Intact yeast (●) nitrogen, (▲) protein; enzyme-treated cells (○) nitrogen, (△) protein.

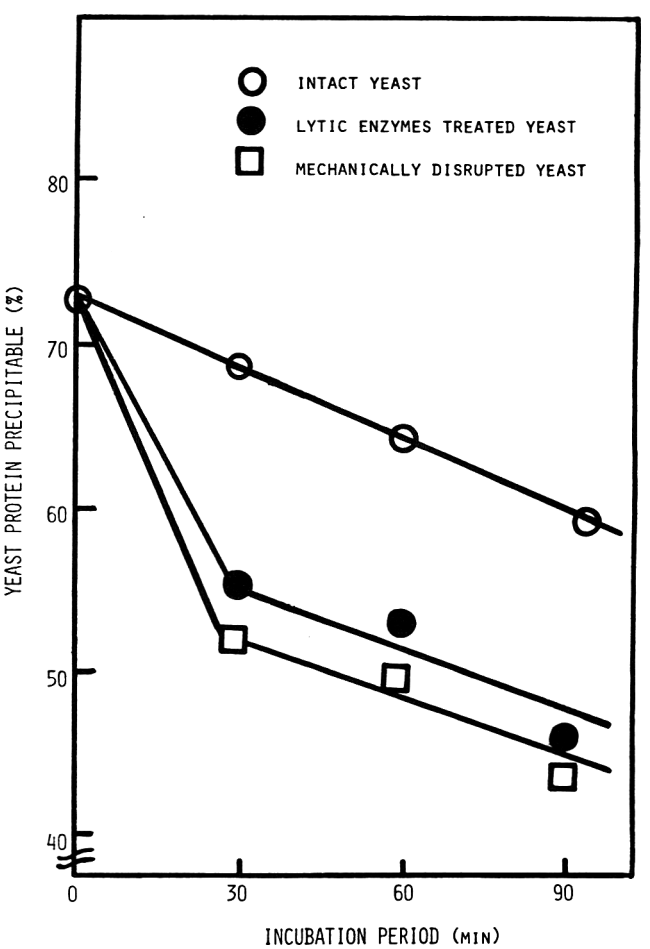

Fig. 3—Influence of incubation (pH 7.5 at 37°C) time on the amount of protein recovered from intact, lytic enzyme treated and mechanically disrupted yeast cells.

TCA was added, cooled in an ice-water bath, and centrifuged. An aliquot from the supernatant was used for estimating the amino groups. A standard curve was prepared using glycine. The amino acid content was expressed in glycine equivalents.

RESULTS & DISCUSSION

Cell integrity.

Electron microscopy was used to determine the integrity of the yeast cell wall after incubating with the lytic enzymes. The electron micrographs of yeast cells (Fig. 1a) showed intracellular constituents surrounded by an intact cell wall. Lytic enzymes hydrolysed the cell wall (Fig. 1b) and allowed the release of intracellular constituents where the cell wall was ruptured.

Effect of lytic enzymes on the release of nitrogenous material from yeast cell

The relative rates of release of total nitrogen and protein during incubation of yeast cell with and without lytic enzymes is shown in Figure 2. Addition of lytic enzymes during incubation of the yeast cells markedly increased the release of soluble nitrogenous material with incubation period.

More than 80% of the total nitrogen content of the yeast cell was solubilized within 90 min of incubation of yeast cells with lytic enzymes. This was almost double that released from untreated yeast cells. The release of soluble protein increased during the initial 60 min of incubation. The marked release of nitrogen with prolonged incubation period (> 60 min) resulted from the increased solubilization of nonprotein nitrogenous (e.g. RNA) components of the cells. The prolonged incubation of yeast cells with lytic enzymes may have caused the activation of endogenous proteolytic enzymes and hydrolysis of the intact protein (Hough and Maddox, 1970; Follows et al., 1971). The effect on the rate of autolysis with the addition of lytic enzymes supports the suggestion that in order to initiate the hydrolysis of yeast protein, intracellular membranes must be disorganized.

Proteolysis

The rate of proteolysis during incubation of yeast cells with lytic enzymes was monitored by determining the amount of acid (pH 4.5) precipitable protein. For comparison, intact yeast cells, and mechanically disintegrated yeast cells were also used. Protein was extracted at pH 9.0 from intact, enzyme treated, and mechanically disrupted cells after incubating for different intervals of times. In all cases the amount of protein precipitable at pH 4.5 decreased with incubation time; however, the extent of decrease varied with the different treatments (Fig. 3). About 72% of the proteins extracted from the unincubated yeast cells was precipitated at pH 4.5. This gradually decreased with increasing incubation period of intact yeast cells. A more significant decrease was observed with other treatments. The decrease in the precipitable protein at pH 4.5 presumably resulted from hydrolysis of proteins by intracellular proteolytic enzymes which were activated following disorganization of the cell. From these data the activation of the intracellular proteolytic enzymes is apparently related to the degree of disorganization of membranous structure of yeast cell (Fig. 3). The activation of proteolytic enzymes and extensive proteolysis during incubation of yeast cells with sulphydryl reagents has been reported (Shetty and Kinsella, 1978). However, in this study, it was observed that the rate of proteolysis of yeast depended not only on the disorganization of the cell wall but also on the extent of disruption of the cell.

The observation that the addition of lytic enzymes during incubation of yeast cells increased proteolysis suggested that this approach could be exploited for the rapid production of yeast autolysate. Therefore, in order to accelerate the hydrolysis of the protein, exogenous proteases were added during the incubation of yeast cells with lytic enzymes.

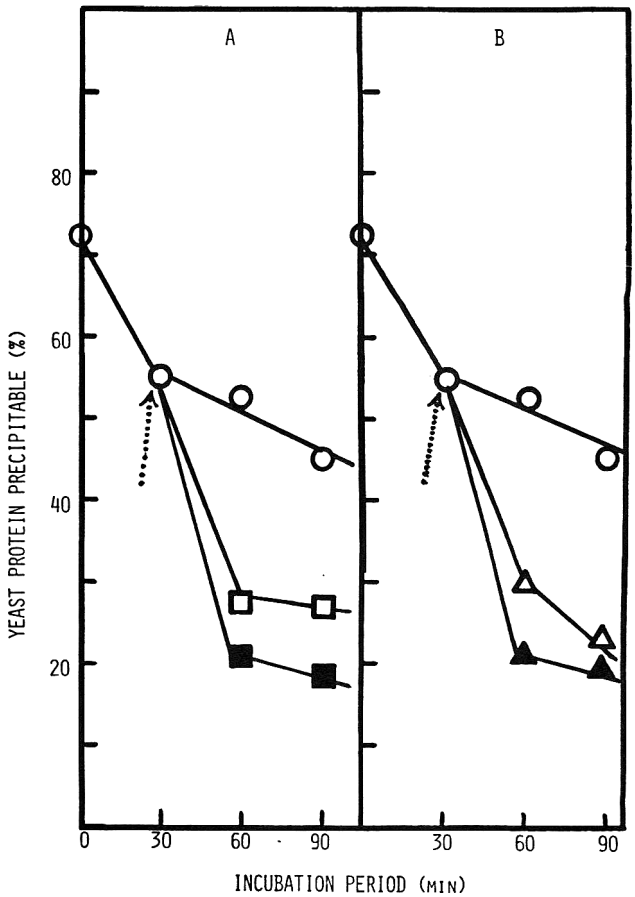

Fig. 4—Influence of lytic and proteolytic enzyme treatments on the amount of protein released from yeast cells following incubation. Arrows indicate addition of pancreatin (A) and pronase (B): ○ zymolase/lysozyme treatment; □ pancreatin addition (4 mg/g yeast); ■ pancreatin addition (8 mg/g yeast); △ pronase addition (1 mg/g yeast); ▲ pronase addition (4 mg/g yeast).

The addition of proteolytic enzymes significantly decreased the quantity of precipitable proteins (Fig. 4). Pancreatin and pronase added separately after 30 min of incubation caused hydrolysis of more than 80% of the protein within 60 min of incubation. Significant hydrolysis of yeast proteins was also observed at lower concentrations of pancreatin or pronase.

Hydrolysis was demonstrated by the release of the amino groups which increased steadily with increasing incubation time (Fig. 5). The amount of amino groups released indicated that only partial hydrolysis of intact yeast proteins (to polypeptide level) occurred during treatment with lytic enzymes of yeast cells. Addition of exogenous proteolytic enzymes greatly increased the release of the amino groups indicating that partially hydrolyzed yeast proteins were digested to a greater extent.

Effect of autolysis on RNA reduction

To determine if RNA was hydrolyzed concurrently by endogenous ribonuclease, the rate of ribose released from the yeast cell during incubation was monitored (Fig. 5). The release of ribose increased with increasing incubation period. The increase in the ribose content during incubation might have resulted from the release of the endogenous ribose of the cell or the hydrolysis of RNA by

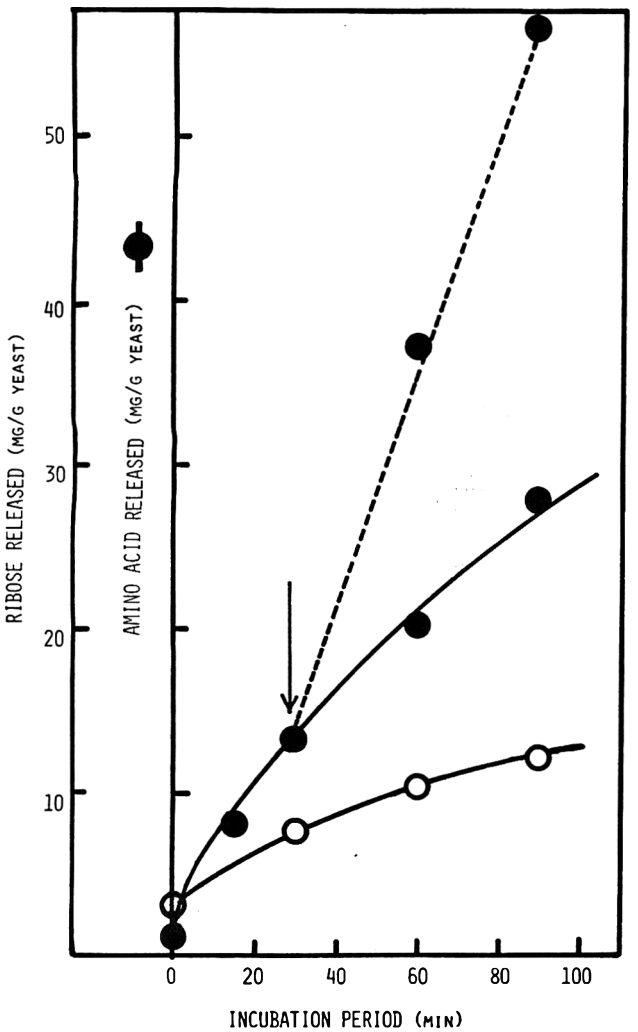

Fig. 5—Influence of incubation time on the release of ribose and of free amino groups from yeast cells after treatment with lytic and proteolytic enzyme. Arrow indicates point of addition of pancreatin.

endogenous RNase. The total amount of ribose in the mechanically disintegrated yeast cells was similar to that of untreated yeast cells. Hence the marked increase in the ribose content during incubation of yeast cell with lytic enzymes apparently resulted from the hydrolysis of RNA by the activated ribonuclease of the yeast cell.

Incubation of yeast cells with lytic and proteolytic enzymes results in the generation of soluble nitrogen compounds, i.e. formation of a yeast hydrolysate comparable to the yeast autolysate prepared by conventional methods (Johnson, 1977). Possible advantages of this treatment are low cost and adaptability to large scale operations. The activation of the intracellular proteolytic enzymes during hydrolysis of the yeast cell walls by lytic enzymes, coupled with the addition of exogenous proteases may be useful for the commercial production of yeast autolysates. The enzymes are inexpensive and the conditions energy saving in comparison to traditional methods of autolysate production (Johnson, 1977). The short incubation period of approximately one hour also minimizes the problem of microbial contamination and other cross contamination which can occur during prolonged incubation periods.

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DIFFERENTIAL THERMAL ANALYSIS (DTA) APPLIED TO EXAMINING GELATINIZATION OF STARCHES IN FOODS

KEIZO WADA, KOJI TAKAHASHI, KUNIO SHIRAI and AKIRA KAWAMURA

ABSTRACT

Gelatinization of isolated starches and starches in food sections can be examined under a wide range of conditions in an air-tight cell of the DTA apparatus. The specific gelatinization parameters of starches can be determined from endothermal peaks of DTA curves. Temperatures at the onset and peak of endothermal curves are characteristic of various starches. Gelatinization temperatures of starches in foods were substantially higher than temperatures of the isolated starches.

INTRODUCTION

VISCOSITY METHODS such as amylography, optical methods such as photopastegraphy, and microscopic examinations of the morphological and polarizing pattern changes are used to determine gelatinization temperatures of starches and examine their changes in physical properties. These methods measure individual parameters and have limitations. For example, amylography is not applicable to studying samples at concentration above 10%. Plastography requires large samples and is applicable to samples with concentration above 40%. Photopastegraphy is applicable only to samples with concentrations below 0.5% and is not suitable for quantitative examination. In all these methods, the examination is carried out in an open or semi-open system; consequently, considerable evaporation of water may change concentration of the sample during measurement. In addition, they are not suitable for determination of gelatinization temperatures above 100°C. The authors applied Differential Thermal Analyses (DTA) to the analysis of starch gelatinization to overcome the described defects and to extend the study by Stevens et al. (1971) who determined temperatures and heats of gelatinization by using Differential Scanning Colorimetry (DSC) and the study by Freke (1971).

Starch in food tissues is surrounded by various matrix components which likely affect gelatinization of the starch. Therefore, it is important to study starch in a food without isolating it from the other constituents. The authors examined gelatinization of *in situ* starch by using food sections.

MATERIALS & METHODS

Starch

Starches were obtained by triturating foods, sieving and washing exhaustively, and air-drying the slurry.

Section of food

Potato, Indian lotus, taro and sweet potato were used and sections 3 × 3 × 1 mm were collected for examination. Analytical data of the materials are shown in Table 1.

Table 1—Chemical composition of foods

Food	Moisture (%)	Nitrogen (%)[a]	Soluble solids (%)[a]
Potato	78.2	1.58	15.1
Sweet potato	73.7	0.60	25.3
Taro	82.2	1.73	25.5
Indian lotus	83.9	1.51	30.1

[a] Dry weight basis

DTA and polarizing pattern

Starch was steeped in distilled water for 20 hr at room temperature and about 10 mg of wet starch was sealed in an air-tight aluminum cell. DTA was carried out by using Shimadzu DT-30 at a heating rate of 5°C/min, a sensitivity of ±25 μV, and a chart speed of 5–10 mm/min, using water as reference. The polarizing pattern was examined at the onset of the heating stage, at the peak, and at the conclusion of the endothermal process in DTA under a polarizing microscope at a magnification of 400×. The same method was used to examine food sections by DTA and polarizing microscopy.

Amylography and photopastegraphy

Amylography was carried out on a Brabender DC-3 Amylograph by heating from 30°C at a rate of 1.5°C/min; the concentrations were 4% for potato starch and 8% for starches from wheat, corn, waxy corn, rice, tapioca and mung bean. For photopastegraphy the concentrations were 0.1% for starches from waxy corn and rice, 0.2% for starches from wheat, corn and tapioca, 0.3% for mung bean starch and 0.4% for potato starch by using transmittance as monitor.

RESULTS & DISCUSSION

DTA curves and polarizing patterns of starch

Figure 1 shows a typical DTA curve of potato starch

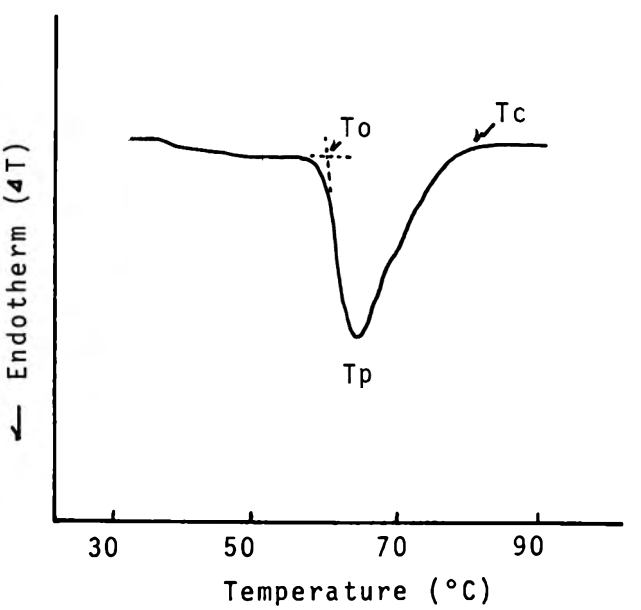

Fig. 1—Typical DTA curve of potato starch. Conditions: analyzer, Shimadzu DT-30; heating rate, 5°C/min; sensitivity, ±25 μV; reference, water; chart speed, 5 mm/min. To = onset temperature; Tp = peak temperature; Tc = conclusion temperature.

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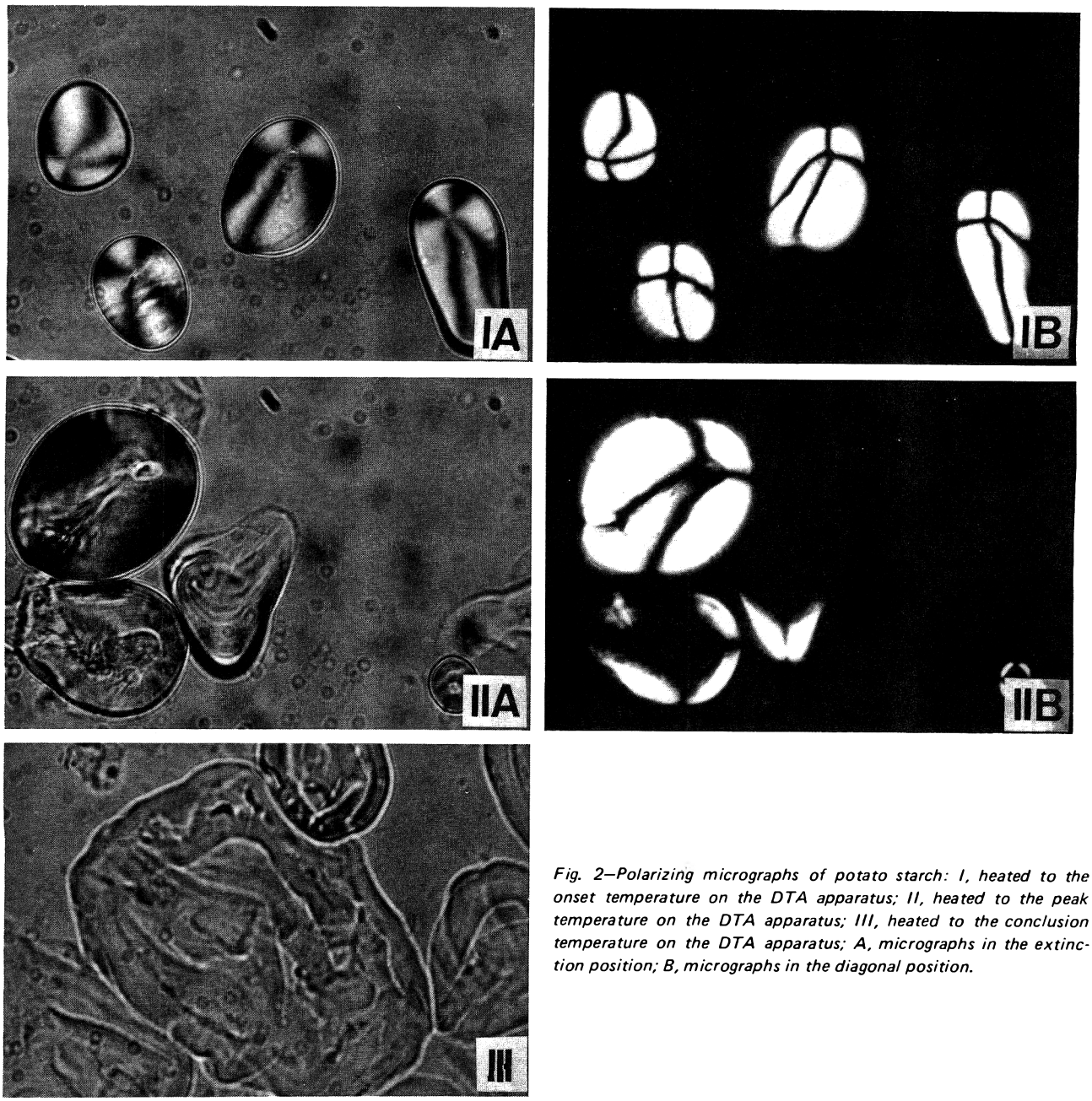

Fig. 2—Polarizing micrographs of potato starch: I, heated to the onset temperature on the DTA apparatus; II, heated to the peak temperature on the DTA apparatus; III, heated to the conclusion temperature on the DTA apparatus; A, micrographs in the extinction position; B, micrographs in the diagonal position.

when it is heated on DTA apparatus. It indicates an endothermal depression that takes off at about 60°C, reaches the peak at about 65°C and recovers the base line at about 80°C. In this paper, the intersection of the largest depression slope by the base line is referred to as onset temperature (To), the base point as peak temperature (Tp) and the point of return to the base line as conclusion temperature (Tc).

Figure 2 shows polarizing micrographs of potato starch heated to temperature To, Tp and Tc on DTA. No change in polarizing pattern and shape of starch particles occurred at To. At Tp, the potato starch particles swelled, were somewhat deformed and had no polarizing pattern at Tc; this indicates the completion of gelatinization of Tc. From these results it is concluded that the DTA curve is suitable to follow gelatinization patterns of starches.

DTA of starches

Typical DTA curves of nine starches are shown in Figure 3. These graphs indicate the uniqueness of the starches with regard to transition temperatures and shapes of the endothermal peaks. Consequently, it is suggested that gelatinization of starches can be characterized by DTA. We then applied DTA to nine starches and measured To, Tp and Tc. The results are shown in Table 2. Standard deviations of To and Tp values are negligible compared to differences between starches and To and Tp values and can be considered characteristic temperatures of starch gelatinization.

In a subsequent experiment, we found that varying heating speed from 2 to 15°C/min, the sample size from 1.8 to 14.3 mg, and sample concentration from 2.6 to 50.6% had no significant effect on To values.

Comparison of DTA with other methods

Temperatures of nine starches measured by DTA, amylography, and photopastegraphy are given in Table 3. Although characteristic DTA temperatures substantially differs from those by amylography and photopastegraphy, the To value by DTA is relatively near the value of photopastegraphy and the Tp value is near values of initial and 20 BU

of amylography. It is tentatively concluded that the DTA curve in the region of To reflects structural changes of starches in the initial gelatinization stage and that in the region higher than Tp, DTA reflects changes that correspond to a viscosity increase.

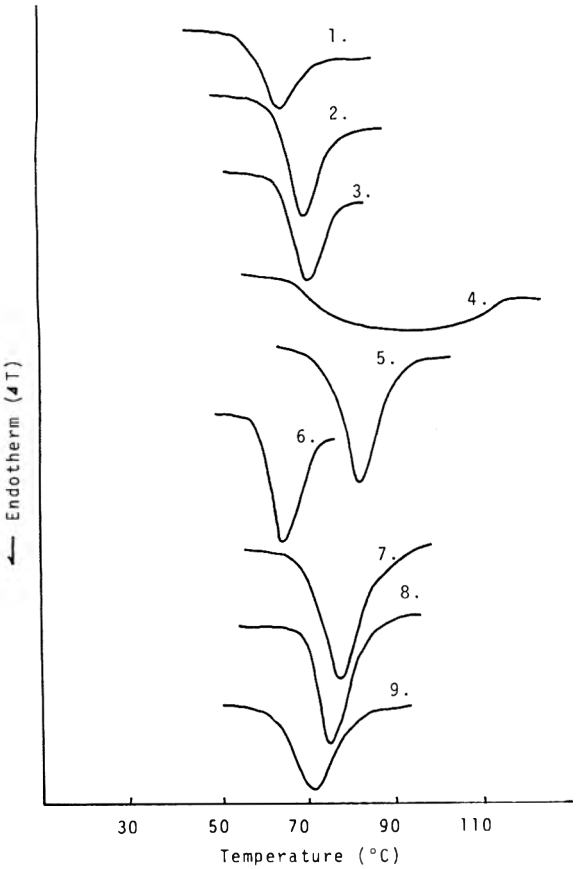

Fig. 3—DTA curves of starches from: (1) wheat, (2) corn, (3) waxy corn, (4) high amylose corn, (5) rice, (6) potato, (7) sweet potato, (8) tapioca, (9) mung bean. For conditions, see Fig. 1.

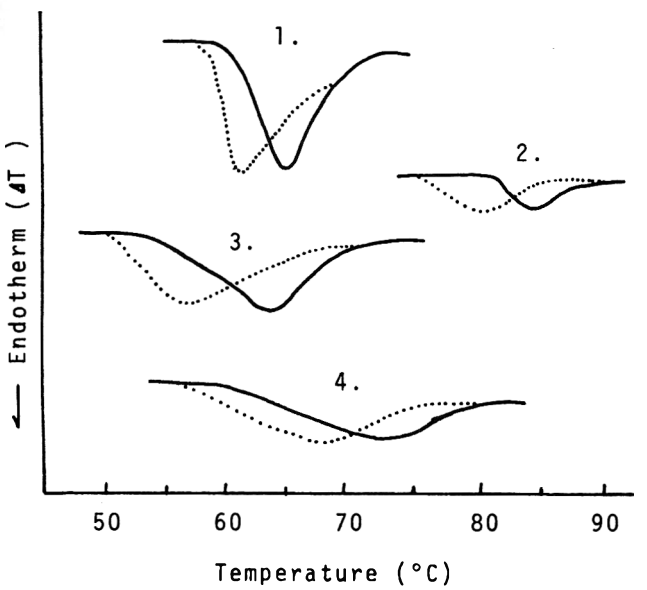

Fig. 4—DTA curves of food sections compared with those of starches isolated from the foods: (1) potato, (2) taro, (3) Indian lotus, (4) sweet potato. Solid lines, food sections; dotted line, isolated starches. For conditions, see Fig. 1.

DTA of food sections

Figure 4 shows DTA curves of sections from potato, Indian lotus, taro and sweet potato and curves of starches isolated from the foods. Curves of starches indicate characteristic patterns and specific gelatinization temperatures. Each curve of the food sections overlaps closely the curve of the corresponding isolated starches after it is shifted by several degrees to a lower temperature. Polarizing micrographs of potato, Indian lotus and sweet potato heated to the stage of To, Tp and Tc on DTA apparatus demonstrated such changes in the pattern of starches accompanying a rise in the heating temperatures from To to Tc as indicate modifications described for heating potato starch (Fig. 2). The results confirm that the curves of food sections represent gelatinization changes of starches in the foods.

Table 4 compares To values of food sections with those of starches. To values of food sections are higher than those of starches by 3.1°C for potatoes and 6.2–7.3°C for the

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Table 2—DTA endothermal temperatures of nine starches

Starches	Avg value (°C)			Std dev (°C)		
	To	Tp	Tc	To	Tp	Tc
Wheat	56.2	64.3	82.6	0.88	1.06	3.79
Corn	63.1	70.1	80.4	0.86	1.05	4.10
Waxy corn	63.9	71.0	85.3	0.63	0.36	4.96
High amylose corn	67.2	89.1	112.9	1.20	1.48	1.64
Rice	69.4	76.3	88.8	0.65	0.56	2.53
Potato	58.9	65.2	79.7	1.21	0.82	5.28
Sweet potato	69.4	76.9	88.9	1.28	0.52	5.59
Tapioca	66.9	73.4	85.8	0.96	1.01	3.08
Mung bean	62.1	71.8	88.6	1.31	1.11	2.21

Table 3—Comparison of gelatinization temperatures by DTA with those by other methods

Starches	DTA (°C)			Amylography (°C)			Photo-paste-graphy (°C)
	To	Tp	Tc	Initial	20BU	Peak	
Wheat	56.2	64.2	82.4	–	–	–	53.2
Corn	63.1	70.1	80.4	70.7	76.1	87.5	61.6
Waxy corn	63.9	71.0	85.3	67.5	69.1	73.4	60.3
Rice	69.4	76.3	88.3	73.5	75.6	93.0	60.0
Potato	58.9	65.2	79.2	62.6	63.2	73.6	58.3
Tapioca	66.9	73.4	85.3	68.4	69.0	73.3	62.5
Mung bean	62.1	71.8	88.6	71.0	73.0	82.9	63.5

Table 4—Gelatinization temperature of starches in food compared with those of isolated starches

Foods and starches	Avg value[a] (°C)	Std dev (°C)
Potato section	60.6	1.33
isolated starch	57.5	1.49
Indian lotus section	58.0	1.36
isolated starch	51.7	0.45
Taro section	81.6	1.00
isolated starch	75.4	0.72
Sweet potato section	60.5	1.78
isolated starch	53.2	1.07

[a] Onset temperature of endothermal peak by DTA

A DYE DIFFUSION TECHNIQUE TO EVALUATE GEL PROPERTIES

G. CURTIS BUSK JR. and THEODORE P. LABUZA

ABSTRACT

A diffusion method is described which can be used to evaluate the properties of macromolecular gelling agents. A dye solution is layered onto the top of a gel prepared to different concentrations and the solution is allowed to diffuse until a concentration gradient has formed. The concentration gradient as a function of distance and time is then evaluated using a scanning densitometer. From these results the apparent diffusion coefficient of the dye using Fick's second law is calculated. Care must be taken when evaluating gel systems using this method to account for dye:polymer interactions and to minimize polymer gel swelling. From the diffusion coefficient an evaluation of the structure of the gel can be made with respect to its degree of spacing.

INTRODUCTION

TAYLOR et al. (1936) was one of the first researchers to note that polymers in solution act as molecular sieves. He noted that the sieving effect of polymer solutions reduced the rate at which molecules diffused through the solution and that the diffusion process in rubber followed Ficks' First Law of diffusion as shown in Eq (1):

$$F = -D\frac{\delta c}{\delta x} \qquad (1)$$

where: F = rate of transfer per unit area of section; D = diffusion coefficient of the molecule; and $\delta c/\delta x$ = concentration gradient measured normal to the section. Taylor et al. (1936) also noted that the diffusion coefficient (D) generally decreased rapidly with increasing polymer concentration and decreased as the concentration of the diffusing material increased in the gel. If the diffusant molecule reacted with the polymer (e.g., adsorption processes) D was not easily predicted due to "strong spreading forces" induced by the adsorption process.

Unsteady state diffusion in which the concentration of the molecule builds up in the gel is best described by Ficks' Second Law. If the diffusion is limited to one dimension, Ficks' Second Law is:

$$\frac{\delta c}{\delta t} = D\frac{\delta^2 c}{\delta x^2} \qquad (2)$$

where: c = concentration of diffusant; t = time; D = diffusion coefficient; and x = distance. The solution to this equation depends on the system geometrics and the initial and boundary conditions determined in the specific method used to measure the diffusing species.

Spacek and Kubin (1967) reported on a device for measuring the diffusion of KCl in gels held between two plates. Eq (2) was intergrated to give a converging series expansion in which a parameter of the concentration difference $[\log(c_2 - c_1)]$ between the diffusant in the gel (c_2) and exterior to the gel (c_1) is plotted vs time to give a straight line. A conductometer was used to measure the salt concentration. It was found that in methacrylate gels the diffusion coefficient for the salt became decreased to a constant above about 30% gel solids.

Wood (1966) studied the diffusion of sodium chloride in pork muscle using a system in which the total salt uptake by the muscle is plotted vs $(\text{time})^{1/2}$ to give a straight line. The apparatus uses a tube of material above which the salt solution is placed. Del Valle and Nickerson (1967) used a similar method for fish muscle and found that the diffusion coefficient for salt went through a minima at about 1–2 moles/liter indicating some change in the fish muscle three dimensional structure.

In a study of diffusion of developing materials in gelatin thin layers, such as for photographic film, Iwano (1969) used a form of Eq (2) in which the diffusing salt reacted to form a layer in the gel. In this case the square root of the time to penetrate a certain thickness gives a straight line from which the diffusion coefficient can be found. Iwano (1969) found little effect of pH but a significant decrease in the diffusion coefficient with increased molecular size. Diffusion was not studied as a function of gel concentration. Polson and Parkyn (1969) used a similar mathematical approach using a special rotating horizontal tube in which a thin gelatin layer was suspended. The diffusion coefficient of sucrose was 25×10^{-7} cm^2/sec and of ovalbumin was 7.5×10^{-7} cm^2/sec in 0.5% agarose gel.

Nixon et al. (1967, 1968) and Georgakopoulos and Nixon (1969) have done extensive studies on diffusion of the dye methylene blue in gelatin. In their technique the dye is made in the gel and the gel is then placed in water. As the dye diffuses out, samples of the water solution are measured spectrophotometrically. The diffusion coefficient was found by plotting the log of the concentration in the solution vs reciprocal time which is an empirical solution to Eq (2). Their results show a linear decrease in diffusion of methylene blue as a function of gelatin concentrations between 10–16% ($D \approx 5 \times 10^{-7}$ cm^2/sec). Addition of glycerol over the range 0–60% caused a maxima in methylene blue diffusion at about 10% which was attributed to structure and viscosity effects. They did not correct for any diffusion of water into the gel which could change its properties.

In all the above cases special apparatus designs were required or the change in concentration as a function of time is needed so that some form of Ficks' Second Law can be used. Another way to measure diffusion is to determine the concentration gradient at any point in time in the gel. Crank (1965) has published a solution for this in the form of Eq (3) which may be easily applied to diffusion of dyes through spectrophotometric measurement.

$$D = -\frac{\frac{1}{2t}\int_0^{c_1} x\,dc}{\delta c/\delta x} \qquad (3)$$

where: D = diffusion coefficient; t = time of diffusion; c_1 =

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Table 1—Dye diffusion results—Gelatin

Gel conc (g/100g H_2O)		
As formulated	Measured after the diffusion experiment (mean value)	Dye diffusion coefficient D X 10^8 ($cm^2 sec^{-1}$)
10	10.05	7.6
10	10.12	7.6
15	13.32	6.7
15	13.51	6.3
20	16.95	5.1
20	17.10	5.5
25	20.10	4.2
25	20.17	3.8
30	22.32	3.8
30	22.37	3.5
35	24.62	2.8
40	25.64	2.8
40	26.27	2.5
50	27.22	2.7
50	28.28	2.7
60	30.69	2.1
60	33.13	2.0

Table 2—Dye diffusion results—κ-Carrageenan

Concentration (% db)	D X 10^6 ($cm^2 sec^{-1}$)
0.69	0.8
0.71	1.2
1.20	0.6
1.26	1.0
2.12	0.7
2.18	0.7
2.31	1.1
2.35	1.3
2.99	0.7
3.01	0.7
3.18	0.8
3.21	0.7
3.98	0.7
4.06	0.7
4.12	0.7
4.16	0.7
4.78	0.6
4.96	0.8
5.07	0.6
5.10	0.7
5.69	0.5
5.87	0.6
5.97	0.6
6.20	0.6

Table 3—Dye diffusion results—Agar

Concentration (% db)	D X 10^6 ($cm^2 sec^{-1}$)
1.16	2.1
1.18	1.7
2.09	2.3
2.12	1.2
3.03	1.3
3.10	1.3
4.17	0.8
4.20	0.9
5.12	0.8
5.15	0.8
6.15	0.8
6.84	0.6
7.33	0.7

concentration of diffusant at distance x which is at midpoint according to Crank (1956); x = distance to diffusion plane from c = 0; $\delta c/\delta x$ = concentration gradient of diffusant at c_1; and xdc = area under curve from c_1 to c = 0. A graph of c versus x at time t will then have all the necessary data to evaluate D according to the method of Crank (1956, pages 232–233). Crank (1956) points out that this is an apparent diffusion coefficient, it is not equal to D_0—the diffusion coefficient of a diffusant in pure polymer. Use of this method of integration simplifies the design and does not require any assumptions as is needed for the other mathematical solutions.

Duckworth (1962) was one of the first researchers to use diffusion methods to evaluate food polymer systems. He applied labeled glucose to carrot and potato pieces and stored them at various relative humidities. In this way, a "mobilization point" with respect to a_w was established. Duckworth noted that diffusion rates at equivalent a_w's were faster for carrots than for potatoes, however he did not present his data in such a way that the diffusion coefficient (D) could be evaluated.

Laurent (1963) stated that the pore size of a polymer network could be evaluated by measuring diffusion coefficients. In this way he established that, between 4 and 8% concentration of agar gels the average pore size is 50 Å in diameter. This work was not carried out on other gels however.

Gillespie and Williams (1966) and Karel (1976) have both stated that diffusion data may be used to characterize gels. Karel (1976) states that reductions in D are due to local viscosity effects as well as molecular obstructions to diffusants. He reports diffusion coefficients for food materials ranging from 1.45×10^{-5} cm^2 sec^{-1} to 2×10^{-8} cm^2 sec^{-1}.

Blanshard (1970) has studied the diffusion of water in a starch:water system. He showed that a 20% starch gel reduced D_{H_2O} by a factor of 100 from that of pure water. Nakayama and Jackson (1963) have also reported diffusion of water in gels.They report that a 1% solution of agar reduces D_{H_2O} to a value of 2.2×10^{-5} cm^2 sec^{-1} from a value of 2.4×10^{-5} cm^2 sec^{-1} in pure water.

From the above discussion it is obvious that the diffusion coefficient of molecules in a gel is an intrinsic property which can be used to evaluate a polymer gel macrostructure. However, few researchers have used this property and even fewer have evaluated the diffusion coefficients in more than a single polymer system.

MATERIALS & METHODS

Materials

Three macromolecular gelling systems were selected for this research. The gels evaluated include gelatin (General Gelatin–250 Bloom, lots #09552 and 09974), carrageenan (Marine Colloids–kappa fractions, lot #RE-7691) and agar (Tragacanth Importing Company–agar-agar, lot #61476). The concentration of gel levels ranged from about 0.5% water basis (wb), which was the minimum necessary to obtain a gel at room temperature, to as high as 60% depending on the gel.

Gels were prepared by modifications of procedures reported by Persidsky and Luyet (1959). The powder was weighed and suspended in deionized-distilled water at room temperature to form a slurry. At high concentrations (e.g., greater than 40% for gelatin) this process was aided by mixing in a Brabender Model D 3002 prep center (C.W. Brabender Instruments, Inc., South Hackensack, NJ) equipped with a Farinograph opposed screw sigma blade mixing bowl. The slurry was then heated using a magnetic mixer, to 65°C for gelatin, 80°C for carrageenan and 98°C for agar. When it was not possible to agitate magnetically, the samples were removed from the heating medium and stirred by hand for 5 min. The samples were returned to the heating medium and the process repeated until the slurry dissolved. After total solvation, as indicated by complete translucence of the solution, the samples were removed from the

heating medium and transferred into test tubes (15 mm × 150 mm). The samples were then returned to the heating medium and held for a sufficient length of time to allow for complete deaeration indicated by the absence of visible air bubbles.

After deaeration, the samples were cooled in an ice/water bath for 2 hr. They were then transferred into a 6°C cooler for aging an additional 24 hr before subsequent evaluation.

Methods

Moisture contents for this study were evaluated using the vacuum oven method. Samples were weighed to an accuracy of 0.01 mg, dried for 24 hr in a vacuum oven at 60°C and a vacuum of 750 mm Hg.

The apparent diffusion coefficients of the gels were determined by warming the gels to room temperature, layering 4 ml of a dye solution on the top, allowing a concentration gradient to form and then reading the gradient with a Quick-Scan Fluro-Densitometer (Helena Laboratories, Inc.)

A standard dye solution of approximately 1% was prepared by mixing solid amaranth dye (lot #Y1595, H. Kohnstamm and Co.) with deionized distilled water buffered to a pH of 4.01. The solution was buffered to this pH to minimize the dye:macromolecule ionic interactions. The exact final concentration was evaluated using serial dilutions and measuring the absorbance at 523 nm on a Coleman III single beam spectrophotometer (Hitachi, Perkin-Elmer).

After the dye was layered onto the top of the gel, the tube was stoppered and stored at room temperature. Characteristic times for the formation of concentration gradients which could be used were 12–18 hr for agar and carrageenan and 1–2 wk for gelatin. The tube size used for the gel was selected to fit in the densitometer for direct scanning.

The diffusion gradient was recorded by setting the flouro-densitometer at manual zero, fast scan speed, wide light beam slit and using a 525 nm light filter. The slope of the resultant gradient curve can then be determined in Figure 1 from the linear portion of the curve where the concentration decreasing. The absorbance of any point can be measured in the gel to get a value of c_1. From this and the time at which these values are measured, the apparent diffusion coefficient is easily calculated by obtaining the area under the curve of c vs. x from the value of c_1 to where it becomes zero. In this case the midpoint was chosen and D was evaluated only at that concentration.

RESULTS & DISCUSSION

THE APPARENT DIFFUSION coefficients of the dye amaranth (Red No. 2) in gelatin, κ-carrageenan and agar gels are shown in Tables 2 to 3. Table 1 lists the concentration of gelatin to which each gel sample was originally formulated as well as the mean concentration of each sample measured at the conclusion of the diffusion experiment. With the exception of the 10% solids concentration, all of the gelatin samples were more dilute at the conclusion of the experiment (measured concentration) than at the beginning of the experiment (formulated concentration). This indicates that all of the gelatin samples studied absorbed water, as well as dye, from the dye solution placed above it. This swelling phenomenon is similar to that reported by Ferry (1948). The D values reported therefore are probably somewhat larger than they would be if it were possible to conduct the experiment at constant concentrations. The long times necessary to carry out the gelatin experiments (469 hr) no doubt contributed greatly to this swelling phenomenon. An uptake of water was not found to occur in either the carrageenan or the agar systems during the 18–24 hr required to develop the concentration gradients in these systems. The change in gel concentration due to water diffusion represents one of the largest sources of error with this method. The absorption of dye solution water leads to changes in both the concentration of the gel as well as changes in the gel structure due to swelling.

Because of the swelling phenomenon outlined above, the gelatin dye diffusion coefficients and those for carrageenan and agar should not be compared on an absolute basis. The results for gelatin however, can be analyzed as a function of initial concentration (10%) as compared to other concentrations. An initial concentration of 10% solids was selected for gelatin because it was the lowest concentration which would form a gel discrete enough to support the dye solution at room temperature.

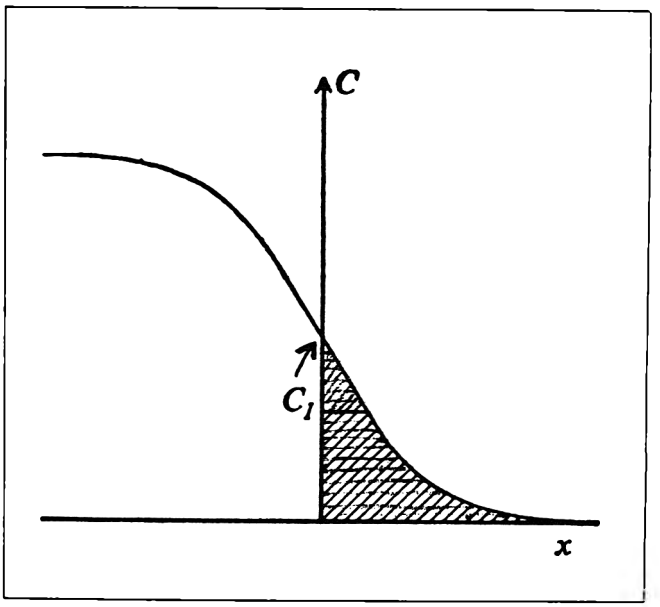

Fig. 1—Concentration (absorbance) vs diffusion distance (x) for dye penetration in gel. At C_1 the diffusion coefficient is evaluated by the shaded area.

It is evident from the results in Table 1 that, at high gel concentrations, the diffusion coefficient for the dye becomes less dependent upon gel concentration. This indicates that the network structure of gelatin gels changes less at high concentrations upon addition of a given amount of gelatin than it does if the same amount is added to a low solids content gelatin gel. This conclusion is supported by model studies if it is assumed that the gelation mechanism is random coil→low energy helix→high energy helix as reported by Eldridge and Ferry (1954). At higher concentrations the chance of high energy helical structures (which are more dense) forming initially should be greater, thus at high concentrations additional polymer would have less effect on the gel structure than it would have if added to a low concentration gel. This same conclusion was reported by Lewicki et al. (1978) and Busk (1978) evaluating gelatin gels by pulsed NMR and suction pressure. As the concentration of gelatin increases, at high gelatin concentrations gelatin:gelatin interactions increase much faster than gelatin:water interactions.

The results for κ-carrageenan (Table 2) and agar (Table 3), (neither of which swelled during the experiment), also show that there are gel concentration dependent effects in these systems. Below about 2–3% solids the diffusion coefficients for carrageenan and agar are highly variable. As the concentration of polysaccharide increases past this range the diffusion coefficients become much less variable indicating increased ordering of the gel structure. If, at low concentrations, these polymers form gels which are a mixture of low and high density helical regions (Arnott et al., 1974), and since the relative concentration of each density region is very dependent upon the temperature history of the sample during gel formation, then one could expect that it would be very difficult to obtain a reproducible diffusion coefficient at low polysaccharide concentrations. If the formation of these helix regions is cooperative then the diffusion coefficient should become more reproducible at higher concentrations.

—Text continued on page 1372

These results also show that diffusion of the dye in carrageenan becomes increasingly restrictive as concentration increases while the diffusion coefficient of agar levels off at about four percent solids. The carrageenan:water interaction results reported in the literature (Lewicki et al., 1978) show increasingly higher interaction energies at high concentrations while the agar results consistantly supports the data published by Obrink (1968), that agar gels at high concentrations have stable pore sizes which are relatively independent of the gel concentration. Finally, in a comparison between gels it is seen that the rate of diffusion of the dye is about 100 times greater in agar and carrageenan than in gelatin. This could be due to absorption on the gelatin or ionic interaction. There seems to be very little difference in magnitude between the agar and carrageenan even though in carrageenan about 25% of the monomers have an $SO_4^{=}$ group. At the pH of the diffusing solution this charge must be neutralized.

CONCLUSION

THIS PAPER describes a method by which the apparent diffusion coefficients of gels may be measured. The coefficients reflect intrinsic concentration dependent properties of the macromolecular structures of the systems studied and may be used to evaluate relative porosities. The apparent diffusion coefficients recorded as a function of concentration also gives some insight into the development of the microstructure of these systems.

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DTA FOR EXAMINING GELATINIZATION OF STARCHES . . . From page 1368

other foods. A possible explanation is that the difference is from the effect of the matrix in which the starches are imbedded; that matrix includes considerable amounts of soluble solids (see Table 1).

To values of potato sections and potato starch were measured in solutions that contained various sodium chloride, sucrose or ethyl alcohol. To in 20% sodium chloride was 10–12°C lower than To of the control (in distilled water); To in 30 or 50% sucrose, or 50% ethyl alcohol, was higher than the To of the control. This is likely the result of instabilization of the starch micelle structure by sodium chloride and the suppressed reaction between starch and water caused by the strong hydration of sucrose and ethyl alcohol.

CONCLUSION

DTA of isolated starches and food sections in an air-tight cell can be used to analyze gelatinization under a wide range of conditions. Gelatinization parameters of isolated starches *in situ* differ. The starch-species specific parameters are affected by the presence of high concentrations of sucrose, sodium chloride, and ethanol.

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GAS CHROMATOGRAPHIC CHARACTERIZATION OF OREGANO AND OTHER SELECTED SPICES OF THE LABIATE FAMILY

HAN Y. RHYU

ABSTRACT

The Labiate family includes many common spices, some so similar that differentiation can be difficult. Available literature on the individual spices does not take into consideration the lot-to-lot variances and does not aid much in differentiation. All major Labiate spices procured from various areas during 1971–1978 were ground and steam-distilled in the laboratory. Gas chromatographic profiles show no differences in qualitative composition of monoterpenes but only in their quantitative distribution. Correlation and multiple correlation coefficients suggest further similar chemistry of compound formation throughout the Labiate family during the life cycle of the plant. Differentiation between Greek and Mexican oregano is demonstrated by the use of linear discriminant function.

INTRODUCTION

THE MEMBERS of the Labiate family that are most commonly used for culinary purposes are sweet marjoram (*Marjorana hortensis* M.), oregano (*Origanum spp.* or *Lippia spp.*), peppermint (*Mentha piperita* L.), spearmint (*Mentha spicata* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L. or *S. triloba* L.), savory (*Satureia hortensis* L.), sweet basil (*Ocimum basilicum* L.) and thyme (*Thyme vulgaris* L.). Although the Federal Specification (Anonymous, 1975a) on oregano recognizes only *Origanum vulgare* L. or *Origanum spp.*, which is generally grown in the Mediterranean region, especially in Greece, a more pungent Mexican oregano, *Lippia spp.* of the Verbena family, is also widely used in the United States, according to governmental statistics (Anonymous, 1975b). While most of these spices have distinctive characteristics in aroma and appearance in unground form as available in bulk trade, a few of them are surprisingly close to one another once they are pulverized. Thus, the Greek oregano cannot always be differentiated readily from the Mexican type. Ground marjoram can be mistaken for oregano, and ground basil, savory and thyme display only subtle differences.

Many of the published studies, such as the ones by Ikeda et al. (1962) on some essential oils and those on oreganum oil by Calzolari et al. (1968), Brieskorn and Brunner (1967), Maarse and van Os (1973) and Buil et al. (1977) have contributed a great deal to an understanding of the composition of the essential oils; however, they do not aid much in differentiation of the Labiate spices currently available in the United States. This is because some of these studies have dealt exclusively with the commercial essential oil, for which the authenticity of the raw material is not always known, while the others deal with the species not currently in use in this country. Moreover, to date, no studies have been published in which the Labiate family is treated as a group, or in which the lot-to-lot variations of a given species are considered. In this paper, therefore, we present gas chromatographic profiles obtained under identical conditions that may be useful for differentiation of several of those Labiate spices listed at the outset of this paper. For obvious reasons, we have eliminated peppermint and spearmint.

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EXPERIMENTAL

A NUMBER OF LOTS of spices (lot sizes varying 10,000–40,000 lb) obtained during 1971–1978 from the various areas of the world through the normal commercial channels were sampled by the ASTA schedule (ASTA, 1968) and ground to pass a screen of 1 mm openings using a Wiley laboratory Mill, Model 4. It should be mentioned that although every attempt was made to assure the authenticity of the products, especially through statistical sampling, the large size lots can become contaminated with different lots in commercial distribution. The ground samples were steam-distilled (ASTA, 1968) within a day after being ground. The volatile oils thus obtained were stored in glass vials with Teflon-lined caps and kept away from light until analysis by GC, usually within 0–6 months. A Varian GC Model 2704 with flame ionization detector was fitted with a 0.01 in. × 200 ft stainless steel capillary tubing (Applied Science Laboratories, Inc., State College, PA), coated with Carbowax 20M by the method of Teranishi (1971). The operating conditions were: column at 90°C for 4 min, linear temperature programmed at 4°/min to 180°C and held; injector and detector at 250°C; nitrogen (carrier gas) flow 1.9 ml/min, hydrogen 25 ml/min, air 300 ml/min and nitrogen make-up gas 17 ml/min; split ratio 1:74; volume injected 0.1 μl. The peaks were recorded with a Varian model A-25 recorder at 1 mV span, 0.25 in./min and integrated with a Varian Data System CDS 101. Most of the major peaks were identified by peak enrichment method as previously reported (Rhyu, 1979). Whenever identities of minor peaks in one spice to another or in one lot to another within a spice were questionable, repeat runs were made at much higher sensitivity by injecting the oils singly or blended. Absence of an extraneous peak on the blended oil, or identity of the retention times of the singly-run pairs was not necessarily taken as evidence for identity of the peaks, but rather as an additional element to the major peaks useful for general pattern comparisons.

RESULTS & DISCUSSION

THE CHROMATOGRAMS of the Greek and Mexican oreganos shown in Figure 1 are typical of those of the Labiate spices studied in this report. In most cases, the chromatograms were identical, down to the trace peaks, with regard to the number of peaks detected and their retention times, but varied greatly in the quantitative distribution of the peaks. The precision of the GC analysis in terms of coefficient of variability (ratio of standard deviation to mean) was in the order of 0.01, never exceeding 0.05 for any of the peaks.

All samples analyzed are listed in Table 1. This table includes, out of more than 100 peaks detected, only those peaks for which at least one of the samples shows more than 0.1% by area. The "+" symbol indicates presence, but in less than 0.1%. The first observation to be made in this table is that all peaks from peak 4 to carvacrol are present, as mentioned above, in every sample with very few exceptions, the major one being peak 17-0 of which 2 oregano samples showed a considerable amount while no other spices showed any.

The second observation is the enormous lot-to-lot varia-

tions of the quantitative distribution within a type. For example, thymol for the Greek oregano varied 0.9–26.7% and for the Mexican oregano, 0.7–40.2%. Yet, the data taken as a whole clearly distinguish the seven spices from each other. This is more apparent in Figure 2, where the histograms were constructed by averaging those peaks present in more than 5%. Consider marjoram and the Greek oregano in this figure. We note that marjoram is considerably higher than the Greek oregano in sabinene, α-terpinene, cineole, γ-terpinene, terpinolene and especially in terpinen-4-ol. Also, marjoram has very little carvacrol whereas it is the major component in the Greek oregano. As expected, the same spices grown in different areas remain much closer to each other than to the different spices of the Labiate family.

Although both the Greek and Mexican oreganos displayed a great deal of lot-to-lot variation, as with *the other* spices, the z values calculated from linear discriminant func-

Table 1—Composition of steam volatile oil of selected Labiate spices, % by peak area

	Lot no.	4	7	α-pinene 9	camphene 11	β-pinene 12	sabinene 13	13-1	13-3	myrcene 17	17-0	α-phellandrene 17-1	α-terpinene 20	d-limonene 22	1,8-cineole 23	γ-terpinene 26	p-cymene 29	terpinolene 30	32	thujone 37	38
MARJORAM																					
Chilian	9097	2.9	1.6	0.7	0.1	0.1	2.4	+	+	1.2	0	0.6	7.1	0.9	1.4	12.6	2.6	2.2	+	0	+
Chilian	4794	2.9	1.8	0.1	0.1	0.2	3.6	+	+	1.8	0	1.7	8.4	1.2	2.5	15.9	3.0	2.8	+	0	+
Egyptian	5367	28.1	18.1	1.0	0.1	0.5	2.4	+	+	0.7	0	0.3	10.1	0.9	6.4	8.6	0.9	1.9	1.2	0	+
Egyptian	4463	3.0	1.7	1.3	0.1	0.3	4.5	+	+	1.5	0	1.1	8.4	1.9	3.7	14.8	4.2	3.3	0.7	0	+
Domestic	5530	41.7	26.7	0.2	+	+	0.4	+	+	+	0	0.2	7.2	0.3	0.6	3.0	0.7	0.7	1.5	+	+
OREGANO																					
Greek	8069	5.4	3.3	0.2	0.1	0.1	+	+	+	+	0	+	0.4	0.2	0.3	1.4	2.7	+	0.2	0.1	+
Greek	5414B	22.8	14.5	0.3	0.1	+	+	+	+	0.5	4.6	0	0.7	0.1	0.2	3.4	5.1	0.1	1.1	0.2	+
Greek	5414A	20.4	12.6	0.3	+	+	+	+	+	4.4	0	+	0.5	0.1	0.2	2.8	4.5	+	1.0	+	+
Greek	8087	6.7	4.2	0.4	+	+	+	+	+	0.4	0	+	0.3	0.1	0.2	1.3	4.0	+	0.3	0.3	+
Greek	76204	+	0.2	0.2	+	+	+	+	+	0.3	0	0.1	0.3	0.2	0.1	0.2	3.5	+	+	0.1	0.1
Greek	90143	0.5	0.3	0.7	0.2	0.1	+	+	+	0.8	+	0.1	0.8	0.2	0.3	3.8	6.0	0.1	+	0.4	0.1
Mexican	76274	0.1	0.1	0.7	0.2	0.2	+	+	+	2.0	0	0.5	1.1	0.6	4.5	5.4	9.7	0.2	0.1	0.1	+
Mexican	8475	0.8	0.6	0.2	0.1	0.1	+	+	+	0.9	0	0.2	0.5	0.4	6.5	3.0	6.2	0.1	0.2	0.1	+
Mexican	9167	0.9	0.6	0.8	0.6	0.2	+	+	+	1.4	0	0.3	0.8	1.1	13.0	1.8	14.9	0.2	+	0.1	+
Mexican	89833	+	+	0.8	0.1	0.1	+	0.2	+	2.5	0	0.2	1.3	0.5	2.1	5.8	11.4	0.1	+	0.2	+
Mexican	5554A	2.3	1.3	0.6	0.2	0.1	0.1	+	+	1.4	0	0.2	1.3	0.4	4.8	5.7	7.7	0.1	+	0.1	+
Mexican	76262	0.1	0.1	0.1	0.2	0.2	+	+	+	1.7	0	0.2	1.2	0.4	2.0	4.5	13.8	0.2	+	0.1	0.1
Mexican	89764	0.2	0.2	0.6	0.2	0.1	+	+	+	1.5	0	0.2	0.9	0.5	4.7	2.6	10.6	0.1	+	0.1	+
Turkish	5413	28.1	19.3	0.3	0.1	+	+	+	+	0.4	6.3	0	0.6	0.1	0.2	2.2	2.1	+	1.6	0.2	+
ROSEMARY																					
French	4996A	3.4	2.0	17.2	6.8	0.6	+	0.2	+	2.6	0	0.1	0.7	3.4	20.5	1.1	2.7	0.1	0.2	0.3	0.2
Portugese	9251	2.9	2.0	6.9	2.4	1.5	+	0.3	+	19.2	0	1.8	0.7	5.4	14.7	1.8	3.7	0.6	0.2	0.5	0.1
Spanish	90202	1.8	1.0	24.5	9.9	0.7	+	0.8	1.1	5.1	0	0.1	0.5	4.3	19.5	0.2	2.7	0.1	+	0.1	0.1
Yugoslavian	4996B	4.8	3.2	15.6	3.2	0.2	+	0.8	+	1.9	0	0.1	1.2	3.3	18.5	0.3	2.0	0.2	0.2	0.5	0.2
SAGE																					
Dalmatian	1776	2.8	1.4	3.8	3.1	0.3	+	+	+	0.5	+	+	0.5	1.0	9.9	+	1.1	+	+	25.8	+
Albanian	7777	5.3	2.6	1.3	3.4	0.7	0.2	+	+	0.7	+	+	0.6	1.8	5.6	0.9	0.7	0.4	0.1	17.3	+
Greek	78210	0.7	+	4.1	4.0	2.3	0.1	+	+	1.5	+	+	+	2.3	40.7	+	1.2	0.1	+	1.0	0.2
SAVORY																					
Spanish	5456	14.3	8.5	1.2	1.7	0.1	+	+	+	0.7	0	2.4	0.9	0.5	0.1	3.8	15.3	0.1	0	0.1	+
Spanish	5189	7.9	4.8	1.3	1.5	0.2	+	+	+	2.3	0	0.1	0.8	0.7	0.8	3.3	13.7	0.1	0	0.2	0.1
Yugoslavian	5020	5.0	3.3	1.3	1.9	0.2	+	0.1	0	0.4	0	1.0	0.5	0.4	0.4	0.8	9.7	0.1	0	0.5	0.1
Yugoslavian	3849	0.2	0.1	1.2	0.8	0.2	+	+	+	0.9	0	0.1	1.0	0.4	0.4	3.7	12.0	0.2	+	0.9	0.1
SWEET BASIL																					
Domestic	4508	25.3	16.1	0.1	0.1	0.2	+	0	0	0.2	0	5.9	0.1	0.2	1.3	0.2	0.3	+	1.6	0.2	+
Domestic	5012	28.0	18.9	0.1	0.1	+	+	0	0	0.2	0	6.0	+	0.1	0.8	0.2	1.2	+	1.4	0.2	+
Domestic	241	16.5	9.4	0.1	0.1	0.1	+	0	0	0.4	0	3.2	+	0.4	2.9	0.1	0.6	+	0.9	0.4	+
THYME																					
French	78577	0.7	0.4	2.9	2.4	0.4	0.1	0.6	0	0.4	0	0.1	0.2	1.2	0.6	0.3	42.5	+	0.1	0.3	0.5
French	89819	0.7	0.4	2.4	1.4	0.2	0.1	1.0	+	0.6	0	+	+	1.3	1.2	2.8	29.7	0.1	0.2	0.2	0.4
French	92498	3.8	1.9	2.8	1.9	0.2	+	0.7	+	+	0	+	+	1.6	1.2	6.5	39.7	0.1	0.2	0.2	0.4
French	8101	12.5	7.5	1.5	1.1	0.2	0.1	0.3	0.2	2.2	0	+	+	0.7	0.5	1.9	21.8	+	0.5	0.1	+
Spanish	92499	10.7	5.7	2.2	2.0	0.5	0.1	0.4	+	1.9	0	+	+	1.1	4.8	1.6	26.4	0.5	0.5	0.1	0.2
Spanish	89813	0.4	0.3	3.7	0.9	0.6	0.1	0.1	+	+	0	+	+	2.1	7.1	5.0	7.7	2.0	0.2	0.1	0.2
Spanish	9418	5.4	3.4	3.0	0.9	0.6	0.5	0.3	+	2.6	0	0.3	+	1.8	7.4	5.1	7.3	0.8	0.3	0.1	0.2
Spanish	9056	12.3	7.4	2.0	1.6	0.1	+	0.5	+	2.4	0	+	0.2	0.9	0.4	0.4	39.9	0.1	+	0.1	0.3
Domestic	5531	3.9	3.1	1.0	0.7	+	+	+	+	0.5	0	0.7	+	0.4	1.4	2.7	25.2	0.1	+	0.8	0.2

tions obtained on three different four-dimensional sets, as shown in Table 2, show no overlaps. This suggests that the linear equations shown as a footnote of the table may be used to yield the z values by means of which the two-way classification may be made with a high degree of success. By way of illustration, consider the sole Turkish oregano analyzed and included in Table 1. The peak distributions appear similar to those of the Greek. Substitutions of % of peaks 4, 7, thymol and carvacrol in x_1, x_2, x_3 and x_4, respectively, of the first equation (z_1) and similar substitutions of the 4-tuple sets in the corresponding equations (z_2 and z_3) gave the z values of 7.37, 1.82, and −0.08, all of which fit the Greek type.

Returning to Table 1, we note that there might be some correlations between certain pairs of the compounds, for example, between peaks 4 and 7. Peak 7 is consistently lower than peak 4. These correlations would suggest similar chemistry of compound formation during the life cycle of

Table 1—Continued

d-camphor	copane	d-linalool				terpinen-4-ol		β-caryophyllene				methyl chavicol	α-terpineol								thymol	carvacrol	
39	45	47	47-1	51	52	53	53-1	54	55	58	59	60	61	62	63	63-1	64	70	80	97	101	102	103	total
0	+	1.2	+	+	+	23.4	+	1.9	+	+	+	+	3.6	0.6	+	+	+	+	+	0	+	9.9	1.3	78.3
0	+	1.3	+	+	+	27.6	+	2.4	+	+	+	+	3.9	0.6	+	+	+	+	+	0	+	15.0	3.0	99.8
0	0.4	1.0	+	+	+	10.7	+	2.1	+	+	+	+	1.2	1.0	+	+	+	+	+	0.1	+	+	+	97.7
0	+	2.6	+	+	+	28.5	+	4.0	+	+	+	+	3.6	1.4	+	+	+	+	+	0.1	+	+	+	90.7
+	0.5	0.3	+	+	+	2.8	+	1.3	+	+	+	+	0.3	+	+	+	+	+	+	+	+	+	+	88.4
+	0.2	0.1	+	+	0.8	+	+	1.7	3.2	0.1	+	+	0.3	0.6	+	+	0.9	+	+	+	+	26.7	46.5	95.5
+	0.4	0.4	+	+	0	0.6	0.1	1.0	0.1	+	+	+	0.3	0.4	+	+	0.7	0.1	0.7	+	+	7.4	32.7	98.6
+	0.3	0.3	+	+	0	0.5	+	0.8	+	+	+	+	0.2	0.4	+	+	0.8	+	+	+	+	8.5	32.6	91.2
+	0.2	0.3	+	0.3	0	0.7	0.1	1.1	1.4	+	+	+	0.3	0.6	+	+	1.1	0.1	0.8	+	+	7.3	62.0	94.5
+	0.1	0.1	+	+	0	1.2	+	0.6	0.2	+	+	+	0.3	1.3	+	+	2.2	+	0.6	+	+	0.9	84.8	97.6
+	+	0.3	+	0.9	0	1.4	+	0.1	+	+	+	0.3	0.6	+	+	+	0.9	+	+	+	+	12.3	67.2	98.4
0.3	+	0.9	0.3	+	1.9	2.3	+	5.9	0.4	+	0.3	+	4.7	0.3	0.4	0.1	0.4	0.3	+	+	+	32.0	21.2	96.7
0.2	+	1.2	0.3	+	1.1	1.0	+	6.8	1.2	+	0.2	+	5.8	0.5	0.4	0.2	0.5	0.2	+	+	+	34.5	21.4	95.4
+	0.2	2.4	0.2	+	0.4	4.3	+	5.7	0.1	+	0.4	0.3	5.7	1.2	+	+	0.4	0.2	0.7	+	+	0.7	29.6	89.2
+	+	0.7	+	+	1.9	0.9	+	5.1	0.8	+	0.2	+	3.0	0.3	0.7	+	0.5	0.2	0.7	+	+	40.2	15.2	95.7
0.2	+	0.8	+	+	0.5	0.8	0.1	4.4	0.7	+	0.2	+	3.2	0.6	0.8	+	0.3	+	0.3	+	+	25.0	28.1	92.3
0.1	+	0.8	0.2	+	0.6	1.3	0.2	4.8	0.3	+	0.3	+	3.0	0.4	0.3	0.1	0.4	0.1	0.4	+	+	15.8	41.2	95.2
+	0.1	1.0	0.1	+	1.0	1.8	+	5.9	0.6	+	0.1	+	4.1	0.6	0.5	+	0.5	0.2	+	+	+	23.5	31.7	94.2
+	0.6	0.2	+	+	0	0.9	+	1.0	0.1	+	+	+	0.2	0.7	+	+	1.3	+	0.5	+	+	0.8	30.0	97.8
0.1	0.1	17.3	+	0.4	+	1.2	+	1.7	+	+	0.2	+	3.0	4.4	+	2.3	+	+	+	0	+	0.7	0.1	93.6
0.2	0.3	14.4	+	0.4	+	1.7	+	2.9	+	0.3	0.3	0.1	3.6	1.4	+	4.1	+	+	+	0	+	0.5	0.5	95.4
0.1	+	14.1	+	0.4	+	0.9	+	0.9	+	+	0.2	+	1.8	3.6	+	3.0	+	+	+	0	+	+	+	97.5
0.6	0.3	14.5	+	1.3	+	1.8	+	2.9	+	0.1	0.3	+	2.8	8.0	0.1	5.3	+	+	+	0	+	1.2	2.0	97.3
12.8	+	18.9	+	0.9	+	0.4	+	0.6	0.1	+	0.3	+	2.1	2.2	0.3	+	+	+	0.1	8.1	+	+	+	97.0
2.7	0.6	28.5	+	2.5	+	1.8	+	4.1	+	+	+	+	6.8	5.7	+	+	+	+	+	3.7	+	+	+	98.0
1.6	0.2	16.8	+	4.2	+	0.7	+	5.8	0.8	+	0.1	+	2.0	4.6	0.3	+	+	+	+	0.3	+	+	+	95.6
+	0.1	3.7	+	+	+	0.6	4.2	1.9	0.5	+	+	+	0.5	3.7	0.3	+	+	+	0.4	+	+	28.9	1.2	95.7
+	0.2	5.3	+	0.2	0.2	1.4	4.2	2.2	0.5	+	+	+	1.2	4.5	0.4	0.4	+	+	+	+	+	34.1	2.9	95.5
+	0.2	1.9	+	+	0.7	0.7	1.3	3.1	0.2	+	+	+	+	7.0	12.5	0.2	+	+	+	+	+	13.4	24.4	91.3
+	+	1.2	+	+	1.5	1.2	2.4	4.4	0.5	+	+	+	0.3	2.8	0.2	+	1.1	0.1	0.2	+	+	13.6	44.1	95.8
+	0.8	10.7	0.1	5.3	1.9	0.7	+	0.3	+	0.6	+	0.9	3.3	0.6	0	+	0.8	3.6	2.4	+	6.8	+	+	90.6
+	0.6	7.6	+	5.1	1.4	0.7	+	+	+	0.2	+	0.1	2.6	0.5	+	+	1.2	2.2	3.9	+	4.9	0.6	0.6	89.4
+	1.2	20.0	+	6.4	2.5	0.7	+	4.1	+	0.2	+	0.1	3.2	0.5	0	+	1.3	4.3	1.2	+	10.2	0.2	0.2	91.4
0.1	+	1.6	0.7	0.3	0.4	2.3	0.1	0.6	+	+	+	0.1	0.7	10.5	+	7.1	+	0.5	+	+	+	15.6	0.5	94.8
+	0.1	4.3	1.0	+	+	1.7	+	+	+	+	+	+	1.0	4.9	+	0.4	+	+	+	+	+	28.2	3.7	88.0
+	0.1	1.3	0.8	+	+	0.9	+	+	+	+	+	+	0.8	5.8	+	0.7	+	+	+	+	+	7.7	4.2	83.5
+	+	2.3	+	0.3	0.3	0.3	1.1	0.5	+	+	0.3	0.1	0.6	4.0	0.5	3.6	+	+	+	+	+	16.7	4.8	86.5
0.2	0.2	2.0	2.6	+	+	1.6	+	1.0	+	+	+	+	1.8	4.6	+	3.6	+	0.4	0.3	+	+	13.4	2.1	92.5
0.1	+	12.5	9.4	+	+	9.5	+	0.9	+	+	+	+	9.4	4.2	+	0.5	+	+	+	+	+	15.9	0.8	93.7
0.1	0.1	6.1	0.5	0.7	+	7.9	+	0.7	+	0.5	0.3	+	4.5	5.3	0.6	3.5	+	+	+	+	+	22.4	2.0	95.2
+	0.2	1.4	0.5	0.2	0.3	1.0	0.3	0.4	0.1	0.3	0.1	0.1	0.5	4.8	3.4	+	+	0.2	0.2	+	+	11.8	1.7	96.1
+	+	3.8	0.4	+	+	0.7	+	+	+	+	+	+	0.4	10.5	+	0.2	+	+	+	+	+	39.9	2.8	99.4

the plant throughout the Labiate family. Squares of correlation coefficients on selected pairs and squares of multiple correlation coefficients on a few triplets listed in Table 3 all show a high degree of correlation. $R^2 = 0.807$ for camphene in terms of peak 4 and α-pinene means the fraction of the variability of camphene that may be explained (in a linear fashion) by peak 4 and α-pinene is 0.807, a remarkably high figure for a collection as varied as the 42 samples in regard to the crop year, area of cultivation and other associated variables.

Figure 2 is rather self-explanatory and further discussion on compositional similarities or differences among the Labiate spices as a whole would be repititious and unnecessary. A more extensive study focusing on commercial sage has been reported recently (Rhyu, 1979).

Finally, data reported on the Greek and Turkish oreganos by Calzolari et al. (1968) are compared with our own in Table 4, in which the compounds listed are those commonly shared by the two works. That is, Calzolari's work included a number of other compounds not identified in the present study. Yet, the agreements are reasonably good, especially in the z values.

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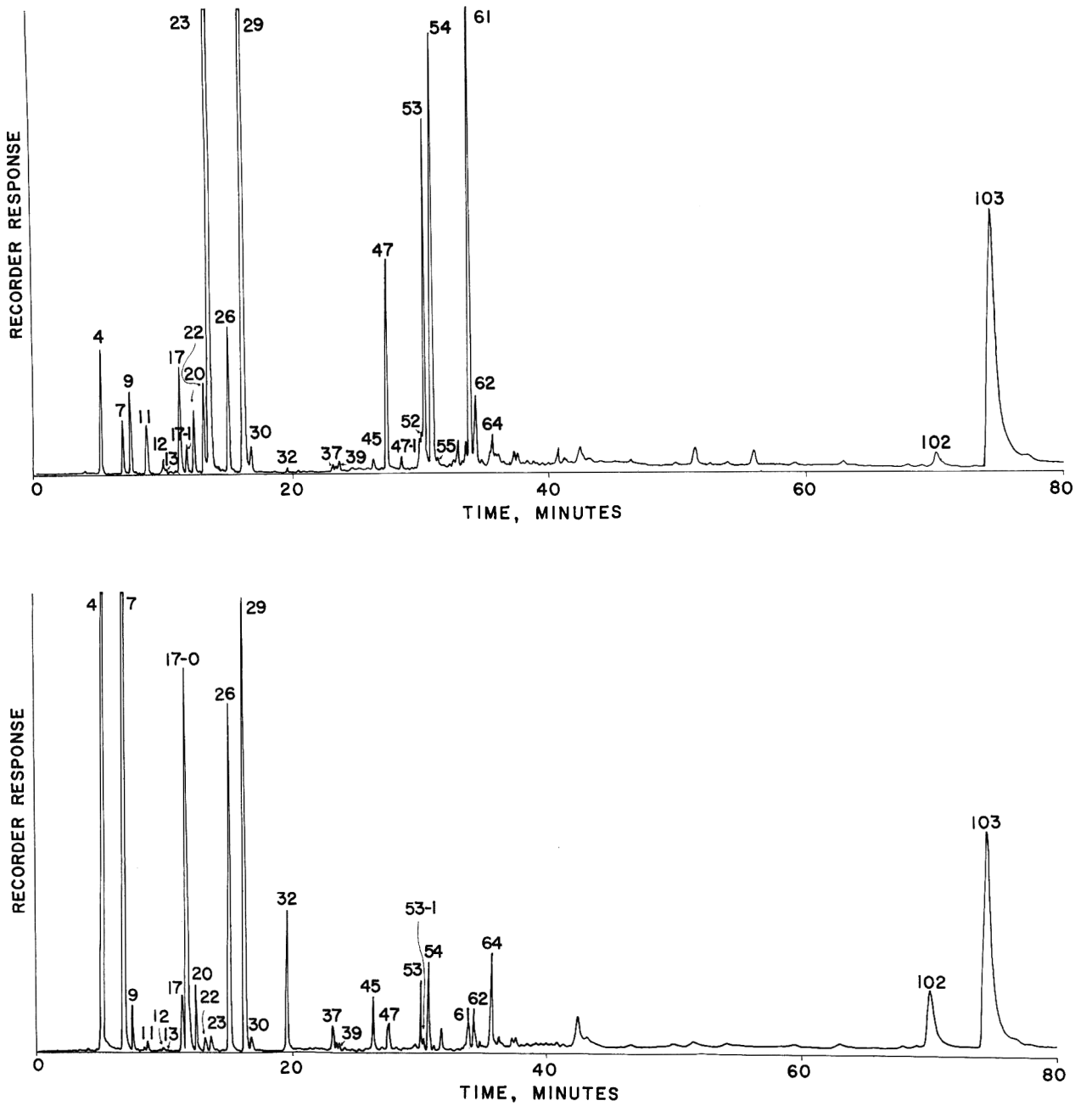

Fig. 1—Typical chromatograms of oregano, top (Mexican, lot 9167), bottom (Greek, 5414B)

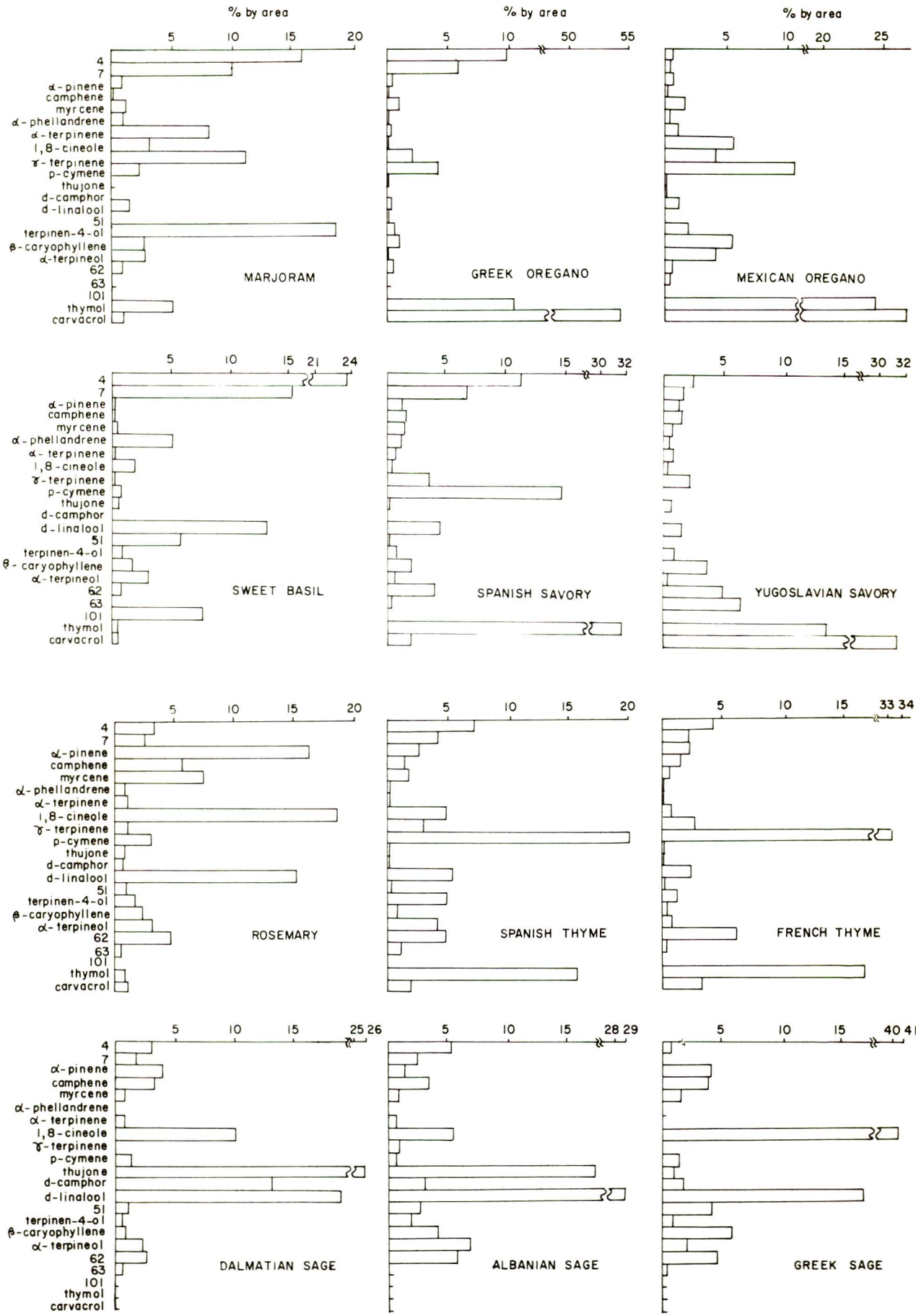

Fig. 2—Major constituents of steam volatile oil of selected Labiate spices.

Table 2—Values of z for linear discriminant function, Greek vs Mexican oregano

Lot no.	x_1 →	z values from [a]	
	x_2 →	peak 4	cineole	α-pinene
	x_3 →	peak 7	cymene	myrcene
	x_4 →	thymol	caryophyllene	limonene
		carvacrol	terpineol	linalool
		Greek oregano		
8069		8.28	2.85	0.20
5414B		9.16	2.67	0.09
5414A		9.28	2.24	0.32
8087		9.07	2.49	0.06
76204		9.47	1.75	0.22
90143		8.53	1.87	0.14
		Mexican oregano		
76274		4.27	10.26	0.70
8475		4.51	10.33	0.50
9167		3.57	10.40	1.26
89833		4.09	9.73	0.59
5554A		5.44	7.49	0.44
76262		5.60	10.05	0.57
89764		4.93	10.34	0.57

[a] x_i denotes the area of the corresponding peak. The linear discriminant functions for columns 1, 2 and 3, respectively, are

$$z_1 = 1.222x_1 - 1.577x_2 + 0.059x_3 + 0.114x_4$$
$$z_2 = -0.150x_1 + 0.277x_2 + 1.222x_3 + 0.221x_4$$
$$z_3 = -0.216x_1 + 0.060x_2 + 1.215x_3 + 0.004x_4$$

Table 3—Squares of correlation coefficient (r^2) and squares of multiple correlation coefficient (R^2)

Between or among	r^2 or R^2
peaks 4 and 7	0.998
α-pinene and camphene	0.914
γ-terpinene and terpinen-4-ol	0.882
camphene in terms of peak 4 and α-pinene	0.807
terpinen-4-ol in terms of peak 4 and γ-terpinene	0.778
terpinen-4-ol in terms of α-pinene and γ-terpinene	0.791

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Table 4—Comparison of oregano oil composition with other published data

	Calzolari et al		Rhyu[a]		
	Greek	Turkish	Greek	Mexican	Turkish
α-pinene	0.95	0.31	0.4	0.5	0.3
camphene	0.14	0.28	0.1	0.2	0.1
β-pinene	0.10	0.03	0.1	0.1	+
sabinene			+	0.1	+
myrcene	0.93	0.41	1.1	1.6	0.4
α-phellandrene			0.1	0.3	0
α-terpinene	0.85	0.47	0.5	1.0	2.2
d-limonene	0.11	0.17	0.2	0.6	0.1
1,8-cineole	0.22	0.23	0.2	5.4	0.2
γ-terpinene	3.67	1.90	2.2	4.1	2.2
p-cymene	6.90	2.27	4.3	10.6	2.1
terpinolene			0.1	0.1	+
thujone			0.2	0.1	0.2
d-camphor			+	0.1	+
copane			0.2	0.1	0.6
d-linalool	0.19	0.39	0.3	1.1	0.9
terpinen-4-ol	0.85	1.44	0.7	1.8	0.9
β-caryophyllene	1.05	0.82	0.9	5.5	1.0
methyl chavicol			0.1	0.1	+
α-terpineol			0.3	4.2	0.2
thymol	5.19	0.86	10.5	24.5	0.8
carvacrol	74.90	83.10	54.3	26.9	30.0
z_1	8.84	9.52	8.87	4.61	7.37
z_2	3.16	1.60	2.33	9.78	1.82
z_3	−0.01	0.17	0.22	0.72	−0.08

[a] For Greek and Mexican, average of all lots.

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AN ANALYSIS OF THE WATER BINDING IN GELS

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ABSTRACT

The water-binding properties of four gelling macromolecules (carrageenan, agar, potato starch and gelatin) were studied through measurement of the vapor pressure of water in the gels, suction pressure and Flory-Huggins χ-value developed by the gel, and reduced mobility of water protons as measured by the spin lattice relaxation time from NMR. The difference in water binding between gels could be explained based on hydrogen bonding of water to specific sites and between water molecules; induced water-water interactions due to hydrophobic regions on the macromolecule; and dipole interactions of water with the ionic sites on the macromolecule. The suction pressure method shows carrageenan and agar to be the best water binders at low concentrations which is illustrated by a decreased χ-value with increased concentration. Gelatin and starch show opposite behavior. The NMR results indicate a different order of water binding due to the difference in cooperative bonding. In this case agar was the best water binder at low a_w and gelatin the poorest. This study shows the difficulty that could arise in using only one index of water binding.

INTRODUCTION

THE WATER-HOLDING properties of macromolecules are of great interest and importance to food scientists. Despite numerous studies conducted on the water-binding properties of gelling macromolecules, the exact nature of the forces by which water is held in gels is still largely unknown. A review of the most recent studies indicates that the water inside a gel is very similar to free water and is largely unrestricted (Labuza, 1977). However, there are measurable differences between pure water and that held in gels.

Formation of a gel is an intrinsic property of many macromolecular molecules. Upon gel formation a certain amount of water is imbibed by the system, and a dilute aqueous solution is transformed into an elastic substance, whose mechanical properties suggest a three-dimensional crosslinked network structure (Ferry, 1948; Morawetz, 1975). The structure formed during gelation is dependent on the composition and molecular weight of the gelling agent (Ferry, 1948; Rees, 1972a, b); however, for such macromolecular substances as gelatin (Harrington and von Hippel, 1961; von Gerngross et al., 1932; Eldridge and Ferry, 1954), carrageenan (Anderson et al., 1969; Child et al., 1970), agar (Arnott et al., 1974; Pine and Prins, 1973), and potato starch (Suggett, 1975), it is likely that gel formation may involve the formation of double or triple helical structures. As a consequence, the gel structure would then be composed of regions with a high degree of crystal-like order, as well as extensive regions of amorphous structure.

Water which is enclosed in the three-dimensional structure of a gel has been divided into at least two different species. Water is usually classified as either some form of bound water or as free water. The amount of water held by a gel under a given set of conditions is traditionally referred to as the water-holding capacity (WHC) or water-binding capacity (WBC) of the gel. The often ill-defined term, bound water, is usually defined as that portion of the water held in the gel which exhibits physical properties significantly different from those of free or bulk water. Some of the characteristics of bound water are lower vapor pressure, high binding energy as measured during dehydration (ΔH), reduced mobility as seen by nuclear magnetic resonance (NMR), unfreezability at low temperatures, and unavailability as a solvent (i.e., solute restriction).

Although each of these characteristics has been used to define bound water, each gives a different value for the amount of water which is bound. Therefore, the quantification of water in a system classified as bound water varies greatly from one worker to another depending on which defining parameter is used. Because of this, as well as the complexities and interactions of the binding forces involved, no universal definition of bound water has been adopted. To avoid confusion, therefore, this study used the term polymer:water interaction when referring to the summation of forces which binds water to macromolecules.

A great deal of work has been done looking at the effect which particular molecules have when dissolved in water (as a solute) on the binding properties and structure of water. It has been found that water may be associated with solutes in a number of ways. Among those postulated are: (1) water which is strongly chemically bound, such as the hydration of salts; (2) water absorbed on hydrophilic sites of the molecule by hydrogen bonding; (3) water attracted to ionic sites by the molecular dipole of water; (4) water held by capillary forces between surfaces of an insoluble network, a colloidal dispersion or a gel network; (5) water held within an amorphous network due to supersaturation of dissolved molecules; and (6) water held in solution or suspension by "long range" forces. The nature of each of these attractive forces is difficult to isolate due to the multiple interactions possible.

Ling (1965; 1971; 1972;) and Drost-Hansen (1972) have suggested that the water in macromolecular systems exists in polarized multi-layers, and hence the relationship between the water content and water activity (a_w) defined as the ratio of the vapor pressure of water in a gel (p) to that of pure water (p_o) at the same temperature should follow the de Boer-Zwikker-Bradley adsorption isotherm. On the basis of experiments conducted by Fiedjakin (Derjaguin, 1970), Ling has calculated that the polymer surfaces could be covered with hundreds of polarized layers of water molecules. In fact, the water shell surrounding the macromolecule may be thicker than 0.1μ.

On the other hand, Lyklema (1977) has used essentially the same data to conclude that beyond the Stern double layer, no highly structured water exists in polymer solutions. In his discussion, Lyklema assumes that polymer solutions are nonideal. He was able to successfully account for the forces described by Ling and Drost-Hansen by assigning them to a combination of changes in liquid viscosity, density, surface tension and the rate of movement of measuring devices over the surface of the liquid.

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Another way to describe how water is immobilized in macromolecular gel systems is to assume it is trapped in sufficiently small channels and pores (Labuza, 1975; Resing et al., 1964; Dransfeld et al., 1962). In addition, it has been proposed that a reduction in temperature during gelation increases cooperative hydrogen bonding in normal water (Clifford and Child, 1971), and that, in the vicinity of hydrophobic groups, water adopts a clathrate-type structure (Tait and Franks, 1971).

The objective of this research was to use a multi-dimensional approach to explore the interactions between water and polymers in a formed gel. With this approach it should be possible to more accurately elucidate the nature of the forces involved in this "binding" process. Specifically, the research explored the binding of water through measurement of:

(1) the vapor pressure depressing effect of macromolecules in a dilute solution as measured by a capillary suction pressure and direct vapor pressure.
(2) the elastic nature of water in a gel as measured by pulsed NMR.

MATERIALS & METHODS

Materials

Four macromolecular gelling systems were selected for the evaluations in this research. The gels evaluated include gelatin (General Gelatin-250 Bloom, lot #09552 and 09974), carrageenan (Marine Colloids – kappa and iota fractions, lots #RE-7691 and #RE-7690, respectively), agar (Tragacanth Importing Company – agar-agar, lot #61476), and potato starch (J.R. Simplot Co.).

The use level varied depending on the experiment and the gelling agent. Generally the use levels ranged from about 0.5% dry basis (db) which was the minimum necessary to obtain a gel at room temperature, to as high as 70%. Specific use levels are listed in the results and discussion.

Gels were made by modifications of procedures reported by Persidsky and Luyet (1959). The macromolecular powder was weighed and suspended in deionized-distilled water at room temperature to form a slurry. At high concentrations (e.g., greater than 40% gelatin) this process was aided by mixing in a Brabender Model D 3002 prep center (C.W. Brabender Instruments, Inc., South Hackensack, NJ) equipped with a Farinograph opposed screw sigma blade mixing bowl. The slurry was then heated, using a magnetic mixer for constant agitation when possible, to 65°C for gelatin, 80°C for carrageenan and 98°C for agar and potato starch. When it was not possible to agitate mechanically, the samples were removed from the heating medium (water was the heating medium for gelatin, carrageenan and starch and steam was used for agar) and stirred by hand after five minutes. The samples were returned to the heating medium and the process repeated until the slurry dissolved. After total solvation as indicated by complete transluscence of the solution, the samples were removed from the heating medium and transferred into the appropriate analytical container (e.g., sample tubes for NMR evaluation). The samples were then returned to the heating medium and held for a sufficient length of time to allow for deaeration as indicated by the absence of visible air bubbles.

After deaeration, the samples were placed into an ice/water bath for two hours. They then were transferred into a 6°C cooler for aging an additional 24 hours. Subsequent treatment and/or evaluation then varied with the experimental method used.

Methods

Determination of capillary suction potential. The capillary suction potential of agar gel was measured by the method recently described by Lewicki and Labuza (1978) and Lewicki et al. (1978a). In this method the suction pressure developed against a standard capillary system (fiber paper) is measured as a function of moisture content.

Water activity measurements. Water activity (a_w) was measured by the vapor pressure manometer (VPM) as described by Lewicki et al. (1978b). One minor modification was employed in that the VPM system was enclosed in a styrofoam insulated box in which the temperature could be held constant to within ±0.1°C.

Determination of spin-lattice relaxation. The spin-lattice relaxation time (T_1) of water protons was measured by use of the $180°$-τ-$90°$ pulse sequence reported by Farrer and Becker (1971). This was done using a Praxis model PR-102 (The Praxis Corp.) Pulsed Nuclear Magnetic Resonance (NMR) Spectrometer equipped with a Tekronix model RM 503 Oscilloscope (Tektronix, Inc.).

Determination of solids content. The dry matter contents of the gels were measured after each evaluation by drying the samples in a vacuum oven at 750 mm Hg and 60°C for 24 hr.

RESULTS & DISCUSSION

IN ORDER TO understand why and how water is held in macromolecular gelling systems, it is necessary to *first* review the proposed structure of water and the types of bonding which water undergoes. One of the most accepted current theories on water structure is known as the "flickering cluster model" (Franks, 1970). The model describes liquid water as composed of extensively hydrogen-bonded H_2O molecules arranged in clusters ranging from tetrahedral (ice-like with no strains on the hydrogen bonds) to 7-sided (with considerable strain on the hydrogen bonds). It is postulated that at any one time as much as 70–90% of the molecules in water may be associated with clusters (Kuntz and Kauzman, 1974). Water derives its peculiar solid-like properties (melting and boiling points, surface tension, heat capacity, etc.) from the structure in these clusters. Water's liquid-like characteristics (density and viscosity) come from the short lifetime of clusters, in the range of 10^{-12} sec.

Two characteristics of this model of water structure are of particular interest in the work presented here. First, the model predicts an average second neighbor hydrogen bond length in water of 2.85 Å. It should be possible, therefore, to introduce molecules capable of forming hydrogen bonds with this length into the structure of water with a net result of considerably enhancing the overall hydrogen bond structure. Secondly, since the lifetime of the clusters is so short, it should take a consiberable perturbation of the system to macroscopically affect the system. At low solute concentrations, then, one would expect to find little or no solute effect on the thermodynamic properties of water. The other widely accepted model of water structure, based on a continuous but perturbated structure as recently analyzed by Stillinger (1977) using quantum mechanics, can lead to the same conclusions about water structure.

A third characteristic of water which must be kept in mind when analyzing water:macromolecule interactions is that water has an electric dipole and subsequently is highly affected by both charged molecules and nonpolar molecules added to a solution. The microscopic nature of the order that arises due to these electrostatic interactions is poorly understood, expecially the "hydrophobic bonding" that occurs with apolar molecules (Fennema, 1976). The net macroscopic result, however, is that both highly charged ionic species and apolar species tend to cause water to form "hydration clouds." These hydration layers are found around proteins in solution (Ferry, 1948; Kuntz and Zipp, 1977) and around charged groups such as the sulfate groups associated with natural polysaccharides like carrageenan (Arnott et al., 1974). These bond types, however, are noncooperative, meaning that the water structure induced by one bond type is not compatible with the structure induced by the second.

In addition to the three bond types that can occur in water as listed above, (hydrogen, hydrophobic and ionic), it has been postulated that both van der Waal's forces and "long range" forces can be important bond types in polymer solutions. While van der Waal's forces are very weak, contributing only slightly to the net water:polymer interaction forces, some researchers have postulated that "long range" forces may be very energetic and may "order" water

in layers as much as 0.1μ in thickness (Ling, 1968; Drost-Hansen, 1975). Labuza (1977) has postulated that these interactions can best be described as a sum of solute effects, as predicted by the Flory-Huggins equation and Raoult's Law, and capillary effects, as described by the Kelvin equation.

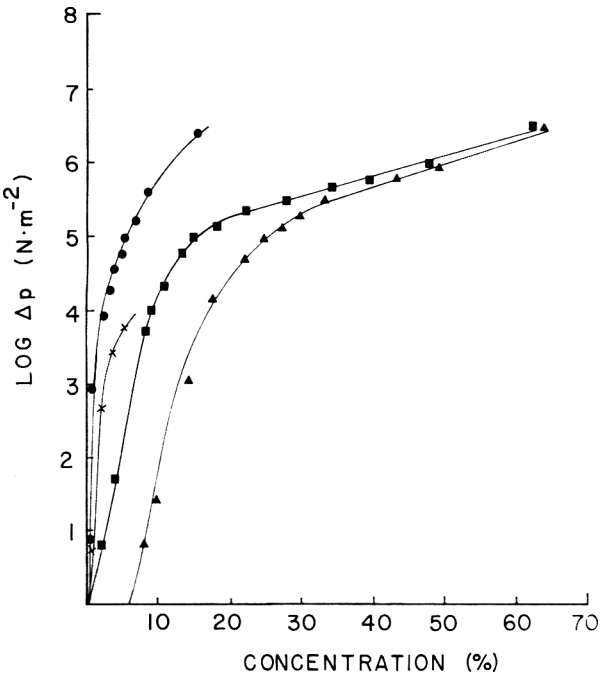

Fig. 1–Suction pressure (N/m²) vs solids concentration for four gelling macromolecules: ●–● carrageenan; x–x agar; ■–■ gelatin; ▲–▲ potato starch.

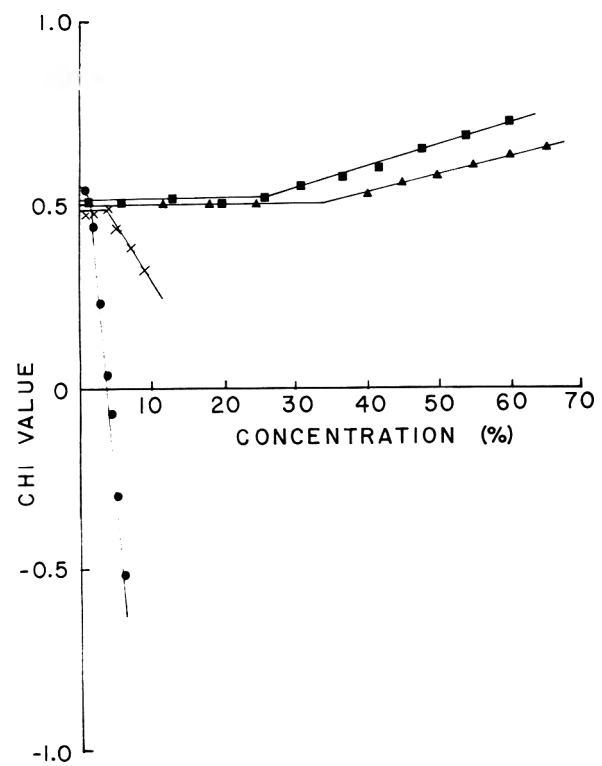

Fig. 2–Flory-Huggins χ parameter as a function of solids concentration for four gelling macromolecules: ●–● carrageenan; x–x agar; ■–■ gelatin; ▲–▲ potato starch.

Lewicki et al. (1978a) have used a suction pressure method to quantify the net water binding effects (the combined effects of the forces described above) of three macromolecules in solution: gelatin, starch and carrageenan. With this method the greater the suction force theoretically, the more tightly the water is held in the system. Figure 1 shows their results along with the suction pressure results for agar solutions found in this study. The net water:polymer interaction of these polymers as measured by this method, may be arranged in the order carrageenan>agar>gelatin>starch with carrageenan having the highest net suction pressure effect, i.e., the suction force necessary to remove water from the gel is largest for carrageenan when compared to the other macromolecules at the same solids concentration.

These results can be analyzed in terms of the Flory-Huggins treatment of macromolecules in solution. The χ-value is derived from the following equations:

$$\ln a_w = \frac{\Delta p \cdot \rho_v}{P_o \cdot \rho_2} \quad (1)$$

where: Δp = suction pressure in $N6m^2$; ρ_v = density of water vapor; ρ_2 = density of liquid water; and P_o = vapor pressure of pure water.

$$\ln a_w = \ln \phi_1 + [1 - \frac{V_1}{V_2}]\phi_2 + \chi \phi_2^2 \quad (2)$$

where: a_w = solvent (water) activity = a_1; ϕ_1 = volume fraction of solvent; ϕ_2 = volume fraction of solute; V_1 = molar volume of solvent; V_2 = molar volume of solute (polymer); and χ = Flory-Huggins interaction parameter.

The theoretical a_w as a function of solids content can be obtained using Eq (1) from the Δp (suction pressure) at the solids content. Then by using published molecular weight and volume data (Lewicki et al., 1978a; and Table 1) χ can be calculated by substitution into Eq (2). Flory (1942) and Huggins (1942a, b) in discussing the change in χ value with respect to concentration indicate that positive χ values (deviations from random mixing character) are due to exclusion of solvent from the macromolecular interior, a decrease in numbers of active solvent binding sites on the macromolecules and increasingly energetic polymer:polymer interactions. Negative deviations from ideality are due to efficient solvent:polymer interactions (e.g., formation of polarized water structures) which are cooperative and become increasingly stronger at higher polymer concentrations.

Figure 2 shows the Flory-Huggins χ parameter for carrageenan, gelatin and starch taken from Lewicki et al. (1978a) and for agar as found in this study presented as a function of polymer concentration. As stated, an increasingly negative χ value indicates higher water:polymer interaction (larger deviations from solution ideality). As seen, agar behaves similar to carrageenan in that it has a decreasing χ value which indicates cooperative polymer water bind-

Table 1–Molecular weight/volume factor used in Flory-Huggins calculations

Macromolecule	Molecular wt MW-(Daltons)	Specific volume cm^3/g
gelatin	75×10^3	0.74
potato starch	1×10^6	0.74
carrageenan	5×10^5	0.51
agar	1×10^5	0.59

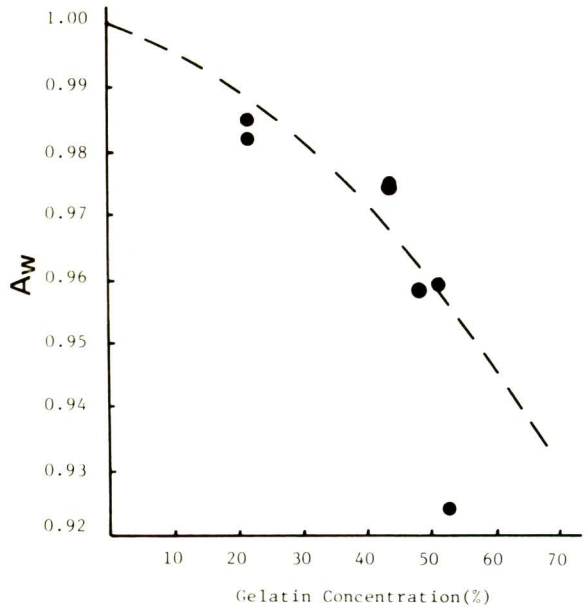

Fig. 3—Predicted and measured water activity of gelatin as a function of solids concentration: • measured by VPM; — — — calculated by Eq (2).

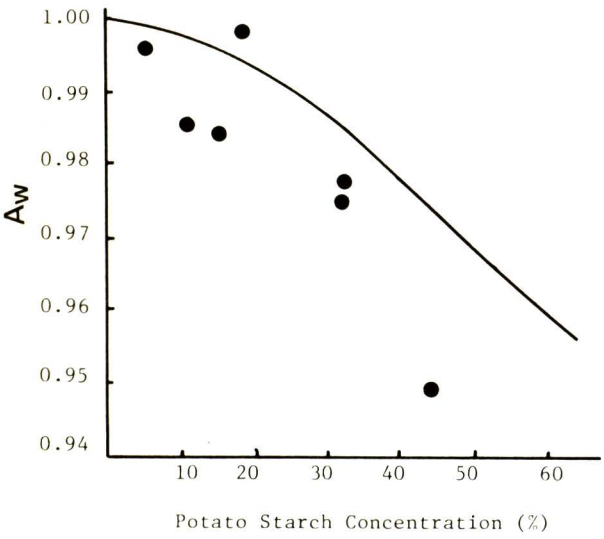

Fig. 4—Predicted and measured water activity of potato starch as a function of solids concentration: • measured by VPM; — — — calculated by Eq (2).

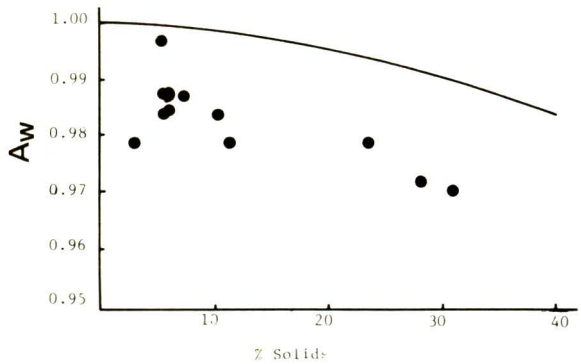

Fig. 5—Predicted and measured water activity of ι-carrageenan as a function of solids concentration: • measured by VPM; — — — calculated by Eq (2).

ing as concentration increases. Thus in comparison to gelatin which has a constant χ value agar should give a higher suction pressure value when compared at the same concentration. This trend is seen in Figure 1 for the low concentration region where all gels can be compared.

The importance of the value of χ has also been investigated by Tobolsky and Yannas (1971). They pointed out that a χ value of 0.5 indicates an ideal solution in which the colligative properties as defined by Raoult's Law would hold. Thus it may be seen by inspection of Eq (2) that if it is assumed that if the ($\ln \phi_1$) term is expanded, Eq (3) results in which the definitions of terms are identical to those of Eq (2).

$$\ln a_1 = -\phi_2 \frac{V_1}{V_2} - (\tfrac{1}{2} - \chi)\,\phi_2^2 - 1/3\,\phi_2^3 - \ldots\ldots \qquad (3)$$

If ϕ_2 is then assumed to be small, the cubed term and above it in the series effectively become zero. A χ value of 0.5 then will result in the interaction term $[-(\tfrac{1}{2} - \chi)\,\phi_2^2]$ becoming zero and the change in a_1 will be due solely to the reduction in entropy of mixing when the polymer is placed into the solvent.

Since the Flory-Huggins Eq (2) relates solvent activity to polymer concentration, direct measurement of the water activity (a_w) can also be used in a Flory-Huggins type of evaluation to investigate water:polymer interactions.

The vapor pressure (or a_w) of gels which synerese, such as agar and κ-carrageenan, cannot be measured directly since the gel surface is always layered with water which gives an erroneous a_w of 1.0 no matter what the polymer concentration of the gel. Gelatin, potato starch and ι-carrageenan do not synerese, however, and the water activity of these gel types may be measured directly, by the vapor pressure manometric technique. In this case however, since the a_w is high the measured values have an error of ± 0.01. The results of these measurements are shown in Figures 3, 4 and 5. Also shown in these figures is the a_w as predicted by Eq (2), assuming ideal water:polymer interactions (i.e., χ = 0 to 0.05). This is the a_w range where the third term of Eq (3) is essentially negligible.

The gelatin results (Fig. 3) show that the Flory-Huggins equation, based on the molar volume of each species in solution and the respective molecular weights, is a very good predictor of gelatin gel a_w's up to the point where χ starts increasing (see Fig. 2). Above about 35 solids as can be seen, the ideal form of Eq (2) no longer accurately predicts the a_w lowering since χ is shown to increase. This is because the ideal form of the Flory-Huggins Eq (2) represents a polymer gel which is a uniform interweaving mass of polymer strands which are not covalently bound to each other. Any deviation of these systems from simple random coil conformation is dominated by changes in entropy and not changes governed by electrostatic considerations.

The starch a_w results measured by VPM (Fig. 4) have a poorer fit to the ideal form of the Flory-Huggins equation. This could be expected since starch like gelatin, is a relatively hydrophobic molecule (Suggett, 1975). However, as seen even in the range when $\chi = 0.5$, some water:polymer interactions may be occurring which causes the measured vapor pressure to be lower. Of course one must also realize that the deviation could be due solely to the error in a_w measurement.

Both water:starch and starch:starch interactions have been reported by other workers. Franks (1977) has shown that the equatorial hydroxyl groups of mono- and disaccharides tend to stabilize the structure of bulk water. These hydroxyls have a molecular spacing about equal to the second neighbor hydrogen spacing of bulk water. Since potato starch is largely composed of unsubstituted glucose

molecules, this same structure enhancing mechanism could account for some of the nonideality noted in Figure 4.

Masuzawa and Sterling (1968a, b) studied starch gels and concluded that at least one of the four free hydroxyls on the starch-glucose monomers is available to interact with water. The other three hydroxyls are either intra or intermolecularly hydrogen bonded. The large number of free hydroxyl groups which could structure water in their vicinity could account for most of the nonideality noted in Figure 4.

The a_w results for carrageenan, shown in Figure 5 indicate a large deviation from the Flory-Huggins model at all concentrations. It should be noted that this was for systems above an a_w of 0.98 which is difficult to measure. The results indicate that the χ value may be a larger negative number then as measured by suction pressure, since in the solids range tested the third term in Eq (2) should be negligible. Unlike starch, *ι*-carrageenan is a highly sulfated polysaccharide. This composition leads to a highly negatively charged polymer which should interact extensively with water via ionic bonding. This ionic bonding, coupled with the two types of interactions discussed for starch, would lead one to predict large deviations from ideality for carrageenan gels with χ values being quite different from 0.5.

The spin-lattice relaxation time gives an indication of the efficiency with which a proton system can dissipate local energy perturbations into the environment. Shorter T_1 times (larger $1/T_1$ times) indicate a more efficient energy dissipating ability and a more highly structured proton system. The spin-lattice relaxation results, graphed as $1/T_1$ vs concentration, for gelatin, starch, agar and *κ*- and *ι*-carrageenan are shown in Figure 6. In this figure, a positive slope indicates a polymer with increasing ability to structure water as concentration increases. The gelatin results show that at high concentrations, the water structuring or binding induced by the polymer increases dramatically. This is opposite to the suction pressure results shown in Figure 1, which indicates only a small increase in the suction pressure at high concentrations. As seen, a sharp change in slope occurs at about 30% solids which is near the point where the χ value increases (Fig. 2). An increase in χ represents increased polymer:polymer interactions or increased helix formation with exclusion of water. In effect, the helix formation in gelatin gels is a cooperative process which is enhanced at high polymer concentrations. This effect has been noted by a number of other researchers (Ferry, 1948; Kuntz and Kauzmann, 1974).

The small change in suction potential above 30% solids indicates that the major network structure has formed and increased addition of gelatin only enhances further helix content development without effecting the overall network pore space. If the cooperative effect of water structuring is, in fact, operational in gelatin gels, then at high concentrations the macromolecular structure around the "void" in which water is trapped becomes increasingly coherent at higher polymer concentrations. Since this "void" would have walls quite hydrophobic in nature (as has been suggested by Nemethey and Scheraga, 1962) the water contained within should be highly structured through hydrogen bonding induced by the hydrophobic shell. These hydrophobic pools then should show the type of NMR results as seen in Figure 6, i.e., a decrease in T_1.

The spin-lattice relaxation results for starch (Fig. 6) show that water in starch gels is more structured than that of gelatin with a similar linear increase as a function of concentration up to 30% solids. Because additional starch from the same lot was not available, it was not possible to measure T_1 beyond 30% solids which is where the χ value measured from suction pressure increases. Presumably a rapid rise similar to that of gelatin would occur. The χ parameter above 30% solids, however, indicates that the polymer:polymer interaction is less than that of gelatin, making starch a slightly better water binder. As noted previously, at low concentration the suction pressure shows starch to be a poorer water binding agent than gelatin which is opposite to the NMR results. These seemingly anomalous results fit together, however, from a theoretical standpoint. Starch binds water mainly by hydrogen bonds which can be cooperative with the formation of the hydrogen bond pools of water induced by the non-polar macromolecular regions. Starch has no charged groups as does gelatin and therefore, can form a weak gel structure which can hold water with the more apolar amylose fraction considered to be insoluble as suggested by Suggett (1975). Thus: (1) at low concentration starch will hold less water at a given solids content; (2) the starch:strach interactions should increase rapidly as solids content increases above a certain concentration as shown by the χ plot; and (3) the interactions with water are more non-ideal than for gelatin since the amylose may not be in solution and thus the T_1 times should be longer.

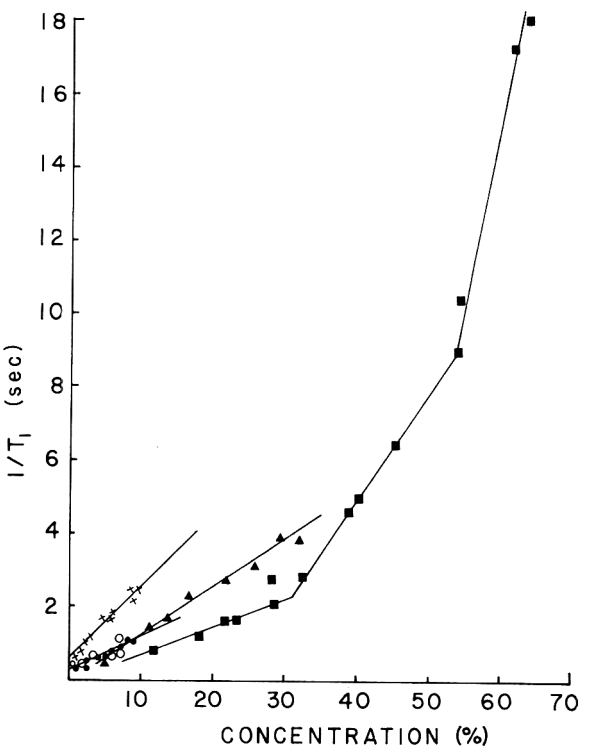

Fig. 6—Spin-lattice relaxation ($1/T_1$) vs solids concentration for four gelling macromolecules: ○—○ κ-carrageenan; ●—● ι-carrageenan; x—x agar; ■—■ gelatin; ▲—▲ potato starch.

The agar results seen in Figure 6 show that water interacts with this polymer more than it does with starch, carrageenan or gelatin at low concentrations. Theoretically the ordering of water induced by agar should be somewhat higher than starch. Since both are unsubstituted polysaccharieds, both can order water through the water structure reinforcing effect of hydrophilic induced hydrogen bonds and pools of H-bonded water induced by hydrophobic regions as noted for starch. Unlike starch, however, agar contains a 3,6-anhydro linkage and thus is sterically hindered and cannot interact with itself as extensively as can starch (Rees, 1969; Rees et al., 1969). Therefore, more free polymer chain is available to interact with water leading to a higher water structuring effect and a greater polymer water interaction as seen in both the suction potential and χ value results.

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Agar is also known to form helix regions via a cooperative effect (Arnott et al., 1974). As concentration increases, this cooperative effect would lead to increased amounts of dense helix regions and reduce the relative amount of free chain available to interact with water. This same effect was noted for gelatin and probably starch. Where gelatin is postulated to form hydrophobic pools, however, with a large increase in water structure, agar should form hydrophilic pools of water with a reduced water structuring effect. At very high concentrations (above 10%), a true gel cannot be made and thus one cannot compare results to gelatin or starch, but it can be postulated that agar would show longer T_1's than gelatin, i.e., $1/T_1$ would be less.

The carrageenan results shown in Figure 6 indicate that $1/T_1$ varies linearly with concentration for both the κ- and ι-fractions. The results show that carrageenan has a water structuring effect similar to that of gelatin at low concentrations and poorer than that of agar. These results are quite opposite to the results found using the suction pressure method and the water activity measurements, where carrageenan shows the highest water binding capacity. This serves to highlight the current problems in the literature in defining what water binding means.

This apparent disparity of results is probably due to the molecular structure of the carrageenans. Since they are polysaccharides they should interact with water by the same hydrogen bonding mechanisms as were described for starch and agar. As noted in the discussion, carrageenan is a highly sulfated molecule which results in the polymer carrying a large negative charge. This negative charge should structure water through ionic interactions. The ionic interactions between water and carrageenans should not, however, exhibit any cooperative effects with the hydrogen bonding induced by hydrophilic groups or hydrophobic regions. Hydrogen bonds and ionic bonds are both orientationally specific but with different orientations (Franks, 1977). Any interface between these types of ordered water, therefore, should result in noncooperative water structuring which would lead to a very long T_1 time. This effect has been noted before as reported by Drost-Hansen (1969). On the other hand the strong polymer:water interaction as noted by the large negative χ value should result in a high water holding capacity and large Δp at low concentration.

SUMMARY & CONCLUSIONS

EVEN THOUGH the polymers studied in this research all gel by similar mechanisms (helix formation), they are very different in chemical composition. Gelatin is an amphoteric protein molecule which is highly hydrophobic. The other polymers are all polysaccharides. Potato starch has a relatively unsubstituted structure which allows a high proportion of intra- and intermolecular hydrogen bonding. Carrageenan, in contrast, is highly sulfated and carries a large negative charge.

The methods used in this research may be used to rank the water structuring effects of these molecules. This ranking, shown in Table 2, may be explained without postulating any "long range" forces or capillary structuring effects. If one postulates that (1) hydrogen bonding is rather weak and that ionic and hydrophobic bonding is relatively stronger (0.5–2 kcal/mole as reported by Kuntz and Zipp, 1977); (2) that the hydroxyl bond lengths found in polysaccharides are such that they fit into the structure of bulk water; and (3) that hydrogen, hydrophobic and ionic bonding are noncooperative, then the orders reported in Table 1 may be easily explained.

When water-holding effects are measured by suction pressure, the steeper the suction potential curve as a function of concentration (Fig. 1), the greater is the water-binding effect. Bearing in mind the fact that this is a macroscopic measurement, one would expect the system with the highest net energy potential to be the best water binder. Carrageenan, with the ability to structure water both ionically and through hydrogen bond reinforcement, does rank highest in suction pressure/water binding effect. Agar, with the capability of reinforcing hydrogen bonds only, ranks second in water structuring effects when measured by suction potential. Gelatin ranks third in suction potential. Even though it is capable of structuring water by forming ionic clouds, gelatin also would largely negate the ionic structure because water would be unable to hydrate all of the chain molecules (i.e., hydrophobic side chains). Starch ranks last since its hydrogen bonding capabilities are largely restricted due to intra- and intermolecular bonding.

Table 2—Water structuring effects of selected polymers

Method	Rank[a]
Suction pressure	Carrageenan Agar Gelatin Starch
Flory-Huggins χ value (from suction pressure)	Carrageenan Agar Starch Gelatin
a_w lowering effect	Carrageenan Starch Gelatin
NMR ($1/T_1$ slope) low concentrations	Agar Starch Gelatin Carrageenan
NMR ($1/T_1$ slope) higher concentrations	Gelatin Agar Starch Carrageenan

[a] In order of decreasing water structuring

The Flory-Huggins χ values give a direct indication of the water structuring effects of polymers since they denote the ideality of the gel solution. With this parameter, the more negative the value, the higher is the water:polymer interactions. The χ value ranking is very similar to the suction pressure ranking. Carrageenan ranks highest since it interacts with water through both ionic and hydrogen bonding. Agar ranks second because it is not capable of forming ionic bonds but has many more hydrogen bonding sites available than does starch. Starch is third through hydrogen bonding, and gelatin is last, although both are similar up to 30% solids. As mentioned above, the hydrophobic nature of gelatin would result in little or no water:polymer interaction, especially at high concentrations where the χ parameter indicates that gelatin no longer exists in solution (at χ values higher than 0.5 as discussed by Tobolsky, 1971).

The a_w lowering effect as measured by the VPM shows the same results as the χ values evaluated from suction pressure results. The same arguments should then hold for the a_w results as holds for the χ value results.

With NMR data, a steeper slope is also indicative of a higher order of water structuring. With this technique, which measures short-time local structure, carrageenan ranks about the same as gelatin and starch at low concentration. This is probably due to the difference in water structuring effects of ions and molecules capable of hydrogen bonding. While they both structure water, one would not expect the water structure to be the same in each case. This would lead to relatively inefficient energy transfer between the two water "states" and a resultant lower $1/T_1$ vs poly-

mer concentration slope. At low concentrations agar ranks highest since it is very efficient at reinforcing hydrogen bonding. Starch with the second highest slope ranks second since it also efficiently reinforces the hydrogen bonded structure of water but has fewer bonding sites available. Gelatin would rank third since its water cloud would be more efficient at dissipating energy than the carrageenan system of bonding. At high concentrations gelatin would be expected to have the highest water structuring effect if hydrophobic pools can form as described earlier.

From the data reported here and from that found in the literature, the water structuring effects of macromolecular gels may be assigned to a combination of three well known bond types – hydrogen bonds, hydrophobic region induced H-bonds and ionic – and thus there is no need to postulate "long range" forces or multilayers hundred of molecules thick. These data also indicate that polymer gels should not be thought of as static networks, but can be treated more correctly by the Flory-Huggins approach. This may be part of the reason the researchers have not been able to identify stable capillary structures in gels despite the many indications that they exist.

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DIFFERENTIAL SCANNING CALORIMETRY (DSC) STUDIES ON THE FREEZING PROCESSES OF WATER-ETHANOL MIXTURES AND DISTILLED SPIRITS

KUNIMASA KOGA and HAJIME YOSHIZUMI

ABSTRACT

DSC measurements of the freezing of water-ethanol mixtures in various ethanol concentrations were made in a temperature range from +20°C to −160°C. One exotherm was observed in the freezing process of each mixture. The freezing point, heat associated with the freezing process and kinetic constant of freezing (k_f) were determined over the entire range of the ethanol concentration from the DSC thermogram. Log k_f was plotted as a function of the reciprocal of the absolute temperature (T^{-1}) of the mixtures. The freezing process of the mixtures in an ethanol concentration of 0~75 (V/V)% obeyed first order kinetics but at a concentration above 75%, the freezing process did not do so. The slope of log k_f versus 1/T plots (d log k_f/dT^{-1}) of the mixture at the concentration below 75% was determined. The concentration below 75% was divided into four ranges (0~18.5%, 18.5~40%, 40~60%, 60~75%) from a variation in the slope of the linear plots. The results of the DSC measurements of freshly distilled spirits (raw whiskey from barley malt and grain spirits) coincided with those of simple water-ethanol mixtures. It is considered that the freezing reaction of distilled spirits proceeded by the same mechanism as that of the simple mixture of the corresponding ethanol concentration. Structures of distilled spirits as a function of ethanol concentration were discussed in terms of the interaction of water and ethanol molecules.

INTRODUCTION

THE PROPERTIES of water-ethanol mixtures are of interest in many fields of inquiry. Franks and Ives (1966) reviewed the physico-chemical properties of the mixture from a thermodynamic point of view. Recently, the structures of water-ethanol mixtures as a function of ethanol concentration have been extensively studied with IR (Taniewska-Osinska and Grochowski, 1970), NMR(Coccia et al., 1975) and light scattering measurements (Kono et al., 1975).

Nakamura (1952) indicated that a matured water-ethanol mixture is associated each other and an unmatured non-associated, with light scattering measurements. Akahoshi (1963) showed by a dielectric method that a strong association between water and ethanol occurred in aged spirits, and suggested that these phenomena are responsible for the maturity of aged whiskey. Measuring the melting process of a frozen water-ethanol mixture with a low temperature DSC, Koga and Yoshizumi (1977) showed that structural alterations of the mixture take place at 40% and 60% ethanol concentrations, and that the strong interaction between water and ethanol in aged whiskey is not destroyed by dilution with water or mixing ethanol.

In the present manuscript, to make the structural alterations of the mixtures clearer primarily from a whiskey-making point of view, the freezing processes of water-ethanol mixtures and distilled spirits are studied with DSC measurements.

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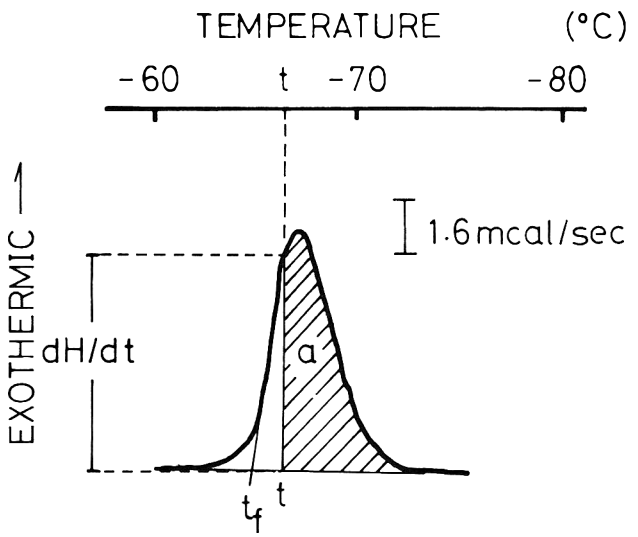

Fig. 1—DSC thermogram representative of freezing of water-ethanol mixtures at 60% ethanol concentration.

EXPERIMENTAL

Ethanol [99.5 (V/V)%, analytical grade] was purchased from Wako Pure Chemical, Co., Kyoto, and was further purified by a fractional rectifying column (120 cm × 2 cm) packed with stainless-steel beads. The ethanol concentration of the distillate was 99.5 (V/V)%. Throughout the measurements, deionized-redistilled water was used. Freshly distilled spirits from barley malt and maize were used for DSC measurements.

The water-ethanol mixture was prepared by weight with 1-mg precision, and the ethanol concentration of a sample mixture is expressed in terms of percentage by volume (V/V%) throughout this paper.

DSC measurements of the freezing of the mixtures and distilled spirits were made with a standard type DSC-meter (Rigaku Denki Co., Tokyo). The mixture was cooled in 10°/min within a scanning temperature range of +20°C through −160°C. The detailed methods of the DSC measurements are described in a preceding paper (Koga and Yoshizumi, 1977).

For analysis of the freezing process of water-ethanol mixtures, freezing point (t_f), heat of freezing (ΔH_f) and kinetic constant of freezing (k_f) were determined from DSC thermogram. Figure 1 shows a DSC thermogram representative of the freezing of water-ethanol mixture at 60% ethanol concentration. The temperature of the initial deflection of the exotherm on the thermogram was taken as a "peak temperature." The heat of freezing was estimated by integrating the peak area between the thermogram and a base-line under the peak, and expressed in terms of joules per unit weight of a sample (J/g). The kinetic constant for the freezing was calculated at various temperatures of the DSC thermogram according to the method of Donovan and Ross (1973). Deflection from the base line of the DSC curve is proportional to the rate of heat flow out of the sample (dH/dt) and this is a measure of the freezing rate. The relative amount of the unfrozen water-ethanol mixture, a, present at a temperature of t°C could be determined by measuring the fractional area under the peak below that temperature. When the freezing process of the mixture obeys first order kinetics, the kinetic constant for freezing at t°C is given by

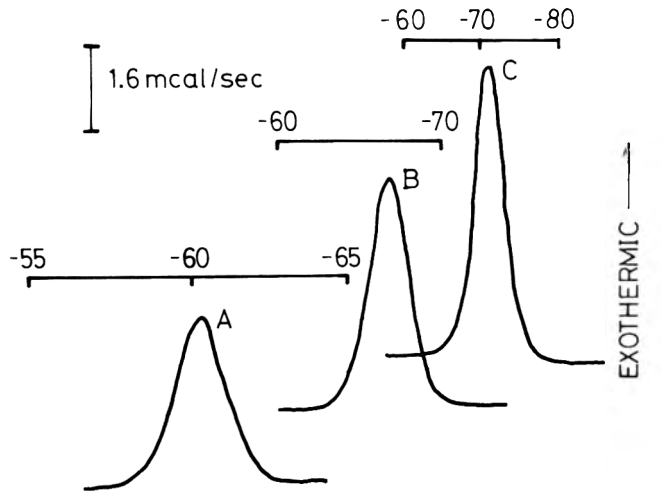

Fig. 2—Effect of the cooling rate of a calorimeter on the DSC thermogram of freezing. Ethanol concentration: 60%; Cooling rate of a calorimeter; A, 5°/min; B, 10°/min; C, 20°/min.

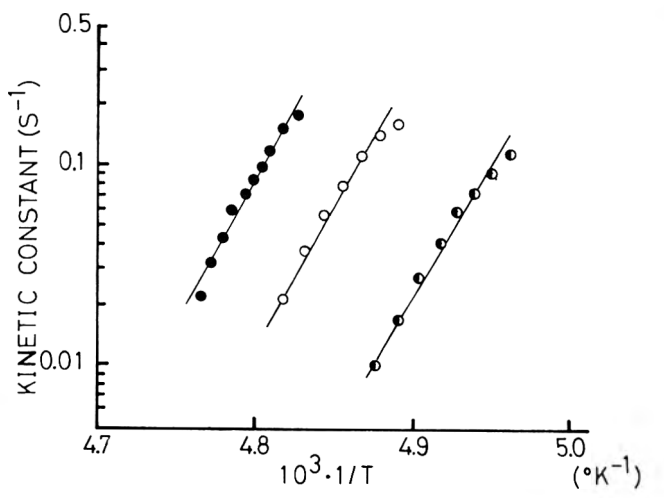

Fig. 3—Log k_f versus 1/T plots for the freezing process of 60% water-ethanol mixtures. Cooling rate of a calorimeter: ●, 5°/min; ○, 10°/min; ◐, 20°/min.

$$k_f(sec^{-1}) = \frac{v_f}{[\text{amount of unfrozen mixture}]} = C \cdot \frac{dH/dt}{a} \quad (1)$$

where C is a constant, and v_f the freezing velocity.

The peak temperature of the thermogram was reproducible within 1°C and the heat of freezing was within 5%. All measurements were repeated at least three times to confirm the reproducibility of the thermograms.

RESULTS

FIGURE 2 illustrates the DSC thermograms of the freezing process of a 60% water-ethanol mixture at cooling rates of 5°, 10° and 20°/min. The temperature of the exotherm, ascribable to the freezing of the mixture, depended upon the cooling rate of the calorimeter. Accordingly, the freezing point was determined by extrapolation to the rate of 0°/min. The heat of freezing did not depend on the cooling rate of the calorimeter, and was obtained as −45.0 kJ/g. On the assumption that the freezing process obeyed first order kinetics, kinetic constant was determined according to Eq (1). In Figure 3, log k_f is plotted as a function of T^{-1} at each cooling rate. As the plots were linear at each cooling rate, it is conceivable that the freezing process of the mixture at 60% thus obeyed first order kinetics. In the case of the chemical reaction in which the rate increases with temperature, the activation energy (E≠) of the reaction could be obtained from the slope of log k_f versus T^{-1} plots (d log k_f/d T^{-1}) according to the Arrhenius equation,

$$\ln k_f = \ln C - \frac{E\neq}{RT} \quad (2)$$

where C is a constant, R the perfect gas constant (1.987 cal mol^{-1} K^{-1}), and T the absolute temperature. However, in the case of the freezing reaction, the reaction rate increases as the temperature decreases and so, the value of (d log k_f/d T^{-1}) is positive. Figure 3 shows that each value of this function does not depend upon the cooling rate, but is constant. Thus this function gives some information on the characteristics of the freezing process of the water-ethanol mixture, itself.

Exothermic heat associated with the freezing process is expressed as a function of the ethanol concentration (10~93%) as shown in Figure 4. The heat of freezing decreases with an increase in the ethanol concentration. The kinetic constant of the freezing was determined from the DSC thermogram of the mixtures for the entire range of ethanol concentration. The freezing process of the mixture for only ethanol concentration less than 75% obeyed first order kinetics. The value of (d log k_f/d T^{-1}) of the mixtures below 75% was obtained in the same manner as the activation energy of the Arrhenius plots and is shown as a function of the ethanol concentration in Figure 5. Within an ethanol concentration range from 0~18.5%, the value of −(d log k_f/d T^{-1}) decreases with an increase in the ethanol concentration. The values for the concentration ranges from 18.5~40% and 40~60% are constant, respectively. In the ethanol concentration range 60~75%, the value decreases with ethanol concentration. The entire ethanol concentration is divided into five ranges [(A)~(E) ranges] from a variation in the value of the function, d log k_f/d T^{-1}.

After adjusting raw whiskey from barley malt and grain

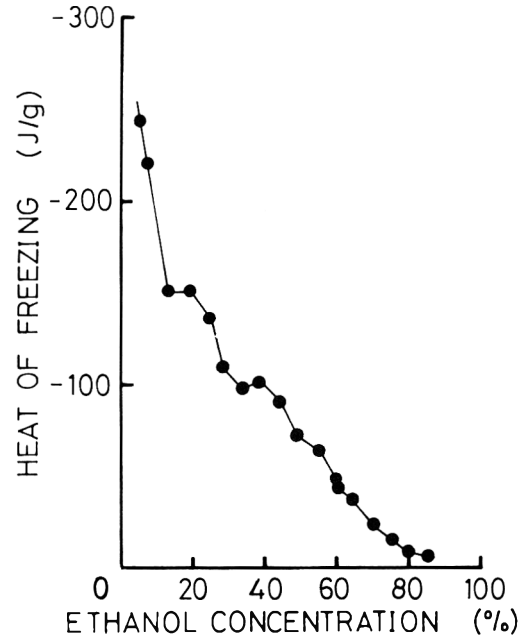

Fig. 4—Relationship between the heat of freezing and ethanol concentration of water-ethanol mixtures.

Table 1—A comparison of freezing points and heats of freezing of freshly distilled spirits (raw whiskey from barley malt and grain spirits) with those of a mixture at a 60% ethanol concentration

	Freezing point (°C)	Heat of freezing (kJoules/g)
Barley malt whiskey	−40.4	−45.4
Grain spirits	−40.1	−45.7
Water-ethanol mixture	−39.6	−45.0

spirits to a 60% ethanol concentration with ethanol, DSC measurements of the freezing of these spirits were made. Freezing parameters (t_f, ΔH_f and k_f) of distilled spirits at 60% are practically identical with those of a 60% water-ethanol mixture as shown in Table 1 and Figure 6. After adjusting the distilled spirits to 20% and 40% with water, these samples were also subjected to DSC measurements. The freezing parameters for each sample were found to coincide with those of simple mixtures at corresponding ethanol concentrations, respectively. The freezing process of distilled spirits proceeded by the same mechanism as for the water-ethanol mixture of the corresponding ethanol concentration.

DISCUSSION

IT IS KNOWN that the viscosity of a water-ethanol mixture has a maximum and the enthalpy of mixing of ethanol and water has a minimum at 40% at 25°C (Akahoshi, 1963; Franks and Ives, 1966; Boyne and Williamson, 1967). A contraction in volume of mixing ethanol and water has a maximum at about 60% at 25°C (Kuppers and Carriker, 1971). These phenomena are considered to closely relate with the structures of water-ethanol mixtures.

In a whiskey making process, after aging in white oak barrels at a concentration of about 60%, the matured whiskey is usually diluted to about 40% at bottling. We tried to investigate the structural alterations of distilled spirits as a function of ethanol concentration by measuring the freezing process of water-ethanol mixtures at various ethanol concentrations. The entire range of the ethanol concentration is divided into five ranges (A range: 0~18.5%; B range:

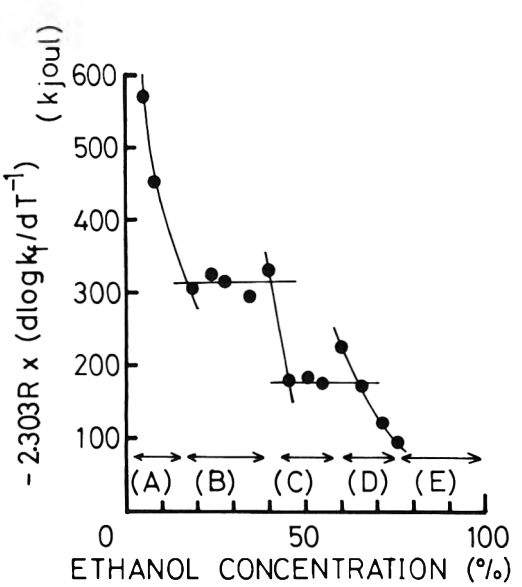

Fig. 5—Relationship between the value of (d log k_f/d T^{-1}) and ethanol concentration of water-ethanol mixtures.

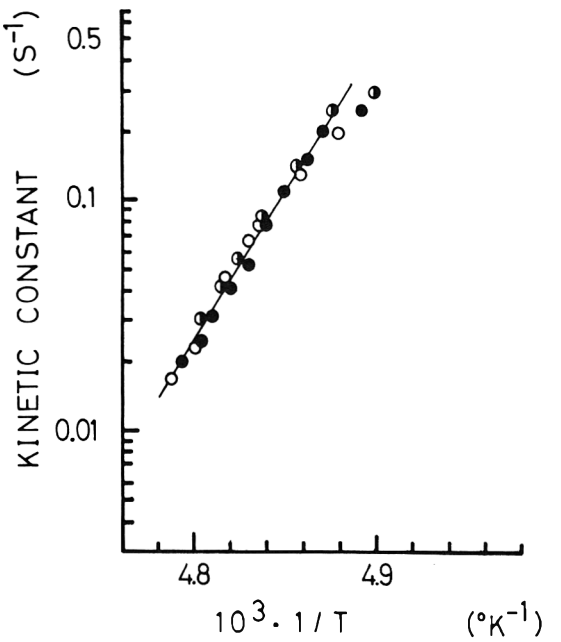

Fig. 6—Log k_f versus 1/T plots for the freezing processes of freshly distilled spirits from barley malt ○ and maize ◑, and water-ethanol mixture ● at a concentration of 60%.

18.5~40%; C range: 40~60%; D range: 60~75%; and E range: 75~100%) from the variation in the value of (d log k_f/d T^{-1}). Considering that this function, d log k_f/d T^{-1}, can be used to evaluate the freezing process of water-ethanol mixtures in like manner of studies on protein denaturation (Multon and Guilbot, 1974), the freezing processes of the mixtures in B and C ranges proceed at a constant course, and the freezing processes in A and D ranges vary with the ethanol concentration.

A preceding paper (Koga and Yoshizumi, 1977) described some of the results on DSC measurements of the melting processes of frozen water-ethanol mixtures. These measurements indicate that structural alterations in the mixtures occur at 40% and 60%. Especially, the temperature of the endotherm, ascribable to the melting of ethanol, shifts from −74°C to −62°C at about 40% ethanol concentration. The result may show that ethanol molecules of the mixtures at an ethanol concentration above 40% are in more intimate interaction with water than those at a concentration below 40%. By NMR measurements of the mixtures, Coccia et al. (1975) studied the changes of ethanol and water OH chemical shifts of water-ethanol mixtures over the entire concentration range. From this experiment, the entire range of the ethanol concentration is divided into five ranges and each range almost agreed with that obtained from DSC measurements. Coccia et al. (1975) also have proposed that water-ethanol mixture at a concentration below about 40% keeps the structure of water, and at a concentration above about 40%, the water structure is progressively disrupted by an increase in the ethanol concentration. This mechanism of molecular association in the mixtures supports our interpretation of the shift in the melting point of ethanol in water-ethanol mixtures at about 40%. Usually, distilled spirits are bottled at about 40% ethanol concentration. This may be related to the structures of eater-ethanol mixtures which keep the water structures in a concentration range from 0~40%.

The relationship between the freezing point and ethanol concentration of the mixture is shown in Fig. 7. This result coincides with that already published (Seidell, 1941). The freezing points of mixtures at concentration above 93% are

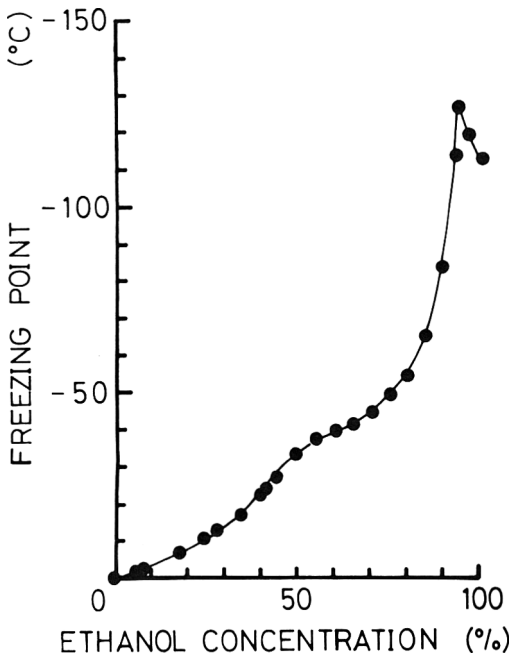

Fig. 7—Relationship between the freezing point and ethanol concentration of water-ethanol mixtures.

lower than that of pure ethanol (−114°C). The vapor pressures of mixtures for the corresponding range of ethanol concentrations are also well known to be higher than that of pure ethanol, e.g. the 95% azeotrope. This may show that the addition of a small quantity of water to ethanol promotes the hydrophobicity of ethanol molecules.

On the other hand, conventionally, whiskey is stored in a white oak barrel at a concentration of 60%. The structure of a water-ethanol mixture at a concentration of 60% has not been understood concretely. However, the present result suggests that structural alterations of the mixture take place at a 60% ethanol concentration (Fig. 5). The preceding paper (Koga and Yoshizumi, 1977) also suggested that the portion of strongly interacted water-ethanol is least at 60% ethanol concentration. In other words, the activity of ethanol in water-ethanol mixtures is maximal at 60%. It is known that the maximum amount of acid materials and phenolic compounds are extracted from the barrel by a water-ethanol mixture of about 60% (Liebmann and Rosenblatt, 1943). When whiskey is stored at 60% ethanol concentration, it may result in an increase in the interaction between ethanol and the white oak barrel, and effectively make the whiskey mature.

In the preceding paper (Koga and Yoshizumi, 1977), the melting process indicated three endotherms, whereas, in this paper, we found that the freezing process has only one exotherm. It is considered that the freezing reaction proceeds by a vitreous transition. Additional studies are in progress to investigate the differences in the melting and freezing DSC curves.

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COMPARISON OF Tetrahymena pyriformis W, Aspergillus flavus, AND RAT BIOASSAYS FOR EVALUATING PROTEIN QUALITY OF SELECTED COMMERCIALLY PREPARED FOOD PRODUCTS

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ABSTRACT

Tetrahymena pyriformis W and *Aspergillus flavus* were used as test microorganisms for evaluating the quality of proteins in commercially prepared foods. The results were compared with those obtained from rat bioassays. There was no significant correlation between the microbiological and rat bioassay values for protein quality obtained for selected food samples under the bioassay conditions in this study. These results are contrary to reports indicating that *A. flavus* can be used to determine the quality of proteins in various cultivars of grains. Microbiological bioassays were conducted using defatted food products and partially purified proteins from these products. There was no apparent improvement in correlation between data obtained using microbiological assays and rat assays when purified proteins were used in place of unfractionated foods in microbiological assays. Variations among replicate analyses were greater when *T. pyriformis* W was used as the test organism than when *A. flavus* was used. Coefficients of correlation between microbiological data and rat % PER data were higher for single-point (S-P) analyses in growth media containing 0.3 mg N/ml than for the four-point slope-ratio (S-R) technique; and higher for the dye-reduction (TPTZ) technique than for direct microscopic count (DMC) for evaluating protein quality.

INTRODUCTION

RAPID METHODS for measuring protein quality in foods have received increased attention in recent years from researchers in food industries, government, and academia. Protozoa and fungi offer potential as test organisms because of their rapid growth rate, ease of handling, and, in some instances, similarity in nutritional requirements to those of higher animals.

Tetrahymena pyriformis W, a protozoan with amino acid requirements similar to those of rats and humans, has been studied for at least three decades for its value in assessing protein quality. Results have been reported recently showing various degrees of potential for using this organism as a biological tool (Landers, 1975; Rølle, 1976; Evancho et al., 1977; Dryden et al., 1977; Butrum and Kramer, 1977; Hegedus and Zachariev, 1978; Wang et al., 1979). In fact, many researchers have already employed this organism for measuring quality of proteins in various kinds of foods (Srinivas et al., 1975; Smith and Pena, 1977; Kramer and Kwee, 1977; Kamath and Ambegaokar, 1968; McCurdy et al., 1978; Wang and Fields, 1978a, b, c). On the other hand, results also have been reported from time-to-time revealing the unsuitability of *Tetrahymena* for use as a test organism in routine bioassays (Evans et al., 1977; Bergner et al., 1968; Niemann, 1977; Lee et al., 1978).

Aspergillus flavus, a fungus commonly found in spoiled grain and legume products, has been investigated recently for its usefulness in screening protein quality of various cultivars of grains and legumes (Mohyuddin et al., 1976; 1977a, b; 1978a, b; Niemann, 1977). Favorable results were reported; the authors found that the fungus could be used to grade protein quality of grain products. The fungal bioassay procedure is simpler than that of protozoan assays.

Many of the foods presently available in the supermarket which might contribute significantly to dietary protein supply are combinations of grains, legumes and other ingredients that have been subjected to various processing treatments and storage conditions. Though chemical interactions among components of the foods are known to occur during processing and storage, there is little information on the ultimate effects of such treatments on protein quality of foods as used by the consumer (Miller et al., 1979).

The objectives of the study reported here were to test the feasibility of using *T. pyriformis* W and *A. flavus* as test organisms for evaluating the quality of protein in commercially prepared foods that contain several ingredients. Specifically, it was desirable to evaluate the response of these microorganisms to complex foodstuffs and to partially purified proteins from these foodstuffs in order to determine their usefulness as a screening mechanism to assess protein quality.

MATERIALS & METHODS

Test materials

Commercially prepared food products were purchased from local supermarkets in the spring of 1978. The products were cooked or baked according to normal procedures for home preparation, ground, freeze-dried (except nonfat dry milk and high-protein cereal), reground, and stored in plastic containers at −18°C until used. Details of procedures for preparation are published elsewhere (Miller et al., 1979). Animal Nutrition Research Council (ANRC) reference casein was obtained from Humko Sheffield (Lyndhurst, NJ). Rat diets contained the complete dried food product, whereas foodstuffs incorporated into *Tetrahymena* and *A. flavus* growth media were defatted or defatted and fractionated. In the latter instance, either the defatted food or the extracted protein fraction was enzymatically hydrolyzed before incorporating into media.

Proximate analyses of food samples

Moisture content of the foods was determined by loss in weight after drying for 24 hr at 70°C in a vacuum oven. Nitrogen in fresh and dried products was measured by Kjeldahl analysis (AOAC, 1975). Lipid content of the dried samples was determined by gravimetric analysis of material extracted by the Bligh and Dyer (1959) procedure. Samples were ashed in a muffle furnace at 450°C for about 16 hr (Miller et al., 1979). Nitrogen content of partially purified proteins was determined with a Technicon Auto-Analyzer II (Technicon Instrument Corp., Tarrytown, NY) using a procedure described by Wall and Gehrke (1975) with minor modifications as devised by Phillips (1978).

Preparation of samples for bioassays using T. pyriformis W and A. flavus

Dried foods were defatted and digested with pepsin as previously reported (Wang et al., 1979), except that samples were defatted first and then weighed for enzymatic digestion. The procedure for extraction and partial purification of proteins from foods was essentially that of Fernell and Rosen (1956), with some modifications. Defatted food samples were extracted for 4 hr with 0.1N NaOH (1:20, w/v, food:NaOH) while being agitated on a gyrotary shaker (New Brunswick Scientific Co., New Brunswick, NJ) at 25°C. The slurry was then centrifuged for 20 min at 10,300 × G and the clarified supernatant fluid was adjusted to pH 5.0 with 1N HCl.

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Precipitation of the protein was completed by adding five volumes of acetone. The precipitate was collected on Whatman No. 1 filter paper under vacuum, washed once with acetone and twice with diethyl ether, and allowed to dry at 37°C for 30 min. The dried protein was ground to pass an 80-mesh sieve and stored at −18°C in a sealed container until used. All microbiological assays were performed in four replicates.

Bioassay procedure using T. pyriformis W

The *Tetrahymena* bioassay procedure described previously (Wang et al., 1979) was used. Growth of the protozoan was measured by direct microscopic count (DMC) using a hematocytometer and a microscope, and by a dye-reduction method using 2,3,5-triphenyl tetrazolium chloride (TPTZ) as an indicator. For the dye-reduction method, 2 ml of test culture were mixed with 0.5 ml of 2.5% TPTZ in 0.1M potassium phosphate buffer at pH 7.7 and held in a water bath at 37°C for 15 min. After incubation, 0.2 ml of 0.1M $HgCl_2$ in 0.24N HCl was added to stop the reaction. The precipitate obtained by centrifugation was dissolved in 5 ml of acetone and percentage of light transmission of the solution was read at 485 nm.

Tetrahymena relative nutritive value (RNV) was calculated by two methods. The regression of growth on nitrogen concentration in the growth medium (0.1–0.4 mg N/ml) was calcualted to pass through the origin of the axes, and the slope of the line was compared to that obtained with casein (slope-ratio, or S-R). Growth in media containing 0.3 mg/N ml, which is the concentration usually recommended for *T. pyriformis* bioassay, was compared to the growth in casein medium with the same nitrogen concentration as single-point (S-P) assay procedure.

Bioassay procedure using A. flavus

A nonaflatoxigenic strain of *Aspergillus flavus* (A-4018b) which had been isolated originally from bran served as the test fungus. A 7- to 10-day-old malt agar culture of *A. flavus* incubated at 28°C was used for inoculum preparation. For measuring the biomass (BM) of *A. flavus* grown in media containing foods, the procedure was similar to that described by Mohyuddin et al. (1977b) but with some modifications. Samples of defatted foods and partially purified proteins containing 6 mg of N were weighed into 250 ml flasks in duplicate. Soluble potato starch (J.T. Baker Chemical) was added to give 1g of total solids. Forty milliliters of Czapek's medium (Thom and Raper, 1945) without sucrose, $NaNO_3$, or agar were adjusted to pH 5.6 and added to each flask. After standing for 30 min, the flasks were stoppered with sponge plugs, autoclaved for 10 min at 121°C and cooled. Each flask was inoculated with 0.5 ml of conidial suspension (approx. 10^7 conidia/ml of suspending fluid containing 1 drop of Tween 20 per 100 ml of distilled water) prepared from inoculum culture. The flasks were incubated at 28°C for 72 hr while being agitated on a rotary shaker (Eberbach Corp., Ann Arbor, MI) operated at high speed. The biomass of *A. flavus* was collected on a nylon sieve by filtering, washing with distilled water, and drying in an aluminum dish at 100°C for 1 hr; dry weight of fungal biomass was then determined.

Table 1—Proximate composition of commercial food items[a,b]

Food	Moisture (%)	Nitrogen (%)	Lipid (%)	Ash (%)
Ground beef	50.5	3.88	21.8	1.1
Cereal, high protein	4.3	3.10	5.1	3.1
Pot pie, chicken	66.1	1.00	9.1	1.7
Pizza, sausage	43.8	1.86	11.5	2.7
Diet bar	3.2	3.20	22.4	4.0
Wiener	51.9	1.54	30.2	3.0
Non-fat dry milk (NFDM)	3.4	5.33	0.7	7.2
TV dinner, steak	78.0	0.74	7.3	1.6
Spaghetti and meat balls	76.3	0.75	4.6	1.7
Baby food, chicken & vegetables	86.9	0.29	1.1	0.2
Food bars	9.8	2.49	20.5	3.2

[a] Proximate composition of food as prepared for eating, except for NFDM which is given as purchased.
[b] Adapted from Miller et al. (1979)

Table 2—Comparison of RNV of commercial foods and partially purified proteins from these foods using T. pyriformis W and A. flavus as bioassay organisms and % PER as determined with rats[a,b]

	Tetrahymena RNV[c]								*A. flavus* RNV[d]				
	Defatted foods				Purified proteins				Defatted foods		Purified proteins		
	S-R		S-P		S-R		S-P						% PER[e]
	DMC	TPTZ	DMC	TPTZ	DMC	TPTZ	DMC	TPTZ	BM	DC	BM	DC	(Rat)
Ground beef	103	93	116	92	91	75	88	89	88	99	92	101	114
Cereal, high protein	95	85	91	81	67	62	69	74	106	103	97	103	107
Pot-pie, chicken	99	72	110	67	69	52	65	60	98	107	93	101	101
Pizza, sausage	115	78	119	77	71	53	67	52	102	102	90	97	101
Casein (ANRC)	100	100	100	100	100	100	100	100	100	100	100	100	100
Diet bar	116	86	121	92	81	55	79	59	104	106	91	105	98
Weiner	107	95	121	102	93	76	96	67	95	103	92	100	97
Non-fat dry milk	96	95	103	82	76	81	80	70	94	101	93	102	97
TV dinner, steak	99	77	103	77	103	64	93	58	99	107	89	101	91
Spaghetti and meat balls	90	85	83	78	62	48	64	48	102	105	93	101	88
Baby food, chicken & vegetables	102	81	117	71	106	70	110	72	93	107	104	104	78
Food bars	107	88	102	81	82	66	81	55	95	103	88	102	73
Pooled SEM[f]	6.6	8.0	18.6	11.2	18.4	19.1	16.8	21.8	6.0	2.3	5.3	2.3	2.2

[a] Growth response relative to that obtained with casein (casein = 100)
[b] Rat diets contained the complete dried food product, whereas foodstuffs incorporated into *Tetrahymena* and *A. flavus* growth media were defatted or defatted and fractionated. In the latter instance, either the defatted food or the extracted protein fraction was enzymatically hydrolyzed before incorporating into media.
[c] S-R = slope-ratio (slope of line relating growth response to N concentration in the growth medium: 0.1, 0.2, 0.3 and 0.4 mg N/ml). S-P = single-point (growth measured N concentration in the growth medium equaled to 0.3 mg N/ml). DMC = growth response measured by direct microscopic count. TPTZ = growth response measured by tetrazolium dye-reduction.
[d] BM = growth measured by biomass production. DC = growth measured by diameter of colony.
[e] Based on PER values; PER for casein was 3.44. Values enclosed by the bracket are not significantly different ($P \leq 0.05$) from that of casein (Miller et al., 1979).
[f] Pooled standard error of a mean for each assay procedure including 11 food samples (treatments) with four replicates per treatment for the microbiological assays and 10 replicates per treatment for the rat % PER ($\sqrt{\frac{\text{Error mean square}}{k}}$, k = 4 or 10).

Table 3—Relationships between % PER determined with rats (Y) and RNVs determined with T. pyriformis W or A. flavus (X)[a]

Microbiological method		Linear regression		
		Slope	Y-intercept	r
Defatted foods				
T. pyriformis W				
Slope-ratio	DMC	0.032	91.7	0.022
	TPTZ	0.270	68.3	0.206
Single-point	DMC	0.158	77.9	0.168
	TPTZ	0.380	63.9	0.320
A. flavus	BM	0.188	76.5	0.104
	DC	1.499	−52.8	0.486
Purified proteins				
T. pyriformis W				
Slope-ratio	DMC	−0.232	114.0	−0.283
	TPTZ	−0.037	92.7	0.034
Single-point	DMC	−0.275	117.3	−0.337
	TPTZ	0.500	63.0	0.492
A. flavus	BM	−0.301	122.9	−0.110
	DC	−1.364	233.5	−0.241

[a] See footnotes in Table 2.

The procedure of Mohyuddin et al. (1976) was followed for measuring the growth of *A. flavus* on agar media containing test foods. Sufficient quantities of pepsin digests of commercially prepared foods and partially purified proteins from these foods containing 3 mg of nitrogen each were deposited into 100-ml flasks. Soluble potato starch was added to each flask to bring total weight of solids to 300 mg. Two percent (0.6g) of agar was then added along with 29 ml of hot Czapek's medium (without sucrose, $NaNO_3$, and agar). The contents were autoclaved for 10 min at 121°C and cooled before aseptically adding 1 ml of sterile vitamin solution (Evancho et al., 1977). The agar (approx. 15 ml each) was poured into two sterile Petri dishes, hardened, and inoculated in the center with one drop of suspension containing conidia of *A. flavus*. The plates were incubated at 28°C, and the diameter of colonies (DC) of *A. flavus* was measured at 24-hr intervals for six consecutive days. RNV of the foods and purified proteins was calculated by comparing growth to that obtained with casein assigned a value of 100.

Rat bioassay

Protein efficiency ratio (PER) was determined by the method adopted by AOAC (1975) with minor modifications as published elsewhere (Miller et al., 1979). Rat growth response was calculated as weight gained divided by protein consumed (PER) using 10 animals per treatment. PER values for food products were converted into % PER values relative to that obtained with casein (casein = 100) to facilitate comparisons with the microbiological assays.

RESULTS & DISCUSSION

AS REPORTED previously (Miller et al., 1979) all of the food products (Table 1) selected for study, except ground beef, are subjected to processing treatments and storage conditions that might reduce the quality of protein in the product used by the consumer. Several of the items have significant amounts of reducing sugars, added in the form of corn syrup solids, invert sugar, or dextrose, which could undergo Maillard reaction with proteins. The processed items were selected as being representative of those that are popular with consumers and that may be used as the major source of protein for a meal. Some of these foods, such as pot pies, pizza, and pasta and meat combinations are based on recipes that were traditionally prepared in the home. Other food items are designed to substitute for a complete meal, either as a convenience (e.g. food bars) or for weight control (e.g. diet bars). Ground beef was included for comparison as a frequently used food item that is not subjected to heat treatment prior to preparation for eating.

Data in Table 1 show the proximate composition of the 11 food items as prepared for eating. The moisture content of the food products ranged from 3.2% in the diet bar to 86.9% in baby food. The nitrogen content ranged from 0.29–5.33%, and the lipid content ranged from 0.7–30.2%, indicating a wide range in proximate composition of foods selected for evaluation.

RNVs of food samples and partially purified proteins of these samples using *T. pyriformis* W and *A. flavus* as test organisms and % PER values obtained by rat bioassay of the foods are shown in Table 2 along with a pooled standard error of the mean for each assay procedure. These standard errors show that variations among replicate analyses of the same test sample were greater when the protozoan was used to assess protein quality than when the fungus was used as the test organism.

No apparent relationships were observed between data obtained using *Tetrahymena* or *A. flavus* and those obtained from rat bioassays. This observation can be made without regard to whether *Tetrahymena* RNVs were calculated by the slope-ratio (S-R) technique or by the single-point (S-P) method. In almost all instances, *Tetrahymena* RNVs calculated from DMC data were higher than those calculated from TPTZ data. Furthermore, RNVs for commercially prepared food items as determined using *Tetrahymena* were generally higher than those for corresponding partially purified proteins.

Observations on *A. flavus* RNVs indicated that only a few food items promoted better growth of *A. flavus* than did casein when biomass (BM) production was used for calculations. When calculations were based on diameter of colony (DC), however, the opposite was true. The rat PER of seven of the eleven food products tested was equal to or higher than that of casein.

Table 3 shows the relationships between RNVs of all food items as determined by *T. pyriformis* W and *A. flavus* to % PER determined using rats. No significant correlation was found in any case. A possible explanation for the lack of correlation between results of microbiological assays and rat PER assays is that most of the food items used in this study contained nonnutritive ingredients such as spices and preservatives which may have adversely affected *Tetrahymena* and/or *A. flavus*. Inhibition or promotion of growth of microorganisms by such ingredients have been reported by other workers (Bullerman, 1974; Satterlee et al., 1977; Warren and Labuza, 1977; Eitenmiller, 1978). Furthermore, *Tetrahymena* may be suitable for screening quality of proteins with gross difference in quality but may not be capable of differentiating small variations (Kaestner et al., 1976). The defatting procedure used in sample preparation for microbiological assays may also have affected quality of test proteins.

The correlations between RNV determined with *T. pyriformis* W and % PER determined with rats were higher when the single-point rather than the slope-ratio assays was used for the protozoan. Furthermore, the dye-reduction method for measuring growth of *T. pyriformis* W gave higher correlation with rat % PER than did the DMC.

A. flavus has no specific amino acid requirement. Its occasional success in screening protein quality is postulated to be based on the availability of the nutrients present in the substrate. Thus, it may be useful as an assay organism for foodstuffs with similar nutritional composition, such as various cultivars of a specific grain but not for food products with a wide range of ingredients as used in this study. In addition, there may be differences in growth responses as a result of inherent differences in strains of *A. flavus* studied; the strain of *A. flavus* used in this study was not the strain used in the study reported by other workers (Mohyuddin et al., 1977b).

Parital purification of proteins from food items failed to result in significant correlations between values determined using microbiological and rat bioassay systems. It is possible

that the procedure used for extraction did not yield proteins identical in composition to those of the original test foods. Therefore, these partially purified proteins may not contain the same amino acid profile and/or the same physical form as those in original foods.

Although bioassays with microorganisms offer potential for rapid and inexpensive methods for evaluating protein quality, findings of this study show that precautions should be taken regarding the absolute nutritional requirements of test organisms and the effects of nonproteinaceous ingredients on their growth when commercial food samples are tested. A recently published paper reported a study using another species of *Tetrahymena*, viz., *T. thermophila* (formerly *T. pyriformis* WH_{14}), for screening protein quality (Baker et al., 1978). Excellent agreement was found between responses of *Tetrahymena* and rats grown on foodstuffs with high or low protein potency. Crucial for such agreement, according to the authors, was predigestion and solubilization of the test samples with bromelain. It is postulated that the solubilized protein could be made sterile by microfiltration to eliminate the destructive effect of high heat during autoclaving on protein quality. Studies are in progress in our laboratory to evaluate the procedure of Baker et al. (1978) for its suitability to assess the quality of proteins in complex, commercially processed foods.

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STUDIES ON THE GELATINIZATION RATE OF RICE AND POTATO STARCHES

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ABSTRACT

The gelatinization rate of rice and potato starches were investigated. The rheological method, using a capillary tube viscometer, was applied for the measurement of the gelatinization degree. The gelatinization rates were measured for the rice and potato starches at temperature ranges of 70~85 and 60~63°C respectively. The rate parameter in the rate equation was given as the Arrhenius equation. The equivalent values of the activation energy for the rice and potato starches were about 14 and 230 kcal/g-mol respectively. We concluded that the gelatinization rate of starches is limited by the chemical reaction rate and/or the physical transforming rate of starch components with water.

INTRODUCTION

IN PREVIOUS PAPERS (Kubota et al., 1976; Suzuki et al., 1976, 1977), we have examined the cooking-rate equations of rice, udon and kishimen. Udon and kishimen are round and flat wheat flour noodles commonly used in Japan. The cooking-rate equations of these foods have two rate parameters of internal diffusion of water and of reaction rate, which is the rate of chemical and physical changes such as gelatinization of starch. These rate parameters were calculated from the experimental results.

If the powdered starches are heated in water, the gelatinization rate may be equal to the rate of reaction since the internal diffusion of water perhaps is negligible, and can be compared with the results of the previous papers. It is expected that the gelatinization rate of starches can be obtained by measuring the flow behavior of heated starch suspensions at various temperatures.

In this paper, we studied the gelatinization rate of rice and potato starches. For measuring flow behavior, we used a capillary tube viscometer operated under various pressures (Kubota et al., 1978).

The flow equation of a pasted starch can be expressed in the following formula:

$$\gamma = (1/K)(g_c\tau - g_c\tau_y)^n \qquad (1)$$

where, $\gamma(sec^{-1})$ is the shear rate, $\tau(g_f/cm^2)$ is the shear stress, $g_c(g\cdot cm/g_f\cdot sec^2)$ is the gravitational conversion factor, $K(g^n/cm^n\cdot sec^{2n-1})$ is the fluid consistency index, n(–) is the flow behavior index and $\tau_y(g_f/cm^2)$ is the yield stress.

The relation between the shear stress and the pressure difference $\Delta P(g_f/cm^2)$ acting on the cylinder of flowing fluid of radius r(cm) and length L(cm) in a circular tube can be expressed as follows:

$$2\pi rL\cdot\tau = \pi r^2\cdot\Delta P$$

$$\therefore \tau = r\Delta P/2L, \quad \tau_w = r_w\Delta P/2L \qquad (2)$$

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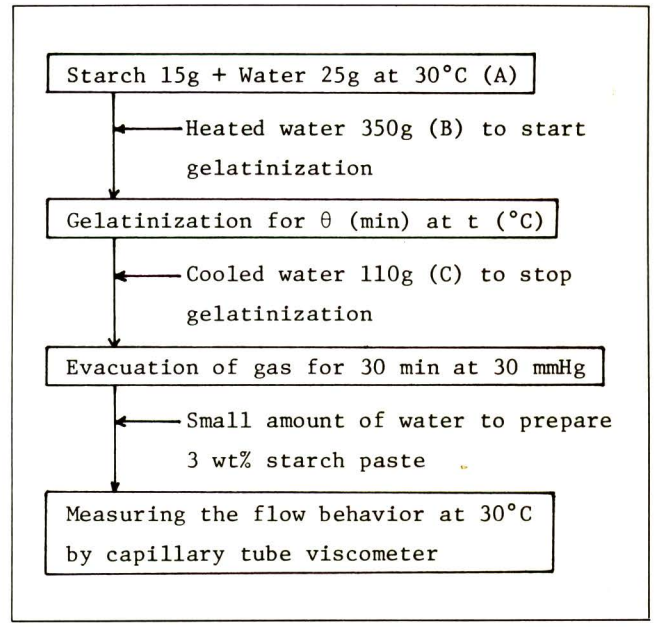

Fig. 1—Flow diagram of gelatinizing potato starch.

where, r_w(cm) is the radius of the tube and $\tau_w(g_f/cm^2)$ is the shear stress at the wall.

The volumetric flow rate $Q(cm^3/sec)$ of flowing fluid is expressed by:

$$Q = \int_0^{r_w} 2\pi r\cdot u\cdot dr = \pi\int_0^{r_w} r^2\cdot\gamma\cdot dr \qquad (3)$$

where, u(cm/sec) is the velocity of fluids at radius r.

Combining Eq (1)~(3), we obtain:

$$Q = \left\{2\pi g_c^n r_w^3(\tau_w - \tau_y)^{n+1}/K\tau_w^3\right\}\left\{(\tau_w - \tau_y)^2/2(n+3) + \tau_y(\tau_w - \tau_y)/(n+2) + \tau_y^2/2(n+1)\right\} \qquad (4)$$

For power-law fluids which is τ_y=0, we obtain:

$$Q = \pi g_c^n r_w^3\tau_w^n/(n+3)K \qquad (5)$$

The viscometric constants n, τ_y and K in Eq (4) and (5) can be calculated using a nonlinear least square method (Kubota et al., 1977) from the experimental data of the volumetric flow rate and the pressure difference. The electric computer program of the calculation of these viscometric constants has been described in a previous paper (Kubota et al., 1978).

EXPERIMENTAL

Samples

The powdered starches used in these studies were commercially prepared in Japan (Potato starch, Katayama Chemical Co.; Rice starch "Micro Pearl", Shimada Chemical Co.).

Experimental procedure

The flow diagram of gelatinization of potato starch is shown in Figure 1. The starch pastes used in the gelatinization were prepared

Table 1—Calculated values of the viscometric constants of gelatinized potato and rice starch pastes (measuring temp = 30° C)

Sample: Potato starch paste (3 wt%); Gelatinizing temp 61° C				
Gelatinizing time (min)	Viscometric constants			
	n(—)	$\tau_y(g_f/cm^2)$	$K(g^n/cm^n \cdot sec^{2n-1})$	$K(g^{1.2}/cm^{1.2} \cdot sec^{1.4})^a$
10	1.211	0.225	4.104	5.084
30	1.208	0.240	5.069	6.094
60	1.183	0.186	5.326	7.502
120	1.190	0.127	6.698	8.387
Sample: Rice starch paste (6 wt%); Gelatinizing temp 75° C				
Gelatinizing time (min)	Viscometric constants			
	n(—)	$\tau_y(g_f/cm^2)$	$K(g^n/cm^n \cdot sec^{2n-1})$	$K(g^{1.3}/cm^{1.3} \cdot sec^{1.6})^b$
5	1.325	2.73×10^{-3}	1.131	1.008
15	1.342	1.91×10^{-3}	1.432	1.119
30	1.346	1.40×10^{-2}	1.495	1.304
60	1.257	3.06×10^{-2}	1.145	1.741
120	1.261	2.97×10^{-2}	1.201	1.754

[a,b] The values fixed τ_y=0.0 and n=1.2 and n=1.3, respectively.

Table 2—Calculated values of the viscometric constants of nongelatinized potato and rice starch suspensions (measuring temp = 30° C)

Nongelatinized starch suspensions	Viscometric constants			
	n(—)	$\tau_y(g_f/cm^2)$	$K(g^n/cm^n \cdot sec^{2n-1})$	$K(ii)^a$
Potate (3 wt%)	0.434	0.00383	0.00182	0.0467
Rice (6 wt%)	0.431	0.00461	0.00197	0.0429

[a] The values fixed τ_y=0.0 and n=1.2 and 1.3 for potato and rice starch suspensions, respectively.

at the desired temperature by agitation of two liquids: one liquid (A) was prepared by vigorous agitation of 15g powdered starch and 25g distilled water at 30°C; the other (B) consisted of 350g distilled water heated to a temperature hotter than the gelatinizing temperature.

After gelatinizing the starch paste (A plus B) under vigorous agitation at the desired temperature for a fixed time, the gelatinized starch was cooled immediately to stop the gelatinization by mixing 110g distilled water (C) cooled to 2°C. By this treatment, we can consider some starch retrogradation, but this effect is small (Hizukuri et al., 1972). The gas in the gelatinized starch obtained (A plus B plus C) was evacuated with a laboratory vacuum drier for 30 min at 30 mm Hg and 35°C. The total weight of the gelatinized starch was adjusted to 500g (to prepare 3 wt% starch paste) with distilled water. The flow behavior of the gelatinized starch obtained was measured by the capillary tube viscometer at 30°C. The rice starch used in these experiments was prepared to 6 wt% starch paste, by the flow diagram similar to Figure 1.

Tube viscometer

The capillary tube viscometer used in these studies was the same as one described previously (Kubota et al., 1978). A glass capillary tube, 0.286 cm i.d. and 26.41 cm long, was used to perform all measurements. The diameter of the capillary tube was calculated from the weight of mercury required to fill it thoroughly. The sample feeder and the capillary tube are made up in a constant-temperature water bath.

As the fluid sample in the sample feeder can be mixed by means of a magnetic mixer, this tube viscometer is also useful for the study of the flow property on various suspensions.

The pressure difference of the fluid through the tube can be obtained by using a vacuum pump, and the volumetric flow rate of the fluid is measured from the time required for the given volume of the fluid to pass through the tube.

RESULTS & DISCUSSION

THE RELATIONS of the volumetric flow rate $Q(cm^3/sec)$ and the pressure difference $\Delta P(g_f/cm^2)$ for all gelatinized starch were determined by the capillary tube viscometer. The value of Hagenbach coefficient which is the corrected factor on ΔP for the contraction and enlargement effect between the large diameter of the sample feeder and the small diameter of the capillary tube, was obtained from the experiments with glycerin-water solutions of known viscosity (Lange, 1967).

The shear stress at the wall $\tau_w(g_f/cm^2)$ can be calculated from each obtained ΔP by Eq (2). The relations of the volumetric flow rate Q vs the shear stress at the wall τ_w for the potato and rice-pasted starches were obtained. By applying the experimental results of Q vs τ_w, it is possible to calculate the viscometric constants n, τ_y and K in Eq (1) using a nonlinear least square method. Table 1 lists the calculated values of these viscometric constants for the potato and rice starch pastes which gelatinized at 61°C for 10~120 min, and at 75°C for 5~120 min, respectively. The digital electric computer in The Computation Center of Hiroshima Univ., HITAC 8700-OS7 was used.

The values of yield stress τ_y may be overlooked in the cases of the 3 wt% potato and 5 wt% rice starch pastes which gelatinized at 60~63°C and 70~85°C respectively; however, it is necessary to take τ_y into account in the case of higher concentrated starch pastes. In these gelatinized starch pastes, a power-law flow equation, as shown in Eq (5), may be applied. The values of flow behavior index n are found to be between 1.15 and 1.25 and between 1.2 and 1.4 respectively for the gelatinized potato and rice starch pastes. The values of K which fixed τ_y=0.0 and n=1.2 and n=1.3 respectively for the gelatinized potato and rice starch pastes are listed in Table 2.

The relations of the volumetric flow rate Q vs the shear stress at the wall, τ_w, of the nongelatinized potato and rice

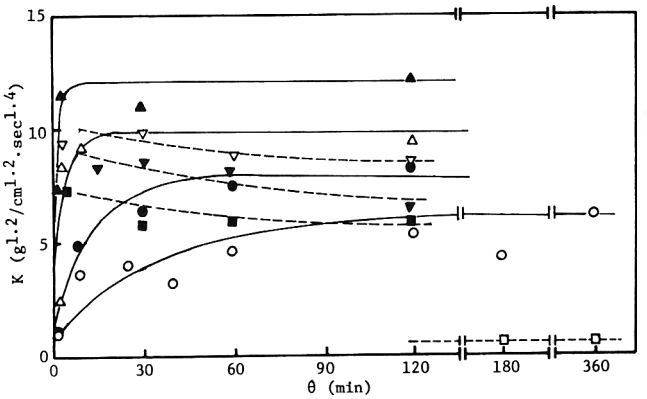

Fig. 2—Relations between the fluid consistency index K and the gelatinizating time θ of gelatinizated potato starch solutions (3 wt%). (Measuring temp 30° C; Gelatinizating temp □ 58° C, ○ 60° C, ● 61° C, △ 62° C, ▲ 63° C, ▽ 65° C, ▼ 70° C, ■ 75° C, Calculated values: ——)

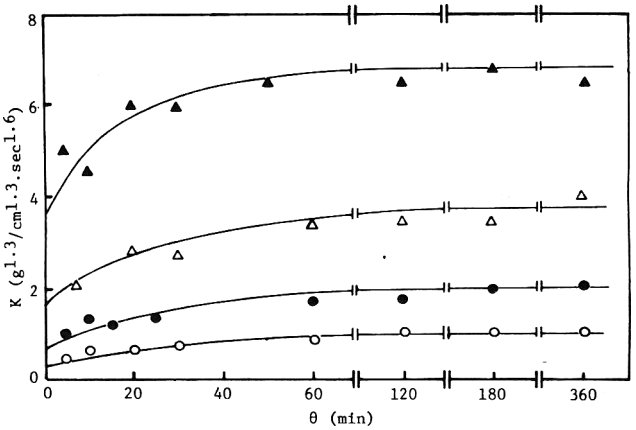

Fig. 3—Relations between the fluid consistency index K and the gelatinizating time θ of gelatinizated rice starch solutions (6 wt%). (Measuring temp 30° C; Gelatinizating temp ○ 70° C, ● 75° C, △ 80° C, ▲ 85° C, Calculated values: ——)

starch suspensions were also obtained. The calculated values of the viscometric constants for the nongelatinized suspensions are listed in Table 2. From the values in Table 2, it is clear that the nongelatinized starch suspensions are dilatant fluids ($n < 1$) and the gelatinized starch pastes are pseudoplastic fluids ($n > 1$).

The relations of the fluid consistency index K($g^{1.2}/cm^{1.2} \cdot sec^{1.4}$) fixed τ_y=0.0 and n=1.2 and the gelatinizing time θ(min) for the gelatinized potato starch pastes at various gelatinizing temperatures are shown in Figure 2. As the values of K at 58°C are nearly zero, the potato starch does not react at around this temperature. The values of K at 60~63°C increase with increasing gelatinizing time and temperature, so we can judge that the gelatinizing reactions of potato starch proceed at these regions. At temperature above 65°C for values of K decrease with increasing gelatinizing time. At these higher temperatures, the gelatinizing reactions take place almost instantly, then it is convenient to imagine that the disintegrations of the gelatinized starch proceed at the observed gelatinizing time region. These phenomena of increasing and decreasing viscosity have been observed as the viscosity changes (Brabender units) of potato flake slurries (Tape, 1965).

The relations of the fluid consistency index K($g^{1.3}/cm^{1.3} \cdot sec^{1.6}$) fixed τ_y=0.0 and n=1.3 and the gelatinizing time θ(min) for the gelatinized rice starch pastes at the gelatinizing reaction region are shown in Figure 3. The values of K at 70~85°C increase with increasing gelatinizing time and temperature.

From the values of K fixed τ_y=0.0 and n=1.2 or 1.3 in the last column of Table 2, the values of K at starting time are nearly zero. However, the K has values slightly higher than zero at starting time due to the instantaneous gelatinizing reaction which takes place by the mixing of hotter water. The values of K_0 at the starting time were approximated by the observed values at 1 min of gelatinizing time completed the mixing phenomena of hotter water.

The values of K_0 at starting time and the values of K_e at equilibrium gelatinizing time of the gelatinized potato and rice starch pastes are expressed as following experimental formulas:

For the potato starch at gelatinizing temperature of 60~63°C:

$$K_0 = \exp(0.7774t - 47.29) \quad (6)$$

$$K_e = \exp(0.2136t - 10.96) \quad (7)$$

For the rice starch at gelatinizing temperature of 70~85°C:

$$K_0 = \exp(0.1640t - 12.63) \quad (8)$$

$$K_e = \exp(0.1209t - 8.349) \quad (9)$$

where, t(°C) is the gelatinizing temperature.

The gelatinizing-ratio x(−) can be expressed as follows:

$$x = (K - K_0)/(K_e - K_0) \quad (10)$$

The gelatinizing-rate equation using the gelatinizing-ratio x is expressed simply as follows:

$$dx/d\theta = k_m(1-x)^m \quad (11)$$

where, $dx/d\theta(min^{-1})$ is the gelatinization rate, m(−) is the order of gelatinization, and $k_m(min^{-1})$ is the rate parameter of m-th order. As the data were scattered, we could not reliably determine the values of m. So, we assumed simply m=1, since the order of many complicated reactions of food materials has been expressed by m=1 such as observed for the cooking of rice (Suzuki et al., 1976, 1977).

The values of $k_{m=1}$ fixed m=1 are obtained by the following integrated equation derived from Eq (11):

$$k_{m=1} = -\ln(1-x)/\theta \quad (12)$$

The values of logarithm of $k_{m=1}$ for potato starch are plotted in Figure 4 against the reciprocal of the absolute temperature. A straight line is obtained. The Arrhenius equation is shown as follows:

$$\log(k_{m=1}) = 148.37 - 4.995 \times 10^4 (1/T) \quad (13)$$

where, T(°K) is the gelatinizing temperature.

The equivalent activation energy for the gelatinization of potato starch, calculated from Eq (13) is approximately equal to 230 kcal/g-mol. This value is comparatively one order larger than the values of under 100 kcal/g-mol for general chemical reactions. The phenomena on the gelatinization of potato starch at 60~63°C is very similar to the thermal denaturation of the white of an egg with a high activation energy. The calculated values of K using Eq (6), (7) and (13) are illustrated by the solid lines in Figure 2.

The values of logarithm of $k_{m=1}$ for the rice starch are plotted in Figure 5 against the reciprocal of the absolute

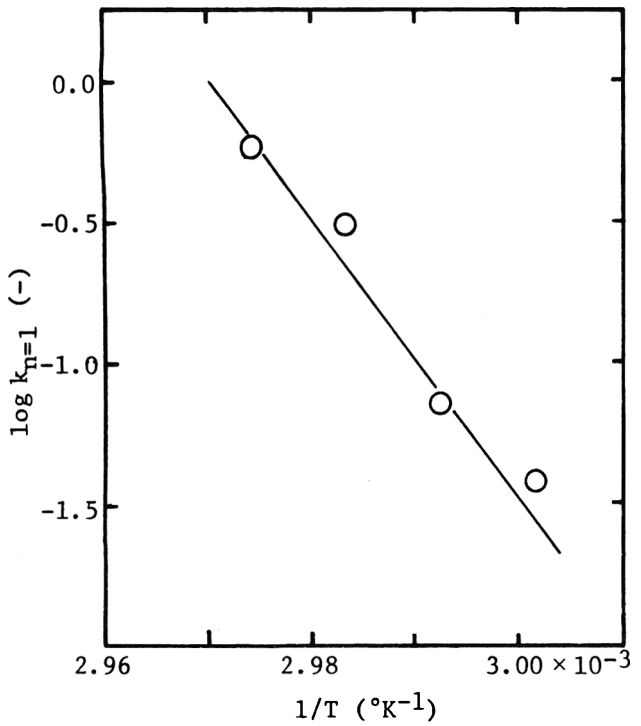

Fig. 4—Arrhenius plot of the gelatinization rate parameter $k_{n=1}$ of potato starch solutions.

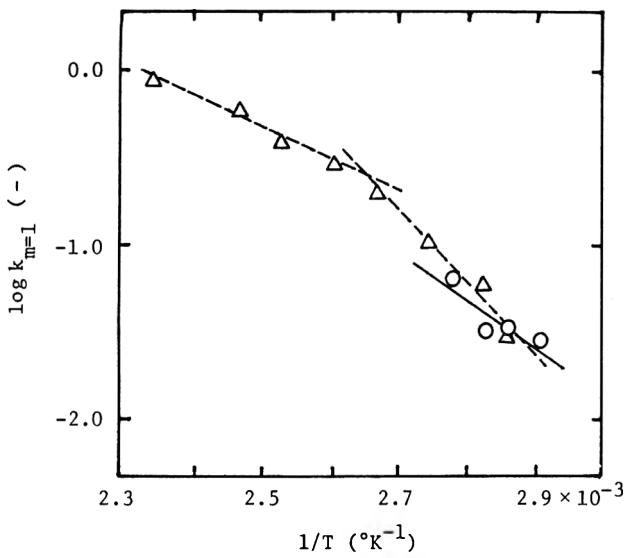

Fig. 5—Arrhenius plots of the gelatinization rate parameter $k_{m=1}$ of rice starch. (This work: ——, The previous work of cooking of rice (Suzuki et al., 1976): ----)

temperature. The Arrhenius equation is shown as follows:

$$\log(k_{m=1}) = 7.092 - 2.990 \times 10^3 \, (1/T) \qquad (14)$$

The equivalent activation energy is approximately equal to 14 kcal/g-mol. This value is of the same order as the value of 19.0 kcal/g-mol at the reaction region of 75~110°C on the cooking of rice studied in a previous paper (Suzuki et al., 1976). The values of the rate parameter of m=1 obtained on the cooking of rice are shown by the broken lines in Figure 5. From the comparison of the solid line with the broken line in Figure 5, we can consider that the phenomena of the gelatinization of rice starch at our experimental conditions appear to be very similar to the cooking of rice which has been studied. The calculated values of K using Eq (8), (9) and (14) are illustrated by the solid lines in Figure 3.

From the discussion mentioned above, we may conclude the following: The values of equivalent activation energy on the gelatinization of potato and rice starch are not smaller than the values of under 10 kcal/g-mol for general diffusion phenomena. Thus, we can conclude that the diffusion rate of water in starch granules is negligible, and the gelatinization rate is limited by the chemical reaction rate of starch components with water and/or the physical transforming rate such as the melting of starch crystalline regions. The value of equivalent activation energy for the gelatinization of potato starch is one order larger than that for rice starch. It can be considered that potato starch granules swell more than rice starch granules and could give rise to larger viscosities and higher thermal values by viscometric method. In order to consider the higher equivalent activation energy obtained for potato starch, and to discuss the gelatinization phenomena, the gelatinization rate must be measured by various method.

NOMENCLATURE

g_c	:	Gravitational conversion factor, ($g \cdot cm/g_f \cdot sec^2$)
K	:	Fluid consistency index, ($g^n/cm^n \cdot sec^{2n-1}$)
k_m	:	Rate parameter of m-th order rate equation, (min^{-1})
m	:	Order of gelatinization rate, (–)
n	:	Flow behavior index, (–)
ΔP	:	Pressure difference, (g_f/cm^2)
Q	:	Volumetric flow rate, (cm^3/sec)
T and t	:	Gelatinizing temperature, (°K) and (°C)
x	:	Gelatinizing-ratio, (–)
γ	:	Shear rate, (sec^{-1})
O	:	Gelatinizing time, (min)
τ	:	Shear stress, (g_f/cm^2)
τ_y	:	Yield stress, (g_f/cm^2)
τ_w	:	Shear stress at the wall, (g_f/cm^2)
Subscripts		
0	:	Initial state
e	:	Equilibrium state

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FOAMING PROPERTIES OF PROTEINS: EVALUATION OF A COLUMN AERATION APPARATUS USING OVALBUMIN

R. D. WANISKA and J. E. KINSELLA

ABSTRACT

A specially designed small-scale foaming apparatus was used to determine dynamic and static foaming properties of proteins. Foam was produced by sparging nitrogen at a known rate through a dilute protein solution. The temperature of the protein solution and protein foam was maintained by a water-jacketed column. Ovalbumin was used as the reference protein. Foaming properties (foaming capacity, foam strength, and stability) were improved when the protein concentration was increased (0.01–0.1%); when sodium chloride was added to the protein solution and when the temperature was decreased from 40 to 2°C. Foaming properties were optimum at pH 3.8–4.0, slightly below the isolelectric point of ovalbumin, and at a gas flow of 20 ml/min. The apparatus permits many of the variables affecting foaming properties of proteins, i.e. pH, temperature, ions, carbohydrates and surfactants to be controlled while quantitatively determining foaming properties of small quantities of protein.

INTRODUCTION

THE FOOD INDUSTRY is constantly seeking new protein ingredients for use in food manufacture. Many proteins are used for specific applications because of their particular functional properties (Kinsella, 1976). An important functional requirement of proteins used in angel food cake, whipped toppings, divinity and souffle-like products is the capacity to form stiff stable foams. In evaluating new proteins as foaming agents an objective method for measuring foam formation and foam stability is needed. Reports in the literature describe three dynamic procedures for determining foaming capacity of proteins viz whipping, shaking or sparging (Cumper, 1953; Yasumatsu et al., 1972). One important difference between these methods is the amount of protein required for foam production. The amount of protein ranges from 3–40% for whipping, around 1% for shaking and ranges from 0.01–2% for gas sparging (Lawhon and Cater, 1971; Yasumatsu et al., 1972; Buckingham, 1970). Another difference between these methods is the manner in which the foam is formed. While shear forces are involved in all three methods, they are most important in whipping and apparently of little importance in the sparging process. Foam bubbles are formed and broken by shear in both whipping and shaking. In the sparging method once the foam is formed the rate of rupture of the bubble is a function of the lamella thickness and interfacial viscoelasticity (Cumper, 1953; Mita et al. 1977). Whipping, the most commonly used method, produces protein foams that can be measured by the increase in foam volume, specific gravity and/or viscosity (Eldridge et al., 1963; McKeller and Stadelman, 1955; Lawhon and Cater, 1971). Rapid shaking of a horizontal graduated cylinder containing a protein solution (1%) produces a foam that can be measured by its volume (Yasumatsu et al., 1972; Graham and Phillips, 1976; Wang and Kinsella, 1976). The sparging of gas into a protein dispersion produced foams from very small amounts of protein (0.01–2%). Foaming capacity can be measured by the ratio of the volume of gas in foam to the volume of gas sparged, or by the maximum volume of foam divided by the gas flow rate (Buckingham, 1970; Mangan, 1958; Cumper, 1953; Mita et al., 1977).

The stability of protein foams is usually measured by the volume of liquid drained from a foam during a specific time at room temperature (Eldridge et al., 1963; McKeller and

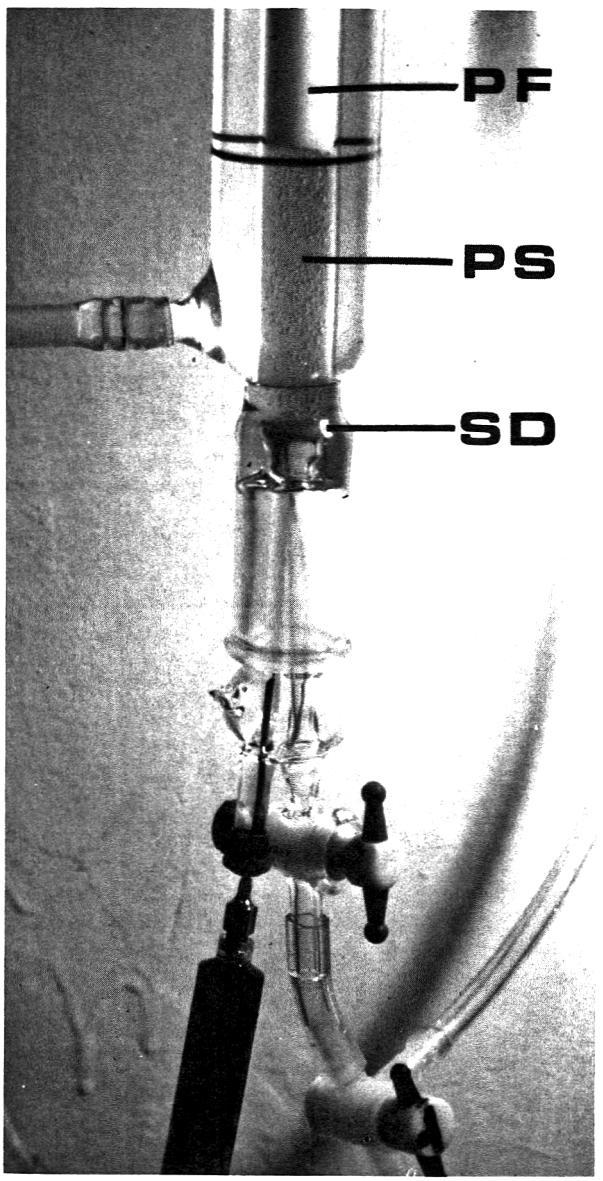

Fig. 1–Lower portion of foaming apparatus illustrating sparging disk (SD), protein solution (PS) and protein foam (PF) in a water jacketed column. Buffer solution is being injected in the rubber septum with a graduated hypodermic syringe.

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Table 1—A summary of the independent parameters used for experiments with the modified foaming apparatus

Nature		
Fixed	Diameter of sparging disc	1.0 cm
	Porosity of sparging disc	4–8μ
	Volume of protein solution	15.0 ml
	Volume of column for foam	70.0 ml
	Mass of weight (six–2.4 mm holes)	25.0g
Varied	Temperature	2.0–40.0°C
	Gas flow rate	4.0–36.0 ml/min

Stadelman, 1955; Mita et al., 1977). Occasionally the decrease in foam volume over time (Wang and Kinsella, 1976) is used to measure foam stability. Dynamic methods that have been employed to measure foam stability include the rate of fall of a perforated weight through a column of foam (Mangan, 1958; Buckingham, 1970), the penetration of a penetrometer cone (McKeller and Stadelman, 1955) or the ability to support a series of specific weights (IAPI, 1956).

Thus a variety of methods have been used to produce and characterize protein foams. The large amount of protein required in the whipping method limits its usefulness in testing experimental proteins and furthermore the incorporation of air increases the temperature of the precooled protein solution during whipping. This affects observed foaming properties. The amount of protein required for shaking is suitable for most experimental situations but the rapid motion quickly increases the temperature of the protein solution and the volume of foam produced is limited by the container. The sparging apparatus of Buckingham (1970) can be modified to maintain the protein solution and foam column at the desired temperature and can be adapted to require small amounts of protein.

As is well documented in the literature the foaming characteristics of proteins are markedly influenced by conditions of preparation, measurement, etc. (Eldridge et al., 1955; Yasumatsu et al., 1972; McKeller and Stadelman, 1955; Kinsella, 1976). Because of the variety of methods employed it is difficult to compare data from different sources.

There is a need for a practical standardized method for determining foaming properties of food proteins. Ideally such methods should provide a measure of the physicochemical properties related to foaming. A standard method for testing foaming properties based on the method of Foulk and Miller (1931) was proposed by Balmaceda et al. (1976). Using a modification of this apparatus we systematically studied the effects of temperature, gas flow rate, protein content, pH, salt and sugar concentration on the foaming properties of ovalbumin under controlled conditions.

MATERIALS & METHODS

CRUDE OVALBUMIN (Sigma Chem. Corp.; lot #94C-0247) was purified by precipitation from saturated NH_4SO_4, dialyzed, and then lyophilized. The foaming apparatus was assembled from available laboratory equipment, a specially designed glass stopper and a machined brass weight. The principle component of the foaming apparatus was a calibrated water-jacketed glass condenser (85 cm long; 1.10 cm i.d.) with a 24/40 joint at one end (Fig. 1 and 2). A Forma Constant Temperature Circulator (cat. no. 2095) maintained the temperature in the condenser within 0.5°C of the desired temperature (2–38°C). A specially designed glass stopper (Fig. 1) which permitted the introduction of gas and liquid was made in the Glass Shop (Dept. of Physics, Cornell Univ., Ithaca, NY). The gas entry port had a fritted glass disc (1.0 cm diameter; 4–8μ porosity; Ace Glass Co. #9436-10) on the inside and a two-way stopcock on the outside. A Perkin Elmer Flow Controller (Serial No. GC17731) regulated the gas flow rate of prepurified nitrogen. The liquid port was

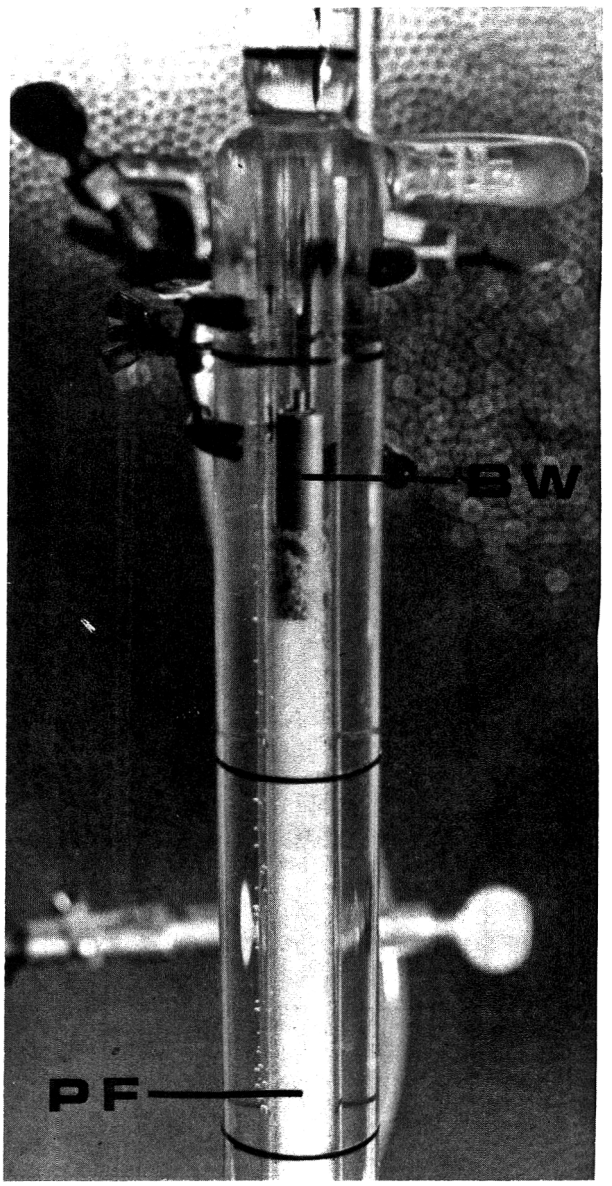

Fig. 2—Upper portion of foaming apparatus illustrating a column of protein foam (PF) with a perforated brass weight (BW) penetrating the foam.

sealed with a rubber septum and protein solutions were introduced with a hypodermic syringe. Foam strength was measured by a brass weight (Fig. 2) which was made at the machine shop (Dept. of Physics, Cornell Univ., Ithaca, NY). The brass weight (25.0g; 1.0 cm diam; 4.6 cm long; six 2.4 mm holes. lengthwise) was attached to a nylon monofilament line to retrieve it after passage through the foam.

The volume of the sparging chamber (15.0 ml) allowed about 0.4 sec for the sparged gas to rise to the air-liquid interface. This time is required for the mass transfer i.e. adsorption of protein from the bulk solution to the gas/liquid interface (Bull, 1972).

The procedure for measuring foaming properties which takes about 30 min per sample is described. The water-jacketed column and buffer solution (50 mM sodium citrate) are equilibrated to the specified temperature (Table 1). The protein is dissolved in the buffer solution with stirring for 5 min to give a concentration of 0.01–1.00% (w/v). The protein solution (15.0 ml) is then injected into the sparging chamber via the septum stoppered inlet. Nitrogen gas is sparged into the protein solution until the foam chamber (70 ml) is filled with foam while simultaneously maintaining the volume of liquid in the sparging chamber by the addition of buffer or protein solution. The time required to form 70 ml of foam and the volume of buffer added to the sparging chamber are recorded. After

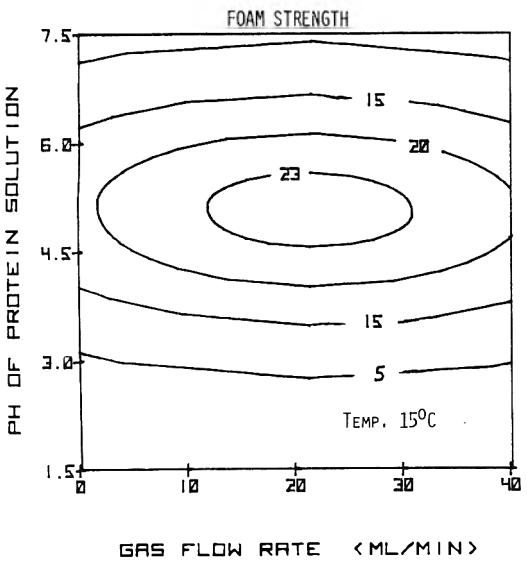

Fig. 3—Effect of gas flow rate and pH on foam strength (sec/ml) at 15°C.

10 min the volume of liquid drained from the foam is recorded. The strength of the foam is then determined by observing the time and distance of fall of the brass weight through the foam (Fig. 2). The measured variables were employed to calculate several parameters (Table 2) used to predict foaming properties.

Experimental design

The characteristics of the foaming apparatus were studied in three experiments. In the first experiment three independent parameters of the apparatus were varied in a central composite design (Cochran and Cox, 1957): gas flow rate (4.0, 10.0, 20.0, 30.0, 36.0 ml/min), temperature (7.5, 15.0, 22.5, 30.0, 37.5°C), and pH (2.5, 3.0, 4.5, 6.0, 6.5). The second experiment was conducted to determine more precisely the effect of pH on foaming properties at the optimum levels of gas-flow rate and temperature as indicated by the results from experiment 1. A protein content of 0.10% (w/v) was used in the first two experiments. The third experiment was conducted to optimize the conditions necessary for determining foaming properties of ovalbumin. Five factors were varied in a central composite design in this experiment. Values for these factors were: protein content [0.010, 0.032, 0.100, 0.316, 1.00% (w/v)], sucrose (0, 25, 50, 75, 100 mM), sodium chloride (0, 100, 200, 300, 400 mM), temperature (2, 10, 20, 30, 38°C) and pH (3.1, 3.8, 4.5, 5.2, 5.9) while the gas flow rate (20 ml/min) was held constant.

Statistical analyses were performed with a Minitab II program (Penn. State Univ., 1976) at the Cornell University Computing Center. Statistical significance was determined by "t-test" (t = bij/SD) where SD is standard deviation. A probability level of 0.01 was used throughout this study.

RESULTS & DISCUSSION

THE FIRST EXPERIMENT was conducted to optimize the independent parameters (Table 1) of the foaming apparatus. Results of statistical analyses provided regression coefficients along with their level of significance (Table 3). The effect of two independent parameters, pH and gas flow rate, on foam strength while holding the third parameter constant is illustrated in Figure 3. The optimum foam strength (FS) was observed around a gas flow rate (FR) of 20 ml/min and a pH of 5.0. At least three response surface graphs for each parameter [(percent of gas initially in the foam (Gi), volume of liquid retained in the foam after 10 min (Vr), percent of the liquid drained from the foam after 10 min (D10), and foam strength, the rate of fall of a weight through the foam, sec/ml (FS)] were plotted and evaluated (graphs not shown). Evaluation of the graphs and significant regression coefficients are summarized below.

Variation in FR (Table 3) significantly affected Gi, Vr and D10. At a fast FR more liquid is initially entrapped in the foam and less gas is lost during sparging. The large amount of liquid initially in the foam was attributed to the inadequate time for the foam lamellae to reach equilibrium with the liquid, hence there was increased drainage. At a slow FR the foam had a longer period to drain before the column was completely filled with foam, thereby decreasing the amount of liquid initially in the foam. Also, the longer period allowed more gas to be lost from the foam. The optimum gas flow rate (20 ml/min) represents a compromise between these effects

Variation in pH (Table 3 and Fig. 3) significantly affected Vr and FS. The optimum pH of 3.8–4.0 is slightly below the isoelectric point (IEP) of ovalbumin (IEP 4.6–4.8). Near the IEP of proteins there is a decreased electrostatic repulsion between protein (Kitchener and Cooper, 1959; Cumper, 1953; Kinsella, 1976) resulting in closer packing of protein at the air/liquid interface and increased viscosity of the adsorbed protein layer (Mita et al.,

Table 2—Equations employed to calculate parameters used to predict foaming properties

$$Gi = \frac{(100)(FR)(Tf)}{70-Vi}$$

$$Vr = Vi - Vd$$

$$D10 = \frac{(100)(Vr)}{Vi}$$

$$FS = \frac{Tw}{Dw}$$

where

Gi	=	percent of gas initially in 70 ml of foam.
FR	=	(gas Flow Rate) — ml/min of nitrogen.
Tf	=	time to fill the column with foam.
Vi	=	volume of liquid in the foam initially.
Vd	=	volume of liquid drained from the foam after 10 min.
Vr	=	volume of liquid retained in the foam after 10 min.
D10	=	percent of the liquid drained from the foam after 10 min.
FS	=	(Foam Strength) — rate of fall of a weight through the foam (sec/ml).
Tw	=	time for the weight to fall through the foam (sec).
Dw	=	ml of foam penetrated.

Table 3—Regression coefficients of surface response model for foaming characteristics of ovalbumin obtained by varying gas flow rate, pH and temperature[a]

Model terms[b]	Gi	Vr	D10	FS
0	98.707+	4.887+	58.95+	17.390+
1	1.900+	−0.373−	11.206+	0.668
2	−0.768	−1.018−	0.10	4.940+
3	−0.284	−0.304	3.88	−3.602−
11	−1.895−	−0.466	−0.48	−1.020
22	1.159	0.385	4.92+	−7.880−
33	−0.244	0.0001	−0.008	1.782+
12	−1.002	0.286	0.453	−0.230
13	0.979	0.534	−3.15	0.309
23	0.323	−0.405	2.37	−1.057
SD	2.49	0.849	4.89	6.19
R^2	57.9%	54.3%	86.7%	60.0%

[a] Coefficients that differ significantly are labelled + or − to indicate whether the T-ratio was larger or smaller than the absolute value of 3.0 ($p < 0.01$).

[b] The numbers refer to subscripts of β (estimators) in the surface response model: $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$ where 0 = mean value, 1 = gas flow rate, 2 = pH and 3 = temperature.

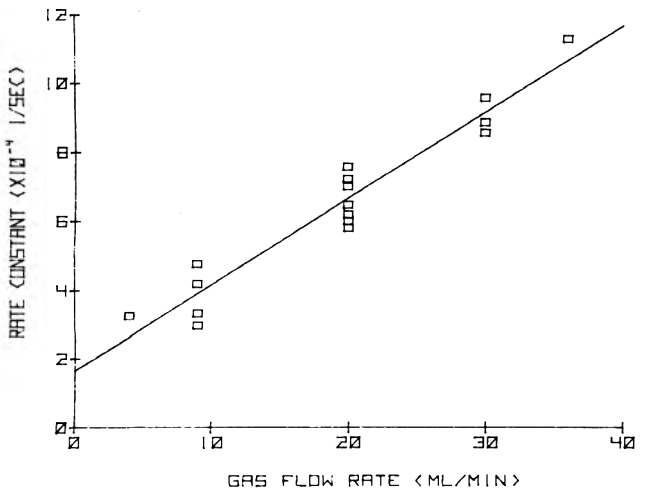

Fig. 4—Effect of gas flow rate on the rate constant of drainage of ovalbumin foam prepared at 22°C and pH 4–5.

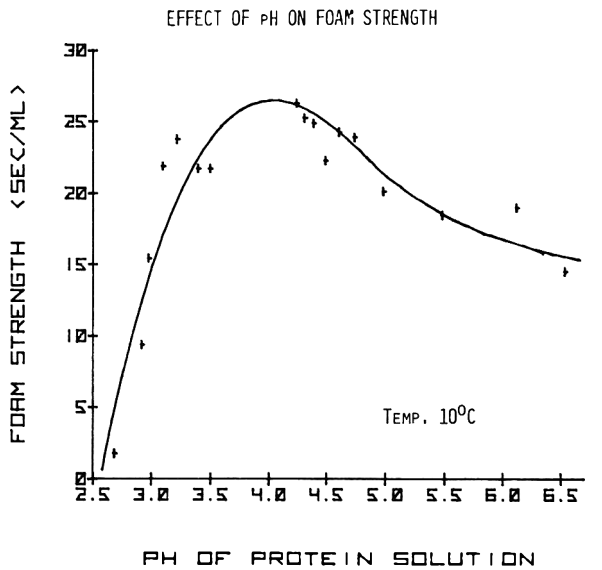

Fig. 5—Effect of pH on foam strength at 10°C 0.10% protein and a gas flow rate of 20 ml/min.

1977). Thus, increased protein-protein interaction near the IEP gave stronger protein films that were less permeable to the entrapped gas.

Variations in temperature (Table 3) significantly affected FS. Results of the surface response design indicated that 15°C was the best temperature, but the optimum temperature was probably below this value. Improved foaming properties at a lower temperature may be related to the increased viscosity of the liquid phase and a decreased rate of protein denaturation (Buckingham, 1970; Mita et al., 1977).

The rate of drainage of liquid from many foams obeyed the equation: $V = Vo \exp^{(-kt)}$ where V is the volume of liquid in the foam and k is the rate constant of drainage (Mita et al., 1977). When log V vs time is plotted, the slope of the straight line is equal to k. For gluten foams, the rate of drainage at the beginning and the end of the foam decay were respectively, greater and less than those calculated by this equation (Mita et al., 1977). The rate constant of drainage was estimated from Vo and Vr, in experiment 1, to compare our results with those of Mita et al. (1977). Slower FR's resulted in protein foams with a lower rate constant (Fig. 4), indicating a more stable foam. Ovalbumin foams near the IEP had the lowest drainage rate constant and percent drainage after 10 min. Only at a FR of 10 ml/min or less did temperature affect the drainage rate constant. At a flow rate of 10 ml/min foam stability decreased with a rise in temperature. A linear relationship was obtained by plotting log k vs 1/T and an activation energy of 1.3 Kcal was calculated. A higher activation energy (3.6 Kcal) was calculated from drainage constants of foams prepared from wheat gluten in 3M urea (Mita et al., 1977).

Closer evaluation of the effect of pH on foam character-

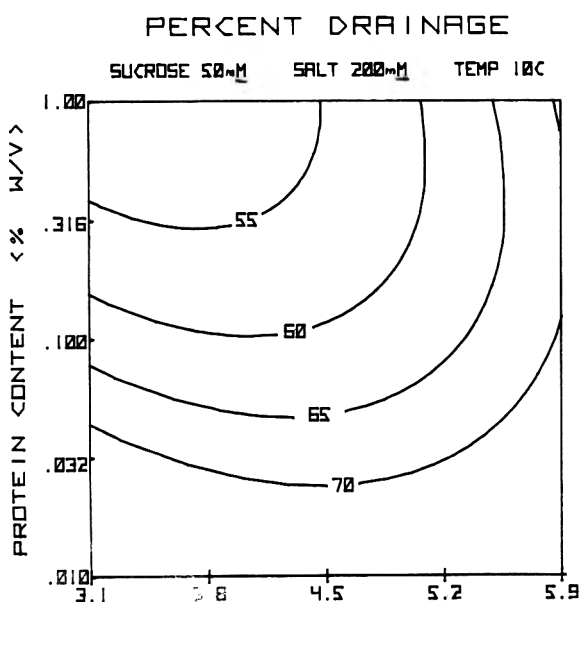

Fig. 6—Effect of pH and protein content on drainage while holding temperature at 10°C, sucrose conc at 50 mM and salt conc at 200 mM.

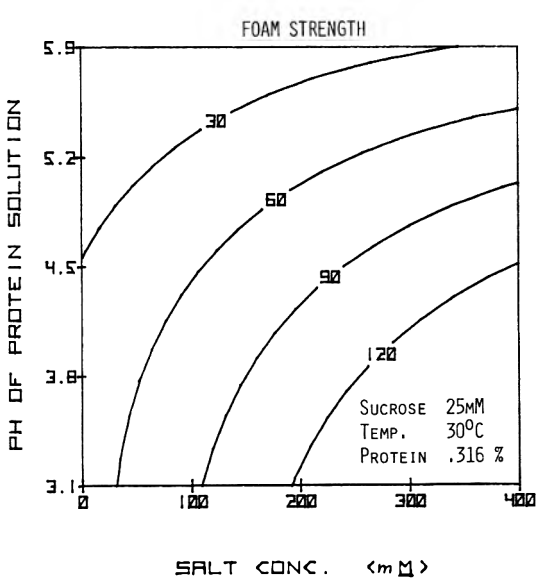

Fig. 7—Effect of salt concentration and pH on foam strength while holding the temperature at 30°C, sucrose conc at 25 mM and protein content 0.316%.

istics in the second experiment had slightly different results. The effect of pH on FS at a FR of 20 ml/min, a temperature of 10°C and a protein concentration of 0.10% (w/v) is shown in Figure 5. While a quadratic model fit the data poorly (correlation coefficient = 0.74), a cubic model had a correlation coefficient of 0.92. However, the optimum value of foam strength was accurately estimated by both models. Optimum values for D10 and Vr were attained around pH 4.0–4.7 while variation in pH did not affect Gi.

The third experiment was designed to determine the effect of five independent variables on foaming properties of ovalbumin using a surface response model. Results of statistical analyses provided regression coefficients (Table 4) for the quadratic models along with their level of significance. The effect of pH and protein content on D10 (Fig. 6) indicates that at the lower level of pH (3.8) and a higher protein concentration (0.316%) optimum retention of liquid in the foam occurred. The effect of salt concentration and pH on FS (Fig. 7) illustrates that at the higher level of salt (300 mM) and at the lower value of pH (3.8) optimum FS was observed.

Ten graphs (not shown herein) for each foaming characteristic were plotted and evaluated to determine the optimum level for each independent variable. The results are summarized below. Variation in sucrose concentration up to 3.44% (w/v) did not significantly affect any measured foaming property. The data revealed trends which showed that a sucrose level of 50 mM (1.72%) produced foams with improved properties.

The stability of wheat gluten foams was not affected with sucrose concentrations less than 12% (w/v); however, increased foam stability resulted with 30 and 50% sucrose (Mita et al., 1977). To stabilize foams made from egg white or protein isolates sucrose concentrations as high as 45% have been utilized (Eldridge et al., 1955; Lawhon and Cater, 1971). Thus for significant effects greater sucrose concentrations need to be tested in this system.

Variation in sodium chloride concentration significantly affected Vr (Table 4 and Fig. 7). The interaction of protein content and salt concentration significantly affected Vr and FS (Table 4). At higher salt levels ($\geqslant$ 300 mM) improvement in foaming properties was probably the result of a more dense protein film (Mita et al., 1977). In the presence of salt, protein solutions have a lower surface tension. Salt causes a more compact protein conformation at an air/liquid interface resulting in a minimum surface area of the adsorbed protein molecules and a viscous, adsorbed film (Mita et al., 1978; Kitchener and Cooper, 1959).

Variation in pH significantly ($p < 0.05$) affected FS(Table 4). At pH 3.8–4.0, slightly below the IEP of ovalbumin, optimum foaming properties were observed (Fig. 6 and 7). Near the IEP proteins lose their effective electrical charge, resulting in minimal electrostatic repulsion and the most compact conformation. Under these conditions more protein orients at the air/liquid interface because the molecules pack closer together. The thickness of a film of bovine serum albumin significantly increased as the pH approached the IEP (Musselwhite and Litchener, 1967). The surface viscosity and elastic potential of the protein film also increase in the isoelectric zone (Cumper and Alexander, 1950; Biswas and Haydon, 1962).

Variations in temperature significantly affected D10 (Table 4). At 10°C less liquid drained from the foam probably because of a slight increase in viscosity and a decreased rate of denaturation of ovalbumin (Buckingham, 1970; Mita et al., 1977; Cumper, 1953).

Variation in protein content significantly affected all measured foaming properties. As protein content of the solution increased, each measured property improved as illustrated for D10 in Figure 5. Similar results were reported by Buckingham (1970). At a protein level of 0.312% ovalbumin produced foams composed of small bubbles. The treatment at 30°C and 300 mM salt at this high protein level showed very good foam properties but after several minutes the foam solidified or gelled. The treatment at 10°C and 300 mM salt at the high protein level also showed very good foaming properties. Small stable bubbles formed quickly and rose in the foam column; however, there was not a clear liquid/foam boundary, hence the volume of buffer added was approximated.

Table 4—Regression coefficients of surface response model for foaming characteristics of ovalbumin obtained by varying sucrose conc, salt conc, pH, temperature and protein content[a]

Model terms[b]	Gi	Vr	D10	FS
0	100.381+	8.538+	62.91+	34.42+
1	0.42	0.31	0.215	−1.39
2	0.366	1.083+	0.244	5.58
3	0.036	0.222	−0.263	−7.55
4	−0.183	0.172	2.779+	−0.40
5	0.565	2.017+	4.918−	28.44+
11	−0.335	−0.284	0.784	−0.21
22	−0.186	0.218	−1.285	−1.27
33	0.053	−0.01	1.492	−2.65
44	0.199	−0.619	0.63	1.27
55	−1.085−	−0.417	1.432	5.36
12	−0.195	0.008	−0.093	−1.99
13	0.033	0.038	−7.48	4.94
14	0.707	0.304	−0.507	0.84
15	−1.286	−0.304	0.403	−7.50
23	−0.074	0.154	2.285	−6.22
24	0.769	0.049	0.110	4.20
25	−0.925	0.281	0.589	10.69+
34	0.169	0.172	−2.13	−6.40
35	−0.164	−0.157	1.43	−7.34
45	1.149	0.390	0.75	4.43
SD	2.60	1.79	4.99	15.8
R^2	70.6%	76.0%	72.6%	83.6%

[a] Coefficients that differ significantly are labelled + or − to indicate whether the T-ratio was larger or smaller than the absolute value of 3.0 ($p < 0.01$).

[b] The numbers refer the subscripts of β (estimators) in the surface response model: $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{55} x_5^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{15} x_1 x_5 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{25} x_2 x_4 + \beta_{34} x_3 x_4 + \beta_{35} x_3 x_5 + \beta_{45} x_4 x_5$ where 0 = mean value, 1 = sucrose conc, 2 = salt conc, 3 = pH, 4 = temperature and 5 = protein content.

From the data obtained in this initial study the foaming apparatus can be used to evaluate the important foaming characteristics of protein i.e. the foaming capacity of protein and the stability and strength of the foam. Foaming ability i.e. Gi denotes the amount of gas retained in the foam reflecting the potential of the protein for foaming. The measures of foam stability, Vr and D10, quantitatively reflects the distribution of liquid in the foam and foam strength FS measures the dynamic stability or resistance of the foam to external force or pressure.

SUMMARY

THE MODIFIED APPARATUS described herein produced foam under controlled conditions. Small quantities (0.10% or 15 mg) of protein produced foams that could be validly evaluated for Gi, Vi, Vr, D10 and FS. Levels of 20 ml/min gas flow rate and 10°C produced foams with optimum properties. The foaming properties of ovalbumin were improved by increasing protein content. Increased salt concentration improved foam strength but was associated with increased drainage. Foaming properties were optimum slightly below the IEP of ovalbumin.

—Continued on page 1411

PHYSICAL AND CHEMICAL PROPERTIES OF POTATO PEEL AS A SOURCE OF DIETARY FIBER IN BREAD

R. B. TOMA, P. H. ORR, B. D'APPOLONIA, F. R. DINTZIS and M. M. TABEKHIA

ABSTRACT

Peelings removed from potatoes (Solanum tuberosum) by four methods, abrasion, steam, caustic, and hand peeling were studied in terms of their physical, chemical and baking characteristics as a source of dietary fiber in bread. Potato peel apparently is superior to a wheat bran in the contents of certain minerals, in total dietary fiber, in water-holding capacity, in its lower quantity of starchy components, and in its lack of phytate. These dietary advantages were not lost in baking quality trials.

INTRODUCTION

RECENT CONCERN about fiber in the American diet has increased the interest in vegetable sources of dietary fiber. In 1971 studies of fiber suggested that the decrease in consumption of plant fiber was related to an increase in certain diseases such as diverticulosis (Painter and Burkitt, 1971) and colonic cancer (Burkitt, 1971). Dietary fiber acts as a bulking agent that increases intestinal motility and moisture content of feces (Forsythe et al., 1976). It was postulated that those effects are important in preventing diseases of the colon (Trowell, 1973). Other studies showed evidence that plant fiber can lower serum cholesterol level (Forsythe et al., 1976; Tsia et al., 1976) and improve oral glucose tolerance in humans (Munoz et al., 1978).

Concerns were reported that dietary fiber might bind certain minerals, as well as bile acids and ingestion of very large quantities would decrease the availability of such minerals as Ca, Zn, Fe, Cu, and Mg (Anon., 1977a). Reinhold et al. (1975) investigated the possible role of fiber in reducing the availability of trace elements and Ca. They suggested that fiber, not phytate, largely determines the availability of di-valent metals for absorption in the intestine. On the other hand, Davies et al. (1977) suggested that phytate, rather than fiber, is the major determinant of Zn availability. Davies and Nightingale (1975) demonstrated that phytate reduced the availability of dietary copper and manganese. Thus the quality of dietary fiber becomes a nutritional factor, particularly concerning phytate content.

Wheat bran is a major source of dietary fiber (9–12% crude fiber) but also is a rich source of phytate (3.39%) (AACC, 1976) that can bind some minerals when fed to mono-gastric animals in large amounts. Also, wheat bran can produce deficiency in some minerals (Branch et al., 1975). However, Sandstead et al. (1978) reported that the addition of 26 g/day of wheat bran (AACC certified food grade obtained from soft white winter wheat and bran obtained from Waldron variety hard red spring wheat) did not adversely affect trace mineral balances when fed to men maintained on a high fat, high protein, and low fiber diet that resembled diets normally consumed by U.S. males.

Potato peel, a by-product from the potato industry, could be considered a new source of dietary fiber that avoids the unfavorable high phytate content found in wheat bran. The processing of potatoes reached 8.5 million tons annually (2/3 of 1976 U.S. crop on a fresh weight basis) according to National Potato Council statistics (Anon., 1978). If 8% were lost in peeling (Smith, 1977), this crop would produce 0.69 million tons of potato peel or 0.2 million tons of dried peel annually (Bloch et al., 1973).

Industrially, most potatoes are peeled with caustics so that peel must be neutralized before use. More NaOH than KOH is used because it is cheaper and nearly as effective (Powers et al., 1977). Potatoes usually are immersed in caustic, then sprayed with water. Potato peel now produced as a waste or by-product of commercial processes, might provide dietary fiber. Use of peel also might significantly reduce waste pollution from commercial potato peeling processes.

One feasible method of incorporating dietary fiber would seem to be the substitution of fiber-containing components for some of the flour in baked products. This has been accomplished by the addition of cellulose and/or bran to baked products (McCromick, 1976; Brochmole and Zabik, 1976) up to a 30% level of substitute without significant change in sensory evaluation (Schafer and Zabik, 1978).

Toma et al. (1979) studied the morphological characteristics of potato peel with scanning electron microscopy. The purpose of our study was to explore the physical, chemical and baking characteristics of potato peel as a potential supplementary source of dietary fiber in bread.

MATERIALS & METHODS

Preparation of peels

Raw potato tubers (Solanum tuberosum) of the Norchip variety were grown under commercial conditions at the Potato Research Farm near Grand Forks, North Dakota. The tubers were stored for 8 months under commercial conditions at 7°C/90% RH at the Red River Valley Potato Research Laboratory, East Grand Forks, MN. In preparation for peeling, the potatoes were soaked in water for 15 min, mechanically washed with roller brushes and water spray (275 kPag), and graded manually. We used approximately 680 kg of potatoes in the study. About 22.5 kg were peeled with a hand-held vegetable peeler and the rest were peeled by the following commercial techniques:

(1) Abrasion–Peel was removed with a Hobart abrasive peeler (Model #6115) for 25–30 sec at 300 rpm with simultaneous washing with water;

(2) Steam–Tubers were exposed to live steam at 690 kPag for 25 sec in a rotary steamer; then peel was removed with a brush peeler and water spray (690 kPag).

(3) Caustic–Tubers were immersed in caustic (NaOH 13%, w/v) at 83°C for 2 min in a rotary lye peeler (A.K. Robins Co., Model #64). Tubers then were immediately soaked in running tap water for 6 min at 14°C to remove alkali. Peel was then removed with a brush peeler and water spray as in the steam method. Additionally, peel was tested for pH to assure removal of alkali residue. Peel from each operation was collected on cheese cloth, rinsed

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Table 1—Comparison of physical properties between potato peels from four peeling operations and wheat bran

Property	Hand peeled	Abrasion peeled	Steam peeled	Lye peeled	Wheat bran[b]
Direct density mg/ml	430.69 ±12.24*	151.05 ± 2.07*	167.39 ± 2.56*	353.1 ± 13.38*	439[b]
Bulk density (packed) g/cc	1.238 ± 0.02	0.277± 0.008	0.250 ± 0.003	1.110 ± 0.005	0.509[b]
Water-holding capacity ml/g	12.1 ± 1.32	6.5 ± 0.09	9.8 ± 0.41	6.8 ± 0.23	9.5[a]
Hydrated density g/ml	2.50 ± 0.01	0	0	1.25 ± 0.02	2.10[b]
Particle size—% of sample retained on U.S. standard sieves:					
20 mesh	1.90 ± 0.0	2.19± 0.17	1.70 ± 0.16	3.23 ± 1.78	9[a]
30	28.22 ± 1.1	16.02± 0.11	21.28 ± 0.31	19.81 ± 0.27	33[a]
40	25.11 ± 0.13	22.51± 1.05	24.19 ± 1.01	19.19 ± 1.14	17[a]
50	19.4 ± 0.25	22.94± 1.70	23.23 ± 0.18	18.45 ± 0.18	9[a]
70	5.11 ± 0.02	9.25± 0.28	9.42 ± 0.12	9.92 ± 0.29	1[a]
>70	20.25 ± 1.86	26.93± 2.27	20.19 ± 0.22	29.13 ± 2.49	Traces[a]

* Mean and S.D. of 3 trials
[a] Official AACC white wheat bran R07-3691, 1976
[b] Source: Parrott and Thrall (1978)

for 1 min and dried for 10 hr at 70°C with a commercial dried (C.G. Sargent, Granitville, MS). Dried peel from each peeling treatment was divided into two samples. For the baking study, one-half was ground in a Wiley mill with a 20 mesh sieve; the other half was blended (Waring Products, Co., Winstead, CT) for use in physical and chemical analyses.

Physical properties

Particle size. A sample of 25g was placed on the largest of a descending series 20, 30, 40, 50, and 70 mesh stainless steel U.S. Standard Sieves that were fitted with a pan and cover. The "nested" sieves were shaken for 10 min, disassembled and contents were stirred lightly, then shaken for an additional 5 min. The residue on each sieve was carefully removed with the aid of a brush and weighed; each residue was expressed as percent by weight of the original sample.

Water-holding capacity. Water-holding capacity was measured by the procedure of McConnel et al. (1974).

Density determinations. For density determination, a calibrated graduate cylinder was filled, with slight shaking, with peel. The contents of the cylinder were weighed and the average of triplicate determinations was expressed as g/ml.

Hydrated density. A calibrated 10-ml graduated cylinder was filled with a known amount of distilled deionized water, and a known weight of peel was added carefully to avoid adhesion of particles to cylinder walls. Results were expressed as grams of sample added per ml of water displaced.

Bulk density. Bulk density was measured with a calibrated graduate syringe (open end packed with cotton). The syringe was filled with a known amount of sample, which varied somewhat depending on particle size and density. Pressure was applied manually until additional pressure would not further reduce the volume.

Chemical properties.

Moisture, ash, and protein (one estimate for protein) were determined by AOAC procedure (1975). Starch was determined by the method of Wood et al. (1977), except released glucose was measured by GC as the alditol acetate (Sloneker, 1971). Macro- and micro-mineral determinations were made by atomic absorption spectrophotometry with the dry ashing method. The freeze-dried sample (2g) was weighed and ashed overnight at 525°C. The ashed sample was added to 5 ml HCl (20%) containing 1% Lanthanum solution as recommended by the manufacturer of the spectrophotometer (Perkin Elmer, Model 303, Norwalk, CT) (Anon., 1971). Total phosphorus was determined by the method of Allen (1940). PH was measured with a pH meter (Sargent Welch, Model 1P, Skokie, IL) after 20g of ground potato peel was mixed with 20 ml of distilled deionized water, stirred well, and allowed to stand for 30 min. Caloric values were determined with an adiabatic bomb calorimeter (Parr Co., Model 1241, Moline, IL). Protein was determined by two methods: (1) Estimated as 6.25 × % total Kjeldahl nitrogen; and (2) calculated in terms of recovered amino acids by techniques according to Cavins and Friedman (1968) wherein amino acid content was measured with a Technicon (Model TSM) amino acid analyzer by methods adapted from Benson and Patterson (1965). Seventeen amino acids, including the sulfur-containing amino acids cysteine and methionine, were determined. Tryptophan was not determined.

Neutral detergent fiber (NDF) was measured by the method of Van Soest and Wine (1967) modified to include an α-amylase starch digestion step (Approved method 32-20 of the AACC, 1978 revision). Cellulose and lignin were determined by the acid detergent fiber (ADF) method of Van Soest (1963) as modified by Holst (1973).

Flour blends and baking

Flours containing 5, 10 and 15% potato peel from each of the four peeling methods were prepared with a commercial hard red spring wheat flour (malted and bromated) containing 12.5% protein, and 0.52% ash on a 14% moisture basis. The formula was:

Flour	100g (14% m.b.)
Salt	2%
Sugar	5%
Shortening	3%
Compressed yeast	3%
Water	Variable

Doughs made from wheat flour and flour blends with potato peel were prepared in triplicate by conventional straight dough procedure. Doughs were mixed in a National 100–200g mixer (National Mfg, C., Lincoln, NB).

Physical properties (absorption and mixing time) of doughs were determined with the farinograph. Samples (50g) of blended flour were tested in a 50g mixing bowl. The best curve obtained in three runs was selected for the farinograph and mixing data.

Fermentation period was 2 hr with the first punch at 55 min and the second 40 min later. The fermented doughs were sheeted with a National sheeter, molded by hand and panned. After a 55 min proof time at 30°C, the bread was baked for 25 min at 230°C. The volume of the cooled loaves was measured by rapeseed displacement.

RESULTS & DISCUSSION

Physical properties

In Table 1, the physical properties of the potato peels are summarized and compared with known values for wheat bran. Densities (direct and bulk) of potato peel were similar between abrasive and steam peeling methods and between hand and lye peeling methods; the higher densities for the two latter types of peel apparently resulted from lesser amounts of crude fiber per gram. This property also affected hydrated density; peel from both abrasive and steam peeling tended to float during measurement.

Distribution of particle size differed between peels and wheat bran (Table 1). High percentages of material were retained on the 30-, 50-, and 70-mesh sieves for potato peel compared to 30- and 40-mesh sieves for wheat bran. Researchers (Kimura, 1977; Connel, 1976; Kirwan et al., 1974) linked diversity in particle size of fiber to increase in rate of water absorption in human colons. In this diversity, potato peel was superior to the wheat bran. Our baking

Table 2—Composition of potato peel as compared with white wheat bran (% dry weight basis)

Constituent	Hand peeled	Abrasion peeled	Steam peeled	Lye peeled	Wheat bran
Moisture	4.9	2.5	4.6	3.7	10.4[a]
Protein (Method A)[e]	17.1	10.5	13.2	11.0	14.3[a]
Protein (Method B)[e]	11	8.1	7.2	6.3	15[d]
Fat[c]	~0	~0	~0	~0	5.2[a]
Starch	12	9.0	12	7.6	23[d]
Crude fiber	9.5	20.6	28.0	18.5	8.9[a]
Ash	8.4	5.0	6.4	5.5	5.12[a]
NDF	18	47	51	28	41[a]
ADF	14	40	40	25	12[a]
Cellulose	8.0	21	19	16	8.0[b]
Lignin	6.2	19	21	8.8	3.3[a]
Hemicellulose as NDF-ADF	4.0	7.0	11	3.0	29[d]
Other fiber[f]	54	38	34	56	40[d]
Dietary fiber[g]	68	78	74	71	52[d]
Caloric value, cal/g	4010	4171	4011	4089	No data
pH Value	5.85	5.59	6.83	9.15	No data
Phytic Acid[c]	~0	~0	~0	~0	3.39[a]
Macro- and Micro-minerals:					
PO_4 %	1.800 ± 0.00	0.95 ± 7.07	1.085 ± 2.12	1.475 ± 6.36	1.03[a]
Ca %	0.156 ± 0.005	0.510 ± 0.001	0.462 ± 0.004	0.494 ± 0.013	0.12[a]
K %	3.090 ± 0.116	1.000 ± 0.048	1.640 ± 0.042	0.472 ± 0.027	1.04[a]
Na %	0.041 ± 0.009	0.055 ± 0.066	0.068 ± 0.088	0.502 ± 0.016	0.10[a]
Mg %	0.150 ± 0.00	0.118 ± 0.084	0.146 ± 0.056	0.198 ± 0.084	0.43[a]
Cu ppm	24.80 ± 2.86	19.80 ± 0.84	26.40 ± 1.52	24.75 ± 11.62	15.5[a]
Fe ppm	178.00 ± 13.04	340.00 ± 61.24	634.00 ± 266.61	608.00 ± 151.06	122[a]
Cr ppm	2.20 ± 1.10	4.00 ± 0.00	7.80 ± 3.11	5.60 ± 0.55	No data
Zn ppm	22.80 ± 4.02	12.80 ± 5.36	124.00 ± 10.84	221.25 ± 34.00	No data
Al ppm	156.00 ± 15.17	288.00 ± 16.43	294.00 ± 20.74	256.00 ± 35.78	54[a]
Si ppm	105 ± 4.17	107 ± 2.81	137 ± 1.9	113 ± 6.8	5.0[a]

[a] Source: The Official AACC white wheat bran R07-3691, 1976.
[b] Source: Bing (1976).
[c] Assumed values of zero for potato peels.
[d] Measured on an 18–30 mesh distribution of AACC bran, not the unsieved material referred to by "a".
[e] A—Protein estimated as 6.25 × % total Kjeldahl nitrogen; B—Protein calculated in terms of recovered amino acids converted to protein.
[f] Other fiber calculated as: 100 − (cellulose + lignin + starch + fat + protein + ash).
[g] Dietary fiber estimated as: other fiber + cellulose + lignin.

tests showed that absorption increased from 3 to 13% as level of potato peel in flour increased from 0 to 15%. Among eight foods, Heller and Hackler (1977) ranked potatoes midway in water-holding capacity between bran (highest) and onion (lowest). However, in that study wheat bran was compared to the raw potato flesh, rather than potato peel.

Chemical composition

Compositions of potato peels and bran appear in Table 2. Two values are listed for protein: protein (method A) values were estimated as % total nitrogen times 6.25; protein (method B) values were calculated from recovered amino acids. The factor 6.25 often overestimates protein content of plant tissues (Tkachuk, 1969), as verified by our data on potato peels. We used method B values in calculations of fiber content.

Various measures of "fiber" appear in Table 2. Crude fiber has been used as a measure of fiber content for many years, but is not suitable for estimating "dietary fiber" because the harsh treatment with strong acid and base remove major amounts of guns, pectins, hemicelluloses, and even important amounts of cellulose. The ADF method, which was developed for forage materials, is often used to measure cellulose and lignin, even though it may remove portions of cellulose from some materials. The NDF method is considered to mainly measure cellulose + legnin + nonsoluble hemicelluloses of plant cell walls. The difference, NDF-ADF, is a valid estimation of cell wall hemicellulose in some plants. For plant tissues that contain considerable amounts of guns, pectins, and NDF soluble hemicellulose, the NDF method is not a suitable measure of dietary fiber and NDF-ADF is not a valid measure of hemicellulose. For some bran materials, the term "apparent hemicellulose" [defined as: 100 − (cellulose + lignin + ash + fat + starch + protein)], has been used to account for possible contents of pectins, gums and NDF soluble hemicelluloses that are valid components of dietary fiber and are present in relatively low amounts (Dintzis et al., 1979). For potatoes, in which recent literature reports cell wall material to contain about 16% pectin on a dry weight basis (Keijbets and Pilnik 1974) the term defined above is perhaps better called "other fiber," since the amount of pectic substances in potato peels is not expected to be relatively low. This term may also contain components such as low molecular weight saccharides. As an estimate of dietary fiber in Table 2 we use the definition: dietary fiber = cellulose + lignin + other fiber.

The white wheat bran differs markedly from the potato peels in several respects. The NDF-ADF value is probably within experimental error of the other fiber value for AACC bran, but is much less than the other fiber for the potato peels. That comparison suggests that soluble hemicelluloses, or gums, or pectins are major components of dietary fiber in potato peels but not in AACC wheat bran. The potato peels are higher in total dietary fiber than the 18–30 mesh distribution of AACC wheat bran. Possibly the proportion of wheat endosperm that is associated with the bran fraction (as indicated by values of starch and protein in Table 2) is higher than the proportion of potato flesh that is associated with the peels. Dietary fiber contents of peels were similar for all four methods of peeling.

Mineral contents of potato peels varied widely among peeling methods (Table 2). Peel content of Na was greater

and of K was lower after lye peeling than after abrasion, hand, and steam peeling. Potato peels from all methods contained more Ca, Cu, Fe, Cr and Al, but less Mg than bran.

Levels of Si and Cr were high in peels. Silicon, which is associated with fiber was a contributing factor in lowering cholesterol, lipid levels, and in binding bile acids in humans (Schwarz, 1974; Anon., 1977b). Chromium facilitated the attachment of insulin to its peripheral receptor sites in diabetes (Jones, 1977; Hambidge, 1978). Potato peel should be studied as a potential source of Si and Cr.

Flour blends and baking

Farinograph and baking data for doughs appear in Table

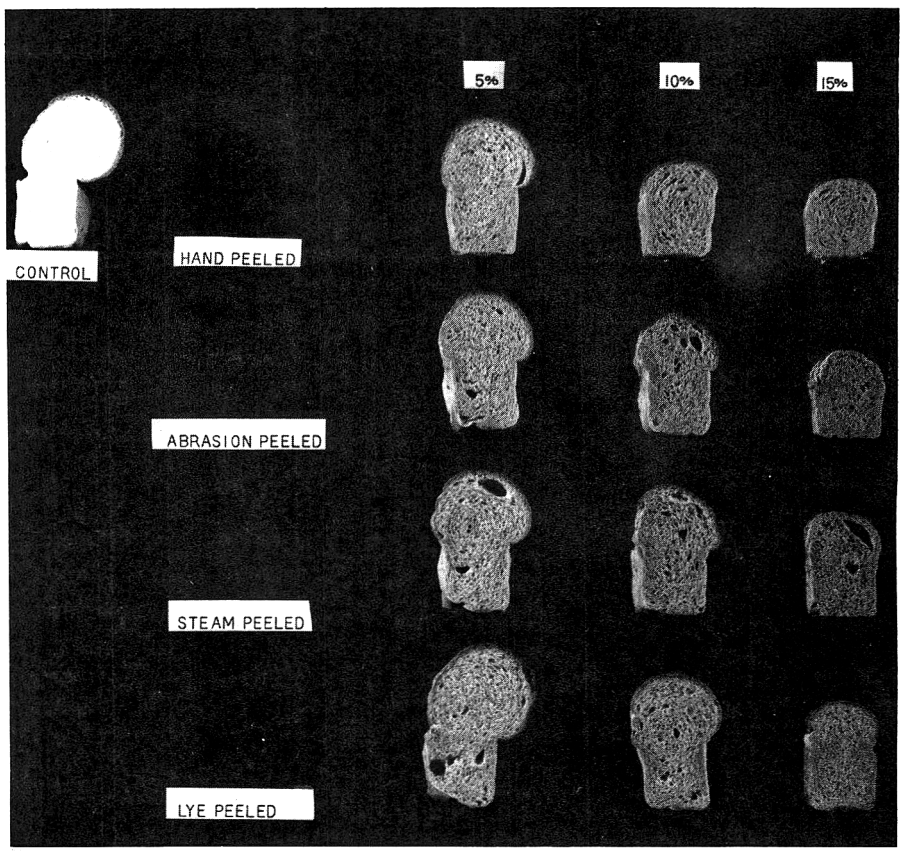

Fig. 1—Internal appearance of bread from different wheat flour-potato peel blends.

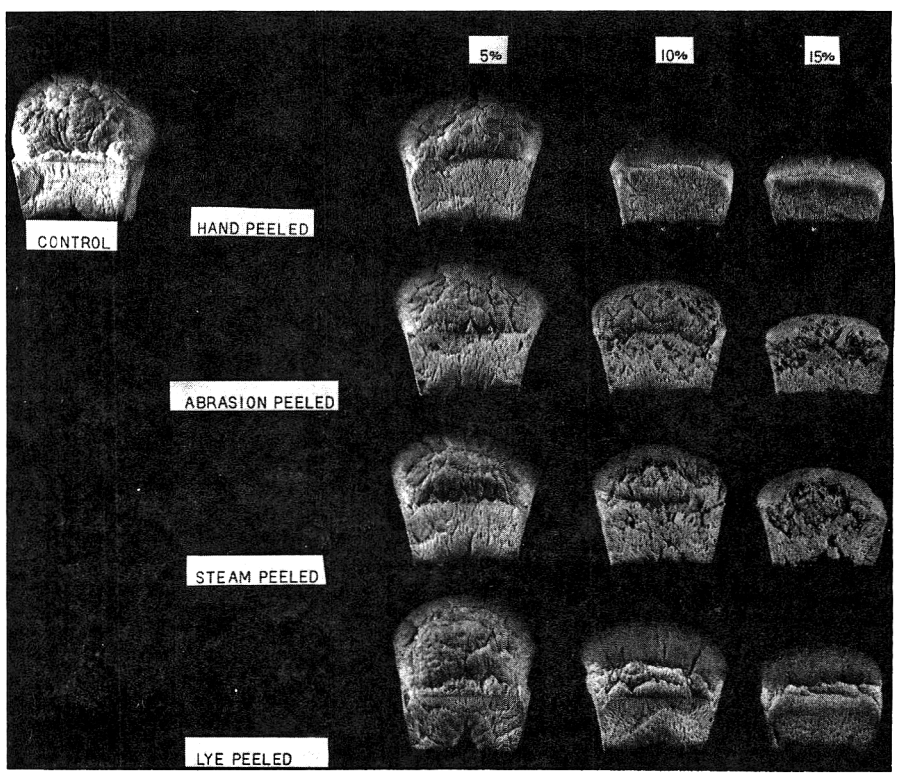

Fig. 2—External appearance of bread from different wheat flour-potato peel blends.

3. In general, absorption and dough development time increased with percent peel; these increases were pronounced with peel from steam or lye peeling.

Apparently the doughs with potato peel required a longer time to hydrate than doughs without peel. All doughs exhibited a weakening of the farinograph curve with incorporation of the potato peel with the exception of the lye-peeled material. The farinograph curves were quite strong with the blends containing the lye-treated peel up to the 15% concentration.

In agreement with farinograph absorption data, the baking absorption also increased with percent peel. Peel did increase hydration time as indicated by increase in mixing time with level of peel. The presence of potato peel in flour causes no problems in terms of dough handling properties.

In Table 4, loaf volume, external appearance, and texture are compared between the control and potato peel breads. Bread was best (compared to the control) in terms of loaf volume and internal and external appearance with flour containing potato peel from the lye peeling method. Loaves containing peel from the hand or abrasive peeling methods gave poor baking results. Figures 1 and 2 illustrate the internal and external appearance of the bread baked from blends of wheat flour with different types and levels of potato peel. Incorporation of abrasion and steam-peeled material at the 10% and 15% levels produced bread with a characteristically old appearance. Of breads with peels, the loaves containing the hand-peeled material had the most appetizing crust color. In crumb color, the bread containing the lye-peeled material most closely resembled bread made from whole wheat flour.

Our research indicated that potato peel is superior to a wheat bran in the contents of certain minerals, in total dietary fiber, in water-holding capacity, in its lower *quantity* of starchy components, and in its lack of phytate. These dietary advantages were not lost in baking quality trials.

Table 3—Farinograph and mixing data for doughs prepared from flour containing 0–15% peel

Sample type & peel Conc	Farinograph data			Baking time	
	Absorption[a]	Dough development time	Mixing tolerance index	Absorption[a]	Mixing time
	(%)	(min:sec)	(Brabender units)	(%)	(min:sec)
Control	62.0	4:30	20	61.0	3:00
Hand peeled					
5%	65.3	7:30	90	64.7	3:30
10%	66.6	8:00	75	65.8	3:30
15%	70.2	8:00	60	69.4	4:15
Abrasion					
5%	66.0	7:00	100	65.8	3:15
10%	71.0	8:00	95	69.5	3:30
15%	71.6	9:30	70	72.5	4:30
Steam treated					
5%	68.5	6:30	85	68.0	3:30
10%	73.8	7:30	135	72.4	3:30
15%	75.1	8:00	120	74.0	4:30
Lye treated					
5%	68.3	9:00	30	68.0	3:30
10%	74.7	11:00	30	72.8	4:00
15%	76.3	15:30	30	74.1	5:00

[a] Expressed on 14% moisture basis

Table 4—Loaf volume, external appearance and texture of bread containing 0–15% potato peel

Sample type & peel conc	Loaf volume[a] (cc)	External appearance[b]	Grain and texture[b]
Control	990 ± 8.16	10.0	10.0
Hand peeled			
5%	800 ± 3.53	8.5	7.0
10%	460 ± 4.02	5.0	3.0 (Dense crumb)
15%	342 ± 1.93	3.0	1.0 (Very dense crumb)
Abrasion			
5%	813 ±20.9	8.5	7.5
10%	598 ± 2.14	4.5[c]	5.0 (Slightly dense crumb)
15%	368 ± 1.32	2.0[c]	2.0 (Very dense crumb)
Steam treated			
5%	810 ± 8.16	8.5	8.0
10%	675 ±10.8	6.0[c]	6.0
15%	503 ± 4.72	3.5[c]	4.5 (Slightly dense crumb)
Lye treated			
5%	940 ± 3.48	9.5	9.0
10%	803 ± 4.72	7.0	8.0
15%	622 ± 4.36	5.0	6.0 (Slightly dense crumb)

[a] Data are an average of triplicate bakes.
[b] Values are based on a score of 1-10 with 10 being the best score.
[c] Loaf had an old appearance.

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PULSED NUCLEAR MAGNETIC RESONANCE STUDY OF WATER MOBILITY IN FLOUR DOUGHS

H. K. LEUNG, J. A. MAGNUSON and B. L. BRUINSMA

ABSTRACT

Transverse relaxation time (T_2) of water protons in wheat flour doughs, an indication of molecular mobility of water, was determined by pulsed nuclear magnetic resonance. The relaxation curves consist of two components with a long and a short T_2, corresponding to a more mobile and a less mobile fraction, respectively. The less mobile fraction has a T_2 of about 20 msec and accounts for about 0.62g water per g dry solid. These values seem to be independent of flour strength and mixing time. The more mobile fraction increased with moisture content and showed T_2 value of about 60 msec for most samples. Mixing and addition of salt to the dough resulted in reduced T_2.

INTRODUCTION

WATER BINDING is of paramount importance in flour dough because it has direct influence on the rheological properties of the dough and its baking performance (Hlynka, 1959; Bushuk and Hlynka, 1964; Bushuk, 1966; Webb et al., 1970). Cereal chemists have relied mainly upon the farinograph in the study of water binding capacity in dough (Jelaca and Hlynka, 1971). These instruments are useful in determining the dough mixing characteristics such as water absorption, mixing time, resistance to mixing, viscosity and stability which are all closely related to the baking performance of the dough. However, these techniques do not provide any direct information on the physical state of water imbibed by the flour. The concept of molecular mobility or degree of "boundness" of water in dough system has not been explored. Such a study should help to elucidate the influence of some important parameters such as flour protein and mixing time on dough properties.

Recently, differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been applied to measure unfreezable, or bound water in dough (Davies and Webb, 1969; Daniels, 1975; Bushuk and Mehrotra, 1977a, b, c). It was found that the bound water content (0.25 g/g sample) in dough was independent of flour protein content, added chemicals, amount of damaged starch, and mixing time. Using the boiling mode of DTA, Bushuk and Mehrotra (1977a) found that the binding energy in dough decreased with increasing protein content. The shortcoming of this technique is that during the heating of the samples, some physical and chemical changes such as protein denaturation and starch gelatinization may occur. Thus the results obtained may not reflect the true water-binding characteristics of the system.

In recent years, pulsed nuclear magnetic resonance (NMR) spectroscopy has been used extensively to study the binding of water molecules in biological systems (Cooke and Kuntz, 1974; Fung, 1977). It can be used to measure the longitudinal (T_1) and transverse (T_2) relaxation times of protons which are characteristics of the molecular mobility of water. When water is bound tightly to the substrate (e.g. flour), it is highly immobilized and shows reduced T_2; whereas free water is mobile and has relatively long T_2. Thus, useful information on the strength or degree of water binding can be obtained. Furthermore, delineation of water fractions based on molecular mobility is possible with this technique (Zimmerman and Lasater, 1958). In NMR studies on food systems, Leung et al. (1976) found that T_1 and T_2 are characteristic of the water binding properties of the food materials. It was reported that the water in some hydrated corn starch samples can be separated into two species with different T_2 values, corresponding to a mobile and a bound water fraction. Capelin and Blanshard (1977) reported in an abstract a pulsed NMR study of the interaction of water with gluten and starch in bread. Since no detail was presented in the summary, it is not clear what they observed in this study. Unlike DTA, pulsed NMR studies require no heating or freezing of the samples and therefore it is a nondestructive technique.

The objective of this study was to study the mobility or binding of water in flour doughs by measuring the transverse relaxation times of water protons. The effects of flour type, moisture content, mixing time and additives on the amount of "bound" water and water mobilities in the flour doughs were investigated.

EXPERIMENTAL

Materials

The wheat flours used in this study included hard, soft and club. The hard wheat flour is a commercial bread bakers blend obtained from Centennial Mills, Spokane. The soft wheat flour is an experimental blend produced by the USDA Western Wheat Quality Laboratory. The club wheat flour is an individual variety (Moro) experimentally milled by the same laboratory. Peak 72, a variety of hard red wheat, was also used in this study. The triticale sample was milled from a commercially available variety grown at Pullman, WA.

Sample preparation

Doughs were mixed at room temperature in a 10-g Mixograph to optimum consistency except when studying the effect of mixing time. Except for doughs used to study the effect of variable moisture, the % water absorption was adjusted to the level suitable for U.S. type bread using distilled water (Finney and Shogren, 1972). Samples of 100% absorption or higher were mixed into slurries by stirring. Sodium chloride and potassium iodate were added to doughs at levels of 2% and 50 ppm on flour basis (14% M.C.), respectively.

Immediately after mixing, a small dough sample was transferred to a 10 mm NMR tube and covered. Duplicate samples were prepared for each treatment. The NMR measurement was performed within 1–2 hr after the samples were prepared. Moisture content of the dough was calculated based on the moisture content of the flour and % water incorporated into the dough. The moisture contents of 20 dough samples were also determined according to the vacuum oven method of AOAC (1975) and they were in good agreement with the calculated values.

Pulsed NMR measurement

The proton relaxation studies were carried out at 90 MH_z using a Bruker WH-90/SXP NMR Spectrometer. The transverse relaxation time (T_2) was determined at 30°C using the Carr-Purcell-Meiboom-Gill (CPMG) technique (Meiboom and Gill, 1958; Farrar and Becker, 1971). The pulse spacing (τ) for all measurements was 2

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msec. A Nicolet BNC-12 Computer System was used for signal enhancement and data processing. Approximately 100 sequences were averaged for each measurement. T_2 was determined from a semi-log plot of the signal amplitude versus the pulse spacing (Farrar and Becker, 1971).

RESULTS & DISCUSSION

Transverse relaxation time

A typical plot of data for determining T_2 for water protons in flour doughs is shown in Figure 1. The nonexponential decay of the signal indicated the existence of two or more species of water molecules on the NMR time scale and the exchange between the different species is slow compared to their relaxation times (Zimmerman and Lasater, 1958). The relaxation curve (Fig. 1) can be resolved into two components characterized by a short and a long relaxation times, T_{2a} and T_{2b}, respectively. The T_{2a} component represents the less mobile, or more tightly bound water fraction, while the T_{2b} component represents the more mobile water fraction (Leung et al., 1976). These two water fractions will be referred to as "immobile" and "mobile" in the following discussion. The phenomenon of multi-phase behavior in water proton relaxation has also been observed in other systems such as hydrated starches (Lechert and Henning, 1976; Leung et al., 1976), hydrated elastin (Ellis and Packer, 1976) and striated muscle (Belton et al., 1972).

The relative population of the two water species can be estimated from Figure 1, as the initial spin echo signal (τ=0) is proportional to the number of protons in each species (Zimmerman and Lasater, 1958). The total moisture content of the dough was 0.88g water/g dry solid. The immobile fraction was calculated to be 0.59g water/g dry solid (DS), or 67% of the total water; while the mobile fraction accounted for the rest. The result indicated that in a wheat flour dough containing 0.88g water/g DS, 67% of the water interacts strongly with the flour, and the excess water exists in a more mobile or "free" state.

Effect of water content

The T_{2a} and T_{2b} values of water in hard wheat flour doughs of different moisture contents are summarized in Table 1. Each of these values is the average of two or more samples. The variation between duplicate samples was usually less than 2 msec for T_2 values shown in Tables 1–5. The amount of water in the immobile (T_{2a}) and the mobile (T_{2b}) fractions are also given in the table. In the range 0.64–0.93g water/g DS, which corresponds to water absorption of about 45–75%, T_{2a} and T_{2b} were approximately 20 msec and 60 msec, respectively. The rather small variation in T_{2a} and T_{2b} in this moisture range indicated that the mobility of each water fraction as measured by pulsed NMR did not change appreciably with water content.

The immobile fraction accounted for 0.62 ± 0.03g water/DS in the moisture range 0.64–0.98g water/DS. Above this moisture content, all of the water added to the doughs is in the more mobile phase. Therefore, the average value of 0.62g water/g DS marks the transitional point between two water species with different physical states. As will be discussed later, this value is also significant in dough rheology.

Using DTA, Davies and Webb (1969) found that 0.33g water/g DS in the dough remained unfrozen down to −50°C, while all the water above this minimal value was freezable. Bushuk and Mehrotra (1977b) reported similar unfreezable water content of 0.30g water/g DS in the flour doughs. However, their DSC study indicated that about two-thirds of the addtional water was freezable and the remainder was of the bound (unfreezable) type. The discrepancy of the two studies remains to be resolved. It is obvious that the amount of the immobile water fraction in the flour dough as measured by pulsed NMR is different from the unfreezable water content determined by DTA or DSC. It should be pointed out that the immobile water fraction classified by T_2 measurement includes the loosely imbibed water in the dough. This water fraction is available for chemical, enzymatic and microbial reaction. Thus, it is not "bound" in terms of total availability.

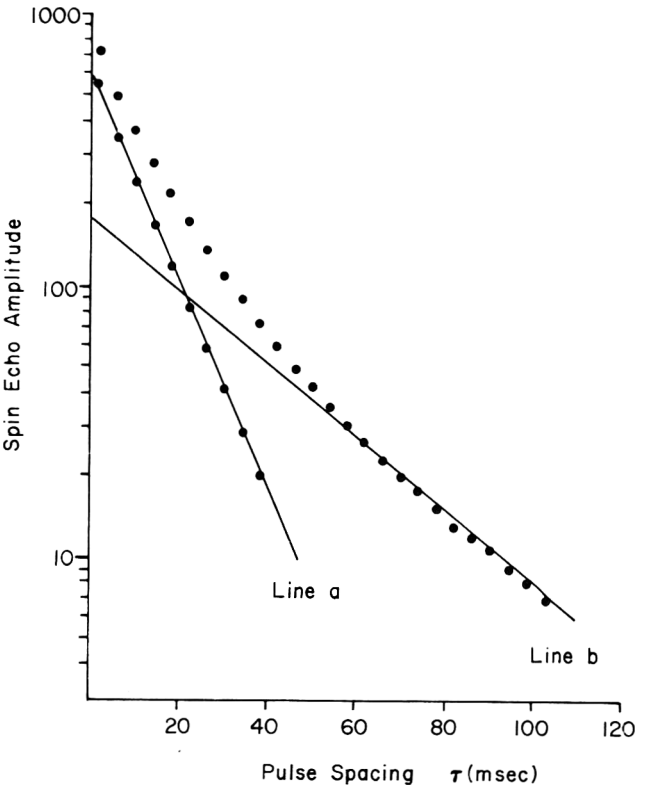

Fig. 1—Transverse relaxation times for water in hard wheat flour dough at 88% moisture, dry basis.

The effects of moisture content on lipid binding and rheology of wheat flour doughs have been investigated by Davies et al. (1969) and Wood et al. (1972). These studies showed that the lipid binding of flour dough increased with moisture content and began to level off at 0.54–0.67g water/g DS. Note that the immobile water content of 0.62 falls within this range. At this moisture level, sufficient free water exists to support the mixing of an extensible dough (Daniels, 1975).

Based on the rheological study of water in flour doughs, Webb et al. (1970) postulated that three forms of water exist in the dough. They are: (a) free water, (b) lightly bound water, and (c) firmly bound water. In light of the

Table 1—Dependence of the transverse relaxation time T_2 on the moisture content of hard wheat flour doughs

Total moisture content (g H_2O/gDS)	Immobile fraction		Mobile fraction	
	T_{2a} (ms)	g H_2O/g DS	T_{2b} (ms)	g H_2O/g DS
0.53	12	0.49	54	0.04
0.59	11	0.47	38	0.12
0.64	16	0.58	62	0.06
0.70	22	0.63	62	0.07
0.76	20	0.65	59	0.11
0.88	21	0.59	58	0.29
0.93	20	0.65	61	0.28
0.98	24	0.64	62	0.34
1.38	34	0.62	71	0.76

Table 2—Dependence of the transverse relaxation time T_2 on the moisture content of soft wheat flour dough.

Total moisture content (g H_2O/g DS)	Immobile fraction		Mobile fraction	
	T_{2a} (ms)	g H_2O/g DS	T_{2b} (ms)	g H_2O/g DS
0.53	17	0.51	116	0.02
0.59	16	0.55	98	0.04
0.64	20	0.56	62	0.08
0.84	18	0.58	58	0.26
0.93	21	0.61	64	0.32
1.06	32	0.53	70	0.53
1.39	31	0.52	74	0.87

Table 3—Effect of flour type on T_2 of flour doughs

Flour	Immobile fraction		Mobile fraction	
	T_{2b} (ms)	g/H_2O/g DS	T_{2b} (ms)	g H_2O/g DS
Hard (Peak 72)	20	0.61	58	0.32
Hard	20	0.65	61	0.28
Soft	21	0.61	64	0.32
Club	20	0.63	58	0.30
Triticale	23	0.69	68	0.24

Table 4—Effect of mixing on T_2 of soft and club wheat flour doughs

Flour	Mixing time (min)	Immobile fraction		Mobile fraction	
		T_{2a} (ms)	g H_2O/g DS	T_{2b} (ms)	g H_2O/g DS
SOFT	0.5	23	0.64	67	0.12
	2.0	20	0.59	62	0.17
	7.0	17	0.62	45	0.14
CLUB	0.5	21	0.63	71	0.18
	1.2	18	0.65	59	0.16
	7.0	18	0.59	52	0.22

foregoing discussion, it seems logical to identify these three forms of water as mobile water, immobile water and unfreezable water. According to the pulsed NMR results, it seems appropriate to modify the term "free water" as very loosely held water in the dough. As shown in the present study, the mobile water fraction exhibited T_2 values of about 60 msec, much shorter than the 1–2 sec for free or bulk water. This implies that even the very loosely held water in the dough is relatively immobile when compared to free water.

The water mobilities of the under- and over-hydrated flour samples were different from those of the smooth doughs. Below a moisture content of 0.59g water/g DS (40% water absorption), the flour samples did not form a smooth dough upon mixing. As shown in Table 1, most of the water in the sample containing 0.53g water/g DS was in the immobile state with a reduced T_{2a} of 12 msec. The small amount of water (0.04 g/g) in the mobile state was probably due to incomplete mixing. The sample containing 0.59g water/g DS had T_2 values of 11 and 38 msec for the immobile and mobile phases, respectively. These values are shorter than those for doughs at 64–98% moisture (dry basis), indicating lower water mobility. The flour containing 1.38g water/g DS exhibited the consistency of a cake batter rather than a dough. The water mobility of this sample was higher than that of the dough, as indicated by the increased T_{2a} and T_{2b} values. Based on these observations, it is suggested that about 0.62g water/g wheat flour is required for the formation of a smooth dough. As the moisture increases above this level, the additional water goes into a more mobile state, while the mobility and quantity of the immobile water fraction remain relatively unchanged. The change in rheological properties of the doughs with increasing water content was probably due to the increase of the more mobile water species.

The T_{2a} and T_{2b} values of soft wheat flour doughs of different moisture contents are summarized in Table 2. The results are similar to those of the hard wheat flour. In the two under-hydrated flour samples (0.53 and 0.59g water/g DS), only a small fraction of water (0.02–0.04g water/g DS) was in the mobile phase. It is not clear why the T_{2b} values of these two samples were exceptionally long. A possible explanation is that a small fraction of the water may not be incorporated into the mixture due to insufficient dough development. The existence of a small amount of relatively free or mobile water may account for the long T_{2b} values observed.

Effect of flour type

Table 3 shows the T_{2a} and T_{2b} values of doughs made from different types of flour. The moisture content of the doughs were adjusted to the same level of 0.93g water/g DS (70% water absorption). Hard wheat flour generally has a higher water absorption, longer mixing time, and more tolerance to mixing as compared to soft and club wheat flours (Pratt, 1971). Triticale flour has a shorter mixing time, lower water absorption and shorter stability toward mixing than those of wheat flour (Tsen et al., 1973). Peak 72 is a variety of hard wheat flour which shows extremely long mixing time and mixing tolerances (Western Wheat Quality Laboratory). Although these flours vary widely in dough characteristics, no distinct difference in water mobility was observed based on the T_2 values in Table 3. This was surprising since hard wheat flour was expected to immobilize water more strongly than the soft or club wheat flours and exhibit shorter T_2. It is likely that the proton nuclear relaxation time was not sensitive enough to detect the different rheological properties of the doughs made with different flours. It is also possible that water mobility may be independent of dough characteristics within a certain range of consistency or viscosity. The different rheological properties exhibited by hard, soft and club flour doughs at the same moisture content could be due to other factors besides water binding. Further study is necessary to elucidate the relationship of dough consistency and water mobility.

Effect of mixing time

It is well-recognized that overmixing of wheat flour doughs, especially for soft and club flours, results in decreased dough consistency and resistance to mixing (Hoseney, 1979). The effect of mixing time on T_2 of water protons in soft and club wheat flour doughs is shown in Table 4. In both cases, T_2 tended to decrease with increasing mixing time. This effect is more pronounced in the mobile fraction (T_{2b}). The decrease in T_2 with mixing probably reflects the increased interaction between water and flour as they were mixed into a dough. However, the reduced T_2 of the overmixed samples (7 min mixing) was unexpected. Soft and club wheat flour doughs reached the maximum resistance to extension around 3 min and began to break down upon further mixing. This resulted in decreased dough viscosity. If decrease in dough viscosity is associated with increased water mobility, one would expect increased T_2. This seems to contradict with the result in Table 4, which indicated that T_2 actually decreased in the overmixed samples. No satisfactory explanation can be given based on the present data. It is possible that water mobility and viscosity of dough may not be directly related.

Table 5—Effect of additive on T_2 of hard wheat flour dough

	Immobile fraction		Mobile fraction	
Flour	T_{2a} (ms)	g H_2O/g DS	T_{2b} (ms)	g H_2O/g DS
No additive	20	0.65	61	0.28
KIO_3, 50 ppm	19	0.68	58	0.25
NaCl, 2%	18	0.59	48	0.34

Effect of additives

Salt (sodium chloride) and potassium iodate have been shown to affect the dough properties (Bloksma, 1973). The effect of these two additives on T_2 of hard wheat flour doughs is shown in Table 5. No change in T_{2a} and T_{2b} of water was observed with the addition of 50 ppm potassium iodate. On the other hand, the addition of 2% salt shortened the relaxation time of the mobile fraction. The reduced water mobility is expected since salt is known to stiffen the dough (Bloksma, 1973). Salt might also promote solubilization of some dough components, resulting in reduced T_2 in the mobile phase.

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CALCIUM AND MAGNESIUM NUTRITIONAL STATUS OF ADOLESCENT HUMANS FED CELLULOSE OR HEMICELLULOSE SUPPLEMENTS

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ABSTRACT

The object of the study was to determine the effect of graded levels of two types of dietary fiber on calcium and magnesium utilization by adolescent boys and girls. The 21-day study was divided into a 1-day introductory nitrogen depletion period, a 2-day adjustment period and three 6-day randomly arranged experimental periods. During the three experimental periods, the eleven adolescent boys and girls and one young adult woman received a basal diet plus 10g or 20g of cellulose or 10 or 20g of hemicellulose supplements. The subjects were divided into two groups of six subjects each. Calcium and magnesium contents of food, feces, urine and blood serum were measured by atomic absorption spectrophotometry. Statistically significant changes in urinary mineral loss and increases in serum values were demonstrated. While receiving the basal diet alone or plus 10g cellulose, 20g cellulose, or no fiber, 10g hemicellulose or 20g hemicellulose, mean urinary calcium excretions (mg/subject/day) were 75.14, 83.14, 76.33, 70.48, 66.64 and 87.58, respectively. During the pre-study and while receiving the basal diet alone, 10g cellulose, 20g cellulose or during the pre-study, no fiber, 10g hemicellulose and 20g hemicellulose supplement, mean magnesium serum values (mcg/subject/dl) were 2.045, 1.938, 1.906, 1.940, 1.995, 1.976, 1.879 and 1.922, respectively.

INTRODUCTION

MINERAL NUTRITIONAL STATUS of human populations both in the United States and abroad is currently a matter of major concern. Magnesium deficiencies have been identified in human populations (Caddell, 1967; Flink et al., 1957; Hammerstein and Smith, 1957; Shils, 1969). A true calcium deficiency syndrome has yet to be identified in human populations. However, secondary calcium deficiency states are thought to be commonplace (Hegsted et al., 1952; Leverton and Marsh, 1942; Schwartz et al., 1973a,b; Malm, 1958). Interactions among calcium and magnesium exist (Carswell and Winter, 1931; McCance et al., 1942).

Dietary fiber has been suggested as a possible causative factor in secondary calcium and magnesium deficiency situations (Reinhold et al., 1976; Ismail-Beigi et al., 1977). Increased intake of dietary fiber by Americans has been advocated (Select Committee, 1978). The beneficial effects of dietary fiber have been attributed in part to its ability to decrease the absorption of harmful substances through increase of fecal transit movement, decrease in intestinal interluminal pressure and/or fiber absorption. A similar action might occur with essential nutrients.

Several studies indicate that mineral utilization may be impaired when high fiber diets are consumed (McCance et al., 1942; Walker et al., 1948; Reinhold et al., 1976; Cummings et al., 1976; Ismail-Beigi et al., 1977). However, this effect is not always similar in direction nor in degree depending upon the mineral under study. Thus, with the minerals known to be competitors for absorption as with copper and zinc or with magnesium and calcium, additions of dietary fiber may have unpredictable results. A further complicating factor is that high fiber diets based on cereal products are usually high phytin diets.

When dietary phosphorus occurs as phytin, there is evidence that calcium absorption is impaired (McCance and Widdowson, 1942). Ten men and women consumed diets containing bread made from 92% extraction flour and bread made from 69% extraction flour. Calcium intake was estimated to be 10 mg/kg body weight. A negative calcium balance occurred initially but calcium equilibrium was eventually reached. Magnesium retention was lowered at first by increasing phytate phosphorus, but later improved. Phytic acid is believed to reduce the stability of magnesium. When phytate was reduced in amount, magnesium retention improved. Calcium was more highly absorbed from the 69% phytate extraction flour than from the 92% extraction flour. A further study of white bread with sodium phytate addition resulted in marked decrease in calcium absorption.

A diet containing less calcium and more phytate phosphorus resulted in immediate negative calcium balances in another human study (Walker et al., 1948). A bread made from 95–100% extraction meal and a bread made from 70% extraction meal were consumed in different dietary periods. The decreased calcium content of the diet resulted in a greater amount of phytate phosphorus hydrolyzed. A gradual adaptation of the body to the lowered calcium intake occurred. Fecal calcium excretion gradually decreased.

Three men were given 2.5g of phytic acid daily as purified sodium phytate together with leavened bread for 28 days (Reinhold et al., 1973). Subjects were then given tanok for 32 days. Tanok is the phytate rich unleavened wholemeal flat white bread which is a staple food of Iranian villages. The purified phytate resulted in negative calcium balances which eventually stabilized. Phytate as tanok caused negative calcium balances in all subjects. Plasma calcium levels decreased with the purified phytate bread and remained below normal for the remainder of the study.

Negative balances of calcium, magnesium, zinc and phosphorus due to increased fecal excretion of each element developed with increased fiber and phosphorus consumption as wheat wholemeal bread (Reinhold et al., 1976). Bazari bread was made from wheat flours with extra dietary calcium, magnesium, phosphorus and zinc added. In a 20-day period of high fiber consumption by 2 Iranian men, mean fiber intake increased when white bread was replaced with wholemeal bread. Increases in fecal and urinary excretions of calcium and magnesium occurred. Calcium balances remained positive, but magnesium balances became negative when wholemeal bread was added.

Wheat fiber was added for 3 wk to a metabolically controlled diet and fed to 6 subjects (Cummings et al., 1976). This diet produced an increase in fecal calcium and magnesium excretion. Fecal magnesium excretion was particularly striking but could not be ascribed to a specific effect of fiber on magnesium absorption as dietary magnesium intake was considerable higher on the high fiber diet.

Addition of 19g of cellulose to a daily diet low in fiber for a 20-day period caused increased fecal calcium, magnesium and zinc excretion (Ismail-Beigi et al., 1977). Three

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Table 1—Experimental plan. Cellulose and hemicellulose supplementation of basal diet

Period[a]	No. of days	N intake[b] g/day	Fiber supplement Kind	Fiber supplement Amount g/day
Study A				
Deplet.	1	0.8	–	–
Adjust.	2	6.8	cellulose[c]	10.0
Pd. 1	6	6.8	cellulose	10.0
Pd. 2	6	6.8	cellulose	20.0
Pd. 3	6	6.8	none	–
	21 days			
Study B				
Deplet.	1	0.8	–	–
Adjust.	2	6.8	hemicellulose[d]	10.0
Pd. 1	6	6.8	hemicellulose	10.0
Pd. 2	6	6.8	hemicellulose	20.0
Pd. 3	6	6.8	none	–
	21 days			

[a] Experimental periods 1–3 were randomly arranged for subjects.
[b] N intake per subject per day: 0.8g N from basal diet, 4.0g N from peanut butter, 20g N from reconstituted dry skim milk.
[c] Alpha-cellulose fiber, approximately 99.5%. Sigma Chemical Corporation, P.O. Box 14508, St. Louis, MO 63178.
[d] Hemicellulose containing pentosans, hexosans and galactans from psyllium. Winthrope Laboratories, Division of Sterling Drug Inc.,

Iranian men participated in the study. Bazari bread was again administered to the subjects, with cellulose added. Increased fecal excretion of calcium occurred along with the development of negative calcium balances. Plasma calcium concentrations increased during low fiber intakes, but decreased on high fiber intakes. Magnesium balances also became negative. An explanation for the dietary effect of fiber may be the impairment of absorption of divalent metals in the presence of wheat fiber, resulting from the ability of fiber to bind these metals firmly in the small intestine.

Thus, evidence exists suggesting that both phytate and fiber inhibit utilization of calcium and magnesium, but the extent of these effects have not been fully explored.

Chemical and physical properties of dietary fibers vary; hence, they probably do not behave identically physiologically. The objective of the current research was to study the effect of dietary cellulose and hemicellulose on utilization of calcium and magnesium by adolescent boys and girls.

MATERIALS & METHODS

THE 21-DAY STUDY was divided into an introductory 1-day nitrogen depletion period, a 2-day nitrogen adjustment period and three experimental periods of 6 days each.

Purposes of the introductory nitrogen depletion period and adjustment period were to deplete subjects of labile nitrogen stores and readjust the subjects to the low protein diet used in the experiment. The technique of feeding a nitrogen-free diet for two days at the beginning of a study has proved useful to this objective in this laboratory. The diet used during the depletion period was the basal diet as described in Table 2 minus peanut butter and milk. Other purposes of these periods were to acquaint subjects with their duties and responsibilities and to determine caloric needs for each individual.

During the three experimental periods, the subjects were fed a basal diet alone or the basal diet plus cellulose or hemicellulose supplements (10.0 g/subject/day or 20.0 g/subject/day).

Calcium intake from supplements and food in the diet averaged 1563.64 mg/day during all experimental periods. Mean magnesium intake was 329.68 mg/day for all subjects.

Some differences in calcium and magnesium intakes existed among subjects because of variation in intake of extra caloric foods. During the study, vitamins and minerals were provided to meet the daily requirements. Energy intake was adjusted to meet the individual needs of each subject.

Table 2—Basal diet

Food	Amount per day
Breakfast	
Starch bread	1/3 daily allotment
Peanut butter	1/3 of 93.3g allowance
Milk	1/3 of 38.7g allowance
Applesauce	100g
Jelly	varied
Butter oil	varied
Vitamin/mineral supplements[a,b]	
Lunch	
Starch bread	1/3 daily allotment
Peanut butter	1/3 of 93.3g allowance
Milk	1/3 of 38.7g allowance
Peaches	100g
Tomato juice	100g
Jelly	varied
Butter oil	varied
Dinner	
Starch bread	1/3 daily allotment
Peanut butter	1/3 of 93.3g allowance
Milk	1/3 of 38.7g allowance
Pears	100g
Green beans	100g
Jelly	varied
Butter oil	varied
Soft drink	varied

[a] Mineral supplements Leverton Mineral Mix, ICN Pharmaceuticals, Inc., Life Science Group, Cleveland, OH provided the following (g/subject/day): calcium, 0.886; phosphorus, 0.538; magnesium, 0.237; iron, 0.0180; copper, 0.0024; potassium, 0.451; iodine, 0.00018; manganese, 0.0024; and zinc, 0.00109.
[b] Vitamin supplement One-A-Day Multivitamin Supplement. Miles Laboratories, Inc., Consumer Products Division, Elkhart, IN supplied the following (subject/day): vitamin A, 5000 IU; vitamin D^2, 600 IU; thiamin, 1.5 mg; riboflavin, 1.7 mg; ascorbic acid, 60.0 mg; pyridoxine, 2.0 mg; niacin, 20.0 mg; folic acid, 0.4 mg; vitamin E, 15 IU; and cyanocobalamine, 6.0 mcg.

The meals consisted of peanut butter, starch bread, peaches, pears, applesauce, tomato juice, green beans and reconstituted nonfat dry milk solids. Calorie intake was adjusted with soft drinks, jelly, honey, hard candy or butter oil for subject weight maintenance. Thus, calorie intake varied among subjects but was constant for any one subject. The subjects were allowed to have sugarless gum as desired. To insure an adequate diet, the subjects were given mineral and vitamin supplements.

Twelve young human beings ranging in age from 10–22 years participated as subjects in the study. Subject descriptions are given in Table 4. All subjects were in good health as determined by their private physicians and carried out their normal daily activities except for eating meals in the laboratory and collecting excreta. Signed consent forms were obtained from each subject, their parents and their private physician. This study was approved for human

Table 3—Individual dietary intake of calcium and magnesium

Subject no.	Calcium mg/day	Magnesium mg/day
556	1563.29	332.91
558	1560.66	327.13
559	1562.99	332.57
921	1562.99	332.57
931	1562.84	332.39
932	1564.61	325.28
937	1564.01	335.11
940	1579.15	326.33
956	1561.52	329.50
957	1559.90	326.26
963	1561.98	330.02
2219	1559.75	326.09
Mean	1563.64	329.68

Table 4—Physical data on subjects

Subject no.	Sex	Energy intake (Cal/Day)	Mg intake (mg/Day)	Ca intake (mg/Day)	Height (cm)	Weight (kg)	Age (Yr)	Ethic/Race Classification
556	M	2800	333	1563	173	70.91	17	Am./White
558[b]	M	2800	327	1561	181	64.21	17	Am./White
559[a,b]	M	2800	332	1563	178	72.27	16	Am./White
921[a,b]	M	2800	332	1563	175	67.27	16	Am./White
931	M	2600	332	1563	152	40.91	13	Am./White
932	M	2800	325	1565	175	55.91	14	Am./White
937	M	2800	335	1564	160	47.27	13	Am./White
940	M	2700	326	1579	155	46.36	12	Am./White
956	F	2000	330	1562	122	38.41	11	Am./White
957[c]	F	1800	326	1560	145	35.68	10	Am./White
963[c]	M	2500	330	1562	170	58.41	14	Am./White
2219	F	1800	326	1560	158	52.27	22	Am./White
Mean			330	1564				

[a] 559 and 921 were twin brothers
[b] 558, 559 and 921 were brothers
[c] 957 and 963 were brother and sister

subject participation by the University of Nebraska Committee on Investigations Involving Human Subjects.

Complete collections of urine and stools were made for the entire study. Urine collections were made by each subject on a 24-hr basis and stools on a 6-day period basis. Toluene and 1% concentrated hydrochloric acid were used to preserve the urine collections. Creatinine was determined daily on the 24-hr composites to determine completeness of the urine collections. Carmine dye markers given to the subjects in capsule form were used as a marker to separate the fecal collections for each experimental period. Fasting blood samples were drawn at the beginning of the study and at the end of each 6-day experimental period.

Urine samples were diluted 1:500 with deionized distilled water for magnesium determination. Urine samples were diluted 1:50 with 5% $LaCl_3$ for calcium determination. Serum samples were diluted 1:50 with 1% $LaCl_3$ for calcium and magnesium determination. Fecal composites samples of each subject for each experimental period and the individual food items were initially prepared by the dry ash digestion method for calcium and magnesium determination. The $LaCl_3$ diluent solution was used only to remove a potential phosphate interference in the calcium determination. Calcium and magnesium contents of the urine, feces, serum and food were lead on a scale of 1 by Perkin-Elmer atomic absorption spectroscopy Model 303.

Statistical analysis of data included analysis of variance and Duncan's Multiple Range Test. The project was approved by the University of Nebraska Institutional Committee on Investigations Involving Human Subjects.

RESULTS & DISCUSSION

INDIVIDUAL and mean fecal excretions of calcium and magnesium of the adolescent subjects while receiving no fiber supplementation or graded levels of cellulose or hemicellulose supplementation corrected for variations in subject mean fecal excretions are given in Tables 5 and 6.

Dry fecal weights of subjects as shown in Table 7 in response to the different experimental diets varied. On the basis of mean values, total dry fecal weight was highest when 20g of cellulose supplements were used and lowest when no fiber supplements were given. A greater increase in dry fecal weight occurred with the increasing levels of cellulose and hemicellulose, with the greatest increase occurring during the 10 and 20g of cellulose supplementation. Due to wellknown errors in division of feces into period lots, absolute values for individuals are less meaningful than are

Table 5—Effect of dietary fiber on fecal calcium excretion

Subject no.	Mean fecal excretion of calcium (mg/day) while receiving diet[a,b]		
	N	LC	HC
556	598.15	461.68	883.46
559	978.39	1068.86	886.19
921	755.31	712.58	813.30
937	621.25	1016.03	658.35
940	840.81	817.35	944.80
957	1164.99	683.10	1051.19
Mean	826.48	793.25	872.88
	N	LH	HH
558	954.79	1043.55	798.86
931	743.56	719.78	717.47
932	563.80	729.14	871.14
956	884.66	1362.18	517.16
963	514.72	1444.29	827.39
2219	906.78	915.46	1059.69
Mean	761.38	1035.73	798.62

[a] Diet code: N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.
[b] Total mean value for all subjects in both studies = 848.06. Standard deviation = 221.36.

Table 6—Effect of dietary fiber on fecal magnesium excretion

Subject no.	Mean fecal excretion of magnesium (mg/day) while receiving diet[a,b]		
	N	LC	HC
556	131.88	138.23	195.78
559	249.77	314.30	226.11
921	188.32	210.70	261.89
937	174.45	262.48	325.28
940	242.64	225.97	258.94
957	312.79	195.06	320.94
Mean	216.64	224.46	264.82
	N	LH	HH
558	258.72	248.38	225.34
931	181.07	71.98	194.88
932	173.48	205.16	211.92
956	219.22	341.35	135.52
963	167.20	453.82	281.66
2219	34.37	278.12	294.65
Mean	172.34	266.47	224.00

[a] Diet code: N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.
[b] Total mean value for all subjects in both studies = 228.12. Standard deviation = 78.99.

group mean values. Hence, group mean values for total fecal weight were used in calculation of daily calcium and magnesium fecal excretions of individuals from dry fecal weights (mg/g).

Fecal calcium excretion of subjects while receiving no supplement 10g cellulose, or 20g cellulose, were 826.48, 793.25 and 872.88 mg/day, respectively (Table 5). Fecal calcium excretion of subjects while receiving no supplement, 10g hemicellulose or 20g hemicellulose were 761.38, 1035.73 and 798.62 mg/day, respectively. Although not statistically different, there was a trend toward increased fecal calcium excretion when 20g of cellulose and 10g of hemicellulose supplements were fed in comparison to values when no fiber and 10g of cellulose supplements were given, with the greatest increase occurring with 10g of hemicellulose supplement.

Fecal magnesium excretion of subjects while receiving no supplement, 10g cellulose or 20g cellulose were 211.64, 224.46 and 264.82 mg/day, respectively (Table 6). Fecal magnesium excretions (mean corrected figures) while receiving no supplement, 10g hemicellulose or 20g hemicellulose were 172.34, 266.47 and 224.00 mg/day, respectively.

—Text continued on page 1416

Table 7—Effect of dietary fiber on dry fecal weight

Subject no.	Mean dry fecal weight (g/period) while receiving diet[a]		
	N	LC	HC
556	72.02	108.22	205.24
559	84.92	202.16	251.61
921	113.86	113.84	237.92
937	59.52	203.02	185.69
940	120.43	204.30	196.62
957	112.64	143.28	190.28
Mean	93.90	162.47	211.23
	N	LH	HH
558	137.86	185.80	251.38
931	121.93	156.54	143.95
932	108.68	136.04	167.02
956	81.40	148.26	67.71
963	93.54	196.84	165.57
2219	94.46	179.16	206.28
Mean	106.31	167.11	166.98

[a] Diet code: N = Basal diet without fiber supplement: LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.

Table 8—Effect of dietary fiber on urinary calcium excretion

Subject no.	Mean urinary excretion of calcium (mg/day) while receiving diet[a,b]		
	N	LC	HC
556	123.33	148.00	145.00
559	80.33	75.00	80.50
921	98.20	101.67	87.83
937	33.50	33.33	37.00
940	41.80	66.83	42.00
957	73.67	74.00	65.67
Mean[b,c]	75.14a	83.14b	76.33a
	N	LH	HH
558	115.83	122.00	130.33
931	36.40	17.33	26.17
932	82.00	58.83	125.17
956	8.83	10.50	10.67
963	96.00	79.83	110.50
2219	83.83	111.33	122.67
Mean[b,d]	70.48a	66.64a	87.58b

[a] Diet code: N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.

[b] Mean values with different letters are significantly different from one another ($P < 0.050$) according to ranking of effect.

[c] Total mean values for subjects on cellulose supplementation = 2.00. Standard deviation = 0.97.

[d] Total mean value for subjects on hemicellulose supplementation = 2.00. Standard deviation = 0.81.

Table 9—Effect of dietary fiber on urinary magnesium excretion

Subject no.	Mean urinary excretion of magnesium (mg/day) while receiving diet[a,b]		
	N	LC	HC
556	211.67	136.60	153.50
559	147.50	157.00	162.33
921	164.60	154.00	153.17
937	76.00	94.17	130.33
940	123.60	148.00	149.17
957	93.33	129.67	92.33
Mean[b,c]	136.12a	136.57a	140.14a
	N	LH	HH
558	142.67	161.50	118.83
931	150.20	93.30	14.33
932	113.67	130.33	166.83
956	71.17	92.50	92.17
963	111.10	129.67	121.50
2219	100.67	116.67	100.50
Mean[b,d]	114.91a	120.66a	102.36b

[a] Diet code: N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.

[b] Mean values with different letters are significantly different from one another ($P < 0.010$) according to ranking of effect.

[c] Total mean value for subjects on cellulose supplementation = 2.00. Standard deviation = 0.86

[d] Total mean value for subjects on hemicellulose supplementation = 1.50. Standard deviation = 0.45.

Table 10—Effect of dietary fiber on serum calcium

Subject no.	Blood serum calcium values per period[a,b] (mcg/dl of blood serum)			
	P	N	LC	HC
556	1.060	1.020	1.110	1.030
559	1.160	1.050	1.140	1.050
921	1.040	1.040	1.050	1.050
937	1.060	1.160	–	1.030
940	1.140	1.180	1.040	1.040
957	1.110	1.160	1.020	1.050
Mean	1.095	1.102	1.072	1.042
	P	N	LH	HH
558	1.110	1.050	1.000	1.110
931	1.000	1.040	1.180	1.050
932	1.060	1.140	1.000	1.030
956	1.060	1.160	–	1.050
963	1.060	1.180	1.050	1.060
2219	1.020	0.970	1.060	1.040
Mean	1.052	1.090	1.058	1.057

[a] Diet code: P = Pre-study without basal diet; N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.

[b] Total mean value for all subjects in both studies = 1.02. Standard deviation = 0.21.

Although not significant, a trend toward increased fecal magnesium excretion occurred when 20g of cellulose and 10g of hemicellulose supplements were given in comparison to the other experimental diets. Fecal magnesium excretion was lowest with no fiber supplementation and highest when 10g of hemicellulose supplementation was given.

Urinary excretion of calcium and magnesium in response to the various experimental variables are given in Tables 8 and 9.

There was a significantly higher urinary calcium excretion ($P < 0.050$) when the subjects were fed 10g of cellulose and 20g of hemicellulose supplements in comparison to the other fiber supplements or no supplements (Table 8). Mean urinary calcium excretion on the diet with no fiber supplement, 10g and 20g cellulose supplement were 75.14, 83.14 and 76.33 mg/day, respectively. Mean urinary calcium excretion with no fiber supplement, 10g and 20g hemicellulose supplement, were 70.48, 66.64 and 87.58 mg/day, respectively.

Mean urinary magnesium excretion on the diet with no supplement, 10g cellulose, or 20g cellulose were 136.12, 136.57 and 140.14 mg/day, respectively (Table 9). There was a trend toward increased magnesium excretion with the cellulose supplement. There was a significantly lower urinary magnesium excretion during the hemicellulose supplementation, with the greatest effect with 20g hemicellulose ($P < 0.010$). The mean urinary magnesium excretion values during no fiber supplement, 10g and 20g hemicellulose supplement were 114.91, 120.66 and 102.36 mg/day, respectively.

Fiber supplementation had no apparent effect on calcium serum levels of subjects while receiving the experimental variables as shown in Table 10. No statistically significant differences were found as a result of variation in dietary fiber. Mean calcium serum values during the pre-study and with no supplement, 10g cellulose or 20g cellulose were 1.097, 1.102, 1.072 and 1.065 mcg/dl, respectively. Mean calcium values during the pre-study and on no fiber supplement, 10g hemicellulose and 20g hemicellulose were 1.052, 1.090, 1.058 and 1.057 mcg/dl, respectively.

Magnesium serum values were statistically different ($P < 0.001$) during the 10g cellulose and 10g hemicellulose supplement in comparison to the pre-study, no fiber or 20g cellulose and 20g hemicellulose supplement. Mean magnesium serum values during the prestudy, no fiber, 10g and 20g cellulose supplement were 2.045, 1.938, 1.906 and 1.940 mcg/dl, respectively. Mean magnesium serum values during the pre-study, no fiber, 10g and 20g hemicellulose supplement were 1.995, 1.976, 1.879 and 1.922 mcg/dl, respectively (Table 11).

Dietary intakes of calcium and magnesium for subjects are given in Table 3. These together with the urinary and fecal excretions were used to calculate calcium and magnesium balances of subjects as shown in Tables 12 and 13.

Table 11—Effect of dietary fiber on serum magnesium

Subject no.	Blood serum magnesium values per period[a,b] (mcg/dl of blood serum)			
	P	N	LC	HC
556	2.055	1.875	1.970	1.870
559	2.095	1.895	2.020	1.900
921	1.935	1.745	1.750	2.010
937	2.140	2.135	–	2.000
940	2.020	2.005	1.910	1.950
957	2.025	1.975	1.880	1.910
Mean[b,c]	2.045a	1.938a	1.906b	1.940a
	P	N	LH	HH
558	1.982	1.900	1.750	1.900
931	2.020	1.935	2.130	2.020
932	2.020	2.095	1.760	1.905
956	2.035	2.130	–	2.035
963	1.900	1.870	1.650	1.650
2219	2.015	1.925	2.105	2.020
Mean[b,d]	1.995a	1.976a	1.879b	1.922a

[a] Diet code: P = Pre-study without basal diet; N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.

[b] Mean values with different superscripts are significantly different from one another ($P < 0.010$) according to ranking of effect.

[c] Total mean values for subjects on cellulose supplementation = 2.20. Standard deviation = 0.66.

[d] Total mean values for subjects on hemicellulose supplementation = 1.87. Standard deviation = 0.40.

Table 12—Effect of dietary fiber on calcium balance

Subject no.	Apparent mean calcium retention (mg/day) while receiving diet[a,b]		
	N	LC	HC
556	841.81	953.60	534.83
559	504.27	419.13	596.30
921	709.48	748.74	661.86
937	909.26	517.65	868.66
940	696.54	695.07	592.35
957	321.24	802.80	443.04
Mean	663.77	689.50	616.17
	N	LH	HH
558	490.04	395.11	631.47
931	782.88	825.73	819.20
932	918.81	776.64	568.30
956	668.03	188.84	1033.69
963	951.26	37.86	624.09
2219	569.14	532.97	377.39
Mean	730.03	459.52	675.69

[a] Diet code: N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.

[b] Total mean value for all subjects in both studies = 639.11. Standard deviation = 222.98.

Table 13—Effect of dietary fiber on magnesium balance

Subject no.	Apparent mean magnesium retention (mg/day) while receiving diet[a,b]		
		LC	HC
556	−10.64	+ 58.08	− 16.37
559	−64.70	−138.73	− 55.87
921	−20.35	− 32.13	− 82.49
937	+84.66	− 21.54	−120.50
940	−39.91	− 47.64	− 81.78
957	−79.86	+ 1.53	− 87.01
Mean	−21.80	− 30.07	− 74.00
	N	LH	HH
558	−74.26	− 82.75	− 17.04
931	+ 1.12	+167.11	+ 23.18
932	+38.13	− 10.21	− 53.47
956	+39.11	−104.35	+101.87
963	+51.82	−253.47	− 73.18
2219	− 8.95	− 68.70	− 69.06
Mean	+ 7.83	− 58.73	− 14.63

[a] Diet code: N = Basal diet without fiber supplement; LC = 10g cellulose; HC = 20g cellulose; LH = 10g hemicellulose; HH = 20g hemicellulose.

[b] Total mean value for all subjects in both studies = −32.19. Standard deviation = 74.29.

The order in which the subjects received the supplements seemingly had no effect upon calcium or magnesium retention, indicating that the subjects were fully adjusted to the diets before beginning the experimental periods. All subjects were in positive calcium balance during the experimental periods. All subjects were in negative magnesium balance during 20g cellulose supplement. All but one subject was in negative magnesium balance during no fiber supplement. Mean magnesium balances were negative, however, throughout the cellulose experimental period. All subjects except one were in negative magnesium balance during 10g hemicellulose supplement. Mean magnesium balances were positive during no fiber supplement and negative during 10 and 20g hemicellulose supplement. A trend toward increased negative magnesium balance occurred during cellulose supplementation, with a greater increase occurring with 20g cellulose supplement. The greatest increase in negative magnesium balance occurred at 10g hemicellulose supplement.

In conclusion, the effect of fiber supplementation of diets of adolescents on calcium and magnesium has been investigated. In spite of the use of short-term periods, some alteration in mineral utilization was noted: 10g cellulose and 20g hemicellulose supplement significantly increased urinary calcium excretion; 20g hemicellulose significantly lowered urinary magnesium excretion. Magnesium serum values were significantly lowered during 10g cellulose and 10g hemicellulose supplement. Results of this project indicate that hemicellulose and cellulose supplements to diets inhibit absorption of calcium and magnesium. This should not be construed that fiber is harmful or should be avoided. Rather, the need for watchfulness in intake of all nutrients should be considered.

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COOLING PACIFIC SHRIMP (Pandalus jordani) WITH SPRAYED REFRIGERATED SEAWATER

EDWARD KOLBE

ABSTRACT

The study's objective was to describe how various factors affect on-board spray cooling of Pacific shrimp. Laboratory samples were sprayed with water to determine the variation of cooling rate with spray rate and depth of sample. Other experiments showed how flow patterns resulting from poorly distributed spray water varied with spray rate and with depth and density of the sample. Results of the study were used to conclude that a spray rate on the order of 0.4 gpm/ft^2 is needed for typical high catch rate conditions.

INTRODUCTION

SHRIMP STOWED onboard Pacific northwest trawlers is traditionally refrigerated with fresh water ice. A recent innovation replaces ice with mechanically refrigerated sea water which is sprayed over the top surface of the bulk-loaded shrimp. Potential advantages include a reduction of expense and effort associated with using ice, and a potential increase in quality and holding time. However, the few vessels using this system have occasionally encountered problems of poor quality which appear to relate to inadequate cooling by the recirculating refrigerated sea water. Some of the spray nozzles commonly used are not capable of delivering a uniform spray pattern (Kolbe, 1979). Rational design recommendations required a better understanding of (1) how the cooling rate of typically loaded shrimp varied with spray rate, and (2) how readily the cooling water from a concentrated spray would spread horizontally as it flowed through typically loaded shrimp.

Other researchers have described the cooling of bulk-loaded food products by various fluids (Bakker-Arkema and Bickert, 1966; Baird and Gaffney, 1976; Hayakawa and Timbers, 1976; Smith and Bennett, 1965). However, it was felt that such models should not be applied to the spray-cooled shrimp case without a confirming study.

The intent of the study reported here was to:

(1) Experimentally determine how spray characteristics affect on-board spray cooling of Pacific shrimp;
(2) Formulate a recommendation on the rate of spray required under some typical operating conditions.

MATERIALS & METHODS

Flow pattern experiment design

The two dimensional horizontal spread of water through a shrimp sample having a concentrated spray at its surface, was observed in samples of various depths. A sample box having a width of 35½ in. and thickness of 5½ in. had a floor made of ¼ in. metal screen. Five flow rates, ranging from an equivalent of 0.14 gpm/ft^2 to 4.0 gpm/ft^2, were concentrated at the center of the upper sample surface. Twelve pans on a sliding tray collected water dripping through the porous floor of the sample box. The 3 in.-wide pans collected all water leaving the sample.

The experiment included two series of tests. In the first series, shrimp were lightly packed in successive 8 in. layers. A range of flow rates, concentrated at the center line, were introduced over each layer. For each run, steady state flow was established before the collector pans were installed for a short collection time interval.

In the second series of tests, a high density sample was created be repeatedly lifting and dropping the sample box. This allowed a comparison of flow patterns in samples of two widely differing densities.

Spray cooling experiment design

Two laboratory samples were prepared by dropping shrimp into a 13½ in. diameter cylinder having a porous base made of ¼ in. screen. Depth and density, typical of newly loaded shrimp in the hold were:

Sample	Depth	Density
1	24 in.	51 lbm/ft^3
2	19½ in.	47 lbm/ft^3

Shrimp for both samples, recently unloaded at a local processing plant, were about 2 days old.

Thermister probes placed at top, bottom, and two intermediate levels of the sample measured water and internal shrimp temperature changes resulting from a step change in the sprayed water temperature. Constant temperature cold water came from a large melting ice bath.

The probes, manufactured by the YSI Co. (Yellow Springs Instrument), were of three types:

(a) General Purpose, model 401; 3/16 in. diameter; 7 sec time constant;
(b) Hypodermic, model 507; 0.048 in. diameter; 0.2 sec time constant;
(c) Tissue Implantation, model 511; 0.024 in. diameter; 0.2 sec time constant.

During the transient tests, temperatures changed slowly enough to allow manual recording using a switch box and telethermometer (YSI model 42). Although absolute accuracy of the system is given as ± 1.0°F, the advertised "reproducibility" of ± 0.1°F would seem to assure a sufficiently accurate measurement of the temperature *changes* important in these experiments.

Single commercial spray heads (Spraying Systems Co.) distributed water over the surface of the shrimp for each experiment. A pressure gauge just upstream of the spray head allowed an accurate measurement of the total flow rate. After each run, spray patterns were measured with a series of 13 collector bottles.

RESULTS & DISCUSSION

Flow patterns

Table 1 shows typical results. Spray rate corresponding to an averaged distribution of 0.60 gpm/ft^2 and shrimp packing of 44 lbm/ft^3 is typical of what might be expected for recently loaded shrimp in the hold. The resulting pattern is similar to that found for two other rates in the range 0.14–0.60 gpm/ft^2: A narrow distribution which establishes itself in the first few inches of penetration and which spreads very little as it flows through the 24-in. deep sample. For the same sample density but with a substantially higher averaged flow rate (2.24 gpm/ft^2) the pattern is wider, again established early, with slightly more spreading through the sample. This result is similar to one higher rate (4.0 gpm/ft^2) that was considered.

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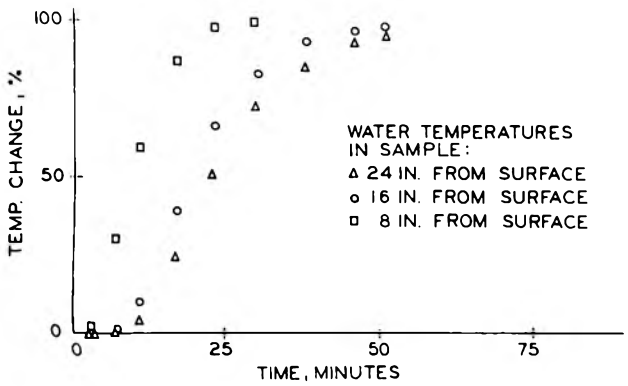

Fig. 1—Temperature response of sample 1 to an averaged spray density of 0.29 gpm/ft².

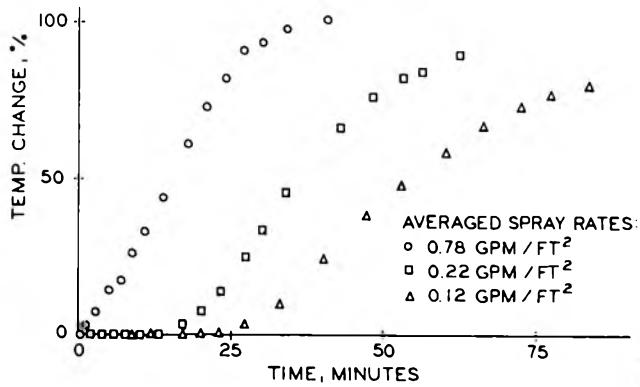

Fig. 2—Exit water temperatures in sample 2 for three spray densities.

The last two lines of Table 1 show the results of increasing sample density from a nominal value of 44 lbm/ft^3 to 60 lbm/ft^3: a substantial increase in the width of the pattern. These results are also typical of the other observed flow rates in a similar range.

Several repeated runs indicated that results were reproducible. No sample flooding occurred for any of the spray rates and sample densities observed.

The results of Table 1 show that for low density samples typical of newly caught shrimp, surface spray concentrated on a line will be likely to spread only 3–4 in. on either side as it penetrates a 24-in. deep sample. Thus the importance of a uniform surface spray is indicated. No channeling of flow was observed for the conditions tested. However, results indicate that as density on the boat increases (due perhaps to vibration and load pressure), increased flow resistance will lead to lateral spread of the cooling water.

Cooling rates

Experiments determined sample thermal response to four different spray rates. Figure 1 gives temperatures at three levels along the center line of sample 1. The data are similar in form to those found for other flow rates which are represented by the water exit temperatures shown in Figure 2.

Because properties of the shrimp and sprayed water are assumed independent of temperature for the ranges considered, only the temperature difference relative to that of the inlet spray is significant. Response is plotted in terms of the dimensionless variable

$$T = \frac{T_w - T_i}{T_s - T_i}$$

where T_w = water temperature at time t; T_s = initial shrimp temperature; and T_i = spray inlet temperature. Actual differences between spray inlet temperature and initial shrimp temperature were generally greater than 20°F.

The spray patterns found for each of the four cooling rate measurements are shown in Figure 3. Each pattern has a sharp peak in the center. Although this peak covers a minor fraction of the surface area, the flow data of Table 1

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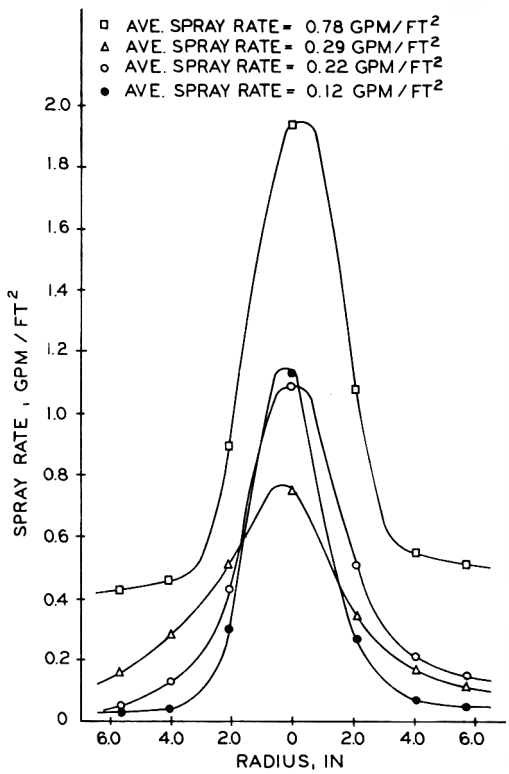

Fig. 3—Spray patterns corresponding to the thermal response measurements represented by Fig. 1 and 2.

Table 1—Distribution of flow (expressed in percent of total) falling from sample

Avg spray density, (gpm/ft²)	Avg shrimp sample density, (lbm/ft³)	Depth of sample, (in.)	Distance (inches) of collector pan center line from center line of sample											
			16.5	13.5	10.5	7.5	4.5	1.5	1.5	4.5	7.5	10.5	13.5	16.5
		8	–	–	–	–	7	55	34	4	–	–	–	–
0.60	44	16	–	–	–	–	10	51	33	5	1	–	–	–
		24	–	–	–	–	10	48	32	8	2	–	–	–
		8	–	–	–	1	13	37	37	13	–	–	–	–
2.24	44	16	–	–	–	1	14	34	35	14	3	–	–	–
		24	–	–	–	2	20	29	30	13	6	–	–	–
0.60	60	24	–	–	4	8	18	18	19	16	11	4	–	–
2.24	60	24	6	9	11	6	9	8	9	7	9	7	8	11

REFRIGERATED SEAWATER SPRAY SYSTEM MODEL FOR SHRIMP VESSELS

EDWARD KOLBE

ABSTRACT

A mathematical simulation of refrigerated seawater spray cooling of shrimp onboard a vessel is described. The simulation describes compressor and chiller performance and heat transfer between shrimp and recirculating spray water. It also accounts for conduction through the hull and for pump energy. A series of temperature measurements onboard vessels verified the model which can be used to evaluate the sensitivity of cooling rates to changes in equipment or operating procedures.

INTRODUCTION

SHRIMP FISHERMEN in the Pacific northwest have recently experimented with refrigerated seawater (RSW) spray as a substitute for ice (Kolbe and Lee, 1978). Figure 1 is a diagram of the system's simplest form: several hundred gallons of seawater, chilled to a temperature close to that of melting ice, recirculates continually through sprayers mounted over the bulk-loaded shrimp. RSW spray has the potential of holding seafoods at a prescribed temperature for a longer period of time than when ice is used. Some systems have worked well, but experience has shown that special design and operational procedures are necessary to ensure consistent performance. In particular, the refrigeration machinery, RSW pump, sprayers, and sump must be properly sized to allow a rate of cooling adequate for the rate of loading. During good fishing periods off Oregon, this rate can result in 5000 lbm loaded at intervals of 1½–2 hr. The model described below is a design aid for proper selection of equipment and for evaluating the effect of changes in capacities or operating schedules.

Model development

The transient process of cooling newly loaded shrimp is modelled by a series of steady state calculations at successive time increments. As illustrated in Figure 1, there are four primary processes:

(1) transfer of heat from shrimp to RSW;
(2) addition of heat to RSW due to pumping and conduction through the fish hold walls;
(3) extraction of heat from RSW by the chiller (heat exchanger);
(4) heat extraction by compressor/condenser.

Experiments describing the transfer of heat from newly loaded shrimp to spray water have been reported by the author (Kolbe, 1979a). This transfer can be modelled using explicit finite differencing equations for one dimensional flow systems, as described by Dusinberre (1961). Convection is assumed to vary with flow rate raised to the power 0.8, as in the case of turbulent flow in closed channels (Kreith, 1958).

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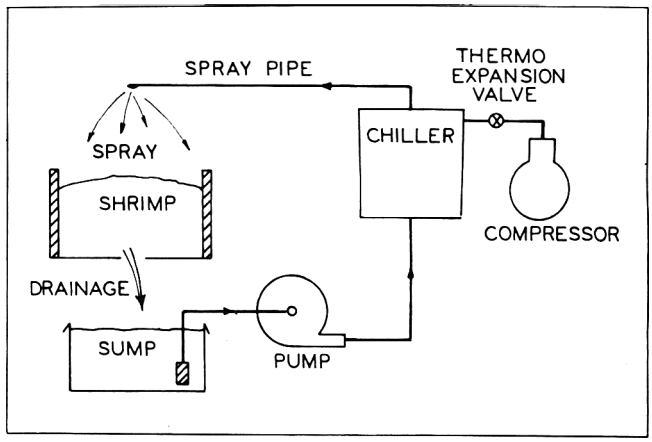

Fig. 1—Closed system for spray cooling of shrimp with refrigerated seawater.

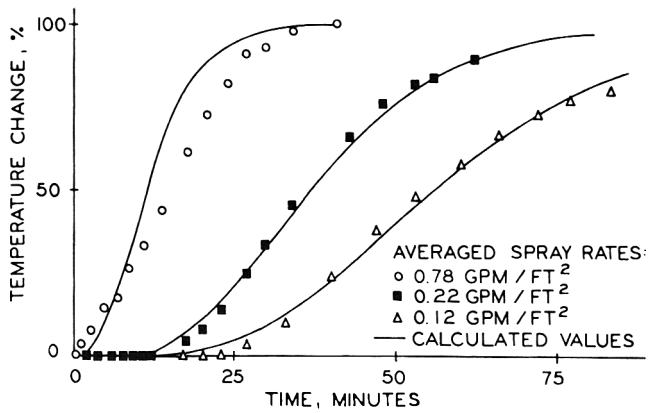

Fig. 2—Finite differencing calculations of spray water leaving a 20-inch layer of shrimp, compared with data reported by Kolbe, (1979a).

The model was used to calculate temperature of water leaving the shrimp mass for flow rates somewhat higher than averaged values measured in tests. This is expected because uneven flow patterns in the tests produced higher-than-average flow rates over the center column, where temperatures were measured. Figure 2 shows a comparison of data and calculated temperature response. The slope of the response curves and the calculated temperature difference between shrimp and water was found to vary with the convection terms. Trials showed that a convection equation producing a good fit with data is:

$$(H)(A_v) = 1380\ (w)^{.8}$$

where H = convection coefficient, Btu/hrft^2 °F; A_v = Heat transfer area per unit volume of shrimp, 1/ft; and w = flow rate, gpm/ft^2. The numerical calculations considered 20 subdivisions or layers of shrimp, and a 0.1-min time step.

The fit of calculations to data (Fig. 2) shows that the

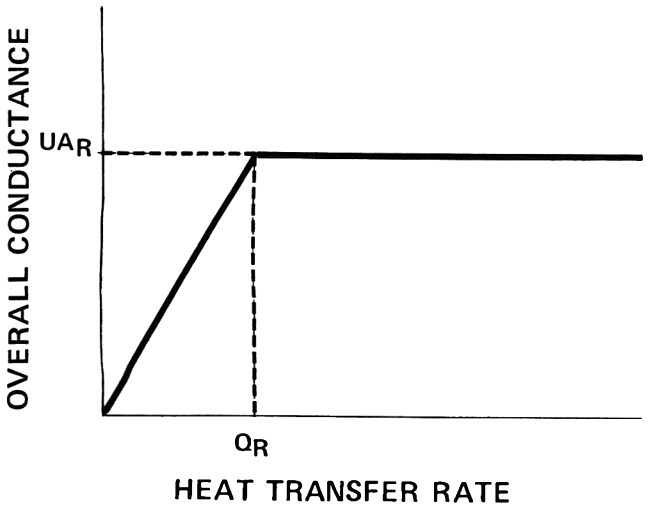

Fig. 3—Model of chiller performance used in the simulation.

model is able to adequately describe heat transfer within the shrimp mass for conditions similar to those which are expected aboard ship.

Heat transfer through the hull walls is described by the equation,

$$Q_L = (U)(A)(\Delta T)$$

where U = overall heat transfer coefficient which depends primarily upon insulation characteristics; A = area of fish hold walls or engine room bulkhead; and ΔT = instantaneous temperature difference between spray water and ocean or engine room.

Pumping heat is the equivalent of water flow energy. It is calculated from the equation:

$$Q_p = (W)(\Delta P)$$

where W = total flow rate through all spray nozzles; and ΔP = pressure difference between pump discharge and atmosphere. Heat exchange in the chiller is predicted by the equation:

$$Q_{HX} = (UA)(\Delta T)$$
$$= UA\left[\frac{T_1 + T_2}{2}\right] - SST$$

where UA = overall heat transfer conductance; T_1 = water temperature entering the chiller; T_2 = water temperature leaving the chiller; and SST = saturated suction temperature of the boiling refrigerant.

The chiller performance is assumed to vary according to Figure 3. As long as heat exchange rate is equal to or greater than the rated value Q_R, the rated conductance UA_R will be used. It is assumed that this coefficient results from the rated water flow and maximum refrigerant flow rates. However, as the load and thus heat transfer rate decreases, the thermo expansion valve which meters refrigerant flowing into the chiller will reduce the amount of refrigerant flowing through the tubing. This will reduce the convection heat transfer coefficient in the refrigerant. The approximation of a linear reduction of UA with Q (as in Fig. 3) is based on the nature of the boiling heat transfer coefficient for refrigerant (Anon. 1972).

The performance of a given compressor is commonly represented by a map, or set of curves giving capacity as a function of saturated suction temperature (in the chiller). Each curve on a map represents a different saturated discharge temperature (in the condenser). Each map corresponds to a given speed and refrigerant. Any map can represent performance at a reduced speed if the capacities are all multiplied by a reduction constant. Note also that a given compressor's performance will depend on the refrigerant used. For example higher capacity will result from the use of Refrigerant 22 (Chlorodifluoromethane) than from use of Refrigerant 12 (Dichlorodifluormethane). This numerical simulation uses maps in tabular form. Interpolation between values is by quadratic equation and is accomplished using Newton's forward difference formula (Sokolnikoff and Redheffer, 1958). The interpolation allows the saturated discharge temperature to vary. However, the present program has no routine to simulate condenser performance, and a constant value of saturated discharge temperature is used.

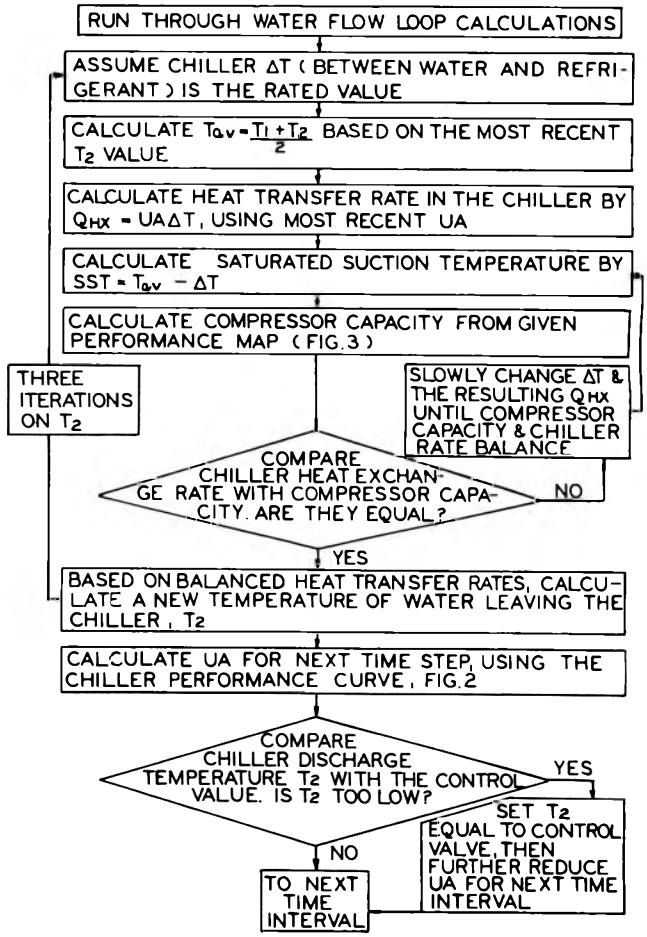

Fig. 4—Iterative logic representing control of compressor.

During each time increment, heat balance equations are solved at various stations around the water flow loop (Fig. 1). It begins at the chiller discharge, followed by temperature increases due to heat transfer through the hull and to pumping energy. It is assumed that the water being pumped flows continually through all of the spray heads. At the beginning of a fishing trip, much of the recirculating water sprays into empty bins, thus picking up no additional heat. A minor fraction of the water sprays onto newly loaded shrimp, picking up heat. The water leaving the bins mixes in the sump, then flows to the chiller. Heat transfer in the chiller must equal the capacity of the compressor after control by the thermo expansion valve. This control and balance is simulated by iterative loops in the calculation as shown in Figure 4.

A computer program solves the sequence of equations

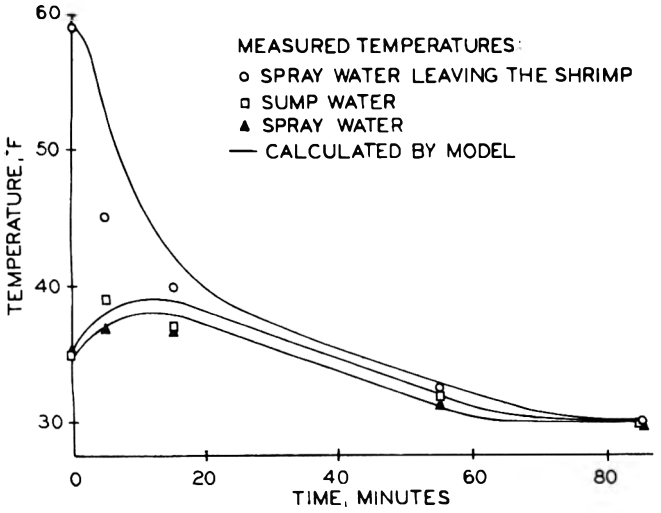

Fig. 5—Comparison of calculated refrigerated seawater temperatures with measured values on boat 1.

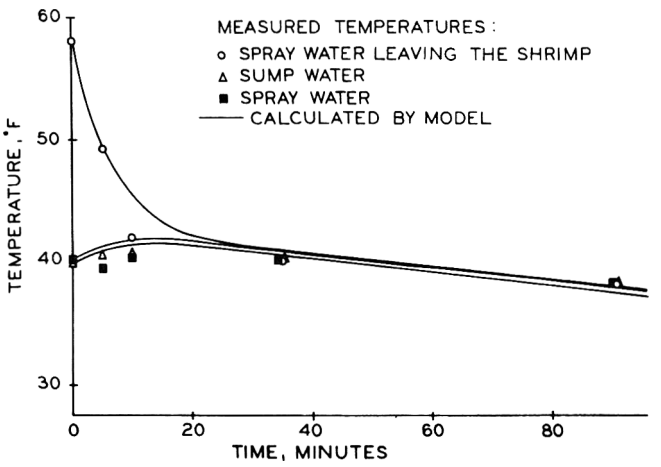

Fig. 6—Comparison of calculated refrigerated seawater temperatures with measured values on boat 2.

making up the simulation. The parameters that need to be entered are:

- compressor map (tables of capacity versus saturated suction temperature for different values of saturated discharge temperature)
- compressor capacity reduction factor (for reduced speed operation, and for superheat, subcool values less than those rated)
- condenser (saturated discharge) temperature
- initial RSW temperature
- initial shrimp temperature
- ambient seawater temperature
- engine room temperature
- heat transfer coefficient for conduction through the hull
- area for heat leakage into fish hold (the program assumes 10% of this to be engine room bulkhead)
- rated UA and capacity of chiller
- total RSW pumping rate
- total mass of seawater in sump
- fraction of spray over shrimp
- sprayed area over shrimp
- pressure out of pump
- weight of warm shrimp
- time increment
- final spray temperature (value used by control loop as described in Fig. 4)

EXPERIMENTAL

TEMPERATURES were recorded on two boats equipped with refrigerated seawater spray systems. Over the duration of the 3-day trips, six YSI (Yellow Springs Instrument Co., Yellow Springs, OH) model 401 thermister probes monitored RSW temperatures at chiller discharge, in the sump, and within the shrimp mass. Leads having a length of 65 ft. connected through a switchbox to a telethermometer; temperatures were manually recorded at appropriate time intervals.

RESULTS & DISCUSSION

TEMPERATURE DATA over two trips lasting 3 days gave profiles for given sets of sensor locations and a range of operating and loading conditions. Two subsets of data were chosen to verify the simulation; both occurred during early loading and initial cool-down. These are shown in Figures 5 and 6. The conditions of each case appear below:

Boat 1

Given:
Compressor displacement: 39.3 in^3
Compressor speed: 1025 rpm
Refrigerant: R-12
Chiller: Shell-and-tube type
Initial shrimp and ambient sea temperatures: 59°F
Initial sump water temperature: 35°F

Calculated parameters:
Outer fish hold wall area: 890 ft^2
Coefficient of heat transfer through hold walls: 0.05 $Btu/hrft^2°F$ (using procedures and data found in Anon. 1972)
Sprayed area of shrimp: 27 ft^2

Estimated parameters:
Rated transfer rate of chiller: 40,000 Btu/hr @ 10°F ΔT
Shrimp weight: 1400 lbm
Total spray rate: 120 gpm
Fraction of spray distributed over shrimp: 0.14
Thermal mass of sump water:
equivalent to 400 gal. of seawater
Engine room temperature: 90°F
Spray pressure: 19 psi
Refrigerant superheat: 15°F
Refrigerant subcool: 0°F
Saturated discharge temperature in condenser: 100°F

Boat 2

Given:
Compressor displacement: 25.9 in^3
Compressor speed: 1380 rpm
Refrigerant: R-12
Chiller type: Tube-and-box
Initial shrimp and ambient sea temperatures: 58°F
Initial sump water temperature: 40°F
Saturated discharge temperature in condenser: 107°F

Calculated parameters
Coefficient of heat transfer through hold walls: 0.04 $Btu/hrft^2°F$ (using procedures and data found in Anon. 1972)
Rated transfer rate of chiller: 11,000 Btu/hr @ 10°F ΔT
Sprayed area of shrimp: 49 ft^2

```
COMPRESSOR = CARRIER 5F4D 39.8 CFM AT 1750
SPEED =  1025.0 RPM      REFRIGERANT = R - 12.      CONDENSER TEMP. = 100.0 F    (CONSTANT)
CAPACITY REDUCTION FACTOR =  .600
15. F SUPERHEAT        0. F SUBCOOL
CAPACITY TABLE, TONS OF REFRIGERATION

        SDT    80.    90.   100.   105.
   SST
   -10.       3.70   3.50   3.20   3.00
    10.       6.60   6.20   5.70   5.50
    30.      10.60  10.00   9.40   9.00


INITIAL RSW TEMP. =   35.0      INITIAL SHRIMP TEMP. =   59.0
AMBIENT SEAWATER TEMP. =   59.0      ENGINE ROOM TEMP. =   90.0
HEAT LEAKAGE TRANSFER COEFFICIENT =  .05 BTU/HR FT2 F      HEAT LEAKAGE TRANSFER AREA =  890. FT2
RATED UA VALUE FOR CHILLER =     4000.BTU/HR F
RATED Q FOR CHILLER =    40000.BTU/HR


TOTAL RSW PUMPING RATE = 120. GPM      TOTAL VOLUME OF RSW =  400. GAL
FRACTION OF SPRAY OVER SHRIMP =  .140      SPRAYED SURFACE AREA =  27. FT2
PRESSURE OUT OF PUMP =19.0 PSI       WEIGHT OF SHRIMP =  1400. LBM


TIME INCREMENT =  .10 MIN      TIME BETWEEN REPORTS =  5.00 MIN      TIME LIMIT =    85. MIN
FINAL SPRAY TEMPERATURE =  30.00 F


QP =  56.37 BTU/MIN


 ELAPSED
TIME, MIN      T2       T1       QL       QS       SST       QC      UA      DELTHX   TSAVE    TSRMP    TS

     5.0     37.0     38.0     18.7   2172.5     23.2     960.9   4000.0     14.3     49.6     55.9     53.1
    10.0     36.0     39.0     17.9   1109.2     24.0     979.2   4000.0     14.5     49.4     49.4     46.3
    15.0     37.9     38.9     18.0    540.7     23.9     976.2   4000.0     14.5     48.3     44.0     41.9
    20.0     37.1     38.1     18.5    319.4     23.3     963.7   4000.0     14.3     38.8     40.8     39.6
    25.0     36.3     37.2     19.1    255.8     22.7     948.3   4000.0     14.1     37.7     38.9     38.3
    30.0     35.4     36.3     19.8    243.6     22.0     932.6   4000.0     13.9     36.8     37.8     37.3
    35.0     34.5     35.4     20.5    241.7     21.3     917.7   4000.0     13.6     35.9     36.8     36.4
    40.0     33.6     34.5     21.1    239.7     20.7     902.8   4000.0     13.4     35.1     35.9     35.5
    45.0     32.8     33.7     21.7    236.4     20.0     888.4   4000.0     13.2     34.2     35.0     34.6
    50.0     31.9     32.8     22.4    232.5     19.4     874.4   4000.0     13.0     33.3     34.2     33.8
```

Fig. 7—Sample output corresponding to simulation of cooldown period on boat 1.

Estimated parameters

Shrimp weight: 1500 lbm
Thermal mass of sump water: equivalent to 900 gal. of seawater
Total spray rate: 200 gpm
Fraction of spray distributed over shrimp: 0.12
Outer fish hold wall area: 1400 ft^2
Engine room temperature: 80°F
Spray pressure: 6 psi
Refrigerant superheat: 15°F
Refrigerant subcool: 0°F

Calculations of cooling rates for the two boats used several estimated values. The weight of shrimp being cooled and the volume of water in the sump were estimated by the fishing vessel captains. The water volume was subsequently increased to account for the effective thermal mass of structural materials, heat exchanger metal and, on boat 2, 2000 lb of previously loaded shrimp. Spray rates were estimated from known pipe sizes, heat exchanger pressure drop characteristics, spray head distribution, and typical pump performance. Heat exchanger performance on boat 1 resulted from first estimating performance based on advertised characteristics, then compensating for badly eroded internal baffle plates that would tend to impair performance.

Several factors may have caused the data to vary from the calculated pattern: nonuniform depth of shrimp; use on both boats of spray heads that tend to produce nonuniform spray patterns (Kolbe, 1979b); poor mixing of water in the sump. Nevertheless, the data and estimated parameters for two boats having substantially different refrigerating power yielded verification of the model.

A sample output is shown in Figure 7 which corresponds to the model prediction of cooling on Boat 1 (Fig. 5). The program first prints out all input data, then prints several calculated temperatures and heat flow rates at preselected time increments.

The model predicts the "worst case" i.e. cooling rate of one batch of shrimp which is put into an essentially empty hold. Because calculations of heat transfer between shrimp and spray water are handled by a computer subroutine, it would be a simple matter to modify the program to consider two batches of shrimp, one on top of the other. Heat transfer in the chiller is also handled by subroutines. These could be used to modify the program to account for variable condenser temperature.

The time increment used in all cases was 0.1 min. This FORTRAN IV program operates on a Control Data Corp. Cyber 73 computer. A 100-min simulation takes about 17 sec of CPU time.

Application

The development of a simulation model allows one to better identify the consequences of using undersized or mismatched equipment. As shown by Kolbe and Lee (1978), it can be used to evaluate the sensitivity of cooling rate to the use of an undersized compressor, heat exchanger or circulating pump, and to show the effects of inadequate precooling of seawater in the sump.

As an example of its application, the cooling of shrimp, subject to varying spray densities, is described in Figure 8. The model uses the following additional parameters:

compressor: 39.3 cu inch displacement, rotating 1200 rpm, using refrigerant-22; superheat and subcooling were both 15°F
chiller capacity: 6 tons at ΔT = 10°F
weight of shrimp: 5000 lb
water flow rate: 240 gpm
sump water: 500 gal. precooled to 31°F.

Figure 8 shows calculations of shrimp temperature occurring at the bottom layers of the pile. (Program output allows monitoring of most calculated shrimp and water temperatures.) The results of this example indicate that with the refrigeration system design specified, between 0.5 and 0.7 gpm/ft^2 are required to bring all shrimp to 32°F within 1½ hours.

CONCLUSIONS

A MODEL simulating onboard refrigeration of spray-cooled

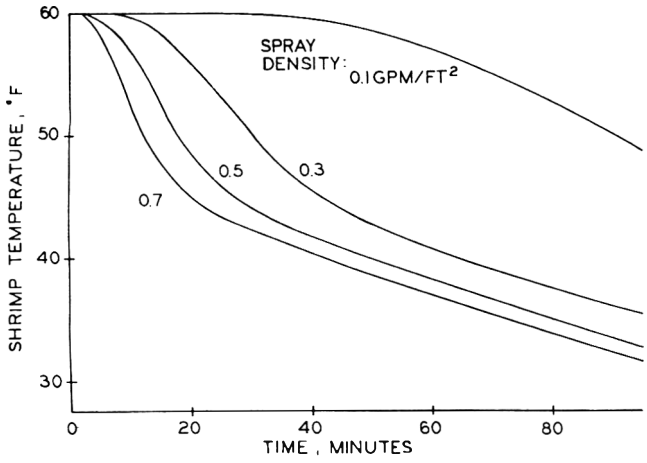

Fig. 8—Example application of model, showing how the calculated bottom shrimp cooling rates vary with spray density.

shrimp has been developed. Using measurements and estimated parameters aboard two shrimp vessels, it has been shown to work well for two cases of different refrigerating power.

NOMENCLATURE

A Heat transfer area of fish hold walls
A_v Heat transfer area per unit volume of shrimp
H Convection heat transfer coefficient between shrimp and flowing water
Q_{HX} Heat transfer rate in the chiller
Q_L Heat transfer rate through the fish hold walls
Q_P Heat production due to pumping
Q_R Rated heat transfer capacity of the chiller
SST Saturated suction temperature
T_1 Water temperature entering the chiller
T_2 Water temperature leaving the chiller
U Overall heat transfer coefficient of fish hold walls
UA Overall heat transfer conductance in the chiller
UA_R Rated conductance of chiller
w Flow rate of spray through shrimp
W Total water flow rate being pumped
ΔP Pressure difference between pump discharge and atmosphere
ΔT Temperature difference

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COOLING PACIFIC SHRIMP . . . From page 1419

would suggest that this excess surface spray would distribute itself only over a 6–8 in. diameter area as it flows through the sample. Thus the central column of shrimp along which temperatures of Figures 1 and 2 were measured, is subjected to an effective spray rate higher than the average. This was confirmed by temperature probes in sample 1 which were distributed over the horizontal cross section at several levels. The response of probes at the outer edges was slower than that of probes placed at the center.

The temperatures plotted in Figures 1 and 2 are water temperatures measured by probes lying in the voids between shrimp. Immediately adjacent to these probes in several locations, additional probes measured the internal temperature of shrimp. For all cases run, the maximum time lag between measured internal shrimp temperature and measured adjacent water temperature was 7 min. Actual times in each case depended on location and spray rate. Lag at the lower sample levels was greater than that above, presumably because of the spreading of flow. The higher flow density at the top of the sample would have produced a higher heat transfer coefficient resulting in a faster response of internal shrimp temperatures.

Repeatability at a given level was tested in sample 2. Two water temperature probes with a horizontal separation of about 1 in. measured response at a level 13½ in. from the surface. In the three cases run on sample 2 (Fig. 2) the maximum difference between readings on these two probes, corresponded to a time lag of 3 min and coincided with the maximum gradient of the response curve.

A desirable cool-down period aboard the shrimp vessel is 90 min, the expected time between loadings during periods of heavy catch-rates. As indicated by the flow distribution data of Table 1, we can assume that the spray pattern peaks (Fig. 3) will distribute uniformly over a circle having a diameter close to 8 in. The resulting flow densities can be compared to the central column temperature responses of Figures 1, 2. This leads to the conclusion that a constant temperature uniform spray rate on the order of 0.4 gpm/ft^2 will cool a 24-in. depth of warm shrimp in 90 min.

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DETERMINATION OF OPTIMUM PARAMETERS FOR PROTEIN ISOLATION FROM KRILL (Euphasia superba) WASTE PRODUCTS

CLAUDIO R. ROMO and CHRISTOPHER G. ANDERSON

ABSTRACT

Waste material obtained from the mechanical peeling of Antarctic Krill (*Euphasia superba*) was studied in order to evaluate its potential use as a raw material for the preparation of protein isolates. A dye-binding method for determination of soluble protein was applied to krill protein and found to correlate well with protein values determined by standard Kjeldahl analysis. The effect of the following parameters on the solubility of protein was studied: pH, ionic strength, solid-to-solvent extraction ratio and time of extraction. Recovery of protein was studied in terms of influence of pH, simultaneous effect of pH and heat, and influence of coagulation time. Optimum conditions for extraction and precipitation of protein were therefore established.

INTRODUCTION

IT IS EVIDENT that production of proteins, at a world level, must increase substantially in order to meet the nutritional needs of an increasing world population more effectively. Although notable progress has been made to increase production of vegetable proteins with high nutritive values by employing modern technology, the increase in production of animal protein still remains relatively expensive and inefficient due to among other things the low conversion ratio of plant protein to animal protein.

One approach to solving the problem of a lack of animal protein is to utilize untapped reserves of fish protein found in the world's oceans in the form of species of fish that up to now have offered little attraction for human consumption due to their lack of appeal to humans, difficult accessibility, etc. The rate of increase in exploitation of conventional fish resources must level off or diminish in the near future because of heavy exploitation of these resources in the past. For example, during the past few decades the quantity of fish caught throughout the world doubled every 10 years reaching a total of nearly 70 million tons in 1970. Estimates of the total potential of conventional fisheries indicate that the catch could reach 100 million tons annually by the turn of the century. This estimated quantity represents slightly less than 50% of the world's available supply of conventional fisheries.

Of the unconventional fisheries which offer a solution to the world's protein problem, the Antarctic Krill seems to be one of the more promising, since methodology has now been developed for not only making krill more accessible, but also for producing food products from krill which are acceptable to humans.

Antarctic krill (*Euphasia superba*) are small Crustacea belonging to the Euphasiaceae order. They are herbivorous, representing the first trophic level in the food chain of the diet of the whale. Adult species of krill, which grow 4–6 cm in length, are found close to the surface in large quantities in areas of the Antarctic Ocean during summer months in the southern hemisphere. Estimates suggest that there could be an annual catch of between 100 and 500 million tons of krill (Anonymous, 1974), an amount which is equal to or exceeds the projected world annual catch of conventional fisheries mentioned above.

In spite of the magnitude of krill availability, it is important to ensure its maximum utilization in order to exploit it in a remunerative way. That is, it is necessary to utilize as much of the whole animal as possible for the manufacture of useful food and nonfood products. Undoubtedly, the highest quality product derived from the crustacean meat must be one which retains to the maximum its original texture, color and organoleptic properties. Therefore, the greatest effort must be made by applying mechanized methods, to obtain peeled tails similar to shrimp, which meet these requirements. Results of our peeling experiments have been good and will be described in a separate report.

Removal of the tail by mechanical peeling results in the production of a large quantity of waste material, which contains a considerable amount of protein that can be further processed by extraction to produce a useful food by-product. Previous investigations on protein extraction from marine sources have shown that the following process variables can influence speed of preparation, quantity and quality of extracted protein: concentration and size of tissue particles in the protein extraction suspension, extraction time, pH of the extraction medium, type and concentration of salts used in extraction and temperature of the extraction medium (Kahn et al., 1974, 1975; Meinke et al., 1972). This paper describes the results obtained from a study of some of these variables as well as others in the extraction, separation and purification of krill proteins.

MATERIALS & METHODS

Materials

Krill. Raw material consisted of krill obtained during an expedition to the Chilean Antarctic in January/March of 1975. Krill was frozen on board ship by the tunnel system and kept at −25°C until it was processed. A period of 6–7 months lapsed between catching and processing. Krill in plastic bags was thawed by submersion in tap water for about 2 hr, drained and peeled using a mechanical system developed in our laboratory. Wastes from this operation were then frozen at −40°C and freeze-dried in an Atlas apparatus, Laboratory Model Ray-1.

Methods

Determination of soluble protein. For the reference method, soluble protein in 20 ml of extract was determined by the Kjeldahl-semimicro method (Pearson, 1973). This method was used to correlate Kjeldahl nitrogen with nitrogen determined by the dye-binding method and for nitrogen determinations on certain samples subjected to thermal treatment for which the dye-binding method was not applicable. The dye-binding procedure was performed as described by Romo et al. 1975. For this, 10 ml of filtered, soluble protein was taken to a volume of 50 ml, and 5 ml of this solution added to 10 ml of dye solution (C.I. Acid Orange 12). The remainder of the method was performed in exactly the same manner as described in the reference cited above.

Proximate analysis. Moisture, ash, and chitin (considered as crude fiber for practical purposes) were determined according to the

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AOAC, 1975. Lipids were analyzed by the extraction method of Bligh and Dyer, 1959, modified by Ederzeel and Ritkes, 1966.

Nitrogen solubility index. Nitrogen solubility index (NSI) is expressed as % protein nitrogen (% N × 6.25), which remained soluble in the extract times 100 and divided by the % total protein nitrogen in the krill waste material (freeze-dried krill after peeling).

Determination of pH influence on protein solubility. Utilizing the dye-binding method described above, soluble protein nitrogen was determined in 100 ml of solution at several pH values between 2 and 12 prepared by extraction of 1.5g of either cooked or uncooked freeze-dried krill samples for 30 min at 22°C. From these data protein solubility profiles were constructed for the uncooked and cooked krill samples.

Protein recovery–influence of pH. The influence of pH on protein recovery was determined from precipitation curves. Data for these curves were obtained by extracting 3g of either cooked or uncooked freeze-dried samples with 200 ml of NaOH solution at pH 12. After centrifugation at 1,500g for 15 min, the solution was filtered and 20 ml aliquots were adjusted to pH values between 2 and 12 with 0.5N HCl. Soluble protein was then determined on aliquots of these solutions using the dye-binding procedure.

Table 1–Proximate composition of cooked and uncooked krill waste products[a]

	Moisture %	Protein[b] %	Lipids %	Ash %	Chitin %
Raw krill waste product	4.7	63.5	12.0	11.2	6.0
Cooked krill[c] waste product	8.7	52.9	20.0	10.2	5.8

[a] Data subject to seasonal variations
[b] Kjeldahl method
[c] Steam cooked

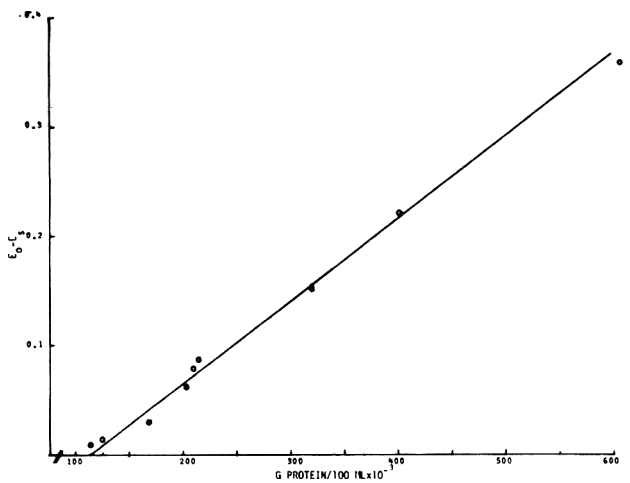

Fig. 1–Relationship between crude protein content of krill wastes and the reduction in absorbance measured by the dye-binding procedure.

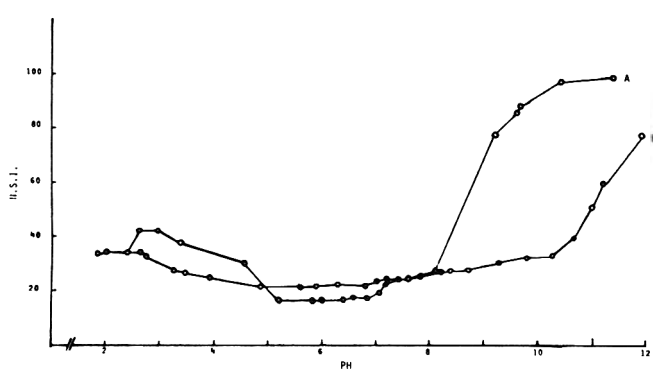

Fig. 2–Solubility profiles for uncooked (Curve A) and cooked (Curve B) krill waste protein.

Protein recovery–influence of the simultaneous use of pH adjustment and heat. The method used for obtaining data in this study was identical to the method described above, except after adjusting the pH to values between 2 and 12, the solutions were heated in a thermostatic bath for 15 min at 90°C, centrifuged, filtered and soluble nitrogen determined in an aliquot of the final solution.

Protein recovery–influence of coagulation time. In order to determine the effect of thermal coagulation time on the yield of precipitated protein from krill protein extracts, samples of extracts were prepared as described above after adjusting pH values to 6.0 and 6.3. Later the samples, which were in test tubes, were placed in a shaker water bath maintained at 90°C and coagulated. The amount of protein which remained soluble was then determined after treatment of 1, 3, 5 and 10 min.

Effect of ionic strength on protein solubility. This determination was performed in the same manner as the method used for determination of protein solubility profiles except that only the uncooked krill samples were studied, and these were extracted with solutions of 0.05, 0.10, 0.20, 0.5, 1.0, 1.5 and 2.0N NaCl solutions adjusted to pH values of 3.0, 5.7, 7.5 and 9.5.

Effect of solid to solvent extraction ratio on protein solubility. The influence of solid-to-solvent extraction ratio on protein solubility was established using uncooked krill samples. Solubility profiles were determined utilizing five different solid-to-solvent extraction ratios in the range 1–5%.

Effect of extraction time on solubility of krill protein. Solubility profiles for uncooked krill wastes were constructed from data obtained in the pH range 3.0–10.5 using extraction times of 10, 20 and 30 min according to the usual procedures described previously.

RESULTS & DISCUSSION

Proximate analysis

The proximate composition of the krill waste product shown in Table 1 indicates a very high protein content, making it an excellent candidate as a raw material for production of protein isolates. It must be pointed out that the proximate composition of the waste material from processed krill is subject to some seasonal variation. For example, waste material from peeled krill caught during a recent expedition in the winter months showed a protein content of 41% and a chitin content of approximately 10%. The differences between proximate values for uncooked and cooked samples is for the most part presumably due to loss of solubles during cooking.

Correlation between Kjeldahl nitrogen and nitrogen determined by the dye-binding method

Soluble protein was determined in aliquots by both the semimicro-Kjeldahl method and the dye-binding method of Romo et al. 1975, which measures reduction in absorbance of a dye solution after reaction with a protein solution. Statistical analysis provided the following regression equation which relates reduction in absorbance of the dye solution to protein content of the solution (plotted in Fig. 1):

$$K_p = 1.36(E_o - E_s) + 0.12$$

where: K_p = crude protein content in the extract expressed as g/100 ml, and $E_o - E_s$ = reduction in absorbance of dye solution.

For the above equation the correlation coefficient, r, is equal to 0.997 with a standard error of estimate equal to 0.0113. The correlation coefficient indicates that reduction in absorbance of the dye solution is highly correlated with the crude protein contents of krill protein extracts determined by the Kjeldahl procedure (see Fig. 1). Furthermore, the standard error of estimate is low; thus, the dye-binding procedure can be used with confidence as a satisfactory

alternative to the Kjeldahl procedure for determination of soluble protein in krill extracts.

Influence of pH on protein solubility

Solubility profiles of extracted krill proteins from both cooked and uncooked krill wastes shown in Figure 2 demonstrates the differences in protein solubility behavior between the investigated treatments. In the case of uncooked wastes, Curve A (Fig. 2) shows a minimum in solubility of proteins in the pH range 5.8–6.4. In this range about 18% of the krill protein still remains in solution. On the acid side, as well as the basic side of this pH range, the solubility of raw krill protein increases; although on the basic side the increase in solubility is much more pronounced. Thus, for example, at pH 9.5 the percentage of solubilized protein reaches roughly 80% of the total and at pH 10.5 practically 100% solubilization is observed. On the other hand at acidic pH values of 2 and 3 the protein solubility reaches a value of only 41%.

Comparing the above values with results obtained with cooked krill (Curve B, Fig. 2), notable changes can be observed. Thermal treatment (cooking) of krill should produce considerable denaturation of proteins. When proteins in the samples are denatured, their solubility is depressed considerably. For example, at extreme pH values such as 10.5 and 2.0 only approximately 40% of the protein becomes soluble. To obtain significant solubilization of denatured proteins, it is necessary to reach relatively high pH values; for instance, at pH 11.25 an NSI of 60 is obtained and at pH 12.0 an NSI of 78 is realized. Minimum solubility still sppears to be in the pH range 4.9–6.3 in which 21% of the proteins remain soluble.

The data in Figure 2 show that a preliminary estimate can be made of the amount of solubilized krill protein expected to be recovered by adjusting the pH of an extract to a value in the isoelectric region of protein solubility. Hence, if raw samples are utilized, essentially 100% of the protein would become soluble at pH 10.5, the majority of which could be recovered by means of isoelectric precipitation. If cooked krill is submitted to a similar treatment, the protein extracted would be about 40% of the total protein present in the sample, and the amount of protein recovered by isoelectric precipitation considerably less. The following section discusses protein recovery.

Protein recovery (precipitation) studies

Influence of pH. Figure 3, Curve A shows the quantity of protein remaining in solution after precipitation by acidification at various pH values of an extract of raw krill initially extracted at pH 10.5–11.0. The beginning of the isoelectric region in precipitation is seen to be at pH 7.5–7.8. Approximately 11% of the total extracted protein remains in the whey (soluble protein fraction) after isoelectric precipitation by adjusting the pH to between 5.5–5.7. The type of precipitate is very fine, a typical characteristic of proteins recovered by this method.

Figure 3, Curve B, shows the results of protein precipitation from an extract of cooked krill obtained by extraction at an initial pH of 11.5. A slow precipitation is observed, which commences at an approximate pH of 6.75 reaching a solubility minimum in the pH range 5.2–5.5. Since more protein remains soluble in this range than in the above experiment, inferior results are obtained with cooked material. In this pH range nearly 22% of the protein remains soluble, a value which is significantly greater than that for raw krill protein solubility in the comparable pH range.

Effect of simultaneous use of pH adjustment and heat to precipitate proteins. With the idea of investigating the possibility of increasing protein yield in the recovery stage, experiments were done employing simultaneously a slow decrease in pH of the extract plus heat. Results shown in

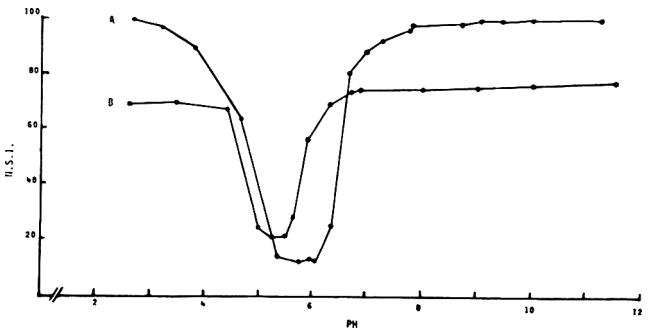

Fig. 3—Precipitation profiles for uncooked (Curve A) and cooked (Curve B) krill waste protein.

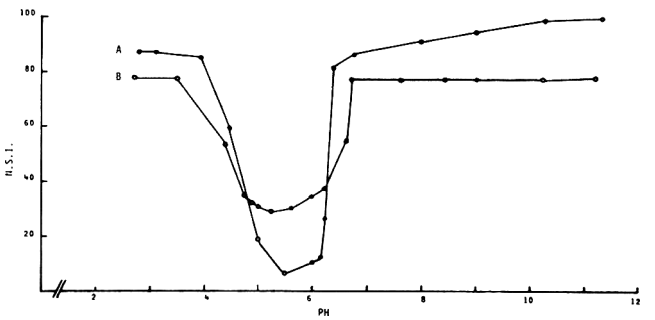

Fig. 4—Heat coagulation/precipation curves for uncooked (Curve A) and cooked (Curve B) krill waste protein.

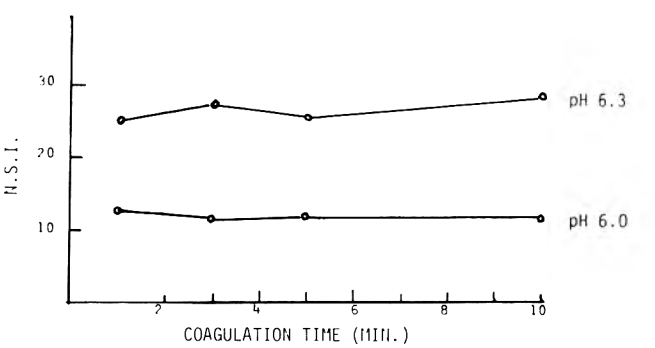

Fig. 5—Influence of coagulation time on protein recovery from krill waste.

Figure 4, Curve A for residues of raw krill indicate that through application of this combined system only 7.5% of total soluble protein remains in solution at pH 5.5. Furthermore, the precipitated protein forms a coagulum which is easily separated and handled. In the case of cooked krill results plotted in Figure 4, Curve B show that use of the system of combined treatments increases rather than decreases the amount of soluble proteins in solution at isoelectric pH to nearly 30% of the total proteins.

Influence of coagulation time on protein precipitation. In order to determine the effect of thermal coagulation time on the yield of precipitated protein from krill protein extracts, samples were prepared whose pH values were subsequently adjusted to values in the isoelectric region. The samples were then thermally coagulated and the amount of protein which remained soluble was determined after heat treatments of specified time periods.

From results shown in Figure 5 for uncooked krill waste,

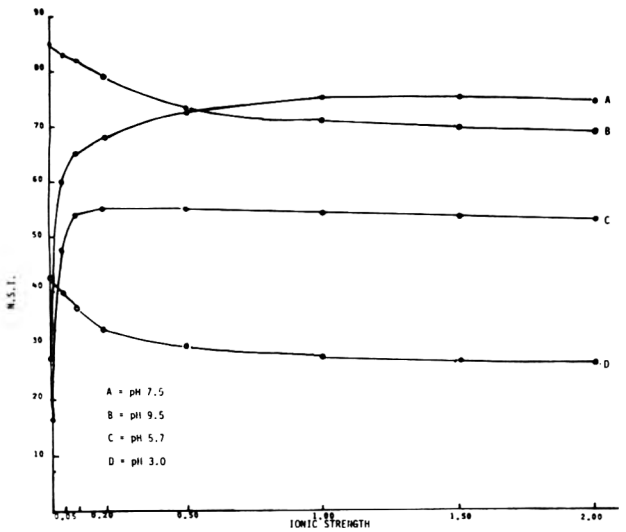

Fig. 6—Effect of ionic strength on krill protein solubility at various pHs.

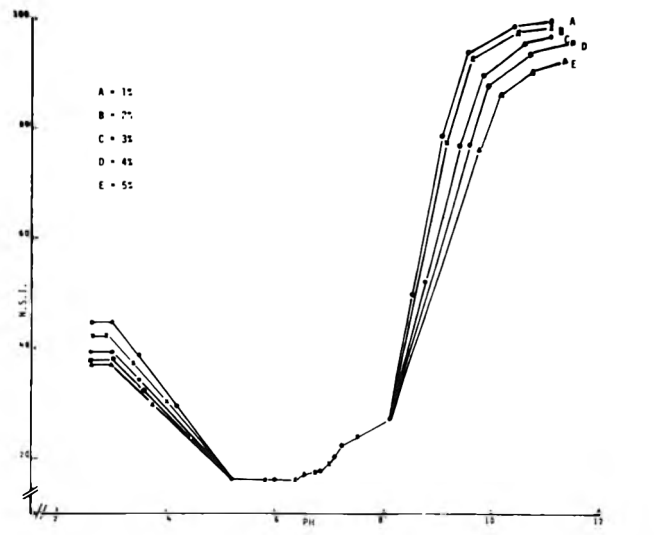

Fig. 7—Effect of solids/solvent extraction ratio on krill protein solubility.

it can be seen that time of coagulation does not appreciably influence the yield of recovered proteins, since after 1 min of thermal treatment at 90°C and pH 6.0, the amount of soluble proteins in solution remains constant and is equal to 13% of the total soluble proteins. If the treatment is done at a slightly higher pH (i.e., 6.3), the results are essentially the same, that is, after 1 min of thermal treatment the amount of coagulated protein does not increase, but the total amount of soluble protein remaining in solution is greater (25%). This allows us to conclude that the time of coagulation does not influence greatly the yield of recoverable proteins by heat, as long as the pH is maintained at a value corresponding to minimum solubility of the proteins.

Influence of ionic strength on protein solubility

In the aforementioned studies, reference has been made to aqueous systems which contain only ions associated with the krill wastes and those added by NaOH and HCl which were used to adjust pH. If krill protein isolates are to be used in food systems, which might contain a variety of ions be these natural or added, the behaviors of these proteins in said systems can depend to a large extent on such ionic environments. Consequently, a knowledge of the effect of ionic strength on protein solubility at various pH values is considered important.

In general, solubility of acid and base soluble proteins is increased on increasing the ionic strength at nearly neutral values of pH. At acid and basic pH values, however, the presence of salts tends to diminish the solubility of proteins (Mattil, 1971). Furthermore, experiments on fish (Meinke et al., 1972) indicate that the influence of NaCl concentration on extraction of proteins depends on the pH at which the solubilization is done. Thus, a decrease in the quantity of protein extracted was observed both at high and low pH values as the salt concentration was increased. Likewise, an increase was shown in the solubility of proteins when the ionic strength was increased at pH values in the region of minimum solubility (approximately pH 6.0). To establish if this effect is also exhibited by soluble proteins from krill, experiments were conducted to determine the influence of ionic strength at several pH values on krill protein solubility.

As seen in Figure 6, a solution of 1N NaCl inhibits to some extent extraction of protein from uncooked krill at pH 3.0, solubilizing only 27% of the total. Without added salt, 43% of the total proteins are extracted at pH 3.0 (Curve D). The influence of NaCl concentration on protein solubility shows a similar pattern at a high pH (9.5). Without the addition of salt, protein solubility is 85%; if the ionic strength is increased up to 1.0N, solubility drops to a value of 71% (Curve B, Fig. 6). At pH 5.7 in the absence of added salt, 15% of soluble protein is found in solution (Curve C, Fig. 6). This value (15%) agrees fairly well with the value of 18% shown in Curve A of Figure 2 at pH 5.7 as would be expected. It can be seen that as the salt concentration increases to a value of 0.1N NaCl, the amount of protein which remains in solution at pH 5.7 increases from the initial value of 15% up to a value of 55%. Similar results were obtained with fish (Meinke et al., 1972).

The solubilizing effect of salt can, therefore, lower the yield of isoelectric precipitated protein after an extraction affected at alkaline or acid pH values. Once the acidic or alkaline extract is obtained and freed of nondispersable solids, the pH is adjusted to the nearest possible value of minimum solubility. For krill proteins this value is approximately 5.7. If adjustment of pH is accomplished by addition of HCl to an alkaline NaOH extract, for example, salt (NaCl) will be formed producing an increase in protein solubility in the isoelectric pH region in comparison to protein solubility in pure water. The yield of precipitated protein obtained from acid or basic extracts is therefore decreased by the presence of generated NaCl.

An alternate approach to alkaline extraction of krill protein from the wastes would be to extract it with a solution of slat at neutral pH followed by dilution with water to cause precipitation (see, for example, Dyer et al., 1950). Curve A of Figure 6, however, indicates that at a pH of 7.5 only 75% of krill protein is solubilized using NaCl solutions of 1–2N; therefore, saline extraction would prove inefficient in the case of krill proteins.

In concluding the discussion of effect of ionic strength on protein solubility, we would like to point out that the depressed solubility of both cooked and uncooked waste proteins in the acidic region (manifested by the large difference in solubility profiles compared to precipitation curves) is probably in part due to solubilization of minerals present in krill shells. The shells make up a large portion of the peeling wastes and at acid pH values, minerals present in the shells (mostly insoluble salts of calcium) would be solubilized, thereby depressing protein solubility. A second factor, which will not be discussed here, is the presence of chitin in

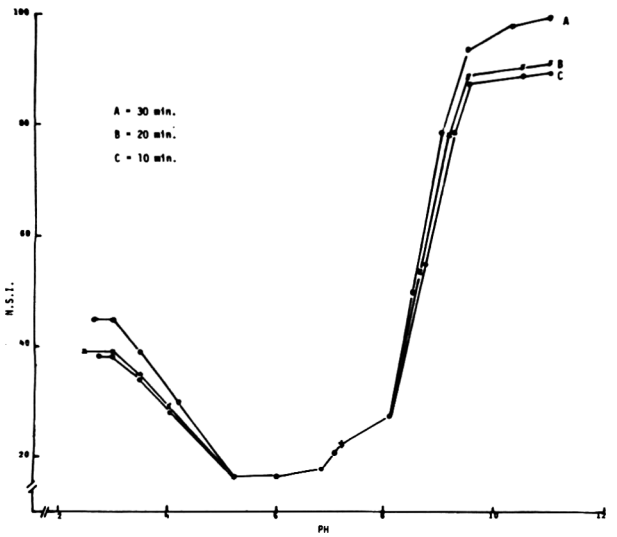

Fig. 8—Effect of extraction time on krill protein solubility.

the suspensions used to obtain data for construction of solubility profiles.

Effect of solid-to-solvent extraction ratio on protein solubility

The ratio of the amount of krill waste to volume of extraction-medium is of prime importance to the economy of a protein isolation plant. The greater the proportion, the less the relative problem will be with control of effluents from the process. Data in Figure 7 show that the proportion of krill protein dissolved in 100 ml of extraction-medium is dependent only to a small extent on the weight of krill waste extracted in the range 1–5% suspensions (Curve A, 1%; Curve E, 5%, respectively) at pH values of about 3.6 and 11.0 in its freeze-dried state. This means that at extraction pH values of 10.5–11.0 and concentrations of 1–5%, the amount of protein in solution averages 90–98%, respectively. Similarly, in the region of minimum solubility (values close to pH 6.00, the protein in solution varies in the range 3–8%, respectively).

From the engineering point of view, it is impossible to recover the whole of the volume of extraction medium; since as the ratio of weight of krill to extraction-medium increases, problems arise with viscosity and gelatinization (concentrations greater than 5%). These factors lead to a smaller recovery volume of extract as the weight of krill increases beyond the value of 5% (approximately 50% on a wet basis).

Effect of extraction time on protein solubility

The length of time used in extraction of krill protein from wastes influences significantly the quantity of protein solubilized. Results of this study shown graphically in Figure 8 indicate that protein extraction from wastes is achieved rather rapidly. After 10 min, 88% of the total protein is dissolved at pH 10.5 and 98% after 30 min at the same pH value.

Other considerations

In view of the fact that at pH values near 10.5 and ambient temperature nearly 100% protein solubilization was achieved without grinding the krill wastes; it was not considered important at this stage to study the effect of particle size or extraction temperature on protein solubility. In the present study tissue particle sizes varied from approximately 0.5 mm to a few hundreths of a millimeter.

Effects of the parameters studied in this paper on the properties of krill protein are in reality applicable only to krill maintained in a frozen state for a long period of time. The material was thawed, mechanically peeled, re-frozen and freeze-dried and no doubt the two freezing processes added to the relatively long storage period caused some loss in protein solubility. This hypothesis has been substantiated in the case of proteins isolated from other marine species (Suzuki, 1974).

CONCLUSIONS

THE RESULTS presented in this paper indicate that alkaline extraction of protein from uncooked krill wastes produced by mechanical peeling is far more practical than extraction of cooked waste material. It was shown that it is possible to extract more than 90% of protein present in uncooked krill wastes by means of alkaline treatment at pH 10.5 and ambient temperature. Extraction at neutral pH renders only 20% of the total protein soluble with both cooked and uncooked waste materials, a value which is low. Extraction time significantly influences the quantity of protein solubilized by alkaline extraction. Protein extraction by means of a saline (NaCl) medium at pH 7.5 renders only approximately 75% of the total protein soluble, making this method unacceptable for krill protein extraction. Added salts such as sodium chloride reduce the yield of precipitated protein by increasing protein solubility in the isoelectric pH region. A solid-to-solvent ratio greater than 5% (dry basis) in the extraction produces problems with viscosity and gelatinization resulting in low protein recovery values. Precipitation of proteins extracted from uncooked krill waste is more effective at pH values in the isoelectric region when a short heat treatment is applied. Based on this study the following process parameters seem most acceptable for efficient protein isolation on a laboratory scale from wastes obtained after mechanical peeling of whole krill: particle size–less than 0.5 mm, extraction time–30 min; extraction temperature–ambient, solute/solvent ratio–1:20 (dry basis); solvent–NaOH solution at pH 10.5 for raw material and pH 11.5 for cooked material, recovery by isoelectric precipitation plus heat (90°C, 5 min, raw krill only) at pH 5.7.

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EFFECT OF CARCASS WEIGHT AND FAT THICKNESS OF LAMB CARCASSES ON SURFACE BACTERIA COUNTS

E. A. SAUTER, J. A. JACOBS, J. F. PARKINSON and S. K. ERCANBRACH

ABSTRACT

Microbial studies were conducted to determine the rate of build-up of surface bacteria during chilling and holding on carcasses of 141 lambs. Samples were obtained using the swab technique. Initial bacterial load was determined on hot carcasses. Chilled carcasses were sampled at 24 hr and after 4 and 7 days post slaughter. Bacterial counts indicated substantial reduction in numbers during chilling, at −2.2°C for 24 hr from 6.4×10^3 to $5.1 \times 10^2/cm^2$ for the total aerobic count on light lamb carcasses. Reduction in numbers of total aerobes during 24-hr chilling was not as great for heavy lamb carcasses, from 5.1×10^3 to $1.7 \times 10^3/cm^2$. Numbers of phychrotrophic organisms after 4 and 7 days appear to be related to the thickness of fat cover. Carcasses having 0.36 cm or less of fat cover had significantly ($P < 0.05$) higher psychrophilic counts. No Salmonella were isolated and numbers of *Clostridium perfringens* in both groups of lamb carcasses were low.

INTRODUCTION

HEAVY LAMBS of high cutability would be desirable for the consumer as well as the producer and processor if they could be produced efficiently. Jacobs et al., (1972) reported that ram lambs slaughtered at 68 kg were equal to wethers at 50 kg in all palatability traits. Both were less tender than wethers slaughtered at 65 kg.

Ingram and Dainty (1971) reported that bacterial growth on meat at chill temperatures is primarily a surface phenomenon and that no definite changes occur in the meat until surface bacterial counts exceed $10^8/cm^2$. Dainty et al., (1975) found that proteolysis could not be detected prior to spoilage of meat. Bacterial numbers were $2 \times 10^9/cm^2$ when spoilage occurred from the natural microflora and $4 \times 10^8/cm^2$ on beef inoculated with slime from other meat. Difference was thought to be due to relatively higher percentage of proteolytic pseudomonads in the slime inoculum.

Gill (1976) reported that bacterial growth on meat was limited by the rate of diffusion to the surface of fermentable substrate from within the tissue. However, while the anaerobic bacteria tested utilized only glucose, the aerobes utilized amino acids and lactic acid when glucose was no longer available. Later work, Gill and Newton (1977), indicated a much more complex relationship. In studies of pure and mixed cultures of *Pseudomonas sp. Enterobacter* and *Microbacterium thermosphactum* they suggest that oxygen availability to the cells may be the limiting factor in growth of aerobic spoilage organisms on sliced meat surfaces.

Hoke et al., (1976) in studies with lamb carcasses of yield grades 2, 3 and 4 found no differences in microbial counts either at the packing house or at the retail distribution warehouse. There were differences among packing houses and these differences continued when sampled at the retail warehouse.

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This study was conducted to compare the microbial build-up during chill (3.3°C) storage on lamb carcasses of different weights and having varying thickness of fat cover.

MATERIALS & METHODS

One hundred forty-one lambs obtained from the U.S. Sheep Experiment Station, Dubois, ID, were slaughtered in two groups at the University meats laboratory. Group one (light) consisted of 72 lambs 6.5 months of age with an average slaughter weight of 50.9 kg. Group two (heavy) consisted of 69 lambs 8.5 months of age with slaughter weight averaging 59.5 kg. All carcasses were quality and yield graded according to USDA standards and data on carcass characteristics recorded. Carcasses from each group were divided into six lots of 12 carcasses based on carcass weight with approximately 2 kg increments except that the lower weight lot from the heavy lambs contained only nine carcasses, all of which weighed less than 25.2 kg.

Bacterial samples were taken immediately after inspection and just prior to weighing the carcass to obtain hot weight data. Areas of 12.9 cm^2 of each leg (biceps femoris) and flank (rectus abdominus), controlled by sterile template, were swabbed using cotton swabs moistened with 0.1% peptone broth. Choice of sampling sites was for uniformity, these sample sites were previously reported by Hoke et al. (1976). Swabs were placed in 9.9 ml of sterile 0.1% peptone, mixed for 2 min using a vortex genie mixer and serially diluted using 0.1% peptone. Plate counts were made in triplicate using Standard Methods Plate Count Agar (PCA) to determine total aerobic bacteria and psychrophilic bacteria. Plates for aerobic counts were incubated at 35°C for 48 hr, those for psychrophilic counts at 7°C for 14 days. Anaerobic Agar (AA) was used to determine numbers of anaerobic-facultative bacteria and Violet Red Bile Agar (VRBA) for total coliform counts. Numbers of *Clostridium perfringens* were determined by use of the pour plate procedure described in Bacteriological Analytical Manual for Foods (FDA, 1972) using SPS agar (BBL) which was allowed to solidify then overlayed with additional SPS agar. Plates were incubated anerobically in gas pak jars for 48 hr at 37°C. The method of Angelotti et al., (1962) was used to confirm the black colonies on SPS agar as *C. perfringens*. Nitrite positive and nonmotile black colonies were considered to be *C. perfringen*. Lactic acid bacteria were enumerated using APT agar (BBL) with 0.02% added sodium azide and numbers of *E. coli* and enterococci were determined using most probable numbers (MPN) techniques (USDA, 1974). Numbers of lactic acid bacteria and enterococci were determined only on the second (heavy) group of lambs after identification of lactic acid bacteria from plates of group 1 carcasses.

An additional series of swab samples was taken from each hot carcass for Salmonella detection. The swabs were incubated in selenite cystine enrichment broth for 24 hr at 37°C then streaked on MacConkey and Salmonella Shigella agar plates to determine presence of Salmonella species. All sampling procedures were repeated at 24 hr and 4 days post slaughter for group 1 carcasses and at 24 hr and 4 and 7 days for group 2 carcasses. Immediately after bacterial sampling, the hot carcasses were moved into an air blast chiller at −2.2°C for 24 hr after which the carcasses were held in a cooler at 3.3°C for the remainder of the experimental period. Count data were converted to logarithms and analyzed by the analysis of Variance (Snedecor and Cochran, 1967) with differences among means evaluated by Duncan's (1955) multiple range tests.

RESULTS & DISCUSSION

AVERAGE surface bacterial numbers/cm^2 for hot and chilled lamb carcasses are summarized in Table 1. Reduction in numbers of surface bacteria during chilling for 24 hr at −2.2°C was somewhat greater for the light lamb carcasses (group 1) than for the carcasses from heavy lambs

Table 1—Bacterial numbers/cm² from hot and chilled lamb carcasses[a]

Type of organism	Light group		Heavy group	
	Hot	Chilled 24 hr	Hot	Chilled 24 hr
Total aerobes	$6.4 \times 10^{3}{}_{a}$	$5.1 \times 10^{2}{}_{b}$	$5.1 \times 10^{3}{}_{a}$	$1.7 \times 10^{3}{}_{a}$
Anaerobic-facultative	$5.8 \times 10^{3}{}_{a}$	$1.3 \times 10^{2}{}_{b}$	$7.0 \times 10^{2}{}_{a}$	$2.5 \times 10^{2}{}_{a}$
Coliforms	$7.2 \times 10^{2}{}_{a}$	$3.9 \times 10^{1}{}_{b}$	$5.0 \times 10^{2}{}_{a}$	$3.9 \times 10^{2}{}_{a}$
Psychrophils	$1.3 \times 10^{3}{}_{a}$	$8.7 \times 10^{2}{}_{a}$	$8.4 \times 10^{2}{}_{a}$	$8.3 \times 10^{2}{}_{a}$
Lactic acid bacteria	–	–	$1.1 \times 10^{2}{}_{a}$	$4.2 \times 10^{1}{}_{a}$
Enterococci	–	–	$3.0 \times 10^{1}{}_{a}$	$1.7 \times 10^{1}{}_{a}$
E. coli	$7.4 \times 10^{1}{}_{a}$	$2.5 \times 10^{1}{}_{a}$	$5.2 \times 10^{1}{}_{a}$	$4.1 \times 10^{1}{}_{a}$
C. perfringens	$<10_{a}$	$<10_{a}$	$<10_{a}$	$<10_{a}$

[a] Values on any line for either group having different subscripts are significantly different ($P < 0.05$).

(group 2) where the reduction was not significant ($P > 0.05$). Numbers of psychrophilic organisms on either group were not reduced by the chilling procedure, likewise, numbers of *C. perfringens* remained constant for both groups.

No Salmonella were detected on carcasses from either group. *E. coli* represented approximately 10% of the total coliform count from hot carcasses for both groups. Micrococci (nonmotile gram positive cocci which do not ferment glucose) were the predominant portion of the microflora from both hot and chilled lamb carcasses.

Effects of carcass weight and fat thickness on the 4-day build-up of total aerobes and of psychrophilic organisms on the surface of light lamb carcasses are shown in Table 2. Standard plate counts/cm² were not affected by either weight or fat thickness. However, numbers of psychrophilic organisms showed a definite trend toward higher counts on carcasses with .36 cm or less of fat cover. Similar data for heavy lamb carcasses at 7 days post slaughter are shown in Table 3. Differences in total aerobic counts were nonsignificant ($P > 0.05$) but the smallest lamb carcasses which had an average of only 0.36 cm of fat cover had significantly higher psychrophilic bacterial counts. The differences in psychrophilic counts are even greater if the comparison is made on a basis of individual carcasses with all having 0.36 cm or less of fat thickness compared to those carcasses having more than 0.36 cm of fat. Numerical averages of 4-day psychrophilic counts from the light lambs were 9.9×10^{5}/cm² for carcasses having 0.36 cm or less of fat and only 1.2×10^{5}/cm² for carcasses having more than 0.36 cm of fat cover. Seven-day psychrophilic counts from individual carcasses of heavy lambs averaged 6.3×10^{8}/cm² from carcasses having less than 0.36 cm of fat cover and 1.8×10^{6}/cm² from carcasses with more than 0.36 cm of fat cover.

If the comparisons are made on a basis of yield grades rather than thickness of fat cover, a similar relationship exists with psychrophilic bacterial numbers as can be noted from Tables 2 and 3. Individual carcasses of light lambs of yield grades less than 3.0 averaged 7.2×10^{5} psychrophils/cm² at 4 days post slaughter whereas carcasses of yield grades 4.0 or greater averaged 9.9×10^{4} psychrophils/cm². This difference was significant ($P < 0.05$). However, 4-day counts of psychrophilic bacteria from carcasses of yield grades 3.0–3.9 were intermediate and not significantly different from either of the other groups. Numbers of psychrophilic bacteria from heavy lamb carcasses by yield grades at 7 days post slaughter averaged 1.9×10^{8}, 4.5×10^{7} and 1.4×10^{6}/cm² for yield grades less than 3.0, 3.0–3.9 and 4.0 or greater, respectively.

These results indicate that fat cover of lamb carcasses may be important in limiting the build-up of psychrophilic bacteria during refrigerated storage. Results also show that fat thicknesses of 0.41 cm were adequate, although somewhat less, than the proposed minimum of 0.51 cm for "consumer preferred lamb," Carpenter (1966). Reasons for reduced growth on carcasses having more fat cover may be reduced surface moisture or that reported by Gill (1976), namely limitation of suitable substrate to the rate at which it can diffuse through the fat cover.

Table 2—Effect of weight, fat thickness and yield grade on 4-day bacterial counts from light lamb carcasses[a]

Carcass wt (kg)	Yield grade	Fat thickness (cm)	Bacterial count/cm²	
			Total aerobic	Psychrophils
19.2	2.7	0.30	$1.6 \times 10^{5}{}_{a}$	$7.3 \times 10^{5}{}_{a}$
22.5	3.1	0.41	$1.6 \times 10^{5}{}_{a}$	$3.0 \times 10^{5}{}_{a,b}$
24.2	3.0	0.36	$1.6 \times 10^{5}{}_{a}$	$8.3 \times 10^{5}{}_{a}$
25.7	3.2	0.41	$1.3 \times 10^{5}{}_{a}$	$2.0 \times 10^{5}{}_{b}$
28.2	4.2	0.64	$1.3 \times 10^{5}{}_{a}$	$1.6 \times 10^{5}{}_{b}$
31.3	4.3	0.71	$1.3 \times 10^{5}{}_{a}$	$1.4 \times 10^{5}{}_{b}$

[a] Values in any column having different subscripts are significantly different ($P < 0.05$)

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Table 3—Effect of weight, fat thickness and yield grade on 4 and 7-day bacterial counts from heavy lamb carcasses[a]

Carcass wt (kg)	Yield grade	Fat thickness (cm)	Bacterial counts/cm²			
			4 Days		7 Days	
			Aerobes	Psychrophils	Aerobes	Psychrophils
23.6	3.2	0.36	$2.6 \times 10^{5}{}_{a}$	$8.5 \times 10^{5}{}_{a}$	$1.0 \times 10^{7}{}_{a}$	$1.2 \times 10^{8}{}_{a}$
26.7	3.3	0.43	$1.8 \times 10^{5}{}_{a}$	$6.4 \times 10^{5}{}_{a,b}$	$1.0 \times 10^{7}{}_{a}$	$1.9 \times 10^{7}{}_{b}$
28.5	3.6	0.46	$1.7 \times 10^{5}{}_{a}$	$2.0 \times 10^{5}{}_{a,b}$	$1.0 \times 10^{7}{}_{a}$	$1.2 \times 10^{7}{}_{b}$
30.9	4.1	0.56	$1.9 \times 10^{5}{}_{a}$	$2.2 \times 10^{5}{}_{a,b}$	$1.1 \times 10^{7}{}_{a}$	$9.0 \times 10^{6}{}_{b}$
33.1	4.1	0.58	$1.4 \times 10^{5}{}_{a}$	$1.3 \times 10^{5}{}_{a,b}$	$6.4 \times 10^{6}{}_{a}$	$5.4 \times 10^{6}{}_{b}$
36.1	4.2	0.64	$1.2 \times 10^{5}{}_{a}$	$1.1 \times 10^{5}{}_{b}$	$4.7 \times 10^{6}{}_{a}$	$3.8 \times 10^{6}{}_{b}$

[a] Values in any column having different subscripts are significantly different ($P < 0.05$).

SENSORY AND COOKING PROPERTIES OF GROUND BEEF PREPARED FROM HOT AND CHILLED BEEF CARCASSES

H. R. CROSS, B. W. BERRY and DAVE MUSE

ABSTRACT

Hot processed ground beef was prepared by one of the following three grinding methods: (1) initial break with kidney plate followed by 0.3 cm final grind; (2) initial break with kidney plate, followed by 1.3 cm grind and 0.3 cm final; and (3) same as No. 2 except that the formulation contained no chilled U.S. Choice plates. Chilled beef (control) was ground through a 1.3 cm plate followed by a 0.3 cm final grind. At 3-hr postmortem, the semimembranosus and longissimus muscles were removed from one side of each hot carcass, while at 24-hr postmortem, the same muscles were removed from the opposite chilled sides. The remainder of the meat from the carcass was used for ground beef fabrication. U.S. Choice plates (conventionally chilled) were added (30% of the formulation) to the formulation in order to bring the final fat content to 21 ± 2%. Total cooking loss was significantly less in the hot processed patties when compared to the chilled patties. Patties from hot-boned beef were more tender and juicy than patties from chilled beef. Method of grinding had no significant effect on any palatability trait except flavor intensity. Hot processed patties had significantly ($P < 0.05$) less configuration change during cooking than chilled patties. Percent diameter change was significantly less in hot processed patties as compared to chilled.

INTRODUCTION

MORE THAN 40 million cattle and calves are being slaughtered in the U.S. each year and require vast amounts of labor and energy for processing, transporting and marketing. Alternate processing methods, such as hot-boning, offer tremendous possibilities in labor, space and energy conservation and increased marketing efficiency (Henrickson, 1975). By 1980, about 50% of the beef slaughtered probably will be consumed as ground beef (Pietraszek, 1975).

Few, if any, data have been reported concerning the feasibility of producing ground beef from hot-boned carcasses. Several potential problems of hot-boning include textural changes, color differences and shelf-life. If processing of ground beef from hot-boned carcasses is to be practical, the hot processing of selected cuts for steaks and roasts from the same carcasses must also be practical. Much of the published data on hot processing have concentrated on the palatability of steaks and roasts from USDA Good and Choice carcasses (Kastner et al., 1973; Falk et al., 1975; Schmidt and Gilbert, 1970). No data have been reported on the effects of hot-boning of mature (> 4 yr) beef carcasses on shelf-life and palatability of the meat. The steak and roast cuts from these carcasses are usually tenderized by mechanical and enzymatic methods. Scientists in this laboratory are currently investigating the effects of hot-boning on the physical, chemical and microbial properties of ground beef and steaks and roasts from mature beef carcasses. We now report on the palatability and cooking characteristics of beef patties prepared from hot and chilled beef.

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EXPERIMENTAL

HOT-BONED GROUND BEEF was prepared by three methods, while ground beef from chilled beef was prepared by the method most commonly used in industry. Two batches of ground beef were prepared for each method and each batch contained four sides of beef except that each batch for the chilled (control) ground beef contained 12 sides of beef (four sides from the opposite sides of the three hot processed methods). Carcasses ranged in USDA quality grade from low to high utility and in maturity from "C" to "E" (> 4 yr). The animals were slaughtered and the ground beef was prepared and stored at a commercial processing plant. At 3-hr postmortem, the top round (semimembranosus), strip loin (longissimus), and ribeye (longissimus) cuts were removed from one side of each hot carcass and at 24 hr postmortem, the same muscles were removed from the sides that had been chilled at 2–3°C. These muscles were set aside for a different study. The remainder of the meat from the boned carcasses was used for ground beef fabrication in this study.

Grinding methods–Chilled (control)

U.S. Choice plates (conventionally chilled) were added (about 30%) to the formulation in order to bring the final fat content to 21 ± 2% using the Anyl Ray Instrument. The U.S. Utility lean and U.S. Choice plates were passed through a 1.3 cm plate, mechanically mixed for 3 min and ground through a 0.3 cm plate for the final grind. Batch size averaged 360–400 kg. The control formulation was not an exact "control" because the grinding systems were not identical for hot and chilled meat. The purpose of the experiment was to compare new systems that might work for hot meat with the most common system used for chilled meat.

Method 1–Hot processed. U.S. Choice plates (2–3°C) were added (about 30%) to the formulation in order to bring the final fat content to 21 ± 2%. The hot lean and chilled beef plates were passed through a large kidney shaped breaking plate (1.3 cm × 1.9 cm) and mixed for 3–4 min. During mixing, CO_2 snow was added at a 1:10 ratio of CO_2 to beef. After mixing, the product was ground through a 0.3 cm plate for the final grind.

Method 2–Hot processed. Method 2 differed from method 1 only in the number of grinds and the manner in which the CO_2 snow was added. The lean and fat were passed through a kidney plate, mixed 3–4 min; passed through a 1.3 cm plate, mixed 3–4 min and passed through a 0.3 cm plate for the final grind. The CO_2 snow was added at a ratio 1:10 with two-thirds added during the first and one-third during the second mix.

Method 3–Hot processed. Method 3 differed from method 2 only in the amount of CO_2 snow added and the absence of U.S. Choice plates. Because no chilled U.S. Choice plates were added, the ratio of CO_2 snow to beef was increased to 1.5:10. Also the carcasses used for this method were slightly fatter than those used in methods 1 and 2 in order for the final fat content to be 21 ± 2%.

Patties

Ground beef from all formulations was formed into 113g (4 oz) patties using a FORMAX model 26 patty machine. Patties were stacked (10 per stack), boxed and frozen in a blast freezer and stored at −10°C for 5 days before shipment to Beltsville, MD for analysis.

Trained panel

In a total of 10 sessions, a 10-member descriptive attribute panel, trained by the procedures of Cross et al. (1978a) and AMSA (1978) evaluated samples from each treatment. Six samples were evaluated per session and each treatment was replicated five times. The panel rated each sample for differences in tenderness, juiciness, connective tissue amount and ground beef flavor intensity with 8 = extremely tender, juicy, no detectable connective tissue, and intense and 1 = extremely tough, dry, abundant connective tissue and bland in ground beef flavor.

Cookery and presentation to panel

Frozen patties were broiled on electric Farberware grills (model 450-A) to an internal temperature of 60°C. Temperature was monitored during cooking with Teflon-coated iron/constantan thermocouples. Total cooking losses were calculated from frozen and cooked weights. Four patties were prepared for each session. Each patty was sectioned into 6 pieces and two of the 24 pieces (4 patties) were randomly assigned to each panelist. The samples were served as warm as possible to the panelist as described in ASMA (1978). Sections also were pictorially scored for degree of doneness (color photographs with 1 = well done and 8 = rare) by a trained laboratory technician.

Shear force

Ten patties from each method/batch/group were used for determination of Instron shear force by the single-blade procedure of Cross et al. (1978b). Four 2.5 cm squares were cut from each patty, so each mean for method/batch represents 40 observations.

Physical and chemical

Height and diameter were measured on the ten frozen and cooked patties used for the Instron. Percent fat and moisture were determined on raw and cooked patties by AOAC procedures. The pH was measured on ten frozen, thawed and cooked patties from each treatment by the procedure described by Nichols and Cross (1978).

RESULTS & DISCUSSION

IN TABLES 1–3, data were combined for methods of hot processing to allow a direct comparison of sensory, physical and chemical properties of ground beef prepared from hot and chilled beef. This is possible since method of preparing hot boned ground beef had no significant effects on sensory and cooking properties. Analysis of variance also revealed that interactions were nonsignificant. Mean palatability and shear force values are presented in Table 1. Ground beef patties prepared from hot processed beef were significantly ($P < 0.05$) more tender (panel) and juicy than patties prepared from chilled beef. Differences in shear force were not significant, but showed the same trend as ratings from the panel. As might be expected, treatment did not significantly affect amount of connective tissue or flavor intensity.

Total cooking loss was significantly less for hot processed patties than for chilled patties (Table 2). The difference was large (33.85 vs 41.06%) and of considerable practical importance. These differences in cooking losses were reflected in ratings for juiciness (Table 1). Hot processed patties changed significantly less in diameter than chilled patties. Percent changes in height and thaw loss did not differ significantly. For institutional use, diameter would be the most important dimension because a constant area of the bun should be covered.

Because the hot-boned beef was processed prerigor, some thaw rigor was a possibility. The possibility was small, however, because our patties were frozen over a 10–20 hr period. Muscle pH was measured to determine whether meat had reached its ultimate pH prior to freezing (Table 3). As expected, pH did not significantly differ between hot and chilled patties. This is also reflected in the lack of significant differences in thaw loss (Table 2). If the hot processed patties had been frozen cryogenically thaw rigor might have been a problem. That and other possibilities are under investigation. Percent fat and water did not differ significantly in the raw patty (Table 3) and percent fat did not differ in the cooked patty. Percent moisture in cooked patties was significantly higher from hot than from chilled carcasses and can be explained by the differences in cooking loss (Table 2). If moisture in raw patties had been higher from hot processed than from chilled beef, their relative nutrient contents might have been questioned. However, the data for cooking losses (Table 2) and for moisture content (Table 3) indicate that cooked patties differed only in water content. Undoubtedly, the hot and chilled patties were quite similar as to composition in the raw state but the chilled patty lost more water during cooking. This water loss was reflected in lower juiciness and tenderness ratings for patties from chilled carcasses and could contribute to more waste on the plate.

Effects of method of grinding on sensory, physical and chemical properties are compared in Table 4 and 5. Data for evaluation by sensory and shear methods appear in Table 4. Method of grinding did not significantly affect any palatability trait except flavor intensity. Flavor of patties was less intense for method 3 than for methods 1 and 2. This difference was probably reflected by the absence of U.S. Choice plates in the formulation rather than method of grinding. In any case, the difference was probably too small to be of practical importance. Regardless of method of grinding, patties prepared from hot-boned beef were

Table 1—Mean palatability and shear force values for cooked ground beef prepared from hot and chilled muscle

	Type of processing	
Trait	Hot	chilled
Tenderness[a]	5.69[e]	5.22[f]
Connective tissue[b]	4.26[e]	4.38[e]
Juiciness[c]	5.47[e]	4.75[f]
Flavor intensity[d]	5.23[e]	5.27[e]
Max shear force, kg	10.99[e]	11.96[e]
n = 30 observations per mean		

[a] 8 = extremely tender; 1 = extremely tough.
[b] 8 = none; 1 = abundant amount.
[c] 8 = extremely juicy; 1 = extremely dry.
[d] 8 = extremely intense; 1 = extremely bland.
[e,f] Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 2—Cooking properties of ground beef patties prepared from hot and chilled muscle

	Type of processing	
Trait	Hot	chilled
Total Cooking Loss, %	33.85[b]	41.06[c]
Degree of doneness[a]	2.32[b]	2.45[b]
Diameter change, %	14.93[b]	19.32[c]
Height change, %	16.06[b]	14.04[b]
Thaw loss, %	5.39[b]	6.21[b]

[a] 8 = rare; 1 = well done.
[b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 3—Chemical properties of ground beef prepared from hot and chilled muscle

	Type of processing	
Trait	Hot	chilled
pH Raw, frozen	5.52[a]	5.46[a]
pH Raw, thawed	5.37[a]	5.32[a]
pH Cooked	5.50[a]	5.46[a]
H_2O, Raw, %	62.11[a]	62.29[a]
Fat, Raw, %	20.01[a]	19.55[a]
H_2O, Cooked, %	52.10[a]	48.60[b]
Fat, Cooked, %	21.10[a]	21.80[a]

[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 4—Palatability traits for patties from three systems of grinding hot, processed beef

Trait	Hot processed beef method of grinding[a] 1	2	3	Control chilled
Tenderness[b]	5.48[f]	5.90[f]	5.68[f]	5.22
Connective tissue[c]	4.06[f]	4.48[f]	4.24[f]	4.38
Juiciness[d]	5.36[f]	5.61[f]	5.43[f]	4.75
Flavor intensity[e]	5.39[f]	5.37[f]	4.93[g]	5.27
Max. shear force, kg	11.19[f]	10.35[f]	11.38[f]	11.96

[a] 1 = kidney plate X 0.32 cm plate; 2 = kidney plate X 1.27 cm plate X 0.32 cm plate; 3 = kidney plate X 1.27 cm plate X 0.32 cm plate (no Choice plates added as in 1 and 2).
[b] 8 = extremely tender; 1 = extremely tough.
[c] 8 = none; 1 = abundant amount.
[d] 8 = extremely juicy; 1 = extremely dry.
[e] 8 = extremely intense; 1 = extremely bland.
[f,g] Means in the same row with different superscripts are significantly different ($P < 0.05$).

more tender and juicy than patties prepared from chilled beef.

Percent total cooking loss, height change, thaw loss and degree of doneness were not significantly affected by method of grinding (Table 5). Percent diameter loss was greatest in patties prepared by method 1 (kidney plate × 0.3 cm). It is difficult to explain why the double grind should cause more shrinkage in diameter than the triple grinds. Possibly, elevated back pressure in the double as compared to the triple grind systems was responsible. Mean pH values of frozen, and thawed and cooked patties; and raw and cooked fat and water percentages were not significantly affected by method of grinding (data not presented).

These data showed that the beef patties prepared from hot processed beef were equal to or superior to patties prepared from conventionally chilled beef in palatability, physical and chemical properties. Patties prepared from hot processed beef were significantly more tender and juicy and lost less water during cooking than those prepared from chilled beef.

Table 5—Comparison of cooking properties of three systems of grinding hot beef

Trait	Hot processed beef method of grinding[a] 1	2	3	Control chilled
Total cooking loss, %	36.48[c]	35.04[c]	30.02[c]	41.06
Degree of doneness[b]	2.05[c]	2.60[c]	2.30[c]	2.45
Diameter change, %	16.53[c]	14.17[d]	14.08[d]	19.32
Height change, %	18.24[c]	20.76[c]	9.17[c]	14.04
Thaw loss, %	5.47[c]	6.23[c]	4.48[c]	6.21

[a] 1 = kidney plate X 0.3 cm plate; 2 = kidney plate X 1.3 cm plate X 0.3 cm plate; 3 = kidney plate X 1.3 cm plate X 0.3 cm plate (no Choice plates added as in 1 and 2).
[b] 8 = rare; 1 = well done.
[c,d] Means in the same row with different superscripts are significantly different ($P < 0.05$).

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QUALITY CHARACTERISTICS OF BRINE-CHILLED AND SMOKED BOBWHITE QUAIL

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ABSTRACT

480 Bobwhite quail were sacrificed at 16 wk of age to study the effect of brine-chilling and smoking on carcass cooling rate during chilling, water uptake, thawing losses after frozen storage, cooking yield after smoking, tenderness and organoleptic characteristics of the smoke meat. Equal numbers of male and female carcasses were chilled in 0%, 5%, or 7.5% brine solutions for 1, 2, 3 or 4 hr, in a 2 × 3 × 4 factorial experimental design. Removal of body heat of the carcass during chilling was accomplished at a much faster rate when brine-chilling was used. Increasing the brine concentration from 5 to 7.5% resulted in a faster cooling rate. Brine chilled carcasses had significantly ($P < 0.05$) greater percent water uptake during chilling, lower thaw losses and higher cooked yield than the slush-ice chilled (control) carcasses. Smoked meat from brine-chilled carcasses had significantly ($P < 0.05$) lower shear force readings (indicating more tender meat) and better organoleptic scores when compared with smoked meat from the control carcasses. Increasing the chilling time resulted in significantly more tender meat and better juiciness scores. Tissue NaCl concentration was increased when higher brine concentration or longer chilling time was used.

INTRODUCTION

FACTORS AFFECTING the quality of processed and further processed poultry meat have been the subject of research in recent years. In a study to determine the effect of salt brining and cooking procedure on tenderness of smoked cornish game hens, Janky et al. (1976) observed a "tenderizing effect" and concluded that this effect was primarily due to the salt brining rather than the smoking process. In a later report by Janky et al. (1978) it was observed that the addition of sodium chloride (NaCl) to chilling water of broilers slightly reduced the microbial flora and improved the organoleptic quality of the cooked broiler meat. Oblinger et al. (1976) reported that the immersion of ready-to-cook broilers in NaCl solutions prior to smoking increased the tenderness of the meat from these birds.

Antemortem injection of sodium chloride into beef cattle had no significant effect on tenderness (Huffman et al., 1967). Injection of beef muscle with solutions of NaCl, however, has been reported to increase water-holding capacity and tenderness (Wierbicki et al., 1954).

With the increased utilization of Bobwhite quail in further processed poultry food products, and as a commercially gourmet food item, Arafa et al. (1978b) studied the quality characteristics of Bobwhite quail scalded at different time and temperature combinations and suggested a temperature of 57.2°C for 30 sec for scalding quail. It was also observed in a later study (Arafa et al., 1978a) that brine chilling in 10% NaCl solution for 16 hr without agitation resulted in an objectionable salty taste in the smoked meat. These authors also reported problems with partial or complete freezing of the quail carcass during chilling when 10% brine was used with agitation.

Prior to the advent of reliable food refrigeration, curing and smoking of meat and meat products was done essentially to preserve the meat and to increase shelf-life. The smoking process accomplishes this by the action of various aromatic compounds with bacteriostatic characteristics and also by the physical effect of partial dehydration of the product surface (Lawrie, 1966). The salt (NaCl), incorporated into the tissues during the curing process is also considered to be a means of preservation (Schaible and Davidson, 1941) due to its effect on the water-activity levels in the product (Scott, 1957; and Lawrie, 1966). With advancement in food preservation techniques, curing and smoking have been used to impart desirable flavor characteristics and improve the appearance of products (Mast, 1978). Although cured and smoked pork and beef products have been more popular than poultry in the past, recently, the demand for further processed smoked poultry products has increased.

Brine chilling of poultry, including quails, before smoking would replace two further processing steps (chilling and salt curing) and would also affect meat quality characteristics. With this fact and other previous findings (Arafa et al., 1978a) in mind, we designed this study to: (1) Determine the best salt brine concentration and chilling time combination for Bobwhite quail which would be smoke, and (2) To study the effect of this combination on the processing characteristics of the carcass and the quality of the smoked product.

EXPERIMENTAL PROCEDURES

Processing Bobwhite quail

Four hundred eighty Bobwhite University of Florida strain quail, 16 wk of age, were slaughtered in two trials. The small size of the bird required a slight adaptation in the shackless system commonly used for broilers. Quail were processed in groups of 16 birds each (8 males and 8 females). They were suspended by placing the shanks in the modified shackles, then, they were killed using an outside cut and were allowed to bleed for 1 min.

Each group of birds was scalded using an Ashley Model #SS-36 scalder. A scalding temperature of 57.2°C for 30 sec as recommended by Arafa et al. (1978b) was used. Scalded birds were picked for 15 sec. using a commercial rotary drum picker (Ashley Model #SP-38). After passage through the mechanical picker, the shanks, head and neck were removed and the carcasses were then eviscerated including the crop and esophagus by splitting the back. The dressed weight of individual Bobwhite quail carcasses were recorded before chilling.

Cooling rates of quail carcass during brine-chilling

Equal numbers of males and females were randomly assigned to each of the three salt (NaCl) brine chilling concentrations (0, 5 and 7.5% sodium chloride solutions). Four different agitated chilling times (1, 2, 3 and 4 hr) were used within each brine concentration. This resulted in a 2 × 3 × 4 factorial arrangement of treatments. Agitation of the chilling solutions was accomplished using a one horsepower enpo-cornell pumps model M-A, type A-6 (Enpo-Cornell Pump Co., Piqua, OH.) This pump circulated the water at a rate of 51 L/min. A 2:1 brine to carcass ratio, w/w, was used. Specific gravity of the salt-brine chilling solutions was held constant during the entire chilling process by the addition of measured portions of

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saturated salt solutions to the chilling tanks to compensate for the added ice during chilling. A 1:1 ratio of ice to water was used. The ratio of chill water volume to carcass weight was kept constant across treatments. Temperature readings of 0.5, −1.5° and −4.5°C were recorded for the 0%, 5% and 7.5% NaCl solutions, respectively, at the end of the chilling process.

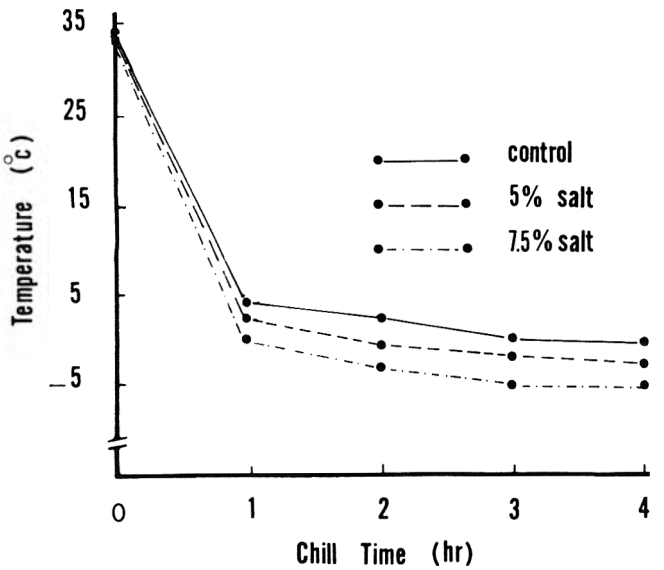

Fig. 1—Cooling curves of Bobwhite quail carcasses chilled in slush-ice, 5% and 7.5% salt (NaCl) brine solutions for 4 hr. "Temperature was continuously monitored from 0–4 hr.

Table 1—Mean % water uptake, % thaw loss, % cook yield, shear force values[a] and organoleptic scores[b] of brine chilled and smoked male and female Bobwhite quail

	Sex	
Variable tested	Male	Female
% Water uptake	7.75	7.69
% Thaw loss	5.00	4.96
% Cook yield	71.94	71.92
Shear force	10.38	10.26
Flavor scores	2.33	2.34
Tenderness scores	2.75	2.79
Juiciness scores	2.75	2.79

[a] kg of force/g of sample
[b] Possible scores ranged from 1 to 5 with 1 being the "best" and 5 being the "poorest."

Quail carcass temperature was continuously monitored from 0–4 hr using thermocouples and was recorded with a potentiometer. Thermocouples were inserted deep in the center of the breast muscle Pectoralis major, and were held in place with tape and rubber bands. After chilling, carcasses were rinsed with fresh water and allowed to drain for 15 min before they were individually weighed, packaged and stored at −10°C.

Percent thaw loss, cook yield, tenderness and organoleptic quality of brine chilled and smoked quail

After 6-wk frozen storage, the carcasses were thawed in a cooler at 3 ± 1°C for 60 hr. The thawed weights were obtained and the thawing losses were computed. Whole ready-to-cook (RTC) carcasses were slowly cooked with smoke in a Koch "Grand Prize" smoke house set at 93°C, with the smoke vent opened. Carcasses were hung in the smoke house and hickory sawdust was used as a source of smoke. After 30 min the smokehouse vents were closed and the temperature was raised to 107°C. The carcasses were then held in the smoke house for 4 hr at this temperature. Carcass internal temperature was monitored during smoking with a single point thermocouple inserted deep in the breast muscle. An internal temperature of 80°C was used as the endpoint. The cooked product was allowed to cool for 45 min before the weights were recorded for cooking yield computations. The smoke birds were then held at 1°C for 24 hr prior to shear force analysis and sensory evaluations.

Objective determinations of tenderness were made utilizing the Food Technology Corporation (FTC) texture press Model TP-1 equipped with a texture gage (TG-2A-0300) and standard shear compression cell (CS-1). A descent speed of 0.31 cm/sec was used. Sections of the Pectoralis major (20 × 40 × 3 mm) from each bird were sheared and the results were recorded as texture gage values (kg force/gram of sample).

Samples for organoleptic evaluation were taken from the Pectoralis major of each carcass, composited within each sex and treatment, and served to 20 experienced sensory panel members for evaluation. Panelists were asked to evaluate the samples for flavor, tenderness and juiciness on a scale of 1–5 with 1 being the "best" and 5 being the "poorest."

Percent NaCl, % water phase NaCl, % moisture and phenolic compounds contents of the brine chilled and smoked quail tissues

Eight carcasses (4 males and 4 females) from each treatment combination were used for these analyses. The skin was removed and meat from the Pectoralis major muscle from both males and females was composited within each treatment. Percent NaCl, % water phase NaCl and moisture content were determined using Association of Official Analytical Chemists procedures (AOAC, 1975). The method of Tucker (1942) was used to determine the concentration of phenolic compounds (expressed in mg/100g) in the smoked meat.

Statistical analysis

Data were subjected to the analysis of variance, Duncan's New Multiple Range Test using procedures described by Steel and Torrie (1961). Contour plots of brine concentrations and chilling times for all tested variables (% water uptake, % thaw loss, % cook yield, shear force values, flavor juiciness and tenderness) were obtained using the

Table 2—Mean[a] % water uptake, % thaw loss, % cook yield, shear force values[b] and organoleptic scores[c] of Bobwhite quail carcasses brine chilled for 1, 2, 3 and 4 hr in different NaCl-brine concentrations

	Chill time (hr)				Salt concentration		
Variable tested	1	2	3	4	0%	5%	7.5%
% Water uptake	6.30c	6.74c	8.33b	9.51a	6.34b	8.59a	8.24a
% Thaw loss	5.00a	4.73a	4.87a	4.91a	7.82a	3.73b	3.38b
% Cook yield	70.89c	71.61bc	72.29ab	72.98a	66.77b	74.31a	74.74a
Shear force	11.44a	10.43b	9.86c	9.54c	13.37a	8.87b	8.72b
Flavor scores	2.52a	2.34a	2.26a	2.19a	2.76a	2.09ab	2.14b
Tenderness scores	3.25a	2.95a	2.53ab	2.35b	3.30a	2.43b	2.57b
Juiciness scores	3.25a	2.83a	2.58ab	2.42b	3.21a	2.52b	2.58b

[a] Means within a row not followed by a common letter are significantly different ($P < 0.05$).
[b] kg of force/g of sample
[c] Possible scores ranged from 1–5 with 1 being the "best" and 5 being the "poorest."

methods described in the Statistical Analysis System of North Carolina State University (SAS, 1976). The benefit obtained by using this statistical tool was that it allowed for investigation of all possible combinations of the two main criteria (NaCl concentration in the chilling water and chilling time) which will yield a specific level of the tested variable. Since no treatment × trial interaction was found, the data from the two trials were pooled.

RESULTS & DISCUSSION

Cooling rates

Regardless of the chilling treatment involved in this study, the greatest reduction in internal carcass temperature occurred within the first hour of chilling (Fig. 1). As would be expected, removal of heat from the carcass was accomplished at a much faster rate when brine chilling was used. Internal carcass temperature decreased from approximately 33–34°C initially to 4°, 2.5° and 0°C for carcasses chilled in slush-ice (control), 5% and 7.5% (NaCl) solutions, respectively. Further reductions of carcass temperature were observed after 2 and 3 hr of chilling; however, increasing the chilling time from 3 to 4 hr had little or no effect on carcass internal temperature.

Increasing the NaCl concentration in the chilling water from 5% to 7.5% resulted in a faster cooling rate during the entire chilling period (Fig. 1). Brine temperature difference is expected to be the caustitive factors. These data agree with and support a previous finding reported by Arafa et al. (1978a). The partial freezing effect observed by these authors, when 10% NaCl brine-chilling was used for 1 hr, was also observed in this study but only after 3 hr of chilling in 7.5% NaCl brine. The immediate removal of body heat from dressed poultry is considered essential for the retention of quality (Tarver et al., 1956). Chilling quail carcasses in 5% brine solution for 2 hr, or in 7.5% brine for 1 hr quickly removed carcass body heat as indicated by the reduction in carcass temperature from approximately 33–34°C to 2.5 and 0°C, respectively.

Statistical analysis of the data obtained from males and females separately for any of the parameter tested did not show any significant difference between sexes (Table 1).

Percent water uptake

Chilling time, regardless of the NaCl concentration in the chilling media, had a statistically significant ($P < 0.01$) effect on the water uptake of the quail carcass during the chilling process (Table 2). Bigbee and Dawson (1963) reported that weight changes, as a result of water uptake, were most closely associated with the length of the chilling period, chilling medium and chilling temperature. Brine chilling in either 5% or 7.5% NaCl solution significantly ($P < 0.05$) increased the amount of water absorbed by the carcasses during chilling over the control. This finding does not agree with that of Gardner and Atkinson (1967), who reported that moisture absorption was not significantly affected by the addition of up to 10% NaCl in the chill water of broiler chickens. These data are in agreement however, with a report on broilers by Janky et al. (1978) and an earlier finding on beef by Wierbicki et al. (1954). A numerical decrease in water uptake from 8.59% to 8.24%, was observed when NaCl concentration was increased from 5% to 7.5% in the chilling water. This confirms a previous report by Arafa et al. (1978) where a similar effect was observed with quail carcasses when NaCl concentration was increased from 5% to 10% in the chill water.

The contour plot for % water uptake as shown in Figure 2 is divided into several areas designated as A, B, C, D, E, F, G, H, I and J. Each area on the plot will have a lower and upper limit (lower and higher % water uptake values in this case). What this plot tells us is that a line such as "line x" will represent numerous possible combinations of salt concentration (SC) and chill time (CT) that will have the water

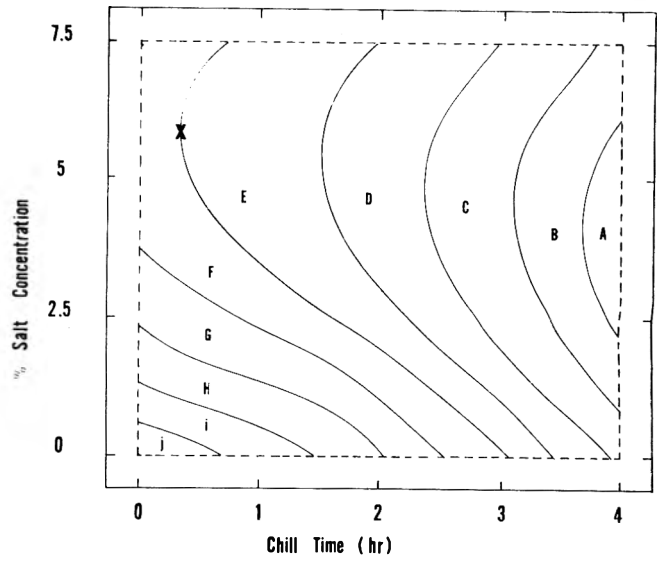

Fig. 2—Contour plot for % water uptake of Bobwhite quail carcasses chilled in different salt (NaCl) brine concentration 1, 2, 3 and 4 hr: A 10.4 – 10.1; B 10.1 – 9.4; C 9.4 – 8.8; D 8.8 – 7.4; F 7.4 – 6.8; G 6.8 – 6.1; H 6.1 – 5.4; I 5.4 – 4.8; J 4.8 – 4.4.

uptake value of 7.41%. Therefore, different points on this line will have different combinations of SC and CT but still the same water uptake. For instance, whether we chill Bobwhite quail in 4.5% salt solution for 1/2 hr or in 2% salt solution for approximately 2 hr, we should end up with the same water uptake value of 7.4 after chilling. A processor, therefore, may choose any combination of SC and CT that best fits his facilities and still expect the same water uptake.

% thaw loss

When the carcasses were frozen for 6 wk and allowed to thaw, numerically lower thaw losses were obtained as the chill time in NaCl solution was increased from 1 to 4 hr (Table 2).

The percent thaw loss from brine-chilled carcasses was

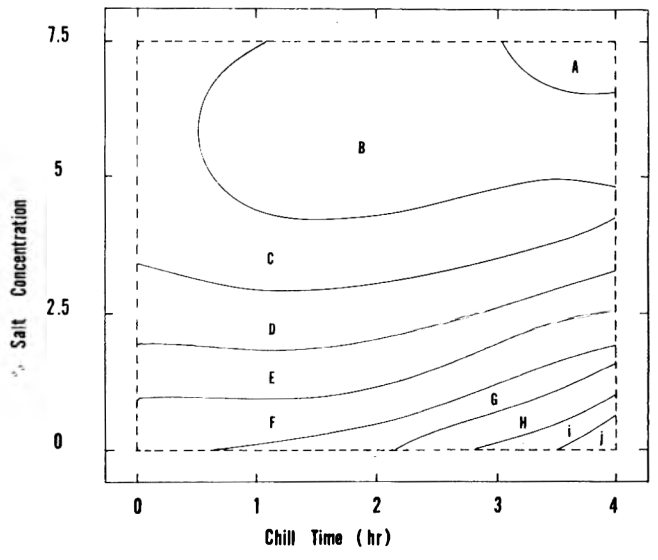

Fig. 3—Contour plot for % thaw loss after frozen storage of Bobwhite quail carcasses chilled in different salt (NaCl) brine concentrations for 1, 2, 3 and 4 hr: A 2.58 – 2.96; B 2.96 – 3.72; C 3.72 – 4.47; D 4.47 – 5.23; E 5.23 – 5.99; F 5.99 – 6.75; G 6.75 – 7.51; H 7.51 – 8.27; I 8.27 – 9.03; J 9.03 – 9.41.

Table 3—Percent sodium chloride (NaCl), % moisture, % water phase salt (WPS) and phenolic compounds (phenols[a]) content of smoked meat from quail carcasses chilled in 5% NaCl for 1, 2, 3 and 4 hr

	Chill time (hr)			
Variable tested	1	2	3	4
% NaCl	.88	1.60	1.77	1.88
% Moisture	70.4	69.8	69.0	68.8
% WPS	1.24	2.44	2.52	2.67
Phenols	0.79	0.77	0.81	0.77

[a] mg of phenolic compounds/100g of meat

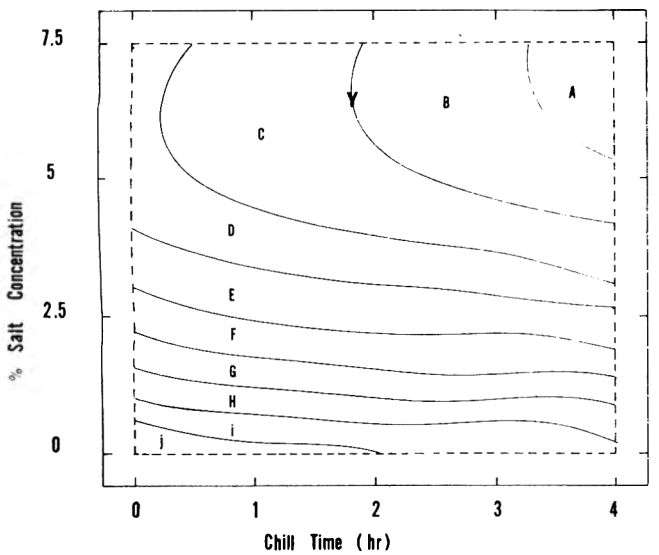

Fig. 4—Contour plot for % cook yield of Bobwhite quail carcasses chilled in different salt (NaCl) brine concentrations for 1, 2, 3 and 4 hr: A 76.3 – 75.8; B 75.8 – 74.7; C 74.7 – 73.6; D 73.6 – 72.5; E 72.5 – 71.4; F 71.4 – 70.4; G 70.4 – 69.3; H 69.3 – 68.2; I 68.2 – 76.0; J 67.0 – 66.5.

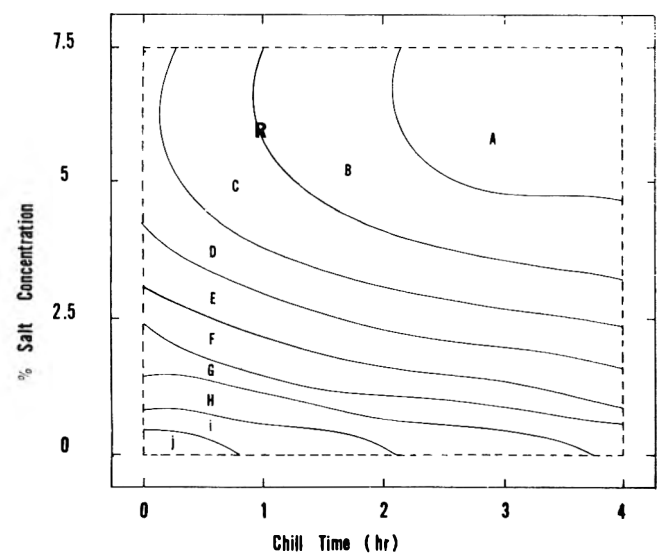

Fig. 5—Contour plot for shear force values of smoked meat from Bobwhite quail chilled in different salt (NaCl) brine concentrations for 1, 2, 3 and 4 hr: A 7.9 – 8.2; B 8.2 – 8.9; C 8.9 – 9.6; D 9.6 – 10.3; E 10.3 – 11.0; F 11.0 – 11.7; G 11.7 – 12.4; H 12.4 – 13.1; I 13.1 – 13.8; J 13.8 – 14.1.

significantly ($P < 0.05$) lower than that obtained with slush-ice chilled carcasses. Immersion chilling in slush-ice results in moisture uptake by the carcass; however, reports by Kotula et al. (1960) and May et al. (1963) have demonstrated that water picked up during chilling in slush-ice is loosely bound and much of it is leaked out during marketing. It is apparent from these data that brine-chilling in either 5% or 7.5% NaCl solution increased water-holding capacity (Wierbicki et al., 1954; Janky et al., 1978) of the tissue. Thus, less thaw loss was observed.

Thaw loss appeared to be lowest from the contour plot (Fig. 3) when at least 3% salt was used for any of the four chilling times tested.

Cook yield

As the chill time increased, a significant ($P < 0.05$) increase in percent cook yield was observed. A cook yield of 70.89%, 71.61%, 72.29% and 72.98% was observed for 1, 2, 3 and 4 hr of chilling, respectively. Cook yield data support and agree with water uptake values and the thaw loss data (Table 2). Brine chilling resulted in significantly ($P < 0.05$) higher cook yields as compared with the slush-ice chilling. A smoked-cooked yield of 66.77%, 74.31% and 74.74% was observed for the control chilled, 5% and 7.5% brine-chilled carcasses respectively. The control chilled (0% salt) group had the highest % drip loss and the lowest cook yield. This finding agrees with and support previous reports on chicken by Bailey et al. (1948) and Arafa and Chen (1978) who indicated that most of the water uptake by the carcass during immersion chilling in slush-ice is lost during storage and cooking.

The contour plot for % cook yield is shown in Figure 4. According to the same basis discussed earlier, it was observed that whether quail carcasses were chilled in 7% salt solution for 1 hr and 50 min or in 4.5% salt solution for approximately 3 hr and 14 min the same cook yield value of 74.71% (line Y) should be expected after smoking. It was also observed that % cook yield increased when longer chilling times and higher salt concentrations were used.

Shear force

Increasing the chilling time from 1 to 2 or 3 hr resulted in significantly more tender meat as indicated by lower shear force values (Table 2). No significant differences in tenderness were observed in meat from carcasses chilled for 3 or 4 hr.

Brine chilling of quail resulted in lower shear force readings, indicating more tender meat. This finding is in agreement with previous reports by Oblinger et al. (1977) on spent hens and Arafa et al. (1978a) on Bobwhite quail.

Figure 5 shows the contour plot of SA and CT for tenderness (as indicated by shear force values) of brine chilled and smoked Bobwhite quail. Line "R" on this plot indicates all possible combinations of SA and CT that should result in a smoke quail with 8.95 kg/g shear force reading. Thus, chilling in 6.0% salt-brine solution for approximately 55 min or in 3.5% salt-brine solutions for 3 hr and 15 min would have the same tenderizing effect and should result in the same shear force reading of 8.95 kg/g.

Organoleptic scores

Organoleptic scores indicated a numerically, but not statistically significant improvement in flavor scores for the smoked quail meat when chilling time was increased (Table 2). Taste panel scores for tenderness and juiciness were significantly ($P < 0.05$) decreased, when carcasses were chilled for either 3 or 4 hr. Flavor, tenderness and juiciness scores indicated that panelists significantly ($P < 0.05$) preferred the smoked meat from brine-chilled quail carcasses as compared to that of the slush-ice chilled carcasses.

Increasing the NaCl concentration from 5 to 7.5% in the

chill water did not significantly effect organoleptic scores; however, the 5% brine-chilled quail were preferred by the taste panelists. The objectionable salty taste of the smoke meat, observed when the brine concentration was increased from 5 to 10% (Arafa et al., 1978a) was not observed in this study as the brine concentration was increased from 5 to 7.5%. It appeared from the contour plots of flavor, tenderness, and juiciness (Fig. 6, 7 and 8) that higher NaCl concentration and longer chill times produced the most tender, juicy, and flavorful smoked meat.

Moisture, sodium chloride, water phase salt and phenolic compounds of brine chilled and smoked quail

The differences in water uptake by the quail carcass during chilling, thaw loss after frozen storage, cook yield and shear force values between the 5% and the 7.5% NaCl treatments were minimal. However, the organoleptic quality of the 5% brine-chilled quail was superior when compared to the 7.5% NaCl treatment. Therefore, moisture, NaCl and phenol analyses were conducted only on the 5% brine chilled and smoked quail. Tissue salt concentration increased as the chilling time increased (Table 3). This finding was observed whether the NaCl was reported as % NaCl or as % water phase salt. A gradual reduction in tissue moisture of the smoked-cooked carcass was observed when the chill time was increased. Tissue moisture for carcasses chilled in 5% salt-brine for 1, 2, 3 and 4 hr was 70.4%, 69.8%, 69.0% and 68.8%, respectively. Chill time had little effect on absorption of phenolic compounds. As the chill time was increased from 1 to 4 hr, phenolic concentrations remained relatively the same.

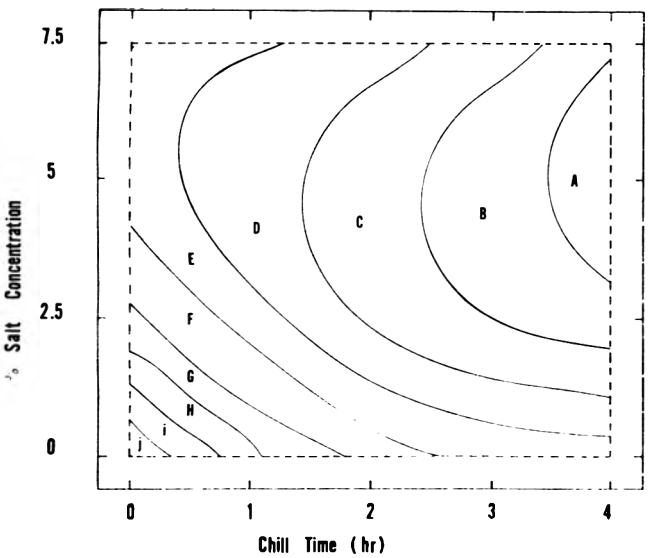

Fig. 6—Contour plot for flavor judging scores of smoked meat from Bobwhite quail carcasses chilled in different salt (NaCl) brine concentrations for 1, 2, 3 and 4 hr: A 1.86 – 1.91; B 1.91 – 2.02; C 2.02 – 2.13; D 2.13 – 2.24; E 2.24 – 2.34; F 2.34 – 2.45; G 2.45 – 2.56; H 2.56 – 2.67; I 2.67 – 2.78; J 2.78 – 2.83.

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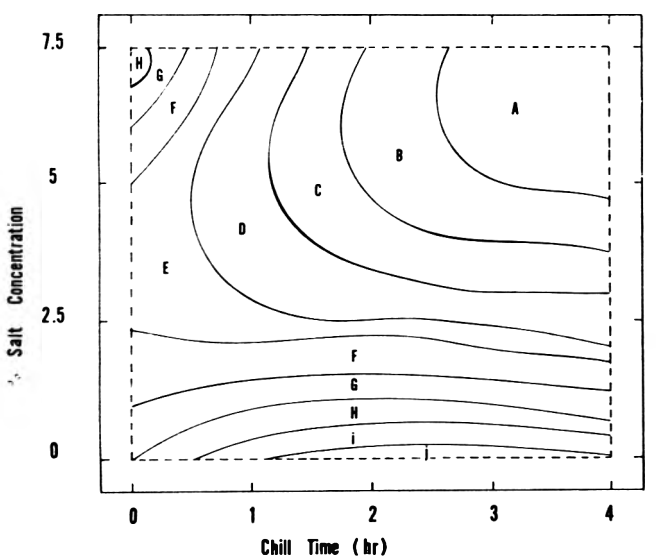

Fig. 7—Contour plot for tenderness judging scores of smoked meat from Bobwhite quail carcasses chilled in different salt (NaCl) brine concentrations for 1, 2, 3 and 4 hr: A 1.99 – 2.10; B 2.10 – 2.32; C 2.32 – 2.54; D 2.54 – 2.76; E 2.76 – 2.98; F 2.96 – 3.20; G 3.20 – 3.42; H 3.42 – 3.64; I 3.64 – 3.86; J 3.86 – 3.97.

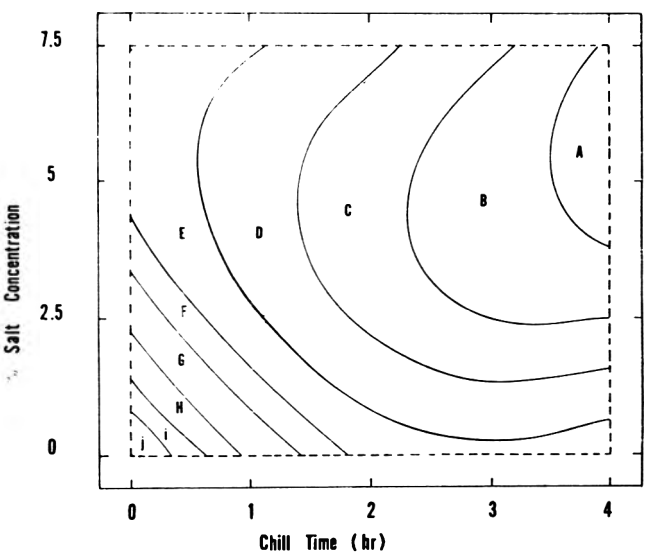

Fig. 8—Contour plot for juiciness judging scores of smoke meat from Bobwhite quail carcasses chilled in different salt (NaCl) brine concentrations for 1, 2, 3 and 4 hr: A 2.12 – 2.21; B 2.21 – 2.40; C 2.40 – 2.59; D 2.59 – 2.78; E 2.78 – 2.97; F 2.97 – 3.16; G 3.16 – 3.35; H 3.35 – 3.54; I 3.54 – 3.73; J 3.73 – 3.82.

YIELD AND COMPARISON OF NUTRITIVE AND ENERGY VALUES: PIGS' EARS

M. W. VAUGHN, D. P. WALLACE and B. W. FORSTER

ABSTRACT

Raw and cooked pigs' ears tissue were compared for yield, proximate mineral, vitamin, amino acid, saturated and polyunsaturated fats, and cholesterol composition and for protein efficiency ratio. The raw yield was 94.7%, a cooking loss of 1.8%, while the total loss was 7.1%. Cooking these connective tissues indicated a significant increase in moisture apparently due to gelatinized collagen. Cooking produced significant losses of protein and caloric values. There were some changes in minerals, vitamins, and amino acids. The raw and cooked tissue data revealed negative vitamins A and C and a significant difference for thiamin, riboflavin, and polyunsaturated fats; but no significant difference for saturated fats and cholesterol. Protein evaluation results clearly indicated that the estimated PER predicted the PER of the pigs' ears tissue within ±0.2 of the corrected rat bioassay PER. The corrected and est. PER for raw ears were 0.60 and 0.71, respectively, while the corrected and est. PER for cooked ears were 0.68 and 0.81, respectively.

INTRODUCTION

SINCE VERY LITTLE has been reported on the nutritive value of selected pork by-products, this study is the second in a series to determine the yield as well as the nutritive value of pigs' ears. In this study all composition values will be determined on paired raw and cooked pigs' ears tissue. These data could be useful for the nutritive data bank (Watt and Merrill, 1963; AMIF, 1964), for consumers, and the general public.

Currently the documented statistics on the consumption of pigs' ears in the United States are very sparse. Our best estimates are that pigs' ears are eaten by 5–10% of the U.S. population.

According to Watt and Merrill (1963), the AMIF (1964), and Orr and Watt (1957), no data have been included on the proximate analyses, caloric values, minerals, vitamins, amino acids, protein efficiency ratio, saturated and polyunsaturated fats, and cholesterol content of pigs' ears.

EXPERIMENTAL

USING STANDARD statistical procedures with a 95% level of confidence, four replications were needed to assure the precision desired.

Three brands of frozen raw pigs' ears were purchased from retail stores on the Lower Eastern Shore of Maryland. These pigs' ears were used to determine yield, total loss and distribution of waste in preparation on the basis of purchased weights. Four composite raw and cooked pigs' ears tissue samples were analyzed for proximate composition, minerals, vitamins, amino acids, saturated and polyunsaturated fats, and cholesterol. Food energy was calculated from the proximate composition. The protein efficiency ratio and digestibility were accomplished by rat bioassay procedures. The estimated protein efficiency ratio (est. PER) was calculated for each replication of raw and cooked products from amino acid data using Eq (3) of Alsmeyer et al. (1974).

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Preparation of samples

There were three available sources of frozen raw pigs' ears. The three brands were labeled A, B, and C. Four samples were purchased for each brand giving a total of twelve (12) purchased samples of 15-lb each in polyethylene wrap.

Yield

The weight of the pigs' ears as purchased was ascertained. Samples were coded with an equal number of raw samples for cooking purposes. Thawing was accomplished in the refrigerator at 1.1°C during a period of 48 hr. The total amount of trim from each coded sample was small and was mainly due to discolorations and hair. The ears were washed twice in cold tap water, then drained 5 min. The drained, washed, trimmed ears were weighed in order to calculate the thawed and trimmed losses.

A 12-qt stainless steel covered pot was used for the cooking process. Cold water was added until ears were covered, heated to a full boil, reduced to simmering temperature (85–98°C), and simmered for 4 hr or until tender. Ears were removed from heat and drained in a drain pan for 5 min. Afterwards, the cooked weight and losses during cooking were calculated (Table 1).

Preparation for chemical analyses

The raw and cooled drained cooked ears were stored in polyethylene freezer bags, air exhausted, sealed, and chilled in a refrigerator at 1.1°C. The chilled ears were chopped rapidly in an electric meat chopper which had 1/8-in. blade and a capacity to chop 10 lb of meat per minute. The ears were passed through the electric meat chopper three times and were mixed thoroughly after each grinding. Individual samples were mixed thoroughly after each grinding. Individual samples were mixed in an extra powerful electric mixer with a 5-qt stainless steel bowl (Kitchen Aid Model K5–A). The composite was mixed in a 60-qt electric mixer (Hobart Mixer, Model H 600 – Timed Mixer). For chemical analyses, 100g composite raw and cooked ears tissue were sealed in polyethylene freezer bags and stored at −23.3°C in plastic freezer bags.

Chemical analyses

All chemical analyses were accomplished by means of four replicate determinations on raw and cooked composite samples as reported by Vaughn et al. (1978). Determinations were made on moisture, protein, fat (petroleum ether extractables), ash, calcium, magnesium, iron, phosphorus, sodium, potassium, vitamins A and C (ascorbic acid), thiamin, riboflavin, niacin, trilaurin, trimyristin, tripalmitin, tristearin, trilinolein, cholesterol, and the amino acid profile. Protein and fat factors from (Merrill and Watt, 1955) were used to calculate the caloric content.

The methods of Sheppard et al. (1974) were used to determine saturated and polyunsaturated triglycerides and cholesterol as previously described by Vaughn et al. (1978). The saturated triglycerides (C_{12}–C_{18}) and cholesterol were determined by gas-liquid chromatography. The saturated triglycerides were determined by fat extraction, saponification and conversion to fatty acid methyl esters. The fatty acid methyl esters were then converted to each of its appropriate triglyceride weight, i.e. (0.995: conversion methyl −16:0 to tripalmitin). From a portion of the solution of fatty acid methyl esters, cholesterol was converted to cholesteryl butyrate, and calculated from the cholesteryl butyrate chromatogram. The polyunsaturated triglyceride (trilinolein) was determined by an enzymatic method. Fatty acids were saponified to potassium salts. The salts of the cis, cis-methylene interrupted (18:2) were then conjugated and oxidized to hydroperoxides. The weight of (18:2) was calculated from the absorbance of the conjugated diene hydroperoxide measured at 234 nanometers.

Protein efficiency ratios and digestibility

Protein efficiency ratios and digestibility were determined on the ANRC protein (casein) as the control as well as the freeze-dried raw and cooked ears' tissue.

Table 1—Yield, proximate composition and caloric content of thawed, trimmed, raw and cooked pigs' ears tissue

Component	Raw		After cooking		Cooking loss	
	%[a] ± S.E.	g/kg	%[a] ± S.E.	g/kg[b]	g/kg[b]	t value[d]
Thaw juice and trim	5.3	53	—	—	—	
Yield	94.7	947	92.9[c]	929	18	
Moisture	62.4 ± 0.33	591	69.7 ± 0.50	648	+57	13.26
Dry Matter	37.6	356	30.3	281	75	
Protein (N X 6.25)	22.3 ± 0.13	211	16.4 ± 0.08	152	59	41.76
Fat	14.0 ± 0.10	133	13.5 ± 0.10	125	8	0.77
Ash	0.6 ± 0.03	5.7	0.4 ± 0.00	3.7	2	0.71
Calories/100g	222.0 ± 0.08		192.0 ± 3.00			7.10

[a] As is basis
[b] Of starting material
[c] Represents a cooking loss of 1.8% and a total loss of 7.1% of the product as purchased
[d] t value significant at 1% level if >3.707

In freeze-drying the samples, 3½ lb were placed on individual stainless steel trays 10½ in. × 35¼ in. The samples in the trays were kept frozen until they were freeze-dried. The samples were freeze-dried in a Stokes shelf freeze-dryer, steam-operated, at 40°C with a vacuum of 1 mm of mercury or less for 14 hr.

Est. PER's were calculated from grams of amino acid per 100g amino acids. Since the equation of Alsmeyer et al. (1974) was based on an amino acid composition including hydroxyproline and hydroxylysine, the percentages of total amino acids and of histidine, methionine, leucine, proline and tyrosine were recalculated taking into account the quantity of hydroxyproline estimated from the proline content of collagenous tissue (10.31% for the raw and 9.74% for the cooked ears) and 1.00% hydroxylysine. Hydroxproline and hydroxylysine were estimated from the hydroxyproline, hydroxylysine and proline content of collagen in beef fatty tissue as reported by Happich et al. (1975). Est. PER was calculated by personnel from the USDA Eastern Regional Research Center, SEA. Comparisons were made between corrected PER values and est. PER's.

Statistical analyses

Statistical analyses of the data were made by the paired "t" test for means. The significance of the difference of the means of the composition values were calculated from the formula of Dixon and Massey (1957).

RESULTS & DISCUSSION

YIELD DATA are presented in Table 1. The percent by weight of thaw-juice and trim of Brands A, B and C combined indicated a mean raw tissue loss of 5.3%, and a raw yield of 94.7%. The cooked yield was 92.9%.

Proximate composition and caloric content are also presented in Table 1. The raw and cooked tissue indicated a significant difference for moisture, protein, and caloric content at the 1% level but no significant difference for fat and ash. Cooked samples showed an increase in moisture content. In cooking these tissues in water at the simmering temperature for 4 hr or until tender, collagen may have been gelatinized in sufficient quantities to absorb water to account for the moisture content in the cooked samples. This method of cooking is adapted to the less tender cuts or those containing large amounts of connective tissue such as pigs' ears tissue. Collagen, the principal protein of connective tissue, is a natural component of meat, and affects the protein quality of meat. Paul and Palmer (1972) stated that long chain molecules such as gelatin and many other proteins have the ability to absorb large quantities of water. Cooking produced significant losses of protein and caloric values. The caloric content of the raw and cooked tissue was due largely to fat. Fat accounted for more than one-half of the calories.

Mineral content data are presented in Table 2. A significant difference of the raw and cooked means at the 1% level was indicated for calcium, iron, sodium, and potas-

Table 2—Mineral and vitamin content of a composite sample of thawed, trimmed, raw and cooked pigs' ears tissue

Component	Raw	Cooked	t value[c]
	Mean value mg/100g ± S.E.		
Calcium	20.5 ± 0.31	18.4 ± 0.27	6.65
Magnesium	7.1 ± 0.10	7.1 ± 0.31	0.24
Iron	2.4 ± 0.16	1.5 ± 0.07	7.82
Phosphorus	40.5 ± 6.08	24.3 ± 3.63	3.19
Sodium	190.6 ±10.40	166.5 ± 0.07	5.18
Potassium	55.3 ± 0.10	40.3 ± 0.29	15.30
Vitamin A[a]	0.00[b]	0.00[b]	
Thiamin	0.08 ± 0.00	0.02±0.00	18.76
Riboflavin	0.11 ± 0.00	0.07±0.00	5.63
Niacin	0.78 ± 0.02	0.56±0.07	3.21
Ascorbic acid	0.00[b]	0.00[b]	

[a] Indicates IU/100g
[b] (0.00) Indicates none
[c] t value significant at the 1% level if >3.707

Table 3—Amino acid composition of a composite sample of thawed, trimmed, raw and cooked pigs' ears tissue (g amino acid/100g amino acid)

Amino acid	Raw	Cooked	t value[a]
	Mean value ± S.E.		
Essential amino acids			
Tryptophan	0.2 ± 0.04	0.2 ± 0.04	0.00
Threonine	2.8 ± 0.02	3.0 ± 0.04	5.20
Isoleucine	2.2 ± 0.04	2.3 ± 0.06	3.35
Leucine	5.2 ± 0.06	5.5 ± 0.08	4.53
Lysine	4 7 ± 0.10	4.6 ± 0.11	1.62
Methionine	0.6 ± 0.37	0.8 ± 0.32	0.67
Phenylalanine	3.2 ± 0.03	3.2 ± 0.11	0.00
Tyrosine	1.8 ± 0.02	2.0 ± 0.05	3.79
Valine	3.7 ± 0.12	4.0 ± 0.14	1.92
Histidine	1.2 ± 0.02	1.2 ± 0.07	0.00
Total	25.6	26.8	
Nonessential amino acids			
Alanine	9.9 ± 0.09	9.6 ± 0.13	3.56
Aspartic acid	7.4 ± 0.08	7.4 ± 0.11	0.75
Glutamic acid	12.5 ± 0.08	12.6 ± 0.05	1.31
Glycine	19.6 ± 0.10	19.7 ± 0.13	0.79
Proline	12.7 ± 0.05	12.0 ± 0.13	7.31
Serine	4.2 ± 0.03	4.3 ± 0.03	3.61
Arginine	8.3 ± 0.06	8.0 ± 0.09	4.89
Total	74.6	73.6	

[a] t value significant at the 1% level if >3.707

Table 4—Protein efficiency ratio and digestibility of a composite sample of thawed, trimmed, raw and cooked pigs' ears tissue

Protein source	Total wt gained (g) ± S.E.	Total feed consumed (g) ± S.E.	PER ± S.E.			% Digestibility	
			Observed	corrected	estimated	Diet	Nitrogen
ANRC protein (casein)	186 ± 10.0	383 ± 24.0	3.40 ± 0.03	2.50	–	95	93
Pigs' ears, raw	70 ± 2.0	169 ± 11.0	0.82 ± 0.04	0.60	0.71 ± 0.00	95	88
Pigs' ears, cooked	72 ± 2.0	173 ± 4.0	0.93 ± 0.09	0.68	0.81 ± 0.01	95	92

[a] PER (protein efficiency ratio) = weight gain/protein intake
[b] Digestibility: Diet = feed intake – fecal weight/feed intake X 100 Nitrogen = N intake – fecal N/N intake X 100
[c] Eq (3) (est. PER) = −1.816 + 0.435 (MET) + 0.780 (LEU) + 0.211 (HIS) – 0.944 (TYR), Alsmeyer et al. (1974)

sium, but no significant difference for magnesium, and phosphorus.

Vitamin contents are also presented in Table 2. The results showed negative vitamins A and C (ascorbic acid) values. There were significant differences of raw and cooked means at the 1% level for thiamin and riboflavin, but no significant difference for niacin.

Data are presented in Table 3 for the amino acid profile and grouped into essential and nonessential amino acids. Histidine is included as an essential amino acid because it is essential in the rat diet. Tyrosine also has been included with the essential amino acids because tyrosine can replace part of the phenylalanine requirement (Rama Rao et al., 1961).

For the essential amino acids, there was no significant difference in the raw and cooked means at the 1% level for lysine, histidine, valine, methionine, isoleucine, phenylalanine and tryptophan but a significant difference for threonine, leucine and tyrosine. Cystine was destroyed during hydrolysis and analysis of the amino acids, WARF Institute (1978). More than 60% of the essential amino acids revealed no significant difference at the 1% level. Tryptophan, histidine, and the sulfur amino acids were limiting in the raw and cooked tissue.

For the nonessential amino acids, no significant difference of the raw and cooked means at the 1% level was indicated for serine, glutamic and aspartic acids, glycine, and alanine but a significant difference for arginine and proline. Raw and cooked pigs' ears tissue showed a high total nonessential amino acid content. The glycine content was 19.6g and 19.7g per 100g amino acids, respectively, for the raw and cooked tissue, which apparently indicated a high amount of collagen.

The proximate analysis indicated moisture, 4.2%; protein, 59.6%; fat, 35.2%; and ash, 1.3% for the raw freeze-dried samples, as compared to moisture, 4.5%; protein, 56.0%; fat, 37.2%; and ash 1.4% for the freeze-dried cooked samples. The results from the microbiological tests indicated that both raw and cooked samples had a plate count of about 10^6 and that the chief microbiological contaminant was *pseudomonas sp.*

Rat bioassay PER was determined with the diet protein adjusted to 10% according to AOAC procedures (1975). Rats of the Sprague-Dawley strain (5 male weanling per group) were used. ANRC protein (casein) was fed as the protein in the control diet. The observed PER values were corrected to that of ANRC protein at 2.5. Standard deviation was determined and Duncan Multiple Range tests were calculated on the observed PER values before correction to 2.5.

Results of the PER tests and digestibility are shown in Table 4. The apparent nitrogen digestibility of these proteins determined were 88% and above, indicating that the nutritional value of the product proteins should be related directly to their amino acid composition. Happich et al. (1975) reported that with a variety of meat products, the observed PER values were directly proportional to the total quantity of essential amino acids present.

Table 5—Fat and cholesterol composition of a composite sample of thawed, trimmed, raw and cooked pigs' ears tissue

Saturated and polyunsaturated fats and cholesterol			
Fat[a]	Raw Mean value g/100g ± S.E.	Cooked Mean value g/100g ± S.E.	t value[c]
12:0[a]	<0.01	<0.01	
14:0	0.07 ± 0.01	0.07 ± 0.00	0.00
16:0	1.65 ± 0.08	1.99 ± 0.15	3.10
18:0	0.57 ± 0.01	0.68 ± 0.05	3.23
18:2[b]	0.80 ± 0.01	0.14 ± 0.00	10.10
Cholesterol	0.082 ± 0.003	0.090 ± 0.007	0.17

[a] 12:0 = trilaurin; 14:0 = trimyristin; 16:0 = tripalmitin; 18:0 = tristearin
[b] Trilinolein
[c] t value significant at the 1% level if >3.707

Alsmeyer et al. (1974) reported that their Eq (3) is a reliable estimator of PER when it is used with products that contain primarily meat, poultry, grain, or yeast origin. Since it is generally assumed that the standard error among duplicate PER determinations in the rat bioassay is 0.2 PER, a prediction equation which can predict PER within ±0.2 such as Eq (3) should be regarded as a reliable and effective estimator of PER.

Our study indicated that the est. PER closely approached corrected PER values. Raw ears showed a difference of 0.11 PER between corrected and est. PER, and the cooked product showed a difference of 0.13 PER between corrected and est. PER. PER's determined by rat bioassay and calculated for the raw and cooked product were less than 1.00. The low PER's were apparently due to the limiting amino acids. These considerations indicate that pigs' ears tissue would be protein nutritionally unacceptable when used alone and is not equal to that of meat (PER 2.3–2.9).

The saturated and polyunsaturated fats as well as the cholesterol data are given in Table 5. No significant difference of raw and cooked means for C12:0, C14:0, C16:0, and C18:0 at the 1% level was indicated. A significant difference in polyunsaturated fat (C18:2) of the raw and cooked means at the 1% level was indicated. No significant difference was confirmed in the cholesterol content of the raw and cooked means at the 1% level for pigs' ears.

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—Continued on page 1454

BINDING OF MEAT PIECES: AN INVESTIGATION OF THE USE OF MYOSIN-CONTAINING EXTRACTS FROM PRE- AND POST-RIGOR BOVINE MUSCLE AS MEAT BINDING AGENTS

R. H. TURNER, P. N. JONES and J. J. MACFARLANE

ABSTRACT

The feasibility of using myosin, extracted from post-rigor bovine muscle, as a binding agent for meat pieces, was examined. Crude myosin was extracted with solutions containing various concentrations of sodium chloride (salt) and sodium tripolyphosphate (TPP). Maximum yield was obtained using 1M salt and about 0.25% TPP in the extracting solution. Binding strength, i.e. the strength with which pieces of meat adhere, of crude myosin extracted with 1M salt and 0.15, 0.25 or 0.5% TPP were not significantly different and were only slightly lower than that for crude myosin extracted from pre-rigor muscle. The myosin preparations had binding strength much greater than that reported previously for actomyosin. These results show that crude myosin extracted from postrigor bovine muscle has a potential use as a meat binding agent.

INTRODUCTION

THE NATURE of the bond between meat pieces in re-formed meat products has been the subject of several recent studies, see, e.g. Theno et al. (1978), Siegel et al. (1978). Macfarlane et al. (1977) compared the effectiveness of myosin, actomyosin and sarcoplasmic proteins as binding agents for meat pieces. To do this, suspensions of these proteins were prepared at several protein concentrations and with various amounts of added sodium chloride up to 1.4M. An aliquot from each suspension was located between two pieces of muscle cut to a standard cross-sectional area, then cooked. The force required to separate the two pieces of meat is referred to as the binding strength. At salt concentrations up to 1M, the binding strength of myosin was superior to that of actomyosin or sarcoplasmic protein. It appeared that even at low salt concentrations myosin could be useful for binding purposes in re-formed meat products. Another study by Ford et al. (1978) indicated that similar binding ability to that found for relatively pure myosin was obtained using a crude myosin preparation. The present investigation was undertaken to ascertain whether a myosin-containing fraction with similar binding properties to the crude and the relatively pure myosins prepared from pre-rigor muscle, could be easily extracted from post-rigor muscle with solutions of salt and polyphosphate.

The first part of the investigation was concerned with the effect of change in salt and polyphosphate concentration on the yield of crude myosin from post-rigor muscle. Subsequently the binding ability of selected crude myosin preparations was assessed and the binding of one of these was compared to that of crude myosin from pre-rigor muscle.

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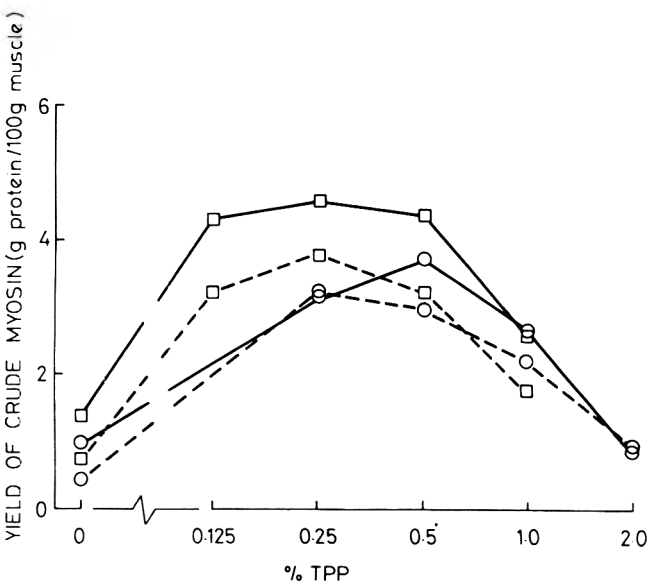

Fig. 1—Effect of sodium tripolyphosphate and sodium chloride concentration in the extraction solution on the yields of crude myosin: □ animal 1; ○ animal 2; Full lines 1.0M NaCl; broken lines 0.5M NaCl.

EXPERIMENTAL

Effect of sodium tripolyphosphate (TPP) and sodium chloride (salt) concentration on extracts from post-rigor meat

Two experiments are reported:

Experiment 1. Semitendinosus muscles were obtained from the carcasses of two 3–4 yr old bullocks approximately 2 days after slaughter at a local abattoir. Each chilled muscle was ground in a mincer with a 4.5 mm plate and then divided into 10 portions of 100g.

Solutions were prepared with NaCl and TPP levels as indicated in Figure 1. A 300-ml aliquot of each solution was used to extract separate 100g ground meat samples. Grinding of samples and preparation of solutions, extractions and subsequent isolation of protein fractions were carried out in a room, temperature-controlled at 1°C. Each mixture was blended using an Ultra Turrax Mixer Emulsifier Type Tp 18/2 (Janke and Kunkel K.G., Staufen i, Br., W. Germany) for 30 sec then allowed to stand for 30 min. Each was then centrifuged at 13,000 × G for 15 min and the supernatant liquid was decanted then filtered through cheesecloth. The protein content of this fraction was estimated by removing an aliquot and measuring protein content by the biuret method described by Gornall et al. (1949). The remaining supernatant liquid was diluted with deionized water to a sodium chloride concentration of approx. 0.03M (24 volumes and 11.5 volumes of deionized water, respectively, to 1 volume of the 1M and 0.5M salt solutions). The protein precipitate that formed (herein referred to as crude myosin) was allowed to settle overnight and the clear liquid was then siphoned off and discarded. The crude myosin was further concentrated by centrifugation at 13,000 × G for 1 hr. Yield of crude myosin was calculated from the weight and protein content (biuret method, Gornall et al., 1949) of the residue and expressed as g protein per 100g of muscle. The protein composition of each crude myosin preparation was investigated by means of sodium dodecylsulphate (SDS) gel electrophoresis (Weber and Osborn, 1969). Actin was identified from mea-

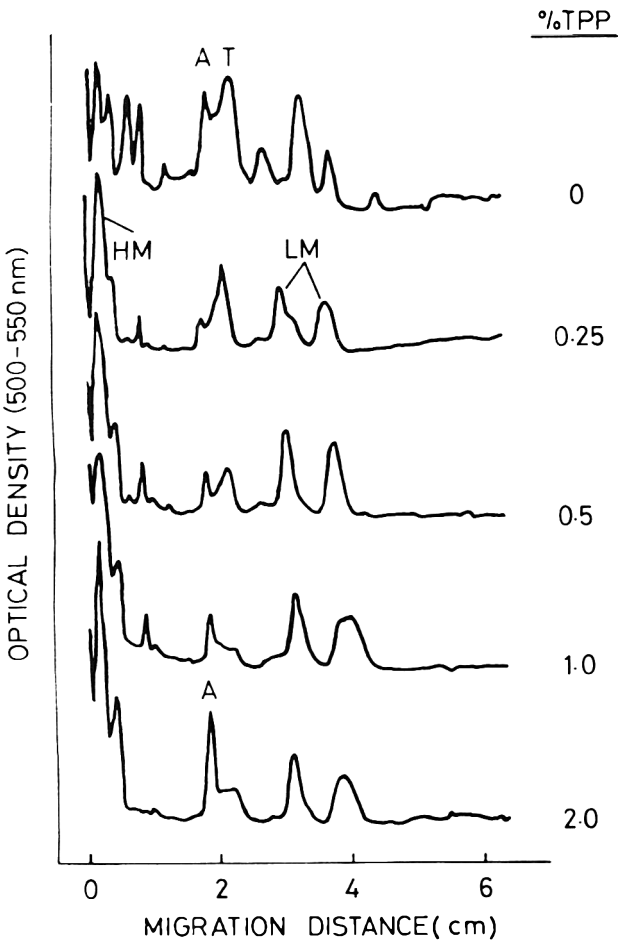

Fig. 2—Densitometric scans of polyacrylamide electrophoresis gels corresponding to some of the crude myosin preparations of Fig. 1. TPP concentrations of extraction solutions, which all contained 1.0M sodium chloride, are indicated on the scans. The same quantity of protein was applied to each gel: A = actin; T = tentatively assigned to tropomyosin; HM = myosin heavy chains; LM = myosin light chains.

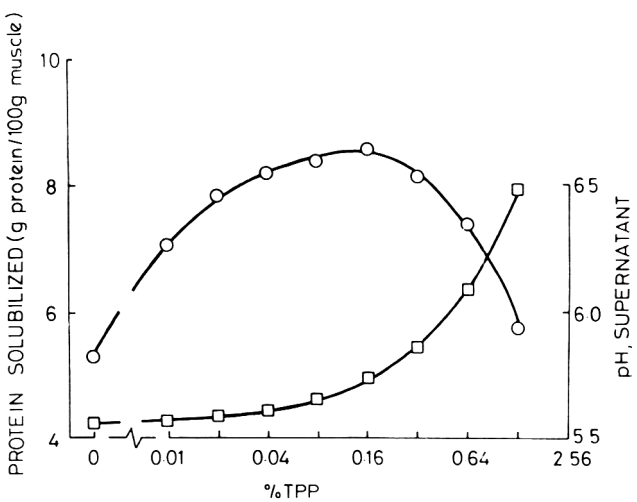

Fig. 3—Change in protein solubilized from muscles with change in the concentration of sodium tripolyphosphate in the extraction solution. All solutions contained 1.0M sodium chloride. The results are the means from experiments on three muscles: ○ g protein solubilized/100g muscle; □ pH, supernatant

surements of the electrophoric migration of a sample of the purified protein.

Experiment 2. Semitendinosus muscles were obtained from three beef carcasses, processed in a similar manner to that described in Experiment 1. The muscles were ground separately and then divided into 10-g portions. Solutions with TPP concentrations as shown in Figure 3 and containing 1M NaCl, were prepared and 30 ml of each was used to extract protein from a 10-g sample of ground meat. Extractions were carried out at 1°C. Samples were blended in an Ultra Turrax Mixer Emulsifier for 30 sec, allowed to stand for 30 min, then centrifuged at 13,000 × G for 15 min and the supernatant liquid was collected. Protein content of the supernatant liquid was determined by the biuret method and is expressed as g protein per 100g of muscle.

Binding strength of crude myosin extracted from post-rigor muscle

Samples of ground meat were prepared from post-rigor beef semitendinosus muscles from four carcasses, using experimental conditions as described under Experiment 1. Extraction, precipitation and concentration of protein also followed the procedure described above, except that only three solutions were investigated for extraction of protein from the ground meat, namely 0.15, 0.25 and 0.5% TPP in 1.0M sodium chloride. Mixtures containing 5% w/w crude myosin in 0.4 and 1.0M NaCl solutions were prepared and binding strength for meat pieces measured as described by Macfarlane et al. (1977).

Comparison of binding strength of crude myosin preparations from pre- and post-rigor muscles

Pre-rigor. Crude myosin was prepared from the semitendinosus muscles from three, 3–4 yr old bullocks using the procedures described by Ford et al. (1978). In brief, within 1 hr of slaughter, 200g of the ground muscle were extracted with 600 ml of a solution containing 0.06M Na_2HPO_4, 0.09M NaH_2PO_4 and 0.3M NaCl (pH 6.5). The extract was diluted with 19 volumes of deionized water to precipitate crude myosin which was concentrated by settling followed by centrifugation.

Post-rigor. The portion of each semitendinosus muscle remaining after sampling for the pre-rigor preparation was stored in a chiller for 1 day. The muscles (pH < 5.6) were ground separately and a 200-g sample taken from each and protein extracted with a 0.25% TPP, 1.0M NaCl solution. An extraction solution of this composition had already been found (see A and B above) to give crude myosin in near-maximum yield and binding strength. Precipitation and concentration was performed as outlined in Experiment 1.

Statistical methods

For Experiments 1 and 2, analysis of variance was performed on protein yield to examine the response to the TPP and salt levels. Binding strength was analysed in a similar way. Binding strength of crude myosin preparations from pre- and post-rigor muscle were compared and polynomical effects of the salt levels were investigated.

RESULTS & DISCUSSION

THE MAIN PURPOSE of this investigation was to explore whether a simple procedure could be used to obtain a crude myosin preparation from post-rigor muscle, with similar binding properties to that obtained from pre-rigor muscle. Other investigators (Yasui et al., 1964) have studied the extractability of myosin B from rabbit muscle myofibrils in the presence of inorganic phosphates and sodium chloride. Their results indicate that a high extractability could be achieved with a solution containing tripolyphosphate (about 0.03M) sodium chloride (0.4M) and calcium chloride (5 mM). High extractability in the presence of tripolyphosphate might be expected because, like adenosine triphosphate, inorganic triphosphates and other polyphosphates can react with actomyosin to form dissociable complexes (Acs et al., 1949). It was therefore decided to follow a procedure similar to that used by Ford et al. (1978) to prepare crude myosin from pre-rigor muscle, the essential difference being that inorganic tripolyphosphate was added to extraction solutions as a substitute for ATP present in pre-rigor muscle. Calcium was not added to the extraction solution. However, from data presented by Lawrie (1974),

Ca^{++} in the aqueous phase of meat is estimated at 2.5 mM approximately although in the presence of the extraction solutions this would be reduced to approx 0.6 mM. Also, the presence of Mg^{++} is thought to be necessary for the dissociation of actomyosin by polyphosphates (Acs et al., 1949). This ion is also present in meat but as for Ca^{++}, its concentration will be reduced in the extraction process. Whether the concentration of these ions under the conditions employed here was sufficient to achieve the above-mentioned effects has not been investigated.

Effect of TPP and salt concentration of extraction medium on yield of crude myosin

In Figure 1 the effect of TPP and NaCl concentration in the extraction solution on the yields of the crude myosin is shown. It can be seen that the yield of crude myosin was greatest at TPP concentrations in the extraction solution of about 0.25%. Also, yields were generally increased when the NaCl concentration in the extraction solution was increased from 0.5M to 1.0M. The densitometric scan of sodium dodecyl sulfate polyacrylamide electrophoresis gels of various fractions are shown in Figure 2. These indicate that at TPP concentration from 0.25% to 1% the actin content of the crude myosin was relatively small. However, judged by changes in the relative size of the peak due to actin and those due to other components, actin content increased markedly in the absence of TPP, or in solutions containing 2% TPP. It was assumed that the increased actin content reflected an increase in the amount of actomyosin in the crude myosin which would impair the binding ability of these fractions because of the inferior binding strength of actomyosin (Macfarlane et al., 1977). From the densitometric scans it appears that increase in TPP concentration in the extraction solution resulted in other changes in the crude myosin preparations, notably a decrease in peak T which is at the position expected for tropomysin. It must be noted that since there was no preliminary extraction of sarcoplasmic proteins, it is possible that errors could occur in assigning bands to individual proteins. These errors could be due to coprecipitation of the sarcoplasmic proteins with the myosin preparations. Aldolase, enolase or creatine kinase for example may contribute to the "A" and "T" bands since their subunit molecular weights are similar to actin and tropomyosin. From the results shown in Figure 1, it appeared that even using an extraction solution containing the lowest concentration of TPP investigated, namely 0.125%, the yield of crude myosin was near the maximum obtained for any of the other TPP concentrations investigated. It was therefore decided to investigate the efficiency of extraction solutions with lower concentrations of TPP. The results of these studies are presented in Figure.3. For these investigations total protein extracted from the muscle was used as an indication of the efficiency of myosin extraction. This procedure was thought adequate for this purpose as in Experiment 1 it was found that total protein extracted was a reliable predictor (correlation coefficient 0.95) of the yield of crude myosin. It is seen that protein extracted increased with TPP concentration up to about 0.16%, then decreased. The decrease in extractability occurs even though pH of the solution is increased, a circumstance that might be expected to promote solubilization of myofibrillar proteins provided their isoelectric pH remains near that in meat, namely pH 5.0 (Hamm, 1960). Yields of solubilized protein were not significantly different ($P > 0.05$) for extraction solutions with TPP concentration ranging from 0.02% to 0.32%.

Binding strength of crude myosin extracted from post-rigor muscle

From the results presented in Table 1, it can be seen that the binding strengths of crude myosin preparations from extractions using solutions containing 0.15, 0.25 and 0.5% TPP in 1.0M NaCl were not significantly different ($P > 0.05$). However, the binding strength of crude myosin when tested in mixtures at the higher salt concentration (1.0M) was greater ($P < 0.001$) than that in those at the lower concentration (0.4M) at all TPP concentrations.

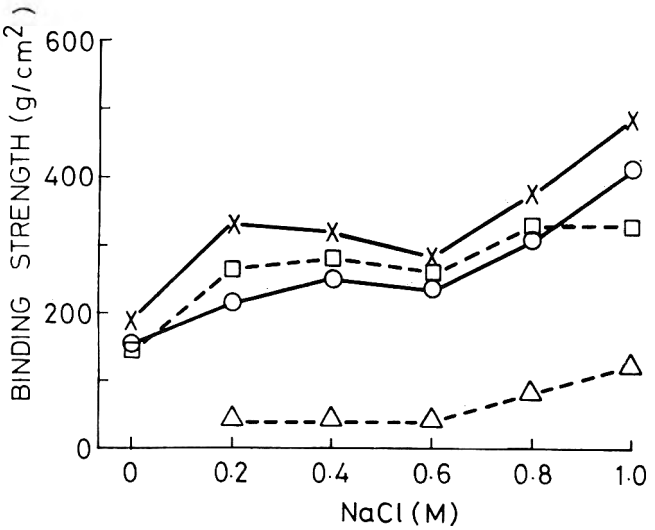

Fig. 4—Comparison of the binding strengths of crude myosin preparations from pre- and post-rigor muscle, tested as 5% suspensions in solutions containing the amounts of sodium chloride indicated. Binding strengths of actomyosin and myosin obtained in another study (Macfarlane et al., 1977) have been included for comparison: X, unbroken lines—pre-rigor crude myosin; ○, unbroken lines—post-rigor crude myosin; □, broken lines—myosin as found in the other study; △, broken lines—actomyosin as found in the other study.

Table 1—Binding strength (g cm^{-2}) of crude myosin obtained from postrigor meat extracted with solutions of various TPP concentrations in 1.0M NaCl

Salt conc (M) in 5% crude myosin mixture	% TPP in extraction solution		
	0.125	0.25	0.50
0.4	231	247	271
1.0	375	394	394
Least significant difference (5%), 51g cm^{-2}			

Comparison of binding strength of crude myosin preparations from pre- and from post-rigor muscles

From Figure 4 it can be seen that at all salt concentrations tested crude myosin extracted from pre-rigor muscle had greater binding strength ($P < 0.05$) than that extracted from post-rigor muscle. The yield of crude myosin from each of the experiments referred to in the experimental section was 3.0, 3.8, 3.0 (mean 3.3) g/100g of pre-rigor muscle, and 4.0, 4.8, 4.6 (mean 4.5) g/100g post-rigor muscle (cf. the value for myosin content of 6.5 g/100g muscle quoted by Lawrie, 1975). Therefore in practice the loss of binding strength of the crude myosin from the post-rigor extract might be offset to some extent by a greater yield. This loss in binding strength is possibly due to the presence of a small quantity of actomyosin in the post-rigor extract. For purposes of comparison the previously reported (Macfarlane et al., 1977) binding strengths of actomyosin and myosin measured under similar conditions to those employed here have been included in the graph. Although the values for binding strength of the crude myosins prepared

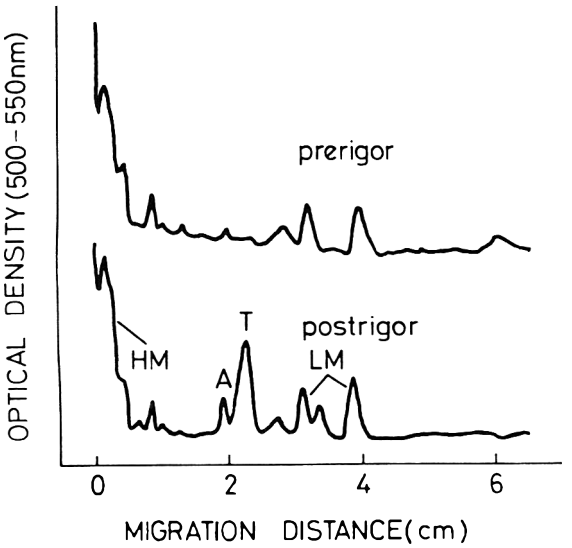

Fig. 5—Densitometric scans of polyacrylamide electrophoresis gels of the crude myosin preparations referred to in Fig. 4. The same quantity of protein was applied to each gel: A = actin; T = tropomyosin; HM = myosin heavy chains; LM = myosin light chains.

from post-rigor muscle were lower than obtained for the myosins prepared from pre-rigor muscle, they were still much higher than those obtained for actomyosin. Figure 5 shows the densitometric scans on sodium dodecyl sulphate polyacrylamide electrophoresis gels of the crude myosin preparations. These indicate proteins of molecular weights intermediate to myosin heavy and light chains, are present in greater amounts in the post-rigor than in the pre-rigor preparation.

Analysis of the means plotted in Figure 4 for the crude myosin preparations indicates that there is a significant cubic relationship for the change in binding strength with increase in salt concentration to 1.0M. The cubic equation was common to the pre- and the post-rigor preparations. In contrast, in the earlier investigation using purified myosin preparations (Macfarlane et al., 1977), at 0.2M NaCl myosin reached a high level of binding which did not change significantly up to the highest concentration investigated, 1.4M.

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LOW WATTAGE MICROWAVE COOKING OF TOP ROUND ROASTS: ENERGY CONSUMPTION, THIAMIN CONTENT AND PALATABILITY

HELEN H. VORIS and FRANCES O. VAN DUYNE

ABSTRACT

Six pairs of top round beef roasts were used to compare microwave cooking at low power levels with conventional roasting. Roasts weighing 3.4–4.7 lb were cooked to an internal temperature of 68.3°C in a microwave oven or in an electric oven at approximately 149°C. Mean total cooking time, 77.6 min, and energy consumption, 0.860 kwh, for microwave cooking were significantly lower ($P < 0.01$) than the respective means, 134.8 min and 1.403 kwh, for conventional roasting. Total cooking losses, moisture, fat and thiamin contents of cooked meat, and shear and press fluid values for semimembranosus muscles did not differ significantly due to cooking method. Microwave roasts were scored lower than conventional roasts for aroma, flavor and exterior color; however, mean scores for tenderness, juiciness and interior color were similar. Results of this study indicate that preparation of a less tender cut of beef in a microwave oven operating at reduced power levels yields a product which is comparable in several parameters of quality to conventional roasting.

INTRODUCTION

A RECENT ADVANCE in microwave oven design, namely development of the variable power feature, provides a range of on-off cycles for the magnetron and consequently the delivery of high to low wattages to the oven cavity. This feature allows greater control of the rate of heating than in the past. Reducing the rate of heating raises the possibility of preparing less tender cuts of meat in the microwave oven, more successfully in terms of yield and palatability, than has previously been feasible. Using such an oven, Korschgen and Baldwin (1978) cooked top round roasts to an internal temperature of 98°C by a moist-heat method at "high" and "simmer" settings.

Earlier research on the microwave cooking of beef was done in older types of microwave ovens and more work has been published on tender cuts of beef than on those characterized as less tender. Microwave and conventional cooking of top round roasts was studied first by Marshall (1960). Cooking losses were much greater for roasts prepared in the microwave oven than for those dry roasted in a conventional electric oven and average scores for appearance, tenderness, juiciness and flavor were higher for the latter.

Other less tender cuts of meat also received lower palatability scores when prepared in microwave ovens than when counterparts were cooked conventionally (Law et al., 1967; Ruyack and Paul, 1972; Ream et al., 1974). Furthermore, in spite of the shorter times required for microwave compared to conventional cooking, cooking losses (either drip, evaporative and/or total) were greater for microwave cooked meat (Law et al., 1967; Ruyack and Paul, 1972; McCrae and Paul, 1974; Ream et al., 1974; McMullen and Cassilly, 1976).

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Moist-heat methods have commonly been recommended for cooking less tender cuts of beef; however, a number of studies including those reported by Dawson et al. (1959) and Berry et al. (1977) indicated that dry roasting at a low temperature was a superior method for cooking certain less tender cuts of beef. Thus in the present study paired top round roasts were dry roasted in a microwave oven with a variable power feature or in a conventional electric oven at 149°C. Parameters of quality investigated included cooking losses, subjective and objective evaluations of palatability, and thiamin content. In addition, energy consumption during cooking by the two methods was measured.

Recent promotional material on microwave ovens emphasizes the potential energy savings when cooking with microwaves, and the figure of 75% savings is widely quoted. However, there has been a limited number of studies on energy consumption and the 75% savings figure is not well documented (Herndon, 1960; Pollak and Foin, 1960; Power, 1967; Davis et al., 1971; McConnell, 1974; Butel, 1975; Jones, 1975; Rhee and Drew, 1977; Drew and Rhee, 1978). Considerably more data are needed to determine if and when the use of microwaves for cooking can save energy in comparison with conventional methods.

MATERIALS & METHODS

SIX PAIRS of top round roasts weighing between 3.4 and 4.7 lb were obtained from the Meat Science Division of the Department of Animal Science at the University of Illinois. Four pairs were from U.S. Choice beef carcasses and one pair each from low Choice and low Good carcasses. After slaughter, the carcasses were hung at 0–3°C for 6–25 days before cutting, then the top round roasts were frozen and stored at −20°C for 3–11 days before thawing.

Prior to cooking, the roasts were thawed 45–48 hr in a 4°C refrigerator in the original freezer wraps. Upon removal from the refrigerator a roast was wiped with paper towels and weighed. Each week one roast of a pair was cooked in a microwave oven, the other in a conventional electric oven on separate days. Three roasts from the left sides of carcasses and three from the right were used for both methods. During the study the microwave roasts were prepared first three times and the conventional ones three times.

Microwave cooking

The roasts were cooked uncovered for 6.5 min per lb at the "Roast" setting, followed by cooking at the "Simmer" setting in a 2450 MHz variable power Litton model 419 microwave oven until an internal temperature of 68.3°C was reached. This method is a modification of the one recommended by Litton (Anonymous, 1976) for cooking 3–4 lb rump roasts to a medium stage of doneness. A Taylor microwave thermometer, number Z4505, was used to monitor the internal temperature of the roasts. They were turned over, reversing the position of the lateral and medial edges of the roast in the oven after cooking on the "Roast" setting, and they were turned 180° in a horizontal plane every 15 min thereafter. The temperature of two roasts rose upon removal from the oven; one increased to 68.9°C and one to 69.4°C.

Prior to cooking, the power input to the oven cavity was measured. The magnetron was prewarmed and the power input in watts was determined by heating 1 liter of distilled water in a 1000 ml Pyrex beaker for 2 min and multiplying the increase in temperature in degrees Celsius by 34.5 (Van Zante, 1959). The power input at the "Roast" setting ranged from 283–317 watts, and on "Simmer" from 182–204 watts.

Conventional roasting

The top rounds were dry roasted, uncovered, in a General Elec-

tric model J406T1WH oven at 149°C to an internal temperature of 68.3°C measured with a Taylor meat thermometer, Number 5930. The oven was preheated 15 min before roasting was begun. The temperature of five of the six roasts rose after removal from the oven, resulting in end point temperatures of 69°C for two roasts, and 70°C, 70.6°C and 72°C for one roast each.

Measurement of energy consumption

Voltage input to the conventional oven was controlled at 240 volts and to the microwave oven at 120 volts by a Variac autotransformer that was part of a multifunctional power control and monitoring unit. A type J-5 portable watt-hour meter, manufactured by Sangamo Electric Company, Springfield, IL, was used to measure energy consumption. Energy consumption for the microwave roasts was measured from the time the roasts were put into the oven until they were removed. For the conventional roasts it was measured from the start of the preheating periods until the roasts were removed from the oven. The door of the microwave oven was opened three to five times during the course of roasting to turn the meat, and the door of the conventional oven was opened slightly three to four times near the end of cooking to determine the internal temperature of the roasts.

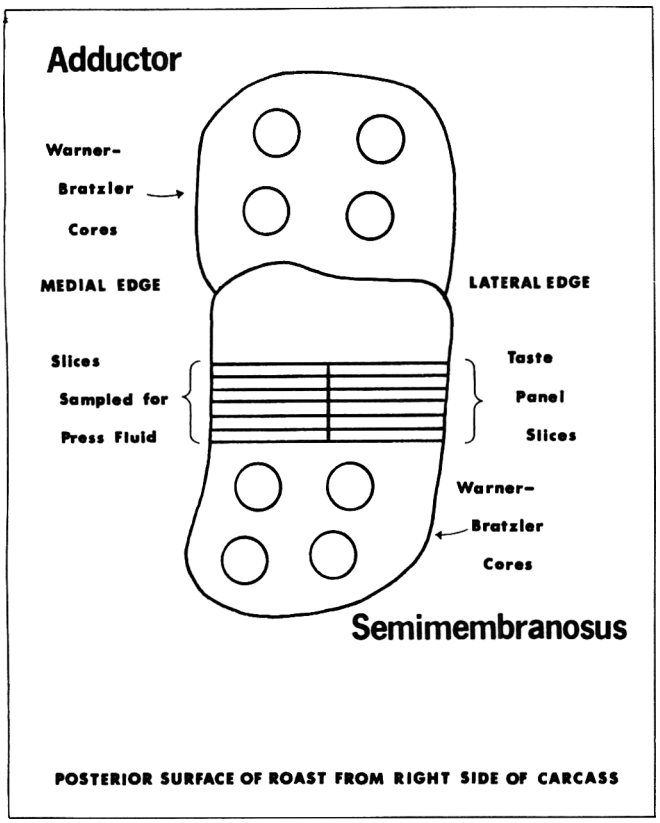

Fig. 1—Positions of Warner-Bratzler cores, press fluid slices and taste panel slices for top round roast from right side of carcass.

Cooking losses

After cooking, a roast was allowed to stand 15 min, loosely covered with aluminum foil, after which the weights of the cooked roast and drippings were determined and the cooking losses calculated. Any rise in internal temperature was recorded during this period of time.

Subjective evaluation

Six 0.6-cm slices were cut perpendicular to the cut surface of a roast from the semimembranosus muscle (Figure 1). The lateral half of each slice was served warm on prewarmed plates to a trained, six-member taste panel. Aroma, interior color, tenderness, juiciness, flavor and exterior color were scored on a 5-point descriptive scale with 1-point increments. A score of 5 points represented the highest quality. Since the microwave and conventional roasts could be distinguished on the basis of external appearance, the panel members scored exterior color of the unsliced portions of a roast after the slices were rated for the other characteristics. The total score was obtained by adding scores for the individual characteristics. Within each week a panelist received slices from the same position in the paired roasts. However, during the study, a panelist evaluated paired slices from all six positions.

Press fluid measurements

A 1-cm wide strip was removed from the upper portion of the medial half of each slice (from center to edge) for the determination of press fluid (Fig. 1). These samples, weighing from 3–5g, represented a range of doneness, as recommended by Sanderson and Vail (1963). They were wrapped in preweighed aluminum foil, weighed, and then pressed between filter paper for 2 min at 2000 psi using a Carver Model B Laboratory Press. The samples were reweighed and press fluid was calculated as the percent loss in weight.

Warner-Bratzler shear values

Four cores were taken from the semimembranosus muscle and four from the adductor (Fig. 1). The 2.5 cm diameter cores were cut perpendicular to the cut surface of the roast and sheared within a temperature range of 29.4–35.0°C. Values for cores that contained visible blood vessels or large amounts of connective tissue or fat were omitted from computation of a mean shear value for a muscle.

Chemical determinations

The sheared cores and any juices released upon cutting were added to the remainder of a roast, and the meat was ground with dry ice in a Hobart electric meat chopper through a plate with 4.8 mm holes. Triplicate determinations for moisture, fat and thiamin were made on each ground cooked meat sample and for thiamin in the drippings. Moisture and crude fat were determined by procedures based on methods of the AOAC (1975). Thiamin determinations were made by the thiochrome method of the Association of

Table 1—Raw and cooked weights, cooking times and energy consumption and cooking losses of microwave and conventionaliy cooked top round roasts

	Microwave cooking	Conventional roasting	F Ratio: Between methods	F Ratio: Among pairs
Raw weight (g)	1906.4 ± 83.1[a]	1841.2 ± 45.2[a]		
(lb)	4.2 ± 0.2	4.0 ± 0.1		
Cooked weight (g)	1396.2 ± 71.4	1360.3 ± 28.0		
Drip weight (g)	187.0 ± 9.1	139.5 ± 7.7		
Total cooking time (min)	77.6 ± 3.2	134.8 ± 4.9	138.51**	1.91
Minutes/lb	18.5 ± 0.5	33.3 ± 1.0	238.43**	1.47
Energy consumed (kwh)	0.860 ± 0.054	1.403 ± 0.402	41.68**	0.33
Drip loss (%)	9.9 ± 0.7	7.6 ± 0.3	8.58*	0.84
Evaporative loss (%)	17.0 ± 0.2	18.5 ± 0.6	6.34	1.12
Total cooking loss (%)	26.9 ± 0.8	26.1 ± 0.8	0.41	0.44

[a] Means and standard errors of the means
* Indicates a significant difference ($P < 0.05$) between means for methods or among pairs
** Indicates a significant difference ($P < 0.01$) between means for methods or among pairs

Table 2—Fat, moisture and thiamin content

	Microwave cooking	Conventional roasting	F Ratio: Between methods	F Ratio: Among pairs
Moisture in cooked meat (%)	60.4 ± 1.2	59.7 ± 0.4	0.48	2.18
Fat in cooked meat (%)	9.2 ± 1.1	9.1 ± 0.9	0.04	5.39*
Thiamin mcg per g cooked meat	0.95 ± 0.05	0.96 ± 0.05	0.08	10.88*
Thiamin mcg per g dry, fat-free cooked meat	3.12 ± 0.14	3.05 ± 0.15	0.26	3.86
Thiamin mcg per g drippings	2.57 ± 0.11	2.64 ± 0.14	2.03	24.43**

[a] Means and standard errors of the means
* Indicates a significant difference ($P < 0.05$) between means for methods or among pairs
** Indicates a significant difference ($P < 0.01$) between means for methods or among pairs

Vitamin Chemists, Inc. (1966) with minor modifications. These consisted of taking larger samples and increasing the amount of reagents for extraction and digestion. In addition, the amount of acid potassium chloride solution used to elute thiamin from a Decalso column was doubled (Pippen and Potter, 1975). A Turner Model 111 Fluorometer operated at its least sensitive setting was used to measure fluorescence of the solutions. The fluorometer was equipped with a general purpose ultraviolet lamp, Number 110–850; a primary filter, Corning narrow pass filter, Number 110–811; and a secondary filter combination of Kodak Wratten sharp cut filter, Number 110–816, and Kodak Wratten narrow pass filter, Number 110–813.

Statistical analysis

Means and standard errors of the means (Snedecor and Cochran, 1967) were calculated on the data obtained with each method. A two-way analysis of variance was used to determine significant differences between means of the data for microwave and conventional cooking and among means for paired roasts.

RESULTS & DISCUSSION

Cooking time and energy consumption

Microwave preparation of top round roasts required significantly ($P < 0.01$) less cooking time, whether comparing total time or min per lb, than did conventional roasting at 149°C (Table 1). The savings in time amounted to 42 or 44%, respectively. Even greater savings in time were reported in two previous investigations with top round roasts (Marshall, 1960; Korschgen and Baldwin, 1978). In the present study the reduced power inputs to the oven cavity on "Roast" and "Simmer" settings probably accounted for the differences from previous studies.

Microwave preparation also required significantly ($P < 0.01$) less electrical energy compared to conventional oven roasting (Table 1). The mean 0.860 kwh consumption associated with the microwave oven is identical to that reported by McConnell (1974), with details supplied by Rennekamp (1977), for microwave cooking of a 4.75 lb rolled roast or a 4 lb sirloin tip roast. In that study, conventional preparation of roasts of the same weights required 2.01 kwh and 2.31 kwh, resulting in energy savings of 57% and 63%, respectively, when cooking with microwaves. The 39% savings obtained in the present study is less than either of these values, but greater than the 25% savings reported for 6 lb beef roasts in a midwestern utility study cited by McConnell (1974). Drew and Rhee (1978) reported a 45% savings of energy when top round roasts were cooked in a microwave oven compared to a conventional one. In contrast to these results, Pollak and Foin (1960) found that a microwave oven required 24% more energy than conventional roasting when 8 lb beef short ribs were prepared. One difficulty in drawing conclusions from these comparisons is that different ovens and/or cuts or shapes of meat were used. Osepchuck (1975) pointed out that differences in size, shape and composition of a food item may affect the rate of heating in a microwave oven and thus the time and energy consumed.

Cooking losses, fat and moisture content

The mean total percent cooking loss for the microwave cooked roasts did not differ significantly from that of conventional roasts; however, microwave cooking resulted in a significantly ($P < 0.05$) higher mean drip loss than did conventional roasting (Table 1). This was partially offset by a slightly but not significantly higher evaporative loss for conventional roasting. In contrast to the results of the present study, the previcus studies comparing microwave and conventional cooking of less tender cuts of beef (with the exception of McCrae and Paul, 1974, and Korschgen and Baldwin, 1978) all resulted in greater total cooking losses for the microwave cooked meat (Marshall, 1960; Law et al., 1967; Ruyack and Paul, 1972; Ream et al., 1974; McMullen and Cassilly, 1976).

The mean percent moisture and fat contents of the cooked roasts were not significantly different after microwave and conventional preparation (Table 2). Significant differences existed among paired roasts in mean percent fat contents in the cooked meat ($P < 0.05$). The fat contents in the roasts ranged from 4.9–13.0% with the lowest percentages, 4.9 and 6.1, in the paired roasts from the low Good carcass.

Thiamin content

The range of thiamin concentration was 0.78–1.10 mcg per g for microwave cooked roasts, and 0.77–1.11 mcg per g for conventionally cooked roasts. Watt and Merrill (1963) reported a value of 0.8 mcg of thiamin per g of cooked Choice grade beef round. No significant differences due to cooking methods were found between mean values for mcg thiamin per g cooked meat, mcg thiamin per g of dry, fat-free cooked meat, or mcg thiamin per g drippings (Table 2). Significant ($P < 0.01$) differences existed among pairs of roasts for mean mcg thiamin per g of cooked meat as determined and for mean thiamin content per g of drippings. However, when thiamin content of the roasts was calculated on the dry fat-free basis, there was no significant difference among pairs. These results are consistent with the few data available on the thiamin content of microwave cooked less tender cuts of beef (McMullen and Cassilly, 1976; Korschgen and Baldwin, 1978).

Sensory evaluation of palatability

The mean total score was significantly ($P < 0.05$) higher for conventional roasts than that for microwave cooked ones (Table 3). Differences between microwave and conventional roasts were significant ($P < 0.05$) for mean aroma scores and significant ($P < 0.01$) for mean flavor scores. With the exception of Korschgen and Baldwin (1978), other researchers have reported that microwave cooked meats scored lower in flavor and/or aroma than those cooked conventionally (Marshall, 1960; Ruyack and Paul, 1972; Ream et al., 1974). MacLeod and Coppock (1976, 1977) found qualitative and quantitative differences in aro-

Table 3—Sensory evaluation scores for microwave and conventionally cooked top round roasts

	Microwave cooking	Conventional roasting	F Ratio Between methods	F Ratio Among pairs
Aroma	4.0 ± 0.1[a]	4.4 ± 0.1[a]	13.43*	2.46
Interior color	4.1 ± 0.1	4.2 ± 0.2	0.20	0.78
Tenderness	3.7 ± 0.2	3.8 ± 0.3	0.12	0.68
Juiciness	3.7 ± 0.1	3.6 ± 0.2	0.03	1.76
Flavor	3.8 ± 0.1	4.4 ± 0.1	24.20**	0.40
Exterior color	3.0 ± 0.1	4.0 ± 0.1	32.65**	0.44
Total palatability	22.2 ± 0.5	24.4 ± 0.5	8.65*	1.09

[a] Means and standard errors of the means
* Indicates a significant difference ($P < 0.05$) between means for methods or among pairs
** Indicates a significant difference ($P < 0.01$) between means for methods or among pairs

Table 4—Percent press fluid and Warner-Bratzler shear values for microwave and conventionally cooked top round roasts

	Microwave cooking	Conventional roasting	F Ratio Between methods	F Ratio Among pairs
Percent press fluid	40.0 ± 1.4[a]	41.1 ± 1.3[a]	0.34	0.90
Shear value of semimembranosus (lb)	8.4 ± 0.5	7.5 ± 0.3	1.67	0.30
Shear value of adductor (lb)	8.7 ± 0.6	9.8 ± 0.7	9.07*	10.78*

[a] Means and standard errors of the means
* Indicates a significant difference ($P < 0.05$) between means for methods or among pairs

matic volatiles of beef cooked by microwave and conventional methods and suggested that certain flavor and aroma compounds might develop only with prolonged heating.

Microwave cooked roasts were lighter in color, and the mean exterior color score was significantly ($P < 0.01$) lower than for conventional roasts. The lower flavor and aroma scores of microwave roasts may be related to this lack of browning, since browning reactions are important in the development of flavor and aroma as well as color (Copson et al., 1955).

Interior color, juiciness and tenderness did not differ significantly due to cooking method. The very similar mean scores for tenderness are of special interest, since they indicate that it is possible to reduce power input to a microwave oven and achieve a degree of tenderness comparable to that obtained in conventional roasting of a less tender cut of meat. Korschgen and Baldwin (1978), using power inputs of 550 and 250 watts, which were higher than those of the present study, reported moist-heat microwave cooked roasts to be less tender than their conventionally cooked counterparts as indicated by sensory and objective measures of tenderness.

Objective measurements of shear and press fluid

Mean Warner-Bratzler shear and press fluid values on the semimembranosus muscles from which taste panel slices were taken did not differ significantly due to cooking method (Table 4). Thus the shear values confirm the similar palatability scores for tenderness. The press fluid results are consistent with the findings that sensory scores for juiciness, total cooking losses, and moisture and fat contents of the cooked meats did not differ significantly due to cooking method. However, the mean shear value for the adductor muscles was significantly ($P < 0.05$) lower for the microwave roasts than for the conventional ones, and there was a significant ($P < 0.05$) difference among pairs. Despite frequent turning, roasts prepared in the microwave oven were more unevenly cooked than the conventional counterparts. In particular, portions of the adductor muscles appeared quite rare in some of the microwave roasts, but not in the conventional ones. This may account for the significant differences in mean shear values on the adductor muscles between cooking methods and among pairs.

CONCLUSIONS

THE USE OF reduced levels of power input to a microwave oven resulted in top round roasts that were similar to conventionally prepared ones in tenderness and juiciness as evidenced by subjective evaluation and objective measurements. In addition, yield and thiamin content were comparable for the two methods while microwave preparation provided substantial savings in time and energy. However, the problem of uneven heating in a microwave oven was not completely solved by the variable power innovation even with frequent turning of the roasts, a procedure which necessitated considerable attention of the food preparer. While conventional roasts were scored higher than the microwave counterparts for exterior color, aroma and flavor, for practical purposes these differences probably would not be detected in the presence of added ingredients.

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EFFECT OF SODIUM NITRITE CONCENTRATION AND PACKAGING CONDITIONS ON COLOR STABILITY AND RANCIDITY DEVELOPMENT IN SLICED BOLOGNA

HWEI-SHEN LIN and JOSEPH G. SEBRANEK

ABSTRACT

Color stability and shelf life of sliced bologna made with various nitrite levels and packaged with different films and vacuum levels have been studied. A maximum (686–737 mm Hg) initial vacuum levels combined with films of low oxygen permeability (7.0 ml/m²/24 hr or less) had higher color scores, greater cured pigment conversion and lower TBA numbers. A lower nitrite concentration (50 ppm) was possible while maintaining product characteristics if good barrier films (less than 7.0 ml oxygen/m²/24 hr) and high initial vacuum levels (686–737 mm Hg) were used.

INTRODUCTION

THE USE OF sodium nitrite for meat curing is largely responsible for the typical characteristics of cured meat products such as formation of cured meat color (Bailey et al., 1964; Fox, 1966), development of cured meat flavor (Cho and Bratzler, 1970), retarding rancidity (Watts, 1954; Cross and Zeigler, 1965) and preventing toxin production by *Clostridium botulinum* (Greenberg, 1974; Hustad et al., 1973). The potential health hazards arising from the use of nitrite for meat curing have been extensively discussed and, consequently, there has been considerable interest in reducing nitrite levels in cured meat.

Cured meat provides an environment conducive to many chemical and biochemical reactions. Consequently, the fading of cured meat color can be affected by lipid oxidation, light dissociation of nitric oxide from the heme, temperature, packaging conditions, bacterial growth, drying of the meat surface, etc. Reduced levels of nitrite probably would require rather specific packaging conditions to prevent discoloration and rancidity development and to maintain a high-quality meat product. Therefore, this experiment was undertaken to study the effects of packaging films, initial vacuum degree and nitrite concentrations on color and rancidity of sliced bologna after five storage periods.

EXPERIMENTAL

LARGE-DIAMETER BOLOGNA was made at the Meat Laboratory at Iowa State University with a mixture composed of 3% salt, 0.5% Heller's number 531 seasoning, 0.05% sodium erythorbate and 10% ice and with three different nitrite levels of 50 ppm, 100 ppm and 156 ppm. Fresh beef and pork were trimmed to desired fat levels, approximately 10–50%. All trim was ground through the 9.5-mm plate of a grinder. Samples weighing 5.9 kg were taken from each batch of ground meat and placed in an Anyl-Ray (Anyl-Ray Corp.) machine for rapid fat analysis. The weights of analyzed meat were prepared in the proper proportions to give a 25% ± 1% fat mixture. The beef trim was first placed in Kramer-Grebe Silent Cutter, followed immediately by ice and salt. After 3 min of chopping, the pork trim and other ingredients were added. The final temperature was about 10–11°C. The emulsion was stuffed (Vemag Robot 1000S Type 116 model) into large-diameter fibrous casing and smokehouse processed to 65°C internal temperature. After processing, samples were sliced (10 mm) and randomly packaged in eight different types of film characterized in Table 1.

Table 1–Characteristics of packaging films[a]

Film code no.	Oxygen permeability ml/m²/24 hr
1	0.1
2	7.0
3	7.0
4	60
5	120
6	0.3
7	0.2
8	1600

[a] All films and typical permeability data provided by American Can Company

Three different initial vacuum levels of maximum attainable, 90% and 70% of maximum, were used in a Multivac Pouch machine MG 2 equipped with heat-sealing bar. Measuring the vacuum levels inside packages immediately showed the three vacuum levels to be 686–737 mm Hg, 584–635 mm Hg and 457–533 mm Hg, respectively. All samples were stored under 200 ft.-c of cool white fluorescent light at 2–5°C in a display case for 1, 4, 10, 21 and 35 days. At the time of packaging, the sliced bologna was very fresh and very bright pink, free of surface discoloration and very desirable in general appearance.

Residual nitrite was determined according to the method of AOAC (1970).

Rancidity measurements were made by the TBA (2-Thiobabituric Acid) number according to the method of Tarladgis et al. (1960). An objective vacuum level inside of packages was determined by placing each package in a plastic bell jar. The equilibrium vacuum pressure between package and chamber was measured by a vacuum gauge while the chamber was evacuated by an electric vacuum pump. As the film separated from the meat surface, a measurement in millimeters was recorded as the equilibrium vacuum level.

Quantitation of nitrosopigment was performed by acetone-water extraction according to techniques described by Hornsey (1956). Pigment conversion was the percentage of total heme pigments converted to nitric oxide haemochrome.

Reflectance spectroscopy also was used to determine the degree of pigment nitrosation. A ratio of K/S values for percentage reflectance readings at 570 and 650 nm was used with a Beckman DK-2A spectroreflectometer (Judd and Wyszecki, 1963; Wodicka, 1956; Giddy, 1966). A photovolt with a green filter also was used to measure color change as total reflectance values.

Subjective evaluation of surface discoloration also was included by use of an 8-point scale scored from 8 = dark, reddish-pink to 1 = brown-green abnormal.

Data were analyzed using analysis of variance (Snedecor and Cochran, 1967). The significance of the difference between means was determined by the least significant difference method.

RESULTS & DISCUSSION

TABLE 2 shows that film 1 had the greatest cured meat color retention resulting from the fact that it is a good oxygen barrier and that it also includes aluminum foil, which makes it nontransparent. This is followed by films 2 and 3, which show quite similar results. These in turn are followed by films 6, 7 and 4. Films 5 and 8 showed the worst color retention as measured by nitric oxide heme

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Table 2—Effect of packaging films on cured color and rancidity of sliced bologna after 35 days storage[a]

Variables	Films 1	2	3	4	5	6	7	8
Nitric oxide heme pigment (ppm)	101.65a ±10.79	76.33b ±6.11	75.62b ±14.76	59.20d ±25.74	38.51e ±26.92	66.42c ±26.79	60.91d ±28.99	9.80f ±9.33
Pigment conversion (%)	72.62a ±10.03	64.11b ±10.55	62.14b ±9.49	48.41d ±20.08	31.85e ±18.34	54.40c ±18.88	51.38cd ±21.27	12.35f ±5.77
Terminal vacuum (mm)	586.7 133.4	623.6 94.7	599.4 103.6	590.0 138.7	608.8 107.7	491.7 260.0	432.8 261.4	562.9 196.3
TBA number	0.67c ±0.17	0.52c ±0.06	0.59c ±0.11	0.98c ±0.79	3.77b ±4.47	0.60c ±0.16	0.78c ±0.47	8.23a ±5.75

[a] All means in a row with different letters are significantly different; X ± S.D. = mean ± standard deviation

Table 3—Effect of packaging films and storage interval on cured color of sliced bologna as measured by reflectance spectra ratios (K/S 570 / K/S 650)[a]

Storage times (days)	Films 1	2	3	4	5	6	7	8
1	6.25 ±0.82	5.60 ±1.02	4.69 ±1.07	4.58 ±0.61	4.24 ±0.88	4.18 ±1.36	3.54 ±1.47	1.60 ±0.08
4	7.08 ±0.48	5.30 ±1.34	4.65 ±1.54	4.03 ±1.06	2.20 ±0.61	3.80 ±1.77	4.03 ±1.77	1.57 ±0.04
10	7.23 ±0.53	4.85 ±1.24	4.63 ±0.85	2.77 ±1.40	1.70 ±0.38	4.85 ±1.43	4.12 ±1.81	1.62 ±0.30
21	6.32 ±0.43	4.17 ±1.10	42.6 ±1.07	2.00 ±0.59	1.32 ±0.09	4.24 ±1.52	4.50 ±1.93	1.33 ±0.03
35	6.46 ±0.96	3.96 ±0.93	3.86 ±0.32	1.67 ±0.53	1.41 ±0.25	3.92 ±1.61	3.91 ±1.52	1.23 ±0.01

[a] X ± S.D. = Mean ± standard deviation

pigment and pigment conversion. The terminal vacuum degree was only slightly different between films except for films 6 and 7, which were significantly lower ($P < 0.05$). This might be due to the basic material of these films, which was more rigid and did not adhere as closely to the product as did the others. Rancidity development was not significantly different between films except for films 5 and 8, which have high values probably resulting from greater oxygen permeability (120 and 1600 ml/m^2/24 hr, respectively). The principle involved in using oxygen-impermeable films is to prevent reentry of oxygen into the package after residual oxygen is converted to carbon dioxide.

Reflectance ratios (K/S 570 / K/S 650) for sliced bologna treated with different packaging films and storage are shown in Table 3. A higher value in this ratio is indicative of greater color development. Spectral reflectance analysis revealed that a ratio of 3.5 or greater for sliced bologna samples indicates acceptable color. Comparisons for different films indicate that the samples in films 4 and 5 seemed brownish-gray at 10 days and 4 days storage, respectively. Samples packaged in film 8 seemed abnormally greenish after only 1-day storage. Slices packaged with film 1 retained pink color even at 35 days storage. Films 2, 3, 6 and 7 were quite similar in that sample color decreased with storage time but was still acceptable at 35 days. This indicates as expected that the better oxygen barrier film will provide longer shelf life and better quality with the storage period. All values were confirmed by extracted pigment concentrations (Table 2) and subjective color score (Table 4), although all values are not shown.

Figure 1 (A and B) shows the significant interaction effect of packaging films and initial vacuum degree on cured meat color retention as measured by reflectance ratios. In general, all films have a higher value for maximum vacuum, followed by 90% and 70% of maximum vacuum. Films 4, 5 and 8 all show very significant discoloration corresponding with initial vacuum level. This suggests that the films with oxygen permeability greater than 60 ml/m^2/24 hr cannot accomplish good color retention even with high initial vacuum. Use of films 6 and 7 result in good cured meat color development as long as maximum vacuum is used; otherwise, they will show complete fading of color. This might be due to loss of vacuum between initial and terminal evaluations, particularly for packages of lower initial vacuum.

Examination of initial vacuum degree also confirmed that these values affect the development of cured meat color and rancidity indicated in Table 4. The maximum and 90% initial vacuum samples showed no significant difference in residual sodium nitrite or TBA numbers; however, they have significantly more residual nitrite and lower TBA

Table 4—Effect of initial vacuum degree on cured color and rancidity of sliced bologna after 35-days storage[a] *(all film types)*

Variable	Degree of vacuum Max	90%	70%
Color score	5.17a ±1.70	3.93b ±1.53	2.50c ±1.40
Nitric oxide-Heme pigment (ppm)	75.99a ±26.08	71.14b ±24.94	47.14c ±26.56
Pigment conversion	60.78a ±13.94	57.52b ±13.43	41.39c ±19.77
$NaNO_2$ residual (ppm)	8.71a ±3.77	8.34a ±3.69	7.12b ±3.37
TBA number	1.74b ±3.50	1.93b ±3.55	2.39a ±3.80

[a] All means in a row with different superscripts are significantly different; X ± S.D. = Mean ± standard deviation

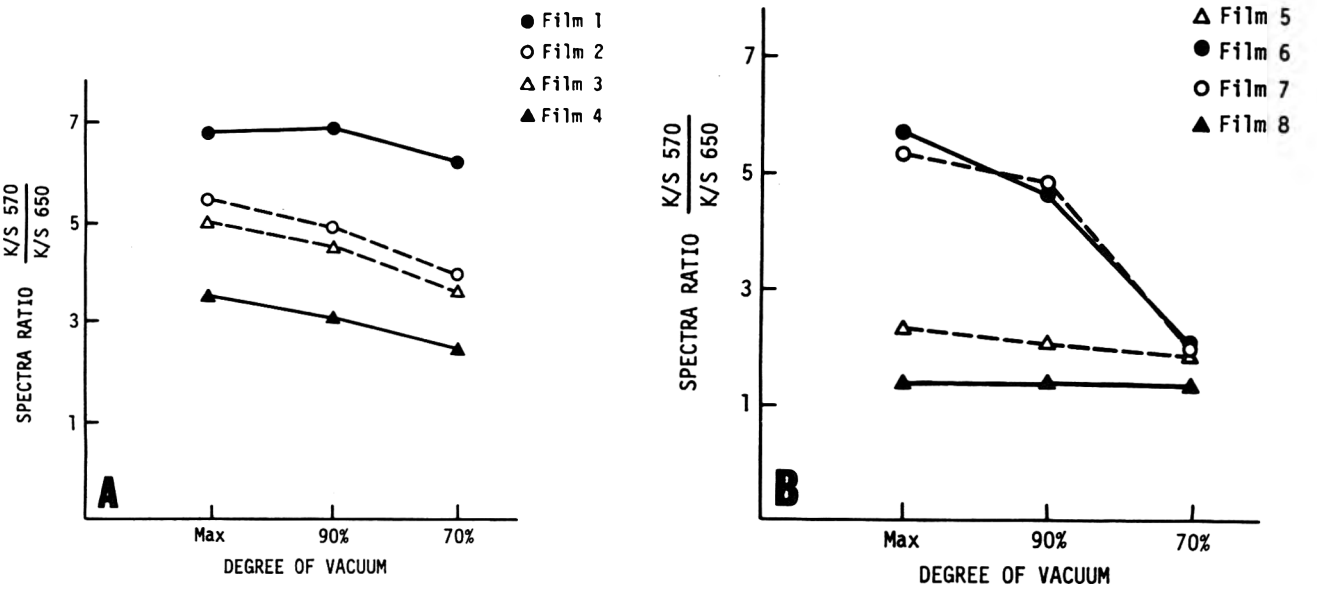

Fig. 1—Effect of packaging films and degree of vacuum on product color.

numbers than those of 70% initial vacuum group. Packages with higher initial vacuum levels resulted in greater film adhesion to the product surface. Generally, cured meat products with good film-to-product contact had less surface discoloration. Bowling et al. (1977) pointed out that the higher initial vacuum levels are associated with more complete removal of residual air, tighter package adhesion, less purge flow over fat surfaces, less browning of lean and fat surfaces and possibly less metmyoglobin formation. When discoloration did occur in the bologna samples, it usually started from the edge of the product surface. This might be due to lack of package adhesion and more residual air spaces. A greater partial pressure of oxygen within the package or perhaps greater bacterial activity in the 70% initial vacuum packages might also be causing the surface discoloration though these possibilities were not examined.

Figure 2 shows that maximum and 90% initial vacuum packages experience only slight change in terminal vacuum within packages. The 70% initial vacuum packages, however, show more loss of vacuum with storage time, which may have resulted from more production of gas inside the bag through greater bacterial activity or from more atmospheric air passing through the film.

Table 5—Effect of nitrite concentration and initial vacuum degree on cured color in all film types as measured by nitric oxide heme pigment (ppm) and spectra ratio K/S 570 / K/S 650[a]

Nitrite conc & Initial vacuum	Color measurement	
	K/S 570 / K/S 650	Nitric oxide heme pigment (ppm)
156 ppm		
maximum	4.75 ± 0.92	85.96 ± 13.19
90%	4.22 ± 0.81	83.18 ± 9.58
70%	2.43 ± 1.08	48.60 ± 23.55
100ppm		
maximum	5.17 ± 1.54	87.90 ± 15.46
90%	4.65 ± 1.23	84.24 ± 12.95
70%	2.88 ± 1.24	50.33 ± 24.07
50 ppm		
maximum	5.16 ± 1.44	60.22 ± 16.46
90%	4.29 ± 1.09	60.19 ± 15.05
70%	3.19 ± 1.54	42.77 ± 20.58

[a] X ± S.D. = mean ± standard deviation

Table 5 indicates the interaction of nitrite concentration and initial vacuum level on cured meat color development. There is slight difference in cured meat color between 156 ppm and 100 ppm nitrite concentration used. Both nitrite concentrations show small differences between maximum and 90% initial vacuum samples, but both nitrite levels show poor cured meat color in the 70% initial vacuum packages. Fifty ppm nitrite results in less cured meat color development at all vacuum levels. Nevertheless, 50 ppm nitrite also produced good cured meat color in the maximum initial vacuum packages as long as a good barrier film was used (films 1, 2, 3, 6 and 7). These results tend to agree with those of Kraft and Ayres (1954) who recommended

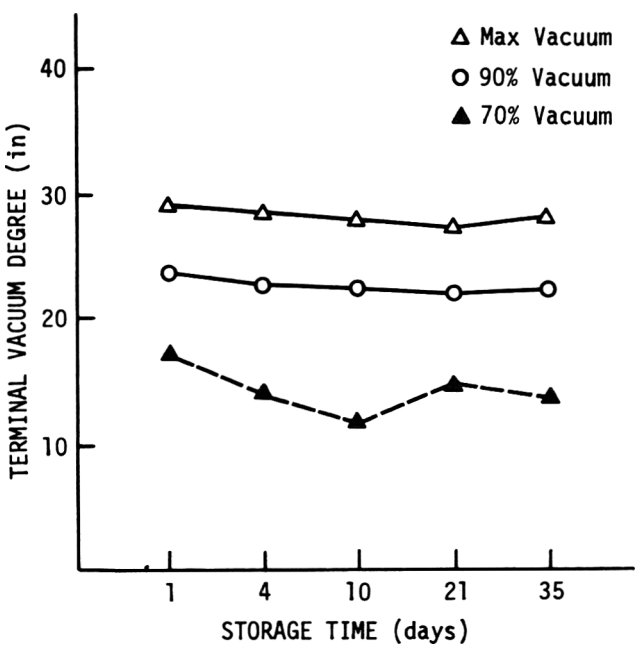

Fig. 2—Effect of initial vacuum and storage time on package terminal vacuum.

29 in. (737 mm) Hg vacuum pressure and with those of Westerberg (1971) whoe recommended that films with oxygen transmission rates less than 1 cc/100 in.^{2}/24 hr ATM be used for packaging sliced bologna.

More importantly, these results show that superior packaging systems (high-barrier films and the highest possible vacuum level) will allow the use of reduced levels of sodium nitrite and still retain product appearance. Levels as low as 50 ppm retained desirable color during storage as long as the package environment was carefully controlled. Therefore, one method for reducing nitrite level in cured meat is to very carefully design the packaging system to remove and exclude all possible oxygen.

This study made no consideration for microbial growth under the various conditions discussed. The microbial question needs to be addressed before any application of this approach is made to commercial practice.

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Reference to brand name does not constitute endorsement by the U.S. Department of Agriculture or the University of Maryland, Eastern Shore over others of similar nature not mentioned.

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WHITE-SPOT FORMATION ON PACKAGED BEEF STEAKS

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ABSTRACT

Bottom round (n=162), eye of round (n=80) and rib (n=126) steaks were used to identify conditions involved in formation of white-spots on muscle surfaces of packaged meat cuts. Conditions investigated were: (a) retail packaging films, (b) angle at which cuts are displayed in the retail case, (c) maximum ambient temperature achieved during defrost of the retail case, and (d) type of film-to-meat surface contact. Neither microbes nor packaging film components (antifogging agents, plasticizer) appeared to be the cause of white-spots. Retail packaging films were not associated with surface discoloration, incidence of white-spots or overall appearance. Incidence of white-spots was increased by: (a) displaying retail cuts at an angle of 15°, (b) temperature abuse between packaging and retail display, (c) defrost cycles in which ambient temperatures in the retail case reached 21°C, (d) wrapping cuts in a manner resulting in "poor" film-to-meat surface contact, and (e) longer (3 days vs 1 day) periods of retail display. White-spots appear to result from physical, rather than chemical, phenomenon with one plausible explanation being that of condensation of moisture between wrapping film and meat surfaces and concurrent washing of color pigments from muscle; such spots most frequently occur proximal to the boundary between areas of "good" vs "poor" film-to-meat surface contact.

INTRODUCTION

A RECENT INCREASE in incidence of white-spot formation on the surface of fresh meat cuts during retail display has caused concern to retailers and to manufacturers of meat packaging film (W.B. Cashion, RJR Archer, Inc., personal communication). Retailers first registered complaints and, when incidence of this anomaly did not decrease, filed financial claims against film manufacturers. Consumers and retailers purportedly relate these white spots to migration of free vinyl monomers from polyvinyl chloride (PVC) films into or onto meat surfaces; the validity of this claim and the consequent implications to human health are not certain (Daun and Gilbert, 1977).

Because specific retail packaging films have been associated with white-spot formation more often than other films, the search for the cause of this problem should include comparisons of these and other meat packaging films. Several studies have compared packaging films used for wrapping retail meat cuts (Ball et al., 1957; Clauss et al., 1957; Kraft and Ayres, 1952); in no case was mention made of white-spot formation. However, dramatic changes have occurred in materials and technology employed in the manufacture of retail packaging films since the 1950's, thus it is appropriate to evaluate newer films with regard to their ability to maintain satisfactory meat appearance during retail display. A second objective of the present study was to identify factors and conditions associated with occurrence of white-spots on muscle surfaces of fresh meats.

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Table 1—Characteristics of films used in packaging study

Film	Plasticizer[a] (%)	MVTR[b] (kg/M²/24 hr)	O_2 TR[c] (liters/M²/24 hr) 4°C	23°C	40°C
I	25	0.23	9	18	NA[d]
II	28	0.24	9	20	39
III	24	0.24	5	12	29
IV	22	0.23	3	7	15
V	0	0.05	5	10	21
VI	NA	0.23	6	13	NA
VII	NA	0.23	8	16	NA

[a] Plasticizer was dioctyl adipate.
[b] Moisture vapor transmission rate (determined in a Mocon apparatus)
[c] Oxygen transmission rate (determined in a Mocon apparatus)
[d] Not available

EXPERIMENTAL

Relationship between film type or film components and occurrence of white-spots

Bottom round (biceps femoris) steaks (n = 144), approximately 2.5 cm in thickness were placed in styrofoam trays. Steaks were then randomly assigned to one of eight groups; the 18 steaks in seven of the groups were then overwrapped with one of seven different packaging films. Films differed in oxygen transmission rate, percentage plasticizer and other film characteristics. The characteristic properties of the films, supplied to us by the manufacturers, are presented in Table 1. The 18 steaks in each packaging film group were displayed under simulated retail conditions at 2°C with all packages tilted at a 15° angle. All retail display conditions were the same except for the maximum ambient temperature attained in the retail case during defrost; for six steaks from each packaging film group the ambient temperature during defrost reached 6°C, for six other steaks the temperature attained 13°C and for six other steaks the temperature reached 21°C.

Three other groups of six steaks (the eighth group of 18 steaks) were used to assist in determining whether certain film components (plasticizers and antifogging agents) were contributing factors to the formation of white-spots. In one of these groups, a thin layer of plasticizer was applied to the surface of each steak; the surface of each steak in the other two groups was thinly covered with one of two antifogging agents (hereafter referred to as antifog 1 and antifog 2). Steaks in each of these groups were packaged in Film VI and displayed as described above, with four defrost cycles (at 6°C) per 24 hr.

All steaks were weighed prior to packaging and reweighed after 72 hr of retail display to determine percentages of weight loss. Each cut was evaluated after 72 hr of retail display by a 5-member trained panel for surface discoloration using a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration), overall appearance using an 8-point scale (8 = extremely desirable; 1 = extremely undesirable) and for severity of white-spots using a 5-point scale (5 = no noticeable white-spots; 1 = extremely noticeable white-spots).

Relationship between film-to-meat surface contact, defrost cycles and temperature abuse between time of wrapping and retail display on the occurrence of white-spots

Rib (longissimus dorsi) steaks (n = 63) were placed in individual styrofoam trays and overwrapped with PVC film with "good" (as nearly complete as possible) film-to-meat surface contact. An additional 63 rib steaks were also wrapped in PVC film after placing a

Table 2—Mean surface discoloration scores[a] after 72 hr of retail display stratified according to defrost cycle temperature and film or film component

Film or film component	Maximum ambient temperature during defrost[b] of retail case 6°C (A)	13°C (B)	21°C (C)	Order of means[c]
Film I	5.4d	5.4d	3.8d	A B C
Film II	5.2d	5.7d	3.9d	B A C
Film III	4.8d	5.4d	3.9d	B A C
Film IV	5.5d	4.7d	4.1d	A B C
Film V	4.9d	4.9d	4.1d	A B C
Film VI	5.1d	4.5d	4.3d	A B C
Film VII	5.0d	4.7d	4.1d	A B C
Treated with plasticizer	5.5d			
Treated with antifog agent 1	4.5d			
Treated with antifog agent 2	4.1d			

[a] Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration). Means in the same column followed by a common letter do not differ ($P > 0.05$).
[b] Defrost cycles were of 30 min duration; the four cycles were equally spaced (every 6 hr).
[c] Means in the same row underscored by a common line do not differ ($P > 0.05$).

Table 3—Mean overall appearance scores[a] after 72 hr of retail display stratified according to defrost cycle temperature and film or film component

Film or film component	Maximum ambient temperature during defrost[b] of retail case 6°C (A)	13°C (B)	21°C (C)	Order of means[c]
Film I	5.5d	5.6d	4.2d	B A C
Film II	5.7d	5.9d	4.1d	B A C
Film III	5.6d	4.8d	3.9d	B A C
Film IV	5.5d	4.1d	3.4d	A B C
Film V	4.6d	5.2d	3.5d	B A C
Film VI	5.1d	5.0d	3.5d	A B C
Film VII	4.8d	4.7d	3.6d	A B C
Treated with plasticizer	5.0d			
Treated with antifog agent 1	3.9d			
Treated with antifog agent 2	3.7d			

[a] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable). Means in the same column followed by a common letter do not differ ($P > 0.05$).
[b] Defrost cycles were of 30 min duration; the four cycles were equally spaced (every 6 hr).
[c] Means in the same row underscored by a common line do not differ ($P > 0.05$)

sterile glass rod diagonally across each steak so that large areas on the surface of each steak had no contact with the packaging film. These steaks are hereafter referred to as having "poor" film-to-meat surface contact. Each group of 63 steaks was divided into three groups of 21 steaks each. Each of the groups of 21 steaks were wrapped and then placed under conditions such that the surface of each steak was at one of three temperatures (2°C, 13°C, 21°C) for 30 min prior to retail display. Seven steaks of each group of 21 rib steaks were then displayed at 2°C with four defrost cycles at 6°C per 24 hr, seven steaks were displayed at 2°C with four defrost cycles at 13°C per 24 hr and the other seven steaks were displayed at 2°C with four 30 min defrost cycles at 21°C per 24 hr.

All steaks were evaluated after 24 and 72 hr of retail display. Subjective evaluations were made by a 5-member trained sensory panel for severity of white-spots using a 5-point scale (5 = no noticeable white-spots; 1 = extremely noticeable white-spots).

Relationship between film-to-meat surface contact, angle of display of retail cuts and occurrence of white-spots

Eye of round (semitendinosus) steaks (n = 80), approximately 2.5 cm in thickness were placed in plastic foam trays. Forty steaks were wrapped with Film II in a manner that assured "good" film-to-meat surface contact; an additional 40 steaks were wrapped with Film II in a manner (use of a sterile glass rod placed diagonally across each steak) that created "poor" film-to-meat surface contact. Twenty steaks with "good" film-to-meat surface contact and 20 steaks with "poor" film-to-meat surface contact were displayed "flat" (the bottom of the tray was at an angle of 0°); the other 40 steaks were tilted so that one end of the tray was higher than the other, to create an angle of display of 15°. Steaks were displayed at 2°C for 72 hr under conditions where the ambient temperature during defrost reached 6°C. Each steak was evaluated at 24, 48 and 72 hr of retail display by a 5-member trained panel for severity of white-spots using a 5-point scale (5 = no noticeable white-spots; 1 = extremely noticeable white-spots).

Microbial counts, pH measurements and pigment concentrations for white-spot areas and for normal tissue

In all phases of this study, when white-spots occurred on steaks, the affected areas and adjacent normal tissue were dissected away from muscle surfaces for subsequent analysis. Total microbial plate counts were obtained from normal (red) and discolored (white) areas of meat by use of the swab method and standard plate count agar. pH measurements and total pigment concentrations also were obtained from normally colored (red) surfaces and adjacent areas of discolored (white) tissue; the total pigment concentration was determined using the procedures of Rickansrud and Henrickson (1967).

Statistical analyses

All data, except percentages, were analyzed using analysis of variance. When significant ($P < 0.05$) main effects were observed, mean separation was performed using the Duncan (1955) multiple range test.

Table 4—Mean weight losses[a] after 72 hr of retail display stratified according to defrost cycle temperature and film or film component

Film or film component	Maximum ambient temperature during defrost[b] of retail case 6°C (A)	13°C (B)	21°C (C)	Order of means[c]
Film I	0.6e	1.3d	0.9d	B C A
Film II	0.9de	1.8d	1.1d	B C A
Film III	2.0d	1.3d	1.2d	A B C
Film IV	0.9de	0.9e	1.4d	C B A
Film V	1.5de	0.9e	0.9d	A C B
Film VI	0.5e	1.1de	1.5d	C B A
Film VII	0.8e	1.2de	1.2d	C B A
Treated with plasticizer	1.0de			
Treated with antifog agent 1	0.9de			
Treated with antifog agent 2	1.2de			

[a] Weight loss, expressed as a percentage, during retail display. Means in the same column followed by a common letter do not differ ($P > 0.05$).
[b] Defrost cycles were of 30 min duration; the four cycles were equally spaced (every 6 hr).
[c] Means in the same row underscored by a common line do not differ ($P > 0.05$).

Table 5—White spot severity data for steaks displayed 72 hr stratified according to defrost cycle temperature and film or film component

Film or film component	Maximum ambient temperature during defrost[a] of retail case														
	6°C					13°C					21°C				
	White spot severity[b]					White spot severity[b]					White spot severity[b]				
	5	4	3	2	1	5	4	3	2	1	5	4	3	2	1
Film I	44[c]	50	6	0	0	33	56	0	11	0	28	50	17	6	0
Film II	39	61	0	0	0	28	72	0	0	0	28	39	22	11	0
Film III	22	61	11	6	0	22	28	28	22	0	33	44	6	17	0
Film IV	33	13	47	7	0	28	50	6	6	11	33	50	17	0	0
Film V	33	50	17	0	0	22	61	17	0	0	28	56	0	6	11
Film VI	28	50	22	0	0	39	44	17	0	0	33	50	17	0	0
Film VII	39	50	11	0	0	22	28	33	6	11	33	33	33	0	0
Treated with plasticizer	19	52	24	5	0										
Treated with antifog agent 1	39	33	11	11	22										
Treated with antifog agent 2	0	33	0	33	33										

[a] Defrost cycles were of 30 min duration; the four cycles were equally spaced (every 6 hr).
[b] 5 = no noticeable white spots, 4 = very slightly noticeable white spots, 3 = slightly noticeable white spots, 2 = moderately noticeable white spots, 1 = extremely noticeable white spots.
[c] Percentage of steaks in a film or film component × defrost cycle group assigned a white spot severity score of 5. Percentages may not total 100 because of rounding.

RESULTS

Relationship between film type or film components and occurrence of white spots

Mean surface discoloration scores after 72 hr of retail display, as affected by film or film component and defrost cycle are presented in Table 2. Surface discoloration was not significantly affected by type of film or by film components at any of the defrost cycle temperatures. Significantly more surface discoloration was usually observed on steaks exposed to defrost cycles at 21°C as compared to defrost cycles at 7°C or 13°C.

Mean overall appearance scores after 72 hr of retail display, as affected by film or film component and defrost cycle temperature are presented in Table 3. As was the case for surface discoloration, overall appearance was not significantly affected by type of film or by film component at any of the defrost cycle temperatures. In general, steaks exposed to defrost cycles at 21°C had less desirable overall appearance than did steaks defrosted at 7°C or 13°C.

Mean weight losses, expressed as percentages, for steaks during retail display are shown in Table 4. Weight losses were lowest ($P < 0.05$) for steaks packaged in Films I, VI and VII when defrost cycle temperature attained only 7°C and were lowest ($P < 0.05$) for steaks packaged in Films IV and V when defrost cycle temperatures reached 13°C. Weight losses were not ($P > 0.05$) increased beyond that of the film group (Film III) with the highest weight loss by treatment with plasticizer or antifogging agents.

White-spot severity data for steaks displayed 72 hr stratified according to defrost cycle and film or film component are presented in Table 5. Steaks wrapped with all seven of the films and steaks treated with plasticizers and antifogging agents exhibited at least very slightly noticeable white-spots at one or more of the defrost cycle temperatures. The severity of the white-spots was substantially higher when steaks were exposed to high temperature (21°C) defrost cycles. The application of plasticizer and antifogging agents also appeared to increase the severity of white-spots. These agents were liquid at room temperature but solidified to a white paste when applied to the meat surfaces and did not cause discoloration of the same type as that typical of the white-spot anomaly under study.

—*Text continued on page 1458*

Table 6—Mean white spot severity scores[a] stratified according to retail display interval, defrost cycle, film-to-meat surface contact and temperature prior to display

Retail display interval (hr)	Maximum ambient temp during defrost[b]	"Good" film-to-meat surface contact			"Poor" film-to-meat surface contact		
		Temperature prior to display[c]			Temperature prior to display[c]		
		2°C	13°C	21°C	2°C	13°C	21°C
24	6°C	5.0[d]	4.9	4.9	4.3	4.7	4.4
24	13°C	4.8	4.8	4.7	4.9	4.9	4.5
24	21°C	4.9	4.9	4.3	3.8	3.6	3.5
72	6°C	4.8[e]	4.9	4.0	3.2	3.4	3.1
72	13°C	4.9	4.9	3.8	3.9	3.8	3.7
72	21°C	4.6	4.5	3.9	2.8	2.8	2.2

[a] 5 = no noticeable white spots; 1 = extremely noticeable white spots.
[b] Defrost cycles were of 30 min duration; the four cycles were equally spaced (every 6 hr).
[c] Steaks were packaged and then subjected to temperatures of 2°C, 13°C or 21°C for 30 min prior to display at 2–3°C for 24 or 72 hr.
[d] There were significant ($P < 0.01$) differences after 24 hr of retail display due to type of film-to-meat surface contact and defrost cycle; differences due to temperature prior to display were not significant ($P > 0.05$).
[e] There were significant ($P < 0.01$) differences after 72 hr of retail display due to type of film-to-meat surface contact, temperature prior to display and defrost cycle.

Table 7—Percentage of steaks having white spots[a] stratified according to retail display interval, defrost cycle, film-to-meat surface contact and temperature prior to display

Retail display interval (hr)	Maximum ambient temp during defrost[b]	"Good" film-to-meat surface contact			"Poor" film-to-meat surface contact		
		Temperature prior to display[c]			Temperature prior to display[c]		
		2°C	13°C	21°C	2°C	13°C	21°C
24	6°C	0[d]	7	11	64	21	32
24	13°C	14	11	25	14	11	25
24	21°C	7	11	36	61	79	64
72	6°C	18[d]	11	64	80	82	86
72	13°C	7	7	54	54	64	71
72	21°C	25	29	64	82	89	100

[a] Percentage of steaks in each treatment that had, at least, very slightly noticeable white spots (scores of 4 or less on a 5-point white spot severity scale).
[b] Defrost cycles were of 30 min duration; the four cycles were equally spaced (every 6 hr).
[c] Steaks were packaged and then subjected to temperatures of 2°C, 13°C or 21°C for 30 min prior to display at 2–3°C for 24 or 72 hr.
[d] Percentages were not compared statistically.

Relationship between film-to-meat surface contact, defrost cycle temperature and temperature abuse between time of wrapping and retail display on the occurrence of white-spots

Mean white-spot severity scores stratified according to retail display interval, defrost cycle, film-to-meat surface contact and temperature prior to display are shown in Table 6. Among steaks displayed for 24 hr, white-spot severity increased ($P < 0.05$) with "poor" film-to-meat surface contact and as maximum temperature attained during the defrost cycle increased. Among steaks displayed for 72 hr, white-spot severity increased ($P < 0.05$) with "poor" film-to-meat surface contact, with higher temperature defrost cycles and with higher temperatures (greater temperature abuse) prior to retail display.

Percentages of steaks having white-spots stratified according to retail display interval, defrost cycle, film-to-meat surface contact and temperature prior to display are presented in Table 7. The percentage of steaks exhibiting white-spots was increased by "poor" film-to-meat surface contact, high defrost cycle temperature, greater temperature abuse between packaging and retail display, and longer periods of retail display.

Relationship between film-to-meat surface contact, angle of display of retail cuts and occurrence of white-spots

White-spot severity data for steaks stratified according to film-to-meat surface contact and angle of display of retail cuts are presented in Table 8. Percentages of steaks with "slightly noticeable" (score of 3) or more severe (scores of 2 or 1) white-spots were much higher for steaks displayed at a 15° angle than for steaks displayed at a 0° angle. Percentages of steaks with "slightly noticeable" (score of 3) or more severe (scores of 2 or 1) white-spots were also higher for steaks wrapped with "poor" film-to-meat surface contact than for steaks wrapped with "good" film-to-meat surface contact.

Microbial counts, pH measurements and pigment concentrations for white-spot areas and for normal tissue

Mean microbial counts, pH values and total pigment concentrations are shown in Table 9. There were no significant differences in microbial counts of normally colored and white-spot areas dissected from the surfaces of rib steaks after 72 hr of retail display. White-spot areas had higher ($P < 0.05$) pH values and lower ($P < 0.05$) concentrations of total pigment than did areas on the muscle surface that were considered normal in appearance and color.

DISCUSSION

Comparison of film types

Ball et al. (1957) reported that the average display life of prepackaged fresh meat is only slightly longer than 48 hr.

Table 8—White-spot severity data for steaks stratified according to film-to-meat surface contact and angle of display of retail cuts

Film-to-meat surface contact	Retail display interval (hr)	Angle of display of retail cuts									
		0°					15°				
		White-spot severity[a]					White-spot severity[a]				
		5	4	3	2	1	5	4	3	2	1
"Good"	24	60[b]	38	2	0	0	40	52	8	0	0
"Poor"	24	68	29	3	0	0	53	21	14	12	0
"Good"	48	64	31	5	0	0	24	50	26	0	0
"Poor"	48	45	40	12	3	0	28	33	32	5	2
"Good"	72	54	43	3	0	0	60	28	8	4	0
"Poor"	72	44	38	16	2	0	33	38	10	17	2

[a] 5 = no noticeable white-spots, 4 = very slightly noticeable white-spots, 3 = slightly noticeable white-spots, 2 = moderately noticeable white-spots, 1 = extremely noticeable white-spots.
[b] Percentage of steaks in a film-to-meat surface contact × angle of display group assigned a white-spot severity score of 5. Percentage may not total 100 because of rounding.

Table 9—Mean microbial counts, pH values and total pigment concentration for normal-colored and for white areas of muscle tissue dissected from the surfaces of rib steaks after 72 hr of retail display

Appearance of tissue	Microbial count ($\times 10^4$)	pH value	Total pigment concentration (mg/g)
Normal-colored	6.09a	5.39a	4.92a
White	6.34a	5.49b	3.80b

[a,b] Means in the same column bearing a common letter are not different ($P > 0.05$).

Sanitation during cutting of retail cuts, temperature of display and type of retail wrapping material are the only factors that the meat retailer controls that can extend shelf-life of fresh meats. Kraft and Ayres (1952) determined that wrapping films that retain carbon dioxide within the package increase shelf-life, however, color desirability is not as good as with more permeable films.

Results of the present study suggest that the commercial films available to the trade have generally similar effects on appearance of cuts during retail display. The adverse effects of temperature abuse both between time of wrapping and retail display, and during defrost cycles are evident, irrespective of the packaging film used.

Factors associated with the incidence of white-spots

Prior to the initiation of this study, white-spots were observed on cuts in commercial retail display cases and were most frequently found: (1) on beef cuts which tend to have high moisture contents—such as those from the round, (2) on beef grading less than U.S. Choice, (3) on cuts in triple-deck retail cases, (4) on cuts in display cases which had long defrost cycles or electrical shut-off defrost cycles, (5) after 24–36 hr of retail display, (6) on the edges of meat cuts in an area where there was an accumulation of moisture at the juncture of "good" and "poor" areas of film-to-meat surface contact, and (7) in stores which had obvious problems with sanitation.

It had been suggested by those in industry that the white-spots may be an accumulation of plasticizer which had migrated from the packaging film. Daun and Gilbert (1977) reported that the amount of plasticizer (diethylhexyl adipate) which migrated onto the surface of meat wrapped with PVC film was in the range of 20 mg/dm^2. Plasticizer concentration in this range would not be visible to the naked eye and is therefore assumed not to be the source of the white-spots being investigated in this report. Butz et al. (1974) studying "white-film" development on sliced, vacuum packaged, country style-ham, reported that "white film" incidence was not of microbial origin and that it was independent of temperature in the range of 4–26°C.

Results of the present study support a hypothesis (Fig. 1) in which myoglobin is washed out of surface areas where

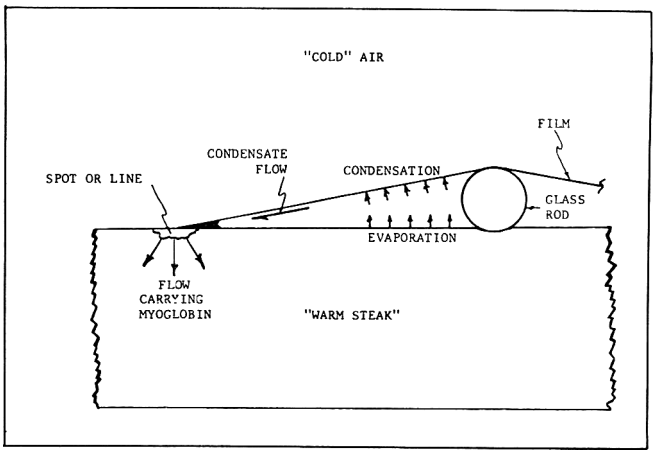

Figure 1—Schematic diagram of water movement in a retail meat package.

condensation collects on the underside of the wrapping film. Factors that cause an increase in the amount of condensation or cause the condensation to collect in certain areas favor the formation of white-spots. Such factors include: (a) displaying retail cuts at an angle of 15°, (b) temperature abuse between packaging and retail display, (c) defrost cycles in which ambient temperatures in the retail case reach 21°C, (d) wrapping cuts in a manner resulting in "poor" film-to-meat surface contact, and (e) longer (3 days vs 1 day) periods of retail display.

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OPTIMIZATION OF LACTIC ACID FERMENTATION OF FROZEN VEGETABLE PROCESS WASTES: MODEL SYSTEMS FOR PRODUCING ANIMAL FEEDS

N. J. MOON

ABSTRACT

Optimum conditions for controlled lactic acid fermentation of frozen food processing wastes to produce a stable ensiled feed are described. Parameters used to determine success of fermentation treatments were rapid pH reduction to < 4.55 and rapid acid production. Treatments investigated included: inoculum; *Lactobacillus plantarum* culture 15A, or 2B, *Lactobacillus bulgaricus* and *Streptococcus faecalis* subsp. *liquefaceins*, and waste fermented. Black-eye peas, potato, green beans, corn and turnip greens represented wastes of different carbon and nitrogen levels. Other treatments included time (0, 1, 2, 5, 12 days); and moisture content (90, 70, 50 and 30%). Moisture level adjustments were made by drying or adding peanut hulls, wheat straw, or cardboard. Optimum conditions were culture 15A, 70% moisture obtained by drying or adding peanut hulls (~ 23%) (v/w), and carbon nitrogen ratio of 20–25:1 (10–15% protein). A pH < 4.55 was obtained after 5–12 days with sufficient acidity present to control stability.

INTRODUCTION

AS MUCH AS 40% of raw vegetables are generated as waste during processing (Jones, 1973). In many cases, these wastes are not used in the final salable product because of size, shape, or blemish, but remain high in nutritional value. Currently, these wastes are often not utilized in the food production system, but are disposed of by various methods including incineration and landfill treatments (Hudson, 1971). Increasing Federal emphasis on curtailing ground water contamination from landfill or other waste disposal sites makes suitable alternatives for waste utilization critical. The 1972 Amendments to the Federal Water Pollution Control Act (PL-500) declare as a national goal that the discharge of pollutants into navigable waters be eliminated by 1985. Ideally, solid wastes could be used or recycled in the food system, thus preserving their maximum economic and nutritive value. By-product recovery has been successfully demonstrated in some food industries (Green and Mattick, 1977; Soderquist, 1972) but is difficult in vegetable processing plants in general because of the variability of products processed during the season.

The solid waste resource might be effectively recycled and utilized in the food system if processed into a ruminant animal feed that has undergone lactic acid fermentation. A vegetable waste silage should have many of the advantages of other silages (McCullough, 1977) and provide a feasible means of disposal for the processor and a nutrient source for cattle producers. Previous attempts at a frozen food processing plant to ensile vegetable wastes had been unsuccessful. Wastes were in general too high in moisture (~95%) causing severe and undesirable seepage during fermentation. Secondly, pH reductions due to lactic acid fermentation in silages weren't reproducible from waste to waste, nor from one trial to the next with the same waste. Vegetables used for ensiling were foods similar to the salable product in composition (as pods, stems, etc. are routinely left in the field) but were designated as wastes because of repeated blanching and/or freezing and thawing; or were otherwise unfit for packaging due to blemishes or mechanical damages. Each of the temperature stresses would alter the normal flora of the wastes and may account for the irregular and often undesirable results experienced in previous attempts at silage production, although other causes for past fermentation failures may exist. In order for vegetable waste silage production to be an attractive waste utilization alternative, increased understanding of the conditions which determine successful controlled lactic acid fermentations of the wastes is needed. A suitable process must be developed for wastes of different nutritional characteristics, water contents, and severe heat stresses (such as freezing and blanching) typical of some frozen vegetable processing plants. In addition, a suitable bacterial culture must be selected that will produce lactic acid, lower the pH and thus control opportunistic flora which cause undesirable fermentations.

Others have demonstrated that certain food processing and other wastes can be used to produce a lactic acid stabilized feed (Hoover et al., 1976; Oelshlegel et al., 1969; Weiner, 1977). Yet, to date, systematic experimental designs to determine optimum parameters for fermentation conditions have been lacking. This report summarizes efforts to identify the parameters controlling lactic acid fermentation of frozen vegetable processing wastes and to determine the optimum model system necessary to produce a stable product that could be used as a ruminant animal feed.

EXPERIMENTAL

Bacterial selection

Suitable inocula for silage production were not readily available. Commercially available cultures of organisms reported (Lesins and Schulz, 1968; Whittenbury et al., 1967) to be beneficial in silage fermentations were used in preliminary screening trials. These included *Streptococcus faecalis* subsp. *liquefaceins, Streptococcus faecalis, Streptococcus faecium, Streptococcus thermophilus* (2 strains), *Streptococcus lactis* (3 strains), *Streptococcus mitis, Streptococcus salvarius, Streptococcus faecalis* subsp. *zymogenes, Lactobacillus bulgaricus* (2 strains), *Lactobacillus acidophilus* (3 strains), *Lactobacillus delbrueckii, Lactobacillus casei* subsp. *casei* (2 strains) and *Lactobacillus plantarum.* These cultures were obtained from commercial sources and culture collections at the Food Science Department, University of Georgia.

In addition, environments likely to yield suitable organisms were sampled and selected for lactobacilli by enrichment, since these organisms were believed to be of primary importance in silage fermentation. These included aged cheddar cheese, fermented refrigerated pickles, a commercial live *Lactobacillus* sp. 'starter' additive for silage, and corn silage that had fermented for one month. For bacterial isolation a 0.5-g sample was added to 10 ml sterile limus milk and incubated at 32°C in an incubator (15% CO_2 atmosphere) for 3 days. Samples from those tubes showing characteristic dye reduction, coagulation and acid production were streaked on LBS agar (BBL, Cockeysville, MD), incubated at 32°C in an incubator (15% CO_2) for 5 days. Isolates were examined for gram reactions, lack of catalase production, motility, and reaction in limus milk.

Samples were also evaluated for lactobacilli by direct plating of

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Table 1—Composition[a] of selected vegetables

Vegetable	% H_2O	% Protein (Dry wt)	Carbon nitrogen ratio[b]
Turnip greens	93.0	33.8	9.0
Blackeye peas	66.0	26.4	11.5
Green beans	90.1	19.3	13.8
Corn	76.2	7.1	23.6
Potatoes	64.0	7.6	44.9

[a] *Nutritional Value of American Foods in Common Units.* Agriculture Handbook No. 456. 1977.

[b] C:N ratios calculated from carbohydrate, fat and protein values reported in *Nutritional Value of American Foods in Common Units.* Agriculture Handbook No. 456.

5-g samples on LBS agar (APHA, 1972). Suitable colonies were examined for lactobacilli as described for enrichment cultures.

Gram positive, catalase negative, nonmotile, microaerophilic rods from LBS agar were further identified as species of lactobacilli by suitable techniques (Bergey's Manual, 1974). These included temperature optima, carbohydrate fermentation pattern and optical rotation of lactic acid (Sigma Chemical Co., St. Louis, MO).

Preparation of inoculum. Inoculum for fermentation was prepared by activating stored refrigerated cultures by two successive transfers in sterile milk (11% solids) incubated at 32°C for 24 hr. A final 18-hr culture was used for 2.5% (v/w) inoculation into vegetables.

Preliminary survey of organisms. Thirty organisms that grew rapidly in milk and/or MRS broth were screened for their ability to ferment five frozen vegetable wastes at their natural water content. Percent lactic acid/or pH reduction at 0, 1, 2, 5 and 12 days were determined and fermentation rates of each organism on each vegetable evaluated.

Selection and preparation of frozen vegetable wastes

The wastes produced from frozen vegetable processing include products that vary widely in moisture and proximate composition. Because a primary objective of this study was to develop systems for wastes of a variety of compositions, common vegetables were placed in groups according to moisture content and C:N ratios calculated from proximate compositions. From these groupings representative vegetables were selected for further study in fermentation trials including black-eye peas, potatoes, green beans, corn, and turnip greens (Table 1). Preliminary production of silages at frozen vegetable processing plants had indicated that the wastes are often closely approximated by the final salable product in composition, as unedible plant parts are routinely left in the field when possible. Vegetables that are unfit for packaging because of size, blemish, heating, freezing, thawing, and mechanical damage at various processing steps are disposed of as waste. The amount of temperature stress received by vegetables to be designated as wastes was difficult to determine in practice as several temperature stress cycles may have occurred in packaging and repackaging operations commonly employed. Accordingly, vegetables that had been processed for retail sale represented a minimum amount of temperature stress and were used as simulated process wastes in this study as actual wastes were not readily available. Silos are often duplicated experimentally when large numbers of treatments are examined by using oxygen impermeable containers (Ohyama et al., 1975; Woolford and Wilkins, 1975). They are disadvantageous because they don't subject the silage to pressures or oxygen tension routinely observed in silos. However, others have indicated that even with these inherent disadvantages, trials successful under these conditions are very likely to be successful in larger batches. In this study individual vegetables were thawed, mixed and treated to reduce moisture content if necessary. Then 20-g samples were tightly packed into sealable 100-ml jars and frozen at −10°C until needed, then thawed, steamed at 100°C for 15 min, cooled, and inoculated at 2.5% (v/w) and incubated at 25°C. At 0, 1, 2, 5 and 12 days jars were opened and 5-g sample removed for analysis.

The extreme temperature treatments of the samples helped to duplicate the severe temperature stresses of vegetable wastes at frozen food processing plants previously observed to undergo inferior fermentations. The procedure also afforded uniform treatment of the large numbers (148) of samples prepared at one time and used in this study. The number of natural flora would also have been altered by the sample treatments and would have afforded the inoculum a competitive advantage.

Moisture adjustments

In the first phase the effect of moisture on the fermentation was assessed by drying the wastes. Turnip greens and green beans normally near 90% moisture were dried to 70, 50 and 30%. The other three vegetables were normally near 70% and were dried to 50 and 30%. All samples were dried in a vacuum oven at 60°C until appropriate weight reductions had occurred.

In the second phase of the study, waste materials characteristically low in moisture were added to vegetables to help absorb moisture. The agents used were: wheat straw cut in a hammer mill to 2.5 cm lengths; paper board, cut to pass a 1.9 cm screen; and peanut hulls ground to pass a 5 mm screen. Moisture determinations (115°C, 18 hr) were performed on each waste and used to determine amounts of drying agent to be added to reduce total moisture content to 70%.

Dewatering wastes using a Carver press was also evaluated and abandoned when much of the solids was expelled with the water if wastes had received heat or freezing treatments.

pH and developed acidity determinations

Five-gram samples were removed from fermentation jars at 0, 1, 2, 5 and 12 days and blended with 95 ml water in a Waring Blendor for 1 min. Measurements for pH were determined on an Electro-Mark Analyzer (Markson Science, Inc., Del Mar, CA).

Acidity measurements were determined by titrating to pH 9.6 with 0.1N NaOH using an automatic titrator (Beckman Instruments, Inc., Fullerton, CA). Sample color interfered with use of phenolpthalein as an indicator, so titration to the pK of this indicator was chosen as a suitable method. Acidity was calculated as percent lactic acid and developed acidity determined using controls at 0 time. At later fermentation times (e.g. 12 days) the total acidity calculated may not reflect actual acidity levels due to lactic acid production, as extensive proteolysis and other degradations may have occurred which would influence the amount of base consumed in the titration.

Experimental design

A factorial treatment design was used for evaluation of main effects and their interactions. A model system was chosen for fermentation which included the determinant variables of inoculum culture, waste composition, moisture content, and time and their effects on pH and developed acidity:

$$pH = a_1(\text{culture}) + a_2(\text{waste}) + a_3(\%H_2O) + a_4(\text{time}) + E_1$$

$$\text{developed acidity} = b_1(\text{culture}) + b_2(\text{waste}) + b_3(\%H_2O) + b_4(\text{time}) + E_2$$

where a_1 through a_4 and b_1 through b_4 are estimates of the coefficients of the regression equation and E_1 and E_2 are estimates of error.

Four selected cultures were evaluated for their fermentative ability on five different vegetables, adjusted to four different moistures by drying. All moisture levels (90, 70, 50, 30%) were not used for all vegetables because only turnip greens and green bean wastes were normally 90% moisture. This design resulted in the fermentation of 116 different treatments. In the second phase of the experiment one optimum moisture level was chosen (70%) and those wastes requiring moisture removal (turnip greens and green beans) were treated by drying or adding dry wastes including peanut hulls, straw and cardboard as previously described. This resulted in an additional 32 treatments for a total of 148 different treatments in this study. The second phase fermentation was evaluated using a similar linear regression model:

$$pH = c_1(\text{culture}) + c_2(\text{waste}) + c_3(\%H_2O) + c_4(\text{time}) + c_5(\text{dewatering AGT}) + E_3$$

$$\text{developed acidity} = d_1(\text{culture}) + d_2(\text{waste}) + d_3(\%H_2O) + d_4(\text{time}) + d_5(\text{dewatering AGT}) + E_4$$

where again c_1 through c_5 and d_1 through d_5 are estimates of the coefficients of the regression equation and E_3 and E_4 are estimates of the error.

Data were analyzed using the SAS statistical program packages

(Barr et al., 1976). A regression estimate (R^2) of 0.70–0.75 was chosen as sufficient evidence of model fit of data variability. To aid in the selection of the most successful treatments among the large number examined, the data were analyzed using a selective ranking procedure in order of pH and/or developed acidity. An arbitrary limit of pH 4.55 was selected as a minimum acceptable level for pH reduction. Fermentations higher than pH 4.55 would probably not have sufficient acidities to prevent undesirable secondary fermentations, particularly those proteolytic in nature. Using this ranking procedure it was possible to construct frequency diagrams for each treatment indicating the percent of total observations with pH < 4.55 each for time observation.

A minimum standard of performance for developed acidities was not used to group successful fermentations. Wastes had different buffer capacities making developed acidity poorer grouping criteria.

RESULTS & DISCUSSION

INITIAL STUDIES with more than 40 microorganisms indicated that not all organisms were suitable for further study because all vegetables were not fermented to the same extent or at the same rate. Thirty organisms were selected that reduced the pH and produced titratable acidity at a maximum rate for each vegetable. Of these, the four organisms that were most frequently successful for all vegetables were used for further study in the complete fermentation model. These organisms reduced the pH of the test frozen vegetable wastes rapidly and produced at least 2% developed acidity on all wastes. The organisms used were *Lactobacillus plantarum* 15A, *Lactobacillus plantarum* 2B, *Lactobacillus bulgaricus* PI, *Streptococcus faecalis* subsp. *liquefaceins*. The first and second cultures were isolated from pickles and corn silage respectively and the latter two were commercially available cultures.

Successful fermentations of traditional forage and waste materials to silage have been characterized by rapid pH reductions to about 3.5–4.5 (Weiner, 1977). This reduction is due to the rapid growth of bacteria which produce primarily lactic acid as an end-product. Previous attempts at silage production at frozen vegetable processing plants were often unsuccessful probably because of inadequate handling procedures and insufficient natural lactic acid microflora. When the four selected cultures used in this study were compared for their ability to grow rapidly, produce lactic acid, and reduce the pH; all were successful in some trials. However, only *L. plantarum* cultures 15A and 2B were successful in a large number of fermentations (Table 2). For example after 5 days culture 15A and 2B had reduced the pH of nearly one-third of all the trials to < 4.55 (Fig. 1). At 12 days this trend continued and nearly 50% of the trials were < 4.55. Culture 15A was slightly more effective than 2B after 12 days of fermentation.

The fermentation pHs of vegetables inoculated with *L. plantarum* 15A were illustrative of the more successful fermentation trials (Table 3). The vegetables having early pH reduction regardless of moisture content were green beans and corn. The pH reductions were a result of acid which accumulated throughout the fermentation period. Figure 2 illustrates the direct relationship between pH reductions and acid accumulations for *L. plantarum* 15A as an inoculum for green beans at 70% moisture content. Similar acid accumulations were observed in other fermentations of low pH. The largest concentration of acid was observed in corn silage. This may have resulted in part from considerable starch hydrolysis by the microflora which survived the heat stress of sample preparation as *L. plantarum* cultures might not be expected to be amyolytic. Some protein hydrolysis may have also occurred although this was not specifically determined. Potatoes, turnip greens and blackeye peas were somewhat slower to ferment having higher pH values after 5 and 12 days than the other vegetables. The trends of superior fermentation of certain vegetables discussed here for *L. plantarum* 15A were also followed by the other three cultures. Figure 3 illustrates the superior fermentation of green beans and corn overall as a large number of trials were < 4.55 after 5 and 12 days.

Table 2—Main effects of culture, time, vegetable waste, and percent moisture on pH and developed acidities using Duncan's multiple range test[a] for significance at p =0.05

Treatment	Mean pH	Mean acidity
culture[b]		
15A	5.44 a	2.61 b
2B	5.25 a	2.91 b
SF	6.22 c	0.95 a
LB	5.75 b	1.45 a
time		
1	5.84 b	0.40 a
2	5.78 ab	−0.33 a
5	5.57 a	2.39 b
12	5.56 a	4.37 c
vegetable waste		
blackeye peas	6.62 c	0.56 a
potatoes	5.87 b	1.14 a
green beans	5.11 a	2.95 b
corn	5.19 a	5.40 c
turnip greens	5.77 b	0.31 a
moisture %		
90	5.00 a	3.91 b
70	5.50 b	4.05 b
50	5.85 c	0.86 a
30	5.93 c	0.25 a

[a] Means followed by the same letter are not significantly different.
[b] Cultures used were: 15A = *L. plantarum* 15A, 2B = *L. plantarum* 2B, SF = *S. faecalis* subsp. *liquefaceins*, LB = *L. bulgaricus*.

A pH reduction to < 4.55 was not in itself always indicative of a stable product or successful fermentation. For example culture SF reduced a substantial number of trials to pH < 4.55 by 5 days but after 12 days some samples rose to pH > 4.55 (Fig. 1). Silages fermented by this culture apparently contained insufficient acid (< 1%) to control secondary fermentations of lactic acid at later stages. Even though the samples received severe temperature treatments in preparation, they were not sterile and undesirable microflora could apparently develop if acidities were inadequate.

Table 3—Fermentation of silages by Lactobacillus plantarum 15A

Vegetable	Moisture	Time (days) 0	1	5	12
Green beans	90%	6.60	5.30	4.05	3.40
Turnip greens		6.51	5.50	4.70	4.70
Green beans	70%	6.40	5.70	4.00	3.75
Corn		7.10	5.90	4.20	3.90
Turnip greens		6.20	5.90	5.00	7.00
Potatoes		6.40	6.80	5.40	4.05
Blackeye peas		7.30	6.70	5.20	4.55
Green beans	50%	5.50	5.55	4.20	4.15
Corn		6.70	6.50	4.95	4.35
Turnip greens		5.55	5.85	5.75	5.15
Potatoes		6.00	5.80	5.85	5.70
Blackeye peas		7.10	6.70	6.70	6.50
Green beans	30%	5.50	5.85	5.65	5.75
Corn		6.70	6.40	4.20	4.00
Turnip greens		6.00	6.00	5.90	6.00
Potatoes		6.05	6.40	6.20	6.00
Blackeye peas		7.00	7.10	7.00	7.00

An attempt was made to correlate the success or failure of fermentation with the composition of the wastes. This would aid in the preparation of optimum mixtures of wastes in future trials at processing plants. Carbon:nitrogen ratios (Table 1) were calculated from carbohydrate nitrogen values. Vegetable wastes that were high in protein (Table 1), such as turnip greens, were in general somewhat slower to ferment and were very low in developed acidity resulting in few samples having a pH < 4.55 after 12 days. Vegetable wastes high in carbohydrate, but low in nitrogen, also showed very slow fermentation rates, minimum acid production and pH reduction (Table 1 and Fig. 4). Those waste products intermediate in this composition range (for example green beans and corn) fermented more rapidly initially and also had a high percentage of samples with pH < 4.55 after 12 days. For example, after 12 days, wastes from corn had more than 65% of all samples at all treatments < pH 4.55. This indicates that there is a direct effect of vegetable composition on pH and acid development. When the ratio of carbon to nitrogen in wastes is in the range 20–25:1 the most lactic acid was produced and the minimum pH reached (Fig. 4). After 12 days wastes near this C:N ratio (green beans) had pH levels of about 4.0 and the absence of putrid odor.

The moisture content of the wastes had a dramatic effect on the fermentation. For example, *L. plantarum* 15A had most rapid pH reductions in vegetables of 90% moisture (Table 3). As the moisture was adjusted to 70%, pH reductions were slower and minimum pHs were higher. At 50 and 30% moisture, pH reduction rates were slowed further and final fermenation pHs were higher. These trends in pH reductions were also observed for other cultures (Fig.

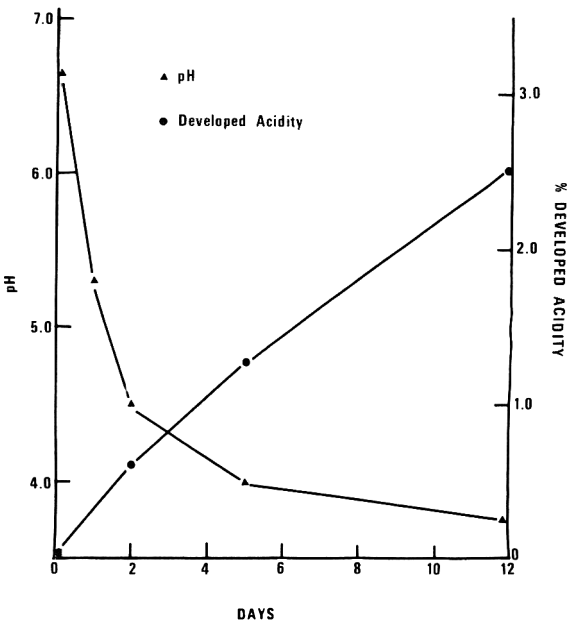

Fig. 2—Example of a successful fermentation of frozen vegetable processing wastes: green bean waste adjusted to 70% moisture by drying and inoculated with L. plantarum 15A.

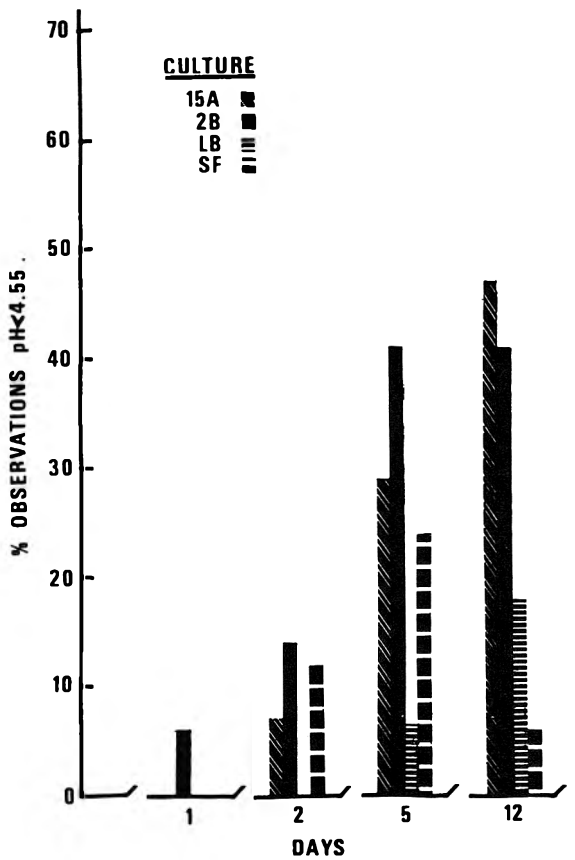

Fig. 1—Effect of inoculum culture on the reduction of pH of treatments (including all 5 vegetable wastes used and all 4 moisture levels) during the 12-day fermentation period. Results expressed as % of the number of treatments inoculated with each culture that had pH < 4.55 at each fermentation period. 15A = L. plantarum 15A. 2B = L. plantarum 2B, LB = L. bulgaricus, SF = S. faecalis subsp. liquefaceins.

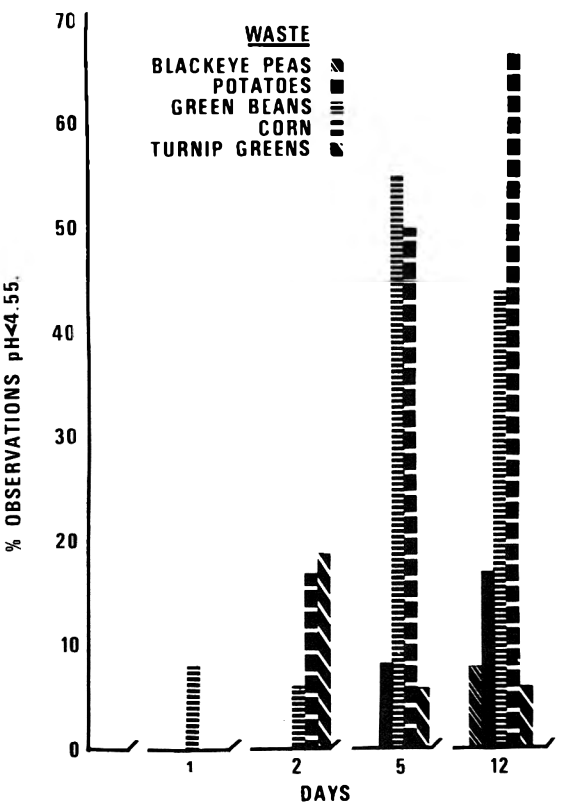

Fig. 3—Effect of waste fermented on the reduction of pH of treatments (including all 4 inocula used, and all 4 moisture levels) during the 12-day fermentation period. Results expressed as % of the number of treatments on each waste that had a pH < 4.55 at each fermentation period.

5). While it might be expected that the buffer capacity of vegetables would increase as moisture was removed, this may not account entirely for the lack of pH reduction. Acid was also in lower concentrations (Table 2) and slow to develop in these samples as well, indicating poorer bacterial growth at lower moistures. Those vegetables that fermented well at higher moistures also had lower pHs at lower moisture content, indicating the advantage of waste composition at lower moisture content.

While it might appear most advantageous to use wastes at 90% moisture, others have indicated silage production is more practical at lower moistures. For example, samples greater than 70% moisture were difficult to handle in practice as they tended to expel water on standing. Since 70% moisture often gave adequate results, wastes that were 90% in moisture were dewatered in various ways and their fermentation assessed. Drying requires energy, which is expensive, so other alternative water reduction methods were tried. It was found that ground peanut hulls, straw and cardboard would absorb about two times their weight in water. This water would not be expelled under pressures typically found in silos (30 lb/in.^{2}). These wastes were added to the two wastes high in water and their effectiveness compared to vegetable wastes dried to 70% moisture and those normally near 70%.

Addition of peanut hulls was the most successful dewatering agent, (Fig. 6). Additions of straw or cardboard were less successful causing prolonged lags in pH reduction and lactic acid production. Addition of straw and cardboard caused great difficulty in packing the containers as their fluffy nature caused inefficient occlusion of oxygen. Drying of wastes was superior to addition of any of the agents for

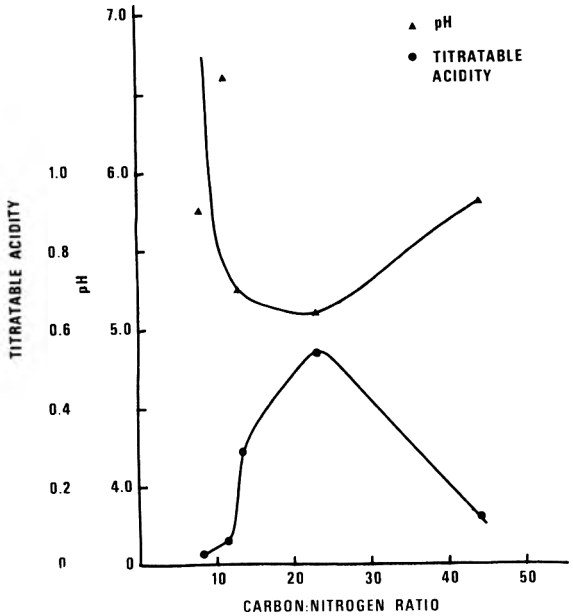

Fig. 4—Effect of carbon:nitrogen ratio (calculated from proximate composition) on the average pH and titratable acidity throughout the 12-day fermentation.

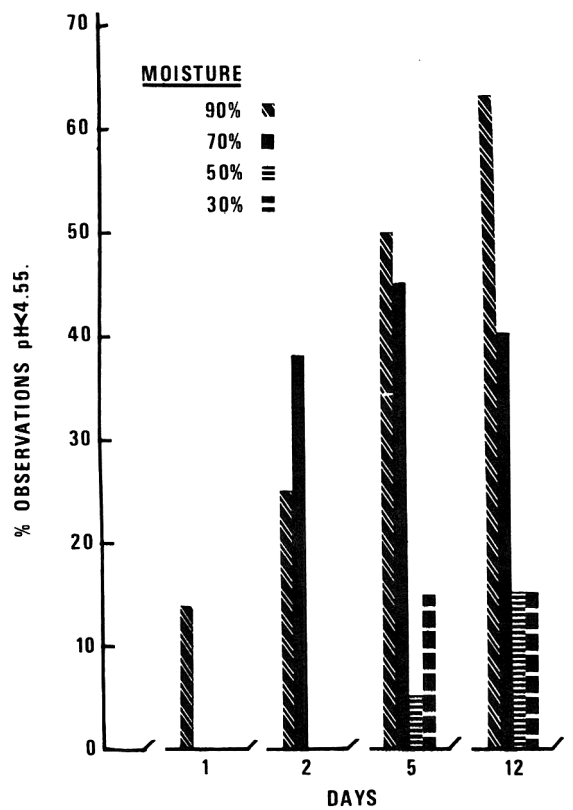

Fig. 5—Effect of moisture content of waste silages on the pH reduction of all treatments (including all 4 inocula used and all 5 vegetable wastes during the fermentation period). Results are expressed as % of the number of treatments at each moisture that were < 4.55 at each fermentation period.

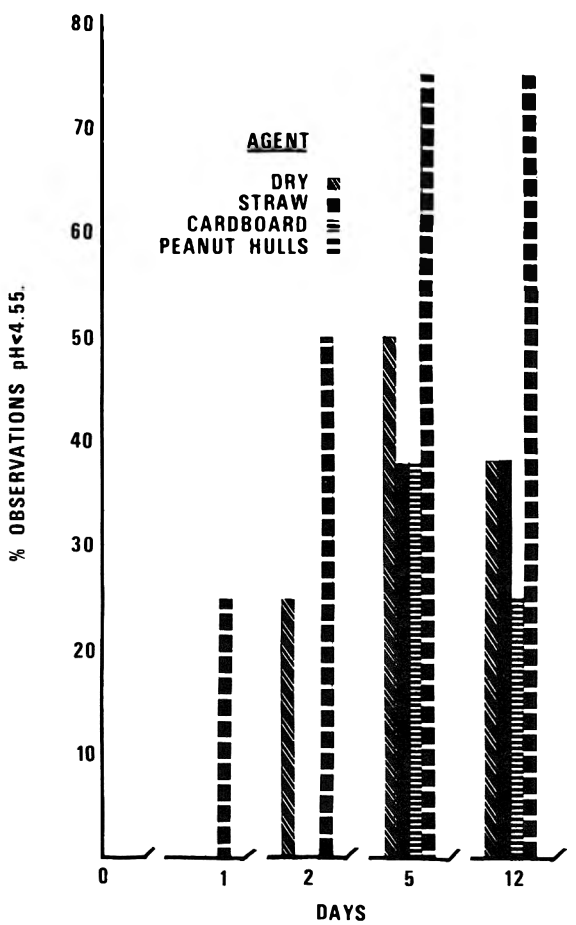

Fig. 6—Effect of water absorbing or drying agent used to adjust moisture to 70% on the pH reduction of all treatments (including all 4 inocula used on turnip green and green bean wastes). Results are expressed as % of treatments treated with each drying agent that had a pH < 4.55 at each fermentation period.

proper acid development and second only to peanut hull additions in effectiveness of pH reduction. The effectiveness of peanut hulls over other dewatering agents may be because of their inherent low pH (5.40) compared with straw (6.50) and cardboard (6.90). Also, if these dewatering agents were adjusted to 70% moisture by addition of water and fermented, only peanut hulls showed final pH levels of 4.55 or lower, thus indicating nutrient additions as well as drying ability. When peanut hulls were added to turnip greens to dry to 70% moisture, pH levels were lower than when the moisture level was 90% or dried. This effect was not observed with green bean wastes which may have near optimum carbon nitrogen balance. Addition of peanut hulls to dewater to 70% total moisture would increase the fiber content of green beans which may be of value as an animal feed (Bartow et al., 1974). Other dewatering agents high in carbohydrates may be suitable for wastes high in fiber such as leafy greens.

CONCLUSIONS

IN SUMMARY, these studies on simulated frozen food processing wastes indicate that fermentations were successful if suitable inocula are added. Others (Lesins and Schulz 1967, Ohyama et al., 1975) have also reported that inoculation of silage with suitable cultures is beneficial if the material has received sufficient environmental stress to inhibit the development of normal lactic acid flora. The use of suitable bacterial inocula in these studies insured a rapid production of acid and consequent reduction of pH < 4.55 in about 5 days. Successful fermentations were characterized by acid concentrations high enough to inhibit undesirable fermentations and maintain low pH levels. These characteristics have been reported to be essential in production of other silages as well (Whittenbury et al., 1967).

Moisture concentrations of the waste should be adjusted to about 70% to insure rapid fermentation and minimum leakage of dissolved solids. Others have shown that similar moisture levels are most beneficial in conventional silage production (Gordon, 1967; McCullough, 1977). Moisture reductions can be accomplished best by drying or by use of a second dry waste product such as peanut hulls. In these studies, use of straw or cardboard were ineffective probably because of their fluffy nature. Some workers have indicated that other dry wastes such as citrus pulp may be successfully used in conventional silage production to dewater grass-legumes silage (Archibald, 1953).

Wastes should be adjusted to, or contain an optimum protein and carbohydrate composition. For best results a carbon nitrogen ratio of about 20:1 to 25:1 should be used; corresponding to a protein concentration of about 10–15%. This composition is difficult to compare with conventional silages because much of the carbohydrate content in these products is unavailable fiber.

These studies do not indicate the cause of past fermentation failures at frozen food processing plants. The wastes used for ensiling at these plants might have been expected to harbor sufficient normal lactic acid flora for rapid fermentation. However, normal procedures for ensiling and assuring anaerobosis were followed, and some silages prepared from near optimum compositions indicated in these studies did not ferment properly. Either sufficient normal flora were not present or some other unidentified causes for inadequate pH reduction occurred. Further studies should be undertaken to determine if the culture used in this study can successfully control fermentations of wastes receiving less severe temperature stresses so that normal flora would be more active. Secondly, the optimum conditions for fermentation determined in this study need to be evaluated in large batches in pilot plant scale silos with special attention to reducing inoculum size to about 1.0% and assessing actual feed value.

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ANALYSIS AND RENOVATION OF RETORT COOLING WATER

R. H. WALTER, R. L. MITCHELL and D. L. DOWNING

ABSTRACT

Retort effluent was found by gravimetry to contain generally 20–40 ppm lubricant in the form of a stable micro-emulsion. Adsorption-filtration columns of aluminum filings, aluminum powder or charcoal removed lubricant from this effluent at a rate of 18–20 lb per 10^3 lb of adsorbent, with possibly greater efficiency being realized by proper attention to a small number of performance characteristics. Aluminum had an advantage over charcoal, because of its ease of regeneration in situ by elutriation with an organic solvent. Absorbance data, obtained from a series of standard emulsions, showed that accurate measurements of lubricant concentrations could be made photometrically, in the absence of interfering matter, at the levels found in retort effluent. Retort effluent was renovated experimentally by a two-stage adsorption-filtration process, involving aluminum (1st stage) and charcoal (2nd stage). The combined eluate had an absorbance (A) of zero until the break-through volume when turbidity increased rapidly at A = 0.005 – 0.010.

INTRODUCTION

THE FEDERAL Water Pollution Control Act Amendments of 1972 require by not later than July 1, 1983, the achievement of effluent limitations for point sources, using the best available technology that is economically feasible (USEPA, 1976). With reference to fruit and vegetable canning, the water used in automated cooling retorts, initially of potable quality, contacts generally clean cans and an otherwise clean lubricated mechanism at a high temperature. It is then discharged mostly to the environment, in New York State, in the form of a stable, oil-in-water emulsion that is presently without prospects for re-use. Such dispersed systems require a relatively high energy input as that supplied by vigorous agitation at the retort operating temperature (121°C). Ghosh and Brown (1975) renovated similar effluents experimentally by filtration and coalescence, using carbon-iron and carbon-aluminum adsorbents to destabilize the residual haze (micro-emulsion). Their explanation of the process was based on electro-chemical theory. They used transmittance data to measure the degree to which initial water quality of the eluate was restored.

The purpose of this study was to ascertain the magnitude of lubricant contamination of this retort cooling water, and to devise a reliable method for its rapid determination, based on characteristics of the cooling water, since present analytical methods for the isolation and quantitation of grease from industrial wastewaters (APHA. AWWA. WPCF, 1971; Maxcy, 1976) are tedious and are subject to large errors of precision and accuracy (Ford and Elton, 1977).

EXPERIMENTAL

Lubricant profiles

Processing discharge samples were collected at the bottom of an adjacent processing-plant's cooling tower, four times daily for 15 days, and hourly on the 16th day, in the summer of 1977. Particulate matter was separated by settling and flotation before the stable emulsions were analyzed for grease (APHA. AWWA. WPCF, 1971) and acidity (pH). Hourly sampling and analysis were repeated in the summer of 1978. Standard emulsions and dilutions were also analyzed as cooling water. The dilutions were extracted with trichlorotrifluoroethane in a separatory funnel. The solvent was afterwards evaporated, and the residue was weighed.

De-emulsification

Two grams of aluminum filings, aluminum powder, and 40–60 mesh activated charcoals with a different equilibrium pH in a water suspension, were packed separately in 20 cm × 2.5 cm (o.d.) glass columns. The stable emulsions were allowed to percolate through each of these adsorption-filtration beds at a rate of approximately 1 ml/min. This rate was achieved by pre-tamping the columns simultaneously with the percolation of water through the beds. Aliquots of 5 ml were collected automatically by a fraction collector until there was visual evidence of eluting lubricant.

Photometry

Absorbance of each 5-ml aliquot was measured on a Spectronic 20 spectrophotometer (Bausch & Lomb, Rochester, NY) in the wavelength region of maximum absorption (460 nm). Standard emulsions were made from the processing-plant lubricants (Lubriplate, Super FML and FMO, Fiske Brothers Refining Co., Newark, NJ), as follows: 100 and 125 mg of the FML and FMO lubricants, respectively, was agitated for 5 min in 1L of 90°C water with the aid of a high-speed blender, and thereafter was diluted 1:2 (50 and 62.5 ppm), 1:4 (25 and 31.3 ppm) and 1:8 (12.5 and 15.6 ppm) with distilled water. These standard emulsions were passed through the adsorption-filtration columns, and an ultraviolet spectrum of each was obtained, before and after percolation through charcoal, on a recording spectrophotometer (Beckman Instruments, Inc., model DB, Fullerton, CA) between 320 nm and 220 nm. The absorbance data for each series of dilutions were plotted against lubricant concentrations.

Adsorption isotherms

One hundred milliliters of a standard emulsion (C'_o = 100 ppm) were agitated with 0.2, 0.4, 0.6 and 0.8g (M) each of aluminum and of charcoal. After 2.5 hr, the supernatant liquid was filtered through filter paper. Concentrations remaining in the liquid phase (C') were measured from a standard curve, and adsorption isotherms (Hassler, 1974) including correlation coefficients were then computed and drawn by the method of least squares. X ($C'_o - C'$) was the concentration of lubricant adsorbed.

Two-stage renovation

One gram of aluminum (1st stage) was packed in series with 1g carbon (2nd stage) in the same column. Effluent was allowed to percolate through the beds until the total volume of eluate showed an incipient turbidity.

RESULTS & DISCUSSION

THE DAILY SAMPLES collected at the food plant were a confluent discharge from many retorts. The collection was made at the cooling tower near the point of entry into a natural stream. The water temperature at this terminus was 20–26°C. The pH was 5.5.

Trichlorotrifluoroethane extraction of the standard emulsions resulted in 90–95% recovery of lubricant. The APHA. AWWA. WPCF method (1971) resulted in 110–115% recovery.

Lubricant profiles

Lubricant concentrations over the 15-day interval in 1977 were relatively constant at 30–40 ppm. However,

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there were occasionally excessive amounts (Fig. 1) that should be controllable through careful applications. Generally, therefore, the retort discharge may be described as being a very dilute emulsion of oil in water.

It became of interest to ascertain changes of lubricant concentration with time after the daily lubrication. Lubricant content of the discharge was again found to be relatively constant at approximately 40 ppm throughout the 16th day, after the initial application when it would be most likely to be in excess (Fig. 2, A). These levels were discussed with the plant's management. Subsequently, in 1978, they fell by 20–30 ppm (Fig. 2, B), thereby entering the range of concentrations found in similar industrial effluents (Jacob, 1978).

Photometry

It was discovered that the standard emulsions were turbid and stable enough to facilitate quantitation by measurements of optical density. These data yielded lubricant concentrations that compared favorably with trichlorotrifluoroethane recovery data which were in the 90–95% range. Such spectrophotometric accuracy was attributed to the constancy of the particle size in the micro-emulsions, that permitted the absorbed, transmitted and scattered fractions of light to be directly proportional to the number of dispersed oil or grease droplets, as these were represented by ppm (Fig. 3). This stability was evidenced not only by the reproducibility of the A vs ppm relationship (Fig. 3), but also by a constant optical density in a given emulsion for many days. The oil or grease content of retort discharges, therefore, appears to be amenable to routine photometric quantitation, in conformance with Lambert-Beer principles of light intensity.

De-emulsification

Aluminum filings, aluminum powder, and charcoal were all capable of removing lubricant from the influent emulsions, and charcoal, although claimed to have limited use in removing oil from industrial wastewaters (Ford and Elton, 1977), consistently yielded efficiency indices (defined as the weight of lubricant withdrawn/weight of adsorbent) that were comparable with those of aluminum. This index was calculated to be 18–20 lb lubricant per 10^3 lb, for those charcoals with an equilibrium pH of 5–7.

Flow rate and particle size were observed to affect the magnitude of the index. For example, within the limits of these experiments, and referring to charcoal only, the greater the tamping density for a given particle size, the slower was the percolation rate, and the higher was the efficiency index. Likewise, the smaller the particle size, the higher was the efficiency index at a given flow rate. Thus, performance characteristics, e.g., bed density, mesh size, column length, etc. (Ghosh and Brown, 1975) which influence the contact time between charcoal and influent will also determine the magnitude of the efficiency index. Additionally, the filtration component of the index should be higher for smaller mesh sizes, inasmuch as these would permit a greater exposure of micropores and capillaries that would effectively retain the relatively large lubricant particles, while allowing the passage of water.

The greater efficiency resulting from the use of smaller mesh sizes may also be interpreted as a response to an increase in the total adsorbing surface that would accommodate more lubricant molecules per unit weight. Moreover, the highly dispersed lubricant phase, tending to decrease spontaneously its surface energy, do so by coalescence (Jirgensons and Straumanis, 1962) at the carbon-water interface. This positive adsorption of lubricant would be maximized at the lowest practicable temperatures of the cooled effluent before percolation through adsorption-filtration beds.

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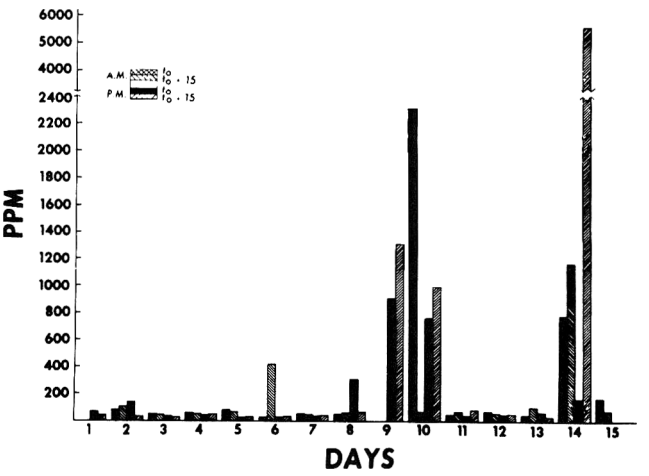

Fig. 1–Lubricant concentrations (by gravimetry) in a retort effluent during a 15-day interval in 1977, sampled daily at 10:00 a.m. (t_o), 10:15 a.m. (t_o + 15), 2:00 p.m. (t_o) and 2:15 p.m. (t_o + 15).

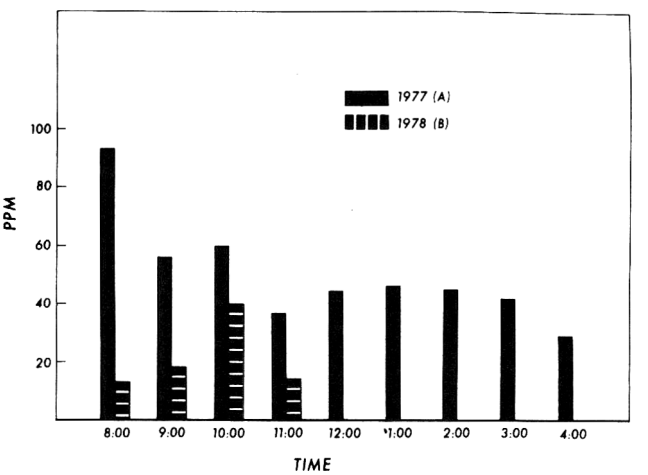

Fig. 2–Lubricant concentrations (by gravimetry) in a retort effluent sampled at hourly intervals, on the 16th day in 1977 (A), and concentrations (by spectrophotometry) in 1978 (B).

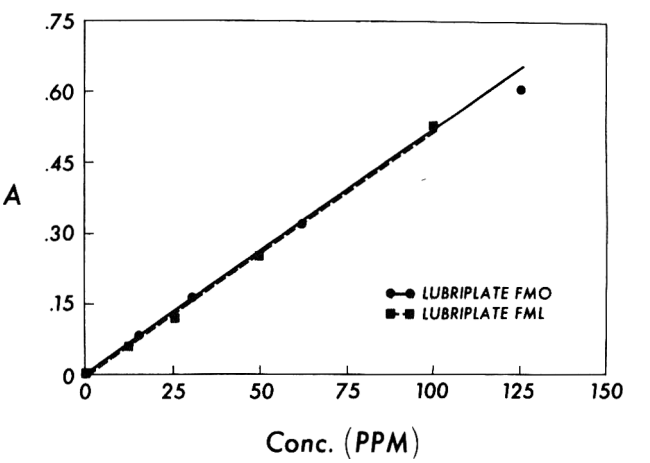

Fig. 3–Absorbance (A) vs concentration (ppm) of a series of standard emulsions of FML and FMO lubricants in water, at 460 nm.

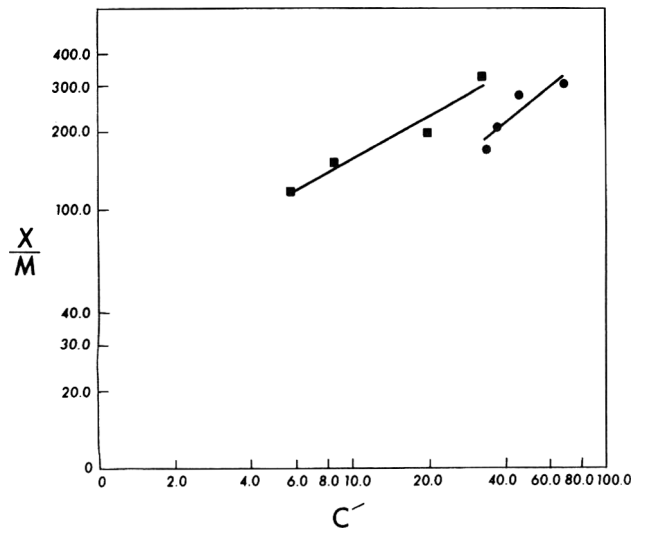

Fig. 4—Adsorption isotherm of aluminum and of charcoal in a standard emulsion containing FMO lubricant at 100 ppm (C'_o = 100%): C' = % remaining in the liquid phase; X = C'_o − C', the % removed by each adsorbent: M = weight of adsorbent.

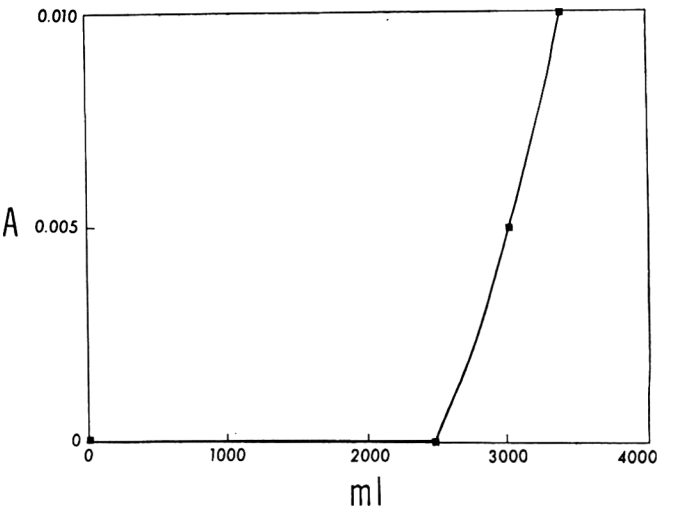

Fig. 5—Absorbance (A) vs volume (ml) of filtrate from a cooling retort discharge (17 ppm), after passage through aluminum powder (1g) and charcoal (1g) (1 ppm lubricant at A = 0.005; 2 ppm lubricant at A = 0.010).

Aluminum had an advantage over charcoal in being easily regenerated in situ by elutriation with an organic solvent.

Adsorption isotherms

Figure 4 represents the best of the aluminum and charcoal isotherms, having correlation coefficients of 0.97 for aluminum and 0.90 for charcoal. Agitating the mixture of emulsion and adsorbent would be expected to cause some deposition of lubricant on the surface of the containers. Minor imprecision notwithstanding, the high X/M values (weight of lubricant adsorbed per unit weight of adsorbent) are evidence of the practicability of renovating cooling water discharges with aluminum and charcoal. The slope of the isotherms (28° and 38° for aluminum and charcoal, respectively), being less than 45° [any interval of log (X/M)/(log C') < 1] indicates that resulting concentrations of lubricant remaining in the effluent (C') were quite low, relative to unit changes in the adsorbed concentrations on the aluminum and charcoal surface. However, it is also noted that while aluminum effected a greater reduction of lubricant concentration (100% to 6%) than did charcoal (100% to 33%), charcoal, on the basis of its slightly steeper slope, would be somewhat more efficient at higher lubricant concentrations.

Two-stage renovation

The capacity of aluminum to effect greater reduction of lubricant concentrations from the oil and grease emulsions was the basis of its selection as the 1st stage. After the break-through volume (2500 ml, Fig. 5), a distinct haze appeared and rapidly intensified at low absorbances (0.005–0.010). The exact break-through volume in any adsorption-filtration system will depend on a number of engineering variables (Ghosh and Brown, 1975).

The ultra-violet spectrum of a sample from the total volume of filtrate that measured 0 absorbance was identical with the ultra-violet spectrum of the reference solvent (water). This was an indication that no measurable amount of benzenoid additives, if present in the lubricant, was transferred to the purified effluent.

Since most oil and grease removal systems are designed for higher concentrations than those existing in retort effluent, one suitable for the fruit and vegetable processing industry might therefore comprise merely a series of charcoal columns, preceded by a series of aluminum columns, or by an agitating or percolating tank containing aluminum powder, in series with a terminal ozone or chlorine-disinfection system.

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A METHOD FOR THE CONCENTRATION OF PROTEINACEOUS SOLUTIONS BY SUBMERGED COMBUSTION

A. H. LUEDICKE, JR., BRUCE HENDRICKSON and G. M. PIGOTT

ABSTRACT

Proteinaceous solutions are difficult to concentrate in conventional facilities, due to denaturation, followed by "bake-on" of insoluble material on heat exchange surfaces. Vessels used are expensive and difficult to clean and are generally heated by steam from high first-cost boilers at low fuel utilization percentages, due to inefficiencies inherent in such units. A technique of concentrating fish hydrolysate (6–8% solids w/w) prepared by enzyme digestion was developed, using submerged combustion. In this method, combustion products of commonly available gaseous fuels are exhausted beneath the liquid surface. Heat transfer is extremely rapid and, since it occurs across bubble film surfaces, there is no bake-on. Use of secondary air cools the burner surfaces and allows regulation of bulk liquid temperature. Spray-dried material prepared from the concentrate is a fully functional protein; PER tests show high indices; fish protein averages 100% of the PER for casein. Fuel utilization is excellent; approximately 86% thermal energy input is used for evaporation. Bulk liquid temperatures were controlled from 158–169°F in concentrating hydrolysate with initial solids contents of 6–8%. Solutions of acidities ranging from pH 2.7–5.9 were concentrated to 30% solids, using commercial propane and compressed air. The cost was approximately $0.02/lb of 30% solids solution. Air-cooled submerged combustion appears to offer a very efficient method of heating and concentrating heat-sensitive solutions without incurring the penalties of "bake-on" and difficult clean-up. Low first-cost equipment may be used; the possiblity exists of using disposable tank liners.

INTRODUCTION

THERE HAVE BEEN many publications dealing with the search for inexpensive methods to produce wholesome protein supplements from animal and vegetable sources. Much of the work has been carried out on a small laboratory scale, often neglecting consideration of the facilities required to economically produce products on a commercial scale. Of particular concern in processes developed at the University of Washington (Pigott and Bucove, 1978; Chu and Pigott, 1973) is the evaporation of low solid concentration solutions prior to spray drying. The work presented in the paper is a description of an apparatus developed to efficiently concentrate (heat sensitive protein or) protein hydrolysate solutions with minimum nutrient degradation.

In the enzymatic hydrolysis method described by Bucove and Pigott (1975), a solution averaging 6–8% solids is concentrated to ca 30% solids before being spray-dried. Lower solids concentrations result in unacceptably high costs in spray drying. It became apparent from the time that the first bulk lots of hydrolysate were obtained that the step of dewatering the solution was critical, since operating costs and equipment first cost could inflate finished product cost.

Heating vessels represent a large capital investment and require a steam boiler. Steam boilers themselves are subject to film difficulties in heat transfer. This means additional requirements for feed water treatment facilities and a condensate system with its traps, drains, pumps, leaks, and entrained air. Since heated water vapor enormously increases its volume when pressure upon it is released, boilers are subject to intense municipal scrutiny, licensing, and inspection. Furthermore, in order to get exhaust gases up the stack, "draft" it usually achieved by the simple expedient of sacrificing some of the heat in the fuel burned for steam production. A maximum of 88% of the fuel consumed in a boiler is used to make steam. A very efficient large-scale central boiler utilizes 85% (Charm, 1971). To concentrate thermolabile solutions at the low temperatures (ca 163°F) used here, ejectors, condensors, low-pressure vessels, and evaporator heat transfer equipment are required. These items contribute additional first cost and operating inefficiencies of the total system.

In addition to nutrient retention, a major problem in conventional heat transfer equipment is "clean-up." In this instance, a clean-up is not simple sanitation, but the necessity of removing "bake-on" - denatured protein. This can be seen when milk is heated in a pan. The proteins in milk, small though they may be in amount, denature and become insoluble as heating proceeds. A film then forms on the interior of the pan, followed by a reduction in heat transfer through the metallic wall, due to this film. Since the heat source available is in excess of the requirement, the temperature of the pan wall will increase. As this occurs, "bake-on" and, often, charring occur. Considerable effort is involved in removing this film by scrubbing, and the possibility of damage to heating surfaces exists.

The principle of submerged combustion is a possible solution to the problems involved with conventional evaporation facilities. In submerged combustion, a premixed explosive gas/air fuel is burned under the liquid surface, with the products of combusion escaping in the form of bubbles. Heat is transferred directly to the bulk fluid through the bubble wall, so that film formation does not occur. Early attempts at designing such appartus date back almost 100 years, when Collier described his unit in a British patent dated 1887. Use of these burners became widespread in chemical and metallurgaical industries in the years that followed, but no evidence existed that such burners were employed in the direct preparation of food products. Submerged combustion has been used to evaporate waste materials. For example, Durkee and Lowe (1973) applied the technique to concentrating waste cucumber pickle liquor.

The units consist of three parts: (a) a mixer for combining the gas and air (liquid fuels are sometimes used in other industries). This may be an elaborate valve, obtaining its inputs from mass flow sensors, or a simple mixing chamber; (b) a velocity tube, through which the explosive mixture passes. The tube is so sized that mixture velocity comfortably exceeds flame propagation velocity for the one particular mixture employed. Premixing ensures efficient combustion; a homogeneous mixture precludes mixture perturbations which lead to flame instability; and (c) a combustion chamber in which the mixture burns. Contrary to what might be taken for granted, flame cannot be sustained when the mixture issues from a simple opening: the flame front is

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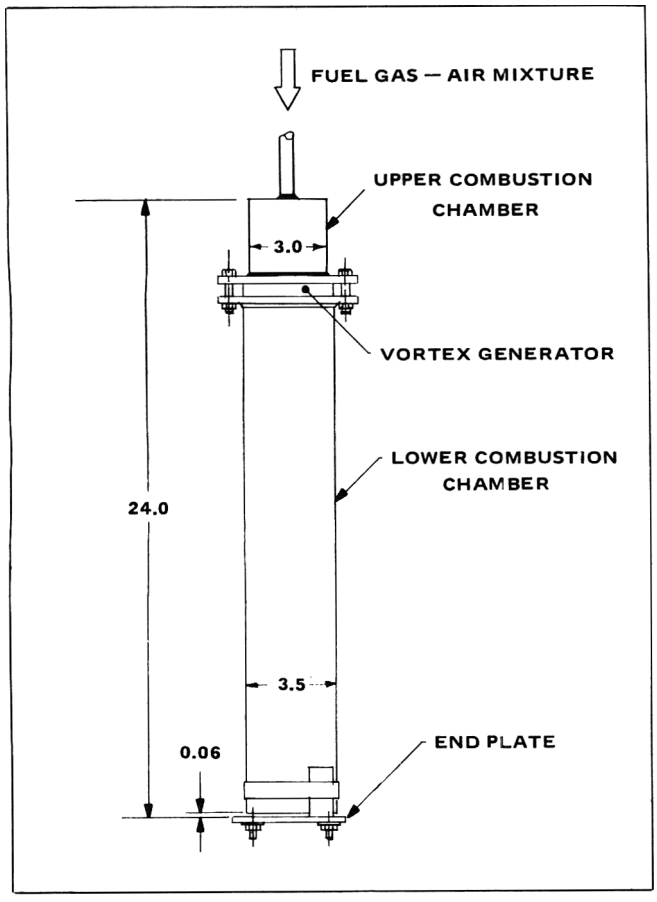

Fig. 1—Submerged combustion unit.

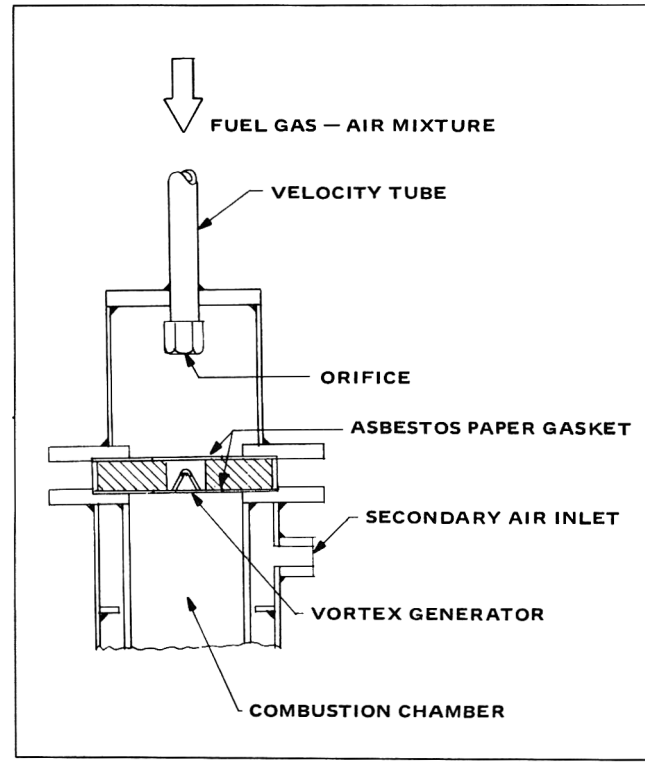

Fig. 2—Submerged combustion unit.

not uniform and the flame is extinguished. In order to have steady state flame, it is necessary to employ a vortex generating device suspended in a turbulent (Re $\geqslant$ 4,000) stream. These may range from a simple rod to complex baffling, but their purpose is to produce eddies in the stream in which the flame remains localized and acts as an ignition source for the incoming mixture. This principle is employed in all aircraft turbine engines.

EXPERIMENTAL

Apparatus

For initial experimentation, the dimensions of Kobe et al. (1933) were used to construct a small unit which differed from the original, however, in the means of ignition. Instead of an incandescent chamber liner, a vortex generator of wire gauze was employed in conjunction with chamber lining of refractory cement. Following initial test runs to evaporate water, a large unit of the Kobe type was designed and constructed to concentrate hydrolysate from the pilot plant. The vortex generator, instead of screen, was a metal angle placed apex up in an orifice. Also, corrosion resistant ("stainless") steel (AISI Type 304) was used to fabricate the combustion chamber. Commercial propane was used for fuel gas.

During the first trials, this unit produced disappointing results. A blackened, tarry residue resulted which smelled strongly of charring, and the spray-dried residue produced low PER's on animal feeding. Investigation revealed two sources of this phenomenon: (a) bulk liquid contact with the heated chamber lining due to splash, as well as external surfaces which heated as the lining became hot; and (b) flame length in excess of combustion chamber length. None of the end baffle arrangements tried entirely prevented splash or prevented flame from extending beyond the combustion chamber. Excessive flame length allowed the liner to reach high temperatures in the same areas in which splash occurred. To overcome this handicap, a combustion chamber surrounded by an annulus for the distribution of cooling air was designed and constructed, with introduction of secondary air into the combustion zone at midlength and at the exit end of the chamber. Sufficient length was afforded for flame containment and an unperforated end plate used at the exit to allow radial travel of the exhaust gas. This unit is illustrated in Figures 1, 2, 3 and 4.

Figure 1 shows the part of the submerged combustion tube which is immersed in liquid. The lower end plate was secured by two bolts which were welded to small angle sections. The angles were, in turn, clamped to the lower combustion chamber with a hose clamp. The end plate was equipped with a pivot hole and a slot so that it could be swung aside for lighting the flame.

Figure 2 shows a cross section of the assembly in the region of the vortex generator. The generator was formed from AISI Type 304 corrosion resistant steel sheet and suspended across a hole in the circular plate separating the upper and lower combustion chambers. Figure 3 shows a cross section of the lower combustion unit. It was constructed of welded AISI Type 304 corrosion resistant steel tubing and plate. The ring immediately below the secondary air inlet formed a plenum to distribute air around the circumference of the combustion chamber wall.

The secondary air nozzles were holes drilled through the inner wall, tangential to the inside surfae. The axis of each hole was at an angle with respect to the centerline of the lower combustion chamber.

Figure 4 shows a cross section of the mixer. It was constructed entirely of standard schedule 40 pipe and fittings. The 1/4 pipe and 3/8 pipe were drilled as shown. The purpose of the holes was to produce thorough mixing of air and gas. One of the ends of each diffuser pipe was welded shut. They were then welded into holes drilled in a standard pipe cap. The velocity tube is projected from the upper chamber. The upper end of this tube was affixed to the mixing unit.

For an evaporating vessel, a 17.0-in. diameter stainless steel tank was used which was 26.0 in. deep. A smaller sump, 10.0 in. in diameter and 11.0 in. deep, was welded to the bottom of the tank. The sump allowed immersion of the combustion chamber when concentration reduced the volume of bulk liquid. The tank was insulated with asbestos cement to reduce heat loss. Drains were

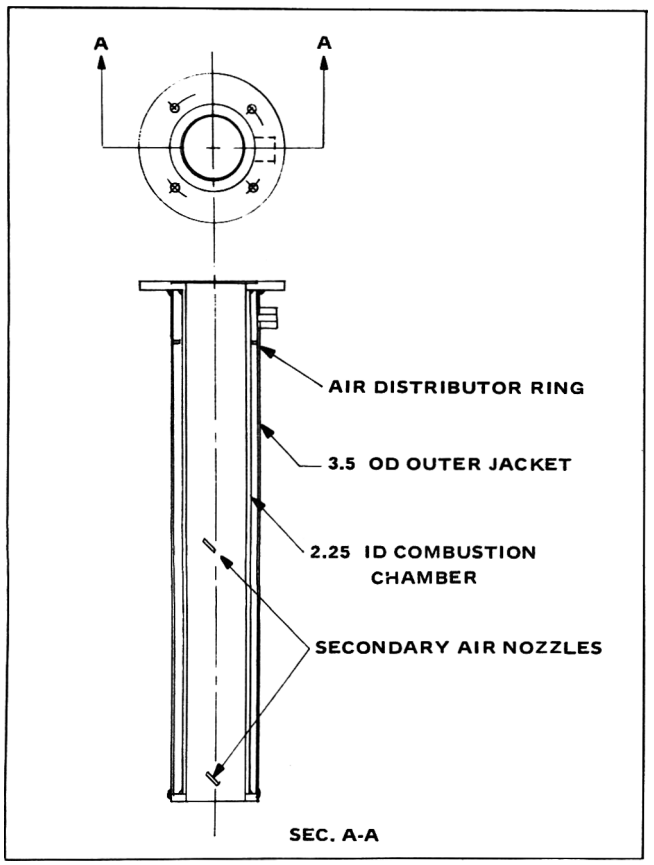

Fig. 3—Submerged combustion unit.

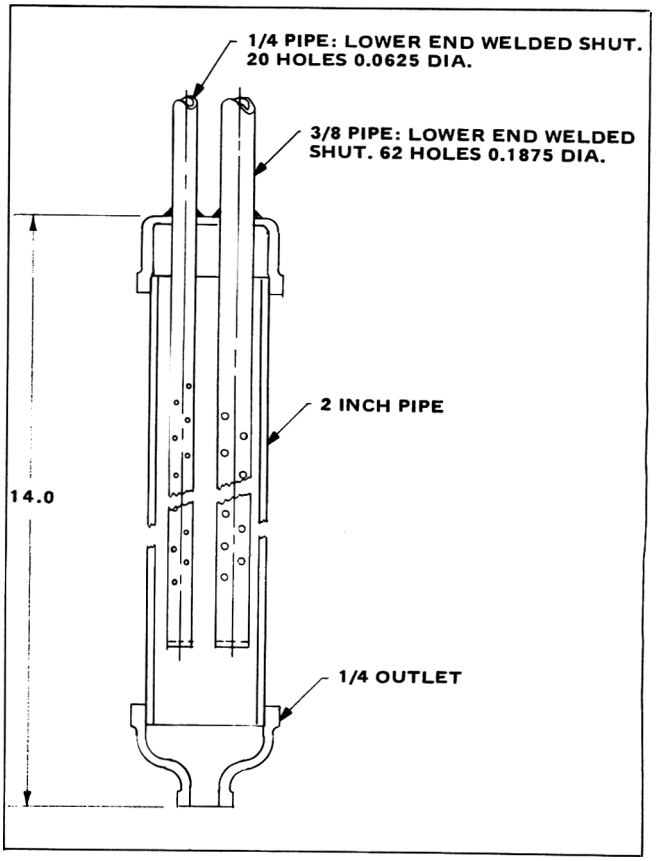

Fig. 4—Submerged combustion unit.

installed in both the upper and lower portions of the tank to allow withdrawal of liquid and cleaning. The tank was covered while evaporation was in progress to prevent liquid cooling from ambient air. Since the liquid surface is quite turbulent from ascending bubbles, cooling can be rapid; a temperature reduction of 18°F was noted when the cover was removed. In large full-scale operations, a duct is usually employed to remove water vapor from the evaporator.

The tank was placed on a small platform scale so that weight could be read directly to facilitate calculations for water removal. The scale and tank were located under a large overhead exhaust hood, through which the water vapor was withdrawn.

During the first few runs concentrating hydrolysate, a thermocouple array was used to measure liquid and vapor temperatures in various regions of the evaporating vessel. Investigation showed that these were within 1 to 2 degrees of each other. Since a clear trace of bulk liquid temperature history was all that was desired, only two thermocouple junctions were subsequently used.

Procedure

Aqueous fish protein solution was prepared by the method of Bucove and Pigott (1975) at pH values of 2.7, 4.6 and 6.0 in 55–80 lb lots so that adequate sample quantities of protein powder would remain available for biological testing after concentration and spray drying. Batch evaporation was carried out, beginning with the solution at room temperature. Gas flow was measured by a roto-meter type flowmeter calibrated by collecting gas over water.

The procedure followed in operating the unit consisted of turning on primary air to approximately half that used during concentration, then turning on propane in excess of the amount to be used. The gas/air mixture was then ignited with a standard industrial welding igniter. Secondary air was next turned on and the gas/primary air flow adjusted to the optimum amount. Experience indicated that this could be attained by watching the flame until a turbulent blue combustion resulted, together with a characteristic howling noise. The end plate was then fastened in place and the burner was lowered into the tank containing the hydrolysate by means of a small block and tackle.

In use, the entire combustion zone was under water and all of the heat liberated from the burning fuel could escape only into the surrounding bulk liquid. It should be appreciated that the combustion zone can be some distance from the liquid, as long as the exhaust gases are liberated *beneath* the surface. This modified burner performed very well, producing a light colored concentrate free of any odor of charring.

The amount of gas used for each run was determined as the product of average flow rate and elapsed time. Time to heat the vessel and hydrolysate to evaporating temperature was calculated, as was the time to evaporate to 30% solids. From recorded flow data, using published heat value for propane, heat to evaporate 1 lb of water, heat to concentrate to 30% solids, and heat required to produce each pound of 30% solids were calculated.

The heat required to concentrate fish protein hydrolysate is shown in Table 1. A typical time/temperature chart recording is shown in Figure 5. Time required to heat a batch from room temperature to evaporating temperature was taken as the point at which the slope of the temperature vs time trace begins deviating from a straight line, just before the "knee" in the trace.

Thirty percent solids concentration was the desired percentage. However, exact target percentages are difficult to achieve in the batch method, since evaporation continues after the heat source is removed. Therefore, heating must be stopped prior to reaching the target value. Weight readings from the platform scale were taken manually and corresponding time noted. Since initial solids concentration was known, the final batch weight could be calculated. As the target weight was approached, the scale beam counterweight was set at the desired weight. As the beam began dropping, the submerged combustion unit was hoisted out of the vessel.

Since the exact target concentration was never achieved, time to reach 30% solids was calculated from the time/weight data. These values are shown in Table 2.

It will be noted that gas used is given in terms of weight (Table 3). These weights are calculated from the cubic feet used, based on published heat value for commercial propane. This was done since sufficiently accurate weighing equipment of the capacity required to support large gas cylinders was not available.

The work was performed in a multiple use area which required

Table 1—Heat required to concentrate fish hydrolysate

Run no.	Elapsed time (min)	Weight (lb)		Heat used (Btu)			Btu to evaporate 1 lb water
		Total	HOH evap.	To warm[a]	Evaporate	Total	
18	120	55.5	47.5	6660	47067	53727	991
19	101	73.5	59.0	8011	56062	64062	950
20	128	55.0	43.0	Interrupted run — weld failure			
21	151	81.0	64.0	8701	69747	78448	1089
22	82	59.5	49.5	Interrupted run — severe foaming			
23	120	64.5	51.0	6192	52013	58205	1019
24	145	76.0	59.5	8360	61971	70331	1041
25	140	72.5	57.5	6525	63992	70517	1112
26	113	60.0	44.0	6960	47849	54809	1088
27	109	59.0	45.0	5900	51036	56936	1134

[a] Heat used to bring bulk liquid to temperature at which evaporation proceeds.

Table 2—Concentration of fish hydrolysate to 30% solids

Run no.	Total soln wt (lb)	Initial Conc (%)	Heat, Btu to 30%	Heat, Btu to warm[a]	Diff.	Pounds 30% solids	Btu/lb 30% soln	Time to reach 30% (min)
19	73.5	6.1	63428	8011	55417	14.94	3709	100
20	55.0	Interrupted run — weld failure						
21	81.0	6.9	77308	8701	68607	18.87	3635	148
22	59.5	Interrupted run — severe foaming						
23	64.5	8.0	56570	6192	50378	17.23	2924	117
24	76.0	7.0	69361	8360	61001	17.93	3402	143
25	72.5	6.1	66936	6525	60411	14.74	4098	138
26	60.0	7.6	55295	6960	48335	15.20	3179	114
27	64.0	6.4	57458	5900	51558	13.65	3777	110

[a] Heat used to bring bulk liquid to temperature at which evaporation proceeds.

disassembly of the equipment each day. Sources of error included leaks and pressure fluctuations. While leakage is not constant or measurable, it would contribute to a lower efficiency than that actually reported. Since the efficiency was quite good, this source of error does not mitigate validity of results.

Another source of variation was air pressure deviation (± 1.5 psi) which caused flow rate variation. Simliarly, there were small deviations (± 0.5 psi) in the fuel supply pressure and flow rate due to imprecision in the relatively crude pressure regulator used.

These deviations were compensated by manual adjustment of controls and averaging of flow rates observed throughout the run.

DISCUSSION

IT IS APPARENT that evaporation of water proceeded at a temperature considerably below boiling. This is true of all submerged combustion burners, but not to the extent possible with the unit described here. It results from the fact that the products of combustion contain considerable amounts of noncondensable gases, mainly nitrogen and carbon dioxide. The water vapor formed by combustion, as well as that liberated from the bulk fluid, is only a portion of the content of each bubble. Hence the partial pressure of the water vapor is less than the total pressure, and the boiling temperature is depressed accordingly.

The use of hot combustion gas has several advantages over dry air. Dry air bubbling through a liquid will cause evaporation to take place. However, evaporation by bubbling hot combustion gases and air is much faster than by bubbling dry air through a solution. The burner used here imparted 490 BTU/min to the solution concentrate, supplying nearly all of the enthalpy of vaporization for the 0.433 lb H_2O evaporated per minute.

Another advantage of using submerged combustion is the high efficiency of evaporation obtained. If the heat contained in the water that is evaporated (including water formed during combustion) and noncondensable gases is divided by the heat content of the gas burned to effect evaporation, plus sensible heat of the combustion air, evaporative efficiency is found to by 86%. It has already been pointed out that in submerged combustion the heat liberated from the fuel can escape only through the surrounding liquid and therefore high efficiencies are to be expected. However, the manner in which the heat transfer occurs proves to be quite different from what one might expect, particularly with a flame temperature of 2000°F.

Table 3—Fuel required to concentrate fish hydrolysate.

Run no.	Pounds water evap	Evaporate temp (°F)	Btu to evap	Pounds gas used[a]	LB water / LB gas
18	47.5	169	47067	2.183	21.75
19	59.0	169	56052	2.599	22.70
20	43.0	Interrupted run — weld failure			
21	64.0	163	69747	3.235	19.63
22	49.5	Interrupted run — severe foaming			
23	51.0	164	52013	2.412	21.14
24	59.5	163	61971	2.874	20.70
25	57.5	158	63992	2.968	19.37
26	44.0	160	47849	2.219	19.82
27	45.0	160	51036	2.367	18.98

[a] Based on published values for commercial propane of 21,560 Btu/lb.

By observation and calculation, it can be shown that 1 lb of dry air will form ca 8×10^6 bubbles 0.25 in. in diameter, with a surface area of ca 6000 sq ft. The experimental unit produced (including secondary air) 43.6 lb of noncondensable gases per pound of propane burned. This results in a bubble cloud with a total surface area of some 2.6×10^5 sq ft. Heat transfer coefficient across a liquid surface is quite low, approximately 2.5 BTU per hr-sq ft-°F (Charm, 1971). Temperature difference across the bubble wall is insignificant—some 0.035°F. The small temperature gradient results from cooling by the secondary air, as well as the rapid heat transfer brought about by the tremendous surface area. Heat transfer is nearly instantaneous, and thermo-

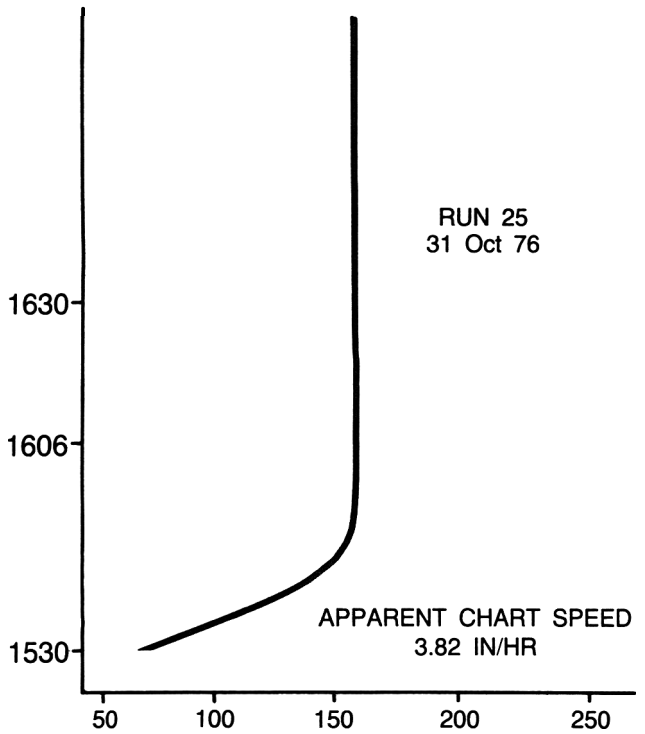

Fig. 5—Typical time/temperature chart recording. The time required to heat a batch from room temperature to evaporating temperature was taken as the point at which the slope of the temperature vs time trace begins deviating from a straight line, just before the "knee" in the trace.

couple arrays did not detect any liquid areas warmer than bulk liquid temperature.

Based on propane cost of $0.11/lb and electricity at $0.04/KWS to operate a blower or compressor, the cost to concentrate 1 lb of 30% solids from an initial concentration of 6–8% was $0.021. Other gaseous fuels such as methane or even hydrogen may possibly be used, as the economics of the future may dictate.

One question which frequently arises concerns foaming. It is true that foaming sometimes did occur but, with one exception, was never severe. When foaming did occur, it began always during heat-up of the bulk liquid, becoming most noticeable at 90–100°F. As bulk liquid temperature rose, foaming decreased and stopped entirely over 150°F, except in one instance when a run had to be discontinued due to liquid loss. Foaming did not seem to be related to pH or initial solids concentration; however, hydrolysate which had been stored for some time seemed, in general, to foam very little or not at all. No investigation was conducted on foam formation in elevated temperature environments, and it would not be prudent to offer predictions at this time beyond remarking that foam containment can most likely be dealt with by evaporating vessel design. In general, while evaporating proteinaceous solutions to 30% solids, removing an average of 26 lb of water/hr, foaming did not seem to be a governing parameter.

As reported by Heggelund and Pigott (1977), the protein efficiency ratio (PER) values for spray-dried supplemental fish protein (SFP) prepared from the concentrated solutions (30% solids) consistently ran well above those of casein, ranging between 110–130%.

SUMMARY & CONCLUSIONS

SUBMERGED COMBUSTION offers a low first-cost unit which can be used for a variety of food processing operations. Operating costs are low and clean-up is minimal.

The experimental unit described in this paper cost approximately $1,000 to construct of corrosion-resistant (stainless) steel in university shops. The 1975 price for a small commercial unit of this type was $15,000 for a unit with an output of 150,000 BTU/hr, including underwater ignition and mass flow monitoring of fuel gas and air. The only equipment required other than a blower is an insulated evaporating vessel which needs no jacket or heat exchanger. It is not inconceivable that supporting structures employing disposable liners might be used. The units operate unattended and, hence, manpower requirements both in terms of numbers and of skill level required are low.

Cleaning requires only brushing and rinsing, since no bake-on occurs. The temperature of the vessel wall is no higher than that of its contents. An additional benefit attained is the vigorous agitation due to the mass of rising bubbles. By the addition of a plain tubular shroud, Hammond (1930) showed how a simple but effective pump, employing the air-lift principle, can utilize the bubble stream as motive power. Thus, these units might be employed for a wide variety of heating and mixing or cooking tasks.

The use of a mixture of air and combustion gasses to concentrate a thermolabile food protein solution has been demonstrated to be highly efficient, effective, and gentle to the material. Many applications of this technology may well be found in the concentration of various heat-sensitive solutions such as milk, sugar, fruit juice, protein solutions, extracts and pharmaceuticals.

Further research and development of air-cooled submerged combustion for the food industry certainly appears needed in this time of energy scarcity and cost abundance.

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COMPARISON OF FARMERS' MARKET AND SUPERMARKET PRODUCE: TOMATOES AND BELL PEPPERS

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ABSTRACT

Undergraduate students made flavor and appearance ratings of tomatoes and bell peppers purchased from certified farmers' market suppliers and from supermarkets in the same cities. Tomatoes from the farmers' markets were less expensive, those from the supermarkets were more attractive, and there was no difference in flavor preference in double-blind taste trials. Bell peppers from a certified farmers' market were preferred in double-blind flavor trials over bell peppers from supermarkets.

INTRODUCTION

THERE IS INCREASING interest in direct market operations as an alternative to the regular commercial marketing system. Programs are being developed at all levels of government to encourage direct marketing. At the federal level, the Farmer-to-Consumer Act of 1976 offered grants to states to develop farm trails associations, U-pick operations, and farmers' markets. In Canada, the Province of Alberta provides grants to market gardeners who sell through U-pick or "farm gate sales" and to qualifying organizations and individuals to sponsor farmers' markets. Numerous advantages have been claimed for farmer-to-grower operations. The reasons most frequently cited by proponents are that they offer the consumer more nutritious, fresher, and tastier food at less cost by eliminating the middle levels of the marketing system. Surveys have found that consumers by and large accept these claims (Zehner and Meldrum, 1969; Bennett, 1974). Surveys by USDA indicated that access to better quality food was the major reason for direct market buying (Jones et al., 1978).

Despite repeated claims of advantages of direct marketed produce to consumers, little empirical evidence on this issue is available. In view of increasing government interest in direct market programs, it seems important to sort out fact from fiction in this area. Schutz and Lorenz (1976) found that there was no difference between organic and nonorganic produce in double-blind flavor trials. The present study is designed to test flavor and appearance attributes of direct marketed produce. Specifically, it involves a comparison in double-blind flavor and appearance trials between two items (tomatoes and bell peppers) commonly sold in direct marketing operations with comparable items purchased in supermarkets. In addition to flavor and appearance, a third attribute of direct marketed produce, price, is also included as a variable in the study.

The flavor and appearance of tomatoes have been the subject of considerable discussion in the popular press. In some sense the tomato has become a symbol of what is wrong (or right) with American agriculture, depending upon which side you are on. Surveys conducted by the USDA in 1974 and 1976 showed a high level of consumer dissatisfaction with fresh tomatoes—more dissatisfaction than with any of the other 32 food products tested. The major focus of the dissatisfaction was on price, taste, and ripeness, and was more concentrated among the younger, better educated, and more affluent consumers (Handy and Pfaft, 1975; Weimer and Stevens, 1974). Consumers were much less critical of the appearance than they were of other characteristics.

Defenders of the store tomato see it as a boon to the customer, combining year-round national availability with adequate flavor and appearance. The problems, its proponents maintain, are caused by its being picked too early and faulty storage practices by stores and customers. Proponents also maintain that criticism is based largely on confusion in the public's eye between tomatoes grown for commercial processing, which are intended for machine harvesting and handling, and table variety tomatoes which, at least in California, are still harvested by hand (Rick, 1978). This confusion is not surprising in that the typical consumer knows little about the origin of tomatoes found in the supermarket. The consumer expects the tomato in the produce bins in the dead of winter, regardless of whether it was grown in a hothouse or imported from another nation, to be the same fruit that is available in the summer. Finally, consumers are given little education on proper storage methods for tomatoes.

The present paper describes four studies designed to compare the flavor and appearance of farmers' market and supermarket produce, two studies each involving tomatoes and bell peppers. Comparisons are made using both hedonic ratings (overall liking or disliking) and paired comparison tests between produce from the two sources. Rather than ask the same subject to make both kinds of judgments, hedonic ratings and paired comparisons were done as separate procedures involving different subjects.

STUDY 1: TOMATOES, HEDONIC RATINGS

Materials and methods

Selection of produce. Two researchers were involved in these studies, Researcher A, who purchased and coded the produce, and Researcher B, who conducted the taste trials and was unaware of the origin of the produce. The first issue to be decided concerned the selection of tomatoes. One possibility was to ask Researcher A to select the best tomatoes from each supplier. This would have introduced considerable subjectivity and the possibility of personal bias (due to a preference to a certain size or shape or color) and also would have yielded unrepresentative produce for the testing sessions. Instead, we opted for a prepared random selection system. Researcher A had a written plan which required him to select the third tomato in the second row, the second tomato in the fourth row, and so on. The only exception would be for the researcher to avoid any tomato clearly unsalable because of blemishes, cracks, etc. On this latter basis, two tomatoes which had met the requirements of the prepared random plan, one from a supermarket and the other from a farmers' market, were rejected.

All purchases were made during the first 2 wk in Sept., 1978. The supermarkets all belonged to large national chains. There was a significant difference in cost between the tomatoes purchased from the two sources. Tomatoes purchased from the supermarkets averaged 49¢ per pound compared to 21¢ for those tomatoes purchased

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from the farmers' market. This difference is consistent with price comparisons we have made over a much larger range of produce (Sommer, 1979).

All tomatoes were purchased by Researcher A and stored at room temperature for 1 day prior to the flavor trials. Then they were transported to tasting laboratories at the University of California, Davis campus, washed, placed on small white paper plates, and coded in series from A1–A6, B1–B6, C1–C6, and D1–D6. Those tomatoes used for the flavor trials were cut into eight sections and those used for the appearance trials were left intact. The times-to-serving for the cut tomatoes ranged from 10–90 min.

Subjects. The subjects were 45 University of California, Davis undergraduate student volunteers recruited from introductory psychology classes. These students all received small amounts of extra credit for participating in experiments, since this was considered a worthwhile learning experience for the students.

Setting. Subjects made their judgments in individual tasting booths specially designed for flavor tests. These booths had small windows through which food samples could be passed without any conversation or visual contact with the experimenter. Each booth contained a small fountain which the subjects could use to rinse their mouths if they wanted.

Rating scales. Two rating scales were constructed prior to the test sessions, one for flavor, and the other for appearance. The respondent was first asked to choose among six adjective pairs (sweet-not sweet, wrinkled-not wrinkled, etc.) and then to give an overall hedonic rating on desirability. The subject could check anywhere along the hedonic scale which ran from *dislike very much* (0 centimeters) up to *like very much* (18 centimeters). The specific adjectives used in the flavor trials were as follows: *texture*, too mushy-too hard-about right; *flavor*, too weak-too strong-about right; *skin*, too thick-too thin-about right; *acidity*, too acid-too bland-about right; *moisture content*, too runny, watery-too dry-about right; *sweetness*, too sweet-too bland-about right. The specific adjectives used to measure external appearance were: wrinkled-not wrinkled, too large-too small-right size; deformed-good shape; unappetizing-appetizing; blemished, spotted-unblemished; and artificial-natural. The subjects were asked to check one adjective in each response category. The subject's score on the hedonic scale was measured to the nearest centimeter and used as an index of overall preference. Each subject completed his or her flavor tests before beginning the appearance ratings.

The study was conducted over two successive days, and involved 27 subjects and 24 tomatoes, four from each of three supermarkets, and four from each of three farmers' market suppliers. Each subject was given six tomato sections, one at a time. Subjects were asked to taste as much as they wanted of the tomato section and evaluate it on the rating sheet. A separate rating scale was used for each tomato section. When the subject had done this, the subject clicked a light and a new tomato slice and a new rating sheet were then passed to the subject through a metal gate by the experimenter who was out of the subject's view. After the subjects had rated the flavor of 6 tomato sections, the appearance trials were begun. The subjects were given whole tomatoes on paper plates, one at a time, to be rated on appearance.

Results and discussion

The first data to be analyzed involved the centimeter measurements of the subject's rating on the hedonic scales. Two analysis of variance tests were run, one for flavor and the other for appearance, followed by t-tests where appropriate. The first analysis compared all tomatoes from the three farmers' market suppliers with all the tomatoes from the three supermarkets. As Table 1 shows, there was no difference in the overall flavor ratings of the tomatoes from the two sources. A more detailed inspection revealed that the highest rated tomatoes came from one farmers' market supplier, but the next two highest rated tomatoes came from supermarkets.

A very different result was apparent in the appearance ratings. The average rating by all subjects of the supermarket tomatoes was 11.84 compared to an average rating for the market tomatoes of 8.22. This difference is highly reliable ($p < 0.001$). The tomatoes from one particular chain store were particularly desirable and received top ratings from most subjects. Curiously, the tomatoes from this particular supermarket had been considered *least* desirable during the flavor trials. However, there was no overall correlation between the average hedonic ratings between the taste and appearance trials (rho = 0.07, $p > 0.90$).

Table 1—Flavor and appearance of farmers' market and supermarket tomatoes

Source	Mean hedonic rating[a]	F	p
	Flavor		
Supermarket	10.22	0.48	N.S.
Farmers' Market	9.72		
	Appearance		
Supermarket	11.84	24.67	0.001
Farmers' Market	8.22		

[a] Scale from 0 (dislike very much) to 18 (like very much)

Table 2—Correlation of individual flavor attributes with overall liking

Attribute	Correlation with overall liking: Farmers' market tomatoes	Supermarket tomatoes
Texture	0.446***	0.568***
Skin	0.309**	0.153
Moisture content	0.203*	0.304**
Flavor strength	0.478***	0.367***
Acidity	0.269**	0.227*
Sweetness	0.360***	0.299**
Sum of all attributes	0.717***	0.718***

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

In addition to the hedonic rating scales, subjects also filled out a six-item adjective checklist on both flavor and appearance. On the flavor checklist there were no differences between tomatoes from the two sources in regards to texture, skin quality, strength of flavor, acidity, or sweetness. There was a significant difference between tomatoes from the two sources in perceived moisture content. Significantly more of the farmers' market tomatoes were too runny and more of the supermarket tomatoes were too dry ($\chi^2 = 12.3$, d.f. = 1, $p < 0.001$).

On the appearance checklist, there were no differences between the judges' ratings of the tomatoes from the two sources in terms of wrinkles, deformations, or blemishes. Significantly more of the farmers' market tomatoes were judged too small while more of the supermarket tomatoes were judged too large ($\chi^2 = 78.97$, d.f. = $p < 0.001$). Supermarket tomatoes were also rated as having a more appetizing appearance ($\chi^2 = 10.41$, d.f. = 1, $p < 0.001$).

Correlations were computed between the overall hedonic rating of flavor and the individual flavor attributes on the checklist. Table 2 shows that all the individual attributes correlated with the hedonic rating to some degree, but that texture, flavor strength and sweetness correlate most highly. The composite responses to all six flavor attributes correlated 0.717 and 0.718 (both significant to the 0.001 level) with overall hedonic ratings for the farmers' market and supermarket tomatoes respectively.

Correlations were also computed between the overall hedonic rating of appearance with the individual appearance

Table 3–Correlation of individual appearance attributes with overall liking

Attribute	Correlation with overall liking	
	Farmers market tomatoes	Supermarket tomatoes
Wrinkles	0.295**	0.187*
Shape	0.472***	0.572***
Blemishes	0.156	0.338***
Size	0.003	0.278**
Appetizing appearance	0.787***	0.683***
Artificial/natural	0.313**	0.324**
Sum of all attributes	0.674***	0.721***

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

attributes on the adjective checklist. Table 3 shows that for tomatoes from both sources, overall liking correlated most highly into appetizing appearance and shape (lack of deformations) and least with tomato size. These results should not be generalized beyond an acceptable range of produce. As a selection criterion, all of our tomatoes had to be considered salable in order to be included in the study. None of the tomatoes were blemished or wrinkled to a degree that it would make them unsalable. It is within this acceptable range of produce that appetizingness and good shape are most highly correlated with overall liking.

The composite response for all six appearance attributes was correlated with overall liking. This resulted in coefficients of 0.674 for the farmers' market tomatoes and 0.721 for the supermarket tomatoes, both significant at the 0.001 level.

The general conclusion is that for these subjects in double-blind testing, there was no difference in preference and acceptance between farmers' market and supermarket tomatoes and that the appearance of the supermarket tomatoes was superior to that of the farmers' market tomatoes. Texture and flavor strength were most highly related to the overall flavor ratings, and appetizing appearance and shape (lack of deformations) were related most highly to the overall ratings for appearance.

STUDY 2: TOMATOES, PAIRED COMPARISON

BECAUSE some researchers believe that a paired comparison procedure is more sensitive to flavor differences than a rating scale procedure (Gridgeman, 1961), a second study was undertaken. Instead of asking subjects to rate single slices of tomato along various dimensions, the paired comparison procedure was employed. Subjects were presented with two tomato segments, each with a code number, asked to taste both and indicate which of the two tasted better. Study 2 involved 18 subjects, and 24 tomatoes, half of which came from three supermarkets and half of which came from three farmers' market suppliers. No appearance ratings were undertaken in Study 2. Instead we used a brief questionnaire to assess the subjects' attitudes towards tomatoes. We wanted to see whether students who expressed a strong liking for tomatoes might show different preferences than those who were indifferent to tomatoes.

Results and discussion

There was no trend for the subjects to prefer tomatoes from either source. An analysis of the attitude scale results also disclosed no significant trends. Those subjects who indicated that they liked fresh tomatoes the most and would go out of their way to buy them directly from farmers, did not show any more preference for farmers' market tomatoes over supermarket tomatoes than did those respondents who were indifferent to tomatoes.

Previous research on this campus has established that tomato tasters can distinguish between vine ripened tomatoes and those picked mature green and allowed to ripen later (Kader et al., 1977). In the present study, which used representative items from two sources–supermarkets and farmers' markets–no firm knowledge was obtained about the origin or picking conditions of the fruit. However, our interviews disclosed that farmers' market suppliers tend to harvest produce the day before or the morning of the market. None of them had sophisticated storage or transporting facilities. Fewer assumptions can be made about the supermarket produce. Whatever its origins and picking conditions–vine ripened or mature green–they were equal in preference in flavor and superior in appearance to the farmers' market tomatoes. Since the tomatoes had been purchased during the peak of tomato harvest season in a major tomato growing area, it seems very likely that they were not hothouse tomatoes.

The second aspect of the study concerned the appearance of the produce. Supermarket tomatoes were rated as more desirable in their appearance than those from the farmers' market. It should be noted that all tomatoes were stored for at least a day after purchase at room temperature. Previous research has indicated that refrigeration can adversely affect tomato appearance and texture (Kader et al., 1978).

To assess the practical significance of these results, it is necessary to include the cost variable which was not a part of the flavor and appearance ratings. Subjects had no knowledge of the cost of any of the produce items they tasted. However, any attempt to develop consumer implications of these findings must include consideration of cost. Interviews with farmers' market customers that were part of our larger study of direct marketing practices (Sommer, 1979) revealed that price savings was a major reason why many consumers, particularly those who are older, retired, and living on fixed incomes, go to farmers' markets. Supermarket tomatoes purchased for the testing sessions were 135% more expensive than those from the farmers' market. This parallels the results from a larger series of price comparisons based on the average price of 26 farmers' market suppliers in three different cities and of the 29 supermarket prices in those same cities. This study showed that the average price of supermarket tomatoes was 118% higher than the average price of direct market tomatoes.

How does one put together this information on flavor, appearance, and price? The purchase decision made by the consumer is one of trade-offs rather than absolute values. Our data make it clear that flavor need not be a factor in the consumer's decision. At least under the circumstances in which this study was undertaken, there was no flavor preference between the tomatoes from the farmers' markets and supermarkets. If the consumer is primarily interested in price, then the farmers' market tomatoes are obviously the best buy. However, this must be traded off against appearance. The supermarket tomatoes look better. Whether this is a result of varieties chosen or of culling or of some combination of the two, we do not know.

The task of consumer research is to generate valid information without making decisions for consumers. Presumably consumers can decide the extent to which they are willing to trade off appearance for cost savings. This raises many interesting questions about marketing and display practice which put great emphasis on attractive appearance on the assumption that "Consumers buy tomatoes on looks." A major wholesale distributor of tomatoes on the East Coast was quoted in the press as saying, "The important thing for us in a tomato is to get it to the consumer looking good. No blemishes, no black spots, no softness" (Whiteside, 1977). Since estimates of the cullage in Califor-

nia tomato packing houses is found to range between 15% and 50% of delivered fruit, with an overall average of 20% cullage (Kader and Morris, 1976), it is an interesting question whether commercial outlets would find it feasible and profitable to provide consumers with additional tradeoffs in price and appearance.

BELL PEPPERS

THE PRESENT STUDY describes flavor trials undertaken with bell peppers (*Capsicum annuum*), another item commonly sold in direct markets. The basic question is whether bell peppers purchased from certified farmers' market suppliers would be preferred in double-blind taste trials to bell peppers purchased from supermarkets in the same city.

Materials and methods

Produce. Three bell peppers were purchased from each of two supermarkets affiliated with large national chains and two suppliers at a certified farmers' market. All purchases were made on the same day. Selection of produce followed a prepared random system in which the researcher had decided beforehand to select peppers in specific locations. Prices paid at the supermarket averaged 64¢ a pound compared with 25¢ a pound at the certified farmers' market. All the bell peppers were stored in bags at room temperature for 2 days before the tasting trials.

Subjects. Subjects were 21 undergraduate students in a class in research methods.

Setting, instruments, and instructions. These were similar to those in Studies 1 and 2.

Experimental design. All flavor trials took place during a single class period. The instructor had cut up the bell peppers beforehand into equal sized pieces, placed them on small plates, and wrote a code letter (A-D, M-P) on each plate. Students worked in pairs testing one another. One student presented the stimuli and the rating sheets, while the other tasted and made the ratings. After the series was complete, the two students switched places, and the second tasted and rated the bell peppers.

Eleven students used the rating scale procedure. Each was presented in succession with a section of bell pepper from each of the four suppliers (two farmers' market growers and two supermarkets). The order of presentation was counterbalanced across subjects. Ten subjects made paired comparisons indicating which of the two taste samples they preferred. Pairings were randomly selected from all possible pairings. Each subject rated three pairs of peppers. Each bell pepper was assigned a score based on the number of times it was preferred over the others.

Results and discussion

The average rating of the farmers' market peppers was 8.8 compared to 3.2 for those from the supermarkets. Analysis of variance showed the farmers' market peppers were preferred over those from the supermarket ($F = 4.1$, d.f. = 1/42, $p < 0.02$).

In addition to the overall hedonic rating, each student rated the pepper slice on five attributes—sweetness, crispness, skin toughness, flavor, and aftertaste. The student could choose one of three categories (for example, sweet-in between-bitter) and the desirable quality was scored 2, the middle category 1, and the undesirable quality 0. The students' ratings of the bell peppers from supermarkets and farmers' market suppliers are shown in Table 4. The farmers' market peppers were significantly better in terms of sweetness and aftertaste.

Similar results were found in the paired comparisons. Peppers from the farmers' market were preferred on 24 occasions, peppers from the chain stores were preferred on five occasions, and on one trial the student did not give a preference. The difference is also highly reliable ($\chi^2 = 12.4$, d.f. = 1, $p < 0.01$). Students in the paired comparison procedure were also requested to write comments indicating the basis of their judgment. Peppers from the farmers' market were described as sweeter, crisper, and less bitter than those from the supermarket.

Table 4—Attributes of bell peppers

Attribute	Mean rating (0–2) Supermarket pepper	Mean rating (0–2) Farmers' market pepper	t ratio	p
Sweetness	0.6	1.7	3.62	0.01
Crispness	1.6	1.8	0.68	N.S.
Skin (tough/tender)	0.5	0.4	0.32	N.S.
Flavor (mild/strong)	1.0	1.4	1.58	N.S.
Aftertaste (sweet/bitter)	0.6	1.4	3.65	0.01

It seems odd that the flavor of fresh tomatoes has received so much criticism in USDA surveys and popular articles, and there has been little public criticism, as far as we know, directed at bell peppers. The reason may be due to the nature of the food item itself. Some of the subjects in the present study expressed surprise when the procedure was initially described to them. The idea of tasting bell peppers somehow seemed odd. They had never really paid attention to bell peppers before. Our results make it clear that a high level of awareness is not necessary for untrained subjects to detect flavor differences among produce items. Taste trials can bring to consciousness differences which are otherwise unrecognized. This is especially likely in the case of a food item that does not play a significant role in peoples' diets.

These results require us to ask why there is so much criticism of commercial tomatoes both in consumer surveys and in the popular press when our double-blind flavor trials show no preference for farmers' market tomatoes over supermarket tomatoes. The answer may lie in the confusion in the consumers mind between different sorts of tomatoes and in seasonal differences. It is apparent that some criticism of the "tough, tasteless, square tomato" is based on a confusion between cannery tomatoes which are meant to be machine harvested and table tomatoes which are, at least in California, almost entirely harvested by hand. There is also a lack of consumer information about differences between hothouse tomatoes and field-ripened tomatoes. A customer walking down the supermarket aisles seeing a tomato at the produce section in the dead of winter is likely to generalize its flavor to all supermarket tomatoes. More realistic perceptions of what can be expected from produce at different times of the year would reduce consumer criticism or at least channel it in the right direction. Problems with some produce items, including tomatoes, may lie in storage conditions. Despite research that has shown that refrigeration impairs the flavor of table tomatoes, some retail outlets continue to store tomatoes under refrigeration.

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EFFECT OF SHORT-TERM HIGH CO_2 TREATMENT ON THE MARKET QUALITY OF STORED BROCCOLI

C. Y. WANG

ABSTRACT

Broccoli was treated at 5°C with 20, 30 and 40% CO_2 for 3 and 6 days soon after harvest, and then transferred to storage in air at 5°C. CO_2 injury was severe in samples treated with 40% CO_2 for 6 days. Offensive odor and flavor also developed by the end of 6 days' treatment with 30% CO_2 but dissipated when the broccoli was transferred to air. CO_2 delayed yellowing and loss of both chlorophyll and ascorbic acid, and retarded ethylene production. Except at the level that was injurious to the tissue, CO_2 retarded mold growth and had no appreciable effect on compactness, turgor, and opening of the florets.

INTRODUCTION

THE SHORT-TERM application of high CO_2 was found to extend the storage life and keeping quality of Golden Delicious apples (Couey and Olsen, 1975), Anjou pears (Wang and Mellenthin, 1975), and strawberries (Harris and Harvey, 1973), It also reduced soft rot in asparagus (Lipton, 1968) and stem-end rind breakdown in grapefruits (Hatton and Cubbedge, 1977).

However, exposure to CO_2 may not benefit all vegetables and fruits. CO_2 enhanced the danger of chilling injury in tomatoes (Parsons et al., 1970), caused development of brown stain in lettuce (Stewart and Uota, 1971; Lipton et al., 1972), induced excessive softening and discoloration in cauliflowers upon cooking (Lipton et al., 1967), and increased decay in potatoes (Nielsen, 1968). With sweet peppers, high concentrations of CO_2 had to be continuously present in the surrounding atmosphere to be effective in retarding senescence, and little beneficial residual effect could be derived from a short-term treatment of high CO_2 (Wang, 1977a). In McIntosh apples, it was found that the potential for injury outweighed the benefits from high CO_2 pre-treatment followed by controlled atmosphere storage (Bramlage et al., 1977).

The usefulness of short-term high CO_2 treatment must, therefore, be evaluated separately for each crop. Considerations important in each evaluation include concentration of CO_2, sensitivity of the product to CO_2 injury, stage of product maturity, treatment temperature and durations and O_2 level in the atmosphere during treatment.

Many factors lower the market quality of broccoli after harvest (Morris, 1947), but one of the most serious is yellowing of the florets (Ryall and Lipton, 1972). Florets turn yellow very rapidly, especially at high temperatures (Lieberman and Hardenburg, 1954). Yellowing of the broccoli head overrides any other factor and limits its storage and shelf life after harvest. Retardation of the yellowing, therefore, is important in maintaining the salability of broccoli. It has been shown that yellowing could be delayed by elevated CO_2 atmosphere (Lebermann et al., 1968a; Lieberman and Hardenburg, 1954; Lipton and Harris, 1974). Although broccoli is considered to be relatively tolerant of high CO_2 (Lipton, 1975), prolonged exposure to high CO_2 or low O_2 were found to induce off-odor and off-flavor (Kasmire et al., 1974; Lipton and Harris, 1974; Wang and Hruschka, 1977). This study was conducted to determine whether a short-term high CO_2 treatment of broccoli could delay yellowing and loss of both ascorbic acid and chlorophyll without causing the problems associated with long-term exposure to CO_2.

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EXPERIMENTAL

FRESHLY HARVESTED 'Waltham 29' broccoli grown at Bridgeton, NJ, were used in the first experiment. Samples of the central flower head were transported under ice directly from the field to Beltsville Agricultural Research Center and immediately placed in 0°C storage until start of the experiment the next day. The experiment was repeated the next year with broccoli grown at Clinton, MD. These samples were placed in the test atmospheres within 8 hr after harvest.

The broccoli were treated for 3 and 6 days at 5°C, with the following atmospheres: air (control), 20% CO_2 with 3 and 21% O_2, 30% CO_2 with 3 and 21% O_2, and 40% CO_2 with 3 and 21% O_2. Each gas combination was supplied, calibrated and pre-mixed. in a gas cylinder. Approximately 2.5 kg of broccoli consisting of 10 heads was placed in each of 14 20-liter glass jars. The jars were then sealed with lids fitted with inlet and outlet tubes and flushed with the designated gases at a rate of 12 liters/hr for 24 hr. The flow rate was then reduced to 6 liters/hr and so maintained for the rest of the treatment. Concentrations of CO_2 and O_2 were monitored with a Fisher-Hamilton Gas Partitioner Model 29. All broccoli were transferred to trays after the treatment and stored in air at 5°C and 90–95% relative humidity. Duration of the tests, which included CO_2 treatment and air storage, was 20 days.

Ethylene production was determined daily during and after the CO_2 treatments until day 8 of the tests. During treatment, effluent gases from the jars were sampled, and ethylene was measured with a gas chromatograph equipped with an alumina column (Meigh et al., 1960). Ethylene production was calculated from the sample weight and flow rate at the time of sampling. During storage in air, a subsample of three heads of broccoli for each treatment was enclosed for 1 hr in a 4-liter plastic jar. The accumulated ethylene was measured as before, and ethylene production was calculated according to sample weight, size of container, and length of time the broccoli was enclosed.

Samples were analyzed for ascorbic acid and chlorophyll at the end of the 3- and 6-day treatments and then on days 10, 15 and 20 of the tests. Ascorbic acid was determined by the AOAC method (1970), and chlorophyll according to Arnon (1949), with 50g and 10g, respectively, of floral tissues.

Color of the broccoli head was measured with a Hunterlab color difference meter and expressed as a/b values. Positive a values indicate redness; negative a values, greenness. Positive b values indicate yellowness; negative b values, blueness. A value of 0 for a or b indicates grayness. The same area—the center of the broccoli head—was measured for color so that variation between measurements would be minimized.

Evaluations were made daily of turgor, color, compactness, opening of flowers, decay, raw and cooked odor, raw and cooked flavor, and salability. Cooked odor and flavor were evaluated after broccoli segments had been cooked in boiling water for 5 min. Numerical values were given to each of the above traits according to the rating scales described in Table 1.

RESULTS & DISCUSSION

Chlorophyll change as affected by high CO_2

Table 2 shows that the chlorophyll content of the control sample started to decline after day 6 at 5°C and was significantly lower than that of any treated sample by day

10. Rates of chlorophyll degradation were greater for the control sample than for the treated samples even after CO_2 treatments had been discontinued. This indicates that a high CO_2 treatment has both a residual effect and an initial effect during treatment of inhibiting chlorophyll breakdown.

Similarly, a residual effect of the modified atmosphere on delaying yellowing of broccoli was also found by Lipton

Table 1—Descriptive equivalents of numerical ratings for broccoli.

	Rating					
Trait	10	8	6	4	2	0
Turgor	Turgid	Less turgid	Trace limp	Limp	Dried	Brittle
Color	Dark green	Light green	Slightly yellow	Moderately yellow	Severely yellow	Completely yellow
Compactness	Compact	Trace of looseness	Slightly loose	Moderately loose	Severely loose	Extremely loose
Decay	None	One mold nest[a]	2 to 4 mold nests	5 or more mold nests	General coverage	Leaky decay
Odor and flavor	Normal	Trace of off-odor	Slight off-odor	Moderate off-odor	Severe off-odor	Nauseating
Opening of florets	None open	20% open	40% open	60% open	80% open	100% open
Salability	Excellent	Acceptable	Barely salable	Not salable	Barely salvageable	Not salvageable

[a] Each mold nest is 2–5 mm in diameter.

Table 2—Total chlorophyll content (mg/100g fresh weight) of broccoli after short-term postharvest treatment with various concentrations of CO_2 and O_2[a]

Initial treatment			Days at 5°C				
Days	% CO_2	% O_2	3	6	10	15	20
3	20	3	48.6 a	–	45.7 a	39.0 de	28.1 ef
	20	21	47.4 a	–	43.9 a	38.2 e	26.6 f
	30	3	47.0 a	–	44.2 a	40.6 cde	27.2 f
	30	21	49.5 a	–	44.8 a	39.5 de	27.8 f
	40	3	46.8 a	–	45.2 a	41.4 bcd	31.4 de
	40	21	48.9 a	–	45.9 a	38.8 de	31.9 d
	Air control		48.0 a	–	37.4 b	26.2 f	12.8 g
6	20	3	–	47.2 a	44.6 a	39.7 de	35.5 c
	20	21	–	48.0 a	45.2 a	41.6 bcd	36.7 bc
	30	3	–	47.6 a	46.5 a	43.1 abc	39.4 ab
	30	21	–	46.3 a	44.8 a	41.6 bcd	38.2 bc
	40	3	–	48.6 a	47.1 a	45.4 a	41.8 a
	40	21	–	46.8 a	46.0 a	43.8 ab	42.5 a
	Air control		–	47.6 a	39.2 b	28.1 f	13.6 g

[a] Each value is based on two analyses each in two replicated experiments. Mean separation in columns by Duncan's multiple range test at 5% level.

Table 3—Ascorbic acid content (mg/100g fresh weight) of broccoli after short-term postharvest treatment with various concentrations of CO_2 and O_2[a]

Initial treatment			Days at 5°C				
Days	% CO_2	% O_2	3	6	10	15	20
3	20	3	115.7 b	–	98.4 d	92.2 cd	73.6 c
	20	21	121.3 ab	–	102.7 cd	95.9 bc	77.1 bc
	30	3	126.4 ab	–	99.3 d	92.8 cd	78.5 bc
	30	21	119.2 ab	–	97.7 d	85.6 d	74.2 c
	40	3	128.8 a	–	105.2 cd	96.1 bc	75.8 bc
	40	21	125.3 ab	–	102.7 cd	91.3 cd	74.2 c
	Air control		102.1 c	–	89.5 e	73.9 e	57.8 d
6	20	3	–	112.5 bc	104.7 cd	98.5 abc	82.9 abc
	20	21	–	108.3 c	109.4 abc	101.8 abc	79.8 bc
	30	3	–	112.0 bc	110.5 abc	103.7 ab	80.6 abc
	30	21	–	117.8 ab	107.6 bc	99.6 abc	85.1 ab
	40	3	–	122.9 a	116.2 a	106.9 a	90.2 a
	40	21	–	118.9 ab	113.8 ab	108.3 a	81.4 abc
	Air control		–	97.4 d	86.6 e	75.1 e	62.6 d

[a] Each value is based on two analyses each in two replicated experiments. Mean separation in columns by Duncan's multiple range test at 5% level.

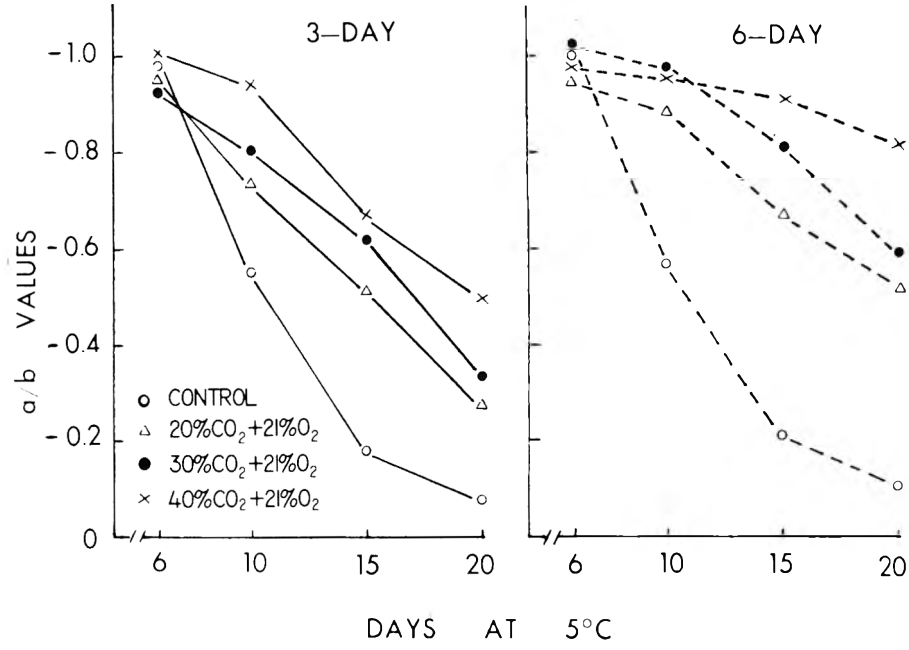

Fig. 1—Effect of short-term high CO_2 treatment on color change of broccoli as measured by Hunterlab color difference meter.

and Harris (1974). They reported that O_2 at 1% or less inhibited yellowing of broccoli curds during storage at 5° or 7.5°C, and that the effect persisted during their subsequent aeration at 10°C.

The samples treated for 6 days lost chlorophyll at a slower rate than those treated for 3 days; and the difference in their chlorophyll contents was especially pronounced on day 20. For each level of CO_2 within the 3-day or 6-day treatment, the effect of CO_2 on chlorophyll content was the same whether the O_2 concentration was 3 or 21%. This finding agrees with that of Lebermann et al. (1968a), who reported that the color of broccoli was the same whether it had been treated with 21 or 2% O_2 with 20% CO_2. The effect of low O_2 may be masked when CO_2 concentration is 20% or higher. It is also possible that the O_2 concentration used in this study was not low enough to exert any effect. Lipton and Harris (1974) reported that the O_2 level has to be lower than 2% to retard yellowing. Lebermann et al. (1968a) reported that a high level of CO_2 was more effective than a low level of O_2 in delaying loss of chlorophyll.

Effect of high CO_2 on ascorbic acid content

Broccoli is one of the richest sources of vitamin C in green vegetables (Watt and Merrill, 1975; Wheeler et al., 1939), but loses it gradually following harvest (Morris, 1947; Wheeler et al., 1939). Table 3 shows that ascorbic acid in broccoli declined steadily in all treatments, but that the decline was delayed by CO_2.

Platenius and Jones (1944) also found that CO_2 reduced the rate of ascorbic acid loss in broccoli during storage. There are other reports, however, indicating that CO_2 has a destructive effect on the ascrobic acid and the content in the commodities stored under controlled atmosphere conditions was lower than in those stored in air (Bangerth, 1977). The effect of controlled atmosphere on ascorbic acid content appears to vary with commodity and temperature. Platenius and Jones (1944) found that CO_2 inhibited the loss of ascorbic acid in broccoli and brussels sprouts stored at 10° and 24°C. The presence of CO_2, however, accelerated the destruction of ascorbic acid in asparagus

Table 4—Ethylene production (nl/100g/hr) of broccoli during and after short-term postharvest treatment with various concentrations of CO_2 and O_2[a]

Initial treatment			Days at 5°C							
Days	% CO_2	% O_2	1	2	3	4	5	6	7	8
3	20	3	29.3 b	22.6 b	22.6 b	35.2 cd	50.4 bc	52.2 bc	56.9 bc	52.2 bcd
	20	21	34.2 b	26.2 b	24.1 b	41.4 b	54.6 b	58.3 b	62.0 b	57.8 b
	30	3	31.5 b	23.7 b	21.7 b	32.8 cd	46.6 c	52.0 bc	59.6 bc	54.1 bcd
	30	21	31.9 b	22.2 b	24.1 b	38.0 bc	52.2 bc	55.6 bc	60.6 bc	52.8 bcd
	40	3	29.0 b	21.8 b	20.5 b	29.6 de	46.4 c	50.2 c	54.5 cd	49.6 cd
	40	21	30.8 b	24.4 b	21.3 b	30.6 d	49.5 bc	53.7 bc	57.7 bc	50.2 cd
	Air control		82.1 a	86.2 a	89.3 a	84.8 a	86.2 a	83.9 a	82.5 a	74.1 a
6	20	3	32.3 b	27.1 b	21.4 b	21.4 f	22.8 d	22.8 d	39.2 f	48.4 d
	20	21	30.8 b	25.6 b	20.2 b	22.5 f	23.7 d	25.6 d	46.3 ef	53.7 bcd
	30	3	33.4 b	24.0 b	21.8 b	24.0 ef	25.7 d	26.2 d	42.6 ef	50.2 cd
	30	21	29.9 b	22.6 b	19.8 b	21.9 f	23.0 d	25.4 d	40.5 f	48.9 d
	40	3	30.2 b	22.2 b	19.4 b	20.6 f	22.3 d	24.1 d	48.4 de	55.1 bc
	40	21	29.3 b	25.5 b	22.0 b	24.8 ef	25.6 d	25.6 d	49.2 de	56.6 b
	Air control		79.6 a	84.2 a	88.1 a	84.2 a	88.1 a	82.6 a	78.8 a	69.8 a

[a] Each value is based on two measurements each in two replicated experiments. Mean separation in columns by Duncan's multiple range test at 5% level.

Table 5—Subjective evaluation of broccoli stored at 5°C for 20 days after short-term postharvest treatment with various concentrations of CO_2 and O_2 [a]

Initial treatment											
Days	% CO_2	% O_2	Color	Turgor	Compactness	Decay	Raw odor	Cooked odor	Raw flavor	Cooked flavor	Salability
3	20	3	5.5 c	5.8 a	7.8 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	6.5 d
	20	21	5.8 c	6.3 a	7.8 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	7.0 cd
	30	3	6.3 bc	6.0 a	8.0 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	7.0 cd
	30	21	5.8 c	5.8 a	7.8 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	6.5 d
	40	3	6.3 bc	6.5 a	8.0 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	7.5 bc
	40	21	6.5 bc	6.3 a	8.0 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	7.0 c
	Air control		3.8 d	5.8 a	7.8 a	8.5 b	10.0 a	10.0 a	10.0 a	10.0 a	4.2 e
6	20	3	7.3 b	6.0 a	7.8 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	8.0 ab
	20	21	7.5 b	5.8 a	8.0 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	8.0 ab
	30	3	7.5 b	6.5 a	8.0 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	8.8 a
	30	21	7.5 b	6.3 a	8.0 a	10.0 a	10.0 a	10.0 a	10.0 a	10.0 a	8.3 ab
	40	3	9.0 a	3.2 b	6.0 b	5.3 c	4.0 b	5.0 b	4.5 b	5.5 b	2.5 f
	40	21	9.0 a	3.1 b	5.8 b	4.5 c	4.0 b	5.3 b	4.8 b	5.3 b	2.0 f
	Air control		3.3 d	5.8 a	7.8 a	7.5 b	10.0 a	10.0 a	10.0 a	10.0 a	3.8 e

[a] Mean separation in columns by Duncan's multiple range test at 5% level. The column on "opening of florets" is omitted because of no difference among treatments.

and spinach at room temperature and retarded it at 10°C. In peas, CO_2 was detrimental to ascorbic acid content at both temperatures. The higher retention of ascorbic acid content in the CO_2-treated broccoli in this study may have been associated with the retention of freshness induced by the CO_2, rather than with the CO_2 itself directly.

In this study, retention of ascorbic acid was often greater in the 6-day-treated samples than that in the 3-day-treated samples. However, level of CO_2 was not consistently related to the extent of ascorbic acid retention. Variation among the samples may be one of the factors minimizing the effect of different CO_2 treatments.

Ethylene production during and after high CO_2 treatment

Ethylene production by the control broccoli remained relatively high for the first few days of the test and then gradually declined (Table 4). During treatment, the CO_2 atmospheres retarded ethylene production. The degree of retardation did not seem to be correlated with the concentration of CO_2 within the range used in this experiment. The production of the treated samples remained lower than that of the untreated control even after the 3-day or 6-day treatment, indicating a residual effect from the short term high CO_2 exposure. In this respect, the response of broccoli to CO_2 treatment is quite different from that of sweet peppers (Wang, 1977a). Production of ethylene by the treated broccoli gradually increased following removal from CO_2 atmosphere but did not return to the level comparable to that of the control.

Color measurement

All broccoli on day 6 of the test had an *a/b* value near −1 (Fig. 1). The color difference meter thus indicated that the broccoli was green, even though it detected some degree of yellowness. Thereafter, the *a/b* values for all the treatments increased. The rate of increase was fastest for the controls, slowest for the 6-day treatments, and intermediate for the 3-day treatments. Thus, by day 20, the controls were predominantly yellow, and the 6-day treated samples were predominantly green.

Market qualities as influenced by short-term high CO_2 treatment

The attributes of market quality—color, turgor, compactness, decay, raw and cooked odor, and flavor and salability—were subjectively evaluated daily but only the evaluations for day 20 are reported (Table 5). All the high CO_2 treated samples were greener than the control samples. The degree of green color, however, were not significantly different from each other between the various concentrations of CO_2 in the 3-day treatments. The broccoli treated for 6 days with 40% CO_2 was greener than broccoli with any other treatment, but the general appearance was not good because of CO_2 injury.

CO_2 had no discernible effect on turgor or compactness, except where it caused injury. The characteristics of CO_2 injury, manifested by broccoli treated with 40% CO_2 for 6 days were wilted curds, shrivelled stems, discolored leaves and off-flavor and off-odor. Broccoli in this treatment were significantly less turgid and compact than that of any other treatment and were susceptible to decay, apparently because of the tissue injury and death of the cells.

A trace to slight amount of mold was present on the cut end of stems and on the flower head of the control samples after 20 days. The decay seemed to be associated with the yellowing and general senescence of the florets. Except for the samples treated for 6 days with 40% CO_2, all the CO_2-treated samples were free from mold when examined on day 20.

The 6-day 40% CO_2 treatment also induced off-odor and off-flavor in both raw and cooked broccoli as a result of the CO_2 injury. Off-odor and off-flavor were also detected in the samples treated for 6 days with 30% CO_2 immediately following the treatment, but they dissipated when the broccoli was transferred to air. Occurrence or nonoccurrence of off-odor depends upon O_2 and CO_2 concentrations, rate of aeration, temperature, and duration of the storage (Kasmire et al., 1974).

In controlled atmosphere storage, a low O_2 concentration usually accentuates CO_2 injury (Hansen and Mellenthin, 1962; Wang, 1977a). In these tests, however, results were similar with 3 and 21% O_2. Injury depended on the CO_2 level. Kasmire et al. (1974) reported that off-odor was not serious until O_2 was depleted to 1% or lower. Lieberman and Spurr (1955) found that the products of anaerobic metabolism increased greatly when O_2 fell below 1% in the atmosphere surrounding the broccoli.

Taking all the market attributes into account, broccoli previously treated with 40% CO_2 for 6 days at 5°C were rated not salable even though it remained green. The untreated broccoli were also rated not salable mainly because of yellowing and decay. All other short-term high CO_2 treated heads of broccoli remained in salable conditions, especially those treated with 20 or 30% CO_2 for 6 days. High CO_2 has an added beneficial effect on broccoli quality

by tenderizing the tissues (Lebermann et al., 1968b). This effect was thought to be directly related to the increase in pH of the tissue after exposure to CO_2.

The results of this study indicate that pretreatment of broccoli with high CO_2 is effective in delaying its loss of chlorophyll, and ascorbic acid, and could maintain its salability. These effects of CO_2 may be related to CO_2 inhibition of ethylene production and ethylene action (Burg and Burg, 1967). Retardation of the deterioration of broccoli also has been demonstrated by use of ethylene inhibitors (Wang, 1977b) and senescence inhibitors (Fuller et al., 1977).

In studying the packaging of broccoli, Wang and Hruschka (1977) found that quality of broccoli was best when stored at 0°C, regardless of types of packaging or concentrations of O_2 and CO_2 in the packages when compared to that stored at 10 or 20°C. While short-term high CO_2 treatment probably will never replace refrigeration as the most effective means for extending the storage life of broccoli, this technique may be useful where refrigeration is not readily available as a good supplementary method for improving the keeping quality of broccoli.

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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.

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CONSUMERS' RESPONSES TO FOOD PRODUCTS WITH INCREASED LEVELS OF POLYUNSATURATED FATTY ACIDS

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ABSTRACT

Pigs were reared on different diets, leading to different percentages of PUFA'S (up to 40%) in their fat. Pork products, mostly cold, were evaluated by a consumer panel for overall preferences, and on various characteristics such as color, etc. Panel members evaluated also the acceptability of the products. Results show that the modified pork products do reasonably well, compared with standard products. The biggest exception is liver sausage.

INTRODUCTION

CORONARY HEART DISEASE is an important health problem in the industrialized countries. Preventive and curative measures usually include nutritional factors.

Although there is still much discussion on this point, many medical and nutritional experts recommend among other things to restrict one's energy intake via fat to 30–35%, preferably equally provided by saturated, mono- and polyunsaturated fatty acids (especially linoleic acid). Well-known are in this respect the advices given by FAO (1977), U.S. Senate (Dietary Goals, 1977) and the Dutch Nutrition Council (1973). Within the present dietary practice in industrialized countries, many people will find it difficult to reach these amounts of PUFA'S in the diet. Changing food habits permanently is a slow and difficult process. However, another possibility is the modification of food products already fitting in an accepted dietary pattern, to such extent that the desired fatty acid composition of the total diet can be obtained. It is known, that the proportion of PUFA'S to total fatty acids in the fat of certain animals can be changed by several factors. Ford et al. (1975, 1976) recently published data on sheep and cattle, together with panel reactions to the flavor of sheep meats and beef, as did Skelley et al. (1975) in relation to pork meat.

The underlying report describes differences in consumer's overall preferences for fresh pork products with normal, moderately high and high content of polyunsaturated fatty acids. Relative preferences for those products based on specific characteristics such as color, smell, taste and consistency are also measured. Furthermore, an attempt is made to get an impression of the relative importance of above-mentioned characteristics for the different products. Finally panel members are questioned about the acceptability of the products.

EXPERIMENTAL

Animals and feed

The products used in this study have been prepared from 30 Yorkshire barrows, selected out of a number of 36, which underwent a dietary experimental feeding program. This feeding program started when the animals reached an average weight of 30.4 kg and lasted until the average weight was 104.1 kg.

The purpose of the experimental diets was to obtain three groups of 10 pigs each, one with 10% linoleic acid in subcutaneous fat (normal group), one group with 20% linoleic acid (medium group) and one with 30% (high group). Variations in the diets were obtained by varying the percentages of maize, soy-products, wheat flour, tapioca-flour and beef fat. The normal group (N) was fed on rations with 4% beef suet, the medium group (M) with 2.0% beef suet + 4.25% soy oil and the high group (H) with 8.5% soy oil. More details have been published by others elsewhere (Houben and Krol, 1978). The energy values of the diets were very similar.

The slaughtering took place in a public slaughterhouse. At slaughtering, the linoleic acid resp. total PUFA content as fractions of the total fat content of the backfat tissue and M.longissimus dorsi were as shown in Table 1.

From the carcasses, a number of products were prepared and selected for chemical and sensory analyses (see Table 2). All products were made according to standard recipes from the Dutch Centre for Meat Technology CIVO-TNO. (For recipes and preparation methods see Houben and Krol, 1978, 1979). Except for Guelders' ring sausage, these products are generally not heated before consumption and are very often used with bread, as cold meats. Ring sausage is frequently used as the meat component in hot dishes.

All samples used for chemical analysis were vacuum packed and stored at −40°C. The gross chemical composition of the products is given in Table 2.

Consumer panel preferences

Preferences were measured for all products, by means of a con-

Table 1—Linoleic acid and PUFA content of some unprocessed pork tissues

Feed	Normal (N)		Medium (M)		High (H)	
Tissue	C18:2	PUFA	C18:2	PUFA	C18:2	PUFA
Backfat	13.8	15.5	26.3	30.1	36.0	41.4
M.longessimus dorsi	6.2	7.3	11.7	13.5	19.9	23.0

Table 2—Gross chemical composition of several pork meat products

		Moisture	Ash	Protein	Fat	C18:2	PUFA
	N	47.8	3.6	13.5	34.5	10.9	12.3
Back	M	46.1	3.3	13.7	36.3	22.6	25.9
bacon	H	48.6	3.7	14.9	33.2	33.7	39.4
	N	67.8	3.5	19.9	9.2	10.4	11.9
Pork loin	M	66.8	2.9	20.5	10.1	23.4	27.4
roll	H	67.4	3.3	20.0	9.6	34.2	40.4
	N	58.7	2.7	18.1	20.8	12.9	15.5
Saxon liver	M	56.8	2.6	17.1	22.5	22.2	26.5
sausage	H	57.0	2.7	16.8	23.0	30.2	36.5
	N	49.8	2.9	13.4	34.5	12.8	16.3
Guelders'	M	47.2	2.9	12.9	37.8	24.3	29.6
ring sausage	H	48.6	3.0	13.4	32.5	39.0	40.4
	N	69.4	2.8	18.0	9.4	10.2	12.1
Shoulder	M	70.0	2.8	17.0	10.4	22.2	25.9
(with fat)	H	67.3	2.6	16.9	12.3	33.5	39.6
	N	41.2	4.7	17.8	35.3	12.7	14.1
Dutch-type	M	42.7	4.6	17.8	36.0	23.2	26.9
cervelat	H	49.3	5.3	20.7	23.5	29.8	34.6

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Table 3—Data of overall preference, color, smell, taste and consistency and of the relative importance of these quality characteristics

	Preference	Color	Smell	Taste	Consistency	Color, Smell, Taste, Cons.
Back bacon	H,N > M	–	–	H,N>M	–	taste>color,cons.,smell
Pork loin roll	–	–	–	M > N	–	taste>color>cons.,smell
Saxon liver sausage	N,M > H	N>H,M	N > M	N > H	N > M,H	taste>color,cons./color>smell
Guelders' ring sausage	–	–	–	–	N > M,H	taste>color,smell,cons.
Shoulder with fat	–	–	–	–	–	taste,smell>color,cons.
Dutch-type cervelat	–	–	–	–	N > H,M	taste>color,smell/color>cons.

sumer panel of 36 women. Panel members did not know the nature of the products until they arrived at the tasting sessions. In each session 12 members participated; for practical reasons split up into two groups of 6 each. The first group started with a product, after which they took a rest, during which time the second group judged the same product. After that, a second product was judged in the same fashion. For each product the three different varieties were available.

The sensory analyses consisted per taster of three tasks: (1) a paired comparison assay on the overall preferences of the products; (2) a paired comparison assay on the preferences per quality attribute; (3) a paired comparison on the relative importance of these attributes. By means of the first experiment, the tasters overall preferences (if any) to one of the three varieties of one product was measured. The second experiment was conducted to study the taster's opinion on the attributes (color, smell, taste and consistency) of the products, while the third experiment should give the authors an impression of the relative importance of these characteristics for judging the involved meat products. As regards the overall preference study and the study of specific characteristics, the subjects were seated in booths and presented with the samples. For the study of the relative importance of different characteristics, subjects were seated at a round table, six at a time. In the middle of the table, a number of samples of the standard product (one product at a time) was placed, within reach of the panel members. Each panel member had a form, on which in random order, all possible pairs of the concepts color, smell, taste and consistency (this last concept was explained), were presented. The instruction was to indicate for each pair which characteristic was thought to be the more important one for the product present at the time.

Before tasting, the ring sausage was heated in a warm-water bath of 80°C. The other products were kept in a refrigerator at 4°C, until a few minutes before presentation. The products were judged from 0.5 to 3 days after preparation. Before presentation, Saxon liver sausage and the ring sausage were cut by hand into slices of 1.5 cm thickness. The other products were cut by a standard slicing machine (thickness varying from 2–4 mm, depending on the product). It was noticed, that products with increased PUFA'S could be sliced better if the thickness of the slices was slightly increased. (The slices at standard thickness showed a tendency to fall apart into two or more pieces). Therefore, the slices of the normal products were also slightly increased in thickness. It is possible, that a different way of slicing could have prevented this problem. This aspect has not been further investigated. As to back bacon, a special problem was met in the sense that the outer rim of fat repeatedly became detached from the central part of the slice. In cases where this happened, both parts were presented.

In all cases, as mentioned, the method of paired comparison was used. The observed frequencies of preference for any of the objects were transformed into arcsin square root deviates. This solution has some desirable characteristics, and hypotheses concerning differences and appropriatenes of the statistical model can then be tested (see Bock and Jones, 1968; Theunissen et al., 1977).

Since for each task only three objects were available (i.e. one product with three levels of PUFA'S in each) it was possible to vary systematically over the six booths the between- and within order of pairs in the presentations.

RESULTS

Panel reactions

Statistical analysis of the observations in the way mentioned above, showed that in only a few cases one could conclude to significant differences. To avoid a too complicated table, which is difficult to review, we give the results in a more qualitative way (Table 3). In this table is given the descending order of preference of the product varieties c.q. of the relative importance of the different quality attributes. When the symbols are divided by a > sign, it means that significant differences ($p \leq 0.05$) exist between the products, or that the tasters are considering the quality attributes before the sign of greater importance than the attributes after it. If the symbols or terms are divided by a comma sign no significant differences appeared to exist between them. Mathematical treatment (i.e. ANOVA procedures, see Bock and Jones, 1968, Ch. 6) of the results showed also that in no occasion was there any reason that the utilized model was inappropriate.

Table 4—Positive acceptance for the various products in percentages (rounded)

	Normal	Medium	High
Back bacon	94	87	99
Pork loin roll	92	94	90
Saxon liver sausage	87.5	94	79
Guelders' ring sausage	96	90	94
Shoulder with fat	93	94	94
Dutch-type cervelate	97	83	86

Disregarding the data on the relative importance of the various attributes, the most noticable feature of this table is probably the large number of nonsignificant differences especially as regards the most important measure, i.e. that for overall preferences. It is not yet possible to give a definite explanation for this result. One hypothesis is, that this is due to the fact that pork products naturally already have a relatively high content of PUFA'S (compared to other meats), and that the increase (which in an absolute sense is roughly comparable to that found for sheep meats and beef) does not lead to changes in appreciation in a linear way, but in a (strongly?) negatively accelerated way. Another explanation could be, that in this study predominantly cold products were used. Certain relevant volatiles could be absent at this lower temperature or be present in relatively insignificant amounts.

The rather unexpected result for the overall preferences for bacon may be due to some panel members disregarding the possibly detached outer edge of fat for the high PUFA sample.

The overall preferences as measured in this study are relative preferences that do not refer to an absolute zero point of preference. Therefore, it is interesting to know whether, even if a product is not preferred, it is at least acceptable. Therefore, panel members were asked "Is this product acceptable to you as a food product?" The affirmative answers are presented as percentages in Table 4. Notice again the peculiar results for back bacon. The relatively low acceptance for the high PUFA product of Saxon liver sausage is also noticeable.

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OPTIMAL RETORT TEMPERATURE PROFILE IN OPTIMIZING THIAMIN RETENTION IN CONDUCTION-TYPE HEATING OF CANNED FOODS

I. SAGUY and M. KAREL

ABSTRACT

The maximum principle theory (Pontryagin et al., 1962) was used to optimize thiamin retention during sterilization of a conduction-heating canned food. The optimal retort temperature profile determined by this procedure improved thiamin retention by more than 2%, as compared with other methods, and showed that a single solution for the temperature profile exists. This optimization method may be applicable to other processes in which retention of nutrients or other characteristics is to be improved.

INTRODUCTION

RESEARCH INTEREST in simulating food deterioration during processing and storage has increased in recent years. Simulation is typically based on kinetic data describing various deteriorative reactions and their interrelationships. Although many kinetic models have been developed, only very limited attempts have been made to optimize actual processing techniques.

Optimization of a process or product would seem to be the next logical step after a kinetic model has been established. The following factors may partially account for the lack of published research on optimization in the food industry:

1. The complexity of the foods and processes of interest makes analytical treatment difficult.
2. There are no quick and easy methods to deal with numerical solutions.
3. A natural time gap exists between the development of modern optimal control theories and their application to food processes.

Very few studies dealing with the optimization of food handling processes have been published. In a review of the subject, Herrmann (1976a, b, c) indicated that all stages of processing could be optimized for many foodstuffs, although he only hinted at ways in which this could be accomplished. Teixeira et al. (1969a, b) and Hayakawa (1969) developed procedures to predict and optimize nutrient retention in a thermal process. Jen et al. (1971) introduced a method to estimate nutrient retention based on the method of Stumbo (1965), which is applicable to foods heated by conduction. Similar approaches were used by Manson et al. (1970) and Mulley et al. (1975). Lund et al. (1972) presented a model based on the activation energy for thermal degradation and a reference rate constant. Thijssen and Kerkhof (1977) and Thijssen et al. (1978) showed that, given a constant heating-medium temperature, quality losses could be minimized if the processing time corresponded to a Fourier value of 0.45–0.55. A similar approach was used by Lenz and Lund (1977a, b).

Teixeira et al. (1975b) applied trial-and-error search techniques to determine the "best" conditions for improving thiamin retention in thermally processed foods. Theirs is the only published report on optimization of a retort process that allows for variations in the temperature of the heating medium.

The advances in both the theory and applications of optimization techniques in the last decade have made an impact on many other industries. In the fermentation industry, for example, Constantinides et al. (1970) and Rai and Constantinides (1973) used the "maximum principle" (Pontryagin et al., 1972) to determine optimal temperatures and pH profiles in batch fermentors. D'Ans et al. (1971a, b) applied Green's theorem to control problems in a continuous cultivation of microorganisms. Takamatsu et al. (1975) developed a method to optimize a semi batch fermentation process for continuously producing an amino acid by using Green's theorem and maximum principle. Ohno et al. (1976) determined the optimal control of a semibatch fermentation, while Yamane et al. (1977) investigated optimum feeding conditions. An on-line optimization technique was developed by White and Gray (1977).

Considering the improved availability of modern computer facilities, the advancements in numerical and mathematical methodology, and the expanding knowledge of kinetic models of food systems, nutrient retention in a heat-conducted sterilization process may be improved by using the optimal control theory.

The overall purpose of this research was to investigate the application of modern optimization techniques to food quality optimization in processes for which kinetic models of deterioration are available. More specifically, the aim was to develop a method of calculating the optimum temperature profile for a reaction in a retort as a function of the time needed to achieve a specified level of sterilization with maximum nutrient retention. To illustrate an application of the optimization procedure and computational scheme described herein, we determined the optimum temperature profile for thiamin retention in canned foods during a sterilization process.

THEORY

The maximum principle

The continuous maximum principle (Pontryagin et al., 1962) was used to determine the optimum temperature profile for nutrient retention during a heat-sterilization procedure for canned food.

Consider a continuous process (represented schematically in Fig. 1) where x(t) is the s-vector of the state variable ($x_1, x_2, \ldots x_s$), $\theta(t)$ is the r-vector of the control variable ($\theta_1, \theta_2, \ldots \theta_r$) representing the decision at time, t. The performance equations for the process are (Constantinides et al., 1970; Fan, 1966):

$$\dot{x} \equiv dx/dt = f \text{ (for } t_0 \leq t \leq t_F) \qquad f = f\left\{x(t), \theta(t)\right\} \tag{1}$$

where t_0 and t_F indicate initial and final times, respectively. The system can thus be described by s-ordinary differential equations. The initial condition of the system is given by:

$$x(t) = \alpha \tag{2}$$

where α is an s-vector ($\alpha_1, \alpha_2, \ldots \alpha_s$) representing the initial values of the state variables.

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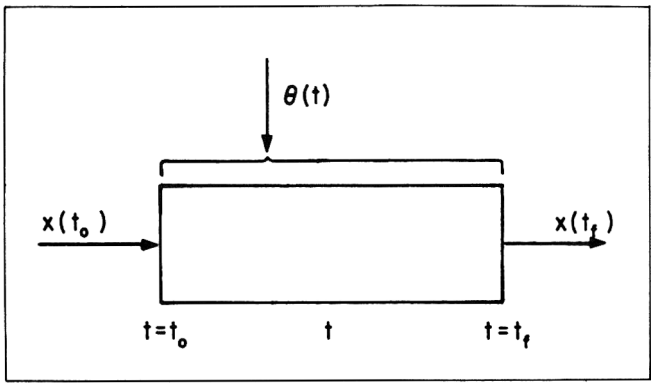

Fig. 1—Schematic process diagram.

With the continuous maximum principle, the objective function, S, is defined as a linear combination of the final values of the state variables, $x(t_F)$.

Thus, $$S = C\,x(t_F) \tag{3}$$

where C denotes a vector of s-constants. To optimize the system (maximum or minimum S), one must find a continuous control vector, $\theta(t)$ (possibly subject to constraints), so that S is maximized. The control vector function so chosen is considered optimal and is denoted by $\theta^*(t)$.

The continuous maximum principle uses an s-dimensional adjoint vector, $\phi(t)$ $(\phi_1, \phi_2, \ldots \phi_s)$, and a Hamiltonian function, H, that satisfy the following relations:

$$H\left\{\phi(t), x(t), \theta(t)\right\} = \phi^1 f \tag{4}$$

$$\dot{\phi} \equiv -(\partial H/\partial x) = -\phi(\partial f/\partial x) \tag{5}$$

$$\phi(t_F) = C \tag{6}$$

where ϕ^1 denotes the transpose of the vector, ϕ; $(\partial f/\partial x)$ is the matrix whose elements are the partial derivatives of the rates of change of the state variables with respect to the state variable [the rates $-f$ are given in Eq (1)]. When the time interval is fixed, and the initial conditions ($\propto$) are given, there are two types of basic problems (Katz, 1960): those with free, unassigned final values of the state variables and those with fixed, preassigned terminal values for some of the state variables. The first type is characteristic of such processes as batch fermentations (Constantinides et al., 1970) and chemical reactions, whereas the second type has prerequisites placed on the final values of the state variables, as in distillation or other processes (Fan, 1966).

The necessary conditions for the objective function, S, to be maximum (or minimum) with respect to $\theta(t)$ are:

$$\frac{\partial H}{\partial \theta} = 0 \text{ (for unconstrained } \theta) \tag{7}$$

$$H = \text{maximum (or minimum) for constrained } \theta \tag{8}$$

The optimum decision vector function, $\theta^*(t)$, is determined by solving Eq (7) for unconstrained $\theta(t)$ or by searching the boundary of the set that meets the requirement of Eq (8).

The algorithm described above may be summarized as follows (Fan, 1966): For the scalar function, S, given in Eq (3) to be maximum (or minimum) for a process described by Eq (1) with the initial conditions given by Eq (2), it is necessary that there exists a nonzero, continuous vector function $\phi(t)$ that satisfies Eq (5) and (6) and that the vector function $\theta(t)$ be chosen so that H is a maximum for every t $(t_0 \leq t \leq t_F)$. [The maximum (or minimum) value of H is a constant for every t]. If, however, the right-hand side of the performance equation depends explicitly on the time, t, the system is called nonautonomous and the value of H is no longer constant for every t.

The use of the continuous-maximum principle produces a two-point, boundary value problem with added difficulties caused by the need for a search technique in which a transcendental equation in the control variable is derived from Eq (7). A Fibanacci search technique may be applicable where only one control variable is being optimized. For several control variables, the "steepest ascent method," which is also referred to as the "gradient in function space" (Storey and Rosenbrock, 1964), is widely used. Other methods include: "quasi-linearization" (Lee, 1966, 1967), "calculus of variations" (Katz, 1960), and "dynamic programming" (Fan, 1966).

Loss of quality and destruction of microorganisms

The rate of destruction of microorganisms and the rate of quality (e.g., nutrient) loss may be expressed as first-order reactions:

$$dx/dt = -2.303\,x/D \tag{9}$$

in which x represents the concentration of microorganisms or of a quality factor at any given time, t; D is the time required to destroy 90% of the concentration. The temperature dependence of D can be expressed as:

$$D = D_0 \exp\left(\frac{T_0 - T}{z/2.303}\right) \tag{10}$$

where D_0 is the time required to destroy 90% of the concentration at a reference temperature, T_0; T is the temperature in the can at any given time and location. z is the temperature increase (°F) needed to reduce the value of D one order of magnitude.

Rewriting Eq (9) and (10) (where 1 denotes the microorganism, and 2 refers to the nutrient) yields:

$$dx_1/dt = -K_1 x_1 \tag{11}$$

$$K_1 = \frac{2.303}{D_{0,1}} \exp\left(\frac{T - T_{0,1}}{z_1/2.303}\right) \tag{12}$$

$$dx_2/dt = -K_2 x_2 \tag{13}$$

$$K_2 = \frac{2.303}{D_{0,2}} \exp\left(\frac{T - T_{0,2}}{z_2/2.303}\right) \tag{14}$$

Eq (11) and (13) may be used to calculate the effect of the heating process on nutrient retention and on the number of viable cells. In an actual thermal process, however, the temperature within the container is a function of both time and location.

The theory of finite differences (Teixeira et al., 1969) whereby a point is taken to be the center of a very small volume element $[\Delta V_{i,j}]$ (where i and j are subscripts denoting the sequence of radial and vertical volume elements) relative to the entire container (V) was used to calculate the temperature $T_{i,j}$ and subsequent concentrations at the center of each volume element ($x_{1,i,j}$ = concentration of microorganisms, $x_{2,i,j}$ = concentration of nutrient). The total concentration is obtained by summing the volume elements.

$$\bar{x}_1 = \sum_{i=1}^{NR} \sum_{j=1}^{NH} x_{1,i,j}\,\Delta V_{i,j} \tag{15}$$

$$\bar{x}_2 = \sum_{i=1}^{NR} \sum_{j=1}^{NH} x_{2,i,j}\,\Delta V_{i,j} \tag{16}$$

$$V = \sum_{i=1}^{NR} \sum_{j=1}^{NH} \Delta V_{i,j} \tag{17}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the concentrations at any given time. $\Delta V_{i,j}$ and V are the element volume and the entire volume, respectively, and NR and NH are the number of radial and vertical elements into which the volume is divided.

The optimization model

According to the maximum principle theory, the model is as follows:

$$d\bar{x}_1/dt = -K_1\,\bar{x}_1 \tag{18}$$

$$d\bar{x}_2/dt = -K_2\,\bar{x}_2 \tag{19}$$

Table 1—Comparison of methods for optimizing thiamin retention during heating in cans

No.	Product	Process time (min)	Can Dimension (in)	Can Name	Thermal diffusivity (in.²/min)	Retort temp (°F)	D 250°F thiamin (min)	Thiamin retention (%)	Reference
1	Pork puree	89	307 X 409	A/2	0.0143	250	178.6	41.0	Teixeira et al. (1975b)
2	Pork puree	84	307 X 409	A/2	0.0143	Variable[a]	178.6	43.0	Teixeira et al. (1975b)
3	Pork puree	89	307 X 409	A/2	0.0143	Variable[b]	178.6	45.3	Present paper
4	Pea puree	85	303 X 406	No. 303	0.0158	250	165.6	49.2	Teixeira et al. (1975a)
5	Pea puree	87	303 X 406	No. 303	0.0158	Variable[a]	165.6	50.2	Teixeira et al. (1975a)
6	Pea puree	87	303 X 406	No. 303	0.0158	Variable[b]	165.6	52.4	Present paper
7	Pea puree	135	401 X 411	A/2½	0.0143	245.4	165.6	37.4	Short-cut method (Thijssen et al., 1978)
8	Pea puree	135	401 X 411	A/2½	0.0143	Variable[b]	165.6	39.9	Present paper

[a] Retort temperature based on sequence of steps (see reference)
[b] Retort temperature determined by the optimal temperature profile; see Fig. 2 and 5

where K_1 and K_2 are functions of temperature [Eq (12) and (14)].

In order to couple the two state variables, a new variable, $\bar{x}_3$, was introduced:

$$\bar{x}_3 = \int_{t_0}^{t_F} [G\bar{x}_2 - \ln(\bar{x}_1)]\, dt \qquad (20)$$

where G is a constant (known as Lagrange multiplier), which must have a numerical value that satisfies the constraints whenever opposed on the system [as in Eq (30a)]. For a given duration of process, t_F, the value of G is a constant throughout the operation. The appropriate value of G is determined by a trial and error search. The above relation was used after several functions were tested that may have compensated for the wide range of possible spore concentrations. The problem of optimization was therefore transformed into one of maximizing the variable, $\bar{x}_3$ (t_F):

$$S = \bar{x}_3(t_F) \qquad (21)$$

From Eq (6) and (21), we have:

$$C_1 = 0 \qquad (22a)$$

$$C_2 = 0 \qquad (22b)$$

$$C_3 = 1 \qquad (22c)$$

and

$$\varphi_1(t_F) = 0 \qquad (23a)$$

$$\varphi_2(t_F) = 0 \qquad (23b)$$

$$\varphi_3(t_F) = 1 \qquad (23c)$$

The Hamiltonian function was therefore:

$$H = -K_1\bar{x}_1\phi_1 - K_2\bar{x}_2\phi_2 + \varphi_3[G\bar{x}_2 - \ln(\bar{x}_1)] \qquad (24)$$

According to the definition of the adjoint vector, we have:

$$d\phi_3/dt \equiv -\partial H/\partial\bar{x}_3 = 0 \qquad (25)$$

It follows from Eq (23c) and (25) that:

$$\varphi_3(t) = 1 \quad (t_0 \leq t \leq t_F) \qquad (26)$$

Hence the Hamiltonian function can be written as:

$$H = -K\bar{x}_1\varphi_1 - K_2\bar{x}_2\phi_2 + G\bar{x}_2 - \ln(\bar{x}_1) \qquad (27)$$

and the adjoint vector as:

$$d\phi_1/d\bar{t} \equiv -\partial H/\partial\bar{x}_1 = (K_1\varphi_1 + 1/\bar{x}_1)\, t_F \qquad (28)$$

$$d\phi_2/d\bar{t} \equiv -\partial H/\partial\bar{x}_2 = (K_2\phi_2 - G)\, t_F \qquad (29)$$

where $\bar{t}$ is a dimensionless time expressed as $\bar{t} = t/t_F$; for $t_0 = 0$, we have $0 \leq \bar{t} \leq 1$.

The optimum temperature profile, $\theta^*(\bar{t})$, for a specified process time, t_F, can be determined once the initial conditions of the state variables are specified [$\bar{x}_1(0)$, $\bar{x}_2(0)$], the kinetic values describing the destruction of spores ($D_{0,1}$ and z_1) and the deterioration of the nutrient ($D_{c,2}$ and z_2) are known, and the relation between the retort temperature (θ) and the temperature within the can (T) can be calculated.

RESULTS

Data

Thermal processing of canned food in a retort was analyzed using three can sizes (Table 1). *Bacillus stearothermophilus* was the food spoilage organism, and the following kinetic data were used: $D_{0,1} = 4$ min at 250°F, and $z_1 = 18$°F (Teixeira et al., 1975a). The sterilization effect (including the cooling cycle) of all the processes defined in this investigation was held fixed and was defined by a five-log-cycle reduction of the initial spore population in the can.

In order to make it possible to compare our results with those of Teixeira et al. (1975a, b) the following assumptions were made: hot fill initial temperature, 160°F; cooling water, 77°F; the cooling cycle was completed when temperature within the can dropped below 160°F. The latter assumption defined the cooling time, t_C.

Thus,

$$\bar{x}_1(0) = 10^5;\ \bar{x}_1(t_F + t_C) \leq 1 \qquad (30a)$$

$$\bar{x}_2(0) = 1;\ \bar{x}_2(t_F) = \text{Maximum} \qquad (30b)$$

Kinetic data describing the effect of the sterilization process on the retention of thiamin in pork puree and pea puree were expressed as the $D_{0,2}$ value at 250°F (Table 1), and $z_2 = 46$°F (Teixeira et al., 1975a).

Temperature distribution within the can was based on heat conduction in a finite cylinder:

$$\frac{1}{a}\frac{\partial T}{\partial t} = \frac{1}{r}\frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial r^2} + \frac{\partial^2 T}{\partial y^2} \qquad (31)$$

where a = thermal diffusivity of the food product; r = radial distance from centerline; t = time; T = temperature at any given point and time; and y = vertical distance from mid-plane.

Temperature distribution was calculated by solving Eq (31) with the computer program introduced by Teixeira et al. (1969b). The volume of the cylinder was divided into 25 parts corresponding to layers of concentric rings with a rectangular cross-section. The time increment for the integration of Eq (31) varied from 0.125 to 1 min.

The mass average temperature (θ_m) was calculated from the following equation:

$$\theta_m = \frac{1}{V} \sum_{i=1}^{5} \sum_{j=1}^{5} T_{i,j} \Delta V_{i,j} \qquad (32)$$

where i and j are subscripts denoting the sequence of radii and vertical volume elements, $\Delta V_{i,j}$, respectively.

Algorithm

The "gradient method in function space" algorithm described by Storey and Rosenbrock (1964) was used. The algorithm was as follows (superscript n denotes the n-th iteration):

1. Let $\theta^{(n)}$ $(\bar{t})$ denote the n-th guess for the retort temperature at any given time, $\bar{t}$ $(0 \leqslant \bar{t} \leqslant 1)$.

2. Solve numerically Eq (18) and (19), for $\bar{x}_1^{(n)}$ $(\bar{t})$ and $\bar{x}_2^{(n)}$ $(\bar{t})$. The temperature within the can at any location and time was determined by solving numerically Eq (31) with the aid of the computer program given by Teixeira et al. (1969b).

3. Using the values of $\bar{x}_1^{(n)}$ $(\bar{t})$ and $\bar{x}_2^{(n)}$ $(\bar{t})$, integrate backwards numerically the adjoint functions $\phi_1^{(n)}$ $(\bar{t})$ and $\phi_2^{(n)}$ $(\bar{t})$ [Eq (28) and (29)] from $\bar{t} = 1$ to $\bar{t} = 0$. This backwards integration avoids the numerical instability encountered if the integration starts at $\bar{t} = 0$. This bidirectional integration method is very useful and improves stability, as was also noted by Constantinides et al. (1970).

4. Compute the value of the analytical derivation of H with respect to θ, i.e.:

$$\frac{\partial H^{(n)}}{\partial \theta}(\bar{t}) = -\bar{x}_1^{(n)} \phi_1^{(n)} \frac{\partial K_1}{\partial \theta} - \bar{x}_2^{(n)} \phi_2^{(n)} \frac{\partial K_2}{\partial \theta} \qquad (33)$$

and replace T with the mass average temperature, θ_m, calculated from Eq (32).

5. Estimate the (n + 1) guess for the optimal program using the following improvement:

$$\theta^{(n+1)}(\bar{t}) = \theta^{(n)}(\bar{t}) + \epsilon \frac{\partial H^{(n)}}{\partial \theta}(\bar{t}) \qquad (34)$$

where ϵ is a small positive number. The value of ϵ is to be determined for each system. ϵ should be small enough so that no instability will result, yet large enough so that convergence will not be too slow. Appropriate values for ϵ in this system were found to be 0.01–0.2.

The initial temperature profile and the value of the Lagrange multiplier, G, were chosen so that the final spore concentration was much lower than the five-log cycle reduction imposed by the sterilization demand. During each iteration, this profile was improved by using Eq (34). The iterative procedure was used until the calculated change in $\theta(\bar{t})$ was very small, and the necessary conditions expressed in Eq (30a) were fulfilled. This constraint was satisfied by guessing the appropriate value of G. The value of this Lagrange multiplier was searched in the range 1,25,.....200. Good convergence was obtained for a constant value of G = 100. This value was then used for all calculations. The process described above is equivalent to the procedure of guessing the value of $\phi(t_F)$ required, whenever a final value for the state variable is preassigned.

A fourth-order Runge-Kutta numerical integration formula with a maximum interval of 0.05 (dimensionless time) was used. The temperature and the values of $\bar{x}_1$ and $\bar{x}_2$ within the interval of the integration were evaluated by a linear interpolation.

The procedure outlined above was programmed on an IBM 370/168 computer at the MIT Information Processing Center.

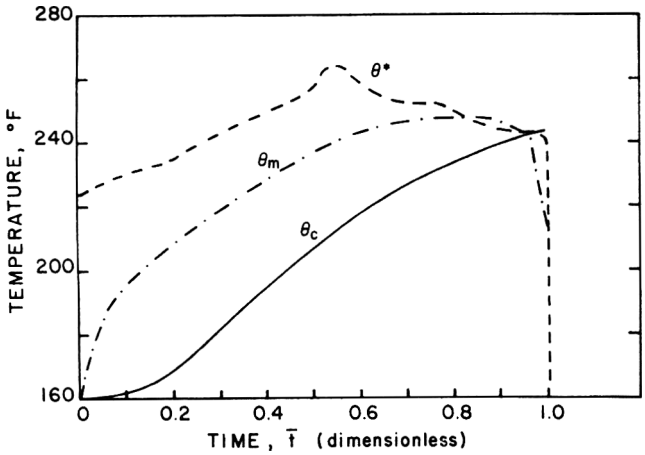

Fig. 2—Optimal retort temperature (θ^*), mass average temperature (θ_m), and central point temperature (θ_c) during the sterilization process (A/2 can).

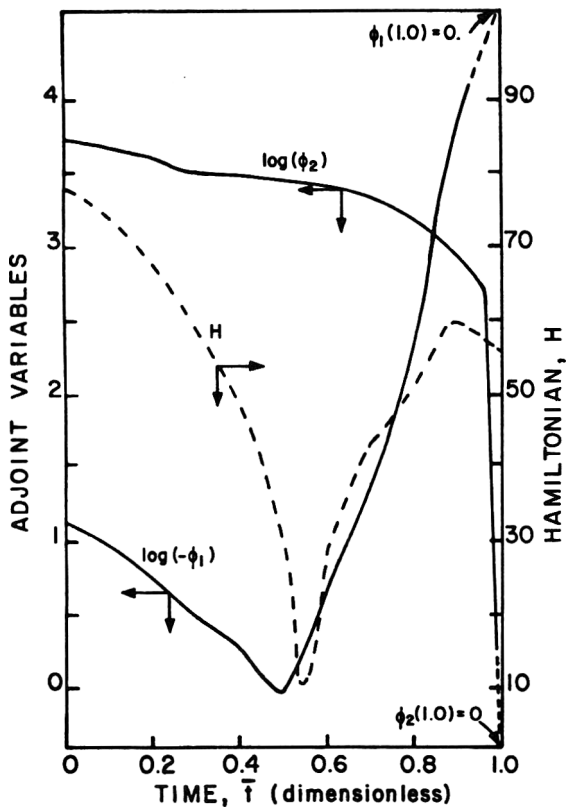

Fig. 3—Optimal profiles of the adjoint variables and the Hamiltonian function during the sterilization process (A/2 can).

DISCUSSION

WE DETERMINED the optimum temperature profile for thiamin retention in canned foods during a sterilization process to illustrate an application of the optimization procedure and computational scheme described herein. Figure 2 shows the optimum retort temperature profile to maximize the objective function given in Eq (21) in an A/2 can of pork puree. The resultant average and mid-center temperatures are also shown. The corresponding adjoint variables and the Hamiltonian profiles are given in Figure 3, whereas the thiamin retention and microorganism destruction corresponding to the above values are given in Figure 4 and Table 1. As expected in this case, where the temperature of the product within the can is a function of time and

location (a nonautonomous system), the derived value of the Hamiltonian was not constant for every t (Fig. 3).

The optimum temperature profiles (Fig. 2 and 5) obtained for the three can sizes studied (Table 1) varied with time and showed that, for conduction heating of foods, an HTST (high temperature/short time) process does not provide maximum nutrient retention. Moreover, each process must be optimized individually.

Table 1 shows that the optimal principle theory improved thiamin retention in all the cases studied. Considering that Teixeira et al. (1975a, b) and Thijssen et al. (1978) have already optimized the process, the fact that any improvement was introduced by the suggested method is very significant. Moreover, the maximum thiamin retention found and the corresponding temperature profiles computed provided a single solution. It may thus be concluded that a best single process to optimize nutrient retention does exist.

Since this research was undertaken to investigate the application of modern optimization techniques, it was beyond our scope to test the industrial applicability of the optimal temperature profile derived here. However, the results show that retention of thiamin in the sterilization process used was limited by heat conduction factors and the dimensions of the cans used. Moreover, it may be concluded that in the cases studied, the constant retort temperature policy was nearly as good as the other methods due to the small differences observed. However, this conclusion was not obvious and only the results derived from the optimal control theory may prove that.

The "gradient method in function space," in which the optimized profile is corrected after each iteration using the maximum principle as recommended here, was found to be fast and reliable, when a good initial guess of the profile has been made. When the initial estimate of the profile is far from the optimum, some difficulties may be encountered, such as slow convergence and instability during the numerical integration. The instability is caused by the high sensitivity of the destruction rate for spores at high temperatures and by high values of ϵ [see Eq (34)]. The latter source of instability may be overcome by either decreasing the value chosen for ϵ or by making ϵ a function of time (Storey and Rosenbrock, 1964).

We also considered a procedure recommended by Fan (1966) in which the initial values of the adjoint function are assumed and the integration is performed only in a forward direction. This procedure resulted in severe instability, which precluded its use.

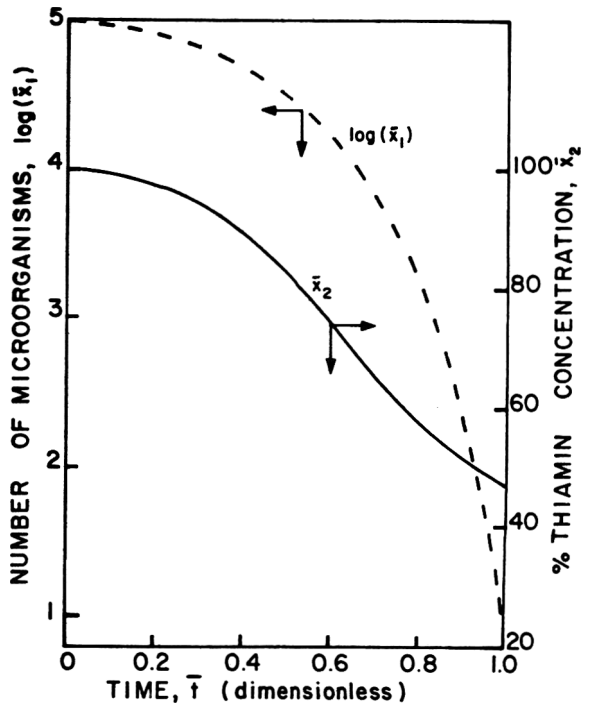

Fig. 4—Optimal profiles of thiamin retention and microorganism survival during the sterilization process (A/2 can).

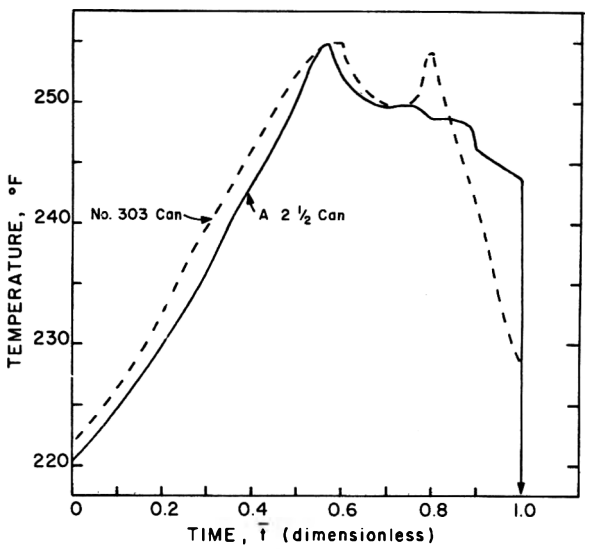

Fig. 5—Optimal retort temperature profiles for the sterilization process of pea puree in #303 can and pork puree in A/2½ can.

NOMENCLATURE

- a Thermal diffusivity coefficient, in.^{2}/sec
- C Vector (C_1, C_2,.....C_s) of constants
- D Time required to destroy 90% of the concentration, min
- f Performance equation defined in Eq (1)
- G Lagrange multiplier, Constant
- H Hamiltonian function
- K_1 Defined in Eq (12)
- K_2 Defined in Eq (13)
- NH Number of vertical elements in a can
- NR Number of radial elements in a can
- r Number of decisions; radial distance, in.
- s Number of ordinary differential equations
- S Target function to be optimized
- t Time, min
- $\bar{t}$ Dimensionless time ($0 \leqslant \bar{t} \leqslant 1$)
- T Temperature within the can, °F
- V Can volume, in.^{3}
- x Vector (x_1, x_2,.....x_s) of state variables
- y Vertical distance from mid-plane, in.
- z Temperature increase (°F) required for reducing the value of D by one order of magnitude
- α Vector (α_1, α_2,.....α_s) of initial conditions
- Δ Volume element, in.^{3}
- ϵ Small positive number
- θ Decision vector (θ_1, θ_2,.....θ_r), °F
- ϕ Adjoint vector (ϕ_1, ϕ_2,.....ϕ_s)

Subscripts

- 0 Reference, initial
- 1 Microorganism
- 2 Nutrient
- c Central
- C Cooling

F Final
i Radial sequence
j Vertical sequence
m average

Superscripts
l Transpose vector
n Iteration number
– Dimensionless; average
* Optimal
· Derivative

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REMOVAL OF RADIOACTIVE STRONTIUM AND CESIUM FROM VEGETABLES DURING LABORATORY SCALE PROCESSING

C. M. WEAVER and N. D. HARRIS

ABSTRACT

Several processing methods were examined for their effectiveness in removing ^{90}Sr and ^{137}Cs from vegetables exposed to the radionuclides through the nutrient solution. The greatest decontamination resulted from a combination of pickling and canning which reduced ^{137}Cs by 94% and ^{90}Sr by 65%. Canning was highly effective in reducing radionuclide concentrations in beans and kale. Freezing significantly reduced radionuclide content of only ^{137}Cs in kale. Blanching of sweet potatoes appeared to result in a transfer of radioactivity from the peel to core, indicating that skins of contaminated potatoes should be removed prior to thermal treatment. Preparatory procedures prior to processing did not significantly reduce radionuclide levels except for ^{137}Cs in beans.

INTRODUCTION

FOOD is a major route by which environmental radiocontaminants reach man. Even with strict controls and containment, releases of radioactive fission products from nuclear power plants and leakages from containers in which radioactive waste is stored or transported are likely to occur. The two abundant nuclear fission products with relatively long half-lives are cesium-137 and strontium-90.

Studies undertaken to investigate decontamination of vegetables have focused on vegetables exposed to radionuclides through aerial deposition and the effectiveness of preparatory steps prior to processing on removal of radioactivity (Rohleder, 1972; Ralls et al., 1971; 1968–69; Thompson, 1965; Dubrovina and Belova, 1964; Food & Drug Adm, 1964). Washing and removal of outer skins readily decreased levels of radionuclides in the vegetables studied, indicating that the radioactive materials were absorbed primarily onto the surfaces. Removal of radionuclides which have been absorbed through the roots of vegetables represent a different problem than when the contamination reaches only the external portions of plants. Ralls (1968–1969) reported the effects of preliminary steps prior to processing on the removal of ^{89}Sr and ^{134}Cs in spinach, peas, and broccoli grown in contaminated soil. A 70% reduction of ^{89}Sr content and a 50% reduction of ^{137}Cs content was achieved in peas by broth flotation and water blanching, whereas, a 75% reduction in ^{89}Sr content was observed after washing and blanching spinach and broccoli. The content of ^{137}Cs was reduced in spinach by 60% and in broccoli by 90% after similar treatment. Use of ion exchange resins substantially reduced radioactivity of internally-contaminated potato and tomato pieces (Ralls et al., 1971; Perkins and Strachan, 1964).

This paper is concerned with the removal of radionuclides in several vegetables representing a variety of edible tissues, including parts which grow above ground and below ground, by a variety of processing procedures including washing, blanching, freezing, canning, and pickling. Unlike previous studies, the vegetable crops were contaminated by supplying them with nutrient solution containing radionuclides from germination to maturity. The results of this investigation can aid in planning emergency actions following accidental releases of nuclear fission products with subsequent contamination of agricultural crops.

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EXPERIMENTAL

Growth conditions

A hydroponicum was constructed inside a greenhouse for growing vegetables with above-ground edible tissues. It consisted of polyvinylchloride piping cut so that 20 plants could be grown in each pipe by inserting seeds into cellulose blocks spaced approximately 20 cm apart. A 1/10 strength Hutner's nutrient solution (Furuya and Hillman, 1964) was circulated to each pipe via pumps and flow of solution was controlled by screw clamps. Solutions were replaced at the end of each week with freshly prepared solution. Losses in nutrient solution due to transpiration and evaporation throughout the week were replenished by the addition of control solution. Ports for entry and exit of solution supplemented with radionuclides were built into the hydroponicum. With such a system the environmental factors which might influence radionuclide uptake, such as temperature, pH, and nutriture, were held constant and uptake potential of plants was maximized.

Kale (*Brassica oleracea* var. acephala D.C. cv. 'Blue Curl') and red kidney beans (*Phaseolus vulgaris* L.) were grown from germination to maturity in nutrient solution containing either 79,400 cpm ^{90}Sr/liter or 70,490 cpm ^{137}Cs/liter. Cucumbers (*Cucumbis sativus* var. Toska-70) were grown in nutrient solutions containing either 94,750 cpm ^{90}Sr/liter or 82,424 cpm ^{137}Cs/liter.

Sweet potatoes (*Iponoea batatas* Poir. cv. 'Georgia Red') could not be grown successfully in the hydroponicum. Consequently, sweet potatoes were grown in styrofoam coolers (2 slips/container) containing sand which was periodically moistened with nutrient solution. The radionuclides were administered by distributing a single dose (25 ml aliquot containing 564,672 cpm ^{137}Cs or 245,250 cpm ^{90}Sr) on the soil surface after planting.

Food processing procedures

Beans were harvested after radionuclide exposure through the nutrient solution, snapped into 3 cm lengths, mixed thoroughly, and divided into three lots. One lot was analyzed without further treatment. The other two lots were washed in distilled-deionized water for 2 min and blanched for 2 min at 88°C. One lot was reserved for analysis and the remaining lot was packed hot into half-pint jars. The jars were filled with boiling distilled-deionized water to which NaCl (1.5g) was added and jars were heated in a retort to 120°C for 12 min. Canned samples were held for 3 months before being analyzed for radionuclides.

Contaminated kale was harvested and the leaves were mixed thoroughly and divided into four lots. One lot was analyzed with no processing treatment. The other three lots were washed under running distilled-deionized water for 2 min, scrubbed in standing water for 2 min, and blanched for 6 min at 78°C. One lot was removed for analysis. One lot was divided in half and each half was deposited in a quart size freezer bag, placed in a labelled freezer carton, and frozen at −11°C. The remaining lot was distributed among half-pint jars. NaCl (1.5g) was added to each jar. Jars were filled with distilled-deionized water and heated in a retort for 40 min at 120°C. Frozen and canned samples were stored for 3 months prior to analysis for radionuclides.

Replicate samples of sweet potatoes were harvested and one-third of each potato was removed and the skins and edible tissue were analyzed separately for radionuclide content. The remaining two-thirds of each potato were washed by immersion in distilled-deionized water for 2 min, scrubbed for 30 sec with a brush, and blanched by heating for 8 min at 115°C. The skin and one-half of the blanched sweet potatoes were held for radionuclide analysis.

—Text continued on page 1492

Table 1—Reduction in ^{137}Cs and ^{90}Sr content of beans by washing, blanching and canning

Treatment	Mean[a] ^{137}Cs content (cpm/g dry wt) ± SD	% Change from controls[b]	Mean[a] ^{90}Sr content (cpm/g dry wt) ± SD	% Change from controls[b]
Raw	7771 ± 482		9766 ± 2038	
Washed & blanched	7011 ± 528	− 9.8*	9743 ± 1942	− 0.2
Canned	2864 ± 523	−63.2**	5228 ± 1489	−46.5**

[a] Each value represents the mean of 10 determinations.
[b] Values followed by * and ** were significant at $P \leq 0.05$ and $P < 0.0005$, respectively.

Table 2—Reduction in ^{137}Cs and ^{90}Sr content of kale by washing, blanching, canning and freezing.

Treatment	Mean[a] ^{137}Cs content (cpm/g dry wt) ± SD	% Change from controls[b]	Mean[a] ^{90}Sr content (cpm/g dry wt) ± SD	% Change from controls[b]
Raw	81153 ± 7509		49727 ± 5537	
Washing & blanching	76927 ± 13322	− 5.2	52311 ± 6183	+ 5.2
Canning	18595 ± 3249	−77.1*	20480 ± 7577	−58.8*
Freezing	60195 ± 8696	−25.8*	46081 ± 6510	− 7.3

[a] Each value represents the mean of 10 determinations.
[b] Values followed by * were significant at $P \leq 0.0005$.

The peel and the edible portion of each sweet potato were analyzed separately. The remaining portion of blanched sweet potato was subjected to a canning process which involved slicing the potatoes crosswise and placing them in half-pint glass jars. Distilled water (50 ml) was added to each jar and the samples were heated in a retort for 45 min at 115°C. Each canned sample was held for 1 month before being analyzed for radionuclides.

Cucumbers were picked and blossoms were removed following growth in a radionuclide-containing nutrient solution. Each cucumber was cross cut into slices 4 mm wide. Every other slice was analyzed with no further treatment. Remaining slices were made into brined dill pickles according to Home and Garden Bulletin No. 92 (USDA, 1968). The brine consisted of 62.5 ml vinegar, 52.8 granulated NaCl, and 1 liter distilled water. Whole mixing pickling spice (18.8 ml) was added. After a 3-wk fermentation period, half the pickle slices were reserved for analysis and half were placed in half-pint jars. Fresh brine was added to each jar and jars were placed into a boiling water bath for 15 min. Each canned sample was held for 1 wk before being analyzed for radionuclides. All samples were drained and homogenized in a Virtis blender prior to analysis.

Radionuclide assays

Each sample was weighed, dried in a vacuum oven at 80°C (170 mm Hg) for 20 hr, ground in a Wiley Mill, and reweighed. Ground material from plants exposed to ^{137}Cs was placed in a test tube and counted by gamma spectroscopy using a NaI well-type scintillation counter. Ground material from plants exposed to ^{90}Sr was ashed in a crucible lined with ashless filter paper in a muffle furnace (800°C) overnight and analyzed by suspension-gel liquid scintillation spectrometry after a 3-wk holding period to ensure attainment of a secular equilibrium between ^{90}Sr and its daughter ^{90}Y. Sample ash was suspended in a thixotropic gel formed by placing 400 mg of Cab-O-Sil in each scintillation vial. The scintillation solution was prepared by dissolving 7.5g 2,5-diphenyloxazole and 0.15g 1,4-bis-2-(5-phenyloxazolyl) benzene in 2 L toluene (Ralls et al., 1971) and 10 ml of this solution was added to each vial. Samples were counted using a Searle Isocap/300 liquid scintillation system. Samples which exhibited quenching were corrected for decreased efficiency by extrapolation from a quench curve prepared by addition of equal aliquots of a ^{90}Sr stock solution to vials with increasing weights of plant ash. Recovery of ^{90}Sr added to plant tissue prior to ashing was 98.8%. The amount of radioactivity was recorded as counts per minute after correcting for both background counts in control samples and fluctuations in counting efficiency, as determined with standards prepared from stock solutions used in each experiment. Aliquots for ^{137}Cs standards were placed in test tubes to be counted with samples on the NaI well counter and aliquots for ^{90}Sr were added to vials containing Cab-O-Sil to be counted with samples by suspension gel liquid scintillation spectrometry.

RESULTS & DISCUSSION

Beans

The extent to which ^{137}Cs and ^{90}Sr are removed from red kidney bush beans (grown hydroponically in radionuclide-containing nutrient solution) by washing, blanching, and canning is given in Table 1. Washing and blanching significantly reduced the concentration of ^{137}Cs by 9.8% ($P = 0.013$), but not the concentration of ^{90}Sr ($P = 0.974$). Cesium is more soluble than strontium which could result in greater removal by procedures prior to processing. Since beans were snapped, the opportunity for loss of water soluble nutrients by leaching was increased. Canning reduced the concentration of ^{137}Cs in beans by 63.2% and that of ^{90}Sr by 46.5%, both of which were highly significant ($P < 0.0005$). Canned snap beans were found to contain 62% less ^{90}Sr than raw samples exposed to natural fallout (Laug, 1963).

Kale

The removal of ^{137}Cs and ^{90}Sr from kale by washing, blanching, canning and freezing is given in Table 2. Washing and blanching did not significantly alter the ^{137}Cs and ^{90}Sr content in kale ($P = 0.422$ and $P = 0.368$, respectively), but reductions of 77.1% for ^{137}Cs and 58.8% for ^{90}Sr by canning were highly significant ($P < 0.0005$). Freezing did not significantly reduce the ^{90}Sr content ($P = 0.290$), but the 25.8% reduction of ^{137}Cs was highly significant ($P < 0.0005$). Cesium is an intracellular ion. Perhaps during the freezing and thawing of the kale, some of the vacuolar sap and accompanying intracellular ions separated from the leaves used for analysis.

Sweet potatoes

The results of experiments designed to determine the effect of washing, blanching, and canning on removal of ^{137}Cs and ^{90}Sr from sweet potatoes are presented in Table 3. The results for raw sweet potatoes indicate that the concentration of ^{90}Sr is much greater in the peel than in the core but that the difference between the core and the peel is much less for ^{137}Cs. These observations could be interpreted to mean that ^{137}Cs is translocated from the leaves and/or roots into the interior of the storage roots, while contamination with ^{90}Sr results from diffusion by direct contact of the storage roots with contaminated sand. Other investigators have reported similar evidence in potatoes. When radiocontamination of potato plants was studied, ^{90}Sr was found in the leaves but it was not translocated from leaves into tubers (Mel'nikova and Baranova, 1967). Only when the tubers were in direct contact with contaminated soil did the tubers contain ^{90}Sr. Most of the radionuclide was concentrated in the first 1 mm layer of each tuber and the concentration of the nuclide gradually decreased into the interior of the tubers (Mel'nikova et al., 1971). In contrast, ^{137}Cs was concentrated in tubers following exposure of potato plants to the radionuclide by painting the leaves with a $^{137}CsNO_3$ solution (Perkins and Strachan, 1964). Removal of a 1 mm layer of peel resulted in a loss of only 25–27% of the ^{137}Cs present in the intact tuber indicating the presence of a significant fraction of the radionuclide in the interior of the tubers.

Washing and blanching resulted in a decrease in the concentration of both radionuclides in the peel and an increase in the concentration of radionuclides in the core of sweet potatoes (Table 3), although the increase observed in the

core was not significant for ^{90}Sr or ^{137}Cs (P = 0.291 and 0.201, respectively). If radioactivity is transferred from the peel to the interior during blanching as is indicated by these results, contaminated potatoes should have their skins removed before processing. A combination of washing, blanching, and canning resulted in a decrease of 1.4% for ^{137}Cs and 25.5% for ^{90}Sr over unprocessed sweet potatoes, although neither of these changes were significant (P = 0.963 and 0.162, respectively). It is conceivable that had the peel been removed prior to thermal treatment, canning would have significantly reduced the radionuclide content of sweet potatoes. This premise is supported by evidence that natural levels of ^{90}Sr in potatoes were reduced 24% by peeling, washing, and boiling (Thompson, 1965) and simple boiling of potatoes reduced the ^{90}Sr level by 20% (Dubrovina and Belova, 1964).

Cucumbers

Pickling reduced ^{137}Cs content in cucumbers 84.7% and canning further reduced the radionuclide content of the pickles making the total reduction 94.1% as compared to the raw cucumbers (Table 4). Pickling and canning reduced the ^{90}Sr content of cucumbers 64.6% (Table 4). All of these reductions were highly significant.

CONCLUSIONS

REGARDLESS of the processing treatment or the vegetable, with the exception of sweet potatoes, the ^{137}Cs content was reduced to a greater extent than ^{90}Sr, which may reflect the greater solubility of ^{137}Cs. If most of the accumulated ^{90}Sr is concentrated in the first 1 mm layer of a sweet potato and if ^{137}Cs is present throughout the interior of the sweet potato as was reported for potatoes (Mel'nikova et al., 1971 and Perkins and Strachan, 1964, respectively), the removal of ^{90}Sr by processing procedures, including the removal of the outer skins, may be greater than the removal of ^{137}Cs even though ^{137}Cs is more soluble than ^{90}Sr.

The reduction in radionuclide content of the vegetables by canning can be accounted for by transfer to the drained liquid. Pickling and subsequent canning had a greater effect on reduction of the radionuclides than did the other processing treatments. The radionuclides are more soluble in acidic solutions and since cucumbers were pickled in a vinegar brine, more of the radionuclides may have leached out into the brine. The pickling process denatured the membrane so that the characteristic of semipermeability was lost and ions became free to move according to their own chemical potential gradients. Slicing of cucumbers increased the surface area which would enhance movement by diffusion.

Although Ralls et al., (1968–1969; 1971) reported a considerable reduction in radionuclide concentration by steps prior to processing such as washing and blanching, only processing treatments substantially reduced radionuclide concentration in the present study. It is not surprising that rinsing or washing would remove substantial amounts of externally contaminated ^{137}Cs or ^{90}Sr since crop samples were harvested and treated 1–2 hr after spraying before the radionuclides had an opportunity to penetrate into the vascular system. However, it is difficult to understand how surface treatments could account for 21–72% reduction in internally contaminated crops. Ralls et al., (1968–1969) did not report actual levels of radioactivity nor statistical analysis, but rather only provided percentage changes which makes interpretation of results difficult. In their other paper (1971), they reported spray rinsing of strawberries removed only 12% of internal strontium contamination, although actual values were not given.

Even though processing procedures reduced the radionuclide content of vegetables, the canning liquor or pickling brine must be discarded in order for the treatment to be effective. The safety of ingestion of contaminated foods following processing procedures would depend on the level of radiocontamination.

Table 4—Reduction in ^{137}Cs and ^{90}Sr content of cucumbers by pickling and canning

Treatment	Mean[a] ^{137}Cs content (cpm/g dry wt) ± SD	% Change from controls[b]	Mean[a] ^{90}Sr content (cpm/g dry wt) ± SD	% Change from controls[b]
Raw	29183 ± 5352		6267 ± 2031	
Pickling	4453 ± 141	−84.7*	c	
Canning	1733 ± 434	−94.1*	2219 ± 828	−64.6*

[a] Each value represents the mean of 10 determinations.
[b] Values followed by * were significant at P ≤ 0.0005.
[c] Samples could not be completely recovered from crucibles following ashing.

Table 3—Reduction in ^{137}Cs and ^{90}Sr content of sweet potatoes by washing, blanching and canning

Sample	Treatment	Mean[a] ^{137}Cs content (cpm/g dry wt) ± SD	% Change from controls[c]	Mean[b] ^{90}Sr (cpm/g dry wt) ± SD	% Change from controls
Core	Raw	142 ± 62		705 ± 330	
	Washed & blanched	163 ± 93	+14.1	1091 ± 282	+54.8
	Canned	140 ± 58	− 1.4	518 ± 249	−26.5
Peel	Raw	237 ± 124		2368 ± 950	
	Washed & blanched	175 ± 70	−26.2*	2013 ± 858	−15.0

[a] Each value represents the mean of 10 determinations.
[b] Each value represents the mean of 5 determinations.
[c] Values followed by * are significant at P ≤ 0.05.

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REGULATION OF FROZEN SNAP BEAN QUALITY BY POSTHARVEST HOLDING IN CARBON-DIOXIDE ENRICHED ATMOSPHERES

R. W. BUESCHER and HELEN BROWN

ABSTRACT

Quality attributes of freeze-processed snap beans (*Phaseolus vulgaris*) were examined after holding the raw product in nonrefrigerated CO_2-enriched atmospheres. Exposure to CO_2 reduced shear resistance and titratable acidity while −a/b values, pH, and sensory color scores were enhanced. Texture and flavor were not significantly affected in most trials, but in some they were improved by the CO_2 treatment. Alterations in color, resistance to shear, pH and acidity induced by CO_2 increased in intensity with increasing conc of CO_2, appeared within 2 hr of exposure, were rapidly reversible when exposed to air, and occurred regardless of temperature or sieve size. CO_2-induced changes were not apparent in the raw product but were observed after heating. Exposure to CO_2-enriched atmospheres resulted in enhanced levels of CO_2 and carbonates in extracted juice even after cooking. The results indicate that handling snap beans in CO_2-enriched atmospheres would be beneficial to freeze-processed quality.

INTRODUCTION

SNAP BEANS destined for canning and freezing are usually transported from production areas to processing facilities in open-bed trailers without precooling or refrigeration. Quality characteristics such as color, texture, blemishes and discoloration of snap beans may be adversely affected after several hours of holding in nonrefrigerated conditions. Although adverse changes which occur during postharvest handling are detrimental to quality of the canned product, these changes are most detrimental to quality of the frozen product.

Holding snap beans in CO_2-enriched atmospheres at 27°C has been shown to inhibit broken-end discoloration, the accumulation of phaseollin and phenolic substances (Buescher and Doherty, 1978; Buescher and Henderson, 1977; Doherty and Buescher, 1978; Henderson and Buescher, 1977; Henderson et al., 1977; Reitmeier and Buescher, 1975). Color, texture and flavor of canned snap beans were not appreciably altered by holding the raw product in CO_2-enriched atmospheres (Buescher and Doherty, 1978; Henderson and Buescher, 1977), but color of the frozen product after cooking was substantially improved (Buescher and Doherty, 1978). Atmospheres enriched with CO_2 along with reduced temperatures have previously been shown to retard deterioration of snap beans (Groeschel et al., 1966), asparagus (Lipton, 1965; Wang et al., 1971; Lougheed and Dewey, 1967), broccoli (Lipton and Harris, 1974; Lebermann et al., 1968), and spinach (Burgheimer et al., 1967).

In this study the effects of holding raw snap beans in nonrefrigerated CO_2-enriched atmospheres on texture, color, flavor, and acidity of the frozen-cooked product were examined. Variables included CO_2 concentration, duration of exposure to CO_2, and duration in air for reversing CO_2-induced changes, as well as the influence of sieve size and temperature on CO_2-induced alterations. In addition, CO_2-induced changes and levels of dissolved CO_2 in raw, blanched, and frozen-cooked products were determined.

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EXPERIMENTAL

Source and preparation

Snap beans ('Early Gallatin' and 'Cascade') were obtained from a local processor (Steele Canning Co., Div. of Pioneer Foods, Springdale, AR) and mechanically separated according to sieve size. Sieve sizes 4 and 5 were used in all experiments except one when sieve size was used as a variable and then sizes 3, 4, 5 and 6 were observed separately.

Treatment

Uniform samples (approx 300g) of snap beans were placed in 8-L chambers, equipped with gas inlet and exhaust ports, and held in thermally controlled conditions. Air or CO_2-enriched air was passed through the chambers at rates of 4–5L per hour which prevented CO_2 accumulation and O_2 reduction from respiration. Modified atmospheres of 20, 40 and 60% CO_2 with 21% O_2 were formed by mixing CO_2 with air and O_2 in the system described by Shaw and Kattan (1971). Oxygen content was adjusted to 21% to avoid the effects of reduced O_2 when CO_2 levels were increased. CO_2 and O_2 levels were monitored by a Hamilton-Fisher gas partitioner. Levels of CO_2, duration of exposure to CO_2 or air, and temperatures during treatment are indicated in the tables of results. Each treatment was performed in duplicate.

Processing

Samples were processed initially (no holding) and after holding for various times in CO_2-enriched air or air by mechanically snipping, cutting into 4 cm pieces, blanching in boiling water for 3 min, cooling in tap water (25°C), sealing in polybags, and freezing at −25°C. After at least 1 month in storage at −25°C, samples were opened, placed in aluminum pans, and prepared for analysis by cooking in steam for 8–10 min.

Analysis

Resistance to shear of 150-g samples of cooked snap beans was determined with a texture test system (Food Technol. Co.) which was equipped with a 454 kg ring. The sheared tissue was mixed and 100g was homogenized with 100 ml of H_2O. Color of the homogenates were determined with a Gardner Color Difference Meter (CDM) standardized with a white color plate (L=93.5, a=−1.4, b=3.2). Additional water (200 ml) was added to 100g of homogenate for facilitating determination of pH and titratable acidity. The pH was recorded and the solution was titrated to pH 8.2 with 0.1N NaOH to determine milliequivalents (meq) of acid.

Color, flavor and texture of the cooked snap beans were evaluated on a hedonic scale from 1 (poor) to 9 (excellent) by a sensory panel consisting of 8 trained members.

Juice was extracted by squeezing pods through miracloth and analyzed for dissolved CO_2 content. The assay for dissolved CO_2 consisted of enzymatically forming oxalacetate from phosphoenol pyruvate and CO_2 and then measuring the decrease in absorbance at 340 nm when reduced nicotinamide adenine nucleotide was oxidized in the presence of malate dehydrogenase and oxalacetate to form malate (Anonymous, 1977). The decrease in absorbancy was proportional to the quantity of dissolved CO_2, bicarbonate and carbonate in the sample.

Each analysis was replicated at least three times.

RESULTS

HOLDING freshly harvested snap beans in air for 24 hr at 27°C reduced −a/b (greeness) values of the processed product, while holding for 48 hr enhanced resistance to shear and titratable acidity and further reduced −a/b values (Fig.

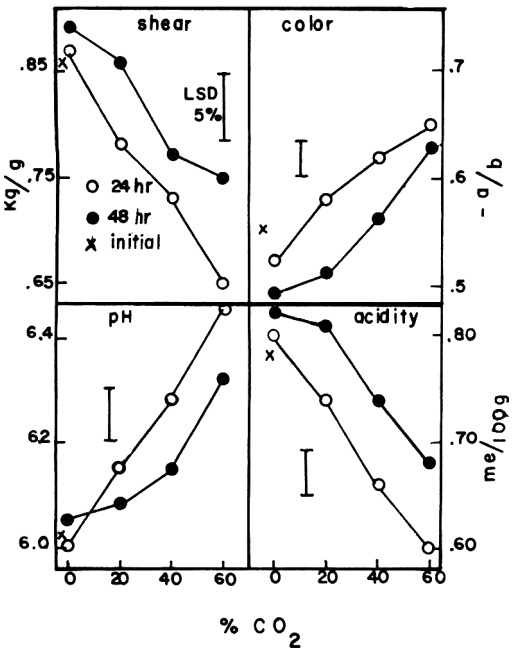

Fig. 1—Influence of holding raw snap beans in elevated CO_2 atmospheres at 27°C on shear resistance, color, pH and acidity after cooking the frozen product.

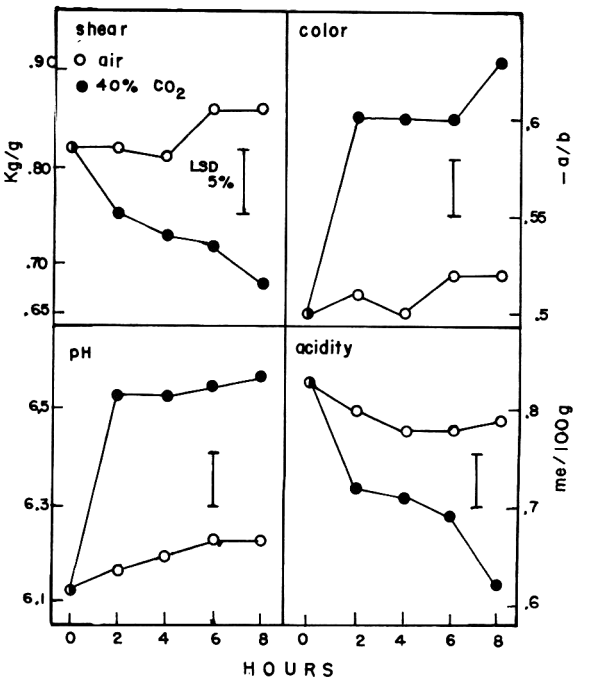

Fig. 2—Changes in shear resistance, color, pH and acidity after cooking frozen snap beans as affected by duration of exposing the raw product to 40% CO_2 at 27°C.

1). CO_2-enriched atmospheres prevented these adverse changes in quality and actually improved certain characteristics. Holding snap beans in CO_2-enriched atmospheres reduced shear resistance and titratable acidity and enhanced −a/b values and pH. Trends for sensory color scores (data not shown) were similar to −a/b values while sensory scores for flavor and texture were not significantly different between treatments. The alterations induced by CO_2 increased with increasing concentration of CO_2 in the holding atmosphere. After 24 hr, 20% CO_2 in the holding atmosphere was sufficient to significantly alter shear, −a/b values, pH, acidity and color scores while after 48 hr of holding, 40% CO_2 was necessary to provide significant alterations.

Within 2 hr of exposing fresh snap beans to 40% CO_2 at 27°C, shear resistance and acidity were reduced and −a/b values and pH were enhanced in the processed product (Fig. 2). With increasing duration of exposure to CO_2, these alterations continued, but at a reduced rate. Sensory color scores (data not shown) closely paralleled changes in −a/b values.

The reversibility of CO_2-induced changes in snap bean quality was observed by holding in air after holding in 40% CO_2 for 24 hr at 27°C (Fig. 3). Shear resistance and titratable acidity were reduced and −a/b values and pH were enhanced by exposure to 40% CO_2. Trends for sensory color scores were similar to −a/b values while texture and flavor were not judged to be different between treatments. As compared to characteristics of snap beans held continuously in air, −a/b, acidity and sensory color scores were still significantly altered in CO_2 treated snap beans when subsequently exposed to air for 5 hr. After 10 hr in air, −a/b values and sensory color scores were the only characteristics altered while after 24 hr in air no differences were detected. Reversibility of CO_2-induced alterations appeared to be more gradual for color than shear resistance, pH and acidity.

With increasing temperature, shear resistance and acidity increased and −a/b values, pH and sensory color scores tended to decline, regardless of holding atmosphere (Table 1). Compared to snap beans held in air, those held in 40% CO_2 had reduced shear resistance and acidity and enhanced −a/b values, pH and color scores at each temperature. The greatest difference in values between initial and CO_2 treated snap beans occurred at 10°C and then as the holding temperature increased, the differences became less. Sensory scores for texture and flavor declined in snap beans held in air as the temperature increased, while scores for those held in 40% CO_2 remained similar to scores for those processed initially. Texture and flavor scores for snap beans previously held at 24°C in 40% CO_2 were greater than for those which had been held in air at 24°C and 38°C.

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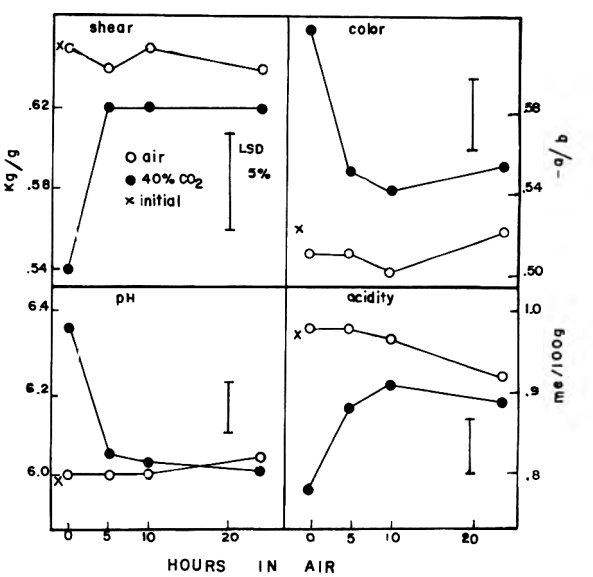

Fig. 3—Duration in air at 27°C after exposure to 40% CO_2 for reversing CO_2-induced changes in shear resistance, color, pH and acidity.

Table 1—Effect of holding temperature on CO_2-induced changes in texture, color, pH, acidity and flavor of subsequently cooked snap beans

Temperature (°C) of holding for 24 hr	Shear Resistance kg/g		−a/b		pH		meq/100g		Sensory scores Color		Texture		Flavor	
	Storage atmosphere													
	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2
(Initial)	(0.56)		(0.49)		(6.08)		(1.04)		(6.8)		(7.3)		(7.5)	
10	0.58	0.48	0.48	0.57	6.07	6.32	0.97	0.82	6.8	8.3	6.9	7.1	6.7	7.2
24	0.67	0.53	0.47	0.53	5.98	6.18	1.01	0.85	6.4	8.1	6.5	7.7	6.6	7.7
38	0.73	0.65	0.43	0.49	5.98	6.13	1.08	0.88	4.5	6.6	6.3	6.7	6.3	7.0
LSD 5%	0.08		0.05		0.11		0.06		1.1		0.7		1.1	

Table 2—Influence of sieve size on CO_2-induced changes in texture, color, pH, acidity and flavor of subsequently cooked snap beans

Sieve size	Shear resistance kg/g			−a/b			pH			meq/100g			Sensory scores Color			Flavor		
	Storage atmospheres																	
	Initial[a]	Air[b]	CO_2[c]	Initial	Air	CO_2	Initial	Air	CO_2	Initial	Air	CO_2	Initial	Air	CO_2	Initial	Air	CO_2
3	0.69	0.91	0.76	0.56	0.49	0.58	6.25	6.25	6.40	0.67	0.77	0.62	7.8	6.5	7.7	7.3	5.3	6.8
4	0.76	0.98	0.76	0.56	0.48	0.58	6.25	6.20	6.38	0.71	0.78	0.69	7.7	6.0	7.6	7.2	5.7	7.0
5	0.79	0.97	0.79	0.53	0.48	0.57	6.13	6.15	6.35	0.82	0.91	0.74	7.0	6.0	7.3	7.0	5.2	7.0
6	0.92	1.18	0.88	0.50	0.45	0.56	6.13	6.18	6.30	0.89	0.90	0.82	6.0	5.0	6.3	7.0	5.6	7.2
LSD 5%		0.14			0.06			0.12			0.07			1.2			1.1	

[a] Samples processed immediately
[b] Samples held in air at 27°C for 24 hr
[c] Samples held in 40% CO_2 at 27°C for 24 hr

Regardless of treatment, shear resistance, color and pH tended to decline while acidity increased as snap bean sieve size increased (Table 2). Holding in air for 24 hr resulted in enhanced shear resistance and reduced −a/b values, as well as reduced color and flavor scores for all sieve sizes. In contrast, holding in CO_2-enriched atmospheres for 24 hr prevented these adverse changes in processed quality. The response to CO_2 appeared to be similar for all sieve sizes.

Holding snap beans in 40% CO_2 for 24 hr at 27°C reduced titratable acidity in the raw product while −a/b values and pH were not significantly different as compare; to snap beans held in air (Table 3). After blanching, both pH and titratable acidity were altered, and after blanching, freezing and cooking, differences in −a/b also became apparent. Apparently, chlorophyll is altered or degradation is retarded in snap beans exposed to CO_2-enriched atmospheres which does not become apparent until the product is cooked for more than 3 min. With heating, pH increased and acidity declined, and in snap beans treated with CO_2, these changes were greater than in those held in air.

CO_2 levels in juice from raw, blanched and cooked snap beans were substantially enhance by holding in CO_2-enriched atmospheres prior to processing (Table 4). As compared to CO_2 levels in raw juice, blanching reduced the CO_2 content and cooking after blanching and freezing reduced the levels further. CO_2 content of juice after cooking was depleted in snap beans held in air, while in those exposed to CO_2-enriched atmospheres, 0.12 meq/L was still present.

DISCUSSION

IN ADDITION to preventing discoloration in snap beans (Buescher and Henderson, 1977; Henderson and Buescher, 1977; Henderson et al., 1977; Reitmeier and Buescher, 1977) color was dramatically improved by holding the raw product in CO_2-enriched atmospheres at 27°C. Texture was also affected; however, the sensory panel did not usually find the tenderization either beneficial or detrimental. It appeared that when snap beans toughened during holding in air, then the tenderization mediated by CO_2 was judged to CO_2 treatments, but when significant differences occurred, it was indicated that CO_2 treatments were beneficial. From these results, it is concluded that CO_2 induced improved color, while texture and flavor may also be improved. These

Table 3—Alteration of color, pH and acidity of raw, blanched and cooked snap beans by prior exposure to 40% CO_2 for 24 hr at 27°C

Product	−a/b		pH		meq/100g	
	Storage atmosphere					
	Air	CO_2	Air	CO_2	Air	CO_2
Raw	0.64	0.64	5.83	5.90	1.85	1.68
Blanched for 3 min at 100°C	0.71	0.73	6.05	6.30	0.86	0.73
Blanched, frozen, and cooked for 10 min in steam	0.53	0.63	6.10	6.40	0.79	0.60
LSD 5%	0.06		0.16		0.11	

Table 4—CO_2 levels in juice of raw, blanched and cooked snap beans held in ambient and 40% CO_2 atmospheres for 24 hr at 27°C

Product	CO_2 conc meq/L of juice		F
	Storage atmosphere		
	Air	CO_2	1% level
Raw	0.66	1.40	**
Blanched for 3 min at 100°C	0.48	1.05	**
Blanched, frozen and cooked for 10 min in steam	0	0.12	**

results are consistent with those of previous investigators that observed the effects of modified atmospheres on fresh market quality of various green vegetables when held for several days at reduced temperatures (Burgheimer et al., 1967; Groeschel et al., 1966; Lebermann et al., 1968; Lipton, 1965; Lipton and Harris, 1974; Lougheed and Dewey, 1967; Wang et al., 1974).

Exposure to biologically active plant tissues to CO_2 has been shown to increase pH and reduce acidity (Fife and Frampton, 1935; Miller, 1936; Thornton, 1933) which results in alterations of color and texture expecially during heating (Lebermann et al., 1968; Lipton and Harris, 1974; Wang et al., 1971). It is well established that pH plays an important role in regulating chlorophyll degradation during cooking (Clydesdale et al., 1971; Sweeney, 1970; Sweeney and Martin, 1961; Van Buren et al., 1964). By increasing the pH of acidic, chlorophyll containing tissues, conversion to pheophytin is retarded, which has led to the recommendation of using buffers for improvement of green color during heating (Clydesdale et al., 1971; Sweeney, 1970). In addition, enhanced pH may cause excessive softening; however, this can be controlled by reducing the time of heating or magnitude of pH increase (Sweeney, 1970; Sweeney and Martin, 1961). From the results presented herein, shear resistance and color alterations were closely associated to CO_2-induced changes in pH and acidity. Regulation of pH and acidity was regulated by the CO_2 conc, time and temperature of exposure or by replacement of CO_2-enriched atmospheres with air.

When exposed to CO_2, changes occurred rapidly, and when CO_2 was replaced by air, the alterations were rapidly reversed. Wager (1974a) has shown that when partial pressures of CO_2 were increased and then decreased surrounding peas, internal gas conc equiliberated in about 3 hr. Malate content declined in the presence of CO_2 and increased when it was removed similar to the rate of change in internal CO_2 contents (Wager, 1974b). Changes in malate levels in snap beans exposed to CO_2 could account for enhanced pH and reduced acidity. Although exposure to CO_2-enriched atmospheres enhanced the CO_2 content dissolved in the juice, it is unknown if its presence directly contributes to enhanced pH and reduced acidity. It appears that it is not important since differences in titratable acidity between air and CO_2-treated snap beans were about the same for each product while substantial differences in dissolved CO_2 levels were observed. Reduction of acidity with blanching and cooking may be accounted for by losses in volatile acids (Lowe, 1955) while reduction in acidity in CO_2-treated products could be attributed to reduced levels of malate.

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PROCESSING FACTORS AFFECTING ACIDIFICATION OF CANNED PIMIENTO PEPPERS

L. F. FLORA and E. K. HEATON

ABSTRACT

Lye-peeled, cored and washed pimiento peppers were separated into three ripeness levels and acidified in a citric acid bath, immersed in three different organic acids, processed at five different temperatures, and evaluated for pH and drained weight at intervals up to 12 months in separate experiments. Pimientos were also acidified at three acid bath concentrations, at three temperatures, for three periods of time and pH was measured at each treatment. Pimientos were also sampled at various points along a commercial processing line and pH and variability were determined at each step. Results showed pimientos at different levels of ripeness exhibited different pH levels after processing when acidified by immersion, whereas pimientos acidified in the jar exhibited no pH differences due to ripeness. Fumaric acid was more effective than citric or malic acids in lowering pH. An equation predicting pimiento pH from acid bath concentration, temperature and duration of dip showed concentration was by far the most important determinant. Processed pimiento pH seemed to decrease slightly as processing time increased. No changes in pH of jar fractions of whole and diced acid bath-acidified pimientos was seen over a 12-month storage period. In-plant studies illustrated higher than normal pH values and high variabilities at intermediate process steps but fairly uniform pH values after processing. A significant negative correlation was observed between pimiento pH and acid bath concentration. Whole pimientos were more sensitive to acid bath variations than diced pimientos.

INTRODUCTION

THE SAFE PROCESSING of pimiento peppers at atmospheric pressure requires acidification to bring pH below 4.6. Published studies have reported the effects of several variables on pimiento acidification and pH. Powers et al. (1950, 1961) reported that as the season progressed, less citric acid was required to lower the pH of pimientos to 4.5. Powers et al. (1950) also reported that direct addition of the acidulant to the pimientos in the container resulted in more uniform pH levels than occurred when pimientos were immersed in an acid bath prior to packing in containers. Consequently, most studies on pimiento acidification have employed acidification in the container. Because the possibility exists that a container may be missed when acidification is done on a per container basis, processors commonly use variations of immersion acidification. Powers et al. (1950) reported effects of type of acidulant, acid strength, and immersion time upon the pH of pimientos in 4-oz tin cans.

Another matter of concern has been the time required for pH in the processed pimientos to reach a state of equilibrium or constancy in the container, and whether minute changes occur during storage. Supran et al. (1966) found that, even after 15 months' storage, the pH of the solids was usually higher than the pH of the liquor in cans of processed pimientos acidified in the container.

In addition to using in-container acidification, the aforementioned studies were performed at a time when pimientos were usually flame-peeled and canned in tin cans. Pimientos are presently lye-peeled and packed mostly in glass jars. Flora et al. (1978) reported effects of several variables on pH and quality of lye-peeled pimientos packed in glass jars and acidified directly in the container.

The purposes of this study were: (1) to reevaluate effects of pimiento ripeness and type of acid on pH when pimientos were acidified by immersion rather than in-jar deposition; (2) to evaluate the relationships of acid bath strength, temperature, and duration of the dip on pimiento pH; (3) to assess the effects of processing temperature and storage time on pimiento pH and equilibration and on drained weight; and (4) to evaluate pH and variability of pimientos at various points in a commercial processing line. The study was designed and conducted to gain a better understanding of some of the factors which may be important in adjusting and controlling pH in canned pimientos.

MATERIALS & METHODS

PIMIENTOS used in the study were commercial field run obtained from a canning line after lye-peeling, washing and coring. Pods were randomly selected and immediately brought to the Food Science pilot plant for the remainder of the processing treatments.

To examine the influence of maturity on acidification requirements, the prepared pods were segregated into three maturity levels based on visual color: deep red (most mature) medium red (intermediate) and orange red (least mature). The pods were blanched in a thermal screw for 2.5 min, acidified by dipping in a 0.75% (w/w) citric acid bath at 63°C for 15 sec, packed by hand (200g/200ml capacity glass jar), exhausted 6 min, capped by hand, and cooked under water at 100°C for 55 min.

For the comparison of the type of organic acid for acidification, prepared and blanched pods that had been previously selected and mixed for uniformity were acidified by dipping for 15 sec in baths containing 0.75% (w/w) citric, malic, or fumaric acid. After acidification they were packed and processed as described earlier.

To estimate the relationships among acid bath concentration, temperature and duration of dip on pH of acidified pimientos, a 3 × 3 × 3 experimental design was implemented which utilized acid bath concentrations of 0.5, 1.5 and 3.0% (w/w) citric acid, at 38°C, 65.5°C and 93°C, and dip times of 15, 30 and 60 sec. The pH was determined on pimientos acidified by each combination of factors. The experiment was repeated for statistical convenience.

To determine whether processing conditions had any effect on final pH, samples consisting of 10 jars each received the following thermal process treatments: (1) 99°C for 55 min; (2) 104.5°C for 24 min; (3) 110°C for 16 min; (4) 115.5°C for 14 min; and (5) 121°C for 13 min.

To ascertain whether small changes occurred in pH of processed acidified pimientos during storage, 30 jars of whole pod and 30 jars of diced (0.5 in.) pimientos were acidified and processed as described above. The pH and drained weights were measured on six jars of whole pod and diced product after storage at 25°C for 0, 3, 6, 9 and 12 months.

In an attempt to better understand the effect, if any, the various processing steps have upon acidification and final pH, samples were taken at several selected points along a commercial pimiento processing line and pH measurements made. The second aspect of this part of the study was a comparison of titratable acidity of the citric acid bath with final pH of processed whole pod and diced pimientos. The readings furnished by the quality control department of a processing plant were taken at 30-min intervals and encompassed a nonconsecutive 7-day period.

pH determinations were made on the liquid, the drained flesh (blended), and the total jar contents (blended) of processed samples

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Table 1—Effect of ripeness and acidification method on processed pimiento pH

Level of ripeness	pH[b] In-jar acidification[a] Liquor	In-jar acidification[a] Blend	Acid bath acidification Liquor	Acid bath acidification Blend
Red	4.53a	4.37a	4.28a	4.29a
Med. red	4.51a	4.36a	4.21b	4.20b
Orange	4.51a	4.41a	4.11c	4.10c

[a] Data taken from Flora et al. (1978).
[b] Values in the same column not followed by the same letter are significantly different at $p \leq 0.05$.

Table 2—Effect of acid bath concentration, temperature and duration of dip on pimiento pH

Factor	Level	Pimiento pH[a]
Acid bath conc, (% w/w)	0.5	4.43c
	1.5	3.94b
	3.0	3.59a
Temperature (°C)	38	4.09b
	65.5	3.94a
	93	3.93a
Time (sec)	15	4.15c
	30	4.01b
	60	3.80a

[a] Values in the column for a given factor not followed by the same letter are significantly different at $p \leq 0.05$.

Table 3—pH and percent drained weights of pimientos processed at five temperatures

Process Temp (°C)	Process Time (min)	pH[a]	% Drained wt[a]
99	55	4.47bc	90c
104.5	24	4.49c	92cd
110	16	4.44b	93d
115.5	14	4.44b	88b
121	13	4.38a	80a

[a] Values in the same column not followed by the same letter are significantly different at $p \leq 0.05$.

using a Beckman Model 3500 digital pH meter standardized to pH 4.0.

Drained weights were estimated from differences between weights of net content and pimientos after draining 2 min on an 8 mesh screen.

Data were subjected to appropriate statistical analyses.

RESULTS & DISCUSSION

THE CURRENT good manufacturing practices established by the FDA specify a pH value of 4.6 as the maximum for acidified canned foods in hermetically sealed containers. The pH values of lye-peeled, washed and cored pimientos was usually around 4.9, with some as low as 4.5 and some as high as 5.3. Therefore pH must be adjusted so that all containers remain below 4.6 throughout processing, marketing, and consumption. Having experienced a considerable amount of variation in pH in the finished product throughout the canning season, pimiento processors have tended to over-acidify to make certain every jar retains a safe pH value. Preliminary experiments established that immersion of the pimientos in a 0.75% (w/w) citric acid bath at 63°C for 15 sec would effectively acidify the pimientos to approximately pH 4.5. Monitoring the acidity of the acid baths in which several batches of pimientos were dipped indicated that at least 10 batches of pimientos could be acidified in the same bath without any appreciable change in acidity or acidification potential when 5 lb (2.27 kg) of pimientos were dipped in 4 gal (15L) of 0.75% citric acid.

The pH of the raw pimientos declined as they ripened (4.67→4.65→4.50), a phenomena observed in an earlier study (Flora et al., 1978). Table 1 also shows that, although pimientos acidified in the jar with constant quantities of acid demonstrated no significant differences in pH related to ripeness level, riper pimientos acidified in an acid bath prior to processing were significantly higher in pH than less ripe pimientos. This suggests that riper pimientos absorb less acid than less ripe pimientos or that the buffering capacity of pimientos decreases as their ripeness or maturity increases.

The pH of the blended jar contents was higher than the pH of the liquor for pimientos acidified in the jar (corresponding to the results of Supran et al., 1966), whereas acid bath-dipped pimientos showed essentially no difference. This suggests that absorption of acid by the pimiento flesh is less effective when acid is deposited in the jar than when the pimientos are dipped in an acid bath prior to processing. Thus, a question arises as to whether acid penetrates the product sufficiently during processing to reduce pH within the individual pieces throughout the container to a level that will control growth of *C. botulinum*.

No differences in percent drained weights were observed due to the level of ripeness.

Pimientos immersed in a fumaric acid bath were acidified to a lower pH ($\bar{x}$ = 4.24) than pimientos acidified in equal strength malic ($\bar{x}$ = 4.45) or citric ($\bar{x}$ = 4.44) acid baths, corroborating the results exhibited when the pimientos were acidified with equal amounts of the acids deposited in the jars (Flora et al., 1978).

Percent drained weight was lower for pimientos acidified in a citric acid bath ($\bar{x}$ = 85%) than for pimientos acidified in fumaric ($\bar{x}$ = 88%) or malic ($\bar{x}$ = 87%) acid baths.

The equation derived to predict pimiento pH from acid bath concentration, temperature and duration of dip was:

$$pH = 5.04 - 0.008\ t - 0.004\ \theta - 0.330\ c,$$

where t = duration of dip, sec; θ = temperature of bath, °C; and c = concentration of acid, % w/w. It is evident from the partial regression coefficients in the equation that the temperature of the acid bath and the duration of the dip are of relative minor importance in determining the pH of the acidified pimientos. The coefficient of determination (R^2) for the regression equation indicated that the three factors together accounted for 86% of the variation in pH, and that concentration alone explained 84% of the variation in pH.

The combinations of acid bath concentration, temperature and dip duration were designed to bring the pH of pimientos to levels near and below the critical pH of 4.6. Any prediction of processed pimiento pH would have to take into account the increase in pH observed during cooking (Flora et al., 1978). The pH values of the pimientos receiving the various treatments are shown in Table 2. Pimiento pH was significantly different at each concentration and at each time. However, pH was not lowered by increasing the temperature of the bath from 65.5°C to 93°C. Since interactions were not significant, it was apparent that bath concentration, temperature and duration of dip each responded independently of the other factors, and that differences associated with each were due to that treatment alone.

Processed pimiento pH seemed to decrease slightly as process time increased, only showing a significantly lower pH for pimientos processed at 121°C (Table 3). The advantage of energy savings and sensory properties preservation offered by processing at atmospheric pressure far offset any small savings in acid used at retort temperature.

Table 3 also indicates that the highest drained weights

Table 4—pH and percent drained weight of processed pimientos over 12-month storage period

Storage time (mo)	pH[a]	% Drained wt[a]
0	4.37a	89.9a
3	4.46c	90.7a
6	4.55d	92.4b
9	4.41b	89.8a
12	4.59e	89.8a

[a] Values in the same column not followed by the same letter are significantly different at $p \leq 0.05$.

Table 5—pH and variability at selected steps in pimiento processing line

	Step	pH	Coeff. of Var.
1	Washed, raw pimientos	4.89d	1.94
2	Peeled, washed whole pods	5.20e	2.27
	(Skins on)	6.48g	8.63
3a	Crushed pieces	4.53d	5.25
3b	Crushed, washed pieces	5.35ef	6.23
4	Cored, washed pods	5.45f	3.93
5	Trimmed, washed whole pods and pieces	4.77d	8.91
6	Blanched pods and pieces	4.86d	7.29
7	Acid-dipped pods and pieces	3.90bc	2.56
8	Pieces to be diced	3.58a	3.08
9	Whole pods in jars, exhausted	3.93bc	3.31
10	Processed whole pods	4.04c	3.12
11	Diced pimientos	3.64a	3.35
12	Diced packed pimientos, exhausted	3.74ab	.58
13	Processed diced pimientos	3.74ab	1.20

occurred in pimientos processed at 99, 104.5 and 110°C and declined relatively sharply for pimientos processed at 115.5 and 121°C.

One factor of interest to government scientists and processors is whether pH changes in various fractions of processed pimientos during warehousing and marketing. The study showed no significant differences in pH occurred between diced and whole pimientos or among jar fractions over the 12-month storage period. However, processed pimiento pH did seem to creep upward during storage (Table 4), with only the measurements at 9 mo making the increase nonconsecutive.

Percent drained weight of the processed pimientos peaked at 6 mo and then dropped off again (Table 4).

The pH and the variability in the samples taken at each step in the processing plant are shown in Table 5. The steps are, for the most part, successive. The pH of the pimientos was raised briefly after lye-peeling and washing (Step 2), particularly in incompletely peeled pods which probably trapped and carried over some lye residue. By step 5, however, after 3 washes, pH was back to normal. Variability was considerably reduced in the pimientos after acidification (Step 7) and pH was about 4 or less. Diced pimientos (Step 13) demonstrated a significantly lower pH than whole pimientos (Step 10) and a significantly lower variance, probably resulting from a maximum amount of mixing in diced pimientos.

Viewing the process in total it is evident that, although lye-peeling raised the pH of pimientos slightly and subsequent steps in the process line exhibited relatively large variances, the finished product showed relatively small variances and seemed not to be affected by prior steps in the process line. Of course these results are valid only for a small given period during the processing season. Differences due to raw product and process line variations related to time were not evaluated here.

However, the data provided by the quality control department of the plant was recorded through changes in raw product loads and through work shift changes over a nonconsecutive 7-day period. A highly significant negative correlation was obtained between pimiento pH and acid bath concentration. The correlation was higher for whole pimientos than diced or sliced pimientos, indicating pH changes in whole pimientos were most sensitive to changes in the acid bath concentrations.

CONCLUSIONS

THOUGH CITRIC ACID is normally used to acidify pimiento peppers during processing, less fumaric acid could be used to acidify the pimientos to the same level. Raw product quality, specifically maturity, influences the acidification potential of pimiento peppers. This factor, in conjunction with concentration of the acid bath used in acidification and with compensation for the increase in pH during cooking, might be used to estimate pH of the final product. Processing temperature has no practical effect on pimiento pH. The pH of processed pimientos appears to increase with storage. It appears that pH equilibration may be more complete in acid bath-acidified pimientos than in pimientos acidified in the jar. Though pH is temporarily raised in early steps of pimiento processing and variability is somewhat high, the final product is quite uniform from a given lot. More effort is probably warranted for identifying the factors inherent in the product itself that cause variations during acidification and processing.

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FORMULATION AND EVALUATION OF A LOW pH EGG SALAD

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ABSTRACT

A low-pH egg salad was formulated as an attempt to control microbial growth and to minimize public health hazards associated with egg salads. Three formulations of salad were developed with different levels of acidity, ranging from pH 4.25–4.30. Commercially prepared hard-cooked eggs and acidic salad dressings were used in the formulation as well as an experimentally prepared soy-based salad dressing. Consumer acceptance was evaluated using a six-member sensory panel composed of persons with prior sensory evaluation experience. The low-pH egg salads were given favorable ratings by the sensory panel, using a hedonic rating scale and triangular testing. After sensory evaluation, the preferred formulation was inoculated with *Salmonella seftenberg* to determine whether or not bacterial inhibition was exerted by the low-pH egg salad. A commercial sample of egg salad also was inoculated and bacterial growth in the two salads was studied for 60 hr, at storage temperatures of 5°C and 22°C. Samples taken from all acidic egg salad samples at 12-hr intervals showed dramatic decreases in *Salmonella* numbers, especially during storage at room temperature (22°C). Shelf life of the product was determined to be in excess of 5 wk under refrigeration at 5°C.

INTRODUCTION

IN RECENT YEARS, sale of ready-to-eat salad type foods in retail outlets has become commonplace, and the market for commercially prepared salads is increasing yearly (Anon. 1969). For many years, egg salad has been notorious for harboring foodborne pathogens, principally *Salmonella* and staphylococci (Geiger and Crowley, 1940; Tanner and Tanner, 1953). Since it is an excellent growth medium with the appropriate water activity and pH (Adame et al., 1960), egg salad can support phenomenal microbial growth when left at room temperature.

Egg salad is a food product that lends itself to gross contamination and unchecked bacterial growth. As a sandwich spread it may be abused bacteriologically in many ways: sandwiches are prepared almost entirely by hand, they are not given subsequent heat treatment, they are usually not refrigerated prior to sale or consumption, and they are carried and left at ambient air temperatures for hours (Longree et al., 1959; Adame et al., 1960; McCroan et al., 1964). Egg salad abused under these conditions may result in growth of foodborne pathogens.

Under normal circumstances, the ingredients used in egg salad formulation initially contain low numbers of bacteria. Oblinger and Angalet (1974) studied the bacteriology of peeled hard-cooked eggs. At 25°C, bacteria developed rapidly and sliminess became evident after 4 days of storage, whereas hard-cooked eggs stored at 5°C showed no significant bacterial development until after 20 days of storage.

Mayonnaise and salad dressings are generally considered bacteriologically resistant products because of their low pH, and progressively lower pH values in salad dressings show increasing capabilities to prevent bacterial growth (Kinter and Mangel, 1953; Wethington and Fabian, 1949). Wethington and Fabian (1949) indicated that mayonnaise and salad dressings are not probable sources of staphylococcus food poisoning because of their high acidity and content of acetic acid; however, although the salad dressing itself is not a good medium, it becomes an excellent medium when combined with ingredients that neutralize the high acidity such as chopped hard cooked eggs.

The effect of pH as a bacterial inhibitor has been studied by Levine and Fellers (1940), Supran (1966); Kinter and Mangel (1953). Most microorganisms grow best near pH 7.0 (6.6–7.5) while few grow below pH 4.0 (Frazier, 1967; Jay, 1970). Townsend et al. (1954) cited pH control as a means of inhibiting growth of *Clostridium botulinum* and reducing the thermal process necessary to prevent spoilage. In two studies on dry sausage (Smith et al., 1975; Geofert and Chung, 1970) a positive correlation between the decline in the pH of dry sausage products during fermentation and the decrease in the number of *Salmonella* organisms was shown.

This research was done to formulate a low-pH egg salad organoleptically acceptable to the consumer that would successfully inhibit the growth of food poisoning organisms, especially *Salmonella*, at room temperature. The experimentation involved formulation of the product, sensory evaluation and bacteriological examination.

Table 1–Source and pH Measurement of Ingredients Used in Egg Salad Formulation.

Ingredient	pH
Chopped Egg (Tip-E-Pak, Inc. Burkett, IN)	5.60
Chopped Egg (fresh)[a]	8.50
Kraft Real Mayonnaise (Kraft Foods, Chicago, IL)	4.20
T.H.S. Dressing (CFS Continental, Inc., Chicago, IL)	2.70
Sara Lee Mayonnaise (Sara Lee Salad Co., Cleveland, OH)	2.85
Soy-base Salad Dressing	3.75
Sweet Pickle Relish (Vlasic Foods, Inc., Lathrop, MI)	3.20
Minced Onions (rehydrated) (Griffith Laboratories, Chicago, IL)	6.20
Corn Syrup, Karo light (Best Foods, Englewood Cliffs, NJ)	5.75
Mustard (yellow) (Plochman, Inc., Chicago, IL)	4.00

[a] Products without a source were prepared in the laboratory.

MATERIALS & METHODS

Formulation

The normal ingredients of an egg salad include diced hard cooked eggs, salad dressing, pickle relish, and mustard. In order to produce salads with low pH the source of ingredients was varied as indicated in Table 1. Fresh eggs were held for 5 days at 18°C before being hard cooked. The cooked eggs were peeled by hand and chopped in a food chopper with a 60 mm opening face plate. The soy-base salad dressing was prepared by placing 100 ml of water in a blender, adding 3g of Supro- 620 and blending for 60 sec. Then 3g of 8% lactic acid were added and the mixture was blended for 20 sec. During this blending 20g of soy oil were added to seed the emulsion and mixed for an additional 10 sec. The balance of the oil, 280g, then was added slowly in a small stream with the Osterizer blender model No. 848-34B set on speed 3 of 8 speeds.

Four egg salads were prepared using the formulae listed in Table

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Table 2—Ingredients and pH of each egg salad formulation

	Formula number			
	I (g)	II (g)	III (g)	IV (g)
Chopped fresh egg	800	–	–	–
Chopped acidified egg	–	800	800	800
Sweet pickle relish	200	200	200	200
Mayonnaise Kraft	200	–	–	–
Salad dressing, T.H.S.	–	200	–	–
Salad dressing, Sara Lee	–	–	200	–
Salad dressing, Soy base	–	–	–	200
Mustard	50	50	50	50
Dehydrated onions[a]	–	75	75	75
Corn syrup	–	50	–	–
pH	5.00	4.28	4.25	4.30

[a] Dry onion powder and water, 1:2 ratio.

Table 3—Comparison of cell means for sensory panel hedonic scale scores[a]

	Formula			
	I	II	III	IV
Flavor**	5.556a	7.833b	7.111b	5.500a
Appearance	7.833a	8.833b	7.722a	7.167a
Texture	7.944ab	8.833b	7.500a	7.500a
Odor	7.056a	8.333a	7.333a	6.889a
Color	7.833a	8.944b	7.500a	7.500a
Sweetness	5.000a	7.222b	6.111ab	5.611a
Overall	6.389a	8.111b	6.889a	6.167a

[a] Highest cell mean of 4 Formulations.
** Means with different letters are significantly different at $P < 0.01$.

2. The ingredients were blended thoroughly to assure coverage of all egg particles with salad dressing. Formula I was used as the control product.

Sensory evaluation

Six-member panels consisting of three males and three females with prior sensory evaluation experience were used to evaluate the formulations of egg salad. Each panelist was provided a private booth with modified lighting and controlled temperature, humidity and air flow. One quarter slice of white bread, including the crust, was spread with egg salad. Each sample was coded using a randomly selected three-digit number. Due to the highly tart flavor of the egg salad, water at room temperature was served to panel members between samples to help rinse the palate and to increase the discrimination of the panelists.

Two forms of testing were used in the study, a hedonic rating scale and a forced choice triangle test. A ten-point hedonic scale (with one representing disgusting and ten representing excellent) was used to independently evaluate each of seven factors: flavor, appearance, texture, odor, color, sweetness and overall acceptability. Eighteen observations per treatment (formulation) were obtained for each of the characteristics judged, with one hedonic evaluation of each of the four formulations on each of 3 days of testing. A series of triangle tests also were conducted to determine the degree of difference between the four formulations.

A total of six triangle tests, pairing each of the four formulations in all possible combinations, were conducted in duplicate, allowing 12 responses for each triangle test. Data from the hedonic rating were analyzed using an analysis of variance for factorial design (Purdue University Computing Center, BMD-8V). Invidivual analyses were conducted on each of the seven quality characteristics being measured (flavor, appearance, texture, odor, color, sweetness and overall acceptability) using a $2 \times 4 \times 6$ factorial design, with replications, treatments and panelists as variables. The Newman-Keuls test (Snedecor and Cochran, 1967) was done on the means when analysis of variance indicated significant differences among the means. Results from the triangle tests were analyzed using a chi-square test and calculated tables from the Manual on Sensory Testing (ASTM, 1968).

Bacteriological methods

The bacteriological methods used in these experiments were taken from publications of the National Research Council (1971) and the Food and Drug Administration (1972).

After sensory evaluation, the egg salad formulation most preferred was evaluated for its capacity to inhibit bacterial growth. Formula II (Table 2) was used in the study and was compared to a commercial egg salad obtained from a local delicatessen. The two egg salads (acid and commercial) were inoculated with *Salmonella seftenberg*, and the microbial count of salmonella organisms was monitored for 60 hr.

A fresh pure culture of *Salmonella seftenberg* (ATCC 8400) was obtained and one *standard loopful* was inoculated into a 99 ml blank of phosphate buffer solution. Parent culture was part of culture collection of Dr. F.J. Babel, and was replated and incubated 24 hr before sample culture was taken to insure an active culture for inoculation. Prior to mixing with the salad samples, the bacterial cultures, as well as the salads, were cooled to 5°C. This was done with the purpose of minimizing the sensitivity of the bacteria to the hostile environment of the acid salad, and thereby obtaining the highest possible initial counts (Holtzapffel and Mossel, 1968). One ml of the solution plus inoculum was added to another 99 ml phosphate buffer blank, and this 100 ml (pH about 7.1) was added to 100g of acidified egg salad and blended for 1 min at the speed setting 8 of an Osterizer blender, model no. 848-34B. The same inoculation scheme was used for 100g sample of commercial egg salad. These two mixtures of salad were each divided in half and two storage temperatures were used, 5 and 22°C. Surface plate counts were made immediately after inoculation and at 12-hr intervals. Salmonella-Shigella Agar (Difco) was used for all plating and plates were incubated at 35°C ± 1°C for 24 and 48 hr. The number of organisms per ml in the initial loopful was determined by serial dilution and plated on Salmonella-Shigella Agar and incubated at 35°C ± 1°C for 24 hr. Uncolored to pale pink, opaque, transparent or translucent colonies were considered as typical *Salmonella* and counts were made of colony forming units per ml (CFU/ml).

The data from the bacteriological study were analyzed using an analysis of variance for factorial design (Purdue University Computing Center, BMD 8-V). A $3 \times 2 \times 2 \times 6$ factorial design was utilized and included these variables: three replications, two treatments (formulations), two temperatures and six time intervals. The Newman-Keuls test was used on the means whenever analysis of variance indicated statistically significant difference among three or more means.

In addition, a shelf-life study was conducted to determine the approximate shelf-life of the product at 5°C.

Freshly cooked eggs were soaked 48 hr using the following:

1 gram citric acid (food grade)
1 gram sodium benzoate (food grade)
2 liters distilled water
72 eggs (hard cooked and peeled)

Egg salad was prepared using Formula II, Table 2, with the substitution of the above prepared hard-cooked eggs, for commercial acidified eggs.

Two hundred grams of the egg salad was stored at 5°C. Total plate counts were made upon the egg salad initially and after each 7 days for a period of 35 days. One-gram samples were plated on Plate Count Agar (Difco) and incubated for 48 hr at 37°C. Plating for specific organisms was not done, as total count was most important. pH was measured every time the sample was plated and pH of egg salad was relatively unchanged during storage period, varying by only 0.02 units.

RESULTS & DISCUSSION

Formulation

Four formulations of egg salad were developed from ingredients listed in Table 1. Three of these were with reduced pH due to type of ingredients and one formulation was a control approximating commercial egg salads available in delicatessens (Table 2). A survey of egg salads sold in Lafayette, IN showed this formula to be a typical deli egg salad.

Table 4—Chi-square analysis of triangle test results comparing egg salad formulations

Triangle combination	No. of judgments	Number correct	Minimum no.[a] required 5%	1%	0.1%	Preference of correct judgments	
I & II	12	6	8	9	10	II – 4	I – 2
I & III	12	8*	8	9	10	III – 6	I – 2
I & IV	12	10***	8	9	10	IV – 10	I – 0
II & III	12	7	8	9	10	II – 4	III – 3
II & IV	12	9**	8	9	10	II – 8	IV – 1
III & IV	12	6	8	9	10	III – 5	IV – 1

[a] Calculated values on number of correct judgments required for significance taken from Table 5, p. 68, of *Manual on Sensory Testing Methods* (ASTM, 1968).

*** $P < 0.01$
** $P < 0.05$
* $P < 0.10$

Table 5—Least-squares analysis of variance with replication as covariate for 60-hr survival study of Salmonella seftenberg in egg salad

Source	D.F.	M.S. (X10^5)	F Value
Treatment (Trt.)	1	135712.81	13.84**
Temperature	1	15207.61	1.55
Time	5	2838.58	0.29
Trt. X Temp	1	11257.51	1.15
Trt. X Time	5	439.41	0.05
Temp X Time	5	906.14	0.09
Covariate (Rep.)	1	63242.26	6.45*
Residual	52	9803.37	

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

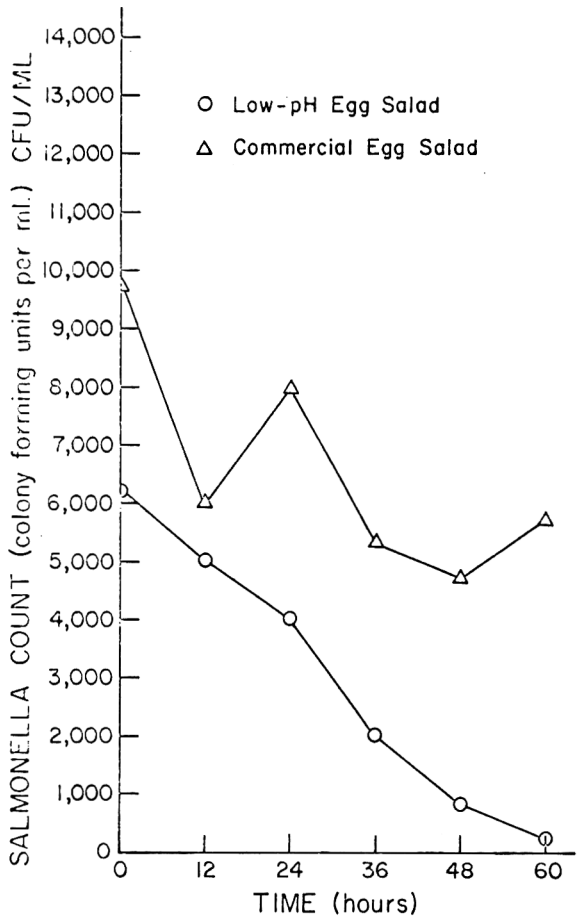

Fig. 1—Changes in Salmonella count during 60-hr survival study at 22°C.

Sensory evaluation

The analysis of variance of the sensory panel hedonic scores showed the only significant effect was the formulation of egg salad used. This factor (formulation) was highly significant ($P < 0.01$) for all quality characteristics evaluated except for odor, where it was still significant ($P < 0.05$). Therefore, it can be concluded that all seven quality characteristics evaluated by the sensory panel using a hedonic scale (flavor, appearance, texture, odor, color, sweetness and overall acceptability) were influenced by variation in formulation. In Table 3, the mean panel scores for each formula are given for each quality characteristic of egg salad evaluated. In all cases, Formula II had the highest mean scores, although they were not significantly different from those of the other formulations in some cases. In the two very important areas of flavor and sweetness, the mean scores for Formula II were significantly ($P < 0.01$) higher than those for Formulae I and IV, and higher, although not significantly different than those for Formula III. When comparing the formulations for overall acceptability, the scores for II were significantly ($P < 0.01$) higher than those for all other formulations.

The results of the triangle test panels are summarized in Table 4. In three combinations of formulations, some significant differences were found. In the triangle test involving formulations I and IV, a highly significant ($P < 0.01$) difference was found. The difference between formulations II and IV also was significant ($P < 0.05$). A slight difference ($P < 0.10$) was indicated between formulations I and III. Formula I had the highest pH (5.0) and was used as a control. When formulae I and IV were compared, all ten correct responses preferred Formula IV over Formula I. Even though Formula IV (soy-based salad dressing) was preferred in this particular test, some comments on score sheets indicated that panelists could detect the soy flavor in the egg salad. The results of the triangular tests indicated that the four formulations appear to be relatively equal organoleptically.

When evaluating the results of both the hedonic scoring and the triangular tests, the low-pH egg salad was as acceptable organoleptically as the egg salad with a higher pH.

Bacteriological

The objective of the bacteriological study was to compare the survival of *Salmonella seftenberg* in a commercial egg salad (pH 4.8–5.1) and in an egg salad (Formula II) of low pH (pH 4.25). The commercial egg salad was obtained from the delicatessen at "Village Pantry" (West Lafayette, IN). *Salmonella seftenberg* was chosen because of its hearty nature, its resistance to heat and other enviromental factors and its known pathogenicity to humans.

Results from the analysis of variance, with replication as covariate, for the 60-hr survival study of *Salmonella seftenberg* in egg salads held at 5° and 22°C are summarized in Table 5. A covariate analysis was used because the inoculation levels varied due to the technique used and were determined simultaneously for each replication by serial dilution. The effect of treatment, either low-pH or commercial egg salad, was significant ($P < 0.01$).

The reduction in numbers of *Salmonella* in the inoculated samples was uniformly greater in the acidic egg salad than in the commercial product. The rate and total of reduction was greater in numbers when salads were held at 22°C than when held at 5°C. Comparative results are shown in Figures 1 and 2.

These results are consistent with the data of Bartolucci

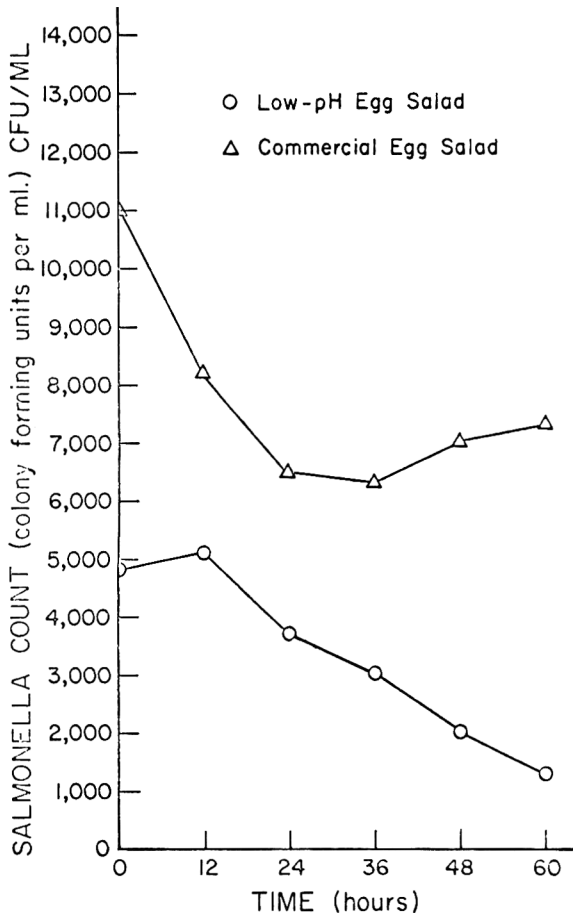

Fig. 2—Changes in salmonella count during 60-hr survival study at 5°C.

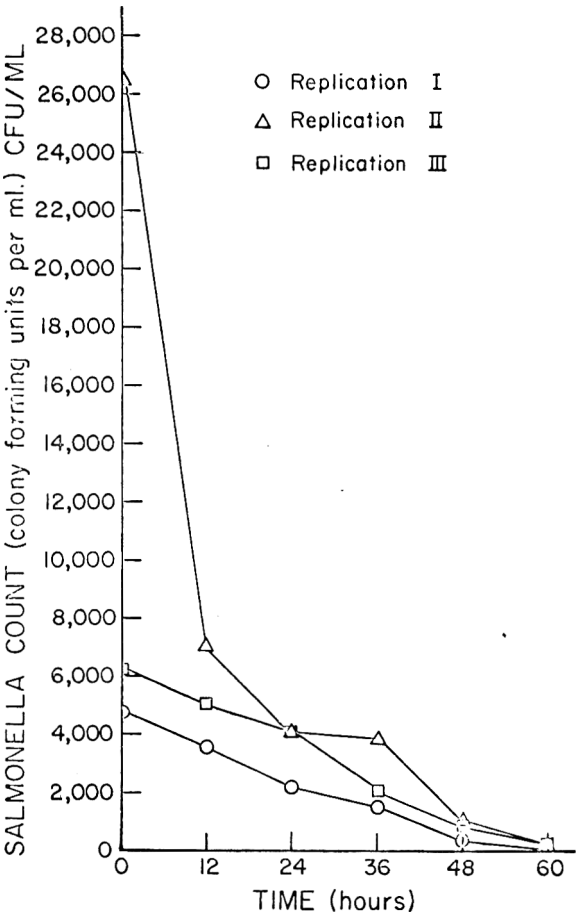

Fig. 3—Changes in Salmonella count during 60-hr survival study at 22°C for low-pH egg salad.

(1975) who reported a decrease in *Salmonella* organisms in a low-pH egg salad during a 24-hr incubation at 25°C and a decrease in the amount of organisms at the end of 48 hr at 25°C. The study concluded that food poisoning organisms such as *Salmonella* spp. can be successfully inhibited in a low-pH egg salad.

The numerical decline in *Salmonella* in acidic egg salads for three replications is shown in Figure 3. Decreases in *Salmonella* counts are shown for all replications of low-pH egg salad held at 22°C. If the reduced pH of the egg salad is sufficient to control *Salmonella* counts even when storage is at ambient temperatures, proper storage at refrigerated temperatures should insure an adequate shelf-life. Since egg salad is bacteriologically abused by storing in picnic baskets and leaving in lunch boxes as well as storage under other unrefrigerated conditions, the bacterial inhibition of low-pH egg salad at ambient temperatures can greatly reduce the public health hazards involved with salads of this type that could be acidified.

The findings of this research are also consistent with studies done by Bryan (1968) and White and Custer (1975) who determined the critical pH range for survival of *Salmonella* organisms to be 4.1 to 4.5. Holtzapffel and Mossel (1968) also reported a decline in *Salmonella* numbers in meat salads of pH's 4.2 and 5.3 held at 20°C. From this study and those of Winter et al. (1953) and Angelotti et al. (1961) the critical pH for *Salmonella* survival in meat and egg salad products lies between 4.0 and 5.5. Luxuriant growth of *Salmonella* in chicken salad was observed at pH 5.6 after 36 hr of storage at 26.7°C.

The results of the 35-day shelf-life study with storage at 5°C, showed that the low-pH egg salad was microbiologically satisfactory when total plate count was considered for up to 5 wk. Initial and 7-day counts were well under 50,000 CFU/ml. After 5 wk of storage at 5°C, total plate counts were under 200,000 CFU/ml.

Reducing the pH is a feasible means of controlling the growth of *Salmonella* in egg salads.

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NATURE OF THE DEPOSIT ON REVERSE OSMOSIS MEMBRANES DURING CONCENTRATION OF PECTIN/CELLULOSE SOLUTIONS

ATSUO WATANABE, SHOJI KIMURA, YOSHIO OHTA, JOHN M. RANDALL and SUSUMU KIMURA

ABSTRACT

Model solutions consisting of varying concentration of the major fouling components of mandarin orange juice, pectin and cellulose, were studied. The deposits were scraped from the membranes and analyzed for weights and concentrations of pectin and cellulose. Suspensions of cellulose with no pectin caused no decrease in permeation flux, but large declines in permeation flux occurred during concentration of solutions of pectin and cellulose. Weights and concentrations of both components in the deposits changed with altering operating conditions and decreased with increasing mean flow velocity. Resistance of the deposit to water permeability (Rg) was related to the concentration of solute at the membrane surface (Cg) by the empirical equation, $Rg = \alpha\ Cg^{1.7}$ (α = constant). Accumulation of deposit on the membrane was in two forms: viscous layer and film layer. The former was a common form of deposit accumulated during the concentration of model solution under all conditions, but the latter was formed only under conditions at higher permeation flux with modelately high mean flow velocity. The film layer was insoluble in water but soluble in 0.1N HCl solution.

INTRODUCTION

THE PREVENTION of permeation flux decrease caused by membrane fouling by solutes in feed is an important subject in the field of membrane technology, and many studies concerning the deposit on the membrane have been carried out. Peri and Dunkley (1971a, b) studied the influence of composition of cottage cheese whey and that of flow conditions, and reported that protein and other macromolecules in whey had a greater influence on performance during reverse osmosis (RO) than smaller solute molecules, and that increasing the flow velocity increased the permeation flux. Lee et al. (1975) and Lee and Merson (1975) studied membrane deposits from whey ultrafiltration (UF) with a scanning electron microscope. Lim et al. (1971) studied the role of protein in the same material, reporting that the major fouling component was casein and that the accumulation was in two forms: a gel-like deposit that resisted removal by fluid shear, and a viscous layer that was readily removed by flushing. However, there are few reports concerning the nature of the deposit from the view point of operating conditions and characteristics of the membrane. Watanabe et al. (1978a) reported that the nature of the deposit from mandarin orange juice on the membrane changed with membrane characteristics and alteriog RO operating conditions. Accumulated deposits were more easily removed from a membrane with higher rejection than from a membrane with lower rejection. Higher feed flow velocity was employed to prevent the concentration polarization and the accumulation of deposit on the membrane, but this feed flow velocity produced a difficult-to-remove deposit on the membrane, while a lower feed flow rate operation produced an easy-to-remove deposit. These results indicated it was necessary to study the nature of the deposit on the membrane with altering operating conditions in order to control the permeation flux decrease by fouling and to restore permeation flux by washing.

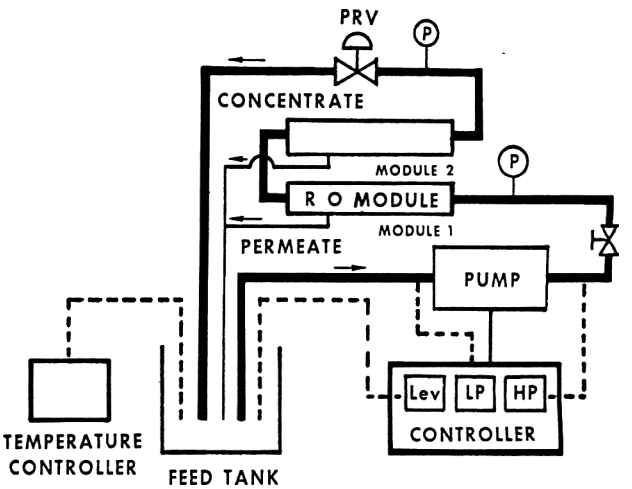

Fig. 1—Flow diagram of reverse osmosis system: P = pressure indicator; PRV = pressure regulating value; Lev = level; LP = low pressure; and HP = high pressure.

Watanabe et al. (1979) reported the main fouling components accumulated during RO concentration of mandarin orange juice were pectin and an insoluble component similar to cellulose, and that pectin is a dominant component producing resistance to water permeability.

The components contained in mandarin orange juice are very complex. Therefore, as a first attempt, pectin/cellulose solutions were used as the simplest model solution, because it is very difficult to estimate the decrease in permeation flux caused by concentration polarization, if the microsolutes, such as sugars and citric acids, are contained in the model solution.

EXPERIMENTAL

Reverse osmosis unit and operating conditions

Four kinds of model solution containing pectin were used: these comprised a solution of either 0.08% or 0.16% pectin (Sunkist Growers, Inc., Pure Citrus Pectin No. 3442, purity more than 90%); solutions with 0.3% cellulose powder (Toyo Roshi Co., Cellulose powder D. 40 mesh) added to each of these solutions; or straight 0.3% cellulose suspension with no pectin, used for examination of adhesiveness of cellulose to the membrane.

The RO unit used in this study was a tubular module (Nitto Denko Co., 1.25 cm i.d., 150 cm length, effective area 581 cm^2). Two different membranes (Nominal NaCl rejections were 80% and 97%, corresponding to modules 1 and 2, respectively, as shown in Fig. 1) were fitted to the RO system in series. The solutions and

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suspensions were recirculated, in order to keep the concentration of solution constant, at 15°C and 50 kg/cm² outlet pressure, at three stages of mean flow velocity: 56 cm/sec, 109 cm/sec and 164 cm/sec. After 90–150 min of operation, when the flux decreased to a steady value, the unit was disassembled, and the solution and/or suspension in the tubular modules were removed with decantation until no droplets came out for 15 sec. Then, the deposits on the membrane which were not removed with decantation, were scraped out by a plunger fitted with a rubber gasket.

Analytical method for pectin and cellulose

Pectin was analyzed by the carbazole colorimetric method of McComb and McCready (1952). Cellulose was analyzed by the gravimetric method with freeze drying: (Weight of cellulose in deposit = weight of freeze-dried deposit − weight of pectin in deposit). Concentrations of pectin and cellulose were demonstrated on a wet basis.

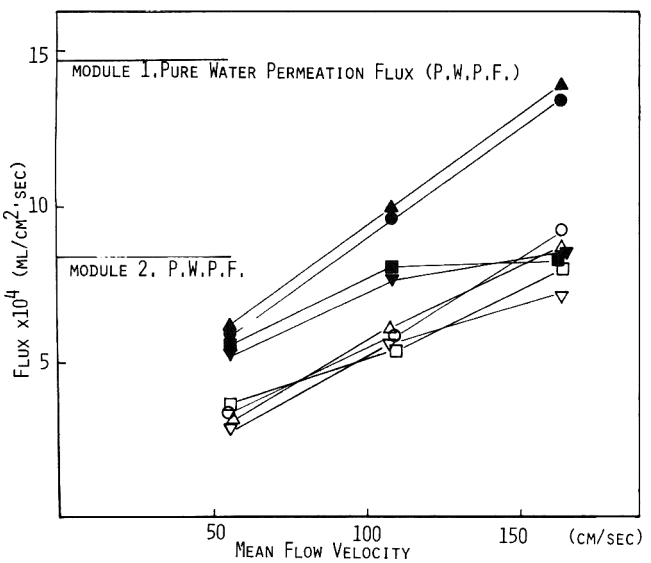

Fig. 2–Influence of mean flow velocity on permeation flux at stationary state: 0.08% pectin, ● Module 1, ■ Module 2, 0.8% pectin + 0.3% cellulose, ▲ Module 1, ▼ Module 2, 0.16% pectin, ○ Module 1, □ Module 2, 0.16% pectin + 0.3% cellulose, △ Module 1, ▽ Module 2.

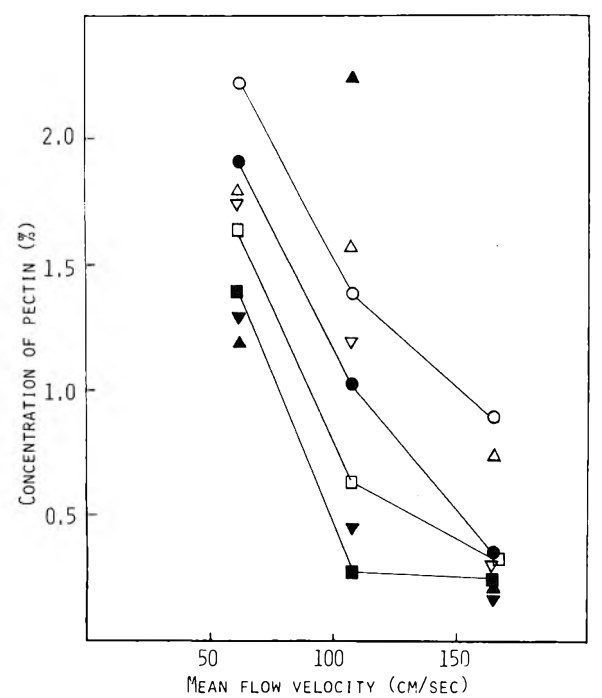

Fig. 3–Influence of mean flow velocity on concentration of pectin in deposit. (Symbols same as Fig. 2)

RESULTS & DISCUSSION

Influence of mean flow velocity on permeation flux

Higher permeation flux was obtained with increasing mean flow velocity as shown in Figure 2, but increasing rate of flux varied with the characteristics of the membrane and the concentration of the solution and/or suspension.

For the higher permeability membrane (module 1), permeation flux increased linearly with the increase of mean flow velocity with all four model solutions. For the lower permeability membrane (module 2), with both solutions with 0.16% pectin, the rate of permeation flux increase with the increase of mean flow velocity was not as high as for module 1, but the flux still increased slowly with higher flow velocity. However, with both solutions containing 0.08% pectin, permeation flux reached an approximate maximum value at 108 cm/sec mean flow velocity and remained constant to the highest velocity used, 154 cm/sec. Concerning the influence of mean flow velocity on permeation flux, Peri and Dunkley (1971b) reported similar results with RO treatment of cottage cheese whey.

Generally, increased permeation flux with the increase of mean flow velocity is attributed to the increase of mass transfer coefficients of solutes because of the decrease in thickness of the boundary layer. Increasing the flow rate decreases concentration polarization and the accumulation of deposits on the membrane. In this study, concentration polarization should be negligible because we used macrosolutes in dilute concentration, so it is assumed that increased permeation flux with higher flow velocity was caused only by decreased accumulation of deposits on the membrane. Therefore, for the looser membrane, we can assume that there is an influence of mean flow velocity at the stage where permeation flux is lower than the pure water permeation flux, because the deposit layer is the determining factor for the permeation flux. However, for the tighter membrane, mean flow velocity had virtually no effect, as the flux showed approximately the same value as the permeation flux of pure water for the 0.08% pectic solution with or without cellulose suspension, because little accumulation of macrosolute occurred on the membrane.

It has been reported that permeation flux was higher during concentration of a suspension which contained suspended solid than during with a solution which does not, because the deposit on the membrane might be scraped away by suspended solids, such as in the case of tomato juice concentration with RO (Uno and Sakaguchi, 1978), UF treatment of bean extract (Timmins, 1972) and spongy ball washing. But in this study permeation flux was independent of the cellulose concentration in the feed suspension, and it was reconfirmed that pectin is a dominant component for resistance to water permeability, as previously reported (Watanabe, 1979). The scraping effect by suspended solids might be demonstrated by using larger scale suspended solids than those used in this study. It is supposed that this effect might be caused by relationships between particle size and thickness of boundary layer. Further research is necessary in this area.

Influence of mean flow velocity on the nature of deposit

Under the condition of accumulation of macrosolute, the solute concentration on the membrane surface has been considered to be constant at Cg (Blatt et al., 1970). It has also been considered that the flux would be expected to vary proportionately with the logarithm of the bulk concentration with a plot of Jv (volume flux through the mem-

brane) vs log Cb (concentration of upstream solute), intercepting the log C axis at a concentration corresponding to Cg, and that differences of permeation flux corresponding to each concentration would be caused by differences in the thickness of the accumulation layer of the deposit. Influences of mean flow velocity on the concentration of pectin in the deposit on the membrane are shown in Figure 3. Concentration of pectin on the membrane was apparently not constant, and decreased with increasing mean flow velocity. Nakao and Kimura (1978) reported that the concentration of gel layer during UF treatment of polyvinylalcohol and ovalbumin was not constant. With 0.08% pectin solution, concentration of pectin on the module 2 membrane was very low at 108 cm/sec mean flow velocity, and did not decrease further with increasing mean flow velocity to 164 cm/sec. However, with 0.16% pectin solution, concentration of pectin on the membrane decreased with the increase of mean flow velocity from 108 cm/sec to 164 cm/sec. This tendency was more apparent with module 1, which had a membrane with higher permeability.

Table 1—Concentration of pectin in viscous and film layer of deposit

	Wt. of deposit (mg/cm^2)	Pectin in the deposit (mg/cm^2)	Conc of pectin (%)
Total	19.41	0.19	0.98
Viscous	14.48	0.06	0.43
Film	4.94	0.13	2.59

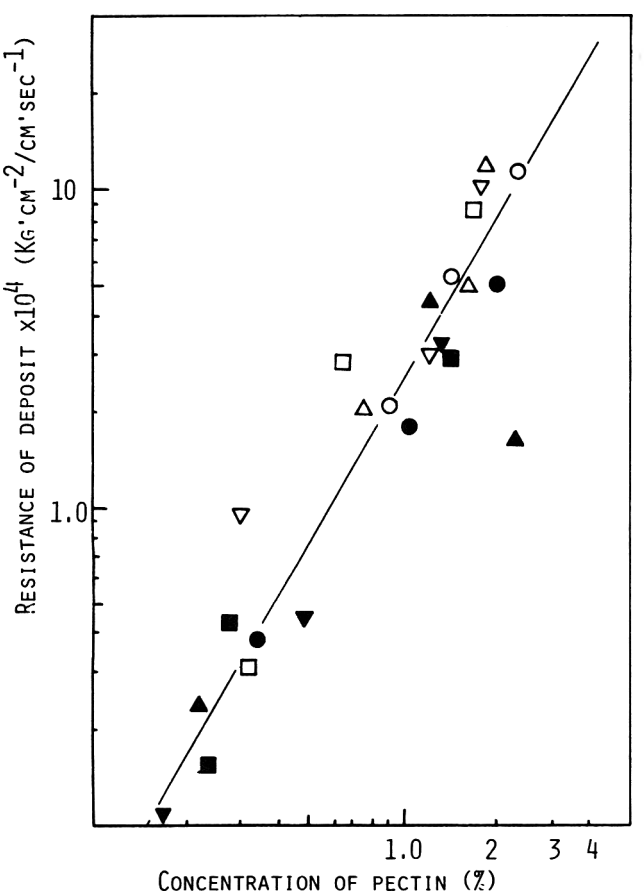

Fig. 4—Relationships between concentration of pectin in deposit and resistance of deposit to water permeability. (Symbols same as Fig. 2)

Influence of mean flow velocity on the weight of deposit on the membrane is not shown in this report, but trends were almost the same as with the relationships between mean flow velocity and concentration of pectin in the deposit, in that weight of deposit generally decreased with increasing mean flow velocity. As little deposit was formed on the tighter membrane (module 2) with 0.08% pectin solution at 108 cm/sec mean flow velocity, there was little further change with the increase of flow velocity to 164 cm/sec. It was concluded that the increase of mean flow velocity caused (1) an increase of permeation flux, (2) a decrease of weight of deposit, which corresponds to a decrease in thickness of deposit, and (3) a decrease of concentration of pectin in the deposit. The accumulation was in two forms: a viscous layer and a film layer. The former was the common form of deposit accumulated during concentration of model solutions, but the latter was formed only at higher permeation flux with moderately high mean flow velocity. The film layer was a rigid film insoluble in water but soluble in 0.1N HCl solution. Pectin concentrations in the deposits are given as the total for both layers in all figures in this report. As an example of how pectin was distributed between layers, weight and concentration of pectin in both layers on the looser membrane (module 1), with operating conditions of 108 cm/sec mean flow velocity and 0.08% pectin solution, are shown in Table 1. In this case, the film layer was separated from the viscous layer by being picked up with tweezers. With the same operating conditions, no film layer was formed on the tighter membrane (module 2). Therefore, it should be considered that permeation flux and concentration of pectin in the deposit plays an important role in the formation of the film layer.

Watanabe et al. (1978) reported that the deposit during operation with a higher feed flow velocity was more difficult to remove on the looser membrane than on the tighter membrane and with a lower feed flow velocity the deposit was easier to remove. Concentration of mandarin orange juice with higher flow velocity operation and higher permeation flux would probably produce a rigid film layer on the membrane. It would be difficult to remove such a rigid film layer from the membrane with shear stress caused by lateral surface flushing.

Relationships between the nature of deposit and resistance of deposit to water permeability

Permeation fluxes can be determined by the flow resistances due to the membrane and fouling layer. Under no fouling conditions, there is only membrane resistance, and permeation flux is expressed as:

$$Jv = Jw = \Delta P/Rm \quad (1)$$

where Jv = volume flux through the membrane; Jw = the permeation flux of pure water; ΔP = transmembrane hydraulic pressure drop; and Rm = resistance of the membrane to water permeation flux.

When the gel layer is formed, it gives resistance to water permeability in series to that of the membrane (Blatt et al., 1970):

$$Jv = \frac{\Delta P - \Delta \pi}{Rm + Rg} \quad (2)$$

where $\Delta \pi$ = transmembrane osmotic pressure drop from bulk solution to permeate; and Rg = resistance attributable to fouling. It can be considered that $\Delta \pi$ is negligible because the model solution, which consisted of the macrosolutes, pectin and cellulose, was in dilute concentration in this study.

The relationship between concentration of pectin in the

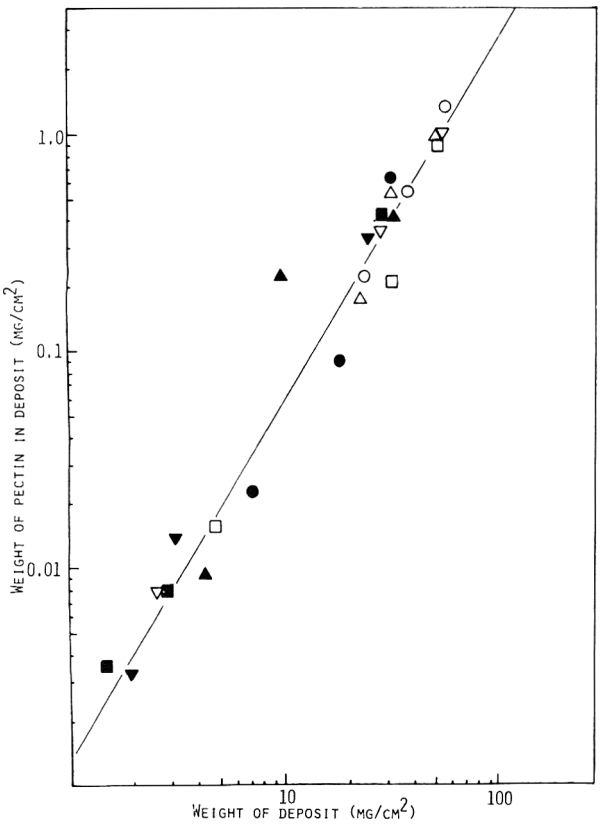

Fig. 5—Relationships between weight of pectin in deposit and weight of deposit on the membrane. (Symbols same as Fig. 2)

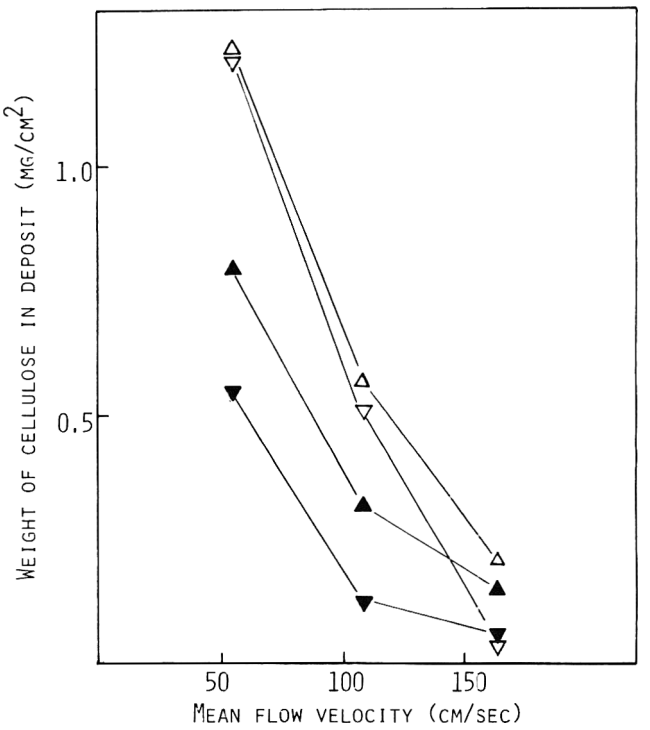

Fig. 6—Influence of mean flow velocity on weight of cellulose in deposit. (Symbols same as Fig. 2)

deposit and resistance of deposit to water permeability (without taking into consideration mean flow velocity and the type of membrane), is shown in Figure 4. Nakao and Kimura (1978) reported that the relationships between concentration of solute in deposit and the resistance of deposit to water permeability with ultrafiltration of polyvinyl alcohol and obalbumin was represented as

$$Rg = \alpha\, Cg^{1.7} \qquad (3)$$

Where α = constant. In this study, the same relationship was shown to be valid for reverse osmosis of pectin solution, with or without added cellulose suspension.

Relationships between weight of deposit and concentration of pectin in deposit

The relationships between weight of pectin in the deposit and total deposit weight, ignoring the mean flow velocity and the type of membrane, is shown in Figure 5. Since the ratio was constant, Cg can be determined from either the total deposit weight or the total pectin weight. Then, the resistance of the deposit to water permeability (Rg) can be calculated from Eq (3), and the permeation flux (Jv) can be calculated from Eq (2), for RO concentration of model solutions of pectin. However, in RO concentration of a natural material such as mandarin orange juice, estimation of permeation flux would be more complicated, for pectin would combine with pulp material. Such a combination would make the accumulation phenomenon of deposit more complicated, and pectin would form a gel on the membrane with the sugars and acids contained in mandarin orange juice.

Characteristics of cellulose in the deposit

The influence of mean flow velocity on the weight of cellulose in the deposit is shown in Figure 6. Weight and concentration of cellulose in the deposit decreased with the increase of mean flow velocity. These trends are the same as those of pectin. However, it could be considered that the mechanisms of accumulation of cellulose and pectin on the membrane are different. Pectin and cellulose are transported to the membrane with solute, which permeates through the membrane. Pectin diffuses back to the bulk solution, but cellulose is mainly returned to the bulk solution by being physically scraped from the membrane area through shear stress generated by the flowing fluid.

Using a straight 0.3% cellulose suspension with no pectin added, permeation flux was not affected by accumulation of cellulose on the membrane. Under the condition of 108 cm/sec mean flow velocity and 50 kg/cm^2 pressure, weight of cellulose in the deposit on module 1 and module 2 membranes were 0.093 mg/cm^2 and 0.018 mg/cm^2, respectively. Weights of cellulose in the deposit with straight cellulose suspension are smaller than those with pectin and cellulose suspension. This fact may mean that cellulose itself is hardly accumulated on the membrane, but that in a cellulose-pectin mixture, cellulose is enveloped by the pectin which is deposited on the membrane.

CONCLUSION

THE SOLUTION concentration on the membrane surface has been considered to be constant at Cg. However, it was apparently changed with altering operating conditions. The characteristics of the deposit was also changed with operating conditions, and the accumulation was in two forms: a viscous layer and a film layer. It is presumed that the film layer is formed when the concentration of deposit and permeation flux becomes higher than a certain value. We are studying the phenomena of formation of two types of deposit in further detail to promote more efficient operation

of RO and better washing efficiency of the membrane for easier permeation flux restoration.

As it may be inevitable to decrease permeation flux by fouling of the membrane with solute, it is a very important subject to restore permeation flux easily by removing the deposit and avoiding formation of a rigid deposit as a film layer. Therefore, the nature of the deposit should be examined with altering operating conditions for each solution to be treated. It is important to select the operating condition for restoring permeation flux easily by removing the deposit with intermittent washing for keeping the total flux high for a long run from the standpoint of energy conservation, because operating conditions with higher flow velocity for preventing the fouling consume much energy. We used pectin and cellulose solution as a simplest model solution of mandarin orange juice. But mandarin orange juice contains sugars and acids, and those solutes might affect gel formation of pectin. Therefore, we are planning to study the nature of the deposit in further detail using solutions containing sugars and acids.

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HYDRATION OF WHOLE SOYBEANS AFFECTS SOLIDS LOSSES AND COOKING QUALITY

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ABSTRACT

Air-dried soybeans absorb water rapidly for the first 2 hr followed by a slower rate of uptake. The beans take up an equal weight of water (100% hydration) after approximately 2.5 hr at 37°C to 5.5 hr at 20°C and reach complete hydration (140% hydration) after 6 hr at 37°C and 16 hr at 20°C. Soluble solids are leached out of the beans at a fairly steady rate throughout the hydration, and the amount is greater with higher temperatures. Temperature is the most important factor in determining the rate of water absorption and of solids losses. Of the total solids lost, 7–16% is protein. The proportion of protein loss increases as the soaking time and temperature increase. About 30–50% of soluble sugars, including fructose, sucrose, raffinose and stachyose, are removed from the beans after overnight soaking at 25°C. On the other hand, the amounts of trypsin inhibitor and hemagglutinin found in the soybean soak are relatively small as compared to that present in the air-dried beans. Hydrating soybeans to 100% prior to cooking reduces the cooking time, increases the tenderness and weight of the cooked beans, and improves their appearance. Complete hydration results in no further improvement in cooking rate or cooking quality of the beans.

INTRODUCTION

THE FIRST AND MOST important step in making traditional soybean foods in the Orient is to thoroughly soak the dry beans. It is a common belief that the soaking process reduces cooking time and improves the quality of the product, although it is not always clear whether the improvement is in the nutritional value, the texture or the flavor of the product. Modern soybean processing technology, on the other hand, employs no soaking so as to reduce processing time and loss. Thus, very few studies have been made on the optimal conditions for soaking and the effect of soaking on soybeans.

Smith et al. (1961) reported that the principal controlling factor in the rate of water absorption by soybeans was the seed coat. They suggested mechanical pretreatment of soybeans to increase the rate of water absorption. However, a recent study on water uptake by soybeans without seed coat (Parrish and Leopold, 1977) indicated that the rate of hydration of soybeans without seed coat was not strikingly different from that of soybeans with intact seed coat. While investigating the effect of soaking beans on the composition and the yield of soybean milk, Lo et al. (1968a) found that soybeans soaked for 24 hr at 1°C lost 5% of their solids, and for 72 hr lost 10%. About 24% of the loss was nitrogenous compounds, of which approximately half was nonprotein nitrogen. The bulk of solids loss was carbohydrates, including raffinose and stachyose, which are causative factors for flatulence. Despite a loss in solids during soaking, soybeans soaked before wet extraction yielded higher milk solids than unsoaked beans and bean flour, because the soaking process made possible a better dispersion and suspension of bean solids during wet extraction (Yan, 1927; Lo et al. 1968b).

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Traditional soybean foods usually are made from whole soybeans, and appearance of beans in some products is important for consumers' acceptance. Thus, mechanical pretreatment of soybeans to improve water absorption or use of processed soybean meals to eliminate soaking does not seem feasible. Furthermore, soybeans, particularly simply processed products, are being used in increasing amounts as human food throughout the world. Therefore, processes that would improve nutritional value and consumer acceptance of the products are warranted.

The present work was undertaken to investigate the conditions for hydration of whole soybeans, the changes that take place during hydration and the effect of hydration on cooking quality.

MATERIALS & METHODS

Hydration

Soybeans of the SRF-200 variety were purchased from a local seed market and stored at 4°C. The beans were hand selected to eliminate the broken ones and those with cracked or otherwise damaged seed coats. All experiments were carried out with the beans in the original dry state containing 6.9% moisture. Twenty-gram portions of sound, whole soybeans were thoroughly rinsed with tap water and then immersed in 100 ml of distilled water for periods of 2, 6, 8, 12, 18 and 24 hr at temperatures of 20, 25, 30 or 37°C. At the end of each period, the beans were removed and rinsed with distilled water. The combined liquid from soaking and rinsing was designated as "soybean soak" and was either freeze-dried or used directly for chemical analyses. The rinsed beans were gently blotted dry and weighed.

Determination of solids and protein

Total solids of the soybean soak were determined by drying aliquots of the soak at 105°C to a constant weight. The soluble proteins were estimated according to the method of Lowry et al. (1951) using albumin (bovine serum) as a standard.

Sugar analysis

Analyses of sugars were carried out by high-pressure liquid chromatography (HPLC) (Conrad and Palmer, 1976). A Waters Associates Model ALC 201 equipped with a differential refractometer was used, and the separation was achieved on a μBONDAPAK/carbohydrate, stainless-steel column (30 cm × 4 mm i.d.) using acetonitrile-water (70:30) as elution solvent.

In determining sugars in the soybean soak, the soak was first freeze-dried and then extracted with hot 80% ethanol. For the analysis of sugars in the beans, the soaked beans were homogenized with 80% ethanol and the extraction was carried out in an 80°C water bath for 30 min with frequent stirring (Black and Bagley, 1978). Unsoaked soybeans were first ground and then extracted with hot 80% ethanol. The filtered extracts were then injected into the HPLC. Once separated, the sugars were identified by comparisons of retention times of peaks with those of known sugars, and their concentrations were calculated based on the peak area. The integration of the peak area was accomplished by computer assistance.

Assays for trypsin-inhibitor and hemagglutinin activities

Trypsin-inhibitor activity was measured with casein as substrate according to the Kunitz method modified by Kakade et al. (1969). The hemagglutinin activity was determined by the conventional procedure of serial twofold dilutions with visual estimation of the end point. The freeze-dried soybean soak or homogenized soybeans were extracted with saline solution. To each concavity of a serological microslide, 100 μl of the serial diluted saline extracts was added to 100 μl of 4% type O, trypsin-treated human red blood cells in phosphate-buffered saline (Gordon et al., 1972). After mixing, the

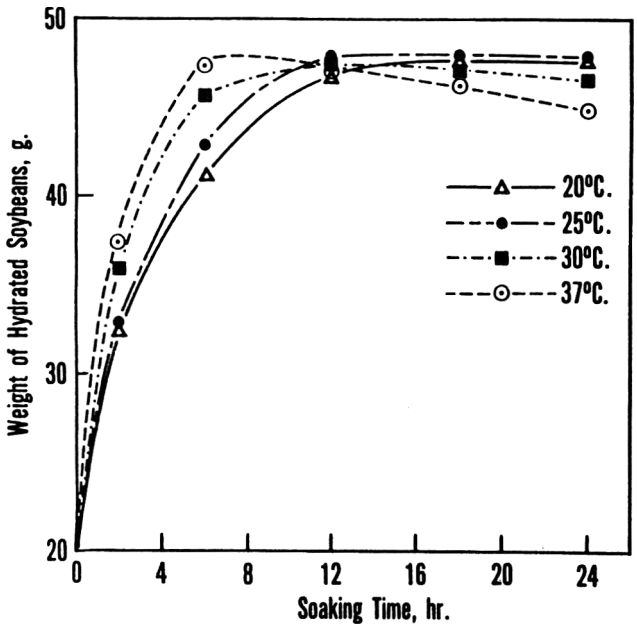

Fig. 1—Rate of water uptake by soybeans with time as affected by temperature.

mixture was allowed to stand for 30 min at 37°C before the end point was determined.

Rate of cooking

Twenty-gram portions of soybeans were soaked, drained and placed in beakers containing 100 ml of boiling water. The beakers were then covered with aluminum foil and placed in a steamer (98°C) for different time periods. After the desired cooking time, the beakers were removed from the steamer and the beans were drained, cooled in ice water and drained again. The tenderness of the cooked beans was measured with an Instron Universal Testing Machine. Bean halves were compressed to 0.13 cm between the upper (mounted beneath the crosshead) and the lower (mounted on the load cell) anvils. The flat, circular anvils were covered with Saran Wrap® for ease of cleaning. Crosshead speed was 2 cm/min and chart speed was 5 cm/min. Load cell sensitivity was set at either 2, 5 or 10 kg, full scale, depending on bean texture. An automatic integrator connected and used in conjunction with the Instron provided a measure of the energy absorbed during the compression test.

All data presented in this report are averages of at least two experiments.

RESULTS & DISCUSSION

Effects of temperature and time on the hydration of soybeans

Figure 1 shows the time course of water absorption by soybeans at different temperatures. The data indicate that the beans absorbed water rapidly for the first 2 hr and then at a declining rate until they became saturated. Also, the rate of water absorption increased as the temperature increased. The soybeans absorbed an equal weight of water (100% hydration) after approximately 5.5 hr steeping at 20°C, 4.5 hr at 25°C, 3 hr at 30°C and 2.5 hr at 37°C. Considering that such other factors as initial moisture content, storage time, size and variety of the beans may also influence the rate of water uptake, our results are comparable to those previously reported, i.e 3–4 hr at 25°C by Smith et al. (1961) and 4 hr at 21°C by Johnson and Snyder (1978). When the weight of the hydrated beans was about 2.4 times that of the original beans or the water uptake was about 140%, complete hydration was approached. The maximum was reached at different times depending on the temperature: 6 hr at 37°C, 12 hr at 30°C and 25°C, 16–18 hr at 20°C. Subsequent losses of weight at 30°C and 37°C could be partly due to the fact that the loss of solids is greater than the water uptake. Compared to other kinds of beans, which generally require 16 hr or longer soaking time at ambient temperature for complete and uniform hydration (Rockland et al., 1970; Quast and da Silva, 1977), soybeans behave similarly.

Losses of solids from soybeans as affected by soaking conditions

The amount of total solids recovered from soybean soaks after soaking beans for various lengths of time at different temperatures is indicated in Table 1. Under the conditions studied, soaking could leach out as much as 10% of the soybean solids. The loss of solids was greater with longer soaking time and higher temperature. Soybeans soaked for 2 hr resulted in a loss of 0.7–1.25% as the temperature increased from 20 to 37°C, whereas soybeans soaked for 24 hr resulted in a loss of 5–10.4%. The rate of the loss was approximately linear for the first 12 hr and then decreased with increased soaking time.

Of the total solids leached out in the soak, approximately 7–16% was Lowry's protein (Table 1). The percentage of protein increased steadily over the first 18 hr of soaking, but it remained fairly constant as the soaking continued to 24 hr. The ratio of protein to total solids was also increased as the temperature increased, suggesting that temperature had a greater effect on the solubility of protein than on that of other solids.

Even though a greater amount of solids, as well as protein, was lost in the soaks when the beans were soaked at a higher temperature, high temperatures facilitated the soaking and made it possible for the beans to reach complete hydration in a much shorter time (6 hr at 37°C vs 12 hr at 25°C in Fig. 1). As shown in Table 1, the amounts of solids and protein leached out during the time period to reach complete saturation at different temperatures were somewhat comparable. However, at lower temperatures (20–25°C), the rate of solids loss beyond the saturation point was much slower than that at high temperatures (30–37°C). Thus, the soaking time became more critical at a higher temperature. To keep soaking losses at a minimum, to avoid extended soaking time and to save energy for cooling and heating, hydration of soybeans at ambient temperature around 25°C would be most suitable.

—Text continued on page 1512

Table 1—Soybean solids and proteins in soybean soak as affected by soaking conditions

Soaking time (hr)	Soaking temp (°C)			
	20	25	30	37
	Total solids g/100g soybeans			
2	0.70	0.70	0.95	1.25
6	1.75	2.10	3.10	4.40
12	3.00	4.40	5.60	7.35
18	4.65	5.00	6.25	9.45
24	5.00	5.20	7.35	10.40
	Lowry's protein g/100g soybeans			
2	0.05	0.06	0.10	0.15
6	0.15	0.24	0.38	0.61
12	0.35	0.62	0.79	1.10
18	0.52	0.71	0.99	1.50
24	0.56	0.75	1.22	1.67
	Lowry's protein g/100g solids in soak			
2	7.10	8.6	10.5	12.0
6	8.6	11.4	12.3	13.9
12	11.7	14.1	14.1	15.0
18	11.2	14.2	15.8	15.9
24	11.2	14.4	16.6	16.1

Table 2—Percentage of soybean sugars remaining in the soaked beans after various periods of soaking at 25° C

Sugars	Soaking time (hr)				
	0	2	6	12	18
	g/100g Original sugars				
Fructose	100	92.3	88.5	76.9	73.1
Sucrose	100	98.5	86.6	74.6	58.2
Raffinose	100	100	91.7	66.7	52.5
Stachyose	100	100	88.2	79.4	67.6

Table 3—Sugars recovered from the soybean soak after various periods of soaking at 25° C

Sugars	Soaking time (hr)			
	2	6	12	18
	g/100g Sugar in original dry soybeans			
Fructose	13.0	25.0	39.8	42.0
Sucrose	1.1	3.1	3.8	4.3
Raffinose	1.0	1.1	1.4	2.8
Stachyose	0.8	1.6	2.4	7.8
	g/100g Dry soybeans			
Glucose	Tr	0.04	0.07	0.14

Table 4—Trypsin inhibitor and hemagglutinating activities of the soybean soak

Soaking time (hr)	Trypsin inhibitor[a]		Hemagglutinin[b]	
	TUI[a]/g bean	TUI/mg protein	HU[b]/g bean	HU/mg protein
2	70	118	2	3.3
6	430	180	6	2.5
12	1280	300	10	2.4
18	2430	339	14	1.9

[a] The trypsin inhibitory activity is expressed in terms of number of trypsin units inhibited (TUI), and 1 unit is defined as an increase of 0.01 in absorbance reading at 280 nm.

[b] One hemagglutinating unit (HU) is defined as the least amount of sample required to produce a positive agglutination under the condition specified.

Effect of soaking on soluble sugars

The SRF-200 soybeans contain 0.46% fructose, 6.81% sucrose, 1.01% raffinose and 3.53% stachyose as determined by HPLC. The amounts of these sugars that remained in the soaked beans and that leached to the soak after various periods of soaking at 25°C are presented in Table 2 and 3, respectively. Partial removal of soybean sugars by soaking, cooking and germinating procedures was reported earlier by Lo et al. (1968a), Hand (1967), Omosaiye et al. (1978), Ku et al. (1976) and East et al. (1972), but it is impracticable to compare the results because conditions for each study were different. Our data indicate that after soaking the soybeans at 25°C for 18 hr, 73.1% of the fructose, 58.2% of the sucrose, 52.5% of the raffinose and 67.6% of the stachyose remained in the beans. The rest was apparently leached out of the soybeans during the soaking process. However, data in Table 3 show that only a small proportion of the decreases in sucrose, raffinose and stachyose could be accounted for in the soak. Similar observations were noted by Hand (1967). The difference, perhaps, can be explained by enzymatic activity that occurred during soaking.

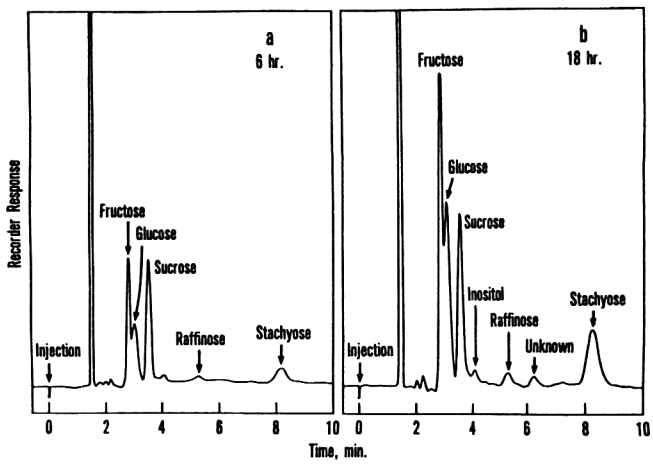

Fig. 2—Chromatograms of sugars in soybean soak after soaking whole soybeans for 6 hr and 18 hr.

As shown in Tables 2 and 3, the total amount of fructose recovered from the soybean soak and the soaked soybeans was greater than that found in the original beans. In addition to increased amounts of fructose, glucose not detected in the original beans also was found in the soybean soak. Although the amounts of fructose and glucose presented in Table 3 may not be precise due to incompletely resolved peaks, the results showed a large increase of these sugars in the soak as compared to sucrose when the soaking time was increased from 6 to 18 hr (Fig. 2). The appearance and increase of the simpler sugars indicate the hydrolysis of higher sugars. When soybeans were soaked in water containing 0.1% tetracycline •HCl to prevent bacterial growth, the same results were observed. On the other hand, when soybeans were first brought to a boil and then allowed to soak, no glucose was detected in the soak and much less fructose was found as compared to that in the unboiled soybean soak. These experiments demonstrated that hydrolysis was due to the endogenous enzymes.

Based on the relative amounts of fructose and glucose in the soak and the absence of galactose, our data did not suggest the complete hydrolysis of raffinose and stachyose. The glucose and increased fructose appeared to be derived mainly from the hydrolysis of sucrose and partial hydrolysis of raffinose and stachyose by invertase.

Effect of soaking on antinutritional factors

Trypsin inhibitor and hemagglutinin are the two well-established antinutritional factors widely distributed among edible legumes. Although they are heat-labile, it has been reported (Honavar et al., 1962) that soaking beans (*Phaseolus vulgaris*) prior to heating was necessary to destroy or inactivate all the toxic effects of *Phaseolus vulgaris*. Therefore, the effect of soaking on these factors was studied.

The trypsin inhibitor and hemagglutinating activities in the soybean soak are shown in Table 4. Both the trypsin inhibitor and hemagglutinin factors were leached out of the beans as a result of soaking; however, the amounts found in the soak were relatively small as compared to that presented in the original beans.

The SRF-200 variety soybeans contain about 38,000 trypsin units inhibited (TUI) per g beans. After soaking the beans for 18 hr at 25°C, 2,430 TUI (6.4%) were leached out to the soak from 1g of beans. But an analysis for trypsin inhibitor activity of the soaked beans showed no significant change after soaking. This, perhaps, indicates the limi-

Table 5—Effect of hydration and cooking time on the weight and tenderness of cooked soybeans

Soaking time (hr)	H_2O uptake (%)	Cooking time (hr)	Weight of cooked beans (g)	Inverse of tenderness TEA[a] (kg-cm)
0	0	1.0	45.4	0.269 ± 0.067[1]
		1.5	46.8	0.169 ± 0.067[2]
		2.0	46.8	0.162 ± 0.052[2]
		2.5	46.6	0.146 ± 0.052[2]
3.5	100	1.0	46.9	0.148 ± 0.049[2]
		1.5	47.5	0.106 ± 0.038[3]
		2.0	47.8	0.094 ± 0.027[3]
18	142	1.0	48.6	0.155 ± 0.049[2]
		1.5	48.6	0.114 ± 0.051[3]
		2.0	48.8	0.089 ± 0.030[3]

[a] TEA, an instron reading, indicates total energy absorbed. Data are means ± S.D. of 50 beans from two experiments. Means without a superscript number in common are significantly different.

tation of methodology for detecting the difference. The specific activity of the leached trypsin inhibitor (expressed as units per mg protein) as shown in Table 4 rose as the soaking progressed, suggesting that other nontrypsin inhibitor proteins leached out faster in the beginning than did trypsin inhibitor protein. Evidently, soaking is not an efficient process for removing trypsin inhibitor from whole soybeans.

Hemagglutinating activity in the soybean soak was also low. After 18 hr of soaking, 14 hemagglutinating units (HU) were found in the soak from 1g of beans that contain 1400 HU. But unlike trypsin inhibitor, the specific hemagglutinating activity in the soybean soak decreased as the soaking progressed. Furthermore, the hemagglutinating activity of the soaked bean was greatly reduced; 25% activity remained in the 18-hr soaked beans. While our data may represent a complex turnover situation, the mechanism is not completely known at present and requires further studies. However, it appears that the hemagglutinin is destroyed or inactivated as a result of soaking and that soaking whole soybeans can greatly reduce the hemagglutinin content.

In 1966, Kakade and Evans found 28% decrease in trypsin inhibitor activity and 75% decrease in hemagglutinating activity after soaking navy beans for 4 days. Recently, Chen et al. (1977) investigated the effect of germination on hemagglutinin content of beans and reported that soybean hemagglutinating activity was reduced to 7.7% after 1 day of germination. Although hydration differs from germination in many respects, hydration is the very first phase of germination.

Effect of degree of hydration on the tenderness of cooked soybeans

As shown in Table 5, the weight and tenderness of the cooked beans were increased by hydration prior to cooking. Unsoaked soybeans required 1.5 hr of cooking to achieve the same degree of tenderness as the soaked beans cooked for 1 hr. Furthermore, longer cooking of the unsoaked beans did not give tenderness approaching that of the soaked and cooked beans. Complete hydration (142% water uptake), on the other hand, did not further increase the tenderness as compared to partially hydrated (100% water uptake) beans. Also, the maize-yellow color of the soaked cooked beans was lighter and brighter than that of the unsoaked cooked beans.

Although very little quantitative information is available on the effect of hydration on cooking rate of the beans, it is a common practice to soak dry beans and peas overnight to facilitate cooking. However, Quast and da Silva (1977) found that soaking black beans prior to cooking did not significantly decrease the required cooking time. Albrecht et al. (1966) reported that high initial moisture in the soybeans was the most important factor favoring rapid cooking, as judged by the low nitrogen solubility index values and the inactivation of urease.

Our data suggest that hydration of soybeans improved the appearance of the cooked beans, reduced the cooking time and increased the weight and tenderness of the cooked beans. However, overnight soaking to complete hydration would not further increase the tenderness of the cooked beans, but would increase the weight.

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NUTRITIONAL AND SENSORY EVALUATION OF BREAD MADE FROM FERMENTED WHEAT MEAL AND CORN CHIPS MADE FROM FERMENTED CORN MEAL

AHMED M. HAMAD and MARION L. FIELDS

ABSTRACT

Fermented wheat meal was significantly higher ($P < 0.01$) in relative nutritive value (% RNV), lysine, isoleucine and riboflavin than the nonfermented wheat meal. Even though there was a decline in % RNV, lysine and isoleucine in bread made from the fermented wheat meal, the % RNV, lysine and isoleucine contents were significantly higher in the bread made with fermented meal than in the control bread. The riboflavin content remained essentially the same in the bread made from fermented wheat meal as in the fermented meal. The loaf volume of bread was less than the control when fermented wheat meal was used. The same trend that was observed in the fermented and nonfermented wheat meal and bread occurred in the fermented and nonfermented corn meal and corn chips. The bread and corn chips were scored acceptable by consumer taste panel.

INTRODUCTION

AROUND THE WORLD, bread is the major food, providing more nutrients than any other food. Pomeranz and Shellenberger (1971) reported that bread consumption varies widely among countries but that it provides 50–75% of the nutritional requirements of the people of most countries of Latin America and several countries of the near and far east.

In many parts of the world, corn or maize constitutes a major item or constituent of the diet of the peoples. Although consumption and popularity of corn chips do not compare with that of bread, they can be introduced into the diets of people in many countries, especially where corn is abundant. In fact, in many developing countries, corn chips could be a way of introducing increased nutrition into the diets of the people.

In previous research, it was shown that wheat and corn meals were improved nutritionally after a natural lactic acid fermentation (Hamad and Fields, 1979). Both the relative nutritive value (% RNV) and limiting amino acids were increased. Fermented wheat meal increased from 73% RNV to 85% RNV and the available lysine and isoleucine increased from 13.9 to 70.6 and 18.8 to 216.3 mg/g N, respectively. The % RNV for corn meal increased from 68 to 80 with the available lysine content increasing from 14.3 to 52.0 mg/g N. Because of these findings, the research reported herein was conducted to determine the acceptability of using fermented wheat meal in bread and fermented corn meal in corn chips.

MATERIALS & METHODS

Bread making

Three types of bread were made, white bread (100% white flour which served as a control bread for volume only), whole wheat bread (50% white flour plus 50% whole wheat flour) and fermented whole wheat bread (50% white flour plus 50% fermented whole wheat flour). The recipe for making bread was that of Lowe (1955), with slight modification. Dry ingredients were placed in the bowl, and were mixed thoroughly. Baking soda was added at the rate of 1% whenever the fermented whole wheat flour was used in the dough in order to adjust the pH to about 5.5 as compared to 5.7 for the control. Dry yeast was weighed (2% W/W flour) into an 80 ml beaker to which 30 ml of lukewarm water (40°C) were added. The mixture was kept at about 35°C for 10 min. The shortening was melted and added to the mix, then the remaining water (100 ml) and the yeast slurry were added. Mixing was done by a Kitchen Aid Mixer (Model K5-A, Hobart Corp., Troy, OH) at speed 2 for 10 min. After mixing, the dough was made into a ball and placed into a greased bowl to produce about 1-lb loaf. The bowl was covered with a damp towel to avoid crust formation on the surface of the dough. Proofing of the dough was done in two stages, 1 hr each at 35°C. Following the first rising, the dough was punched down and worked lightly to allow the gas to escape and to redistribute the gas cells. The dough was allowed to rest for 15 min at room temperature before being shaped to fit the baking pan, which was greased lightly. The loaf was then allowed to rise for 1 hr. The pan was covered with a damp towel. Baking was done in an electric range set at 204°C for 30 min. Loaves were placed on a stainless steel wire holder and allowed to cool to room temperature. After cooling the loaves were placed in plastic bags and kept for sensory evaluation on the second day.

The samples for nutritional evaluation were allowed to dry at room temperature for 2–3 days. Representative samples were passed through a 1 mm screen in a Thomas-Wiley Laboratory Mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA). Samples were stored at 4°C in tightly closed Mason jars until analyzed.

Preparation of corn chips

The corn chips were made from nonfermented meals (control) and from 100% fermented corn meal. Chips were made according to the following recipe: 100g whole corn meal, 4g salt, 60 ml water and 1% baking soda (used with fermented meal). Water was added gradually with continuous working of the dough with a spoon against the sides of the bowl. The dough was shaped into small balls and rolled thinly (1/8-inch thick) with a rolling pin. The dough was divided into strips about 1 × 1 inch and deep fried at 160°C for 1 min. The fried chips were spread on a clean paper towel to absorb the excess oil.

Sample preparation

The chips for sensory evaluation were prepared on the same morning which the consumer panel met. Samples for nutritional evaluation were allowed to dry at room temperature, then were ground with a Kitchen Aid mill (Model K5-A, Hobart Corp., Troy, OH). The ground samples were extracted with ether and alcohol to remove the oil and were allowed to dry at room temperature. The samples were reground in a Wiley Laboratory mill by passing through a 1 mm screen. The finely ground samples were stored in tightly closed Mason jars at 4°C until they were analyzed.

Moisture determination

The finely ground bread and corn chips samples were tested for moisture contents (AOAC, 1975). Moisture content was needed because nitrogen and the results of other assays were computed on a dry weight basis.

Nitrogen determination

The micro-Kjeldahl method (AOAC, 1975) was used to determine the nitrogen content of all test samples.

Relative nutrition value (RNV) and available lysine, isoleucine and tryptophan

A microbiological assay using *Tetrahymena pyriformis* W was used to measure the relative nutritive value (RNV) of both bread and corn chips samples. The method used was that of Stott et al. (1963). Certified casein (Fisher Scientific Co. Fairlawn, NJ) was the

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Table 1—Means[a] percent moisture, percent nitrogen and total crude protein content of bread and corn chips

Bread	% Moisture	% N	Total crude protein %
Bread			
(whole wheat) control	9.15	1.97	11.33
(whole wheat) fermented	9.30	1.98	11.28
Corn Chips			
(nonfermented) control	8.30	1.34	8.37
Fermented	7.50	1.39	8.69

[a] Means of four replications; data calculated on dry basis.

Table 2—Mean[a] RNV, available lysine and isoleucine and riboflavin contents of whole wheat meals (control and fermented) and whole wheat bread

		Available amino acid (mg/g N)		
Product	% RNV[d]	Lysine	Isoleucine	Riboflavin
Whole wheat meal (Control)	72	13.92	18.75	0.14
Whole wheat meal (Fermented)	86[e]	70.62[e]	216.15[e]	0.22[e]
Whole wheat bread[b] (Control)	69	2.60	8.75	0.14
Whole wheat bread[c] (Fermented)	76	21.20[e]	109.00[e]	0.21[e]

[a] Means of four replications, data calculated on dry basis
[b] Made with 50% white flour and 50% whole wheat meal
[c] Made with 50% white flour and 50% whole wheat meal (fermented)
[d] Assayed with *Tetrahymena pyriformis* W
[e] Significant at $P < 0.01$. Values that are significant are read vertically.

reference protein. The percent RNV was calculated according to the formula derived by Helms and Rølle (1970):

$$\%RNV=\frac{\text{Log(count for test protein)}-\text{Log(count for inoculum)}}{\text{Log (count for casein)} - \text{Log (count for inoculum)}}\times 100$$

The microbiological assay method of Difco (1977) was used to determine available lysine and isoleucine in bread and tryptophan in corn chips.

Samples were pre-digested with 1 ml of 2% (W/V) papain solutions for 3 hr at 56°C as described by Ford (1964). Standard curves were prepared for each of the individual amino acids.

Riboflavin content of bread

Since riboflavin was the only vitamin that was increased during fermentation of whole wheat meal, this vitamin was assayed for its retention in bread. The microbiological assay method of Difco (1977) was used to determine the riboflavin content of the samples. Preparation of the test samples for the assay was done according to the method of the Association of Vitamin Chemists (1966). Riboflavin obatined from International Chemical and Nuclear Corporation was used in preparing the standard curve.

Loaf volume studies

Loaf volume is considered one of the major attributes of good-quality bread (Kent, 1975). It is well documented that glutin is the major constituent of the wheat protein that plays a vital role in giving volume to the loaf. Accordingly, it was of great importance to check on the functionality of this protein following fermenting of the whole wheat meal. A comparison was made by measuring the volume of the loaf obtained from whole wheat flour, with that made with fermented whole wheat flour, and that made from 100% white flour.

The rape seed method was used for loaf volume measurement. The loaf was placed in a gallon glass jar and enough rape seeds were added to fill the jar. Then the loaf was removed from the jar, and the volume of the seeds required to fill the jar was considered as the loaf volume.

Sensory evaluation of bread and corn chips

The consumer panels consisted of 60 college students. The time of serving extended from 8:30 a.m. to 4:30 p.m. A pre-recorded tape was played to each group of judges to provide an introduction and instructions for the panel. The bread samples were served first, followed by the chips.

The 5-point preference test was used with consumer panels. The data for each product were analyzed by the paired sample t-test (Snedecor and Cochran, 1956).

RESULTS

Nutritional evaluation of bread and corn chips

Moisture contents. The moisture contents of bread and corn chips samples are shown in Table 1. The moisture contents of the two types of bread were fairly close, while there was about 1% difference between the two types of corn chips.

Percent nitrogen and crude protein. The percent nitrogen and crude protein of bread and corn chips samples are shown in Table 1. There was no noticeable difference in the percentage nitrogen between the two bread samples. This applies also to the two corn chips samples, although the moisture of chips from fermented meal was slightly higher than that of the control.

RNV, available lysine, isoleucine and riboflavin content of whole wheat meals and bread. The RNV of whole wheat fermented bread was 7% higher than the whole wheat control bread (Table 2). The increase was highly significant ($P < 0.01$) as compared to the whole wheat bread containing nonfermented meal. However, the RNVs of bread were lower than those of the flours from which the bread was made with the largest difference occurring with the fermented wheat.

The quantities of lysine and isoleucine retained in the bread in which the fermented meal was included were higher than those of the control (Table 2). The increases in lysine and isoleucine were highly significant ($P < 0.01$) in the bread containing the fermented meal when compared to bread (control) containing nonfermented meal (Table 2). On the other hand, there was a sharp decrease in the lysine content of bread as compared to the flours from which it was made. The lysine dropped from 13.92 mg/g N in the control meal to 2.60 mg/g N in the bread made from that meal, while the fermented meal value dropped from 70.62 mg/g N to 21.20 mg/g N for the bread made from that meal. The same applies to the sharp decrease of isoleucine upon making bread from fermented meals when compared to that of bread made with nonfermented meal. The isoleucine contents of both types of bread dropped to about 50% of the original amounts present in the meals.

Table 2 shows that 100% of the riboflavin content of the meals was retained following bread making. Also, the increase in the content of the bread from fermented meal was highly significant ($P < 0.01$) as compared to bread from nonfermented meal.

RNV, available lysine and tryptophan of corn chips. The RNV of the corn chips made from 100% fermented corn meal (77%) was slightly lower than the RNV (80%) of the fermented meal. However, there was a significant ($P < 0.01$) increase in the RNV of the chips made from fermented corn meal (77%) as compared to the nonfermented meal (69%).

The lysine content of corn chips dropped to about 33% of the original amount present in the meals from which they were made. Control corn chips had 5.0 mg/g N as opposed to 18.0 mg/g N for chips made from fermented meals. The increase in the lysine content of the chips from the fermented meal was highly significant ($P < 0.01$) when compared to that of chips made from control meals.

The tryptophan content of the chips from the corn meal

(control) was as high as that of the meal from which it was made. However, the tryptophan content of chips made from the fermented meal dropped to about 57% of the original content of the meal from which they were made. The increase in tryptophan of the chips made from fermented meal was significantly higher ($P < 0.01$) as compared to tryptophan in control chips.

Loaf volume studies of bread

Bread made from 100% white flour, which was included only as a check on bread volume, had the largest volume followed by control whole wheat bread and then the fermented wheat bread. Although white bread was included in the study as a control, the main comparison was made between whole wheat breads. The loaf volume of whole wheat bread (control) was 1087 ml/200g of flour, as compared to 687 ml/200g flour (fermented.). The volume of the loaf from fermented meal was about 2/3 of that made from nonfermented (control meal). This suggested that fermentation affected severely the gluten of the meal. The 100% white bread had an open texture and large volume whereas the whole wheat fermented bread was slightly compact and had a small volume.

Sensory evaluation of bread and corn chips

The only criterion that was used to evaluate bread or corn chips was the flavor acceptability. The mean score for bread from nonfermented meal was 3.68 as opposed to 3.53 for bread from fermented wheat meal, as judged by 60 panelists. These values are halfway between "like" and "dislike" categories on the hedonic scale. Statistical analysis using paired sample t-test showed no significant difference between the two bread samples due to taste.

The mean score for corn chips made from fermented corn meal and those from nonfermented meals were 3.13 and 2.95, respectively; these means were from 60 observations. Thus, chips made from fermented meal scored slightly higher than the control. However, both scores were in the category of "neither like nor dislike." The paired sample t-test showed that there was no significant difference in taste between the two types. The data for both showed that acceptable products can be made that are more nutritious than their nonfermented counterparts.

DISCUSSION

THE RESULTS of the nutritional evaluation of whole wheat bread made from fermented wheat meal and the chips made from fermented corn meal showed that these products were more nutritious than products made from nonfermented meals. The RNV of the fermented flour dropped from 86 to 76 upon bread making at 204°C for 30 min probably due to the destruction of lysine. The % RNV of the bread made from this flour was significantly ($P < 0.01$) higher than % RNV of bread made from the control flour. The increase in the % RNV of chips from fermented meal, following deep-frying for 1 min was highly significant ($P < 0.01$) as compared to chips from nonfermented corn meal. The retention of the RNV in the products made from fermented meals can be best explained by the higher available lysine, isoleucine and tryptophan contents of these meals over their nonfermented counterparts.

The lysine content of the products dropped sharply during making bread and deep-frying of the chips to about 33% of the original contents of the meals of both wheat and corn. This might be caused by Maillard reaction caused by the lysine interaction with the sugars present in the system. The isoleucine content dropped to about 50% following the baking of both types of bread. The tryptophan content of the chips from fermented meal also dropped sharply following deep frying to about 57% of the original content. Tryptophan was fully retained in chips from nonfermented corn meal. The fact that the amino acids decreased more in chips from fermented meal than they did in the control meals suggest that the amino acids were free in the fermented meals and hence more reactive than the bound amino acids in the control meals. The PER of the flours can be increased by enrichment with some of the essential amino acids such as lysine and isoleucine which are considered limiting in most cereal grains (FAO, 1970). The utilization of proteins of some foods was increased by fortification with L-lysine-HCl (Hegested, 1969; Jansen, 1969). This means that natural fermentation can be used as a cheap alternative for the expensive way of fortification with some of the essential amino acids to improve the nutritive values of cereal grains.

The results of the consumer taste panel on whole bread made from fermented and nonfermented meals indicated that the bread from fermented wheat meal was liked equally as well as the control bread. Hence, an acceptable product can be made from the fermented wheat meal.

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PROTEIN, OIL AND GOSSYPOL CONTENTS OF A VEGETABLE CURD MADE FROM OKRA SEEDS

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ABSTRACT

Seeds of okra were ground by five techniques, extracted with water, and filtered to yield a milk-like emulsion. Proteins and oils were precipitated with six substances at 80°C. Two precipitating agents were tested at five concentrations. The curd was filtered from the whey, washed, pressed, tasted, and then analyzed for oil, protein, and gossypol contents. The highest yield of curd was obtained from seeds soaked 20 hr in water and then ground in a household blender. Six techniques all resulted in satisfactory precipitation of protein, but the best quality curd was obtained by precipitation with lime juice. Variations in concentration of precipitating agent affected protein, oil, and gossypol contents. A higher percentage of the oil of the original sample was recovered in the curd than the percentage recovered of the protein. Gossypol content of the curd was much higher than that of the entire seed. Okra seed curd is an attractive food that can be prepared with home-scale techniques or by large-scale processing.

INTRODUCTION

OKRA, *Abelmoschus esculentus* (L.) Moench, appears to have potential as a high protein crop of the tropics, when grown for its seed (Karakoltsides and Constantinides, 1975). Okra is presently used as a warm season vegetable, the young pods of which are used boiled, in soups, or fried. It is produced throughout the tropics and warmer parts of the temperate zone, and is especially popular in India and West Africa.

The seeds of okra have often been used for various purposes. They have been roasted and ground as a coffee substitute. Edible oil has been extracted from the seeds. The seeds have been ground into meal and used in cooking. These under-documented uses have seldom been the object of scientific attention. Martin and Ruberté (1979) have reviewed such uses and have added still another, the preparation of vegetable curd from the seeds.

Vegetable curd prepared from okra seeds has many advantages as a food. It is easy to prepare, even with household appliances and without the use of electricity. It is attractive in taste and appearance. It is versatile in its uses, and can be substituted for cheese in recipes. Further, it is rich in protein and oil. The production of okra seed and its use in vegetable curd could enrich diets in many parts of the tropics.

Seeds of the Malvaceae, such as cotton seeds, often contain gossypol, a toxic polyphenolic compound. Gossypol irritates the gastrointestinal tract, leads to pulmonary edema of the lungs, paralysis, and in extreme cases, death (Goldblatt, 1969). Okra seeds are known to contain gossypol. An analysis by Karakoltsides and Constantinides (1975) revealed 3.2 mg gossypol per 100g dried seed. This amount is believed to be too low to be toxic. Okra seed meal, presumably including hulls and kernels, was as efficient as casein, a standard test protein, in supporting the growth of rats. Thus, it appears that gossypol levels are not limiting growth in such meal, although other possibly deleterious effects of gossypol were not considered in the above study. Recent analyses of gossypol content reveal varieties with much higher and much lower gossypol contents (Telek and Martin, unpublished). Thus, by selection of varieties it should be possible to eliminate the question of gossypol.

In developing a new food product from okra seeds, it is desirable to measure protein, oil and gossypol contents. In this paper we report levels of these substances as influenced by grinding and extraction techniques, and by the technique of precipitation of the vegetable curd from the extracted seed. We also report some observations of vegetable curd quality.

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METHODS & MATERIALS

THE SEEDS USED for the first two experiments were obtained from a multiple source population based on a world collection. The seeds were sieved to remove small seeds and impurities, washed to remove floating seeds, and dried at 40°C for 24 hr. All test lots weighed 100g. All tests mentioned were performed on three replicated samples.

In the first trials, five different techniques were used to grind or extract seed, as follows:

1. Dried seeds were ground for 1 min in a household blender; 5 volumes of water were added and the mixture was thoroughly stirred.
2. Dried seeds were ground as finely as possible with a hand-operated seed grinder; 5 volumes of water were added, and the mixture was thoroughly stirred.
3. Seeds were soaked in 5 volumes of water for 20 hr, rinsed, and ground for 1 min in a household blender in the same volume of water.
4. Seeds were soaked in 5 volumes of water for 20 hr, rinsed, and ground without water with a hand-operated grain seed grinder. The same volume of water was added and the mixture was thoroughly stirred.
5. Seeds were prepared as described in 4, but the ground mixture in water was soaked for an additional 24 hr.

All of the mixtures were filtered through a cotton cloth. The filter was squeezed by hand to remove excess liquid. The residue was dried at 40°C until weight was stable, and then weighed. The milky liquid was heated to 80°C. Ten grams of commercial epsom salt (magnesium sulphate·$7H_2O$) was then added and the liquid was removed from the heat. After 10 min the liquid was filtered through three layers of cheesecloth. After filtering, water of approximately two volumes of the wet precipitate was poured over the filter twice to remove salts and nonprecipitated substances. The filtered curd in cheesecloth was pressed between two blocks of wood on which a 2-kg weight was placed for 1 hr.

In the second experiment, technique 3 was used to prepare the quantity of "okra milk" needed for all experiments. This milk was well mixed and then separated into 250-ml portions. These were then heated to 80°C and precipitated with varying agents as follows:

1. Hydrated magnesium sulphate (epsom salt), 10g.
2. Magnesium chloride, 10g.
3. Sodium chloride (table salt), 10g.
4. Calcium chloride, 10g.
5. Dilute acetic acid (household vinegar), 20 ml.
6. Fresh lime juice, 20 ml.

The curds were recovered as previously described.

Seeds from the third experiment were of the variety White Velvet. The effect of concentration of two precipitating agents was tested. Duplicate samples of 200 ml of "okra" milk were precipitated with 5 concentrations each of hydrated magnesium sulphate and household vinegar. The pH of the reaction emulsion was mea-

sured. Curds were removed by centrifugation, and washed. The whey from each sample was treated with trichloroacetic acid to precipitate the remaining proteins.

All curds were weighed, tasted, and frozen until analyzed. The whey was observed for clarity. The original seed, "milk," residues and curds, and in the last experiment, the wheys, were tested for protein, oil and gossypol contents.

Dry matter of the frozen samples was determined in a commercial microwave oven at low energy settings. The sample was cut finely and distributed over a glass plate to avoid heating, and dried to constant weight.

Protein was determined on defatted samples. The extracted oils, however, were considered part of the dry weight of the sample. Nitrogen was determined by the micro-Kjeldahl technique (AOAC, 1965). Protein content was calculated as 6.25 × N.

Fatty substances were extracted with N-heptane in a soxhlet apparatus. The solvent was evaporated, and the residue was dried to a constant weight to determine oil content. The oil containing gossypol was determined by the official method of the American Oil Chemists Society (1975).

Table 1—Effects of grinding and extracting techniques on amount of residue, amount of vegetable curd, and amount of sample lost in whey (g/original 100g)

Techniques		Residue not extracted (g)	Wet wt (g)	Dry wt (g)	Amt unaccounted for (g)
Mill	Seed condition				
1 Blender	Dry	60.5	40.7	20.2	19.3
2 Hand	Dry	58.3	44.3	17.8	22.9
3 Blender	Soaked 20 hr	54.4	50.8	23.6	22.0
4 Hand	Soaked 20 hr	62.2	53.2	22.3	14.8
5 Hand	Soaked 20 hr before, 24 hr after grinding	61.8	34.9	14.7	23.5

Table 2—Content of protein, oil and gossypol in vegetable curds made using different grinding techniques, and percent of original oil and protein recovered

Grinding technique	Percent content[a]			Percent recover	
	Protein	Oil	Gossypol	Protein	Oil
1	34.4 b	49.9 ab	0.014 b	27	56
2	43.0 a	40.9 b	0.011 b	29	40
3	35.1 b	50.5 a	0.020 b	32	66
4	33.0 b	52.6 a	0.017 b	28	65
5	39.0 ab	42.0 b	0.039 a		

[a] Differences followed by the same letter are not statistically different (p = 0.05).

Table 3—Relation of precipitating agent to certain characteristics of okra seed curd

Precipitating agent	Apparent texture of curd	Ease of filtering	Color of filtered curd	Taste of the curd[a]
$MgCl_2$	Medium	Average	Dark cream	2
$CaCl_2$	Fine	Difficult	Cream	3
Lime juice	Large	Easy	Light cream	5
Vinegar	Medium	Medium	Cream	4
$MgSO_4 \cdot 7H_2O$	Medium	Medium	Cream	3
NaCl	Fine	Difficult	Dark cream	4

[a] Rated 1–5 in which 5 is best.

Table 4—Dry weight, protein, oil and gossypol contents of okra vegetable curd as influenced by precipitating agent, and as compared to contents of seed and of seed meal residue after milk extraction[a]

Precipitating agent	Dry wt (%)	Protein[b]	Oil[b]	Gossypol[b]
(Seed control)		26.0 c	18.1	0.007 bc
$MgCl_2$	43.6 a	35.8 b	50.8 ab	0.020 a
$CaCl_2$	40.9 ab	37.5 b	48.3 ab	0.020 a
Lime juice	38.2 b	46.7 a	51.2 ab	0.018 a
Vinegar	41.2 ab	42.7 a	51.9 a	0.022 a
$MgSO_4 \cdot 7H_2O$	40.8 ab	45.2 a	50.2 ab	0.014 ab
NaCl	42.1 a	40.8 ab	44.2 b	0.012 bc
(Residue after extraction of milk)		9.64		0.001

[a] Differences followed by the same letter are not statistically different (p = 0.05).

[b] As percent of dry weight

RESULTS

Effects of grinding and extraction techniques

Grinding dry seeds with a household blender obviously produced a finer and more uniform product than grinding by hand. Soaked seeds were more easily ground with the household blender than by hand because the wet meal was resilient, and particles appeared to be larger. The meal soaked for an extra 20 hr fermented, as evidenced by a foul odor and turbid appearance.

Effects of different techniques on extraction of dry matter from the sample can be shown in 2 ways, by dry weight of nonextracted residue, and by dry weight of vegetable curd. Grinding of wet seeds with a household blender (technique 3) resulted in a lighter residue and a heavier vegetable curd (Table 1) than did the other techniques. Although grinding of dry seeds with a hand blender resulted in a low residue, the vegetable curd was lighter than that produced by techniques 1, 3 and 4. The lightest curd, produced in the case of technique 5, was associated with the fermentation of this sample. These results were not analyzed statistically.

The protein and oil contents of the curds, dry weight basis, were impressive (Table 2). Surprisingly, although protein content (26.0) of the seed was higher than oil content (18.1%), the protein content of the vegetable curd was lower than the oil content. Protein recovery ranged from 27–32%. Some of the differences were statistically significant. Protein and oil recoveries were highest in the case of the soaked seeds ground by blender (technique 3), recoveries were also appreciable for soaked seeds ground by hand.

The calculations of recoveries clearly show that technique 5, soaking of seeds after grinding, resulted in a much reduced recovery of oil, and a somewhat reduced recovery of protein (Table 2).

Gossypol content of the curds varied from 0.011% to 0.039%, compared to a gossypol content of 0.007% in the dried seeds. The highest gossypol content was found in the case of the fermented sample (technique 5) and may reflect both more efficient extraction by fermentation and increased concentration due to loss of oil and protein. High gossypol contents in the case of the first 4 techniques were related to efficiency of extraction. The highest gossypol content occurred in the case of technique 3, the most efficient extraction technique, although the differences were not statistically significant.

Effects of different precipitating agents

Heating okra seed milk results in the formation of a fine curd. However, it is difficult to isolate this curd because it rapidly breaks up when filtering is attempted.

The six precipitating agents used all precipitated a light tan curd from okra seed milk, leaving a cloudy or a clear, light yellow whey. The agents differed, however, in apparent texture of the curd precipitated, in ease of filtering,

Table 5—Effects of concentration of two precipitating agents on pH of solution, curd weight and protein, oil and gossypol content of the curd, protein content of whey, and percent recovery of seed protein

Precipitating agent	Amount in 200g milk	pH of soln	Curd wt (g)	Contents of curds (g)			Contents of whey (g)	% of protein and oil of milk recovered as curd	
				Protein	Oil	Gossypol	Protein	Protein	Oil
Hydrated $MgSO_4$	1.25 g	6.6	9.60	4.15	1.71	0.004	0.72	85	33
	2.5 g	6.3	9.74	4.20	2.65	0.008	0.85	82	50
	5 g	5.9	8.91	3.70	3.55	0.013	1.46	73	68
	10 g	5.8	10.02	4.10	4.05	0.015	1.16	80	77
	20 g	5.2	8.82	3.22	3.07	0.010	1.60	67	58
Household vinegar	2.5 ml	5.8	7.36	4.40	2.81	0.018	0.62	87	54
	5 ml	5.4	7.00	4.45	3.24	0.021	0.40	90	62
	10 ml	4.8	7.08	4.82	3.10	0.020	0.17	97	59
	20 ml	4.6	7.84	5.04	3.02	0.021	0.09	98	58
	40 ml	4.2	8.24	5.30	2.83	0.021	0.09	99	54

with respect to the exact color and the taste of the filtered curd, and in appearance of the whey (Table 3). Recovery of curd was not complete in the case of lime juice and vinegar treatments. The whey obtained by filtering was turbid and contained fine protein particles. The mineral salts left yellowish and transparent wheys.

Very fine curds were difficult to filter. Thus, although table salt can be used to precipitate okra seed protein, the very fine precipitate clogs filters and makes home production difficult.

It is important to wash filtered curds on the filter before pressing to extract water because traces of the precipitating agent tend to remain with the curd and flavor the final product. Thus, the flavor imparted by magnesium chloride was particularly disagreeable in all trials. The flavor of curds precipitated with NaCl is pleasantly salty, and with magnesium sulphate neutral and agreeable.

The protein contents of the vegetable curds varied from 29.0–48.77%, dry weight basis (Table 4). The differences in some cases are statistically significant. All differed significantly from the original seed, which had protein content of 26.0%. Thus, protein contents of the curd were about twice that of the seed. The oil contents of about 50%, differed slightly, depending on precipitating agent used. Oil contents of the curds were about 2.5–3 times those of the seed.

Gossypol contents were 2.5–3 times higher in the curd than in the seed. Thus, gossypol content was correlated with oil content. This is not surprising, since gossypol is an oil soluble compound. The differences in content were sometimes significant. Interestingly, table salt as a precipitating agent resulted in the least oil and gossypol in the curd.

The residue of the okra seed after extraction still contained an appreciable quantity of protein (about 10%). The wet or dried residue could be used in the feeding of household animals.

Effects of concentration of precipitating agent

The seed used for this experiment contained 21% protein, 18.2% oil and 0.012% gossypol. The "okra milk" contained 72.0% of this protein, and 74.8% of the oil.

Hydrated magnesium sulphate (epsom salt) was at least as effective at the least concentration (6.25 g/liter milk) as at the highest (Table 5). In fact, unprecipitated protein later recovered in the whey increased as salt concentration increased. Oil and gossypol content of the curd increased with increasing concentration of salt and reached a maximum at about 50 g/liter of salt. The pH of the solution lowered by 6.8–5.2 with increased salt concentration. The protein of the milk recovered in curd ranged from 67–85%, or for the seed protein as a whole, 48–61%. Oil in curd ranged from 33–77%, or for the oil in the seed, 25–56%.

Household vinegar was a more effective protein precipitating agent than magnesium sulphate. Effectiveness increased with concentrations from 12.5 ml/liter to 200 ml/liter as shown by steadily increasing protein contents of the curd and decreased protein in the whey (Table 5). Oil and gossypol in the curd were not much affected by vinegar concentration. The least concentration of vinegar was more effective in precipitating protein than the highest concentration of epsom salt tested. The protein precipitated in curd represented 87–99% that of the milk or 63–71% of the seed protein. The oil precipitated in curd ranged from 54–62% of that in the milk or 40–46% of that present in the original seed.

For small scale or household use, we rated the different precipitating agents as follows: Excellent, lime juice; very good, $MgSO_4$, vinegar; good, $CaCl_2$, NaCl; poor, $MgCl_2$. Lime juice is a common household ingredient in the hot, humid tropics and would often be easier to obtain and less expensive than vinegar or epsom salt.

DISCUSSION

THESE EXPERIMENTS have shown that an attractive high protein, high vegetable oil product can be made at the household level from okra seeds, using simple techniques. The oil and protein contents as well as flavor of the curd can be varied according to precipitating agent used and its concentration. Since okra seeds can be easily produced on the small farm and can be stored in sealed containers for months or even years, okra seed curd could be a new nutritious food for the small farm or the individual household. Techniques can be easily adapted to large-scale production as well.

The gossypol content of the okra seed curd is a potentially serious problem. Practically all gossypol research has concentrated on cottonseed meal, used both as a food and a feed. The U.S. Food and Drug Administration has established a tolerance level of free gossypol in cottonseed meal of not more than 0.045%. Food and Agriculture Organization of the United Nations recommends no more than 0.06% free or 1.2% total gossypol in meal for human use (Pons, 1976). While measurements of gossypol content of okra seed curd are well within this indicated tolerance, a more thorough study of the possible long-term toxicological implications of gossypol should be done with test animals. Recent investigations at our laboratories suggest that gossypol can be avoided by selection of low gossypol varieties.

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ACIDITY OF HALF-SOUR DILL PICKLES

G. M. SAPERS, J. CARRÉ and O. PANASIUK

ABSTRACT

Factors affecting the acidity of half-sour dill pickles prepared by recipes published in cookbooks were investigated. Recipes varied widely in ingredient proportions and procedures. The pH of cucumber-brine homogenates simulating unfermented products exceeded pH 4.6 with 10 of 14 recipes examined. The pH of fermented half-sour pickles was excessively high if products contained insufficient vinegar and were prepared with boiling rather than cool brine.

INTRODUCTION

HALF-SOUR dill pickles, also referred to by such names as new or overnight kosher dill pickles, have gained widespread acceptance in the United States in recent years (Etchells et al., 1976). These products originated as traditional eastern European specialties, and many recipes for their preparation at home have been published.

Because of the relatively low acidity of half-sour dill pickles, with some commercially prepared samples exceeding pH 5.2 (Etchells et al., 1976), the Food and Drug Administration has expressed concern about the safety of this product (Gardner, 1976). Several outbreaks of botulism have been associated with home-made pickles in the United States (Meyer and Eddie, 1965; Mundt et al., 1966). Ito et al. (1976) have demonstrated that *C. botulinum* is capable of growth and toxin formation in cucumber puree adjusted to pH values of 5.0 or higher, but not at pH 4.8 or below.

Because of these indications of potential hazard, we conducted studies to determine whether published recipes for the preparation of half-sour pickles, or inappropriate modifications thereof, might yield high pH products. The results of these studies are reported herein.

MATERIALS & METHODS

PICKLING-TYPE cucumbers were purchased at local produce markets or obtained from Fordhook Farms, The W. Atlee Burpee Co. experiment station in Doylestown, PA (cultivar specified). Fourteen published recipes for half-sour dill pickles, representing typical formulations and procedures, were selected for study. A model system was devised to simulate unfermented products made according to these recipes and to permit the estimation of their equilibrium pH, acidity, and salt content. Cucumber puree, homogenized in a Waring Blendor at high speed for 2 min, was combined with brine containing water, salt and vinegar (50 grain; all vinegar additions described herein refer to this strength) as specified by the recipes. Cucumber-brine combinations were mixed with a magnetic stirrer for 10 min before being analyzed. This model system yielded pH and titratable acidity values similar to those obtained with corresponding unfermented brined cucumbers which had been pasteurized by immersing jars in a boiling water bath for 10 min, cooled and equilibrated at 25°C for 7–12 days prior to analysis.

The effect of using hot or cold brine on acid formation in fermenting half-sour dill pickles was investigated with recipes D and E (Table 1) and two cucumber cultivars: Burpee Pickler and Liberty Hybrid. Brine was prepared by pouring boiling or cool distilled water over the cucumbers and other ingredients contained in quart jars. Jars were loosely closed to permit free gas exchange or tightly closed to obtain a gas-tight seal. Samples were incubated at 21° or 25°C for 3 and 7 days before being analyzed for pH and titratable acidity.

Pickle samples for analysis were homogenized for 2 min at low speed in a stainless steel semi-micro blendor jar. The pH of brine or homogenate samples was measured with an expanded scale pH meter and glass and fiber junction calomel reference electrodes, standardized with pH 4.00 buffer. The titratable acidity of brines and pickle homogenates was determined by titrating a 10-g sample, diluted with 40 ml distilled H_2O, with 0.1N NaOH to a pH 8.1 end point. Sodium chloride was determined as chloride by $AgNO_3$ titration with a chromate indicator (2.109, AOAC, 1970).

RESULTS & DISCUSSION

KEY ELEMENTS of 14 typical recipes for home-made half-sour dill pickles are summarized in Table 1. These recipes vary greatly in specifications for added vinegar (if used at all), added salt, and the proportion of cucumbers to brine (pack-out ratio). The recipes also differ in their approach to certain important steps of the pickling process, i.e., the addition of hot or cool brine to cucumbers, the use of crocks, loosely closed or tightly sealed jars, and the use of fermentation times varying from 2–3 days to 3 wk at room temperature.

Since half-sour dill pickles may be consumed after little or no fermentation, we estimated the pH, acidity and salt content of unfermented products corresponding to the recipes described previously by means of the model system (Table 1). The pH of many of these simulated products, including some containing added vinegar (F, G and J), would be high enough to permit the growth of *C. botulinum*, assuming other conditions to be favorable for growth. Etchells et al. (1976) recommended an acidification level sufficient to lower the product pH to 4.5–4.6 or below for satisfactory preservation during refrigerated storage. Only 4 of the 14 recipes yielded unfermented products having pH values of 4.6 or lower. These corresponded to acidification levels equivalent to 1–2 tablespoon (15–30 ml) of vinegar per quart. Etchells et al. (1976) estimated that 15–20 ml of vinegar per quart would be required for the smaller sizes of pickling cucumbers which are higher in pH, more highly buffered, and can be packed at a higher cucumber-to-brine ratio than larger cucumbers. We have found that the quantity of vinegar required to lower the pH of blended cucumbers to 4.6 in the model system can be as high as 5.3 ml per 100g for some highly buffered cucumber samples. This is equivalent to a level of addition of 28 ml vinegar per quart of brined cucumbers for a pack-out ratio of 60/40 or 33 ml per quart for a pack-out ratio of 70/30, the highest encountered in this study.

The salt content of simulated unfermented half-sour dill pickles varied between 0.7 and 3.1% for the unacidified products which had pH values between 5.5 and 5.7. Roberts and Ingram (1973) reported that at least 5–6% salt would be required to inhibit the growth of *C. botulinum* in this pH range. Data cited by Riemann et al. (1972) suggest that the salt contents of the higher pH acidified products (recipes F, G and J) would probably be sufficient to inhibit the growth of *C. botulinum*.

Since the acidity of half-sour pickles depends in part on

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Table 1—Published recipes for half-sour dill pickles

Recipe	Reference	Proportions (parts by weight)				Brine addition	Container & tightness of seal	Fermentation time (days)	Composition of unfermented products[c]		
		Cucumber	Water	Vinegar	Salt				pH	Titratable acidity (meq/100g)	NaCl (%)
A	Stechishin (1975)	135	100	0	7.5	Boiling[b]	Jar, tight	2–3	5.5	0.8	3.1[d]
B	Levinson (1965)	155	100	0	4.0	Cool	Crock	3–4	5.6	0.9	1.5[d]
C	Berg and Waldo (1955)	135	100	0	5.0	Cool	Jar, NS[a]	10	5.6	0.8	2.1[d]
D	London and Bishov (1971)	135	100	0	5.0	Cool	Jar, tight	14	5.6	0.8	2.1[d]
E	Woman's Day (1966)	135	100	0	1.6	Cool	Jar, tight	14	5.7	0.8	0.7[d]
F	Bar-David (1973)	138	100	2.1[b]	8.4	Cool	Jar, NS	"few"	5.0	1.4	4.0
G	Froud (1972)	220	100	3.0[b]	9.0	Cool	Jar, NS	7–21	5.0	1.6	3.3
H	Edlin and Spector (1964)	216	100	8.4	5.6	Hot	Crock	NS	4.5	2.9	2.2
I	Lesem (1975)	94.5	100	7.1	6.4	Boiling	Jar, loose	NS	4.1	3.4	3.9
J	Grossinger (1958)	194	100	1.6	8.4	Cool	Crock or jar	5	5.2	1.4	3.4
K	Leonard (1951)	216	100	3.7	8.4	Boiling	Jar, loose	8–10	4.7	2.3	3.0
L	Leonard (1951)	130	100	3.8	5.0	Boiling	Jar, loose	10	4.6	2.4	2.6
M	Reasoner et al., (1963)	73	100	6.3	8.4	Cool	Crock	14	4.2	3.3	5.0
N	Etchells et al., (1976)	181	100	4.2	5.8	Cool	Jar, NS	Refrigerated	4.7	2.3	2.6

[a] NS = not specified
[b] Optional
[c] Simulated with cucumber puree-brine combination
[d] Calculated from recipe

the fermentation process, we investigated the effects of variations in pickling methods, as described in Table 1, on acid formation during fermentation. We found that products prepared with boiling brine were consistently higher in pH and lower in titratable acidity than were the same products prepared with cool brine (Table 2). Fermenting cucumbers lagged behind the brine in pH and titratable acidity but followed the same trends. We observed the hot brine effect with two cucumber cultivars—Burpee Pickler and Liberty Hybrid, when recipe D was used instead of recipe E, and when samples were fermented at 25°C rather than at 21°C. Pickles prepared with hot brine remained above pH 5.0 even after 1 wk at 21°C. At the higher incubation temperature, the fermentation proceeded more rapidly, pH values falling below 4.6 after 1 wk. Lid tightness had no effect on pH or titratable acidity in these experiments. The addition of boiling water to jars containing cucumbers, salt and other ingredients might be expected to greatly reduce their microbial load, thereby delaying and perhaps modifying the fermentation and inhibiting acid formation in the product. These results suggest that the practice of preparing unacidified half-sour pickles with boiling brine may be unwise since the product pH may be above 4.6 for an extended period of time.

Several recent publications for home picklers have indicated that "...all pickled products should be heat processed" (i.e., home canned) as a "safety measure" (McNair, 1975) or to prevent spoilage (Kendall, 1976). Guthrie and Guthrie (1974) suggest that picklers wishing to home can a pickled product for which no processing time is specified might use 5–15 min in a boiling water bath. Such instructions are inappropriate for half-sour pickles which may exceed pH 4.6 if insufficient vinegar is added and the fermentation is retarded, i.e., by the addition of hot brine or by refrigeration (Etchells et al., 1976). To avoid a potential risk of botulism, cookbooks and other sources of home pickling information should make a sharp distinction between half-sour dill pickles which should never be thermally processed at 100°C and other more acidic pickled products which may be heat processed.

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Table 2—Effect of hot brine on the acidity of fermented half-sour dill pickles[a]

Brine added	Trial	pH				Titratable acidity[b]			
		Brine		Pickle		Brine		Pickle	
		3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Hot	1	5.2	5.2	5.6	5.2	1.7	2.9	1.1	2.0
	2	5.4	5.4	5.6	5.4	1.4	2.8	1.2	1.9
Cool	1	4.2	3.9	5.1	4.3	2.2	5.0	1.7	2.8
	2	4.2	4.1	5.0	4.5	3.3	4.8	1.9	2.9

[a] Prepared with Liberty Hybrid cucumbers according to recipe E and fermented at 21°C in loosely closed jars
[b] Milliequivalents per 100g

AIR CLASSIFICATION OF DEFATTED, GLANDED COTTONSEED FLOURS TO PRODUCE EDIBLE PROTEIN PRODUCT

R. S. KADAN, D. W. FREEMAN, G. M. ZIEGLER JR. and J. J. SPADARO

ABSTRACT

A modified air-classification technique for producing a low-gossypol, edible cottonseed product from defatted glanded cottonseed flour was investigated. Several milling methods, including fixed hammer disintegrating, pin milling, and air-gun pulverizing, were used to prepare flour from defatted flakes. The milled flours were evaluated for particle size distribution prior to air classification. The yields and the proximate composition of the final fractions indicated that both are affected by the method of milling. With a fixed-hammer disintegrator, a low gossypol edible product was produced. The other milling methods ruptured excessive amounts of pigment glands or did not sufficiently comminute the defatted material. The edible fraction yield can be increased if heat is used to lower the free gossypol content of the final product. Heat converted free gossypol into bound gossypol, therefore total gossypol was unchanged.

INTRODUCTION

THE IMPORTANCE of cottonseed proteins for human foods has been recognized for several years (Bressani et al., 1966; Martinez et al., 1971). Worldwide, nearly 3.5 million tons of cottonseed proteins are potentially available from glanded seed, often in countries where there is great need to improve the protein quality of the local diet (Harden, 1975). However, the cottonseed proteins are rarely utilized as a source of edible foods. The presence of pigment glands, which contain toxic gossypol and other pigments, have been a major obstacle in the exploitation of this valuable source of nutritious protein.

Pigment glands are distributed throughout the kernel. They vary in diameter from 50–400μ, the majority being 80–120μ (Boatner, 1948). Moore and Rollins (1961) showed that the center of pigment glands consists of a complex net-like structure, enmeshing globules of gossypol. The globules were found to be less than 1μ in diameter. The pigment glands must therefore be removed intact, if a food grade protein product is to be made from glanded cottonseed. The outer structure of the pigment glands is fairly rigid, thus allowing properly conditioned kernels to be processed without appreciable damage and subsequent ejection of gossypol (Gardner et al., 1976). The differences in density, size, and shape of pigment glands, and of other extraglandular seed material, particularly in solvent media, have been employed in a number of procedures to obtain a gland free, low gossypol protein product (Gardner et al., 1976). However, the inherent technical problems of classification in liquid medium may hinder its commercial applicability.

Air classification to remove pigment glands from solvent-extracted, glanded cottonseed has been investigated by Meinke and Reiser (1962, 1964) and Vix et al. (1972). Although reductions in free gossypol were substantial, free gossypol of the air-classified product without subsequent treatment was not sufficiently low to meet the Food & Drug Administration (1974) standard. Gracza (1959) and Schaller and Lapple (1971) showed that air classification of solids results in a fines fraction and a coarse fraction that have overlapping particle sizes, unlike sieving that produces two fractions with a sharp particle size cut point. The wide range of particle sizes of air classified fractions suggested that successive air classification might remove intact and partially broken pigment glands. This study explores the feasibility of producing an edible cottonseed protein product by modifying the air classification procedure to keep the amount of coarse fractions small. The role of heat to lower the free gossypol of final air classified product, without affecting its nutritional quality has also been studied.

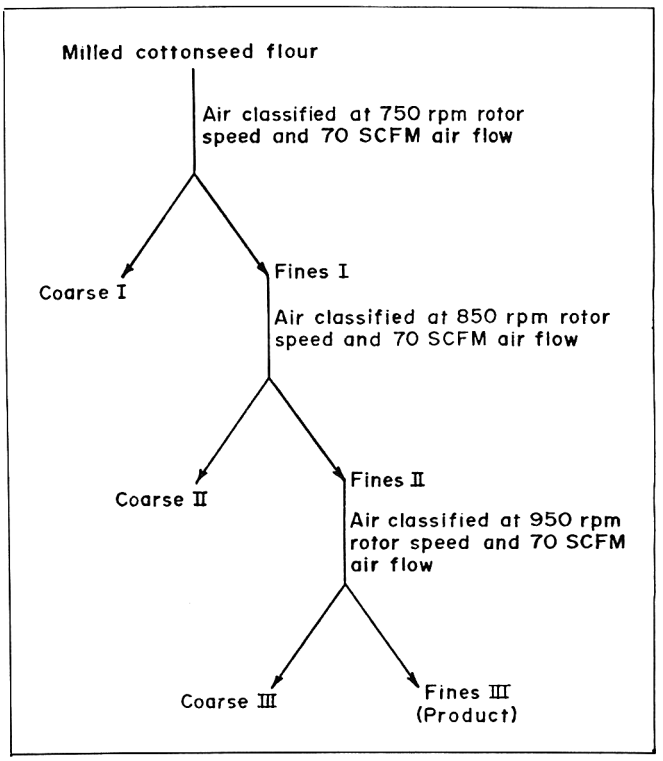

Fig. 1—Flow diagram of air classification steps.

MATERIAL & METHODS

PRIME QUALITY, delintered, dehulled, mill-run quality, consisting of Stoneville and Delta Pines varieties, of glanded cottonseed kernels with 1.1% free fatty acids and free and total gossypol contents of 1.06% and 1.22%, respectively, were used.

Whole and partially broken kernels were flaked to 0.015 in. thickness on smooth Ross Rolls and dried to about 2% moisture in a high-velocity, through-flow type oven at 83°C. The dried flakes were batch extracted in a basket extractor with hexane at ambient temperature and desolventized for 2 hr in a vacuum oven at 83°C. The desolventized flakes were milled by one of the following methods: Rietz mill equipped with either a 0.016 in. or a 0.032 in. sizing screen operated at 10,500 rpm; a Kolloplex mill at 11,500 rpm; a Majac twin-gun air pulverizer at 100 psig air pressure. The Rietz mill is a fixed-hammer disintegrator. Pulverization in this unit is accom-

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Table 1—Comparison of parent, defatted cottonseed flour[a] and its air classified fines fractions

Flour	Moisture (%)	Lipid[b] (%)	Protein[b] N X 6.25 (%)	Gossypol[b] Free (%)	Gossypol[b] Total (%)	Fiber[b] (%)	Ash[b] (%)	Sugar[b] Total (%)	Sugar[b] Reducing (%)	Yield[b] (%)
Starting flour	3.0	4.7	54.6	1.97	2.01	2.40	8.3	7.6	0.10	–
Fines I	7.5	3.8	57.8	0.17	0.24	1.9	9.1	7.8	0.08	57
Fines II	7.6	3.7	58.9	0.09	0.13	1.9	9.2	7.7	0.06	46
Fines III	7.6	3.6	60.4	0.05	0.11	1.5	9.7	7.6	0.06	33

[a] Rietz mill (0.016 in. sizing screen)
[b] On dry basis

Table 2—Effect of milling techniques on air-classified Fines III fractions

Milling equipment	Protein[a] N X 6.25	Gossypol[a] Free	Gossypol[a] Total	Yield[a]
	%	%	%	%
Rietz mill (0.016")	60.4	0.05	0.11	33
Rietz mill (0.032")	62.3	0.03	0.08	17
Majac air pulverizer	62.1	0.13	0.18	28
Kolloplex	61.7	0.08	0.16	27

[a] On dry basis

Table 3—Particle size distribution of defatted cottonseed flours milled by different methods

Milling method	% passed through Alpine air-jet sieves 15μ	25μ	45μ	75μ	106μ
Rietz (0.016")	32	60	69	85	93
Rietz (0.032")	20	30	36	50	61
Majac Air Pulverizer	31	45	47	56	51
Kolloplex	31	53	61	76	84

plished both by the impact of the hammer on the particle and the shearing action of particles against particles. The Kolloplex is a pin mill wherein milling is accomplished by the impact of particles against rotating and fixed studs. In Majac air pulverization, milling is accomplished by the impact of particle against particle in a stream of air. Milled flours were analyzed for particle size distribution on an Alpine AJS-200 Air-Jet Sieve.

The milled flours were air classified in a Donaldson Acu-cut A-12 classifier. A flow diagram of the classification steps is shown in Figure 1. The three rotor speed settings given in the figure were arrived at after preliminary evaluation of the fines fractions by direct microscopic examination for presence of intact and damaged pigment glands. About 5g of fines fraction was spread uniformly in a petri dish and about 1 ml of mineral oil was sprinkled over the sample. The sample thus emersed in mineral oil was examined at 40× magnification by using incident illuminated light. The morphology of the pigment gland is such that one can readily discern the whole glands and fragments of broken glands at the 40× magnification. The presence of broken glands indicate that the gossypol within the gland has dispersed throughout the fraction. This method of microscopic examination was consequently used as preliminary evaluation of the effect of the processing conditions. Final evaluation of the products were based on actual analyses for free and total gossypol of the samples.

A portion of an air-classified product representing a yield of 48% of the starting Rietz milled (0.016 in. screen) flour was heated at 121°C in an open pan in a forced air draft oven for up to 4 hr to determine the effect of heat on free gossypol and available lysine contents. This particular air-classified product had 7.6% moisture, 0.118% free gossypol, and 0.170% total gossypol before heating. Samples were withdrawn at 1-hr intervals for moisture, gossypol, and available lysine assays. Official AOCS methods (1975) were used for proximate analysis and gossypol assays. Available lysine was determined according to the method of Rao et al. (1963).

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION of the starting defatted, glanded cottonseed flour milled by the Rietz mill and of the three air-classified fines fractions are given in Table 1. A comparison of the values indicates that the lipid, gossypol, and fiber contents decreased, whereas moisture, protein, and ash contents increased. Sugar remains relatively unchanged during air classification. The shifts in the proximate composition, with the exception of increased moisture in the fines fractions, agree with the observations of earlier workers (Gracza, 1959; Martinez, 1969). The parent flour used by the earlier workers had a much higher initial moisture (9–12%) than the flour in the present study (3%). Since relative humidity of our laboratory varied between 70–95% during this study, the dry parent flours picked up an additional 5% moisture from the air during classification. However, the additional moisture pickup did not cause any significant coalescence of fines and the material retained its free flowing characteristics during air classification.

There is a significant increase in protein in the fines fractions during classification. The fines fraction had 8.8% more protein (moisture- and lipid-free basis) than the starting parent cottonseed flour (Table 1). Boatner (1948) reported that the gossypol content of pigment glands varied between 20.6–39.0% of the weight, so the reduction of total gossypol from 2.01% to 0.11% should increase the protein contents from 4.87% to 9.22% suggesting that the increase in protein of final product is probably brought about primarily by the removal of pigment glands. The decrease of fiber contents from 2.4% to 1.5% and lipid contents from 4.7% to 3.6% during air classification contributed only slightly to the increase in protein content of fines fraction. Cater et al. (1977) showed that decrease in fiber content of the fines fraction increased the protein contents by 5% over that of the parent glandless flour. Apparently, the starting material used by these workers had a higher fiber content than the flour used in the present study.

The effect of milling techniques on the yields, protein, and gossypol contents of air-classified Fines III samples are given in Table 2. The type of milling clearly affects gossypol contents and yields. Lowest gossypol contents and apparently the least gland damage were from flour milled in the Rietz mill equipped with 0.032 in. screen openings. However, the air classified yield of this sample was very low. Decreasing the screen opening size to 0.016 in. decreased particle sizes (Table 3), thereby increasing slightly both the Fines III yields and gossypol content. Use of the Majac air pulverizer at 100 psig resulted in appreciable increase in gossypol (increased gland breakage), even though the milled flour was still relatively coarse (Table 3). In separate experiments, we found that higher air pressure increased the degree of milling but also increased gland breakage. Decreasing the air pressure lowered the amount of gland breakage, but the resultant flour was too coarse to give acceptable air-classified fines yields. Kolloplex milling

Table 4—Effect of heating air-classified cottonseed flour[a] at 121°C

Heating time (hr)	Moisture (%)	Gossypol		Available lysine (g/16g N)
		Free (%)	Total (%)	
0	7.6	0.118	0.170	3.96
1	2.8	0.079	0.168	3.93
2	2.0	0.061	0.163	3.95
3	1.8	0.058	0.160	3.91
4	1.6	0.045	0.163	3.89

[a] Air classified fines II fraction

resulted in about the same air classified yields as Majac air pulverizing but with apparently less pigment gland breakage.

The particle size distribution of flour is an important criteria of differentiation during air-classification. The weight percentages of milled parent defatted flours passed through Alpine air-jet sieves are shown in Table 3. Flours from the Rietz mill (0.016 in sizing screen) were lower in particle size than from the other milling methods. Kolloplex samples were next in order, followed by the Majac air pulverizer and the Rietz mill (0.032 in. sizing screen). Particle size distribution can be used to predict the potential yield under given classifying conditions. For example, for each of the grinding methods, the amounts of flour that passed through the 15μ Alpine Air-Jet sieve are comparable to the Fines III yields in Table 2.

Some free gossypol can be complexed with cottonseed proteins, i.e., converted into bound gossypol by heat in presence of moisture (Knoepfler et al., 1951). The data in Table 4 show that free gossypol can be significantly reduced by heat at low moisture levels without significantly lowering available lysine or nutritional quality. About 60% of the free gossypol was converted into bound gossypol in 4 hr at 121°C, thereby meeting the U.S. standard. Only 2 hr heating was required to reduce the free gossypol to meet the Protein Advisory Group of United Nations System's (1970) standard.

Identification and optimization of the processing conditions that affect the successful production of low gossypol products by air classification is still needed. Work is in progress at Southern Regional Research Center and studies indicate that existing processing equipment and practices for direct extraction of oils from seeds can be modified to produce suitable defatted flour for air classification.

In summary, air classification of glanded, defatted cottonseed can produce edible cottonseed protein products if there is minimal pigment gland damage during lipid extraction and milling. It is essential to pulverize the defatted material as fine as possible without significant breakage of pigment glands. The defatted flour thus prepared can be fractioned into an edible, low gossypol containing fraction, by multiple air classification steps; wherein in each air classification step, a small coarse fraction is obtained and the fines fraction is re-air classified.

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SEPARATION OF FLAVOR COMPOUNDS FROM LIPIDS IN A MODEL SYSTEM BY MEANS OF MEMBRANE DIALYSIS

K. F. BENKLER and G. A. REINECCIUS

ABSTRACT

The separation of flavor compounds from lipids by membrane dialysis was studied using a model system consisting of 11 flavor compounds and corn oil dissolved in solvent. Several solvent systems and three perfluorosulfonic acid membranes were studied. The most effective solvent system was a mixture of 70% acetone and 30% pentane while the more effective membranes were ones with equivalent weights of 1200 and 1100g and thicknesses of 5 and 10 mils, respectively. Diffusion of the corn oil was less than 0.12% of the oil added. Calculation of permeances showed that diffusion decreased with increased molecular size. The diffusion of 2-methoxypyrazine was hindered by either adsorption on to or reaction with the dialysis membrane. Reaction of the membrane with acetone (solvent) resulted in the formation of two artifacts.

INTRODUCTION

THE ISOLATION of flavor compounds from foods high in fat involves the separation of these volatile compounds from the nonvolatile fraction. The nonvolatile fraction may involve only the fat portion, since the volatile flavors and fat are mutually soluble (Arnold and Barnhart, 1972). The presence of fat in food adds problems to the isolation procedure that are not encountered with aqueous systems. For example, in lipid-free systems, solvent extraction can sometimes be used in lieu of distillation in the isolation of flavor volatiles (Brooks and Reineccius, 1976; Hunter et al., 1961), but solvent extraction cannot be used with fatty foods because lipids as well as flavors will be extracted.

Many other methods have been used for the isolation of flavor volatiles from fatty foods (Teranishi et al., 1971; Weurman, 1969) such as steam distillation (Dulley and Grieve, 1974) and vacuum steam distillation (Chang et al., 1977; Forss and Holloway, 1967). The high temperatures used in steam distillation may cause thermal changes, and the extraction of the distillate with solvent and subsequent evaporation of the solvent used in both steam and vacuum steam distillation can cause losses of the flavor compounds and concentration of impurities present in the solvents (de Bruyn and Schogt, 1961). The method of flushing with an inert gas (Dornseifer et al., 1965; Kramlich and Pearson, 1960) is not useful in the recovery of very volatile or high boiling compounds, and impurities in the inert gas may condense in the cold trap and contaminate the sample (Angelini et al., 1967). Headspace analysis is fast and simple (Merritt et al., 1967; Nawar and Fagerson, 1962), but unless an enrichment technique is used such as the one described by Nawar and Fagerson (1960) in which the headspace is recycled through a cold trap, the method cannot detect many trace compounds (Dupuy et al., 1973).

Solvent extraction has been used occasionally as a first step in the isolation of flavor volatiles, but the method has usually been used on fat-free systems (Brooks and Reineccius, 1976; Hunter et al., 1961; Nelson and Hoff, 1968). The procedure is simple and does not require elaborate equipment as do most distillation procedures. However, some researchers note that while the less volatile compounds can be concentrated, the more volatile ones are lost, and that the solvent can contribute impurities to the sample (Angelini et al., 1967; de Bruyn and Schogt, 1961). Solvent extraction has its merits in that the higher boiling flavor compounds can be isolated when these are of interest, and that acidic, basic, and neutral compounds can be selectively isolated.

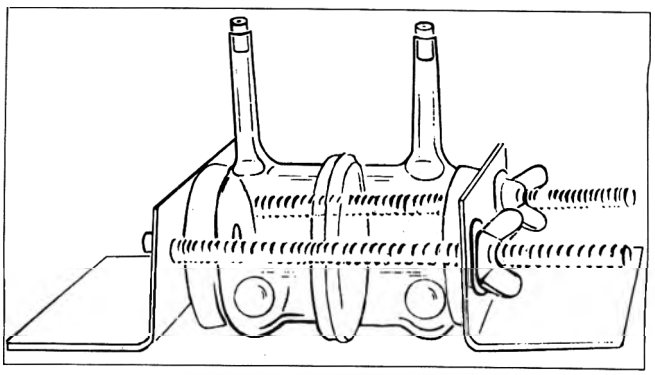

Fig. 1—Dialysis unit.

Dialysis is a separation technique based on molecular size which has had extensive use in the separation of globular proteins from low molecular weight solutes. Since lipids and flavors generally differ in molecular size, it was postulated that dialysis could be used as a separation technique for these also. Most dialysis membranes currently available can be used to separate only very large molecules such as proteins from much smaller molecules, and/or are stable in only aqueous systems. The membranes used in this research were intended for use in ion-exchange applications, but were chosen because of their stability in both water and organic solvents (Grot, 1972).

The present study had several objectives: (1) to determine whether membrane dialysis could be an effective means of separating flavors from lipids, a problem which might occur when solvent extraction is performed on a fatty food; (2) to compare the diffusion rates of flavor compounds across the membrane by the calculation of permeances (the diffusion coefficient divided by the membrane thickness); and (3) to determine the effects of the type of membrane and the variation in solvent on the permeance.

MATERIALS & METHODS

Membrane

Three perfluorosulfonic acid membranes, A, B and C (Nafion, E. I. du Pont deNemours and Co.) were used, each differing in equivalent weight (1200, 1100 and 1200g) and thickness (10, 10 and 5 mil, respectively). The equivalent weight is defined as the weight of the polymer in grams which, when in the acid form, will neutralize one equivalent of base.

Prior to use, each membrane was cut into circles of a diameter

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approximately 1 cm larger than that of the dialysis unit. The membranes were expanded by soaking them in distilled water at room temperature overnight and were patted damp-dry before placement into the unit.

Dialysis unit

A diagram of the unit is shown in Figure 1. It consisted of two heavy-walled glass half-cylinders (i.d. 4 cm), each with a ground-glass edge and an access tube. The membrane was placed between the two half-cylinders and the entire unit was held securely in place by two metal brackets which were screwed together tightly. The glass was cushioned from the metal by #10 rubber stoppers which had been cut to a thickness of approximately 6 mm. The solution containing the flavor compounds was dialyzed against solvent by placing the solution and solvent on opposite sides of the membrane (hereafter designated as the "mixture" and "solvent" sides) through the access tubes by 10-ml syringes until the level of the solution reached approximately halfway up the access tubes. The access tubes were stoppered with cylindrical septa which contained a small hole through which a syringe could be inserted for sample removal. Stirring was accomplished by two Teflon stirring balls which rolled back and forth in the glass depression when the unit was placed on a reciprocating shaker set at 80 oscillations per minute.

Model flavor system

Selection of the compounds used in the model system was based on the complete separation of peaks on the gas chromatograph and the representation of a number of different chemical classes. The system was composed of the following compounds, listed in the order of elution from the gas chromatograph: 1-butanol, 1-hexanal, 2-octanone, 2-methoxypyrazine, n-tetradecane, benzaldehyde, butanoic acid, 2-dodecanone, 1-nonanol, hexanoic acid, and 1-decanol. Dodecane was used as an internal standard. Stripped corn oil (Eastman Kodak Co.) was used as a representative lipid system.

Preliminary experiments showed that the best solvent system based on maximum diffusion of flavor compounds and minimum diffusion of the oil was a mixture of acetone and pentane. Three different acetone: pentane mixtures were studied: 80% acetone: 20% pentane (v/v), 70% acetone: 30% pentane, and 60% acetone: 40% pentane.

Preparation of solutions for dialysis

A general scheme for the preparation of the solutions to be dialyzed is as follows: 100 ml of the appropriate acetone-pentane solvent per dialysis unit was prepared and internal standard was added to make a final concentration of 1 μl/ml of solvent. Half of this solution was used as the solvent against which the solution was dialyzed, and flavor compounds were added to the other half in amounts dependent on the experiment.

Part 1: Separation of flavors from lipids

In order to determine whether dialysis could be used to separate flavor compounds from lipids, an experiment was conducted in which a solution containing both corn oil and flavor compounds was dialyzed against solvent. Each of the flavors described above was used in the concentration of 1 μl/ml. The concentration of flavors that diffused through the membrane was measured on the solvent side by gas-liquid chromatography (GLC) using the formula described in Part 3. Before dialysis, 1 ml of corn oil was weighed in a small beaker and added quantitatively to the flavor solution in the dialysis unit (the mixture side). After dialysis, the amount of oil on either side of the membrane was measured by pouring each side into separate tared beakers which had been dried overnight in a 100°C oven and cooled in a desiccator. The excess solvent was evaporated on a steam bath, and then the beakers were placed in a 100°C oven for 24 hr, cooled in a desiccator, and weighed.

Part 2: Lipid diffusion experiment

Since incomplete evaporation of flavor compounds interfered with the gravimetric determination of oil in the previous experiment, a second experiment was conducted in which 3 ml of corn oil was weighed, placed in one side of the unit in solvent and dialyzed against pure solvent. The three different solvent systems were used with membrane C. The oil was allowed to diffuse for 8 days, the maximum time used in previous experiments. After diffusion, the percentage of oil on either side of the membrane was measured according to the procedure described in Part 1.

Part 3: Diffusion of flavor compounds

The study of the diffusion of the model flavors was conducted in two parts: the first studied the effect of type of membrane on diffusion of the flavors, and the second studied the effect of the solvent on diffusion. The first study was conducted using three different membranes with 70% acetone: 30% pentane, whereas the second was conducted using membrane C with three different solvent systems. In each case a 0.01M concentration of each flavor compound was used and no oil was added.

Diffusion of the flavors was measured by GLC. Prior to the start of each experiment, the solution containing the flavors (the "standard solution") was injected into the gas chromatograph (GC) and the area of each peak was recorded. During an experiment, a sample was withdrawn daily from the solvent side of the dialysis unit (the "sample solution") and injected into the GC. The concentration of flavor diffused was calculated with the following formula:

$$\frac{\text{Area of IS}_{STD}}{\text{Area of IS}_{SAM}} \times \frac{\text{Area of flavor}_{SAM}}{\text{Area of flavor}_{STD}} \times 100\% = \text{Conc of flavor diffused (\% diffused/2 } \mu\text{l)}$$

where IS = internal standard; STD = standard solution; SAM = sample solution.

This formula accounts for differences in the size of injection or in gas chromatographic response (Nawar and Fagerson, 1962) on the concentration of flavor which has diffused. The mixture side was sampled immediately after the solvent side and the concentration of flavor was determined in the same way. The elapsed times from the start of the experiment were noted at the times the solvent and mixture sides were injected and these two times were averaged.

The diffusion was allowed to continue for approximately 8 days, at which time the volume of each side was measured by pouring each simultaneously into two 50-ml graduated cylinders. Permeances for each compound were calculated according to the following formula. If v_1 and v_2 are the volumes of the mixture and solvent sides respectively, c_1 and c_2 are the concentrations of flavor compound (% diffused/2 μl) in the mixture and solvent sides respectively, P is the permeance, A is the area of the membrane, and t is the elapsed time from the start of the experiment, then according to the derivation from Fick's law (Benkler, 1978).

$$\ln 1 - \frac{c_2(v_2 + v_1)}{c_1 v_1 + c_2 v_2} = \frac{-PA(v_2 + v_1)t}{v_1 v_2}$$

A plot of $\ln 1 - \frac{c_2(v_2 + v_1)}{c_1 v_1 + c_2 v_2}$ vs t gives a straight line with a slope equal to $\frac{-PA(v_2 + v_1)}{v_1 v_2}$. The best straight line was found by linear least squares regression. The value of the slope in units of reciprocal hours was used in the calculation of the permeance.

$$P = \frac{D}{x} = \frac{(\text{slope in hr}^{-1})(v_1 \text{ in cm}^3)(v_2 \text{ in cm}^3)}{(3600 \text{ sec/hr})(v_1 + v_2 \text{ in cm}^3)(A \text{ in cm}^2)} = \frac{\text{cm}}{\text{sec}}$$

Gas chromatographic conditions

A Hewlett-Packard 7620A Research Chromatograph equipped with a flame ionization detector, and a Hewlett-Packard 3380A Integrator were used in the study. Separation of the compounds was accomplished on a 1.3m × 2 mm (i.d.) glass column packed with 10% Carbowas 20M on 80/100 Gas-Chrom Q (Applied Science Laboratories). Helium was used as a carrier gas at a flow rate of 30 ml/min. The injection port and detector temperatures were 280°C and 250°C, respectively. The GC was temperature-programmed from 60°C to 200°C at 6°/min with a 2-min post injection hold and a final hold at 200°C until all the compounds had eluted. A 2-μl sample was injected each time.

Statistical analyses

Permeances obtained in the model system study (Part 3) were treated by a two-way analysis of variance to test for significant differences among the permeances according to compound, and solvent or membrane. When significant differences were found, a multiple range test (Duncan, 1955) was used to compare differences in the mean permeances of compound, solvent, or membrane type after accounting for both main effects and two-way interactions, but only after two-way interactions were found not to be significant ($p > 0.05$).

RESULTS & DISCUSSION

THE FEASIBILITY of separating flavor compounds from oil was shown in the results from Part 1 of the study. Figure 2 shows that all of the flavor compounds diffused through the membrane, while an examination of the residue remaining in the beakers after the solvent had been evaporated revealed that very little of the oil diffused through the membrane.

The amount of oil that diffused through the membrane could not be quantitated due to the incomplete evaporation of flavor compounds at 100°C. Therefore, a second experiment was conducted (Part 2) in which oil without flavor compounds was dialyzed against pure solvent. An analysis of variance of the results (Table 1) showed no significant differences ($p > 0.05$) in the amount of oil that diffused as the solvent was varied. In no case was the amount of oil that diffused greater than 0.12% of the amount of oil that was added. The membrane thus acts as an effective barrier to oil diffusion in this system.

Part 3 of the study dealt with the quantitative measure of the diffusion of flavor compounds by the calculation of permeances. Permeances are not reported for four of the eleven added flavor compounds—butanoic and hexanoic acids, butanol and 2-methoxypyrazine. The GLC peaks of butanoic and hexanoic acids tended to "tail," which resulted in inaccurate integration of the peak areas. Butanol is not reported, because it was found that the peak areas on the solvent and mixture sides eventually exceeded the initial area of butanol on the mixture side at time zero. The increase in area was due to the formation of an artifact which eluted at the same time as butanol. The membrane contains sulfonic acid groups which could catalyze certain reactions. This hypothesis was supported by the results of an experiment in which the unused membrane alone was soaked in acetone. Two compounds were formed, one of which eluted at the same time as butanol and was identified by high resolution mass spectrometry, NMR and reactions with dinitrophenylhydrazine and potassium permanganate as 4 methyl-3-pentene-2-one. This compound presumably was formed by dehydration of the second compound, 4-hydroxy-4methyl-2-pentanone. These compounds were probably formed through the acid-catalyzed aldol condensation of two molecules of acetone.

The last compound not reported is 2-methoxypyrazine. Figure 2 shows that the concentration of 2-methoxypyrazine reached a maximum approximately at the time the first sample was taken and then gradually decreased after that time. The decrease was attributed to either 2-methoxypyrazine's adsorption to or reaction with the membrane. Soaking the used membrane overnight in 1N NaOH and then extracting the solution with diethyl ether revealed a GLC peak in the extract which was found to be 2-methoxypyrazine. Other compounds were found in the extract which could not be positively identified by mass spectrometry. Since 2-methoxypyrazine is a basic compound, it can be postulated that it reacted with the acidic sites on the membrane. 2-Methoxypyrazine was the only basic compound tested, and it is not known whether other basic substances would behave in a similar manner. If so, perhaps the membrane could be used as a means of separating basic compounds from acidic and neutral compounds. One would expect that the adsorption of 2-methoxypyrazine and formation of artifacts formed by acid-catalyzed reactions such as hydrolysis, esterification and aldol condensation could be prevented by soaking the membrane in base instead of water before diffusion was carried out. This would change the sulfonic acid groups to sulfonate groups. However, a cursory examination of peak areas showed that diffusion of the compounds was greatly decreased compared to the peak areas found when membranes were soaked in 1N KOH.

Evidence that the presence of 2-methoxypyrazine hindered the diffusion of other flavor compounds was provided in an experiment in which one dialysis unit contained

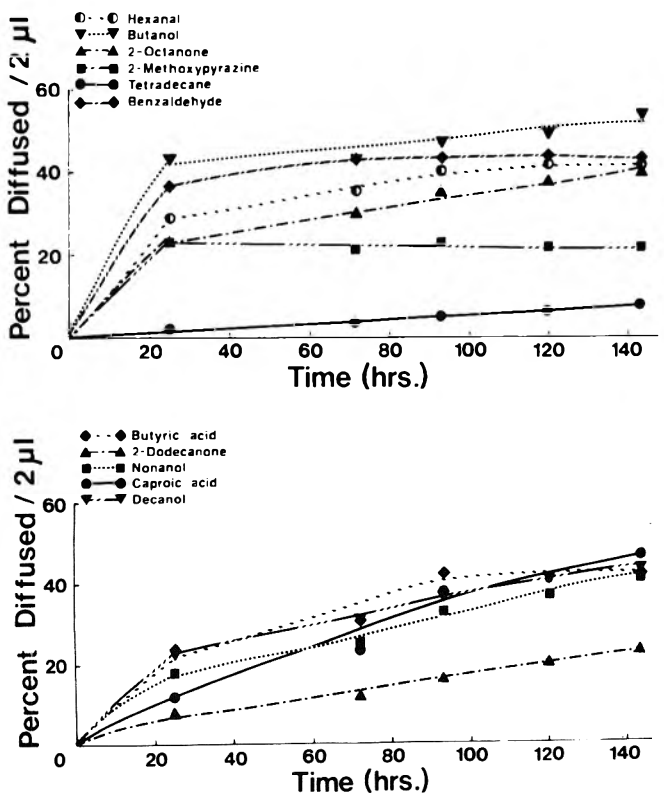

Fig. 2—Diffusion of selected compounds through dialysis membrane as a function of time (50% diffusion represents equilibrium).

Table 1—Recoveries and percentages of oil on either side of the membrane obtained in the lipid diffusion experiment

Solvent[a]	Expt. no.	% Recovered	% Mix. side	% Sol. side
60/40	1	97.84	97.77	0.07
	2	97.49	97.39	0.10
70/30	1	97.68	97.61	0.08
	2	97.76	97.68	0.08
80/20	1	98.59	98.48	0.11
	2	96.98	96.87	0.12

[a] % acetone/% pentane (v/v)

Table 2—Effect of 2-methoxypyrazine on the diffusion of other flavor compounds

Compound	Permeances X 10^5 (cm/sec)[a] Without 2-MP[b]	with 2-MP
Hexanal	0.9718	0.6572
2-Octanone	1.0915	0.5395
Tetradecane	0.1315	0.0415
Benzaldehyde	1.5300	0.8373
2-Dodecanone	0.4690	0.1434
Nonanol	1.1229	0.3625
Decanol	0.8361	0.4950

[a] Each permeance has been multiplied by 10^5 to give the values shown in the table.
[b] 2-Methoxypyrazine

all eleven of the flavor compounds in 70% acetone: 30% pentane and another dialysis unit contained all of the flavors except 2-methoxypyrazine. A 0.01M concentration of the flavors was used in both cases with membrane C. A paired comparison (Snedecor and Cochran, 1967) of the permeances (Table 2) showed that there was a significant difference ($p < 0.01$) in the two systems. The decrease in diffusion in the presence of 2-methoxypyrazine may be due to obstruction of the pores of the membrane.

The effect of the solvent on the diffusion is shown in Table 3. A 70% acetone: 30% pentane solvent was found to be best overall based on the mean permeances of all the compounds. The other two solvents (60 acetone: 40% pentane and 80% acetone: 20% pentane) were not found to be significantly different from each other ($p > 0.05$).

Table 3—Effect of solvent on diffusion of flavor compounds

		Permeances X 10^5 (cm/sec)[a]			
		Solvent[b]			
Compound	Expt. no.	60/40	70/30	80/20	Mean[c]
Hexanal	1	0.8097	1.0139	0.7757	0.8371CD
	2	0.7309	0.8674	0.8252	
2-Octanone	1	0.7120	0.8944	0.7325	0.8308CD
	2	0.8590	0.9664	0.8206	
Tetradecane	1	0.0247	0.0500	0.0727	0.0674A
	2	0.0538	0.1027	0.1003	
Benzaldehyde	1	1.0085	1.2680	0.7573	0.9427D
	2	0.7839	0.9246	0.9137	
2-Dodecanone	1	0.1298	0.1975	0.2328	0.2542B
	2	0.2900	0.3943	0.2811	
Nonanol	1	0.4955	0.6200	0.5715	0.7269C
	2	0.8216	1.1342	0.7186	
Decanol	1	0.8412	0.8261	0.8178	0.9309D
	2	1.0275	1.2496	0.8232	
Mean[c]		0.6134A	0.7506B	0.6031A	

[a] Each permeance has been multiplied by 10^5 to give the values shown in the table.
[b] % acetone/% pentane (v/v)
[c] Means in rows or columns followed by the same letter are not significantly different ($p < 0.05$)

Table 4—Effect of type of membrane on the diffusion of flavor compounds

		Permeances X 10^5 (cm/sec)[a]			
		Membrane			
Compound	Expt. no.	A	B	C	Mean[b]
Hexanal	1	0.6710	0.7694	1.0139	0.7375D
	2	0.5081	0.7795	0.6830	
2-Octanone	1	0.4577	0.6604	0.8944	0.5823CD
	2	0.3477	0.5996	0.5341	
Tetradecane	1	0.0243	0.0375	0.0500	0.0363A
	2	0.0233	0.0437	0.0388	
Benzaldehyde	1	0.8706	1.0205	1.2680	0.9208E
	2	0.6745	0.8998	0.7915	
2-Dodecanone	1	0.0985	0.1506	0.1975	0.1356A
	2	0.0845	0.1510	0.1316	
Nonanol	1	0.2824	0.4017	0.6200	0.3743B
	2	0.2148	0.3773	0.3495	
Decanol	1	0.3642	0.5136	0.8261	0.4959BC
	2	0.2802	0.4899	0.5012	
Mean[b]		0.3501A	0.4925B	0.5643B	

[a] Each permeance has been multiplied by 10^5 to give the values shown in the table.
[b] Means in rows or columns followed by the same letter are not significantly different ($p < 0.05$).

The results of the experiment on the effect of membrane type on the diffusion of the flavor compounds (Table 4) show that membranes B and C were more effective than membrane A. The greater effectiveness of membrane C is probably because it is half as thick as the other two. Increasing the number of sulfonic acid groups on the membrane apparently had a positive effect on the diffusion, since the diffusion of compounds through membrane B with an equivalent weight of 1100g was significantly better than the diffusion of the compounds through membrane A, which has an equivalent weight of 1200g. This result was unexpected, since it would seem probable that the membrane with fewer sulfonic acid groups would have fewer sites with which 2-methoxypyrazine could react and less occlusion to the membrane pores, if this action is indeed occurring.

The diffusion of the various compounds through the membrane can be examined with the data in Tables 3 and 4. A comparison of the effect of size on the diffusion showed that, in general, increasing the molecular size decreased the diffusion. The diffusion of tetradecane was less than that of 2-dodecacone (Table 3) which was less than that of all the other smaller molecules. Linear molecules tended to diffuse less than cyclic ones, which have a more compact shape. For example, even though benzaldehyde has one more carbon atom than hexanal, the diffusion of benzaldehyde was significantly better (Table 4) than that of hexanal. The data of nonanol and decanol show an apparent anomoly. The diffusion of decanol, which has 10 carbon atoms was significantly better (Table 3) than that of nonanol, which has 9 atoms. This result may be due to improper integration of the decanol peak, since the peak tended to have a broad base. It is difficult to determine the effect of the class of compound on the diffusion, since no two compounds of different chemical classes had the same number of carbon atoms.

SUMMARY & CONCLUSIONS

THE RESULTS of this study show that membrane dialysis can be an effective means of separating flavor compounds from lipids. Less than 0.12% of the added oil passed through the membrane, while many of the flavor compounds approached the theoretical maximum (due to equilibrium) of 50% diffused. Comparison of permeances showed that the most effective solvent was 70% acetone: 30% pentane and membranes were those with an equivalent weight of 1100 and 1200 and a thickness of 5 and 10 mils respectively. Comparison of permeances of the flavor compounds showed a trend in which the larger molecules diffused less than the smaller ones, but no conclusion concerning the effects of chemical class on diffusion could be drawn based on the data obtained.

The diffusion of 2-methoxypyrazine was hindered possibly by either adsorption to or reaction with the membrane. Data was not available to determine whether other basic compounds would behave in a similar manner. The reversible adsorption of methoxypyrazine on to the membrane suggests the possibility of separately recovering basic compounds from the flavor isolate.

The formation of artifacts through membrane catalysis of acetone condensation was a problem. We currently are examining the use of other solvents for dialysis. The possibility of artifact formation due to membrane catalysis must be further considered.

A number of questions remain concerning the use of this

technique for flavor isolation. However, the concept was proven feasible and represents and entirely new and unique basis for flavor isolation.

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A NEW PACKAGING METHOD FOR PEANUTS AND PECANS

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ABSTRACT

An economical method for packaging low-moisture peanuts and pecans for long-term storage is presented. The method utilizes the CO_2 adsorption properties of these commodities and involves placing them in plastic pouches impervious to air and CO_2, flushing them with CO_2 and then heat-sealing the pouches. CO_2 is adsorbed into the pores of the commodities forming vacuums inside the pouches. Both shelled, raw and shelled, roasted and blanched peanuts were protected from any significant deterioration of flavor and other quality factors for 12 months; pecans were protected for 27 wk and roasted salted-in-the-shell peanuts were protected for 4 months. This method of packaging protects perishable commodities for extended periods of time, is inexpensive and requires little energy.

INTRODUCTION

WHILE CONDUCTING RESEARCH on underground and underwater storage of cereal grains, Mitsuda et al. (1971) reported that grains and oilseed sealed in an environment of CO_2 adsorbed large amounts of the gas. This adsorption phenomenon is similar to that observed in the sorption of gases by charcoal and silica gel. The CO_2 adsorbed by porous tissues in these commodities is believed to remain in solid solution. As the CO_2 is adsorbed into the pores in the seed, a vacuum is formed inside the sealed environment. The complete adsorption process takes about 24 hr, as does the desorption process when the commodity is exposed to air. We measured a vacuum of 381 mm Hg inside a pouch of raw peanuts flushed with CO_2 and sealed for 24 hr.

We examined the quality of raw and roasted peanuts and pecans flushed with air or CO_2 and then sealed in plastic pouches for long-term storage. Preliminary data on packaging other commodities with CO_2 were also obtained during this study.

MATERIALS & METHODS

THE PLASTIC POUCHES used in this study were obtained commercially and were made of a laminate of nylon and ethyl vinyl acetate (EVA) which has a low permeability to most gases and moisture. For a film thickness of 25μ, the laminate has an oxygen permeability of about 304 cc/m^2/24 hr/L atm and water vapor permeability at 90% R.H. and 40°C of 9–12 g/m^2/24 hr.

The nylon provides strength to the film and serves as a gas barrier, whereas the EVA serves primarily as a water vapor barrier and sealer. The vacuum that is formed inside the pouch after flushing with CO_2 was maintained throughout the 1 yr storage period. This indicates that the gas permeability of the laminate was negligible, otherwise the vacuum inside the pouch would not have been maintained. Mitsuda et al. (1973) measured the gas composition in a pouch made from a laminate of nylon and polyethylene containing rice immediately after being flushed with CO_2 and again after 1 yr of storage. The initial gas composition was as follows: CO_2 –91.8%; O_2 –1.5%; N_2 –6.0%. The composition after 1 yr was as follows: CO_2 –93.2%; O_2 –0.1%; N_2 –5.9%. The increase in CO_2 and decrease in O_2 is attributable to the respiration of the rice. If any pouch developed a leak during the test, which was indicated by the loss of the vacuum inside the pouch, it was not used in the quality evaluations.

The CO_2 flushing device consisted of a 60-liter tank of the gas equipped with a regulator and a flow meter. A solenoid valve was attached to the flow meter and a plastic hose with a metal tube in one end was connected to the solenoid valve. A timing device controlled the solenoid valve. The timer and flow meter were set and then the metal tube was inserted into the product to the bottom of the pouch and the solenoid valve was activated. After the product was flushed for the designated time, the metal tube was withdrawn and the pouch immediately sealed with a heat-sealer.

Eleven samples of freshly dug, shelled, raw, unblanched Flo-

Table 1–Raw peanut treatment groups

Group no.	Treatment after placing peanuts inside pouch	Storage conditions
A1	Evacuated to 530 mm Hg then backflushed with CO_2 to 330 mm Hg and sealed	Ambient
A2	Not evacuated, flushed with 7600 cm^3 of CO_2 and sealed	Ambient
A3	Evacuated to 530 mm Hg then backflushed with air to 330 mm Hg and sealed	Ambient
A4	Not evacuated, flushed with 7600 cm^3 of air and sealed	Ambient
Control A1	Placed inside burlap bag and sealed	1.7°C and 65% R.H.

Table 2–Roasted peanut treatment groups

Group no.	Peanut kernel surface treatment	Treatment after placing peanuts inside pouch	Storage conditions
B1	Blanched	Flushed with CO_2 and sealed	Ambient
B2	Blanched	Flushed with CO_2 and sealed. Seal broken after 24 hr and then resealed	Ambient
B3	Unblanched	Flushed with CO_2 and sealed	Ambient
B4	Unblanched	Flushed with CO_2 and sealed. Seal broken after 24 hr and then resealed	Ambient
B5	Blanched	Sealed without CO_2 flush	Ambient
B6	Unblanched	Sealed without CO_2 flush	Ambient
Control B1	Blanched	Sealed without CO_2 flush	1.7°C and 65% R.H.
Control B2	Unblanched	Sealed without CO_2 flush	1.7°C and 65% R.H.

Table 3–Pecan treatment groups

Group no.	Condition of pecans	Treatment after placing pecans inside pouch	Storage conditions
C1	Raw	Flushed with CO_2 and sealed	Ambient
C2	Roasted	Flushed with CO_2 and sealed	Ambient
Control C1	Raw	Sealed without CO_2 flush	1.7°C 65% R.H.
Control C2	Roasted	Sealed without CO_2 flush	1.7°C 65% R.H.

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Table 4—Effects of packaging and storage time on selected quality parameters of raw peanuts

	Flavor	Optical density (450 nm)	Oxygen bomb (min)	Iodine value	Blanch-ability (%)	Raw kernel color			Butter color		
						L	aL	bL	L	aL	bL
3 months of storage											
Control mean	2.5	0.1891	133	96.9	99.0	39.0	12.6	12.7	44.3	11.7	22.5
Treatment means											
A1 (in CO_2)	2.9	0.1814	125	96.4	99.1	38.5*	12.9	12.0*	44.4	11.6	22.5
A2 (in CO_2)	3.0	0.1749	123	96.7	99.3	38.5*	13.0	12.3	44.2	11.7	22.4
A3 (in air)	2.9	0.1775	129	96.4	99.5	39.1	12.9	12.3	44.1	11.8	22.4
A4 (in air)	2.5	0.1757	128	96.7	99.6*	39.1	12.9	12.4	45.1	11.5	22.7
6 months of storage											
Control mean	2.7	0.1881	132	96.2	99.1	38.2	12.8	12.3	46.6	11.0	23.0
Treatment means											
A1 (in CO_2)	3.0	0.1647	132	97.1	99.5	37.8	12.9	12.2	44.3	11.9	22.5*
A2 (in CO_2)	2.8	0.1664	127	96.7	99.1	37.4	12.9	12.3	44.6	11.8	22.5*
A3 (in air)	2.5	0.1640	125	96.4	99.4	38.0	13.1	12.3	47.1	11.1	23.1*
A4 (in air)	2.6	0.1779	128	96.3	99.3	37.8	13.0	12.2	47.2	10.8	23.0
12 months of storage											
Control mean	2.6	0.1797	128	95.5	99.1	35.1	13.0	11.9	53.0	8.4	23.5
Treatment means											
A1 (in CO_2)	3.1	0.1517	121	95.7	99.0	35.2	13.9*	12.2	51.3	9.4	23.2
A2 (in CO_2)	3.4	0.1425	122	95.6	98.5	34.8	14.0*	12.5	49.7	10.0*	23.0
A3 (in air)	2.9	0.1478	124	95.0	99.2	35.1	13.7*	12.3	51.8	9.1	23.3
A4 (in air)	2.7	0.1565	121	95.1	99.2	35.7	13.7*	12.2	51.9	9.0	23.4

* Significantly different from the control mean at $P \leq 0.05$

runner peanuts (1976 crop) weighing 11.36 kg were placed in 62 × 72 cm pouches and subjected to the treatments outlined in Table 1. In the CO_2 flushing treatment where no initial vacuum was employed, more than the 0.5 cm^3 of CO_2/g of kernel that peanuts adsorb, as reported by Mitsuda et al. (1973), was used in order to sweep all air from the pouch. Three replications of the treated samples and the control were tested at 3, 6 and 12 months according to the following procedures: (1) iodine values of the oil, according to the tentative methods of APREA (1971); (2) optical densities (at 450 nm) of the oil (an indicator of peanut maturity, >0.1 is considered immature), the tentative methods of APREA (1971); (3) blanchability tests (ease of skin or testae removal or percent of kernels that can be deskinned) according to the method of Barnes et al. (1971); (4) oxygen bomb tests (120 min is an acceptable shelf-life for raw peanuts and 85 min is acceptable for roasted peanuts) according to the method of Blankenship et al. (1973); (5) color tests of both raw, unblanched kernels and peanut butter made from the roasted and blanched kernels were made on a Hunter Color Difference Meter (darkening, or lower L readings, and increase in redness, or larger aL readings, are signs of aging); (6) flavor tests were made on roasted peanut kernels which had been blanched, degermed and ground to a fine paste. This paste, or peanut butter, was evaluated by 10 panelists in individual booths illuminated by red masking lights to minimize color differences in the peanut butter samples. The samples were rated on a 5-point hedonic scale (excellent - 1, good - 2, fair - 3, poor - 4 and very poor - 5). The scores assigned to each sample by the panelists were averaged and the means treated statistically using analysis of variance and Dunnett's two-way tests at the 0.05 level to identify treatment means significantly different from their controls (Steel and Torrie, 1960).

The roasted peanuts used in this study were also Florunners from the 1976 crop and had been harvested about 30 days prior to roasting. Three-hundred-gram samples of the roasted peanuts were packed in 17 × 19 cm pouches. Because no information was available on the amount of CO_2 sorbed by roasted peanuts, we assumed that it was approximately the same as for raw nuts and the roasted nuts were flushed with about 200 cm^3 CO_2. Three replicates of the treated samples and the controls were analyzed at 3, 6 and 12 months for flavor, peanut butter color and shelf-life (oxygen bomb). These tests were the same as used on the raw nuts. The other tests used on the raw peanuts are not appropriate for roasted peanuts. The treatments for the roasted peanuts are presented in Table 2. Data were subjected to analysis of variance and Dunnett's tests (Steel and Torrie, 1960).

The pecans used in the study were harvested and shelled the middle of November, 1976, and had been held in cold storage (1.7°C 65% R.H.) 4 months prior to the CO_2 treatments. Three-hundred-gram samples of raw and roasted pecan halves were packaged in 17 × 19 cm pouches and flushed with 200 cm^3 CO_2; (we assumed they had the same adsorptive properties as peanuts). The pouches were heat-sealed and stored at ambient conditions. The treatments are presented in Table 3.

Three replications of the treated samples and the controls were removed for testing at 6, 10, 14, 27 and 52 wk. Because pecans become rancid more rapidly than peanuts, more frequent tests were made in order to pinpoint the time of the onset of rancidity. Flavor and color tests were run on the raw pecans and only the color tests on the roasted pecans because the other tests that were used on peanuts have not been developed for pecans. Data were subjected to analysis of variance and Dunnett's tests (Steel and Torrie, 1960).

A preliminary evaluation of the keeping quality of roasted salted-in-the-shell peanuts under a CO_2 atmosphere was also made. Florunner (1977 crop) peanuts were prepared according to Woodroof (1973). Duplicated samples of approximately 150g were packaged in 17 ×19 cm pouches which were flushed with 400 cm^3 of CO_2. Because of the bulk of in-the-shell peanuts, additional gas was used in flushing. A taste panel, consisting of 13 panelists, evaluated the nuts at 1, 2, 3, 4 and 9 months. Because salted-in-the-shell peanuts are known to have a short shelf-life, more frequent flavor tests were made than were made on the other peanut samples in order to pinpoint the time of the onset of rancidity. The results of this test were obvious without statistical evaluation.

RESULTS & DISCUSSION

Raw peanuts

Results from tests on raw peanuts are presented in Table 4. Flavor scores of treatments did not differ significantly from control values, even after 12 months of storage, nor were there any significant changes in optical density, oxygen bomb results, iodine values and the blanchability of the treated samples from the control samples. The blanchability on treatment 4 (the air flush treatment) increased after 3 months of storage. Although the data showed no striking pattern, color of raw peanut kernels and peanut butter appeared significantly affected by some treatments. Compared to the controls, the significantly decreased L values

Table 5—Effects of packaging and storage time on selected quality parameters of blanched and unblanched roasted peanuts and butter made from them.

	Control mean		Treatment means					
	B1 (blanched)	B2 (blanched)	B1 (blanched)	B2 (unblanched)	B3 (unblanched)	B4 (unblanched)	B5 (blanched)	B6 (unblanched)
3 months of storage								
Flavor	2.2	2.3	2.4	3.7	2.8	2.7	2.6	3.3
Oxygen bomb (minutes)	87	83	91	80	108*	96	79	91
Butter color L	52.2	52.3	51.4	52.0	50.6*	51.5	52.0	51.5
Butter color aL	9.0	9.0	9.3	9.1	9.3	9.4	9.1	9.3
Butter color bL	23.9	23.8	23.8	24.0	23.5	23.9	23.9	23.8
6 months of storage								
Flavor	2.6	2.7	2.8	4.0	3.5	3.5	3.3	3.7
Oxygen bomb (minutes)	90	90	87	84*	106*	100*	78*	93
Butter color L	51.7	51.8	52.0	52.4	50.6	48.9	52.2	51.5
Butter color aL	9.2	9.4	9.3	9.3	9.6	9.8*	9.2	9.5
Butter bL	23.7	23.7	23.7	24.2	23.6	23.1	23.9	23.7
12 months of storage								
Flavor	2.8	2.8	3.3	4.0*	3.7*	3.8*	3.9*	4.1*
Oxygen bomb (minutes)	88	87	77*	75*	97*	90	73*	91
Butter color L	51.3	50.4	51.3	51.8	50.9	50.5	51.5	50.5
Butter color aL	9.4	9.5	9.6*	9.5	9.6	9.7*	9.5	9.6
Butter color bL	23.6	23.4	23.7	23.8	23.6	23.6	23.8	23.5

* Significantly different from appropriate control means at $P \leq 0.05$

(the darkening) seen in peanut kernels stored 3 months in CO_2-flushed pouches were not seen after 6 or 12 months of storage. The redness of peanut kernels (aL) after 12 months in groups A1, A2 and A4 were significantly higher than controls.

Thus, raw peanut kernels flushed with CO_2 or air could be stored up to 1 yr in nylon/EVA pouches and still maintain flavor; most other quality attributes were not significantly different from those of peanuts stored under refrigeration at 1.7°C.

Roasted peanuts

Results from tests on roasted peanuts are presented in Table 5. After 3 months of storage, peanut butter made from Group B3 was significantly darker in color than its refrigerated control (Control B2) and the shelf-life prediction of the peanuts (oxygen bomb) was significantly increased. Peanut skins apparently offer some resistance to oxidation, for unblanched kernels have longer shelf-life (according to the oxygen bomb) than do the corresponding blanched kernels except when refrigerated. No significant differences in flavor between any of the treatment groups (including the control) were detected after either 3 or 6 months of storage. Peanuts stored for 6 months also showed that unblanched peanuts had a longer shelf-life (oxygen bomb) than blanched nuts and that peanut butter made from peanuts from Group 4 (unblanched) also had significantly redder color than the control.

The data from peanut samples stored for 12 months showed several significant changes when compared with the control. Peanuts from groups B2 through B6 had significantly poorer flavor than peanuts from refrigerated controls B1 and B2, whereas the peanuts from group B1 had about the same flavor as the refrigerated controls. Maintaining an acceptable flavor of the roasted and blanched peanuts for 1 yr will be of considerable benefit to the peanut industry. The redness in the peanut butter also generally increased in the CO_2 treatments, significantly so in groups B1 and B4. The oxygen bomb results again demonstrated that the unblanched nuts maintained resistance to oxidation as compared to their control (significantly for B3) and blanched nuts. It should be noted, however, that the flavor of the unblanched ambient-stored nuts deteriorated significantly from the refrigerated control after 12 months, whereas the flavor of the blanched nuts in group B1 was still about the same as the refrigerated controls. This apparent contradiction between flavor and shelf-life may be due to a thin film of oil in the skin that is more susceptible to oxidation than the oil in the peanut kernel. Oxygen bomb measurements are probably unaffected by the small amount of oxidized oil in the skins.

Table 6 shows the separate effects of interaction between atmosphere, peanut skins and storage period on roasted peanuts. Since flavor differences were significant only after 12 months of storage, the effect of atmosphere on the flavor of the ambient samples for the combined storage-periods data was not significantly different from the refrigerated control. As can be seen, however, the time in storage is significant. Storing roasted peanuts with their skins left on caused the peanuts to produce a significantly darker and redder peanut butter. Increased time in storage also significantly increased the redness of the peanut butter. The oxygen bomb data (shelf-life prediction) of the peanuts were significantly affected by the storage atmospheres, peanut skins, storage time and the interactions between atmospheres and peanut skins and between atmospheres and time of storage. Here again, the oxygen bomb data illustrate

Table 6—F values for main effects of atmosphere, peanut skin, storage period and their interactions on roasted peanuts[a]

Quality parameters		ATM	PS	PER	ATM/PS	ATM/PER	PS/PER	ATM/PS/PER
Flavor	F	2.72	0.32	6.92	3.17	0.14	0.04	0.34
	P	NS	NS	0.59	NS	NS	NS	NS
Peanut butter color L	F	0.77	16.15	0.60	1.31	1.20	2.00	1.03
	P	NS	0.08	NS	NS	NS	NS	NS
Peanut butter color aL	F	2.71	27.94	25.00	3.69	1.21	3.69	0.30
	P	NS	0.01	0.01	NS	NS	NS	NS
Peanut butter color bL	F	0.61	9.27	0.95	1.19	0.64	1.39	1.28
	P	NS	0.07	NS	NS	NS	NS	NS
Oxygen bomb	F	106.55	835.52	70.27	3.99	10.65	0.96	0.92
	P	0.01	0.01	0.01	3.68	0.01	NS	NS

[a] ATM = atmosphere, PS = peanut skins, PER = storage period, ATM/PS, PS/PER, ATM/PS/PER = interactions of the indicated variable, NS = difference not significant at $P \leq 0.05$.

Table 7—Summary of results for raw and roasted pecans

Storage period (wk)	Quality parameters	Cold storage means	CO_2 treatment means	F	Level of Significance*
		Raw pecans			
6 wk	Flavor	2.23	1.93	10.13	3.35
	Color L	30.97	30.10	1.45	NS
	Color aL	9.73	10.50	10.80	3.03
	Color bL	14.10	13.93	3.28	NS
10 wk	Flavor	2.43	2.57	2.00	NS
	Color L	30.50	29.30	5.61	NS
	Color aL	10.13	10.90	27.84	0.62
	Color bL	14.07	13.43	3.28	NS
14 wk	Flavor	2.20	2.67	3.38	NS
	Color L	30.80	28.17	60.59	0.15
	Color aL	10.07	11.13	51.20	0.20
	Color bL	14.20	12.73	33.38	0.45
27 wk	Flavor	2.50	3.27	7.56	NS
	Color L	29.97	28.37	10.57	3.13
	Color aL	10.33	11.30	27.13	0.65
	Color bL	13.73	12.67	32.00	0.48
52 wk	Flavor	2.00	3.37	54.23	0.18
	Color L	28.60	27.13	39.51	0.33
	Color aL	10.27	11.77	1012.50	0.01
	Color bL	12.70	12.30	21.05	1.01
		Roasted pecans			
6 wk	Flavor	2.17	2.03	0.70	NS
10 wk	Flavor	2.93	2.83	0.22	NS
14 wk	Flavor	2.60	2.63	0.06	NS
27 wk	Flavor	2.70	3.50	6.86	NS
52 wk	Flavor	2.17	3.53	38.20	0.35

* Level of significance of difference from refrigerated controls; NS = not significant at $P \leq 0.05$.

Table 8—Number of panelists rating roasted salted-in-the-shell peanuts good, fair, or poor

Storage period (months)	No CO_2			CO_2 Flushed		
	Good	Fair	Poor	Good	Fair	Poor
1	13			13		
2	13			13		
3	1	12		13		
4			13	13		
9			13		6	7

that, when the skins are left on, the peanuts resist oxidation better than when they are blanched.

Pecans

Table 7 shows the data from the pecan tests. Flavor of raw pecans stored for 6 wk in the CO_2 atmosphere was significantly better than the flavor of the refrigerated controls. Flavor of roasted pecans stored for 6 and 10 wk in the CO_2 atmosphere was better than that of the controls but not significantly so. The flavor under the CO_2 treatment through the 27th wk for both the raw and roasted pecans was about the same as the cold storage control at the 5% level or lower. This is a considerable increase in the shelf-life of pecans since they normally become rancid in 2–4 wk when stored under ambient conditions. Beyond 27 wk, the flavor in all treatments became significantly worse than the refrigerated control. The color of the raw pecans became gradually darker with storage time in the CO_2 atmosphere and was significantly darker after 14 wk of storage. The redness increased significantly after only 6 wk.

Roasted salted-in-the-shell peanuts

Table 8 shows the results of flavor testing the roasted salted-in-the-shell peanuts. The results indicate that with CO_2-flush treatment they can be stored for at least 4 months and still maintain a good flavor. Further testing will be required to determine exactly how long CO_2-flushed nuts can be stored and still maintain good flavor.

Other commodities

Other commodities, including rice, rough rice, corn, black-eyed peas, beans, wheat, peanut flour and wheat flour, have all been flushed with CO_2 at this laboratory and, in every case, the commodity adsorbed the CO_2 and a vacuum was formed inside the bag. No formal quality tests have been run on these commodities; however, after 6 month's storage at ambient conditions, no change in appearance or insect infestation has been noted.

Benefits of CO_2 packaging

In none of the CO_2-treated samples was there any evidence of insect activity or mold growth. The CO_2-flushed method of packaging perishable commodities is simple and inexpensive; it requires little energy because no mechanical vacuum-producing equipment or refrigerated storage is required. It is adaptable to production line operations and should be practicable for both small and large installations. Another advantage of the method is that the laminated plastic pouch can be molded into just about any shape before the vacuum is produced. Because the kernels are free-flowing when first flushed with CO_2, the pouch can be placed into a container (such as a cardboard box) of the desired shape and, when removed after 24 hr, still retain the shape of the container because the vacuum inside the pouch holds the grains rigid. It is anticipated that the rigid pack could reduce skinning, splitting and breaking of fragile commodities during shipping and handling.

Subsequent reports will present an in-depth study on the effects of CO_2 on the biology and the chemistry of peanuts and other commodities.

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LACTIC FERMENTATION OF PEANUT MILK

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ABSTRACT

The ability of cultures of 19 lactic acid bacteria to ferment a peanut milk (PM) prepared from blanched, full-fat Runner Variety peanut kernels was examined. *Lactobacillus acidophilus, L. brevis, L. xylosus, L. plantarum, Pediococcus acidilactici* and *Streptococcus thermophilus* after 48 hr produced the highest levels of acid in plain PM, producing respectively 0.25, 0.20, 0.42, 0.42, 0.17 and 0.27% titratable acidity (TA), expressed as lactic acid. Chemical analysis indicated that sucrose at 0.60% (w/v) was the major fermentable carbohydrate present in the PM. Supplementation of the PM with 1% levels of glucose, sucrose, whey, tryptose or yeast extract increased acid production by most cultures. Glucose supplementation of PM enabled *L. delbrueckii, L. plantarum, L. helveticus* and *L. casei* to produce 0.69, 0.17, 0.75, and 0.73% TA, respectively. Invertase treatment of the PM led to increases in acid production of 0.23–0.30 TA units by cultures previously unable to ferment the untreated PM. In a model system, the addition of 2.3% (w/v) oleic, 1.3% linoleic and 0.4% palmitic acid to a basal Elliker's broth with 1% sucrose (EB-S) as the sole carbohydrate source, led to 100% inhibition of acid production after 48 hr, compared to controls, for cultures of *L. acidophilus, L. cellobiosus* and *S. thermophilus.* For cultures of *L. brevis, L. xylosus, L. plantarum* and *P. acidilactici,* the inhibition of acid production by the fatty acids after 48 hr was much less, only about 5%.

INTRODUCTION

RISING COSTS and demand for animal protein have led to increased interest in the utilization of vegetable proteins for human nutrition. Already, much of the world consumes vegetable protein as an extension or replacement of animal protein (Jonas, 1975). Vegetable extended dairy products and vegetable protein based dairy analogues, including peanuts, appear to be likely candidates as new food products (Jonas, 1975).

Some low cost, high protein peanut milks have been developed (Chandrasekhara et al., 1971; Mitchell, 1950). Peanut-based cheese, spread and beverage food products using lactic starter cultures also have been prepared (Krishnaswamy and Johar, 1960; Krishnaswamy and Patel, 1968; Krishnaswamy et al., 1971; Ramamurti et al., 1964; Woodroof, 1973).

The behavior of lactic acid bacteria in milks prepared from soybeans has been studied in detail (Angels and Marth, 1971; Hang and Jackson, 1967; Mital and Steinkraus, 1974, 1975; Schroder and Jackson, 1971; Stern et al., 1977; Wang et al., 1974). However, there is comparatively little published information on the activity of lactic acid bacteria in peanut milks (Beuchat and Nail, 1978). Thus, this study was undertaken to determine the amounts of acid produced by 19 different lactic acid bacterial cultures in peanut milks prepared with and without added nutrients. The effect of the oleic, linoleic or palmitic acid concentrations normally occurring in the peanut milk on acid and pH changes produced by cultures from glucose or sucrose in a broth medium was also tested. A preliminary report of this work was presented earlier (Bucker et al., 1975).

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EXPERIMENTAL

Cultures

The sources and incubation temperatures used for lactic acid bacteria tested are listed in Table 1. Inocula for experiments were prepared by transferring cultures three times at daily intervals into fresh Elliker's broth (EB; Ellker et al., 1956). A 100-ml amount of sterile test medium in a 200-ml screw cap bottle, prewarmed at the appropriate incubation temperature, was inoculated with a 1-ml inoculum from the third 16-hr old culture to give an initial cell concentration of about 1.5×10^6/ml and then incubated quiescently at the appropriate temperature.

Preparation of the peanut milk

The same lot of blanched, deskinned, full-fat Runner Variety peanut kernals (Seabrook Blanching Corp., Edenton, NC) was used throughout this study and the peanuts were stored at 5°C prior to use. One volume of peanut kernals was steamed for 30 min in a rice steamer, ground in a kitchen food grinder (Osterizer) and mixed with 9 volumes of distilled water. The resulting slurry was comminuted twice in a 10.3 cm (4 in.) diameter Premier Colloid Mill, first at a setting of 0.20 mm (0.008 in.) and then at 0.10 mm (0.004 in.). This peanut milk was filtered through three layers of cheesecloth and the residue discarded. Tween 80 (J.T. Baker) was added to the filtrate to give a final concentration of 0.08% (v/v). This mixture

Table 1—Cultures and incubation temperatures used to ferment peanut milk

Culture	Source[a]	Incubation temp (°C)
Lactobacillus acidophilus NRRL B 629	A	37
Lactobacillus brevis 1834	B	30
Lactobacillus casei NRRL B 1445	A	37
Lactobacillus cellobiosus NRRL B 1840	A	37
Lactobacillus delbrueckii NRRL B 455	A	42
Lactobacillus fermentum NRRL B 585	B	37
Lactobacillus fermentum 42-7	A	37
Lactobacillus helveticus NRRL B 176	A	42
Lactobacillus xylosus 124-2	B	30
Lactobacillus plantarum ATCC 8014	C	30
Leuconostoc cremoris 8081	D	30
Leuconostoc cremoris 91404	B	30
Pediococcus acidilactici Accel R	E	37
Streptococcus cremoris R6	B	30
Streptococcus cremoris US3	B	30
Streptococcus diacetilactis DRC1	D	30
Streptococcus lactis C-6	D	30
Streptococcus lactis C-10	B	30
Streptococcus thermophilus ST-4	B	45

[a] A is Northern Regional Research Laboratory, Pecria, IL. B is Dr. E.H. Marth, Department of Food Science, University of Wisconsin, Madison, WI. C is Dr. G.L. Powell, Department of Biochemistry, Clemson University, Clemson, SC. D is Dr. E.B. Collins, Department of Food Science & Technology, University of California, Davis, CA. E is Merck and Company, Inc., Rahway, NJ.

was blended in a 4-L (1 gal) stainless steel Waring Commercial Blendor at low speed for 2 min and then dispensed in 150-ml amounts into 200-ml screw-cap dilution bottles. These bottles of PM were autoclaved at 121°C for 15 min and after sterilization, while still warm, they were vigorously shaken to redistribute an oil-like layer which had separated during sterilization.

Fermentation of plain and supplemented peanut milks

Peanut milks were supplemented with 1% (w/v) levels of several different materials to see if they might promote better acid production. Dried whey (Kraft-Krafen®), tryptose (Difco) or yeast extract (BBL) were added and dissolved in the PM prior to sterilization. Ten percent solutions of glucose or sucrose were filter sterilized and appropriate amounts aseptically added to sterile PM to give an added 1% concentration of that sugar in addition to the 0.6% sucrose existing in the PM. Sterile PM was also treated with invertase, 5.3 units/ml of PM (Sigma Chemical Co.) for 15 min at 55°C. Replicate bottles of PM, supplemented PM, invertase treated PM or EB were inoculated with cultures and incubated at the temperatures shown in Table 1. Duplicate 10-ml samples from each replicate bottle were aseptically withdrawn at various time intervals, frozen and stored at −4°C. Changes in pH values and titratable acidities were determined, using methods described below, and compared to values obtained for uninoculated controls.

Effect of fatty acids on lactic acid production

The concentrations of oleic, linoleic and palmitic acids determined to occur in the PM (Table 2), namely 2.25, 1.28 and 0.41% (w/v) respectively, were added singly or in combination to a basal Elliker's broth with 1% glucose (EB-G) or 1% sucrose (EB-S) as the sole carbohydrate and containing 0.08% Tween 80 and the pH adjusted to 6.8. Oleic acid was from Fisher Scientific Co., linoleic from Nutritional Biochemical Co., and palmitic from Sigma Chemical Co. The broth media were comminuted, blended, dispensed and sterilized in the same manner as the PM. Inocula were prepared using *L. cellobiosus, L. xylosus L. plantarum, P. acidilactici,* or *S. thermophilus* as previously described except that inocula to be tested in EB-G were grown in EB-G and those to be tested in EB-S were grown in EB-S. Samples were withdrawn and frozen as previously described and changes in pH and titratable acidity determined as described below.

Chemical analyses

Moisture, fat, ash and protein nitrogen (N) content of the PM were determined by standard procedures (AOAC, 1970). Protein was calculated using the equation N × 5.46 = % protein (Watt and Merrill, 1973). The total carbohydrate content was determined by difference. Glucose was determined by the semi-micro Glucostat method (Worthinton Biochemical Corp.). Sucrose was determined as the difference in the amount of reducing sugars (Nelson, 1944) obtained before and after a 15-min treatment of the PM at 55°C with 40 units of invertase/ml of PM. Pentosans were determined by the method of Bates (1942) and starch by the method of McCready and Hassid (1943).

Fatty acids were extracted from the PM (Newlander and Atherton, 1964) and methyl esters prepared (Stoffel et al., 1959). These were chromatographed at 190 and 200°C in a Microtec model MT220 gas-liquid chromatograph equipped with two 83 cm (6 ft) glass columns, one with 8% butanediol succinate on Chromsorb W and the other with 3% OV-17 on Chromsorb W. Fatty acid methyl esters were identified by comparison with standards.

The pH values and titratable acidities (TA), expressed as percent lactic acid, were determined for 10-ml samples of each fermented test medium. Each frozen sample was thawed and mixed with 100 ml of distilled water. The pH was determined and the sample then titrated with 0.1N NaOH to an endpoint of pH 8.3. The percent lactic acid produced was calculated as the difference in TA values of the inoculated sample and of the uninoculated, homologous control sample titrated to the same endpoint (Keller et al., 1974).

Table 2—Composition of peanut milk

Constituent	% of Constituent in Peanut Milk
Moisture	90.6
Ash	0.2
Protein	2.8
Carbohydrate	2.0[a]
(glucose	0.05)
(sucrose	0.63)
(pentosans	0.20)
(starch	0.20)
Fat	4.4
palmitic (16:0)	0.40
stearic (18:0)	0.07
oleic (18:1)	2.30
linoleic (18:2)	1.30
arachidic (20:0)	0.07
gadoleic (20:1)	0.06
behenic (22:0)	0.1
lignoceric (24:0)	0.1

[a] Total carbohydrate was determined by difference after determining moisture, ash, protein and fat.

RESULTS & DISCUSSION

Peanut milk fermentation

Peanut milks were prepared to simulate the protein and fat content of cow's milk. The sterilized, uninoculated PM was milky white in color and had a pH of 6.8. In preliminary tests, a more stable PM emulsion resulted if the PM was blended after, rather than before, autoclaving. However, the PM used in our fermentation tests was blended before sterilization since a suitable device for the aseptic homogenization of small quantities of the PM was not available.

Whole peanut kernels were steamed for 30 min to minimize the development of a nutty taste (Newlander and Atherton, 1964). Autoclave sterilization further retarded this problem (Krishnaswamy and Patel, 1968) and insured that competition from the natural flora was eliminated prior to fermentation studies. The PM viscosity increased after sterilization as noted by Mitchel (1950).

The proximate composition of the PM is presented in Table 2. Sucrose at 0.63% was the major fermentable carbohydrate in the PM and glucose was present at quite low levels (0.05%). Starch and pentosans were each present at approximately 0.2% but these are not known to be fermented by any of the cultures surveyed here (Deibel and Seeley, 1974; Garvie, 1974; Rogosa, 1974).

The main fatty acids in the PM, presumably present as glyceride esters in the fat and expressed as a percent of the lipid phase, were oleate, 51.2%, linoleate, 29.2% and palmatate, 9.4%. These fatty acids were calculated to be present in the PM at concentrations of 2.3, 1.3 and 0.4% (w/v), respectively (Table 2). Low levels of stearic, arachidic, gadoleic, gehenic and lignoceric acids were also detected.

The acidity change rather than pH change was the more sensitive method to follow fermentation since the titratable acidity (TA) continued to increase after the pH no longer declined. All of the cultures surveyed produced significant amounts of acid (generally 0.3–0.5%) after 48 hr in EB, the control medium, Table 3. However, only the *L. xylosus, L. plantarum, S. thermophilus, L. acidophilus, L. brevis* and *P. acidilactici* cultures produced much acid in the unsupplemented PM after 48 hr, producing 0.42, 0.42, 0.27, 0.25, 0.20 and 0.15% acid, respectively. In growth studies, acid production lagged behind culture growth with maximal TA occurring after the late logarithmic or during the early stationary phase (unpublished data). Two of these cultures, *L. xylosus* and *S. thermophilus,* were previously reported to produce from 0.42 to 0.50% acid in a soy milk (Angels and Marth, 1971).

Acid production by cultures in plain PM was less than expected. With the exception of *L. brevis* and *L. cremoris,* the test cultures were homofermentative (Rogosa, 1974) and theoretically should have converted over 90% of the sucrose to lactic acid to yield about 0.54% lactic acid in the

Table 3—Acidities produced by cultures in Elliker's broth (EB), peanut milk (PM), and supplemented PM after 48 hr

Organism	% acidity[a] produced in		Change in % acidity[b] produced in PM supplemented with					
	EB	PM	1% glucose	1% sucrose	1% whey	1% tryptose	1% yeast extract	Invertase[c]
L. acidophilus NRRL B 629	0.80[d]	0.25[d]	0.02[d]	0.00[d]	0.25[d]	0.16[d]	0.30[d]	0.07[d]
L. brevis 1834	0.37	0.20	0.09	0.11	0.02	0.09	0.07	0.12
L. casei NRRL B 1445	0.37	0.00	0.73	0.01	0.54	0.00	0.16	0.30
L. cellobiosus NRRL B 1840	0.55	0.08	0.06	0.00	0.23	0.02	0.29	0.15
L. delbrueckii NRRL B 455	0.36	0.00	0.69	0.01	0.55	0.00	0.21	0.27
L. fermentum NRRL B 585	0.33	0.00	0.30	0.00	0.10	0.00	0.19	*0.32*
L. fermentum 42-7	0.12	0.00	0.10	0.00	0.08	0.00	0.00	0.30
L. helveticus NRRL B 176	0.37	0.00	0.75	0.00	0.50	0.14	0.24	0.29
L. xylosus 124-2	0.31	0.42	0.07	0.10	0.09	0.04	0.09	–0.10[e]
L. plantarum ATCC 8014	0.51	0.42	0.17	0.17	0.20	0.00	0.11	–0.10[e]
L. cremoris 8081	0.29	0.00	0.33	0.00	0.06	0.00	0.14	0.27
L. cremoris 91404	0.30	0.00	0.36	0.00	0.06	0.00	0.13	0.27
P. acidilactici	0.35	0.15	0.00	–0.01[e]	0.05	0.25	0.05	0.16
S. cremoris R6	0.44	0.07	0.24	–0.05[e]	0.22	–0.07[e]	0.07	0.22
S. cremoris US3	0.40	0.09	0.26	–0.09[e]	0.19	–0.09[e]	0.04	0.20
S. diacetilactis DRC1	0.30	0.00	0.30	0.00	0.28	0.00	0.14	0.33
S. lactis C-6	0.39	0.00	0.33	0.00	0.36	0.00	0.17	0.26
S. lactis C-10	0.38	0.00	0.36	0.00	0.36	0.00	0.14	0.26
S. thermophilus ST-4	0.45	0.27	0.02	0.00	0.07	0.11	0.07	–0.09[e]

[a] Titratable acidity (TA) expressed as % lactic acid

[b] Each value was calculated as follows: the mean TA of the supplemented PM minus the mean TA of the plain PM at the same time.

[c] Enzyme was added to PM to give a final concentration of 5.3 unit/ml. PM was then incubated at 55°C for 15 minutes, tempered to the fermentation test temperature and inoculated.

[d] Each value represents the mean of four determinations which resulted from two experiments each with duplicate samples. A difference exceeding 0.05% acidity units between the supplemented PM and unsupplemented PM values (excluding the data for EB) in either horizontal rows or vertical columns is statistically significant by the least significant difference (lsd) test, $P < 0.05$, (Steel and Torrie, 1960).

[e] Acidity decreased by amount shown, compared to the control.

PM. Yet, no more than 0.42% lactic acid was produced by any of the test cultures in PM, suggesting an incomplete fermentation of sucrose. However, the enzymes for sucrose fermentation were apparently fully operable in these cultures since good levels of acidity were produced by these cultures when samples of inocula were tested in EB-S at the time these inocula were transferred to PM (unpublished data).

Fermentation of supplemented PM

To facilitate comparisons of the effects of supplementation of PM on acid production by each test culture the term "change in TA units" will be used and defined as the mean TA value of the supplemented PM minus the mean TA value of the unsupplemented PM. Sucrose supplementation of PM led to increases of 0.1–0.2 TA units by *L. brevis*, *L. xylosus* and *L. plantarum*, Table 3. There were apparent decreases of 0.01–0.09 TA units for *P. acidilactici* and the two strains of *S. eremoris* and essentially no changes for the other cultures. These results suggested that something other than sucrose concentration in the PM limited acid production.

In test of the six cultures which produced at least 0.15% acid in PM (*L. acidophilus*, *L. brevis*, *L. xylosus*, *L. plantarum*, *P. acidilactici* and *S. thermophilus*, Table 3), glucose supplementation of the PM led to a moderate increase of 0.17 TA units for *L. plantarum* and to either no or slight increases of 0.02–0.09 TA units for the other five cultures. Conversely, the remaining 13 cultures, which produced little or no acid in plain PM or PM with 1% added sucrose (Table 3), produced increases of 0.10–0.75 TA units in PM supplemented with 1% glucose. The most notable increases in TA units (with values shown in brackets) were for the following 11 cultures: *L. casei* (0.73), *L. delbrueckii* (0.69), *L. fermentum* NRRL B585 (0.30), *L. helveticus* (0.75), *L. cremoris* (0.33–0.36), *S. cremoris* (0.24–0.26), *S. diacetilactis* (0.30), and both strains of *S. lactis* (0.33–0.36) cultures. Smaller increases in TA units were observed for the other two cultures: *L. cellobiosus* (0.06); *L. fermentum* 42-7 (0.10). Excluding the EB data, a difference exceeding 0.05 TA units between the supplemented PM and the unsupplemented PM values in either horizontal rows or vertical columns in Table 3 is statistically significant by the least significant difference (lsd) test, $P < 0.05$ (Steel and Torrie, 1960).

In tests with other PM supplements, acidity increases of 0.2–0.55 TA units in PM with whey were produced by *L. acidophilus*, *L. brevis*, *L. casei*, *L. cellobiosus*, *L. delbrueckii*, *L. helveticus*, *L. plantarum*, *S. cremoris*, *S. diacetilactis* and *S. lactic*, Table 3. For the other three cultures this supplement led to small increases of 0.05–0.10 TA units. The addition of tryptose to PM led to increases of 0.1 or more TA units by four cultures: *L. acidophilus* (0.16), *L. helveticus* (0.14), *P. acidilactici* (0.25) and *S. thermophilus* (0.11). For these four test cultures the acidity increases with tryptose addition were greater than with glucose addition, suggesting for these cultures that the availability of peptides and amino acids in the PM was possibly more limiting to acid production than was availability of fermentable carbohydrate. The addition of peptides and free amino acids in the form of tryptose and similar preparations is known to increase the growth of lactic acid bacteria (Deibel and Seeley, 1974; Garvie, 1974; Rogosa, 1974).

Of the ten cultures which produced no acid in PM, nine yielded increases of 0.13–0.21 TA units in PM with yeast extract and only *L. fermentum* 42-7 did not show an increase in TA units (Table 3). For the three cultures that produced between 0.07–0.09% acid in PM, yeast extract addition led to an increase of 0.25 TA units for *L. cellobiosus* but none for the two *S. cremoris* strains. Of the six cultures that produced 0.15% acidity or more in PM, yeast

Table 4—Effect of fatty acids on titratable acidities produced by cultures in basal Elliker's broth medium with sucrose or glucose as the sole carbohydrate.

Culture	Time (hr)	% acidity[a] produced in EB-S[c]	% inhibition in acid production[b] EB-S + oleic	EB-S + lino-leic	EB-S + palmi-tic	EB-S + all 3 f.a.	% acidity[a] produced in EB-G[c]	% inhibition in acid production[b] EB-G + all 3 f.a.
L. brevis 1834	4	0.12[d]	100.0[d]	100.0[d]	100.0[d]	100.0[d]	0.16[d]	100.0[d]
	8	0.18	50.0	100.0	72.2	100.0	0.22	54.5
	16	0.39	35.9	23.0	48.7	43.6	0.49	18.4
	48	0.53	1.9	0.0	20.8	5.7	0.72	16.7
L. xylosus 124-2	4	0.08	100.0	100.0	100.0	100.0	0.11	100.0
	8	0.14	100.0	100.0	57.1	100.0	0.15	73.3
	16	0.40	40.0	77.5	50.0	65.0	0.35	54.3
	48	0.59	25.4	27.1	15.3	16.9	0.66	15.2
L. plantarum ATCC 8014	4	0.08	100.0	100.0	100.0	100.0	0.12	83.3
	8	0.19	100.0	52.6	10.5	100.0	0.19	57.9
	16	0.39	25.6	25.6	41.0	48.7	0.45	42.2
	48	0.53	11.3	13.2	20.8	5.7	0.68	14.7
P. acidilactici	4	0.09	100.0	100.0	44.4	100.0	0.10	80.0
	8	0.14	100.0	100.0	64.3	100.0	0.16	62.5
	16	0.35	51.4	25.7	37.1	57.1	0.33	36.4
	48	0.51	13.7	3.9	5.9	5.8	0.46	39.1

[a] Titratable acidity (TA) expressed as % lactic acid.

[b] Each value was calculated as follows: $\frac{\text{(mean TA for control minus mean TA for test)}}{\text{(mean TA for control)}} \times 100$.

[c] EB-S and EB-G are Elliker's basal broth media with 1% sucrose or glucose as the sole carbohydrate. The fatty acids were tested singly or in combination at the concentrations (w/v) found in the PM as follows: oleic, 2.3%; linoleic, 1.3%; palmitic, 0.4%

[d] Each value is the mean of four determinations which resulted from two experiments each with duplicate samples. Values within the same horizontal row that are underscored by the same number of lines are not statistically different by the least significant difference (lsd) test, $P < 0.05$, (Steel and Torrie, 1960). The lsd test was performed on the data expressed as % acidity and the results then applied to the data expressed as % inhibition in order to compare values between treatments of the same culture and time interval.

extract addition to PM led to increases of 0.30 and 0.34 TA units for *L. acidophilus* and *P. acidilactici* but to increases of only 0.07–0.11 TA units for the other four cultures. It was not determined whether these increases were due to the increased amounts of vitamins, minerals, carbohydrates or other nutrients added to the PM via the yeast extract. Recently Smith et al. (1975) reported the increased growth of *S. lactis* in a yeast extract supplemented medium was actually due to an increased amount of free amino acids this material provided.

Thirteen of the test cultures required that PM be supplemented with glucose, whey, tryptose or yeast extract in order to produce more than 0.1% acid. Similar results were reported for cultures tested in supplemented and unsupplemented soy milk (Angels and Marth, 1971). In a recent report, peanut milk required lactose addition before any significant amounts of acid were produced by *L. acidophilus* and *L. bulgaricus* cultures (Beuchat and Nail, 1978).

Fermentation of PM after invertase treatment

The 13 cultures which produced 0.0–0.09% acidity in plain PM produced no higher acidities in PM with 1% sucrose. These tests suggested these cultures were unable to ferment either the endogenous sucrose in the PM or exogenously added sucrose. Treatment of the plain PM with invertase prior to culture inoculation led to acidity increases by these cultures of 0.15–0.33 TA units (Table 3). Of the six cultures which produced 0.15% or more acid in plain PM, three produced more TA units (shown in brackets) in invertase treated PM: *L. acidophilus* (0.07), *L. brevis* (0.12) and *P. acidilactici* (0.16). The remaining three cultures (*L. xylosus, L. plantarum* and *S. thermophilus*) apparently produced decreases of about 0.1 TA units in the invertase treated PM. Additionally, all these cultures (except for *L. fermemtum, L. helvetius, L. xylosus* and *S. cremoris*) required just 16 hr to produce maximal acidities in the invertase treated PM versus 48 hr to do so in the untreated PM (data not shown).

Sucrose fermentation in presence of fatty acids

Since saturated and unsaturated free fatty acids can inhibit acid production by lactic acid bacteria (Kulsrestha and Marth, 1975) it was suspected that acid production in the PM may have been inhibited by the fatty acids present. However, essentially all the fatty acids present in sound, full-fat peanuts should exist not in free form but in ester linkages in di- and triglycerides (Woodroof, 1973) and analysis of our peanuts did not indicate otherwise. Also, oleate in small concentrations can also serve as a growth factor for the lactobacilli (Williams et al., 1947). Tween 80, the emulsifier used in this study, is a polyoxyethylene derivative of sorbitan esterified with oleic acid and can replace the biotin requirement of lactobacilli (Amber et al., 1951). The 0.08% concentration of Tween 80 used as an emulsifier in PM was not itself inhibitory to any of the 19 cultures tested in broth (unpublished data).

Whether the fatty acids present in the fat of peanuts could inhibit acid production by the starter cultures was tested as follows. The six cultures which produced at least 0.15% acidity in PM and *L. cellobiosis* were tested for their ability to produce acid in a model system using sucrose or glucose in the basal EB medium to which were added singly or in combination the amounts of the three major fatty acids theoretically present in the fat of PM (Table 2). These fatty acids were oleic (2.3%), linoleic (1.3%) and palmitic (0.4%).

In EB-S with all three fatty acids (EB-S-OLP) or with either oleic (EB-S-O) or linoleic (EB-S-L) alone, acid production by *L. acidophilus, S. thermophilus* and *L. cellobiosis* was completely inhibited for 48 hr (data not shown). Acid production by *L. brevis, L. xylosus, L. plantarum* and *P. acidilactici* in EB-S-OLP (Table 4) was completely in-

hibited after 8 hr, compared to EB-S, and inhibited 43–65% after 16 hr. By 48 hr, acid production was only inhibited by about 6% except for *L. xylosus* which was inhibited by 17%. Values for each culture within the same horizontal row in Table 4 that are underscored by the same number of lines are not statistically different by the least significant difference (lsd) test, $P < 0.05$, (Steel and Torrie, 1960).

In EB-G with all three fatty acids (EB-G-OLP) acid production after 48 hr by *L. acidophilus* and *L. cellobiosus* was inhibited by 43.4 and 56%, respectively, compared to EB-G (data not shown). Acid production from glucose by cultures of *L. brevis, L. xylosus, L. plantarum* and *P. acidilactici* grown for 48 hr in EB-G-OLP was inhibited about 15% except for *P. acidilactici* which was inhibited about 39% (Table 4).

These results indicate that the levels of oleic, linoleic and palmitic acids present in the fat of PM could, if free, inhibit acid production. However, the majority of the fatty acids in the PM are probably in the form of triglycerides which are not as inhibitory as free fatty acids (Kulsrestha and Marth, 1975). Some increased levels of free fatty acids might occur through the use of poor quality peanuts, poor storage conditions, or as the result of lipase activity by the cultures. The peanuts we used were of high quality, fresh and stored at 5°C so the first two possibilities do not seem likely. The third possibility of lipase activity producing autoinhibition of cultures is currently being investigated.

In summary, this study indicated that six of the 19 cultures tested could ferment the PM. Additional supplementation with carbon or nitrogen sources, or treatment of the PM with invertase, increased acid production by most cultures. The effect of the free fatty acid concentration (rather than the total fatty acid concentration) or the effect of other inhibitors in the PM on the lactic acid bacteria as well as whether the cultures surveyed here in addition to *Lactobacillus bulgaricus*, will produce more acid in peanut milks prepared from partially and completely defatted peanuts is presently under investigation.

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SENSORY, NUTRITIONAL AND STORAGE PROPERTIES OF COWPEA POWDERS PROCESSED TO REDUCE BEANY FLAVOR

JAMES C. OKAKA and NORMAN N. POTTER

ABSTRACT

Acidified water soaking of cowpeas followed by blanching reduced the beany flavor of drum-dried cowpea powders. A taste panel preferred the low-beany cowpea powders in a porridge-like baby food formula but not in bread where the reduction in beaniness was less apparent or in moin-moin, an African traditional dish, where beany flavor is desirable. The amino acid compositions and available lysine contents of the low-beany powders did not differ appreciably from those of raw cowpeas or from powder not reduced in beaniness. Biological value, as measured by the PER test, was not decreased in powders lowered in beaniness and was enhanced by methionine fortification. Storage stability of low-beany powders evaluated in the baby food formula was excellent for at least 16 wk at 30°C.

INTRODUCTION

THE PROCESSING and properties of various legume products have been reported by Kon et al. (1970, 1971, 1974) and others. Kon et al. (1970) found that the oxidative off-flavor development caused by lipoxygenase activity in California Small White beans and Lee soybeans could be controlled by bean slurry acidification. In a subsequent study, Okaka and Potter (1979) described the production of cowpea powders with reduced beany flavor. Beaniness was decreased by soaking cowpeas in acidified water, dehulling, and blanching in 100°C steam prior to grinding and drum drying. Data on physicochemical and functional properties of such powders in model systems also were reported. Since removal of beaniness by soaking and blanching could result in other changes in the cowpea, it was considered important to further evaluate the low-beany powders in selected food systems, and with respect to nutritional and storage properties.

EXPERIMENTAL

Preparation of powders

Three kilogram batches of cowpeas (*Vigna sinensis*), obtained from Chidsey and Schroeder Co., Rome, Ga., were made into powders by the method of Okaka and Potter (1979), which is summarized in Figure 1. Chemical analysis of raw cowpeas and the cowpea powders (Table 1) were determined by the methods of the AOAC (1975). Protein values are those of Kjeldahl nitrogen multiplied by the factor 6.25 and carbohydrate was calculated by subtracting percentages of protein, fat, ash and moisture from 100. All values are averages from determinations on duplicate samples. Powders passed through a U.S. standard seive with a Tyler equivalent of 50 mesh.

In-product functionality and organoleptic evaluation

The properties of cowpea powders were studied in a warm porridge-like baby food formula, in breadmaking and in moin-moin, an African traditional dish. The taste panel included 13–15 members of the Cornell community, most of whom were Nigerians and were familiar with cowpea products.

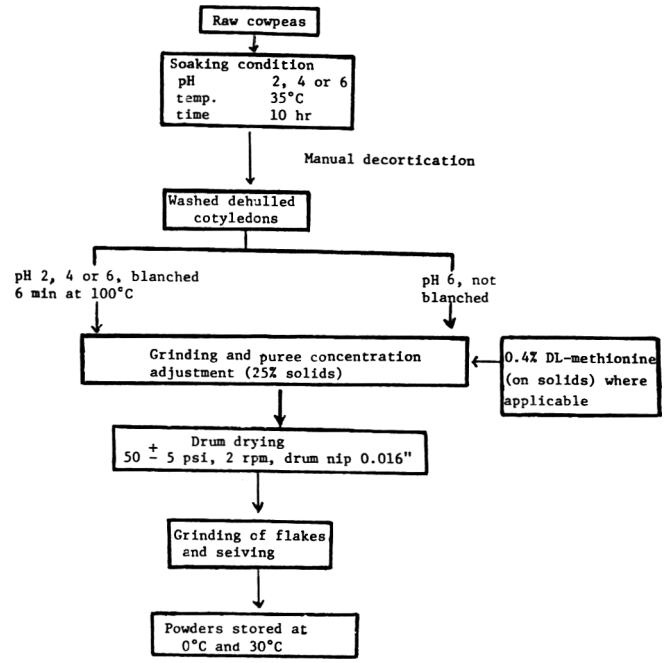

Fig. 1—Process conditions in the preparation of cowpea powders.

Baby food study. Fifty grams of each cowpea powder, 5.5g corn starch and 1.5g table salt were dispersed in 400 ml cool distilled water and brought to a boil. The porridge-like product was boiled for an additional 5 min. Ease of dispersion in cool water was noted and organoleptic evaluation of the finished product for preferences by a 15-member taste panel was determined using the seven-point hedonic scale: 1 = dislike extremely and 7 = like extremely. The panel was also asked to indicate differences in beany flavor intensity of the samples on the scale: 1 = very beany and 7 = not beany.

Breadmaking study. The baking ingredients used are given in

Table 1—Percentage compositions of raw cowpeas and cowpea powders

Component	Samples[a]				
	A	B	C	D[b]	E
Total solids	98.57	98.27	98.57	98.34	89.90
Moisture	1.43	1.73	1.43	1.66	10.10
Protein	24.19	24.51	24.35	24.50	23.10
Ether extract	1.29	1.26	1.25	1.29	1.30
Total ash	2.85	2.91	2.93	2.92	3.09
Crude carbohydrate (by diff.)	70.24	69.59	70.04	69.63	62.41

[a] Samples A, B, C and D were powders from cowpeas soaked at 35°C for 10 hr at pH's of 2, 4, 6 and 6, respectively. Sample E was unsoaked raw cowpea.

[b] All powders except D were produced from dehulled cowpeas that were blanched for 6 min at 100°C. Powder D was made from unblanched cowpeas. All values are averages from duplicate determinations.

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Table 2. Duplicate white pan breads were made with cowpea powders replacing 20% of wheat flour in the formula by the method of Okaka and Potter (1977), except that a 30-min initial fermentation instead of a 90-min fermentation was used. A 90/10 wheat flour/cowpea powder bread was also produced for comparison. Loaf volumes (rape seed method) and loaf weight were measured 1 hr after baking. A taste panel evaluation of wrapped breads was performed 24 hr later by 15 panelists rating for loaf appearance, crumb color, texture, flavor and overall acceptance on a nine-point hedonic scale.

Moin-moin study. The five formulas shown in Table 3 were made into moin-moin by mixing the ingredients, wrapping in folded aluminum foil and steaming (100°C) for 20 min. The moin-moin slurry made from raw cowpeas was given an additional 20 min steaming. A sixth sample provided as a control was a second moin-moin made from raw cowpeas. All samples after cooling to room temperature were presented to 13 panelists. The control (traditional) moin-moin was assigned a score of 5 for texture, flavor and overall acceptance on the seven-point scale: 1 = dislike extremely and 7 = like extremely, and panelists were asked to score the five coded samples relative to this control.

Table 2—Breadmaking formula[a]

Ingredients	Parts by wt
Wheat flour[b] (WF)-cowpea powder (CP) blend	100.0
Sugar	5.0
Salt	1.5
Compressed yeast	3.0
Dough conditioner[c]	0.5
Vegetable shortening[d]	3.0
Water	57 – 66

[a] Formula for 80/20 WF-CP breads contained 0.5 parts sodium steroyl-2-lactylate and 0.2 parts polysorbate (Tween 80).
[b] Seaboard Allied Milling Corp., Buffalo, NY
[c] Reddi-Sponge, Foremost Foods Co., San Francisco, CA
[d] Blue Label, Distributor Enterprises, Inc., Pittsburgh, PA

Table 3—Moin-moin formulas

	Sample				
Ingredients (grams)[a]	I	II	III	IV	V
Soaked, dehulled, raw cowpea (45% solids)	239.6	—	—	—	—
Cowpea powder[b]	—	110	100	100	109.6
Corn Starch	—	—	10	9.6	—
k-carageenan	—	—	—	0.4	0.4
Peanut oil	6	6	6	6	6
Salt	5	5	5	5	5
Water	271	400	400	400	400

[a] Each sample contained 119g solids
[b] Cowpea powder produced from pH 6 soaked, blanched cowpeas

Nutritional evaluation

Since a major tenet for producing low-beany cowpea powders is to extend cowpea use as a protein source, the protein quality of such powders was studied.

Amino acid analysis. Amino acids of raw cowpeas and cowpea powders were determined in the Technicon TSM Auto-analyzer after acid hydrolysis (AOAC, 1975). Tryptophan was determined by the colorimetric method of Horn and Jones (1945) using L-tryptophan as standard. Methionine was determined by the method for amino acids above as well as by the colorimetric method of McCarthy and Paile (1959) as modified by Ascarelli and Gestetner (1962). All results are expressed as grams amino acid residue per 16g nitrogen.

Available lysine. The method of Finley and Friedman (1973) which employs methyl acrylate as the ϵ-amino group alkylating agent was followed. Lysine before and after alkylation was determined by the above amino acid analysis method. The difference in lysine content of each sample before and after alkylation was the available lysine.

Rat feeding study. The method of AOAC (1975) was followed for the biological protein evaluation of cowpea powders, with a rat feeding period of 21 days. Fifty-six Sprague-Dawley weanling rats weighing approximately 45g each were randomly assigned to eight experimental diets (10% protein level) after feeding them for 3 days on a commercial stock diet. The rats were kept in individual wire bottomed cages and were fed ad libitum from porcelain cups cov-

Table 4—Evaluation of cowpea powder baby food

Sample[a]	Ease of dispersion	Taste panel mean scores[b] Flavor	Texture/ mouthfeel	Overall acceptance
A	rapid and complete	5.1**	5.2**	5.3**
B	rapid and complete	4.0	5.2**	5.1*
C	rapid and complete	4.7*	5.6*	5.3*
D	slow with lumps	3.7	3.3	3.7
Standard error of difference (Dunnett's test; DF = 41)		0.456	0.411	0.477

[a] All samples were as in Table 1.
[b] Scores were on the scale: 1 = dislike extremely, 7 = like extremely. Low flavor scores were said to be beany.
* Significantly different from unblanched sample D; $P < 0.05$
** Significantly different from unblanched sample D; $P < 0.01$

Table 5—Breadmaking properties of wheat flour (WF)/cowpea powder (CP) blends.

Bread samples	Loaf vol[a] (cc/500g bread)	Loaf sp. vol (cc/g)	Taste panel mean scores[b] Crumb color	Loaf appearance	Texture	Flavor	Overall acceptance
90 parts WF + 10 parts CP from pH 6 soaked, unblanched cowpeas	2964	5.93	6.5	7.5*	7.0	6.9	7.2
80 parts WF + 20 parts CP from pH 6 soaked, unblanched cowpeas (control)	2517	5.03	6.7	6.7	6.9	6.6	6.8
80 parts WF + 20 parts CP from pH 2 soaked, blanched cowpeas	2537	5.08	6.5	6.8	6.7	6.3	6.2
80 parts WF + 20 parts CP from pH 4 soaked, blanched cowpeas	2526	5.05	7.0	7.1	6.9	6.4	6.7
80 parts WF + 20 parts CP from pH 6 soaked, blanched cowpeas	2522	5.04	7.1	7.1	6.9	6.5	7.0
80 parts WF + 20 parts CP from pH 6 soaked, blanched, 0.4% methionine-supplemented cowpeas	2489	4.98	6.8	7.0	6.9	6.3	6.3
Standard error of difference (Dunnett's test; DF = 69)			0.2576	0.2296	0.2256	0.2044	0.2579

[a] Loaf volumes are averages of duplicate bakes.
[b] Taste panel mean scores based on the scale: 1 = dislike extremely, 9 = like extremely.
* Significantly different from control: $P < 0.05$.

ered with wire screens to minimize spillage. Drinking water was always available. Data on body weight gain, feed consumption, protein efficiency ratio, and efficiency of feed conversion were monitored. Apparent protein and dry matter digestibilities were calculated from protein and dry matter intake data and the fecal protein and dry matter outputs during the last 9 days of feeding.

Storage test

As a measure of storage stability of cowpea powders the baby food described earlier was made from powders stored in medium density polyethylene bags for 16 wk at 30°C and a relative humidity of 40% outside the package. The samples were evaluated as previously described for this freshly made product, but using 13 panelists.

RESULTS & DISCUSSION

THE TASTE PANEL data on the warm baby food are presented in Table 4. The mean flavor scores for the different cowpea powder products followed a pattern similar to that for cold water dispersions of the powders reported in a previous study (Okaka and Potter, 1979). The drum-dried powder from pH 6 soaked, unblanched cowpeas (sample D) imparted the greatest beany flavor impact. The mean texture/mouthfeel and overall acceptance scores showed this same cowpea product to be further inferior to the others. The low texture score was due to lumpiness from poor dispersability of this cowpea powder.

The data presented in Table 5 indicate that any differences in flavor between the different cowpea powders were not significant in bread. This may be due to oven-developed flavors that are capable of masking small flavor differences. Other organoleptic attributes also were not significantly different for the various powders in bread. The clear limitation to the use of these powders in breadmaking is related to the familiar bread volume depression as the level of wheat flour decreases beyond a critical level (Pomeranz et al., 1970 and Okaka and Potter, 1977).

In subjective evaluations, the consistency of cowpea powder moin-moin slurries prior to steaming was more firm than that of comparable slurries made from soaked, dehulled, raw cowpeas. From the results of tests in modified recipes (Table 6), texture and mouthfeel appear to be less preferred with cowpea powder than when raw cowpeas were used for moin-moin, although the differences were not statistically significant. However, flavor scored significantly lower and would have to be restored if cowpea powder moin-moin is to receive high consumer acceptance. Moin-moin is a traditional product where the beany flavor is desirable.

The amino acid profiles of the various cowpea powders (Table 7) did not vary from that of raw cowpeas, nor were they affected by acid soaking and blanching which reduced beaniness. The low methionine levels of all products are evident.

From Table 8 it is apparent that the available lysine contents of all powders were quite high (97–98%) and compare very favorably with the available lysine of raw unprocessed cowpeas. Onayemi and Potter (1976) found no significant change in the available lysine of drum-dried cowpea powder even after storage for 24 wk at 37°C. Since lysine is one of the most likely amino acids to be made unavailable during processing, principally by the Maillard mechanism, the high availability of lysine in the cowpea powders is indication of good retention of protein quality through processing.

Biological data from the feeding test are presented in Table 9. The values for PER have been adjusted to a casein value of 2.5 from an unadjusted casein value of 3.41. These results show unfortified cowpea powders to have lower PER values than casein. This is not from processing but due to the low methionine content of cowpeas. When cowpea slurry was fortified with 0.4% methionine (solids basis) the PERs of the resulting powders were improved beyond that of casein. PER values greater than for casein have been reported for methionine-fortified soybean (Meyer, 1966) and other bean products (Boloorforooshan and Markakis, 1977). The apparent dry matter and protein digestibilities of cowpea powders were 89 and 81%, respectively. Apparent dry matter and protein digestibilities were not affected

Table 6—Evaluation on moin-moin

Sample[a]	Taste panel mean scores[b] Flavor	Texture/ mouthfeel	Overall acceptance
I	5.3	5.0	5.1
II	3.5**	4.3	3.7*
III	3.2**	4.3	3.6**
IV	3.6**	4.1	3.8*
V	3.4**	3.8	3.5**
Standard error of diff. (Dunnett's test; DF = 47)	0.3619	0.4203	0.4434

[a] Sample formulas were as in Table 3.
[b] Scores were on the scale: 1 = dislike extremely, 7 = like extremely.
* Significantly different from sample I; $P < 0.05$
** Significantly different from sample I; $P < 0.01$

Table 7—Amino acid composition of raw cowpea and cowpea powders (g/16g N)

Amino acid	Sample[a] A	B	C	D	E
Aspartic acid	12.12	12.07	10.83	11.41	11.48
Threonine	3.00	3.43	3.18	3.46	3.27
Serine	4.00	3.89	3.74	3.90	3.83
Glutamic acid	18.46	17.47	16.70	18.05	17.64
Proline	4.18	4.12	3.80	4.10	3.94
Glycine	3.83	3.60	3.41	3.71	3.80
Alanine	4.13	4.18	4.07	4.20	4.27
Valine	6.10	5.85	5.62	6.01	5.81
Methionine[b]	1.56 (1.50)	1.56 (1.49)	1.33 (1.40)	1.42 (1.51)	1.52 (1.59)
Isoleucine	4.96	4.72	4.45	4.68	4.57
Leucine	7.89	7.58	7.25	7.75	7.57
Tyrosine	3.02	2.89	2.82	3.08	2.98
Phenylalanine	6.06	5.75	5.49	5.83	5.67
Tryptophan[c]	1.66	1.52	1.61	1.64	1.65
Ammonia	1.73	1.69	1.69	1.70	4.04
Cysteine[d]	–	–	–	–	–
Lysine	6.90	6.85	6.27	6.54	6.74
Histidine	3.07	2.90	2.73	2.91	2.95
Arginine	6.88	7.19	6.78	7.11	7.41

[a] Samples A, B, C, D were as in Table 1. Sample E was whole raw cowpeas.
[b] In addition to the chromatographic determinations, values in parenthesis were determined spectrophotometrically.
[c] Tryptophan was determined spectrophotometrically.
[d] Cysteine was largely destroyed by the acid hydrolysis method used in this analysis.

Table 8—Available lysine content of raw cowpea and cowpea powders

Sample[a]	Lysine content (g/16g N) Total	Bound	Available	% Available lysine
A	6.74	0.20	6.54	97.03
B	6.90	0.17	6.73	97.54
C	6.85	0.10	6.75	98.54
D	6.27	0.17	6.10	97.36
E	6.54	0.10	6.44	98.47

[a] Samples A, B, C, D were as in Table 1. Sample E was whole raw cowpeas.

Table 9—Biological response of rats fed various cowpea powder diets[a]

Dietary[b] group	Protein source for experimental diet	Wt gain (g)	Feed consumed (g)	PER	Apparent dry matter digestibility (%)	Apparent protein digestibility (%)	Wt gain/ day (g)	Wt gain/g feed (g)
I	Casein	80.8	237.1	2.5	90.4	87.0	3.84	0.34
II	Cowpea powder with 0.4% methionine from pH 2 soaked cowpea	99.5	241.7	3.0	88.8	81.5	4.74	0.41
III	Cowpea powder from pH 2 soaked cowpea	60.2	217.6	2.0	88.5	80.8	2.87	0.28
IV	Cowpea powder with 0.4% methionine from pH 4 soaked cowpea	99.6	239.3	3.1	88.8	80.0	4.74	0.42
V	Cowpea powder from pH 4 soaked cowpea	64.0	232.2	2.0	89.6	82.5	3.05	0.28
VI	Cowpea powder with 0.4% methionine from pH 6 soaked cowpea	98.9	251.6	2.9	89.3	82.3	4.71	0.39
VII	Cowpea powder from pH 6 soaked cowpea	63.2	222.2	2.1	89.3	80.2	3.01	0.28
VIII	Cowpea powder from pH 6 soaked cowpea, unblanched	50.9	192.5	1.9	90.0	80.3	2.42	0.26

[a] All cowpea powders were from blanched cowpeas except for group VIII.
[b] 7 rats per dietary group; duration of experiment 21 days

Table 10—Evaluation of baby food made from cowpea powders stored for 16 wk at 30° C

Sample[a]	Ease of dispersion	Taste panel mean scores[b] Flavor	Texture/ mouthfeel	Overall acceptance
A	rapid and complete	4.8**	5.4**	5.2**
B	rapid and complete	4.5**	5.3**	5.0**
C	rapid and complete	4.3**	5.2**	4.8**
D	slow with lumps	2.9	2.8	2.8
D_f	slow with lumps	3.0	2.7	3.1
Standard error of diff. (Dunnett's test; DF = 47)		0.4723	0.4305	0.4061

[a] All samples were as in Table 1. D_f was the same as D but was stored at 0°C for the 16 wk as a "fresh" control.
[b] Scores were on the scale 1 = dislike extremely, 7 = like extremely for texture and overall acceptance; 1 = definitely beany and 7 = definitely not beany for flavor.
** Significantly different from D_f control; $P < 0.01$

by methionine fortification or by blanching. Comparable values for bean products have been reported by Mitchell (1948), DeGroot and Slump (1960), and others.

The stability of cowpea powders stored 16 wk at 30°C, as judged in the baby food product, is seen in Table 10. These data can be compared with the data in Table 4, obtained in the same baby food prepared with the freshly dried cowpea powders. The baby food formula made from heat blanched cowpea again gave higher flavor and texture scores than powders made from unblanched cowpea. Further, storage stability of the powders for this application was excellent.

In conclusion, cowpea powders of reduced beaniness with excellent nutritional and storage properties can be prepared by drum drying acidifed water-soaked, blanched cowpeas. Although the low beaniness of cowpea powders so produced can be demonstrated in cold water slurries and in simple porridge-like recipes, preference for products made from these powders depend largely upon the product evaluated. In the present study, low-beany blanched cowpea powders were favored over the more beany unblanched cowpea powders in the baby food, no preference was evident in bread, but in moin-moin the more beany product was preferred.

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DESIGN OF A GENERATOR FOR STUDYING ISOTHERMALLY GENERATED WOOD SMOKE

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ABSTRACT

A laboratory size smoke generator, working under controlled conditions, has been constructed. The generator is basically a closed system to allow control over the supply of air, and it can produce smoke at a specific, desired temperature (within ± 10°C) in the region 300–550°C. This makes possible meaningful and systematic studies of important properties of smoke generated at various temperatures, such as its antimicrobial, antioxidative, flavor- and color producing effects, and content of polycyclic aromatic hydrocarbons.

INTRODUCTION

CONTROL of the smoking process is a prerequisite for full control of the meat processing line. The generation of smoke is not easily controlled, however, and the smoking equipment commercially available is generally inadequate in this respect. The newer generators based on the principles of friction or super-heated steam offer certain advantages, but it remains a fact that the process of smoking foods has neither been optimized nor brought under adequate control.

Wood smoke is a very complex physico-chemical system (Foster and Simpson, 1961; Foster et al., 1961) which is subject to influence by several generation parameters. It is generally recognized (Polymenidis, 1977) that the temperature of generation is of prime importance for the smoke quality. As far as the content of undesirable polycyclic aromatic hydrocarbons (PAH) is concerned, it is claimed that the lowest possible temperature is preferable (Tóth and Blaas, 1972). Considerable disagreement exists, however, as to which is the optimum generation temperature in terms of the active components of curing smoke (phenols, carbonyl compounds, carboxyl acids).

Spanyár and coworkers (1960) found the concentrations of phenols and carboxyl acids to be largely unaffected by temperature variations within the range 180–440°C; the concentration of carbonyl compounds increased with temperature. Tilgner and coworkers (1960) observed exponential increases in the concentrations of phenols and organic acids when the temperature was raised from 300 to 400°C, while Rusz (1960) drew the conclusion that smouldering at 280–350°C is optimal for the generation of active smoke components. In 1972, Tóth and Blaas claimed 400–500°C to be the desirable generation temperature for obtaining smoke giving good flavor, whereas Kersken (1974) has stated that 300–400°C is particularly optimal with regard to smoke quality and the lowest possible content of PAH. Recently, Potthast (1978) found the concentrations of the three groups of active components to pass through a maximum at about 600°C. He also found that increasing the temperature of generation increased the relative concentration of the phenols with the lowest boiling points.

Undoubtedly, differences in experimental setups and procedures have caused the diversity of results in this area. A major problem, clearly, rests with the difficulty of varying *only* the temperature of generation. For example, the work by Potthast (1978) is based on an experimental generator where the temperature is increased by increasing the supply of air to the smouldering zone. The conditions are thereby changed both in terms of temperature, availability of oxygen, and rate of smouldering. Similarly, commercial smoke generators are available which produce smoke at adjustable temperatures, but where the supply of air is difficult or impossible to control.

Smoking affects both the color, surface texture and shelf life of products in addition to their aroma. However, aside from investigations of smoke composition and general statements arrived at on smoke quality and flavour no work seems to have been done on the significance of the generation temperature. Meaningful studies of variations in the specific properties of curing smoke requires smoke to be produced at well defined generation (smouldering) temperatures, while all other relevant parameters are kept constant or are varied separately in a controlled manner. It is also desirable that experimental smoke be produced in a way which can be adapted to become an industrial procedure.

We describe in this paper the construction and operation of a laboratory smoke generator that essentially fulfills these demands.

EXPERIMENTAL

Preliminary studies

It was considered a vital question to establish if different samples of the same batch of wood chips give smoke of similar chemical compositions when heat treated in the same way. This was checked by dry distillation of randomly sampled beechwood chips (sieved to a size of about 700μ), and gas chromatography of the distillates.

Construction of the generator

In planning the generator, three requirements were put forward:

1. The generator should be able to work continuously over a sufficient period of time (8 hr).
2. The temperature in the smouldering zone should be kept constant within acceptable limits ($\leqslant$±25°C).
3. The air supply should be controlled.

Two different principles were selected as promising: Rotary kiln and fluidized bed. This paper describes the rotary kiln based principle.

In constructing the generator, standard units and assembly parts were used, if available. All the construction work was designed and performed at the Institute workshop.

Description of the laboratory generator

Instead of letting the tube rotate, a feed screw is used to convey the wood chips. This permits a more simplified construction with the same principal function (Fig. 1).

The hopper (3) is charged with wood chips, and a flow stirrer (4) prevents packing. The feed screw (10) conveys the chips through the brass generator body, whereby the chips will pass into the smoke generation zone which is heated by 6 cylindrical heat elements (total effect 1.2kW) contained in a steel mantle (7). While passing the generation zone the wood chips are more or less decomposed. The

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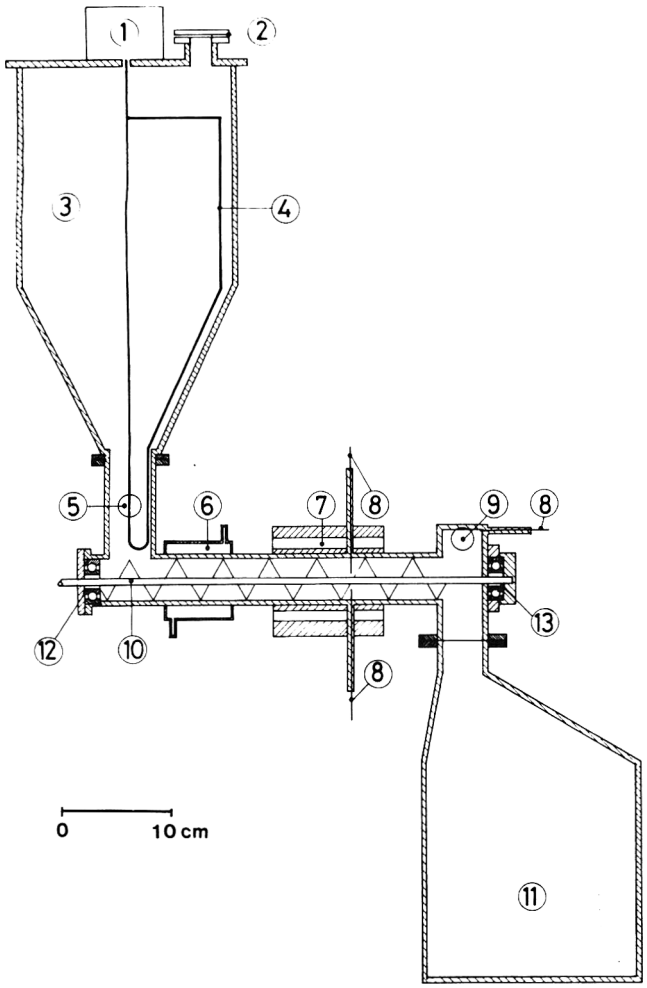

Fig. 1—Laboratory smoke generator: (1) Stirrer motor; (2) Filler cap; (3) Hopper (plexiglass); (4) Stirrer; (5) Air intake; (6) Cooling jacket; (7) Steel mantle with six heat elements embedded; (8) Thermocouples; (9) Exhauster; (10) Feed screw; (11) Ash collector; (12) Feed-screw-housing shaft seal; (13) Rear ball bearing.

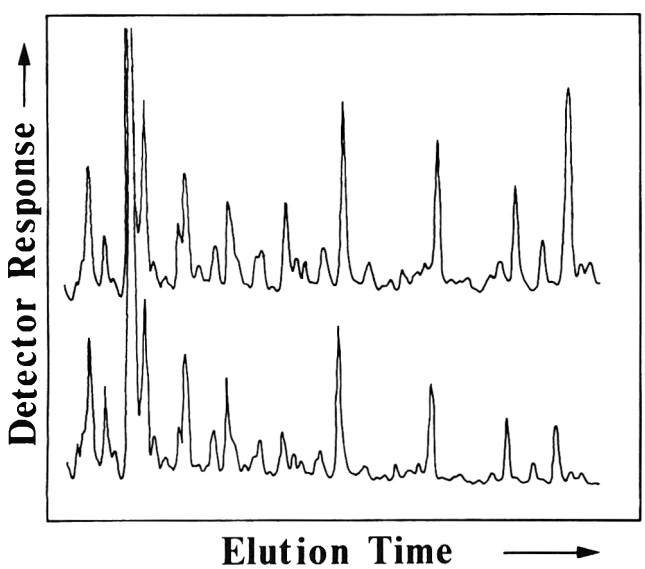

Fig. 2—Typical chromatograms obtained by gas liquid chromatography of condensates from dry distillation of different batches of wood chips.

remaining material is collected in an ash collector (11). The smoke is let out through an exhauster (9).

The feed screw has a maximum speed of 3.1 rpm and is driven via a cog belt by a shunt coupled gear motor. The speed of the motor is regulated steplessly by a tyristor unit.

A full profile helix was chosen as feed screw. For such small dimensions the pitch should equal the helix diameter. By calibration of the screw, values were obtained which give a degree of fill (ϕ) of 0.4. ϕ=0.45 has been given for materials of low viscosity and density (Vierling, 1974). At top speed the screw transports about 400g chips/hr.

The heating by the mantle (7) is controlled by a proportional-derivative regulator. One of the two recorded temperatures in the generation zone, measured by thermocouples (8), was used as the control signal.

Air is supplied at a constant pressure of 1.9 atm. abs. A needle valve regulates the flow to the air intake (5), and a meter displays the flow at any one time.

Condensation and collection of smoke

The smoke is conducted from the exhauster (9) via a short piece of silicon tubing to a trapping system which consists of standard glassware units. This setup will be described in detail in a forthcoming paper.

RESULTS & DISCUSSION

Preliminary studies

Gas chromatography of condensates obtained by dry distillation yielded chromatograms as examplified in Figure 2. The homogeneity of different batches of wood chips was found satisfactory as judged by the similarity of the chromatograms.

The laboratory generator

By careful maintenance (inspection of ball bearings and removal of pitch) the smoke generator proved to work very satisfactorily and could be used in full-day programs. Dismantling for inspection and general interior cleaning is recommended after 40 hr of running.

Preventing air leaks turned out to be the main problem. The following measures gave good results:

(a) Feed-screw-housing shaft seal [Fig. 1 (12)]: A spring loaded seal ring combined with an abundance of ball bearing grease proved sufficient, as the temperature at this point is rather low due to the cooling jacket [Fig. 1 (6)]. The spring-loaded seal ring also prevents the ball bearing from being contaminated with wood chips.

(b) Generator body/ash collector: Adequate tightness was obtained by layering high temperature grease between the flanges each time the collector had been disconnected.

(c) Hopper: The junction between the hopper and the generator body was fitted with an asbestos paper seal. Since the lid of the hopper is rarely removed, it was sealed to the wall by a silicone jointing compound. A rubber o-ring provided tightness when the filler cap [Fig. 1 (2)] was fastened to the lid by screws.

The rear ball bearing [Fig. 1 (13)] is subject to smoke and high temperatures (200–300°C). The bearing seems to stand the temperature, but pitch and tar will block it after a while. This problem, if judged too serious, can be overcome by reconstructing this part of the generator. One possibility is to use the counter current principle for chips and air, as is done in some commercial generators. The generated smoke would then be filtered through fresh chips to lose its content of tar droplets.

At higher temperatures ($\geqslant$450°C) exothermic reactions will cause severe temperature fluctuations (Spanyár et al., 1960) if the smouldering conditions allow them to. To avoid this, the air flow passing through the generator had to be lowered to about 1/10 of the volume required for total combustion. The overall reaction will then remain endothermic, and the need for heat to be supplied allows the smouldering to be controlled. Thus, the generator has been

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A Research Note

INHIBITION OF AFLATOXIN-PRODUCING FUNGI BY ONION EXTRACTS

ARUN SHARMA, G. M. TEWARI, A. J. SHRIKHANDE, S. R. PADWAL-DESAI and C. BANDYOPADHYAY

ABSTRACT

Various extractives of onion were tested for their inhibitory activity towards the growth of the aflatoxin-producing fungi, *Aspergillus flavus* and *A. parasiticus*. Ether extract and lachrymatory factor (LF), which has been earlier identified as thiopropanal-s-oxide, were found to have potent antifungal activity. Steam-distilled onion oil, which is devoid of LF, was not as potent as ether extract and LF. Its major component, dipropyldisulfide was ineffective as a fungal inhibitor. Ethyl acetate extract containing phenolics was also ineffective. Exposure of onions to gamma-irradiation at a sprout-inhibiting dose (6 krad) did not alter the inhibitory potency of the onion extractives which, however, appeared to be heat-labile.

INTRODUCTION

THE WIDESPREAD USE of onion (*Allium cepa* L) as a flavoring agent is well-known. Onion is also known to have medicinal properties. Recent studies have shown antibacterial activity of onion components (Abdou et al., 1972, Lewis et al., 1977). The inhibition of *Aspergillus niger, Penicillium italicum, Tryptophyton gypseum* and *Microsporon audouini* by different homologues of synthetic thiosulfinates was indicated by Small et al. (1949). Boiled water extracts of onion were also reported to cause inhibition of *Alternaria tenuis, Helminthosporium* sp. and *Curvularia perniseta* (Shekhawat and Prasada, 1971). However, it remains unclear as to whether onion has any antifungal activity towards aflatoxin-producing fungi. Earlier work from this laboratory has identified the lachrymatory principle and flavoring compounds of onion (Bandyopadhyay and Tewari, 1973). The effect of these compounds was therefore examined on the growth of two aflatoxin producers, *Aspergillus flavus* and *A. parasiticus*. The present study was also an attempt to ascertain the effects of some treatments such as gamma-irradiation and heat on antifungal properties of onion components.

MATERIALS & METHODS

Treatment of onions

Freshly harvested onions of Nasik Red Globe variety were procured from a local market and divided into two equal lots. One served as an unirradiated control and the other was treated with a sprout-inhibiting dose of gamma-irradiation (6 krad), in a Food Package Irradiator (Atomic Energy of Canada Ltd., 87000 Ci). Various onion extractives were then prepared as described below and their antifungal activity was assayed. In a second set of experiments, 1 kg each of control and irradiated onions were autoclaved (121°C/15 min). Ether extracts were then prepared in each case and tested for antifungal activity. Extracts were prepared once immediately after irradiation and then after storage for 2 and 4 wk at ambient temperature (28–32°C). In each case (unless specifically mentioned) 1 kg of onion was used after removing outer scales and discs.

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Preparation of the spore suspension

Aspergillus flavus (ATCC 15517) and *A. parasiticus* (NRRL 2999/3145) were subcultured on potato-dextrose-agar (Difco Laboratories, Detroit, MI) from the stock cultures. The slants were incubated for 7 days at ambient temperature. The spore material from the slants was suspended in 10 ml sterile water and transferred for surface culture to Roux bottles containing potato-dextrose-agar (200 ml, 4%), which were subsequently incubated for 10 days at ambient temperature. The harvesting of the spores from Roux bottles and preparation of the spore suspension (ca 10^6 spores/ml) was carried out according to the method described earlier (Padwal-Desai et al., 1976).

Assay of antifungal activity

The antifungal activity of onion extracts was assayed using two techniques as follows:

(a) Standard cup assay (Booth, 1972): 1 ml of the spore suspension of one of the three cultures described above, was used to seed the assay plates, prepared by pouring 20 ml of potato-dextrose-agar. After allowing the agar to solidify, cups were prepared using a sterilized glass tube and a propipette. The various extractives were then added to the cups in appropriate quantities in triplicate. In each case, a separate cup with pure solvent was employed as a control. The diameter of zone of inhibition was measured after incubation for 48 hr at ambient temperature.

(b) Assay in flask cultures: 1 ml of the spore suspension containing ca 5×10^3 spores/ml of *A. parasiticus* (NRRL 3145) was inoculated in 100 ml conical flasks containing 10 ml glucose-salt medium (pH 6.5) of Shih and Marth (1973). On the basis of the preliminary information obtained from cup assay, appropriate quantities of various extractives were added in triplicate to the flasks containing spores and medium. Flasks not containing onion extractives served as a control. Flasks were incubated at ambient temperature for 15 days. Thereafter mycelium was separated from the culture broth, washed with water, dried at 85°C for 24 hr and weighed. The flasks which did not show any sign of visual growth were further observed up to a period of 35 days.

Preparation of onion extractives

Ether extract. Pre-chilled onions were cut into small pieces and macerated with 200 ml of water using a Waring Blendor. The macerate was subsequently extracted with chilled peroxide-free diethyl ether according to the method of Bandyopadhyay and Tewari (1973). Aliquots of 2% aqueous solution (W/V) prepared from vacuum dried ether extract representing 0.01, 0.025 and 0.05 ml were used in cup assay, whereas for flask cultures 0.05 and 0.1 ml quantities were used.

Steam-distilled onion oil. This fraction was obtained by steam-distilling the chopped onions for 30 min. Aqueous condensate so obtained was extracted with peroxide-free diethyl ether. Ether was removed by flash-evaporation and the yield of oil was determined by weighing. For assay, 10% aqueous suspension (W/V) of this oil was prepared and 0.01, 0.025 and 0.05 ml, were used for cup assay, whereas 0.05, 0.1 and 0.5 ml aliquots were employed for inhibition studies in flask cultures.

Ethyl acetate extracts. To determine whether phenolics, which are extractable in ethyl acetate have any role in inhibition of aflatoxin-producers, these extracts were made free of all other expected inhibitory volatile sulfur compounds by inhibiting the activity of alliinase. This enzyme is involved in the biosynthesis of various volatile sulfur compounds including lachrymatory factor from precursors such as alkyl (alkenyl) cysteine sulfoxide (Schwimmer, 1969). About 200g of control and irradiated onions were used for extraction. To inhibit the activity of alliinase, intact onions were chilled in a dry ice-ethanol bath for 1 hr followed by crushing into fine pieces in 500 ml of chilled ethanol. The macerate was shaken for 10–12 hr

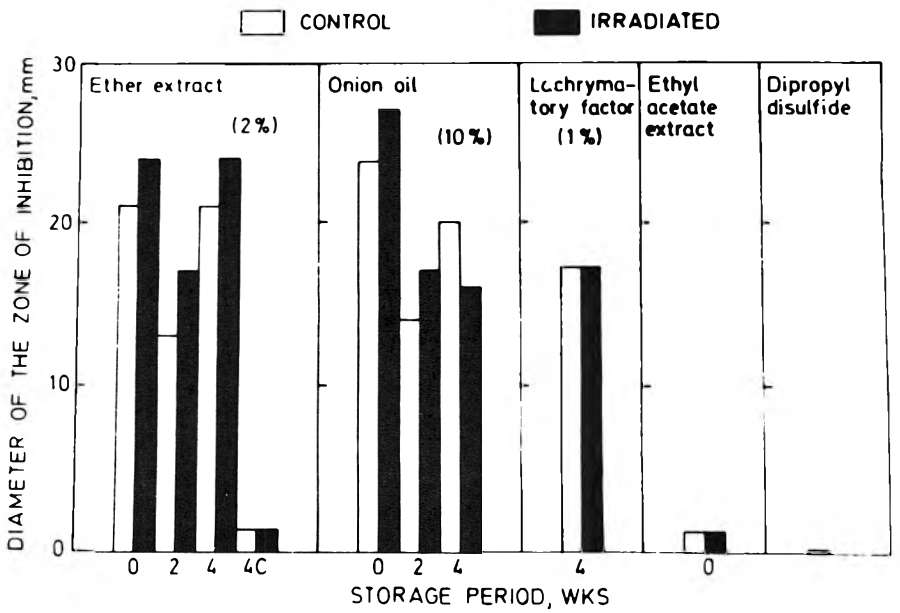

Fig. 1—Diameter of the zone of inhibition (average of six replicates) produced by various extracts from control (unirradiated) and irradiated onions. Concentration used: ether extract, 1 mg; onion oil, 5 mg; LF, 0.5 mg; ethyl acetate extract, 0.1 ml (concentrate); Synthetic DPDS, 10 mg. Cups containing 0.1 ml of sterile distilled water or pure ethyl acetate (in case of ethyl acetate extract) served as control for which zone of inhibition was zero (not depicted in diagram). Samples 4C represent those heat-treated at 121°C/15 min.

on a mechanical shaker and then allowed to stand for 1–1½ hr at ambient temperature. It was then filtered and from the filterate the major portion of ethanol was removed by flash-evaporation. The residual aqueous solution (30–40 ml) was extracted twice with 75 and 50 ml of ethyl acetate, respectively. The two ethyl acetate extracts were combined and flash-evaporated to a volume of 2–3 ml. In cup assay, 0.1 ml of this extract was employed, while aliquots of 0.1 and 0.5 ml were used in flask culture.

Lachrymatory factor (LF)

The lachrymatory component was isolated from ether extracts of onion by high-vacuum distillation followed by preparative thin-layer chromatography of the distillate (Tewari and Bandyopadhyay, 1975) and estimated by the glycine-formaldehyde reagent method, as described by Tewari and Bandyopadhyay (1978). Aliquots of a 1% aqueous solution (W/V) of this isolate at levels of 0.01, 0.025 and 0.5 ml were used for cup assay and 0.05 and 0.1 ml were used in flasks.

Synthetic dipropyldisulfide (DPDS)

This compound is the pungent principle which is nonlachrymatory. It was procured from Fluka AG, Buchs SG, Switzerland. Aliquots of 0.05 and 0.1 ml from a 10% aqueous suspension (W/V) as well as undiluted synthetic DPDS were used in cup assay, whereas in flask culture 0.1 ml and 0.5 ml aliquots from a 10% aqueous solution (W/V) were used.

RESULTS & DISCUSSION

IN THE PRESENT STUDY, different extractives of onion have been investigated in an attempt to identify the nature of the compound(s) that may be effective in inhibiting the growth of aflatoxin-producing fungi. The LF has been identified as thiopropanal-s-oxide, extractable in diethyl ether (Brondnitz and Pascale, 1971; Bandyopadhyay and Tewari, 1973). It has been estimated to be present to the extent of 8% in the ether extract. Besides LF, ether extract also contains dipropyldisulfide (DPDS) and other di- and tri-sulfides. Onion oil, which is devoid of LF, contains DPDS and other di- and tri-sulfides amongst which the main components were identified as cis- and trans-propenyl propyl disulfide, methyl propyl trisulfide and propyl trisulfide (Tewari and Bandyopadhyay, 1978). The diameter of the zone of inhibition of growth of *A. flavus* (ATCC 15517) produced by different extractives is shown in Figure 1. Although the diameter of the zone of inhibition observed with different extractives may appear similar in magnitude, the concentrations of these extractives were significantly different as indicated in Figure 1. Similar results were obtained with *A. parasiticus* (NRRL 2999/3145). Studies on inhibition of growth in flask cultures were undertaken to determine the efficiency of inhibitory components, screened by cup assay, in flask cultures during prolonged incubation. Figure 2 shows the growth inhibition of *A. parasiticus* (NRRL 3145) in flask cultures after incubation for 15 days. Our studies with flask cultures confirm the observations made in cup assay. The flasks which contained the effective inhibitory concentrations i.e. 0.1 ml ether extract or 0.1 ml LF or 0.5 ml onion oil did not show any signs of visual growth after 30 days of incubation at ambient temperature (28–32°C).

Since onion oil also possesses inhibitory activity, though not as pronounced as ether extract or LF, it was interesting to note that its major component, DPDS failed to show inhibition in both cup assay and flask cultures. It is clear that LF and ether extracts have stronger inhibitory activity as compared to onion oil, as the concentrations of LF, ether extract and onion oil are 1%, 2% and 10% (W/V), respectively (Fig. 1). It appears that LF is one of the major inhibitory components of ether extract.

Ethyl acetate extract, which consisted mostly of phenolics and flavonoides, was found to be ineffective against the fungal strains tested. Phenolics from other vegetable sources have been found to be inhibitory towards the growth of aflatoxin-producing fungi (Swaminathan and Koehler, 1976, Sharma et al., 1977).

Results in Figure 1 also indicate that inhibitory potency of various extractives was not influenced by exposure of onion to a sprout-inhibiting dose of gamma-rays (6 krad) and subsequent storage up to one month. On the other hand results with autoclaved onions revealed that the antifungal activity was susceptible to heat (Fig. 1, 4c). Therefore, onions gamma-irradiated for sprout inhibition (Lewis

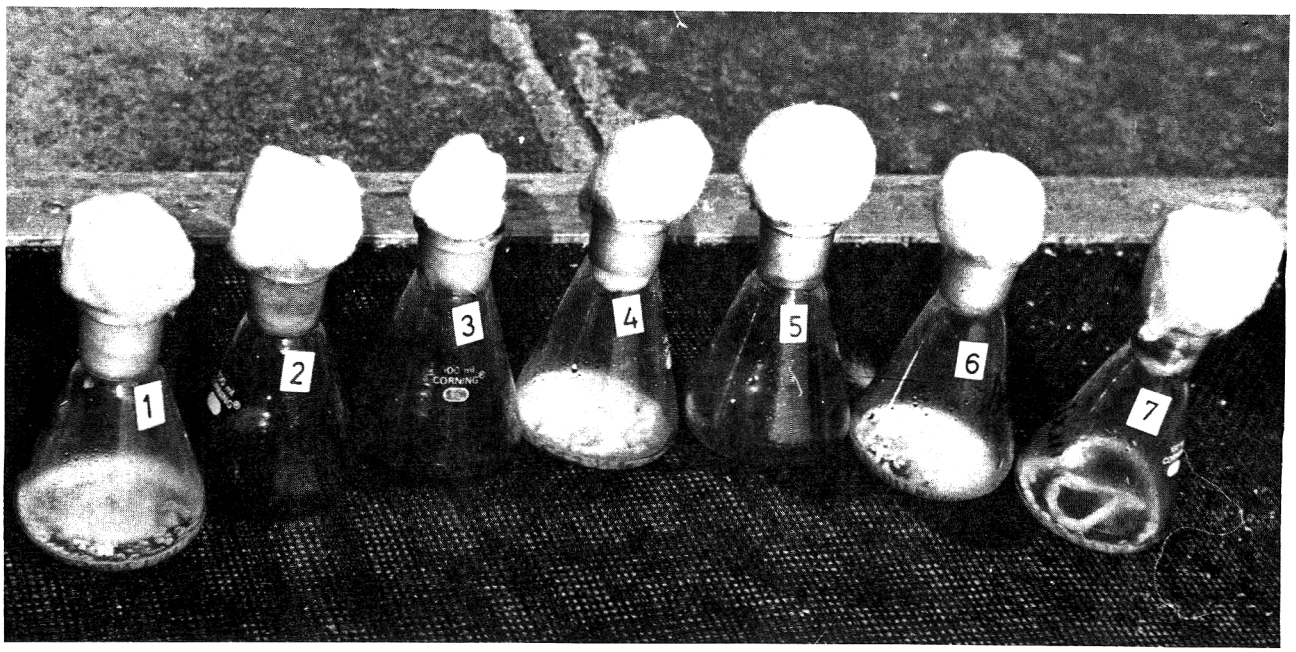

Fig. 2—Growth inhibition in flask cultures. Dry weight of mycelium is represented in parentheses (Avg of 3 replicates): (1) Control (336 mg); (2) Ether extract, 2 mg (no growth); (3) LF, 1 mg (no growth); (4) Onion oil, 10 mg (305 mg); (5) Onion oil, 50 mg (no growth); (6) Synthetic DPDS, 50 mg (394 mg); and (7) Ethyl acetate extract, 0.5 ml of the concentrate (315 mg).

and Mathur, 1963) and stored thereafter, may not promote the growth of aflatoxin-producing fungi, while heat-treated bulbs could be more prone to the attack of such fungi (Priyadarshini and Tulpule, 1976) presumably because of the heat-lability of antifungal principles. Complete inhibition of growth for more than 1 month has been observed under the present experimental conditions, which was the aim of our present studies. The influence, if any, of subinhibitory concentrations of the onion extractives on the growth of these fungi and aflatoxin production, is of further interest.

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A Research Note

CHARACTERIZATION OF BAKED BEAN PROCESSING WASTEWATER AND ITS ASSIMILATION BY *Aspergillus foetidus*

Y. D. HANG and E. E. WOODAMS

ABSTRACT

Baked bean processing wastewater is high in BOD (biochemical oxygen demand) and produces noxious odors when treated in municipal activated sludge systems. The present study describes a process for the assimilation of this wastewater by the mycelium of *Aspergillus foetidus* NRRL 337. The fungus is capable of rapidly digesting over 80% of the BOD and produces no foul odors. The mycelium has a crude protein content of greater than 50% and is readily harvested by simple filtration. The optimal conditions for the fungal process are as follows: pH, 3.3; temperature, 30°C; incubation time, 24 hr; and aeration rate, 2 mMO_2/liter/hr.

INTRODUCTION

LARGE VOLUMES of wastewater are generated daily by the baked bean processing industry. These waste effluents are high in biochemical oxygen demand (BOD) and produce foul odors when treated in municipal activated sludge systems. There is thus a need for an improved method of treating them.

In recent years, efforts have been made to treat food processing wastes with filamentous fungi because (a) they are capable of rapid growth on a variety of substrates, (b) they require less oxygen to metabolize food wastes than does a bacterial system, (c) they have a high protein content, and (d) their mycelia are dewatered easily. Church and Nash (1970) have reviewed the literature on the use of filamentous fungi in waste control. Recently, Church et al. (1973) found *Trichoderma viride* to be one of the most effective organisms for digesting corn canning waste and Hang et al. (1975a) demonstrated the use of *Aspergillus niger* for reducing the BOD in brewery spent grain liquor.

The objective of this study was to determine the ability of *Aspergillus foetidus* (formerly *A. niger* NRRL 337) to digest baked bean processing wastewater.

MATERIALS & METHODS

SAMPLES of wastewater were provided by a commercial baked bean processing plant. They were stored at −10°C until all tests could be made.

Aspergillus foetidus NRRL 337 (Northern Regional Research Laboratory, Peoria, IL) was used for all experiments. The culture was maintained on potato dextrose agar slant at 1°C. The inoculum was prepared as described previously (Hang et al., 1975a).

The standard treatment procedure involved the digestion of 3 liters of baked bean processing wastewater with 300 ml of a freshly prepared inoculum in a New Brunswick laboratory fermentor at 30°C for 24 hr. Dow Corning Antifoam A was used occasionally to depress foam formation.

All tests were made by the Methods described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1975).

Mycelial dry weight was determined by filtering, washing with distilled water and drying at 105°C overnight.

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Table 1—Characteristics of baked bean processing wastewater

	mg/liter
BOD	1,850–29,500
Kjeldahl N	172– 2,760
Total P	55– 590
Total solids	3,800–70,400
Volatile solids	3,140–56,100
Suspended solids	165– 680
Ash	660–14,300

RESULTS & DISCUSSION

THE CHARACTERISTICS of baked bean processing wastewater are shown in Table 1. The wastewater samples differed considerably with respect to BOD, Kjeldahl nitrogen, total phosphorus, solids and ash. Most of the pollution loads in the wastewater originated in the soaking and blanching operations. The large difference in strength between samples was attributable to (a) processing methods and conditions, and (b) factory activity at the time the samples were taken.

The pH of baked bean processing wastewater ranged from 4.4–6.7. To provide a more favorable environment for fungal growth, it was necessary to acidify the wastewater with conc sulfuric acid to lower the pH. The optimal pH for fungal assimilation of baked bean processing wastewater was 3.3. The ability of *A. foetidus* to metabolize the wastewater at low pH is a valuable feature, for it permits a more successful competition with bacteria and other undesirable microorganisms. Church and Nash (1970) have also observed that the optimal pH for reducing the BOD in corn canning waste lay between 3.2 and 3.5.

A. foetidus was capable of digesting baked bean processing wastewater over a wide range of temperatures. This is considered to be another desirable characteristic of this fungus since heating and cooling facilities must be avoided to minimize the cost of waste treatment. The optimal temperature for reducing the BOD was 30°C.

The optimal aeration rate for the reduction of BOD was 2 mM O_2/liter/hr. Thus, the fungal digestion process requires less oxygen to metabolize food wastes than does a bacterial system. Reduced oxygen requirement would be major savings in energy and treatment cost. Church and Nash (1970) have shown that the fungi incorporate much of the organic content of the waste into the mycelium rather than oxidizing it.

There was, however, no appreciable effect of aeration on the protein content of mycelium (greater than 50%). Similarly, the protein content of yeast cells grown on sauerkraut brine and molasses has been found to be independent of aeration (Hang et al., 1975b; Singh et al., 1948).

The effect of incubation time on fungal digestion of baked bean processing wastewater is shown in Figure 1. The greatest removal of BOD was achieved in 24 hr. Prolonged fermentation did not result in further reduction of the BOD. This may be caused by the (a) presence of compounds not readily metabolized by the organisms, (b) exhaustion of essential nutrients, or (c) accumulation of metabolic inhibitors.

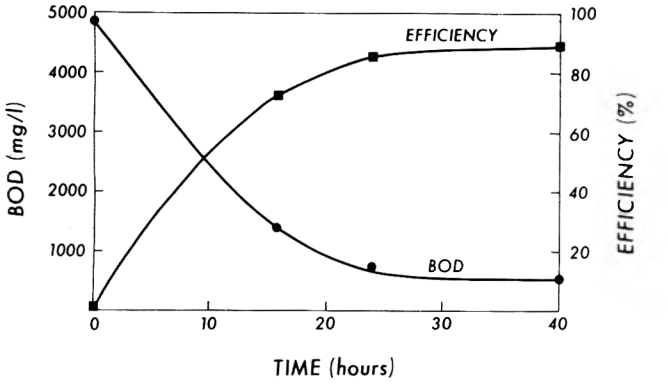

Fig. 1—Effect of incubation time on fungal digestion of baked bean processing wastewater.

The variations in the inoculum (3–20%) did not have much effect on BOD reduction at 24 hr of incubation. Thus, the fungus would be able to compete successfully with other organisms in the wastewater at even the lowest inoculum level.

Under optimal conditions (pH 3.3; temperature, 30°C; incubation time, 24 hr; aeration rate, 2 mMO_2/liter/hr), *A. foetidus* assimilated about 80–88% of the BOD depending on the samples of wastewater. Thus, the reduction in BOD obtained in baked bean processing wastewater was only slightly less than those obtained in corn canning waste (Church et al., 1973) and brewery spent grain liquors (Hang et al., 1975a).

The resulting waste effluents usually had a pH of 6.2 or above and did not produce noxious odors when they were further treated aerobically with activated sludge organisms obtained from a municipal waste treatment plant. This indicates that *A. foetidus* has eliminated the compounds responsible for the foul odor problems in municipal activated sludge systems.

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operated at 500°C without exceeding a temperature variation of ±10°C in the generation zone. Generation temperatures above ca. 550°C must be expected to be hard to control.

Heat economy is not good, evidently, when working in accordance with the above principles; it can be improved, however, only at the cost of temperature control. Incomplete utilization of the wood chips can be avoided by suitable recirculation.

By our judgement, the described generator is a suitable instrument for investigating questions related to smoke and the smoking of foods. All relevant parameters can be varied and/or kept under control. (Obviously, the consequences of large variations in wood chip size cannot be studied). We take only a limited interest in the composition of smoke, however; our research will primarily focus on the effects of smoke on foods. In a forthcoming paper we will report on how the generation temperature of the smoke influences its antioxidative and antimicrobial effects. If the results turn out favorable, possibilities exist for adapting the generator for industrial use.

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A Research Note

COEFFICIENT OF SLIDING FRICTION FOR NONUNIFORM SURFACE PROPERTIES

JERALD M. HENDERSON

ABSTRACT

The determination of the sliding coefficients of friction of the two major parts of squid moving on a metalic surface required using the whole squid. The investigation of the relationship between the coefficients of friction of the individual parts and the average coefficient for the whole lead to the derivation of a general relationship analogous to the relationship for the center of mass of a body. Specific results from the squid investigation are included to illustrate the application of the general friction relationship.

INTRODUCTION

A RECENT STUDY utilized the familiar impending motion on a sloping surface test to determine the coefficient of sliding friction μ of squid on a wet sheet metal surface (Brooks and Singh, 1977). The two major body parts of the squid, the body and the head, had distinctly different values of μ and these properties were utilized to orient the squid as it slid on a sloping surface. To determine the two values of interest the surface slope at impending motion was measured for the smaller value (the body) and the overall (average) value. The larger value was then calculated. This approach was used because the friction between the head and supporting surface was relatively large making it difficult to slide individual parts (heads) without rolling.

The objective of this investigation is to extend the results of the squid friction problem to a general result that can be used in research and design situations where the frictional properties of nonuniform bodies are of interest. Specific details of the squid investigation are presented by Brooks (1977) and Brooks and Singh (1977).

METHODS

THE SQUID PROBLEM leads one to consider the general situation when there are more than two body parts, each having distinctly different values of μ. Consider the case when the body of interest is modeled by differential mass elements as shown in Figure 1. Impending motion for the entire mass takes place at the friction angle θ (Meriam, 1975). The mass is assumed thin and flexible enough so that the only forces acting on the differential element are its weight (dm)g, where g is the acceleration due to gravity, contact forces parallel with the surface from adjacent elements dR, the friction force dF, and the normal force dN. If the local coefficient of friction μ is defined as a function of its position on the contact surface S, the average coefficient of friction μ_{avg} is then

$$\mu_{avg} = \tan\theta = \frac{\int_S dF}{\int_S dN} = \frac{\int_S (dm)g\cos\theta\,\mu}{\int_S (dm)g\cos\theta}$$

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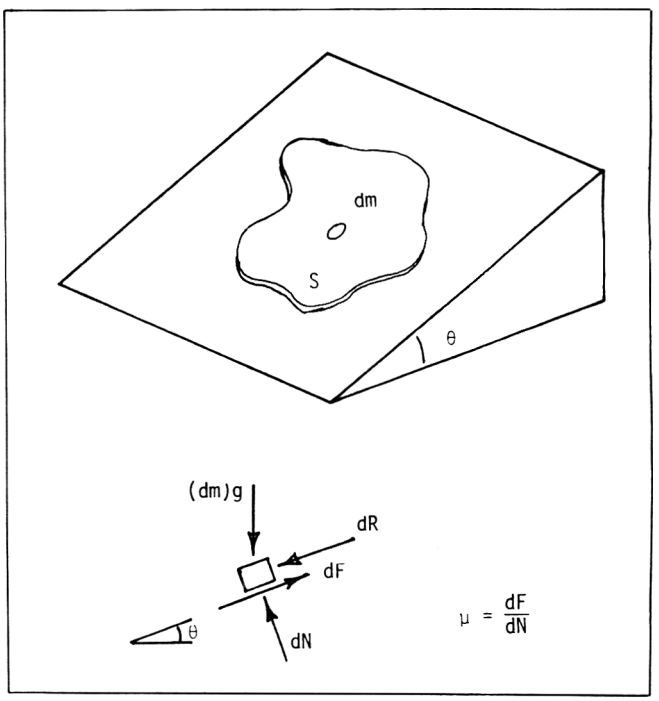

Fig. 1—The model.

where the integration is taken over the surface S. The quotient reduces to

$$\mu_{avg} = \frac{\int_S \mu dm}{\int_S dm} = \frac{\int_S \mu dm}{m} \qquad (1)$$

where m is the total mass of the body. Note that the resulting expression, Eq (1), is analogous to the definition of the location $\bar{x}$ of the center of mass of a body.

$$\bar{x} = \frac{\int x\,dm}{\int dm}$$

where x is the location of the differential mass dm. The centroid of an area is a similar relationship

$$\bar{x} = \frac{\int x\,dA}{\int dA}$$

For a body having n distinct parts Eq (1) can be rewritten as

$$\mu_{avg} = \frac{\Sigma_i \mu_i m_i}{\Sigma_i m_i}, \quad i = 1,n \qquad (2)$$

Figure 2 contains an example with three parts (n = 3).

In addition to the general form of Eq (1) and (2) note that the average coefficient of friction (impending motion angle) is a direct function of the products of the coefficients of friction and the masses of the individual portions of the body. In addition, a composite body with various friction coefficients necessarily will have parts with values of μ both above and below the average value.

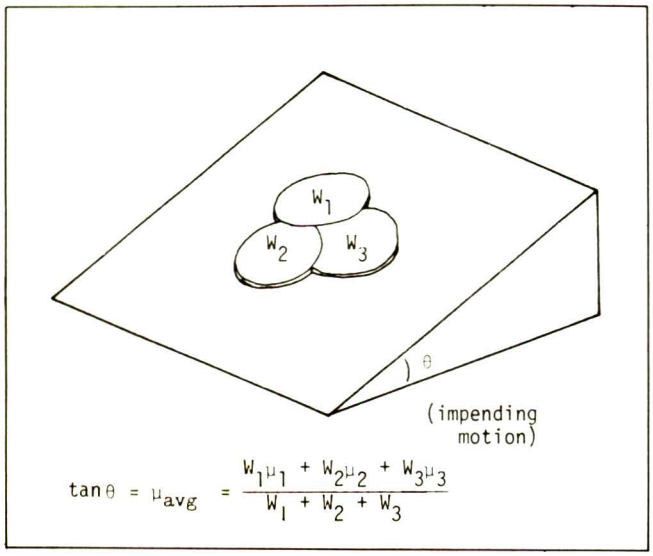

Fig. 2—Three-part example.

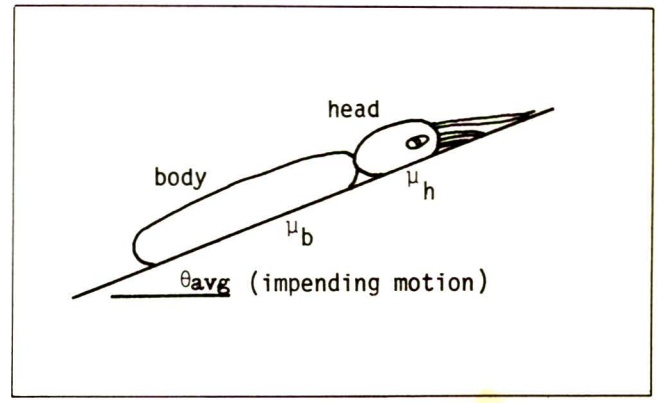

Fig. 3—Squid example.

RESULTS & DISCUSSION

FOR THE SQUID PROBLEM impending motion of the whole squid, as illustrated in Figure 3, can be investigated using Eq (2) giving

$$\tan\theta = \mu_{avg} = \frac{\mu_b m_b + \mu_h m_h}{m_b + m_h} \qquad (3)$$

Brooks (1977) reports the following average values corresponding to the nomenclature in Figure 3 and Eq (3)

$$\begin{aligned} \mu_{avg} &= 0.46 \\ \mu_b &= 0.234 \\ \mu_h &= 1.03 \\ m_b &= 41.67g \\ m_h &= 15.57g \\ m_b + m_h &= 57.1g \end{aligned}$$

Data from 21 squid were averaged to obtain these values and Brooks used the approach mentioned at the beginning of this note. Any one of the three values of μ in Eq (3) could be the unknown of interest. To check Brooks' data, Eq (3) gives

$$\mu_{avg}(m_b + m_h) \stackrel{?}{=} \mu_b m_b + \mu_h m_h$$

$$0.46(57.1) \stackrel{?}{=} 0.234(41.67) + 1.03(15.57)$$

$$26.27 \approx 9.75 + 16.04$$

There is not an error in these values; the coefficient of sliding friction can be greater than one. The relationship between friction force F, normal force N, and coefficient of sliding friction μ is

$$F = \mu N$$

which indicates that a coefficient of friction greater than one will result if $F > N$.

CONCLUSION

THE ECONOMICS of time and money often cause food handling and processing equipment to be designed primarily from experience and intuition rather than experimentation and analysis. The center-of-mass-like Eq (2) developed directly from the principles of mechanics, not only can be used as a direct analytical tool for problems like Brooks' (1977) squid machine design, but also can be used to more accurately estimate, for example, the slope of a surface on which a multi-parted commodity will slide, when one is forced to design by intuition and estimation. The form of Eq (2) allows easy determination of the influence and the relationship of the parameters of the system, masses and friction coefficients, as illustrated with Brooks' data.

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A Research Note

DETERMINATION OF HEXOSAMINES IN CHITIN BY ION-EXCHANGE CHROMATOGRAPHY

J. D. HUBBARD, L. M. SEITZ and H. E. MOHR

ABSTRACT

An ion-exchange chromatography method is described for the determination of glucosamine and galactosamine hydrolyzed from fungal chitin. The method was designed to handle relatively large samples and to minimize the volume of hydrolyzate needed to be evaporated. Final extracts were analyzed on an automatic amino acid analyzer programmed to inject samples alternately onto two columns. Analysis time on the analyzer was 30 min per sample. As little as 30 μg of glucosamine per gram of sample was detected.

INTRODUCTION

ESTIMATES of the degree of fungal invasion in plant tissue could be made if the amount of fungal material present were known; and such estimates have been made, based on the analysis of chitin produced during the fungal growth. Chitin, a polymer of N-acetyl-D-glucosamine found in spores and mycelium of fungi, is usually measured as its hydrolysis product, glucosamine.

Generally, the analysis is carried out colorimetrically (Van de Loo, 1976; Ride and Drysdale, 1972; Donald and Mirocha, 1977) or chromatographically (Wu and Stahmann, 1975; Zacharius, 1976). A colorimetric determination of chitin is not specific for glucosamine, and substances present in plant and seed hydrolyzates often interfere. Donald and Mirocha (1977) used a colorimetric method to estimate the degree of fungal invasion in corn and soybean seeds. Wu and Stahmann (1975) determined chitin as glucosamine, using an amino acid analyzer with a single ion-exchange column 18 cm long and a buffer flow rate of 45 ml/hr. Zacharius (1976) reported a method for the separation and determination of glucosamine and galactosamine, following the removal of interfering substances, in plant glycoprotein that was similar to the method of Wu and Stahmann. Ion-exchange chromatography has the advantage of providing sensitive and specific quantitation of hexosamines from chitin hydrolyzates, which consist mainly of glucosamine, but also a small amount of galactosamine.

We describe a method for determining chitin by ion-exchange chromatography. The method is faster and more convenient than methods previously reported, includes the use of an automated amino acid analyzer, and incorporates some of the techniques reported by Wu and Stahmann (1975). We have used this method to measure fungal components as an indicator of fungal invasion in grains.

MATERIALS & METHODS

Instrumentation and columns

The analysis was performed with a Beckman Model 121 automated amino acid analyzer equipped with a linear absorbance spectrophotometer, a computing integrator and two 9 × 125 mm columns packed with PA-35 spherical resin supplied by Beckman Instrument Co. The resin is a sulfonated styrene copolymer, nominally 7.5% crosslinked with a particle size 16 ± 6 microns mean diameter (Benson et al., 1966).

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Standard hexosamines

D–(+) glucosamine hydrochloride was purchased from Eastman Kodak Company, Rochester, NY and D–(+) galactosamine hydrochloride from Sigma Chemical Company, St. Louis, MO.

Sample preparation

Ten grams of grain sample with fungal growth were weighed into a 250-ml flat bottom, short-neck, boiling flask with an outer 24/40 standard-taper ground-glass joint. The flask was equipped with a Teflon sleeve for the joint to prevent sticking and a 24/40 standard-taper adapter containing a 2-mm Teflon-plug stopcock. The sample was mixed with 40 ml of 6N HCl, and the flask was flushed with nitrogen. Then, the adapter was inserted, the flask evacuated and the stopcock closed. The sample was hydrolyzed for 3 hr at 110°C in an autoclave.

After hydrolysis, the sample was vacuum filtered with a glass fiber disc. The filter was washed with distilled water, and the filtrate diluted with distilled water to 250 ml in a volumetric flask. An appropriate size aliquot (5 ml) was pipetted into an 18 × 150 mm test tube and evaporated to dryness on a 10-sample rotary evaporating apparatus (Hubbard and Finney, 1976). The residue was diluted to an appropriate volume (usually 5 ml) with 0.2N sodium citrate buffer (pH 2.2) and filtered with a 0.22-micron membrane filter before analysis.

The analysis

The automatic sample injector of the amino acid analyzer was

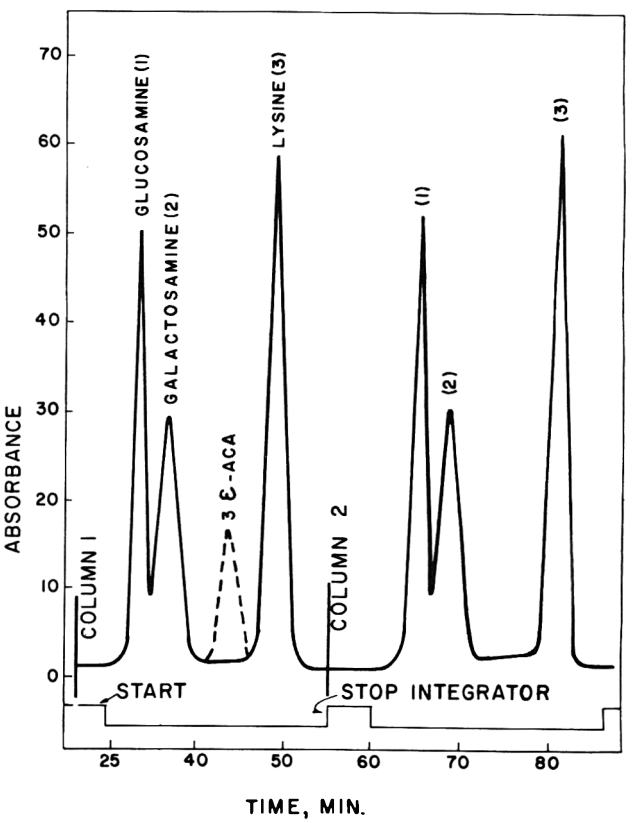

Fig. 1—Standard glucosamine (1), galactosamine (2), and lysine (3) retention times vs absorbance. The concentration of each compound was 0.5 μmole/ml. Column 1 and column 2 indicate points where column switching occurred. Start and stop refers to automatic integration of peak areas.

programmed to inject sample extracts alternately onto the two columns every 30 min. Glucosamine and galactosamine were eluted from the column before lysine (Fig. 1) by 0.35N sodium citrate buffer (pH 5.26) at 70 ml/hr. Column temperature was 52°C and ninhydrin flow rate was 35 ml/hr. Peak areas were determined with a computing integrator. The integrator was standardized with glucosamine and galactosamine solution at a standard concentration of 0.5 μmole/ml.

RESULTS & DISCUSSION

FIGURE 1 SHOWS a typical separation of glucosamine, galactosamine and lysine standards. Glucosamine was eluted 30 min after the sample injection. The analyzer was programmed to integrate peak areas during the elution of the hexosamines, the beginning and the end of the integration period being indicated by an event marker as shown in Figure 1. Peak-to-valley separation of the two hexosamines was about 80%, which was adequate for quantitation, especially considering that glucosamine is the predominate component in chitin. While mannosamine was reported to be eluted with galactosamine on a similar column (Zacharius, 1976), it is reportedly not found in significant amounts in plant materials.

If an internal standard is desired, ϵ-aminocaproic acid could be used because its elution peak is midway between galactosamine and lysine peaks (Bates, 1971).

Figure 2 is a typical chromatogram for a sample of milled whole rice contaminated with *Aspergillus flavus*. The fungus was allowed to grow for 10 days under conditions of 30% moisture and 25°C. Glucosamine concentration was 6.4 mg/g, whereas galactosamine concentration was quite low (<0.2 mg/g). Relatively low background eliminated the need for pre-column clean-up such as that described by Zacharius (1976). Filtration with a 0.22-micron membrane prior to loading the sample into the automatic sample injector removed suspended material which would have plugged the top of the column. Glucosamine levels as low as 0.03 mg/g could be detected in the milled rice substrate before initiation of fungal growth. Analysis of milled rice with *Alternaria alternata* growth gave chromatograms very similar to those shown in Figure 2. At present, we are also investigating chitin production by other fungi usually found on grains.

A sample of 10g was chosen to insure a representative sample of chitin from each fungus culture. It is suggested that chitin hydrolysis may not be complete and that some decomposition of glucosamine may occur. Therefore hydrolytic conditions were selected to maximize recovery of glucosamine (Wu and Stahmann, 1975). Duplicate analyses showed excellent agreement; each result was usually within ±3% of the average. Excellent agreement amoung duplicates held true even though glucosamine levels ranged from 0.030 mg/g in uninfested rice to 26 mg/g in rice heavily infested by *A. flavus*.

Instead of the hydrolysis apparatus of Wu and Stahmann (1975) we used commonly available glassware with a standard 24/40 ground glass joint. A Teflon sleeve was necessary to prevent the joint from sticking. The glassware with 24/40 ground glass joints, grooved for an O-ring, discussed by Wu and Stahmann, is apparently not available from most commercial suppliers.

Also, rather than evaporating the entire hydrolysis mixture to remove excess acid according to Wu and Stahmann (1975), we found it easier and faster to filter the hydrolyzed mixture, adjust the filtrate to known volume, and analyze an aliquot. The aliquot (usually 5 ml) was small enough that 10 samples at a time could be evaporated on a rotary evaporating apparatus developed for routine handling of samples for amino acid assays (Hubbard and Finney, 1976).

Analysis time per sample on the analyzer (30 min) was much less than the time required by the Wu and Stahmann method. Time was saved by using shorter columns, higher flow rate, and by alternating injections onto two columns. Alternating columns allowed us to shorten the analysis time by 50%. After each analysis the ion-exchange column must be washed with 0.2N sodium hydroxide, to remove bound substances, and regenerated with 0.35N sodium citrate buffer (pH 5.26). By alternating columns, regeneration of one column can be performed while sample separation is proceeding on the other column.

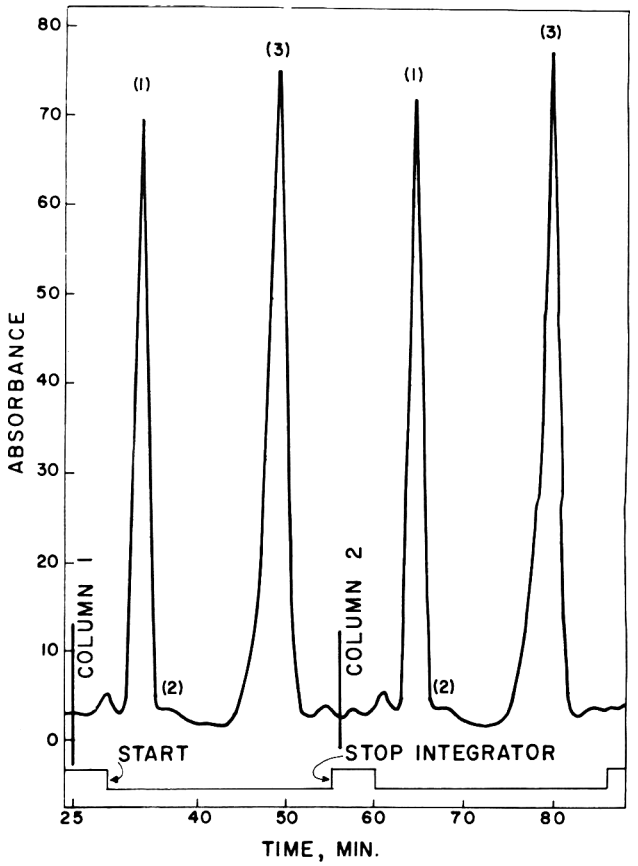

Fig. 2—Chromatogram for a sample representing 10 day's growth of Aspergillus flavus on milled rice at 30% moisture and 25°C. (1), (2) and (3) refer to glucosamine, galactosamine and lysine retention times vs absorbance. Column 1 and column 2 indicate points where column switching occurred. Start and stop refers to automatic integration of peak areas.

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Mention of specific instruments or trade names is made for identification purposes only and does not imply any endorsement by the U.S. Government.

A Research Note

THERMOSTABILITY OF RED BEET PIGMENTS (BETANINE AND VULGAXANTHIN–I): INFLUENCE OF pH AND TEMPERATURE

I. SAGUY

ABSTRACT

The thermostability of red beet pigments (betanine and vulgaxanthin–I) was investigated in beet juice under atmospheric conditions at 61.5, 75.5 and 85.5°C and at pH range 4.8–6.2. The degradation of both pigments followed first-order reaction kinetics. The energies of activation were 19.2 ± 0.5 and 16.3 ± 0.6 Kcal/mole for betanine and vulgaxanthin–I respectively, and were independent of pH. Maximum thermostability was observed at pH 5.8 for both pigments. In beet juice under atmospheric conditions vulgaxanthin–I was more sensitive than betanine.

INTRODUCTION

AS A CONSEQUENCE of intensified safety awareness and restrictions imposed on synthetic color compounds for use in foods, considerable attention has been focused in recent years on natural colorants. Only recently (Anon., 1978), the National Cancer Institute released a report which concluded that p-cresidine, basic to the manufacture of FD&C Red No. 40, one of the two (FD&C red No. 40 and No. 3) remaining red food colors on the GRAS list, is carcinogenic in the rats and mice in the NIC's bioassay program.

Red and golden cultivars of beet root appear to be one of the most logical sources of water-soluble colorants. The color of beet consists of two major pigments betanine (red) and vulgaxanthin–I (yellow).

The effect of pH on the stability of these pigments has been studied in a variety of conditions and processes, such as: sterilization (Habib and Brown, 1956; Lusas et al., 1960); food products (von Elbe et al., 1974a); solutions (Kopelman and Saguy, 1977; Peterson and Joslyn, 1960; Sapers and Horenstein, 1978; Saguy et al., 1978a; Savolainen and Kuusi, 1978; von Elbe et al., 1974b); extraction in air or nitrogen (Wiley and Lee, 1978; Wiley et al., 1979). However, the reported data are in some cases difficult to evaluate because factors such as enzymatic activity, dilution effect, oxygen concentration, pH, cultivar, and light were not always considered.

This paper presents data on pH and temperature effect on betanine and vulgaxanthin–I thermostability in beet juice.

EXPERIMENTAL

BEET JUICE was obtained by homogenizing blanched beet slices in a Waring Blendor with an equal weight of McIlvaines 0.1M buffer solution of various pH ranging from 4.8–6.2 at room temperature. The slices were blanched in steam for 4 min to prevent enzymatic activity. The juice was filtered (Whatman No. 1) and 20 ml were filled into vials. The unsealed vials were placed in a thermostatically controlled (± 0.1°C) shaking water bath, for the heat treatment exposure. The vials (triplicates) were withdrawn periodically and cooled down immediately in an ice water bath. The samples were further diluted with the appropriate pH buffer to allow direct absorbance measurements within the range 0.2–0.8 optical density. Color content was analyzed according to the method described by Saguy et al. (1978b).

Regression coefficient determined on the kinetic data describing the pigment losses were obtained by BMD O2R (Dixon, 1971).

RESULTS & DISCUSSION

THE CONCENTRATION of the retained betanine and vulgaxanthin–I monitored in the beet juice under atmospheric conditions exposed to various heat treatments and time, generated a straight line when plotted on a semilogarithmic scale, for each pH tested, indicating a first-order reaction for both pigments studied. The first order kinetics are in agreement with previously reported data (von Elbe et al., 1974b; Sapers and Hornstein, 1978; Savolainen and Kuusi, 1978; Saguy et al., 1978a). The first-order reaction kinetics permitted reaction rates to be expressed alternatively in terms of half-life values, $T_{1/2}$.

Temperature and pH effects on betanine and vulgaxanthin–I thermostability are illustrated in Tables 1 and 2 respectively. Maximum stability was observed at pH 5.8 for both pigments. Previous estimates of optimal pH were reported as "between 5 and 6" (von Elbe et al., 1974b; Savolainen and Kuusi, 1978).

Temperature effect on pigments lability is expressed by the energy of activation or alternatively by the "so called" Q_{10} value. Energies of activation showed different values for the two pigments, (Tables 1 and 2), e.g. 19.2 ± 0.5 and 16.3 ± 0.6 Kcal/mole for betanine and vulgaxanthin–I, respectively. The pH effect within the range 4.8–6.2 was negligible. The energy of activation for betanine is substantially higher than reported by von Elbe et al. (1974b) (at pH 5), e.g. 12.5 ± 2.0 and 10.0 ± 2.0 Kcal/mole in model system and beet juice, respectively. Sapers and Hornestein (1978) also reported betanine degradation rate constant at 25°C and pH 5 which was significantly lower than reported by von Elbe et al. (1974b) (e.g. 0.066 vs 0.88 day^{-1}). A possible reason for the discrepancies is betanine decoloration due to oxidation, which is particularly important at relatively low temperatures and long times of exposure. Under these conditions differences in dissolved oxygen concentration can furnish an explanation of the differences in observed rates. Moreover, the excellent agreement found when comparing the half-life time obtained at 75.5°C with values reported recently by Pasch and von Elbe (1979) supports the assumption, that discrepancies exist mainly at low temperatures and are due to differences in oxidative decoloration.

Unblanched beet juice under atmospheric conditions and moderate temperatures is very susceptible to enzymatic decoloration. The activity (polyphenol oxidase) was the topic of numerous studies (Lee and Smith, 1978; Soboleva et al., 1976; Viner, 1977). Results indicated a maximum enzyme potency in the range 25–43°C; however, residual activity was noted even after a relatively high temperature long time exposure (Lee and Smith, 1978; Wiley et al., 1979). Betacyanines are more susceptible to this enzymatic decoloration than betaxanthines (Kopelman and Saguy, 1977; Wiley et

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Table 1—Effect of temperature and pH on betanine thermostability in beet juice (k_b first order rate constant, min^{-1}; $T_{1/2}$ half life, min)

Temperature (°C)	pH 4.8		pH 5.2		pH 5.8		pH 6.2	
	$k_b \times 10^3$	$T_{1/2}$	$k_b \times 10^3$	$T_{1/2}$	$k_b \times 10^3$	$T_{1/2}$	$k_b \times 10^3$	$T_{1/2}$
61.5	6.3	110.1	5.6	124.5	4.5	154.3	5.5	126.6
75.5	24.3	28.5	16.5	41.9	14.6	47.4	16.8	41.2
85.5	40.5	17.1	37.4	18.5	32.0	21.7	40.5	17.1
100.0[a]	113.0	6.1	98.0	7.1	94.6	7.3	117.7	5.9
Energy of activation (Kcal/mole)	18.8		18.8		19.6		19.7	
Q_{10} (70–80°C)	2.19		2.19		2.27		2.27	

[a] Calculated

Table 2—Effect of temperature and pH on vulgaxanthin–I thermostability in beet juice (k_v–first order rate constant, min^{-1}; $T_{1/2}$ half-life min)

Temperature (°C)	pH 4.8		pH 5.2		pH 5.8		pH 6.2	
	$k_v \times 10^3$	$T_{1/2}$	$k_v \times 10^3$	$T_{1/2}$	$k_v \times 10^3$	$T_{1/2}$	$k_v \times 10^3$	$T_{1/2}$
61.5	11.9	58.2	9.5	72.6	8.8	78.6	9.1	76.5
75.5	34.1	20.4	25.1	27.8	23.4	29.7	24.3	28.5
85.5	56.0	12.4	49.7	13.6	45.6	15.4	49.3	14.0
100.0[a]	133.7	5.2	120.4	5.8	114.6	6.1	123.9	5.6
Energy of activation (Kcal/mole)	15.5		16.3		16.5		16.8	
Q_{10} (70–80°C)	1.91		1.97		1.99		2.01	

[a] Calculated

al., 1979) while the opposite behavior was observed in blanched juice (Tables 1 and 2).

The energy of activation values for vulgaxanthin–I were slightly affected by the beet juice pH (Table 2), however, the difference observed was not significant. The calculated values at 100°C of vulgaxanthin–I degradation rate constant, k_v, and the half-life, $T_{1/2}$, are in general agreement with previously reported values for purified pigment solutions (Savolainen and Kuusi, 1978) but differ substantially from those reported for beet juice. This discrepancy may be to some extent attributed to different atmospheric conditions (air vs nitrogen).

It might be worth emphasizing that substantial thermostability differences were found in beet pigments as affected by different systems (solution, beet juice and slices), water activity, atmosphere (oxygen, nitrogen), pH, light, beet variety, organic acids, metals and other factors. Thus, special attention is required when data are compared or used. Moreover, further research is required in order to furnish some explanation to the forementioned phenomena.

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A Research Note

LOW OXYGEN RECYCLED VAPOR (LORV) FOR FOOD DRYING

J. P. MORGAN, E. L. DURKEE and J. R. WAGNER

ABSTRACT

An exploratory study has been made where drying was done by reheating the vapors of evaporation which were then recycled with the combustion gasses through a partially closed system containing a low oxygen atmosphere. Fresh carrots dried in this manner were simultaneously dried and blanched and appeared to have more flavor and color and a more natural texture compared to conventionally air-dried carrots when reconstituted. Drying temperatures greater than 143°C throughout drying caused heat damage to the drying product, but higher temperatures may be possible in the initial drying stages by replacing the batch system used in this investigation with a two-stage continuous system.

INTRODUCTION

One of the more successful attempts to improve on conventional methods of food dehydration has been the belt-trough through flow dryer developed at the Western Regional Research Center (Lowe et al., 1955). The 149°C dehydration temperature of the belt-trough dryer approached the lowest transition point (170°C) for superheated steam reported by Yoshida and Hyodo (1970); a practical application of superheated steam would be desirable because of the heat economy it offers (Lane and Stern, 1956).

Success with the belt-trough dryer stimulated the idea of a dryer design using recycled vapors of dehydration in a partially closed system. Also, because oxidation is one of the causes of nutrient losses during food processing (Harris, 1975), a drying atmosphere containing minimal oxygen could be an aid in the retention of greater nutritive values for dried foods.

This is a report of the exploratory study made of low oxygen, recycled vapor drying.

MATERIALS & METHODS

THE EXPERIMENTAL DRYER for low oxygen, recycled vapor (LORV) drying was a wire mesh rotary cage enclosed within a sheet metal cabinet, a gas-fired combustion chamber located at the inlet of a squirrel cage blower, and duct work for directing the flow of heated vapor from the blower to the rotating cage and returning the vapors of evaporation to the combustion chamber for reheating (Fig. 1). Entrance to the cabinet for loading food material to be dried was through a removable cover held in place by fast opening latches.

The recycled vapors were heated to the drying temperature by direct contact with the combustion flame of the burner. Natural gas at 5 psig and compressed air at house pressure (80 psig), each monitored by calibrated flowrators to maintain the proper gas/air ratio, were delivered to the combustion chamber through a special spray nozzle for premixing before entering the burner. The burner design provides almost complete combustion and only relatively small amounts of oxygen (less than 1%) are present in the combustion gasses.

To prevent air leakage into the partially closed system, the unit was operated with a slight positive pressure maintained by discharging the excess vapors through 5/8-inch copper tubing at the bottom of the cabinet into a vessel of water with the discharge about 2-inches below the water surface. A moisture trap in the exhaust line condensed the water and cooled the exhaust stream ahead of a Beckman Model 777 oxygen analyzer (polarographic type) which measured the oxygen content in the drying chamber.

Four thermocouples inserted in the ductwork of the dryer monitored air temperatures: one, located in the vapor return inlet to the combustion chamber, measured the temperature of the recycled vapors before reheating; one, located in the discharge duct from the blower, measured the temperature of the reheated vapor just prior to entering the rotary cage; two more, located in the vapor return line, measured dry and wet bulb temperatures of the vapor leaving the rotary cage. These measurements were recorded on a Brown Electronik recorder.

Approximately 15 lb of fresh carrots were prepared for drying by caustic peeling, dicing (3/8-inch), and sulphiting; moisture content of the fresh diced carrots was 89%. The carrots were dried in 2.2-lb batches. With the cabinet preheated to 143°C and the oxygen content reduced to less than 1%, the blower was turned off, the preloaded rotary cage inserted into the cabinet, and the blower turned on again. Loading required 20 sec; the temperature dropped to 93°C and the oxygen content increased to 6% but recovered to 116°C and 1%, respectively, after 3–5 min drying time. At 40% weight reduction (30 min), the temperature was reduced to 93°C to prevent heat damage. After 2 hr total drying time, drying was discontinued; final weight reduction was 86% and moisture content was 20%.

The experimentally dried carrots were then tested for peroxidase content to determine blanching efficiency and the carotene content was measured since it is known that carotene is easily oxidized (Feinberg, 1973). To ensure adequate storage stability, the moisture content of the LORV-dried carrots was reduced to 2–4% by finish drying overnight in a regular cross-flow dryer at 49°C.

As a control, fresh-diced carrots were blanched and air-dried in a cross-flow dryer for 5 hr at 71°C with drying continued overnight at 49°C until the moisture content was reduced to 2–4%. A quantity of the control and of the LORV- and finish-dried carrots were rehydrated and the reconstituted products subjectively compared with each other and with fresh cooked carrots with regard to their appearance, flavor, and texture.

RESULTS & CONCLUSIONS

AFTER a 2-hr drying time, the LORV-dried carrots had an 86% weight reduction and a moisture content of 20%. Peroxidase tests were negative showing complete enzyme inactivation of the experimentally dried carrots which indicates that simultaneous blanching and drying is feasible. This could effectively eliminate leach losses and the expense of a separate blanching operation. Flavor and texture of the reconstituted LORV plus finish-dried carrots appeared to be more nearly like that of fresh cooked carrots than was the reconstituted air-dried only carrots; greater volatiles retention as a result of drying in a partially closed system having a low purge-to-recycle ratio may account for the increased color and flavor of the experimental product.

Drying temperatures higher than 143°C scorched the carrots, which confirmed results reported in an earlier investigation (Lazar, 1972). However, higher drying temperatures could be used in the first stage of a two-stage continuous LORV design without heat damage to the food being dried; it may be possible to use superheated steam in the first stage (Yoshida and Hyodo, 1966).

The LORV system was found to have the capability of reducing and maintaining the oxygen content inside the

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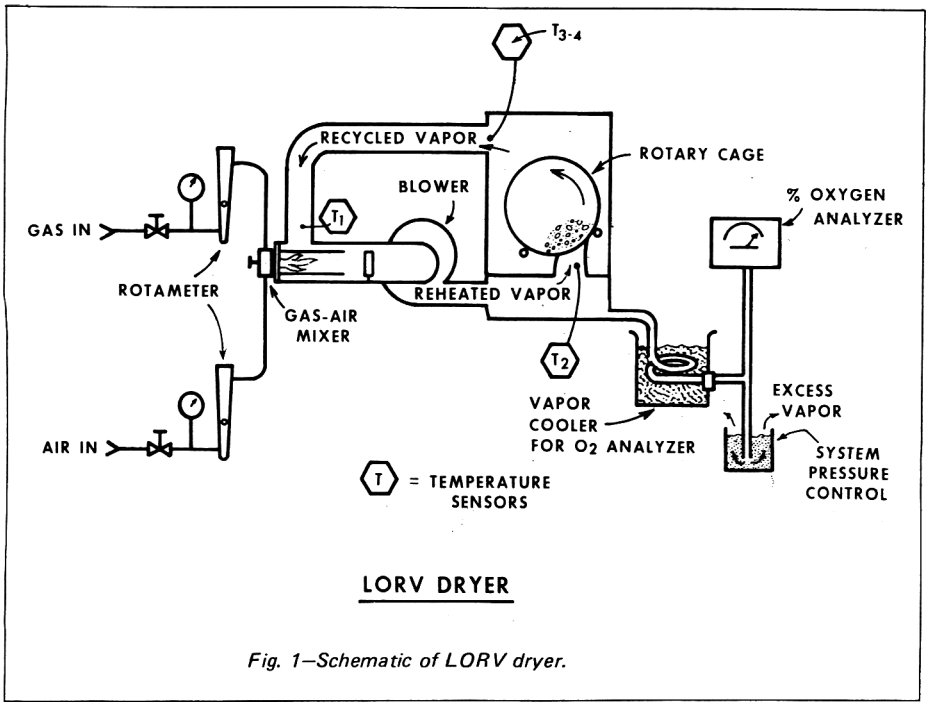

Fig. 1—Schematic of LORV dryer.

system to less than 1% except for the time interval immediately following loading of the dryer; an efficient air-lock design in a continuous drying system would minimize this deficiency. The analysis of β-carotene content showed, on a moisture free basis, about 50 mg/100g for the fresh carrots, 50 mg/100g for the LORV and finish dried carrots, and 80 mg/100g for the conventionally air-dried carrots. The seeming inconsistencies in these data might be explained by the results of an earlier study (Nutting et al., 1970) showing an apparent increased retention of β-carotene in parsley after heating. The possibility that this phenomenon is also occurring in the case of carrots makes it advisable to consider other tests methods to determine the extent of nutrient protection that may be provided by drying in a low oxygen atmosphere.

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Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

A Research Note

EFFECT OF AMMONIATED GLYCYRRHIZIN ON THE MINERAL UTILIZATION OF RATS

LESLIE G. WEST, BECKY J. NONNAMAKER and JANET L. GREGER

ABSTRACT

Administration of ammoniated glycyrrhizin for 14 days in the diets of rats at a 2% level caused a significant increase in iron excretion and a significant depletion in liver iron stores as compared to pair-fed control rats. Zinc and magnesium fecal and tissue levels were not significantly influenced by this dietary saponin. Body and fecal weights were also significantly increased in animals consuming the saponin-containing diet.

INTRODUCTION

AMMONIATED GLYCYRRHIZIN, a saponin obtained from aqueous extracts of licorice root, is added to numerous consumer products ranging from beverages to chewing gum (Life Sciences Research Office, 1974; Federal Register, 1977). This compound is important to the food industry because of its sweetness, its ability to potentiate certain flavors and also its foam stabilization properties (Life Sciences Research Office, 1974). An estimate for average daily adult (2–65+ yr) consumption of ammoniated glycyrrhizin has been reported to be 19 mg (Life Sciences Research Office, 1974); however, this amount can be exceeded with less than 13g of certain soft candies (Life Sciences Research Office, 1974; Federal Register, 1977). Although many biological activities have been attributed to ammoniated glycyrrhizin (Informatics, Inc., 1972), we recently reported the in vitro capacity of this compound to chelate minerals (West et al., 1978). Since it is well known that certain chelators, such as phytate (Oberleas, 1973), can adversely influence mineral availability, we undertook studies to evaluate the potential of ammoniated glycyrrhizin to decrease mineral absorption in vivo.

EXPERIMENTAL

MALE Sprague-Dawley weanling rats (Laboratory Supply Company) were fed purified diets consisting of 63% cornstarch, 20% egg albumen, 8% corn oil, 5% mineral mix (Rogers and Harper, 1965), 2% vitamin supplement (Total Vitamin Supplement, United States Biochemical Corp.), and 2% Alphacel (ICN Pharmaceuticals). The control group (n = 6) was pair-fed to animals (n = 6) receiving a 2% level of ammoniated glycyrrhizin (Dr. Madis Laboratories) which replaced an equivalent amount of cornstarch in the diet. This intake of ammoniated glycyrrhizin parallels that amount necessary to produce other pharmacological effects in the rat (Macabies et al., 1963).

After a 4-day adjustment period feeding a commercial ration (Purina Laboratory Chow), animals were randomly assigned to the two groups. Rats were individually housed in stainless steel metabolic cages and all diets were placed in stainless steel feed cups. Animals were allowed deionized water ad libitum. Rats were fed the diets for 14 days, weighed twice weekly and feces collected from day 7 to day 14. Fecal samples were pooled for each rat, homogenized, and frozen until analyzed. Following a 24-hr fast, rats were sacrificed on day 15 by heart puncture.

The left tibia from each animal was removed, cleansed of all adhering matter, weighed, and frozen until analyzed. The livers were likewise removed, weighed, homogenized, and frozen.

Triplicate samples of feces, tibia, and liver were weighed and dry-ashed overnight at 450°C. One ml of concentrated AR grade nitric acid was then added to each sample before heating for an additional hour at 200°C. Three ml of concentrated AR grade hydrochloric acid were added to each sample and all samples were allowed to stand undisturbed for at least 1 hr. Samples were diluted to volume and analyzed with atomic absorption spectrophotometry (Perkin-Elmer Model 360 atomic absorption spectrophotometer). Standards were prepared by appropriate dilution of 1000 ppm atomic absorption standard solutions (Fisher Scientific Company).

Table 1—Effect of ammoniated glycyrrhizin on growth and fecal mineral losses

Treatment	2% Ammoniated glycyrrhizin	Pair-fed control
Weight gain[b] (g/day)	5.0 ± 0.3*	4.5 ± 0.4
Fecal weight[a] (g/day)	1.0 ± 0.1	0.5 ± 0.03
Fecal Zn (μg/day)	24.6 ± 3.2	18.0 ± 2.4
Fecal Mg (mg/day)	1.5 ± 0.2	1.2 ± 0.1
Fecal Fe[b] (μg/day)	245.1 ± 25.1	143.2 ± 12.4

* Mean ± standard error of mean.

[a] Means are significantly different at the $p < 0.01$ level (student's t test).

[b] Means are significantly different at the $p < 0.05$ level.

RESULTS & DISCUSSION

RATS receiving ammoniated glycyrrhizin were evaluated with pair-fed control rats receiving the basal diet to exclude the possibility that unequal nutrient intake was responsible for differences reported in the saponin-fed group. Liver was analyzed primarily for iron concentration since it is known to be a chief storage depot for iron in the body (Bothwell and Finch, 1962). Recent findings have shown that bone is a potential indicator of zinc status and was thus used to evaluate in vivo zinc changes (Deeming and Weber, 1977). Magnesium is found throughout the body and no one particular tissue is known to be a specific indicator of magnesium status (Aikawa, 1978). Therefore, all tissues collected for iron and zinc determinations were analyzed for magnesium. Fecal samples were analyzed for iron, magnesium and zinc.

The rats fed ammoniated glycyrrhizin were significantly heavier ($p < 0.05$) than the pair-fed rats (Table 1). It is known that glycyrrhizin stimulates the renal tubules to reabsorb water (Gibson, 1978), which could explain the observed weight differences. The ammoniated glycyrrhizin-fed rats did excrete significantly more feces ($p < 0.01$) as compared to controls (Table 1) and also excreted significantly more iron ($p < 0.05$). There was no significant change in the excretion of either zinc or magnesium.

Liver weight was not significantly altered by the administration of ammoniated glycyrrhizin. The iron concentration and total iron content of the livers were significantly lower ($p < 0.01$ and $p < 0.05$, respectively) in rats consuming this substance (Table 2), which is consistent with the

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Table 2—Effect of ammoniated glycyrrhizin on tissue mineral content

Treatment	2% Ammoniated glycyrrhizin	Pair-fed control
Liver weight (% body wt)	3.6 ± 0.3	3.2 ± 0.1
Liver Fe (μg/g)[a]	63.5 ± 3.90	130.7 ± 8.46
(μg/organ)[b]	265.7 ± 22.8	470.2 ± 47.6
Liver Zn (μg/g)	28.9 ± 1.8	31.9 ± 2.3
(μg/organ)	119.0 ± 6.8	110.2 ± 8.1
Liver Mg (μg/g)	213.0 ± 5.7	220.3 ± 14.0
(mg/organ)	0.9 ± 0.05	0.8 ± 0.07
Tibia weight (% body wt)	0.27 ± 0.01	0.26 ± 0.01
Tibia Zn (μg/g)	81.4 ± 3.8	81.0 ± 4.8
(μg/bone)	25.3 ± 1.1	25.0 ± 0.9
Tibia Mg (mg/g)	1.8 ± 0.1	1.9 ± 0.05
(mg/bone)	0.6 ± 0.05	0.5 ± 0.04
Tibia Fe (μg/g)	52.4 ± 6.5	50.5 ± 1.6
(μg/bone)	16.3 ± 2.0	14.7 ± 1.2

* Mean ± standard error of mean.

[a] Means are significantly different at the $p < 0.01$ level (student's t test).

[b] Means are significantly different at the $p < 0.05$ level.

previous observation of increased fecal iron loss for the ammoniated glycyrrhizin-fed rats. Liver, magnesium and zinc concentrations were not influenced by ammoniated glycyrrhizin.

No differences between groups were observed for either tibia weight or tibia mineral concentration (Table 2). This result, coupled with the finding that there was no significant increase in zinc excretion, suggests that zinc availability was not adversely affected by the inclusion of the saponin in the diet. We also observed no significant interference with magnesium absorption as evidenced in the feces or tissues of rats fed the test substance.

In the in vitro studies mentioned previously, we reported that iron was chelated to a greater extent than zinc or magnesium. The in vitro difference, however, between iron and zinc chelation was not nearly so great as observed in the in vivo study. This indicates either the in vitro conditions do not correlate precisely with the proposed in vivo chelation, or other mechanisms to prevent iron absorption by ammoniated glycyrrhizin are operating.

It is already known that consumption of ammoniated glycyrrhizin will cause edema and alter blood pressure in man (Informatics, Inc., 1972; Life Sciences Research Office, 1974). Our experiment suggests this substance may adversely influence human iron status and further studies are needed to fully evaluate this activity with quantities approximating human daily consumption. Additional losses of iron could compound problems in certain populations, such as adolescents and pregnant women, who already have marginal iron stores (Abraham et al., 1974; Radhi, 1978).

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A Research Note

EFFECTS OF ELECTRICAL STIMULATION AND SHROUDING METHOD ON QUALITY AND PALATABILITY OF BEEF CARCASSES

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ABSTRACT

For evaluation of the effects of film overwrap and electrical shock postmortem on carcass quality traits and palatability of meat, 24 sides of beef were assigned to one of four treatments: (1) control–no electrical shock and cloth shroud only; (2) electrical shock and cloth shroud only; (3) no electrical shock and cloth shroud plus PVC film overwrap; and (4) electrical shock and cloth shroud plus PVC film overwrap. Metal pins were placed in the muscle of the round near the achilles tendon and in the muscles between the scapula and the thoracic vertebrae. Carcasses were shocked within 1 hr postmortem and before chilling. Sides were chilled 18 hr at 2–3°C prior to ribbing. Following ribbing and a 15 min bloom time, each side was evaluated for quality grade and yield grade characteristics and scored for heat-ring, color, texture and firmness. Electrical stimulation significantly decreased heat-ring and improved lean color, texture and tenderness. PVC film overwrap contributed little above the effects of electrical stimulation. These data suggested that electrical stimulation significantly decreased the incidence of heat-ring.

INTRODUCTION

SEVERAL WORKERS have reported on the effect of postmortem electrical stimulation on muscle tenderness (Chrystall and Hagyard, 1976; Davey et al., 1976; Savell et al., 1977; Shaw and Walker, 1977; Savell et al., 1978a; Sorinmade et al., 1978). Research suggests that electrical stimulation accelerates postmortem pH decline, hastens rigor development and improves tenderness. Dutson et al. (1979) and Sorinmade et al. (1978) suggested that some portion of the tenderization benefit derived from electrical stimulation, results from enhanced activity of the autolytic enzymes of muscles in treated carcasses. Savell et al. (1978b) suggested that physical disruption of muscle fibers resulting from the massive contractions during stimulation might be a mechanism for the tenderness improvement associated with electrical shock.

Accelerated glycolysis in postmortem muscle could affect certain quality factors such as lean color, texture, firmness and color uniformity. Factors affecting lean color are important to industry in grading and in retail acceptance by the consumer. Savell et al. (1978a) monitored the effects of electrical stimulation on quality-indicating traits of beef. Electrically stimulated sides had brighter colored longissimus muscles and less severe heat-ring formation than control sides (nonuniform color of muscle). Savell et al. (1978a) postulated that the heat-ring was due to a differential rate of chilling and subsequent pH decline in the muscle such that the outside portion of the muscle had an accelerated rate of temperature decline, elevated pH (due to temperature/pH relationship) and dark color. Possibly covering the carcass with an insulative material (such as PVC film) might retard the rate of chill on the outside portion of the muscle and approximate the effect of electrical stimulation. Correspondingly, the objective of this experiment was to evaluate the singular and combined effects of PVC film overwrap and postmortem electrical stimulation on beef carcass quality and palatability.

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EXPERIMENTAL

TWENTY-FOUR SIDES of beef were assigned to one of four treatments as follows: (1) control–no electrical shock and cloth shroud only; (2) electrical shock and cloth shroud only; (3) no electrical shock and cloth shroud plus PVC film; and (4) electrical shock and cloth shroud plus PVC film. Sides were electrically shocked within 1 hr postmortem and before chilling. Metal pins were placed in the muscles of the round near the achilles tendon and in the muscles between the scapula and the thoracic vertebrae. Sides received a 1.5 amp of AC (60HZ) current through the carcass for 3 min with impulses of 5 per min, each of 10 sec duration.

The PVC film overwrap was applied over the outside of the cloth shroud extending from the sirloin and to the third rib. The PVC film was wrapped completely around the side. Sides were chilled 18 hr at 2–3°C prior to ribbing. Following ribbing and a 15 min bloom time, each side was evaluated for quality grade and yield grade characteristics and subjectively scored for heat-ring (15=none, 1=extreme), lean color (8=light grayish-red, 1=very dark red or purple), lean firmness (8=very firm, 1=very soft), lean texture (8=very fine, 1=very coarse); and degree of fat shrinkage away from the rib-eye surface (15=none, 1=extreme). Temperature was recorded for the longissimus muscle (12/13th rib interface) at the time of ribbing. Immediately after ribbings, a 0.60 cm slice of longissimus muscle was removed from the rib-end of the loin and its pH was determined as described by Carse (1973). After 48 hr postmortem, a 15.0 cm section of the posterior end of the rib was removed, frozen and shipped to Texas A&M University (TAMU) for sensory and shear force analyses.

Upon arrival at TAMU, two steaks (2.5 cm in thickness) were cut, double-wrapped in polyethylene-coated paper, frozen and stored (–34°C) for 3 wk. Each steak was removed from the freezer, thawed at 2°C for about 24 hr to an internal temperature of 2°C and broiled on a Faberware broiler to an internal temperature of 70°C (monitored by use of copper constantan thermocouples and a recording thermometer). One cooked steak from each side was evaluated by a 10-member trained (Cross et al., 1978) descriptive sensory panel; the second steak was used for Warner-Bratzler shear force determinations in the manner described by AMSA (1978).

Statistical analyses

Data were treated by analysis of variance as outlined by Snedecor and Cochran (1967) and by the mean separation technique of Scheffé (1959).

RESULTS & DISCUSSION

MEAN VALUES for certain carcass traits are presented in Table 1. Neither shrouding nor electrical shock treatments significantly affected any of the carcass grade traits. In the present study effects of electrical stimulation on USDA grade traits were similar to those Savell et al. (1978c) reported except that they found that electrical stimulation improved lean maturity. It should be noted that paired sides of a given carcass were either assigned to the cloth shroud only or cloth shroud with PVC film overwrap treatments; data can be compared between sides from the same carcass in columns 1 and 3 and in columns 2 and 4 of Tables 1 and 2.

Mean values for certain longissimus muscle and subcuta-

Table 1—Mean values for certain carcass and longissimus muscle traits

Item[a]	Cloth shroud only		Cloth shroud-PVC film overwrap	
	Electric shock	No electric shock	Electric shock	No electric shock
Longissimus muscle area (cm^2)	77.70[g]	78.60[g]	77.30[g]	77.20[g]
Fat thickness, 12th rib (cm)	0.37[g]	0.18[g]	0.38[g]	0.16[g]
USDA Marbling	Small[o g]	Small−[g]	Small[g]	Small+[g]
USDA Lean maturity	A[o g]	A−[g]	A[o g]	A[o g]
USDA Quality grade	Choice−[g]	Choice−[g]	Choice−[g]	Choice+[g]
USDA Yield grade	2.60[g]	2.00[g]	2.60[g]	2.00[g]
Longissimus muscle pH	5.8[g]	5.9[g]	5.8[g]	5.7[g]
Longissimus muscle temperature (°C)	3.5[g]	1.8[g]	5.2[g]	3.8[g]
Longissimus muscle heat-ring[b]	6.5[g]	12.3[h]	6.3[g]	10.3[h]
Longissimus muscle firmness[c]	5.8[g]	6.2[g]	5.8[g]	6.2[g]
Longissimus muscle color[d]	4.2[g]	2.8[h]	5.2[g]	4.3[g]
Longissimus muscle texture[e]	6.7[g]	4.5[h]	6.7[g]	4.7[h]
Longissimus muscle shrink[f]	2.2[h]	5.7[g]	2.2[h]	3.3[gh]
Subcutaneous fat shrink[f]	3.5[h]	3.5[h]	4.8[gh]	6.3[g]

[a] = All traits were evaluated and all measurements were obtained at 18 hr postmortem.
[b] = 15=extreme heat-ring; 1=no heat-ring.
[c] = 8=very firm; 1=very soft.
[d] =8=light grayish-red; 1=very dark red.
[e] = 8=very fine; 1=very coarse.
[f] = 15=no shrink; 1=extreme shrink.
[gh] = Means in the same row bearing a common superscript letter are not significantly different ($P < 0.05$).

Table 2—Mean values for cooking loss, sensory panel ratings and shear force values

Item	Cloth shroud only		Cloth shroud-PVC film overwrap	
	Electric shock	No electric shock	Electric shock	No electric shock
Cooking loss (%)	31.7[d]	29.4[d]	30.2[d]	28.7[d]
Juiciness rating[a]	5.0[d]	4.7[de]	4.8[d]	4.4[e]
Muscle fiber tenderness rating[b]	4.9[de]	3.8[f]	5.0[d]	4.0[ef]
Amount of connective, tissue rating[c]	6.3[d]	5.8[d]	6.4[d]	5.8[d]
Overall tenderness rating[b]	4.9[de]	3.8[f]	5.0[d]	4.1[ef]
Shear force value (kg)	5.4[d]	7.0[e]	5.8[d]	7.0[e]

[a] = 8=extremely juicy; 1=extremely dry.
[b] = 8=extremely tender; 1=extremely tough.
[c] = 8=no panel-detectable connective tissue; 1=abundant amount of connective tissue.
[def] = Means in the same row bearing a common superscript letter are not significantly different ($P < 0.05$).

neous fat characteristics are also presented in Table 1. Neither pH nor temperature of longissimus muscles was significantly ($P > 0.05$) affected by treatment, but longissimus temperature tended to be higher in those sides that were overwrapped with PVC film. Apparently longissimus muscles in all groups had reached their ultimate pH at time of ribbing. These results confirm those of Ray et al. (1978) who reported on the effects of carcass insulation on pH decline, temperature and palatability.

Incidence of heat-ring was significantly ($P < 0.05$) reduced by use of the electrical shock treatment (Table 1). This confirms research reported by Savell et al. (1978a,c). Heat-ring formation was slightly, but not significantly, reduced in sides that were overwrapped with PVC film and not electrically shocked. But the combination of electrical shock and PVC film overwrap was no more effective in reducing heat-ring formation than was use of electrical shocking alone. Lean color was significantly improved by use of electrical shock with cloth shroud only and by use of PVC film overwrap in sides that were not given electrical shock. Texture of the longissimus muscle was significantly improved in sides that were electrically stimulated, but not by use of PVC film overwrap.

Two anomalies that normally accompany heat-ring formation are longissimus muscle shrink (muscle surface is pulled downward toward the neck) and subcutaneous fat shrink (fat cover is pulled downward toward the neck). Electrical shock had no significant effect on ratings for subcutaneous fat shrink (Table 1) whereas the PVC film overwrap significantly reduced ($P < 0.05$) fat shrink on sides that were not electrically stimulated. Neither PVC film overwrap nor electrical shock reduced longissimus muscle shrink but apparently tended to increase it.

Mean values for cooking loss, sensory panel ratings and shear force values are presented in Table 2. Cooking losses tended to be slightly higher in steaks from carcasses that were electrically stimulated suggesting that possibly the increased rate of pH decline decreased water-holding capacity, but the differences in cooking loss were not consistent enough for statistical significance. Steaks from electrically stimulated sides were significantly ($P < 0.05$) more tender than were those from sides that were not electrically stimulated in comparisons of muscle fiber tenderness ratings, overall tenderness ratings and shear force values for sides that had common shroud treatments. Steaks from sides that were not electrically stimulated and were overwrapped with PVC film were rated as high in tenderness as steaks from sides that were electrically stimulated and covered with

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A Research Note

AMINES IN FERMENTED FISH PASTE

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ABSTRACT

The following amines were tentatively identified in fermented fish paste: ethanolamine, 2-methylbutylamine, 2-mercaptoethylamine, 2-phenylethylamine, cadaverine, tyramine, dopamine, octopamine, tryptamine, and histamine. The concentration of these amines ranged from 0.5–64 mg/100g. Histamine and 2-phenylethylamine were the major amines found with maximal amounts of 64.0 and 60.0 mg/100g, respectively.

INTRODUCTION

FERMENTED FISH PASTE is a condiment for rice dishes eaten almost everywhere in Southeast Asia (van Veen, 1953). Traditionally, it is made from small fish or shrimp. The preparation of the paste involves mixing with salt (10–15%), sun drying, kneading to form a paste, and fermentation at ambient temperature for several weeks. Microorganisms responsible for the fermentation originate from the fish, the salt and the environment. Since many microorganisms contain amino acid decarboxylases (Boeker and Snell, 1972), the fermented fish paste is likely to contain amines.

Some of the amines are toxic, especially to patients treated with monoamine oxidase inhibitory drugs (Horwitz et al., 1964; Walker, 1965). Tyramine and 2-phenylethylamine are known to precipitate migraine headaches in susceptible subjects (Hanington, 1967; Sandler et al., 1974). Furthermore, in the presence of nitrite salts, amines may form carcinogenic nitrosamines (Warthesen et al., 1975; Magee et al., 1976).

EXPERIMENTAL

SAMPLES of six different brands of commercial fermented fish paste were purchased from local stores. The products had been imported from Malaysia, Thailand and the Philippines. Two were prepared from anchovies, two from shrimp, one from prawn and one from oysters. The contents of five cans or jars from each brand were thoroughly mixed and, after a series of treatments previously described by Silverman and Kosikowski (1956), an ethyl ether extract of the amines of the fish paste was obtained.

The ether extract was dried in a vacuum oven at 50°C and the residue, which contained the amine hydrochlorides corresponding to 3g of original sample, was mixed with 1 ml trifluoroacetic anhydride and 1 ml anhydrous ethyl ether. The mixture was agitated in a closed tube until the residue was dissolved (ca 2 hr). The solution was evaporated by bubbling nitrogen through it. The N-trifluoroacetyl derivatives of the amines were dissolved in anhydrous ethyl ether and 5 μl aliquots were analyzed using a Perkin-Elmer 900 gas chromatograph with a flame ionization detector. Good separation of the amine derivatives was achieved in a 6 ft × 0.125 in. o.d. column of 3% SP-2100 on 100/200 mesh Supelcoport (Supelco, Inc.). The column was conditioned at 275°C for 4 hr, the injection port was heated at 235°C, the flame ionization detector at 250°C and the column temperature was programmed from 60 to 240°C at 8°C per min. Nitrogen was used as a carrier gas at a flow rate of 18.5 ml/min. Thirteen authentic amines were used for identification of the fish paste amines. For quantitative determination, authentic amines were separately dissolved in water at various concentrations and subjected to the entire analytical procedure applied to the paste samples (ether extraction, derivatization, chromatography). Reference curves relating gas chromatographic peak heights to concentration were prepared for each authentic amine and the curves were used for estimating the concentration of the corresponding amines

Table 1—Corrected retention times and concentrations of amines in fermented fish paste

	Corrected ret. time (min)	Concentration,[c] mg/100g					
		Sample 1 (Anchovy)	Sample 2 (Anchovy)	Sample 3 (Shrimp)	Sample 4 (Shrimp)	Sample 5 (Prawn)	Sample 6 (Oyster)
Ethanolamine	3.73	1.5	ND	4.1	1.9	10.7	ND
2-Methylbutylamine	4.15	0.5	ND	ND	1.3	ND	ND
2-Mercaptoethylamine	6.48	ND[b]	ND	ND	ND	3.5	1.3
2-Phenylethylamine	10.15	1.9	13.0	11.6	4.0	60.0	ND
Cadaverine	13.10	ND	ND	ND	3.5	ND	ND
Tyramine	13.48	3.4	5.4	16.0	8.8	37.6	ND
Dopamine	14.48	1.8	2.8	6.6	ND	30.1	2.6
Octopamine	15.35	1.7	2.7	2.2	0.8	5.0	ND
Tryptamine	17.85	ND	2.3	ND	ND	16.3	4.6
Histamine[a]	–	14.4	40.4	15.9	58.0	64.0	7.8

[a] Determined by fluorometry
[b] ND, not detected
[c] Average of triplicates differing by less than 3% from the mean

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in the samples. Histamine was determined separately by the fluorometric method of Lerke and Bell (1976).

RESULTS & DISCUSSION

THE COMMERCIAL fermented fish paste samples showed chromatographic peaks differing in number from 7 to 17. In total, nine amines were tentatively identified on the basis of gas chromatographic retention times. These amines are listed in Table 1, along with their retention times and concentrations in the fish paste samples. Histamine and 2-phenylethylamine were present in the highest concentrations and 2-methylbutylamine in the lowest. Fermented prawn paste was found to contain the highest concentration of total amines (227.6 mg/100g), while fermented oyster paste contained the smallest amount of total amines (16.3 mg/100g). Large differences in amine concentration were also reported among fermented sausage and cheese samples (Vanderkerckhove, 1977; Koehler and Eitenmiller, 1978). These differences are probably due to variations in raw materials, microflora and conditions of the fermentation.

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cloth shrouds only. Differences in juiciness were significant ($P < 0.05$) but the magnitude of the difference was not of practical importance.

In conclusion, electrical stimulation significantly decreased the incidence of heat-ring and improved ($P < 0.05$) lean color, texture, and tenderness. PVC film overwrap contributed little over and above the effects of electrical stimulation.

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A Research Note

RESTRUCTURED FRESH MEAT CUTS FROM CHILLED AND HOT PROCESSED PORK

D. L. HUFFMAN and J. C. CORDRAY

ABSTRACT

Mechanically tenderized chunks and thin meat slices were combined to form restructured pork chops. A comparison was made among five pork products: 1–restructured pork chop (RPC), no additives; 2–RPC, salt added; 3–RPC, tripolyphosphate (TPP) added; 4–RPC, salt TPP added; and 5–control – boneless pork loin chops. Evaluation of chilled and hot processed pork included tenderness, juiciness, flavor, connective tissue, color, cooking loss, Instron compression and Instron tension. Restructured pork products using the process described have a more desirable eating quality than pork loin chops whether processed from pre-rigor or post-rigor pork. Products containing salt were superior to other restructured products for every variable except color. Color of restructured products was less desirable than loin pork chops. Cooking loss was decreased by the addition of salt alone (for pre-rigor chops) and salt and TPP in combination for both pre- and post-rigor chops.

INTRODUCTION

INTEREST in restructured fresh meat has increased recently due, in large part, to rapid development in the fast-food industry. Forming of restructured meat products has become feasible with the use of hydraulic meat presses that have a wide variety of die shapes.

Considerable work has been done with flaked and formed products since the development of suitable comminution equipment such as the Comitrol (Urschel Laboratories, Valparaiso, IN).

Most of the work done to date with fresh restructured meats has dealt with ground or flaked and formed products. This study deals with meat chunks and slices which give the cooked product a bite and mouth feel more typical of muscle meat than can be obtained from either ground or flaked products. The objectives of this study were (1) to compare sensory properties and Instron evaluation of restructured pre-rigor and post-rigor chops with boneless loin pork chops, and (2) to compare sensory and physical properties of restructured chops containing salt and TPP.

EXPERIMENTAL

Post-rigor study

The raw materials used in this study were obtained from 90 kg market hogs slaughtered at the Auburn University Meats Laboratory. Boneless boston butts were removed from carcasses 5 days postmortem, wrapped with PVC film and frozen at –26°C for 24 hr. After tempering at –4°C for 48 hr, they were sliced to a thickness of 1–2 mm on an automatic slicer.

Hams and sirloin ends were removed from the carcass 24 hr postmortem, boned, then aged an additional 3 days at 2°C. Most of the fat, connective tissue and gristle were removed from the muscle cuts. The cuts were then tenderized 4 times at the 2.54 cm belt advance setting on a Bettcher TR-2 tenderizer to assure total cell disruption and cubed by hand into approximately 2–3 cm^3 chunks. A total of 22.7 kg of each component of the meat block was prepared.

The four restructured treatment groups for both pre- and post-rigor pork were: 1–restructured pork chops (RPC), no additives; 2–RPC, salt (0.75%) and H_2O (2%) added; 3–RPC, tripolyphosphate (TPP) (0.25%) and H_2O (2%) added; and 4–RPC, salt (0.75%), TPP (0.24%) and H_2O (2%) added. Each treatment batch was formulated by placing equal portions of slices and chunks with the appropriate additives in a Hobart horizontal mixer and mixing for 15 min. Ten kilograms of each treatment were prepared in this manner, subdivided into four equal parts, preformed by hand into logs, wrapped with PVC film and placed in a sharp freezer until the internal temperature reached –26°C. The logs were tempered at –4°C for approximately 48 hr and removed when the internal temperature reached –4°C. The logs were unwrapped and pressed at 344 Newtons/cm^2 on a Bettcher hydraulic meat press using a 350 die (pork chop shape). The formed logs were sliced 2 cm thick using a Model 39 Bettcher power cleaver with a 20° blade. Boneless pork loin chops from the same hogs provided the 5th treatment group and were sliced the same thickness (2 cm) on the power cleaver. All chops were placed four to a package in plastic foam trays, PVC overwrapped and held 3–4 days at –15°C for subsequent sensory panels and Instron evaluation.

Color was evaluated on two chops from each treatment group on a 1–8 scale where 1 was extremely undesirable and 8 was extremely desirable. Chops were removed from the freezer, the PVC film removed and allowed to stand at room temperature for 30 min prior to evaluation by a 6-member trained sensory panel. The procedure was repeated on two consecutive days to provide two replications.

Samples were prepared for the sensory panels and Instron evaluation by griddle broiling from the frozen state on a Toastmaster model 350 M griddle. The griddle was preheated 1 hr to a surface temperature of approximately 150°C. Samples were cooked to an internal temperature of 71°C according to the procedures outlined in Meat in the Food Service Industry (1975). Cooking losses were calculated on eight chops per treatment group. The chops were evaluated by a trained 6-member sensory panel for juiciness (1–extremely dry, 8–extremely juicy), tenderness (1–extremely tough, 8–extremely tender), flavor (1–extremely undesirable, 8–extremely desirable) and connective tissue (1–abundant, 8–none). Sensory panel sessions were conducted on three consecutive days to provide three replications for each variable within each treatment group.

Restructured chops for Instron evaluation were prepared in the same manner as the sensory panel chops. For tension evaluation three chops per treatment group were cooled to room temperature (23°C), sliced into 1.4 × 5.0 cm strips and placed in the pneumatic jaws of the Instron Model 1122 which were 3 cm apart at the beginning of the test. A crosshead speed of 100 mm/min was used. For Instron compression tests, samples 5 cm × 5 cm were cut from each of five chops per treatment group and placed in the Lee Kramer compression cell on the Instron. Peak force (kg) was recorded using a crosshead speed of 500 mm/min.

Pre-rigor study

The pre-rigor study was done independently of the post-rigor study using the same procedures except that boneless ham and sirloin chunks were removed from the carcass and manufactured into preformed logs 1½ hr postmortem. Boneless pork loins were removed from the same carcasses and frozen (–26°C) 1½ hr postmortem. Loins were tempered to an internal temperature of –4°C, sliced 2 cm thick, packaged in PVC overwrapped plastic foam trays and stored at –15°C for evaluation.

Statistical analyses

Data were treated by analysis of variance. Where significant differences were found, means were separated by Duncan's multiple range test (Steel and Torrie, 1960).

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Table 1—Sensory evaluation, cooking loss and Instron scores for post-rigor pork chops[a,b,c]

Treatment	Sensory panel evaluation[d]					Cooking loss %	Instron[e]	
	Tenderness	Juiciness	Connective tissue	Flavor	Color		Compression	Tension
No additives	6.57a	5.20a	6.03a	6.10a	5.05b	26.9a	217.6b	0.46b
Salt	7.13a	6.17a	6.37a	6.63a	3.45c	19.3b	182.8c	0.53ab
TPP	6.67a	5.63a	6.27a	6.10a	5.55b	19.7b	215.6b	0.53ab
Salt + TPP	6.83a	6.03a	5.87a	6.53a	5.20b	14.6c	187.0c	0.62a
Pork loin chops	2.27b	3.30b	4.23b	4.70b	7.05a	25.1a	358.2a	–
n	3	3	3	3	2	8	5	3
Standard deviation	0.48	0.68	0.62	0.61	0.37	3.15	13.92	0.070

a,b,c Means in the same column with the same superscript letter are not significantly different ($P < 0.05$)
d 1 = extremely undesirable; 8 = extremely desirable
e = kg force

Table 2—Sensory evaluation, cooking loss and Instron scores for pre-rigor pork chops[a,b,c,d]

Treatment	Sensory panel evaluation[e]					Cooking loss %	Instron[f]	
	Tenderness	Juiciness	Connective tissue	Flavor	Color		Compression	Tension
No additives	6.47a	5.47ab	5.87a	5.97a	4.45b	27.9a	297.2b	0.23c
Salt	6.50a	5.87ab	5.63a	6.03a	4.40b	20.1c	246.6c	0.34ab
TPP	5.87a	5.60a	5.63a	6.07a	4.65b	23.9b	270.8bc	0.29bc
Salt + TPP	6.40a	6.20a	5.43a	6.23a	5.00ab	14.8d	228.8c	0.43a
Pork loin chops	4.20b	4.43b	6.77b	5.37a	6.55a	18.6c	349.6a	–
n	3	3	3	3	2	8	.5	3
Standard deviation	0.75	0.72	0.36	0.62	0.61	2.58	31.92	0.046

a,b,c,d Means in the same column with the same superscript letter are not significantly different ($P < 0.05$)
e 1 = extremely undesirable; 8 = extremely desirable
f = kg force

RESULTS & DISCUSSION

Post-rigor pork

There were no significant differences (Table 1) among the restructured products for tenderness, juiciness, flavor or connective tissue. The boneless pork loin chops were less desirable ($P < 0.05$) in terms of tenderness, juiciness, flavor and amount of connective tissue than restructured chops. The restructured chops containing only salt were significantly lower in color score than any of the other chops, while boneless pork chops were significantly higher. Cooking losses for treatments containing no additives and control pork loin chops, were significantly higher than those chops with additives. Chops containing salt plus TPP had less cooking loss than other treatment groups which agrees with results obtained by Neer and Mandigo (1977), Shults and Wierbicki (1973) and Schnell et al. (1970).

Instron values for the Kramer compression test were higher ($P < 0.05$) for the pork loin chops which agrees with sensory panel evaluation. Restructured chops with added salt had lower compression values than those without added salt while the addition of TPP had little effect. Instron evaluation for tension showed chops with no additives to be significantly lower in binding compared to the salt plus TPP chops. Since salt solubilizes muscle proteins of such products as sectioned and formed hams, it was expected that the addition of salt would provide additional binding in this system. However, this system differs from other processed meats systems where the solubilized protein and meat matrix is immediately heat stabilized.

Pre-rigor pork

There were no significant differences (Table 2) among the restructured pork chop treatments for tenderness, juiciness, connective tissue and flavor. The boneless pork loin chops were significantly less desirable than restructured chops for the sensory attribute tenderness. The boneless pork loin chops were somewhat lower in juiciness than restructured pork chops, but not significantly different from those containing no additives and the salt treatments. Pork loin chops had a higher ($P < 0.05$) score for connective tissue reflecting the lack of desired tenderness. The addition of TPP, alone or in combination with salt, affects juiciness. Color evaluation of pork loin chops rated significantly higher than all restructured products except the salt plus TPP treatment.

Cooking loss was significantly higher for control restructured chops than all other treatment groups. Addition of TPP alone lowered the cooking loss ($P < 0.05$) and the addition of salt alone resulted in a decrease ($P < 0.05$) over TPP alone, but not significantly different from pork loin chops. When salt and TPP were combined the cooking loss (14.8%) was significantly lower than all other treatment groups. There appears to be a slight synergistic effect of TPP and salt in this study which concurs with reports by Neer and Mandigo (1977), Shults and Wierbicki (1973) and Schnell et al. (1970).

Instron compression scores for pork loin chops confirmed sensory panel evaluation that pork loin chops were less tender than restructured products. Control restructured chops had lower ($P < 0.05$) compression ratings than chops containing salt or salt plus TPP, but were not different from chops containing TPP only.

Tension scores for restructured chops provide evidence of improved binding with the addition of salt over control chops or chops containing TPP. This finding confirms results of the post-rigor study regarding salting-out of protein during the mixing phase that are later stabilized during cooking.

CONCLUSIONS

RESTRUCTURED pork products using the process described have more desirable eating quality than pork loin chops whether processed from pre-rigor or post-rigor pork. Color of restructured products is less desirable than loin

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A Research Note

ROLE OF SOME FATTY ACIDS ON THE GROWTH AND LIPASE PRODUCTION BY *Streptococcus faecalis*

HARISH CHANDER, B. RANGANATHAN and JASJIT SINGH

ABSTRACT

Incorporation of short chain fatty acids into the growth medium of *Streptococcus faecalis* was found to be stimulatory for lipase production. The long chain fatty acids were inhibitory to growth and lipase production.

INTRODUCTION

LIPASES are distributed in microbial systems and are responsible for the breakdown of lipids by hydrolysis. The hydrolytic products thus formed contribute to desirable flavors in food products. The microbial lipases are reported to have been stimulated by the addition of lipids to the growth medium (Khan et al., 1967; Shen et al., 1975 and Vadhera and Harmon, 1969), although in few cases, lipids reduced lipase production (Eitenmiller et al., 1970; Mates and Sudakevitz, 1973). The present study was therefore undertaken to investigate the role of different fatty acids on the growth and lipase production by *S. faecalis*.

EXPERIMENTAL

Culture

A strain of *S. faecalis* used in this investigation was isolated from Khoa, a concentrated Indian milk product and the culture was maintained in chalk litmus milk.

Method of cultivation

The organism was grown in a basal medium at pH 7.5 having the following composition: peptone (Oxoid), 2%; Yeast extract (Oxoid), 0.3%; Glucose (B.D.H.), 1%; NaCl (B.D.H.), 0.5%; fresh tomato juice (10.0%).

A 16-hr old broth culture of *S. faecalis* was centrifuged, cells were washed thrice with normal saline and the cell suspension was standardized to provide 5×10^6 cells per ml. One ml of the washed cell suspension was added to 100 ml of the basal medium.

Growth in defined medium

The role of different fatty acids on the growth and lipase production was studied using a defined medium with the following composition:

Dextrose	2.0g
Sodium acetate	0.1g
Sodium citrate	2.0g
Ammonium chloride	0.3g
Dipotassium hydrogen monophosphate	0.5g
Thiamine, riboflavine, pyridoxy, nicotinic acid and pantothenic acid	100 μg each
Para-amino-benzoic acid	20.0 μg
Biotin	1.0 μg
Vitamin B_{12}	0.01 μg
Adenine, guanine, uracil and xanthine	1.0 mg each
Alanine, glutamic acid	100.0 mg each
L-arginine, L-lysine	20.0 mg each
Histidine, isoleucine, leucine, methionine, threonine, tryptophane, valine, serine and glycine.	L-isomer 100 mg each
pH	6.8–7.0

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Table 1—Effect of addition of different fatty acids (0.5%) to the defined medium on the growth and lipase production by S. faecalis

Fatty acids	Growth counts/ml	Lipase activity[a]	Relative activity[b]	Stimulation or Inhibition
Control	50×10^7	5.2	100	–
Propionic acid	42×10^8	7.5	144	+44
Butyric acid	30×10^8	7.2	138	+38
Caproic acid	20×10^8	6.5	125	+25
Capric acid	20×10^8	6.0	115	+15
Lauric acid	45×10^6	4.8	92	– 8
Myristic acid	4×10^6	4.7	90	–10
Palmitic acid	25×10^6	4.5	86	–14
Stearic acid	20×10^6	4.3	83	–17
Oleic acid	95×10^5	3.5	65	–35

[a] μmoles of free fatty acids liberated from the substrate/ml of both
[b] Average of three trials

The fatty acids after sterilization that were added in 0.5% concentration to the sterilized defined medium, consisted of proprionic butyric, caproic, capric, lauric, myristic, palmitic, stearic and oleic acids. The media in 100 ml quantity was inoculated with standardized cell suspension and incubated at 30°C for 12 hr. Growth studies were conducted by plating on basal medium with 2% agar (Oxoid) and incubating at 30°C for 48 hr. The culture was grown in defined medium at 30°C for 10 hr and the cells were harvested by centrifugation at 9000 × G for 15 min. The supernatent was then adjusted to pH 7.5 at 4°C by the addition of 0.01N alkali, which constituted the source of extracellular enzyme.

Assay of lipase activity

Lipase activity was determined by the method of Oi et al. (1969) with some modifications with regard to substrate and buffer. The reaction mixture contained 5.0 ml of 5% butter oil emulsion in 7% gum acacia, 5.0 ml of 0.2M Tris HCl buffer (pH 7.5), 2.0 ml 0.2M $CaCl_2$, 1.0 ml enzyme solution and 2.0 ml glass distilled water.

RESULTS & DISCUSSION

SHORT CHAIN fatty acids such as proprionic, butyric, caproic and capric enhanced the growth and stimulated yield of lipase to the extent of 44, 38, 25 and 15% respectively (Table 1). Higher saturated fatty acids like lauric, myristic, palmitic and stearic acids slightly suppressed growth with corresponding decreased enzyme production (8, 10, 14 and 17% respectively), while oleic acid inhibited lipase synthesis. by 33%. It appears that short chain volatile fatty acids were utilized by *S. faecalis* in preference to other long chain saturated fatty acids for the growth and synthesis of lipase.

Results of the present study indicate that long chain fatty acids, on the other hand, were not properly utilized by the organism thereby retarding growth and lipase synthesis. Smith and Alford (1966) have also reported inhibition of lipase production by lard in *Pseudomonas fragi* presumably due to accumulation of unsaturated fatty acids on hydrolysis of fat. The inhibitory effect of unsaturated fatty acids on growth and lipase activity has also been attributed to the bacteriostatic effect of the above compounds (Eitenmiller et al., 1970). Maxcy and Chandan (1962) suggested that inhibition may be a physical phenomenon of surface tension, in which certain fatty acids interfere with the metabolism of a bacterium. The adsorption of fatty acids at the bacterium menstrum interface may be

critical in the inhibitory process (Maxcy and Dill, 1967), Oishi (1969) found that long chain unsaturated fatty acids (linoleic and oleic acid) had very little effect on growth, while lower volatile fatty acids showed fairly strong growth promoting activity.

Lipase production by *S. faecalis* does not appear to be adaptive in true sense. *S. faecalis* lipase resembles that of *Pseudomonas fragi* (Nashif and Nelson, 1953) as some of the triglycerides were stimulatory while others inhibitory. According to Davies (1963) an enzyme can be categorized as either constitutive or adaptive. Constitutive formation is not a completely fixed property, since it is known to vary depending upon the conditions of the growth of organism.

Several workers have confirmed the adaptive nature of microbial lipases, since the latter are known to be affected by the addition of lipid substrates to the growth medium (Cutchins et al., 1952; Formisaano et al., 1975 and Yoshida et al., 1968). The inhibitory action of lipids on lipase synthesis has also been recorded in some microorganisms like *Pseudomonas fragi* (Smith and Alford, 1966); *Penicillium roqueforti* (Eitenmiller et al., 1970) and *Staphylococcus aureus* (Mates and Sudakevitz, 1973). In the present study short chain fatty acids stimulated the lipase production. This finding may be of considerable value in enhancing the flavour production in the dairy and food products.

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pork chops. The addition of salt reduces color desirability. Cooking loss is decreased by the combination of salt and TPP.

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A Research Note

A BETACYANINE DECOLORIZING ENZYME FOUND IN RED BEET TISSUE

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ABSTRACT

The activity of a betacyanine decolorizing enzyme present in raw beet tissue has been confirmed. The enzyme is postulated to be bound to the inner membrane of the cell wall. Comparison between the rate of betacyanine degradation for solutions containing the crude enzyme extract and solutions of identical pH and temperature void of enzyme indicated optimum enzyme activity at pH 3.4 and 40°C. Sample evaluations were made from 22–55°C over a pH range 2.0–4.8. Minimal enzyme activity was noted at pH and temperature extremes. The activity of this enzyme must be considered as an important factor in the extraction of raw beets and during storage and food product utilization of beet juices in that residual enzyme activity may decolorize substantial amounts of the betacyanine pigments. The enzyme has been found present in diffusion-type beet juice.

INTRODUCTION

THE USE OF the betacyanine pigments as natural colorants in food products has received much consideration in recent years. Recent studies have investigated the efficiency in recovery of the betalaines from red table beets (Wiley and Lee, 1978; Wiley et al., 1979). The color stability of betanine has been shown to be affected by such factors as pH, air, light and heat (Von Elbe et al., 1974). The levels of recovery and retention of betacyanines is thus of major importance in determining extraction process parameters and possible utilization in food products. The presence of a betacyanine decolorizing enzyme has been reported to be bound to subcellular components of beet tissue by Soboleva et al., 1976. The activity of the decolorizing enzyme has been studied and its possible impact on a diffuser-type extraction system was investigated.

EXPERIMENTAL

Enzyme extraction from tissue

Whole beets of the Garnet cultivar from cold storage were chopped finely and placed in a cold solution 0–5°C 0.01M phosphate buffer (pH 4.7). The chopped beets were homogenized in cold buffer solution with the ratio of 100g beet to 50 ml solution. The homogenate was filtered through cheesecloth. Cell fragments were centrifuged out using a Beckman Model J-21B centrifuge at a force of 200 × G for 5 min. The supernatant was further centrifuged at 1000 × G for 4½ min. This sediment was repeatedly washed and centrifuged into fresh buffer and finally resuspended for use in the enzyme analyses.

Preparation of substrate

The betacynaine solutions were made up in phosphate buffer (4.7, 0.01M) from a stock of freeze-dried pigment which was purified using a SP-Sephadex C-25 cation exchange column (40–120μ) and eluted with a 1 mM solution of Na_2HPO_4. The solutions were stored at refrigerated temperatures in the dark. The pH was adjusted to an appropriate value by the addition of a few drops of HCl with stirring. The solution was also equilibrated in a water bath to the various temperatures studied. The actual concentration of betacyanine was determined by spectrophotometric analysis using absorbance at 476, 537 and 600 nm according to Nilsson's recommendations (1970).

Enzyme activity measurements

In factorially designed experiments approximately 1-g samples of crude extract were weighed out into an aluminum weighing dish and added to centrifuge tubes containing the equilibrated betacyanine solutions. After given time intervals the tubes were transferred to a centrifuge and the crude extract was centrifuged down. The supernatant was then examined at the wave-length of 476, 537 and 600 nm to determine the rate of betacyanine decomposition. A control tube containing only betacyanine solution accompanied the enzyme extract in every step of the analysis. Spectrophotometric readings were taken at intervals up to 24 hr. The protein content of the crude extract was determined by the Lowry method according to Gerhardt and Beevers (1968).

RESULTS & DISCUSSION

BECAUSE of the multiplicity of factors affecting the stability of the betacyanine pigment, it was of vital importance that control tubes be run under conditions with similar stress. An evaluation of the betacyanine decomposition at 22–55°C and in the pH range 2.0–4.8 indicated significant differences in decomposition when contrasting the crude enzyme extract and the control samples. The mean value for the protein content of the crude extract was 134.2 ± 8.3 mg/g crude extract. Data suggested the enzymatic activity was in the subcellular portion of beet tissue in that the very finely divided portions provided substantial enzyme activity. Data show that the pH 3.4 is optimum for this activity (Fig. 1). The controls also shown in Figure 1 indicate that the rate of decomposition is increased by as much as 14.8 times by the presence of the centrifuged subcellular enzyme components. Figure 2 shows the effect of temperature of degradation of betacyanines over time. It was noted that either high or low temperatures i.e. 55°C or 22°C as compared to 40°C were temperatures for better control of betacyanine degradation during diffusion.

In light of the results shown in Figures 1 and 2, it may be necessary to re-evaluate some operations in the slope diffusion-extraction process for beet pigment recovery. When freshly cut raw beets were added to the apparatus they were in contact with a dilute acidified hot beet juice solution which was ready for evacuation from the extraction system. The temperature of the juice at this time should be at its highest level and acidities near pH 3.4 should be avoided or there will be enzymatic destruction of the betacyanines. Given the processing conditions, it was seen that subcellular portions of the beet tissue might be carried over into the collection apparatus without having been exposed to a time-temperature treatment that would bring inactivation of the enzyme.

In subsequent experiments, a crude extract was obtained by centrifuging samples of diffusion-type beet juice at 1000 × G for 4½ min. The extract was found to have a protein content of 496 μg/g crude extract. Comparison between the extract from homogenized beet tissue and from diffusion-type beet juice using the same method as described above indicated that both exhibited betacyanin decolorizing activ-

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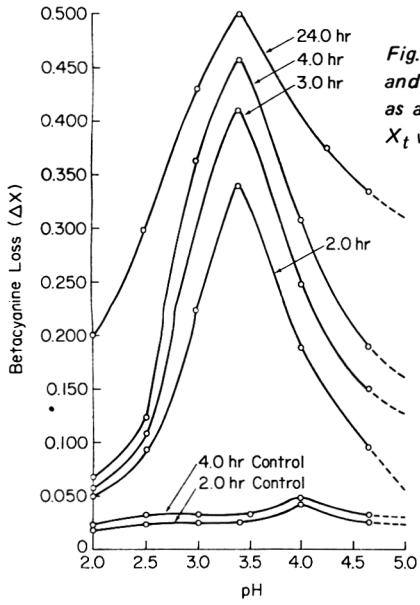

Fig. 1—Corrected ΔA_{537nm} (ΔX) in enzyme and control solutions over several time periods as a function of betacyanine loss. $\Delta X = X_o - X_t$ where $X = 1.095 [A_{537} - A_{600}]$

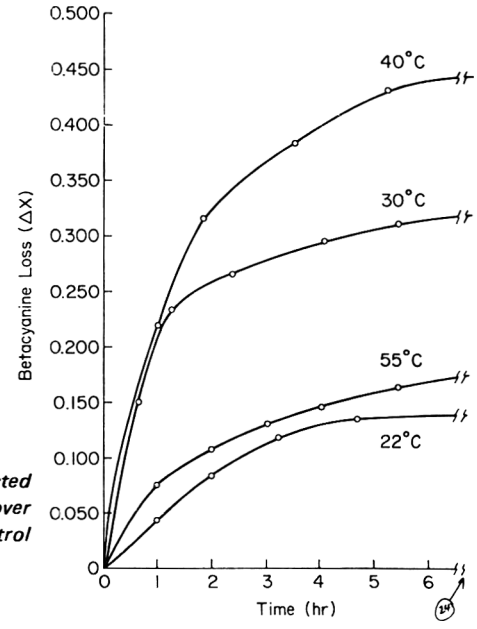

Fig. 2—Influence of temperature on corrected ΔA_{537} as a function of betacyanine losses over a 24-hr period (Includes enzyme and control solutions).

ity in the pH range 2.0–4.8. Failure to give consideration to the presence of this enzyme could result in lowering of betacyanine recovery in the diffusion-extraction system. High enzyme activity in the juice extracted from press-type operations would also be anticipated. This enzyme, though not at full activity, could manifest its presence via accelerated betacyanine losses in refrigerated beet concentrates or other products which provide opportunity for its activity.

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A Research Note

FERMENTATION OF CASSAVA (Manihot esculenta Crantz)

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ABSTRACT

Microorganisms were isolated from traditionally fermented cassava from Cameroon and from fermented cassava in the laboratory. These isolates were examined for their abilities to produce gari flavor. Cassava fermentation was mainly due to lactic acid bacteria. *Lactobacillus* sp. and to a lesser degree *Streptococcus* sp. were responsible for the acid production and gari flavor development.

INTRODUCTION

IN CAMEROON, gari is made by grating cassava and removing the excess water by placing the mash in a burlap sack which is then sewn up. The sack is squeezed between 3 or 4 poles bound tightly in parallel around the sack. This countrivance is then left outside for up to 96 hr during which fermentation takes place. The resulting cake is heated on an oiled hot plate and the granular yellowish material thus obtained is called gari.

In 1959, Collard and Levi proposed that the gari fermentation was a two-stage process involving *Corynebacterium* sp., which broke down starch into acids, and *Geotrichum candida* sp., which produced esters and aldehydes responsible for the gari aroma. The two-stage hypothesis was later supported by Akinrile (1964). Recently, Okafor (1977) questioned the validity of the two-stage fermentation concept as he was unable to isolate *Corynebacterium* sp. in appreciable quantities from fermenting cassava.

This investigation was carried out to isolate and identify the microorganisms associated with the fermentation of cassava and to conduct controlled fermentation using gari isolates and other lactic starter cultures.

EXPERIMENTAL

Isolation of typical gari flavor producing microorganisms from native gari

Cassava mash made by the traditional method in Cameroon was streaked on brain heart infusion (BHI) agar (Difco), L agar (BBL), KF *Streptococcus* agar (Difco), AK agar (BBL) and acidified potato dextrose (PD) agar (Difco). Four sets of plates were incubated at 25 and 37°C, both aerobically and anaerobically, from 48 hr to a week. Various colony types were isolated in pure cultures and maintained on BHI slants. Ten to 15g of autoclaved cassava mash were inoculated with each of the above cultures and incubated for up to 96 hr at 25°C. Six students from Cameroon who are familiar with gari flavor were asked to score the fermented cassava mashes for the typical gari flavors. Only those isolates which scored high in the test were examined further.

Isolation of microorganisms during cassava fermentation in the laboratory

One hundred fifty grams of freshly grated cassava were aseptically transferred into a sterile beaker and closed with autoclaved foam plus. The mash was agitated at regular intervals. After 4, 14, 24 and 48 hr, 1g of the mash was removed, diluted, and spread-plated in duplicates on BHI, PD and L agar and incubated at 25 and 37°C for up to a week. Various colony types were again isolated.

Use of starter cultures for gari fermentation

Lactobacillus plantarum A_8 and *Streptococcus* sp. Dd, isolated from fermenting cassava, were used to inoculate cassava mash, which were then incubated at 35°C with constant agitation. One of the samples consisted of the above cultures plus 0.1% (v/w) of potassium sorbate. The control consisted of noninoculated mash. The pH was recorded every 2 hr for 24 hr and at less frequent intervals thereafter.

Three lactic acid bacteria cultures, *L. lactis*, *L. acidophilus* N and *L. acidophilus* 3532, obtained from the Department of Microbiology, Oregon State University, were also used as starters for cassava mash fermentation. A six-student panel scored the different mashes containing the above cultures and their mixtures, on a nine-point hedonic scale ranging from one (no gari odor) to nine (extremely desirable gari odor). The data were analyzed by analysis of variance ($p < 0.01$).

RESULTS & DISCUSSION

THIRTEEN of the isolates recovered from traditionally fermented cassava produced the gari flavor. Nine of those were short stubby to long thin rods. Eight of them (A_3, A_4, A_5, A_8, A_9, B_3, B_7, B_8) were *Lactobacillus plantarum* and one (A_6) was *Lactobacillus buchneri* according to the classification scheme of Sharpe et al. (1967). Three of the isolates (A_{10}, D_C, D_d) were cocci, and had properties similar to those of *Leuconostoc* sp. described by Sharpe (1962) and Sharpe et al. (1967) except that they produced NH_3 from arginine. Both rods and cocci produced curd in reconstituted skim milk. The sole yeast isolate was identified as *Bettranomyces* sp. according to the master key proposed by Barnett and Pankhurst (1974).

During 4–14 hr of laboratory fermentation of cassava, *Lactobacillus* sp., *Streptococcus* sp., *Moraxella* sp. and *Acinetobacter* sp. were isolated in almost equal proportions. After 48 hours, however, only *L. plantarum* sp., *Lactobacillus* sp. and *Streptococcus* sp. were isolated. No yeast or mold was isolated. The cassava fermentation, thus appears to follow the typical lactic fermentation in which a variety of microorganisms are present at the beginning but, as the fermentation progresses, the other species are gradually outgrown by the lactic acid bacteria.

Among the microorganisms isolated from native gari, *L. plantarum* A_8 produced the most gari-like flavor and a combination of *L. plantarum* A_8 and a *Streptococcus* sp. Dd produced the most gari flavor among the mixed cultures.

Figures 1 and 2 show the pH values obtained at various times during the controlled fermentation of cassava mash, which was inoculated with various starter cultures. When *L. plantarum* A_8 was the only culture inoculated, the final pH at the end of the fermentation was 3.65. When *Streptococcus* sp. Dd was used alone, the final pH was 3.95.

It is generally believed that the lower the final pH of the cassava mash, the better the quality of the gari obtained therefrom. Fermented cassava mash with a pH of 3.9 or above is considered undesirable (Akinrile, 1964).

When a mixture of *L. plantarum* A_8 and *Streptococcus* sp. Dd was used as starter culture, the final pH did not drop below 3.75. Thus it would seem that *Streptococcus* sp. Dd should be used in combination with a strong acid producer. When potassium sorbate was used, it impaired acid produc-

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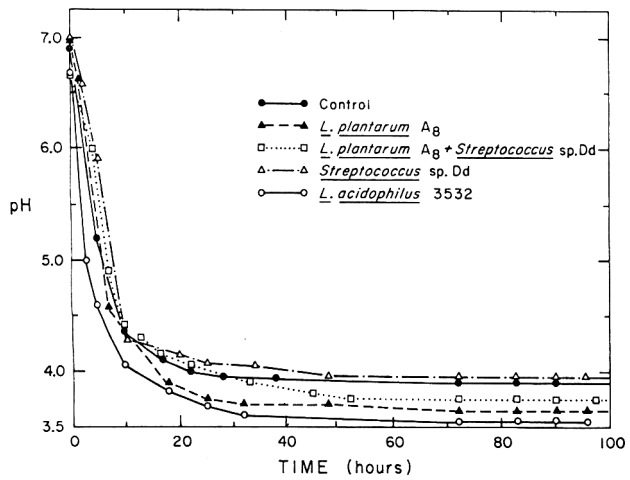

Fig. 1—pH changes in nonautoclaved cassava mashes inoculated with lactic acid bacteria.

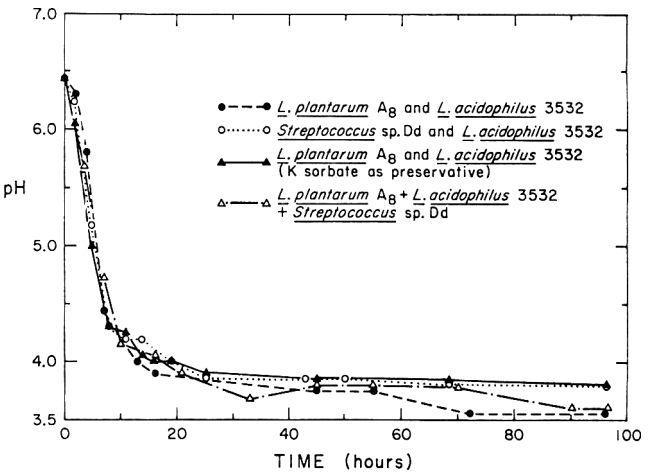

Fig. 2—pH changes in nonautoclaved cassava mash inoculated with mixtures of lactic acid bacteria.

tion (Fig. 2). Thus it would not be suitable as a preservative in controlled cassava fermentation.

Among the three dairy cultures tested for their possible use as "gari culture," two of them, *L. lactis* and *L. acidophilus* N, imparted an objectionable brownish yellow color to the mash. *L. acidophilus* 3532 did not produce the undesirable color but it did impart a dairy flavor which is foreign to gari. However, a combination of the latter with *L. plantarum* A_8 appeared to be more effective in lowering the pH of the mash than with *L. plantarum* A_8 alone (Fig. 2), without significantly affecting the flavor ($p < 0.01$).

The dairy culture (3532) out-competed the gari isolate during controlled fermentation. This undoubtedly is the result of an age-old selection and manipulation received by the dairy starter cultures. Similar opportunity should exist for the development of desired gari starters.

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