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Research Notes

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Volume 50: Number 6

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Memo *FROM THE SCIENTIFIC EDITOR*

The public image of the scientist has changed in recent years — and not necessarily for the better. In the past, scientists were perceived as pursuers of truth, as honest and reliable. There is more cynicism today about science — for a variety of reasons. One of these is a question of the integrity of those who do research. There have been some instances recently in which falsification of data, or actual fabrication of data, have been revealed. In other instances, more impressive to the public, groups with agendas of their own have impugned the work of scientists with whom they do not agree. This has become true among investigators themselves; where disagreements were once voiced at scientific meetings, they are now argued in the press and often with political overtones.

Rushing to publication, and the pressure to publish many papers, has been attributed to personnel policies in which numbers of papers published are counted for promotion. While such pressures may lead to overt scandals (which are those that have been exposed), more often there are minor breaches of ethical behavior. Fragmentation of research reports is one of these. A completed study should be reported in its entirety and not in several papers. Publication of the same data, in slightly revised form, in more than one journal is also considered unethical.

Authors of manuscripts have an obligation. All of the pertinent data should be reported, not just the values that support a desired hypothesis. The data should be interpreted correctly. Statements in the text are sometimes not supported by the values in the tables or figures. And there is a tendency for some authors to read a trend into differences among treatments that their own statistical analyses have shown to be nonsignificant. Such practices, while gaining the author another publication, may be damaging to scientific investigation in the long run. Other investigators may waste considerable time and effort in trying to conduct research based on improper data in the literature. Unfortunately, once published, such data continue to be referenced, while errata or retractions, even if published, are glossed over.

Listing as co-authors department heads (for political reasons) or technicians doing routine analyses is also a deceptive practice. Each co-author should be sufficiently involved in the work to be able to defend the procedures and results, if necessary.

While the *Journal of Food Science* reviewers and the Scientific Editor and his staff try to evaluate a manuscript, it is the responsibility of the principal investigator or the department head to assure the integrity of the information submitted.

To make possible timely publication of a journal of this size requires the dedicated efforts of a number of people. I which to thank Ruth Zabarsky, in my office, Dr. Kazeniac and Anna May Schenck, JFS Assistant Scientific Editors, for the time and care they have devoted. John Klis (Director of Publications), Barney Schukraft (Managing Editor), and Gladys Anderson (Editorial Department Secretary) of the IFT Publications Office have been very helpful and I am pleased to acknowledge their support and assistance.

The Editorial Board has also been very helpful, and I wish to express my thanks to the retiring Board members and welcome the new members as they begin their three-year term.

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The reviewers are the backbone of the Journal of Food Science. These are a group of dedicated scientists who, in spite of their busy schedules as researchers or teachers, read and provide helpful comments for the manuscripts that have been submitted. To these reviewers, listed below, I express my thanks and appreciation, and to anyone whom I may have inadvertently omitted, I offer my apologies.

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In Vivo Digestibility of Insoluble Collagen from Bovine Tendon as Influenced by the Inhibition of Gastric Acid Secretion

ANITA LASER REUTERSWÄRD and STEFAN FABIANSSON

ABSTRACT

Insoluble collagen (bovine Achilles tendon), gelatin and meat were each mixed with whole egg protein (about 50% of the nitrogen) and fed to rats. True digestibility was evaluated after inhibiting gastric acid secretion with omeprazole. Insoluble collagen, supplemented with small amounts of essential amino acids, was also given but without inhibiting gastric acid secretion. Egg protein was fully digested and not affected by inhibition. The true digestibility of insoluble collagen/egg was 85%, corresponding to 71% true digestibility for insoluble collagen alone. This was significantly lower than the value of 95% obtained for insoluble collagen without inhibiting gastric acid secretion. True digestibilities for gelatin/egg were 99% and meat/egg 98%. Inhibition of gastric acid secretion thus did not affect digestion of gelatin and meat.

INTRODUCTION

COLLAGEN normally constitutes a very small part of the total intake of food proteins. The content of intramuscular collagen varies from about 2–20% of the protein in meat (Lawrie, 1979). In some products, e.g. Swedish sausages, 35% of the protein can be of collagenous origin (Fuchs and Kuivinen, 1980). Native collagen is considered to be more or less indigestible (Loewit, 1970; Cheftel, 1977; 1979; Kies, 1981; Ashgar and Henrickson, 1982) but denaturation and solubilization to gelatin, which can occur after e.g. cooking, makes it susceptible to proteolytic enzymes and thus digestible (Paul, 1972; Rogowski, 1980; Ashgar and Henrickson, 1982). It has been reported, however, that unheated samples such as collagenous unscalded pigskin and raw bovine tendon derived from both young and old animals have a true digestibility of more than 92% in rats (Laser Reuterswärd et al. 1985).

The initial step in the normal digestion of food proteins is their denaturation at acidic pH and the action of pepsins in the stomach (Gitler, 1964; Bender, 1978; Silk and Dawson, 1979). *In vitro* studies have shown that pepsin can, to a large extent, solubilize collagen fibers by cleaving peptide bonds in the nonhelical telopeptide region (Weiss, 1976). In a previous *in vitro* study solubilization of old unscalded pigskin (75% collagen of the protein) and insoluble collagen (extracted from bovine tendon) were both found to be highly pH-dependent during incubation with pepsin in hydrochloric acid (Laser Reuterswärd, 1985). The results showed that, when the pH was increased a decrease in solubility was obtained at pH 2.5 and further treatment with pancreatin had only a small effect on solubilization. Etherington (1977) indicated that denaturation of insoluble collagen fibers occurred only after solubilization of the monomers. The denaturation temperature of the monomer was around 39°C (Bailey, 1983) but was pH-dependent and decreased at a lower pH (Dick and Nordwig, 1966). Experiments *in vitro* thus indicate a strong relationship between pH and collagen solubilization and denaturation.

The present study was performed to determine whether gastric acid secretion was critical for the digestion of insoluble collagen *in vivo*.

Author Laser Reuterswärd is with the Swedish Meat Research Institute, P.O.B. 504, S-244 00 Kävlinge, Sweden, Author Fabiansson, formerly with the Swedish Meat Research Inst., is presently with Ferrosan, P.O.B. 839, S-20180 Malmö, Sweden.

MATERIALS & METHODS

Materials

Beef, *M. psoas major*, was freed of visible fat and connective tissue, minced in a Moulinex blender and freeze-dried. Gelatin from swine skin (175 Bloom) and insoluble collagen from bovine Achilles tendon, prepared according to Einbinder and Schubert (1951), were purchased from Sigma Chemical Co., USA. ANRC Reference Protein High Nitrogen Casein (Sheffield Chem., USA) was used as the reference protein. Whole hen's egg was diethyl ether-extracted, lyophilized and used for determination of faecal metabolic nitrogen. The amino acids L-methionine (Biochemische Zwecke), L-tryptophan (chromatographically homogenous), L-isoleucine and histidine × HCl (all from BDH Chem. Ltd., England) were used for supplementation.

Rat assay

True digestibility of the proteins was evaluated by nitrogen balance studies on growing rats as follows (Eggum, 1973): Each diet was tested on five to seven male rats of initial weight of 85g (purchased from Anticimex, Sweden). The rats were placed in individual metabolic cages. After a 4-day adaption period the nitrogen balance study was performed for 5 days. The temperature was held at 26°C and the relative humidity at 65%. Every 12th hour, light and darkness were exchanged. Faeces were collected separately from urine in 5% H_2SO_4 during the five days and analyzed for nitrogen.

To some groups of rats omeprazole (Hässle, Sweden), which inhibits gastric acid secretion, was administered by intragastric intubation twice a day at 8 a.m. and 8 p.m.; 0.5 mL of a suspension of 8 μmol/mL was given. Intubation, performed from the second day of the adaption period and throughout the experiment, was done after light diethyl ether anesthesia. The rats received a portion of 50g dry matter of the diet every day. Corrections for feed residues were made. The diet, according to Eggum (1973), was modified with vitamins and minerals according to Forsum et al. (1973). The composition of the diet was as follows (% of dry matter): protein corresponding to 1.5% nitrogen, amino acid supplements as described below, 5% maize oil, 5% cellulose powder, 4.8% mineral mixture, 0.8% vitamin mixture, 0.2% choline chloride, and 10% saccarose. Maize starch was used as the carbohydrate source and was added up to 100%.

Three groups of rats were tested without using omeprazole; one with an egg diet for estimating faecal metabolic nitrogen, one with a reference casein diet, and one with an insoluble collagen diet. Faecal metabolic nitrogen excretion was determined with the egg diet containing 0.72% nitrogen (Eggum, 1973). The other components of the diet were added as mentioned above. The reference casein diet (1.5% nitrogen) was supplemented with methionine, 0.22% of the diet. The insoluble collagen diet (1.5% nitrogen) was supplemented with 0.15% methionine, 0.10% tryptophan, 0.10% isoleucine and 0.10% histidine. Supplementation with these amino acids was performed to provide a better amino acid balance and thus ensure a more reliable determination of true digestibility.

Four groups of rats were tested with administration of omeprazole, one with an egg diet, and three diets where egg was mixed with either meat, gelatin or insoluble collagen, respectively. The egg diet contained 0.72% nitrogen and was used for the estimation of faecal metabolic nitrogen. In the mixed diets egg was used at a level of 0.72% nitrogen and meat, gelatin and insoluble collagen at a level of 0.78%, respectively; altogether 1.5% nitrogen was thus added in each of these diets.

Chemical analyses and calculations

Nitrogen (N) contents of original samples, diets and faeces were analyzed using the Kjeldahl method. Calculation of true digestibility was performed with the Thomas-Mitchell equation as described by Eggum (1973): [N intake - (faecel N - metabolic N)]/(N intake) × 100.

Hydroxyproline was analyzed on a Technicon Auto-analyzer according to Stegemann (1958) as modified by Weber (1973). Calculations of collagen content were made according to Laser Reuterswärd et al. (1982) based on nitrogen and hydroxyproline analysis.

Stomach content

Each rat was killed on the 5th day, by diethyl ether. For the rats given omeprazole the killing was done 12 hr after they received the last dose. The stomach was removed and put into 20 mL de-aired ice cold water, cut open and emptied of its contents by mincing carefully. The pH was measured and the H^+ concentration was estimated, in duplicate, by titration with de-aired 0.001N NaOH to a final pH of 8.00 (Davenport, 1977).

Statistical evaluation

Significance of differences were evaluated using Student's t-test.

RESULTS

THE AMOUNT of faecal metabolic nitrogen obtained for egg protein when using omeprazole was 71.0 ± 8.4 mg which was not significantly different ($p>0.05$) from the value of 63.9 ± 2.8 mg obtained under ordinary conditions.

Table 1 shows pH and amount of titrable acid in the stomach contents suspended in 20 mL water. The omeprazole group ($n = 24$) had a significantly higher ($P < 0.01$) pH value than the reference group ($n = 5$) and the amount of acid was significantly ($P < 0.001$) lower.

The meat sample contained 2.4% collagen of the total protein. The amino acid compositions of insoluble collagen and gelatin were similar (Laser Reuterswärd, 1984). Table 1 shows the results of true digestibility. The true digestibility values of meat/whole egg and gelatin/whole egg were not statistically different from the value of whole egg protein ($P > 0.05$). The calculated true digestibility value for meat, assuming egg protein 100% digestible, was 97.1%. The corresponding calculated true digestibility value for gelatin was 98.8%.

For the insoluble collagen sample a significant ($P < 0.001$) decrease in digestibility due to the effect of omeprazole was observed (Table 1). The calculated true digestibility of insoluble collagen with omeprazole, assuming egg protein 100% digestible, was 71%.

DISCUSSION

STUDIES of Larsson et al. (1983) have indicated that omeprazole inhibited gastric acid secretion by blocking the parietal cell proton pump — the H^+, K^+-ATPase. Omeprazole has a very long duration of inhibition, a feature unique among gastric antisecreting agents. Repeated oral administration of omeprazole, in the conditions of the present study (3 days of adaption, 40 μmol/kg, every 12th hr) resulted in anacidity for 6–8 hr after each dose and at least 80% inhibition of gastric acid output over 24 hr (Carlson, personal communication). The values presented in Table 1 were estimated 12 hr after the last omeprazole dose i.e. when the inhibitory effect of the drug could be expected to be minimal.

The high digestibility values for egg and meat proteins in this study agree well with previously reported findings by Mauron (1973) using rat assay under normal conditions evaluated with a nitrogen balance method. Since egg and meat are main protein sources in normal diets the present results agree with the findings that total gastrectomy does not increase nitrogen in the faeces of rats (Welbourn and Doggart, 1956) and that partial gastrectomy in man does not increase faecal nitrogen (Taylor, 1968; Silk & Dawson, 1979; Freeman *et al.*, 1979; Johnson, 1981).

Our calculated digestibility for gelatin (98.8%) is somewhat higher than that reported by Mauron (1973), who found 90% digestibility when given to rats under normal conditions.

The present study shows that *in vivo* pH has a greater influence on the digestion of insoluble collagen than on that of meat proteins and gelatin. As mentioned in the introduction an earlier *in vitro* study (Laser Reuterswärd, 1985) showed that solubilization of insoluble collagen (same material as in this study) was highly pH-dependent during incubation with pepsin in hydrochloric acid. The solubility at pH 1.5 was 90%, but started to decrease at pH 2.5. For the gelatin and meat samples a much less pronounced effect of pH was obtained *in vitro*. At pH 1.5 both proteins were completely solubilized and at pH 4 the gelatin sample was 95% soluble and the meat sample 80% soluble. The results found *in vivo* and *in vitro* are thus in accordance with each other.

In the human stomach, pH during digestion varies depending on individuals and the composition of their diet (Gitler, 1964; Davenport, 1977). In general, the role of hydrochloric acid is simply to denature proteins so that peptide bonds shielded inside the native protein become more accessible to proteases (Gitler, 1964; Bender, 1978; Silk and Dawson, 1979; Cheftel, 1979). In the case of collagen, however, four different effects of hydrochloric acid should be considered: (1) breaking of acid-labile crosslinks; (2) swelling of collagen fibers; (3) providing an optimum pH for pepsin activity; and (4) lowering of the denaturation temperature of solubilized monomers (tropocollagen).

First, hydrochloric acid could act by breaking one of the intermolecular crosslinks of collagen, the 'aldimine', which is acid labile. The 'aldimine' occurs in tendon collagen in equal proportions to the other major crosslink, the 'keto', but the latter form is stable against acid (Weiss, 1976; Sims and Bailey, 1981). However, this effect is probably of minor importance since less than 10% of the collagen in tendon is acid soluble (Carmichael and Lawrie, 1967). Secondly, hydrochloric acid has a swelling effect on hide collagen and this has

Table 1—Influence of omeprazole inhibition on gastric acid production and true digestibility of rats fed different proteins. Standard deviations within brackets. pH and H^+ concentration were measured after dilution of stomach content in 20 mL water, at the time of the completion of the rat experiment

Diet protein	Nitrogen in diet %	n	Gastric acid secretion pH	Gastric acid secretion H^+[a] mL	True Digestibility %
Without omeprazole					
Casein (reference)	1.50	5	3.5 (0.1)[a]	39.0 (18.5)[c]	100.0 (0.3)
Insoluble collagen	1.50	5	-	-	95.2 (2.2)[e]
With omeprazole					
Whole egg	0.72	7	6.0 (1.2)	16.4 (2.3)	99.9 (2.4)[g]
Meat/Whole egg	0.78 + 0.72	6	4.5 (1.2)	13.7 (3.8)	98.4 (2.0)[g]
Gelatin/Whole egg	0.78 + 0.72	5	5.8 (1.2)	18.8 (15.6)	99.4 (1.9)[g]
Insoluble collagen/Whole egg	0.78 + 0.72	6	5.4 (1.0)	11.9 (7.6)	85.0 (3.2)[f]
Average value 'with omeprazole'		24	5.46 (1.2)[b]	15.16 (8.3)[d]	

[a] Titrated against 0.001 NaOH to pH 8.0

[a-g] Values with different superscripts in the same column are significantly different ($P < 0.05$).

been shown to be maximal at pH 2 *in vitro* (Gustavson, 1956). Above pH 4.5 insoluble collagen is totally resistant to degradation by cathepsins *in vitro*, probably due to lack of swelling which has been shown to be necessary for interfibrillar attack on collagen by enzymes (Bailey and Etherington, 1980). The action of pancreatin and elastase on undenatured collagen, is known to occur only in the nonhelical telopeptide region (Weiss, 1976; Bailey and Etherington, 1980), and the action of these enzymes in the small intestine would therefore be weak because of the high pH and lack of swelling. The only enzymes known to attack the collagen molecule in the intact helix at neutral pH are collagenases (Bailey and Etherington, 1980). Fullmer et al. (1966), however, stated that they could find no collagenolytic activity in the alimentary tract of rats.

In the present study inhibition of acid secretion clearly would have resulted in reduced swelling of collagen fibers because of the high pH values recorded. Nevertheless the true digestibility of 71% found for collagen indicates that swelling, if any, was sufficient for pepsin action. It thus seems that swelling in the stomach is a contributing factor in collagen digestion.

The third effect of hydrochloric acid could be to provide an optimum pH for pepsin activity for degradation of the collagen fibers. Pepsin action on undenaturated collagen is known to occur only in the nonhelical region (Weiss, 1976). The inhibition of hydrochloric acid by omeprazole and the increase in pH should have resulted in a change of the conditions for pepsin activity. Pepsinogen is converted to pepsin only below pH 5 and the optimal pH of pepsin action on most proteins is around pH 2 (Taylor, 1968). However, several mammalian gastric proteinases with different pH-optima have been found (Foltman, 1981). Degradation of fibrous bovine tendon collagen by human pepsin 1 and 3 active up to pH 3.5 and 3.8, respectively, at 37°C has been reported. Pepsin 1 is a minor and pepsin 3 a major pepsin in humans (Etherington et al. 1980). Parapepsin I, a minor component of gastric proteinases (Foltman, 1981) has been shown to have high activity towards gelatin (Ryle and Porter, 1959) and it is probably equivalent to the gelatinase as indicated by Etherington and Taylor (1967). However, this enzyme does not have a high activity against collagen (Steven, as cited by Ryle, 1964). It is therefore possible that in the rat gastric proteinases, which are also active at a higher pH than 2, are important in solubilizing collagen.

Denaturation of collagen fibers can only occur after solubilization of the monomers (Etherington, 1977). The fourth effect of hydrochloric acid on collagen degradation in the gastrointestinal tract could thus be denaturation/gelatinization of pepsin solubilized monomers (tropocollagen). The denaturation temperature of tropocollagen, i.e. where half of the helix unfolds into random chains, is around 39°C for mammalian collagen according to Bailey (1983). The denaturation temperature for different soluble collagens, i.e. tropocollagen, varies between 36° and 41°C at pH 3.7 depending on the species (Bailey, 1968; Privalov, 1982). However, the exact denaturation temperature for bovine tendon used in the present study is not available in the literature. Moreover, the denaturation temperature is pH dependent, decreasing from 36°C at pH 3.7 to 32°C at pH 2 for soluble calf skin (Dick and Nordwig, 1966). The body temperature of rats is 37° to 38°C (Falkmer and Waller, 1972). In the present study, the inhibition of hydrochloric acid for the insoluble collagen sample, could thus have resulted in a less extensive denaturation after the solubilization process when compared to the control sample. However, in the *in vitro* study of the same material it was shown that all insoluble collagen solubilized by pepsin was also degraded into trichloroacetic acid soluble peptides by further treatment with pancreatin and that this effect was independent of pH (Laser Reuterswärd, 1985). The denaturation of solubilized collagen is therefore probably not a limiting step in the digestion of collagen fibers.

CONCLUSIONS

THE SECRETION of hydrochloric acid *in vivo* was shown to affect the digestion of insoluble collagen but not gelatin and meat proteins. The most important effect of hydrochloric acid on digestion of collagen is probably the swelling of collagen fibers, which facilitates subsequent enzymic attack on the native collagen. It also seems probable that gastric proteinases active at higher pH than pH 2 are important in solubilizing collagen fibers.

The relevance of the present data for collagens other than insoluble collagen from bovine Achilles tendon as well as their relevance for collagen digestion in man needs further study.

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Surface Activity of Food Proteins: Relationships Between Surface Pressure Development, Viscoelasticity of Interfacial Films and Foam Stability of Bovine Serum Albumin

S. H. KIM and J. E. KINSELLA

ABSTRACT

The relationships between film formation and viscoelastic properties and foam stability were studied using bovine serum albumin. The rate of surface pressure development showed a two-phase behavior — a rapid initial increase followed by gradual increase to a value of 20 dyne/cm^2 after 24 hr. In the isoelectric pH range surface pressure developed most rapidly. Surface viscosity, surface yield stress and film elasticity showed maxima in the range pH 5–6. Foam stability, as measured by drainage rates also showed maximum in the same pH range, indicating a relationship between these characteristics.

INTRODUCTION

DURING FOAM FORMATION and stabilization several molecular properties of the surface active proteins are involved (Cumper, 1953; Kinsella, 1976; Halling, 1981). The ability to form a strong cohesive viscoelastic film which retains moisture is a prerequisite for formation of stable foams (Kinsella, 1981; Halling, 1981). A limited amount of research has been done to establish relationships between film forming and foaming properties of proteins (Graham and Phillips, 1976; MacRitchie, 1978; German et al., 1985). Little is known about the effects of molecular size, flexibility and the nature of secondary interactions on the physical characteristics of films in foams. Research to elucidate relationships between the surfactant properties of food proteins and film forming properties is necessary to determine which particular structural features are desirable and how food proteins may be modified to improve surface active properties for food applications. To conduct such research and evaluate appropriate methodology it is helpful to examine the surface active properties of proteins whose physical properties are reasonably well characterized, e.g., β-casein, bovine serum albumin (Graham and Phillips, 1979a), β-lactoglobulin (Waniska and Kinsella, 1985). In conjunction with the development of standardized methods (German et al., 1985) the objective of this study was to ascertain relationships between surface properties (surface pressure, surface viscosity, film elasticity) and foam stability of bovine serum albumin (BSA) as a function of pH. BSA was chosen because its molecular properties are well characterized (Walton and Maenpa, 1979) and it may be involved in foaming of whey proteins (Kinsella, 1984).

MATERIALS & METHODS

ALL CHEMICALS used in this study were of reagent grade. Crystallized and lyophilized bovine serum albumin was obtained from Sigma Chemical Co. (St. Louis, MO).

Surface pressure

The Wilhelmy plate method was used to determine surface pressure of protein solutions (MacRitchie and Alexander, 1963; Waniska and Kinsella, 1985). Protein solutions, 100 mL (5×10^{-3}% (w/v)) were poured into a clean petri dish. After an initial equilibrium period (2 min), the surface of the protein solution was carefully cleaned by suction using a capillary tube, and the platinum plate ($1.0 \times 1.8 \times 0.05$ cm.) (Serial No. 269, Cahn Instruments, Cerritos, CA) was contacted with the surface of the protein solution. The changes in force(s) pulling the platinum plate were continuously monitored by a Chan electrobalance (model 2000, Serial No. 38614) until the surface reached equilibrium. The force F required to maintain the position of the platinum plate is proportional to the surface tension (γ), i.e., $\gamma = F/2L$ were L = length of plate in contact with surface (Hiemenz, 1977). Surface pressure, the decrease in the surface tension, represents the difference between γ of water and γ of protein solution (MacRitchie, 1978).

Surface viscosity

The surface viscosity (η_s) or resistance to shear stress of the surface film is an index of its mechanical strength. The surface viscosity of protein films was measured using a rotating cylindrical rotator attached via a torsion wire to a Brookfield synco-electronic viscometer (Model LVF, Brookfield Engineering Lab, Stoughton, MA). The torque constant of the wire was 673.7 dyne/cm for full deflection of the viscometer scale. The rotator (16g) consists of a cylindrical aluminum disc (50 mm diameter) with a knife edge that is placed in contact with the protein film on the surface of protein solutions (Blank et al., 1970; Waniska and Kinsella, 1985). Petri dishes (58 mm diam.) containing 10 mL of protein solution (0.1%) were carefully positioned on a moveable platform beneath the viscometer. The knife edge adaptor attached to the viscometer was lowered using a Helipath Stand (Model C, Brookfield Engineering Lab.) until the knife edged disc just touched the surface of the solution, i.e., the protein film. Rotation of the disc in a viscous fluid causes a spring deflection in the viscometer. The deflection of the disc, the torque constant of the suspension wire, the dimensions of the disc and rate of rotation are used to calculate η_s (Goodrich, 1973). The magnitude of the deflection is proportional to the force required to overcome the viscous resistance to rotation of the disc in the surface protein film. Surface viscosity readings of protein solutions were taken at four different shear rates, i.e., 6, 12, 30, 60 rpm of the knife-edged disc in the surface film of the protein. Surface viscosity measurements were recorded at specific time intervals. The units of viscosity using the Brookfield viscometer were read as percent of total scale, (i.e., 100 divisions per 673.7 dyne/cm full scale deflection).

The surface yield stress, f_s, the non-Newtonian component of surface viscosity for viscoelastic protein films, was estimated by measuring surface viscosity as a function of angular velocity (rpm). When surface viscosity values were plotted vs rpm, the resulting regression line extrapolated to zero angular velocity or percent torque (T'). The surface yield stress, f_s, was then calculated from the equation of Buckingham and Reid (1974):

$$f_s = \frac{T\,T'}{4\pi \ln (b/a)} (a^{1/2} - b^{1/2})$$

where T is the torque constant of the wire, T' extrapolated value of percent torque, a and b radii of disc and trough, respectively.

Film elasticity

Film elasticity (ϵ), the increase (or decrease) in γ per fractional increase (or decrease) in area (A) of the film may be an important parameter in determining film stability [$\epsilon = -d\pi/d(\ln A)$]. Of the methods for determining ϵ (Blank et al., 1970; Graham and Phillips, 1980), tensiolaminometry (Eydt and Rosano, 1968) which monitors the forces generated during extension and compression of surface films formed in a square wire loop was used. The observed hysteresis effect reflects some elastic properties of the film. In this study the slope in

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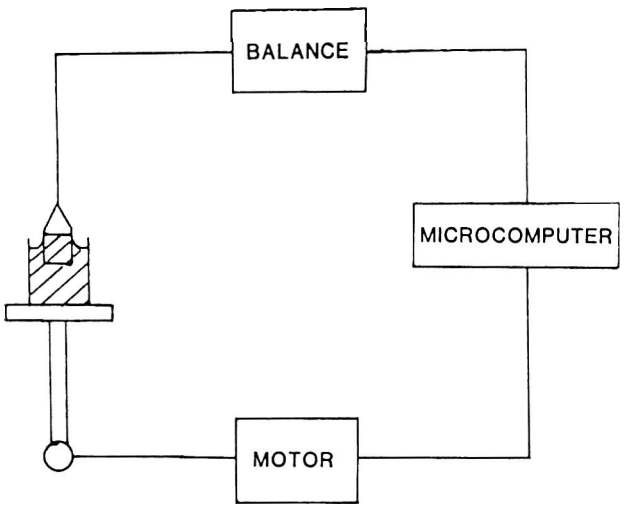

Fig. 1A–Major functional components of the tensiolaminometer. The stepper-motor, platform and balance are coordinately, controlled by the microcomputer which records the data.

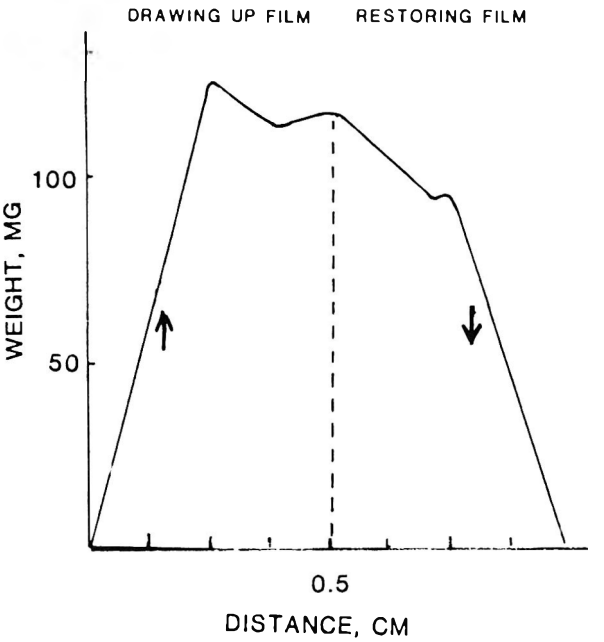

Fig. 1b—Typical recording of weight changes (corresponding to surface tension alterations) during formation and deformation of bilayer lamina of protein film formed while raising and lowering the tensiolaminometer loop from and into the protein solution. (See text for details).

the strain stress curve during the film compression cycle, which reflects the change in γ, corresponded to film elasticity (German et al., 1985). The tensiolaminometer was made of a square (2.5 cm.) frame of platinum wire (0.01 cm diam) which was attached to a Cahn microelectrobalance. For routine measurements the microprocessor controlled system described by German et al. (1985) was used. The platinum wire frame was totally immersed in the fresh protein solution (20 mL of 0.1% protein in 25 mL beaker). The beaker was located on a moveable platform driven up and down by a stepper motor. Both motor and balance are synchronized by a microcomputer (German et al., 1985), which recorded the weight changes (Fig. 1A). Upon activation of the motor the platform supporting the beaker moves down at a constant speed (0.014 cm/sec) and as the frame emerges from the solution a two-sided protein film with a sandwich layer of lamellar water (lamina) is formed on the frame. This is recorded as an increase in weight (Fig. 1B). When the frame is raised 0.51 cm. the film detaches from the meniscus (solution) and is compressed by its own weight with loss of weight via drainage. The direction is then reversed and the frame is slowly reimmersed into the solution during which time the preformed film is compressed and eventually collapses with an apparent loss in weight as the frame is reimmersed in solution. From the force distance coordinates thus obtained, the rate of film formation, weight of film and rate of film collapse are parameters that can be estimated with this technique. The decrease in weight, i.e., γ decrease during the period when the film is out of contact with the solution, reflects elasticity (ϵ) which) was estimated using the equation: $\epsilon = -d_w\, g/2L$, where d_w = weight decrease; g = gravitational acceleration, and L = width of platinum frame. The fractional change in area was assumed to be unity.

Foam stability

Foams were prepared by the method of Waniska and Kinsella (1979) To determine if there were any relationships between film properties of protein and foaming properties, the foam stability was measured by monitoring the rate of drainage (Halling, 1981). By plotting the log of liquid volume remaining in the foam against time a linear first order plot resulted (Fig. 2) and the rate constant of drainage or half-life of liquid in the foam was determined.

RESULTS & DISCUSSION

Surface pressure

The development of surface pressure of bovine serum albumin solutions as a function of time at pH 4.O and pH 5.5 is shown in Fig. 3. There was a rapid initial increase in surface pressure especially at pH 5.5 during the first 60 sec and then the rate slowed considerably. The equilibrium values of π attained after 24 hr were 22.0 and 22.4 dyne/cm at pH 4 and 5.5, respectively. A plot of log rate of surface pressure development ($\pi_x - \pi_t / \pi_x$) versus time gave a biphasic line (Fig. 4) showing an initial rapid rate of adsorption over 20 min and a slower rate subsequently. This behavior reflects the processes of adsorption and rearrangement of protein molecules and is consistent with the observations of Graham and Phillips (1979a) and Waniska and Kinsella (1985) for lysozyme and β-lactoglobulin.

The initial surface pressure at 5 min (π_5) and at equilibrium (π_{24}) were measured at different pH values (Fig. 5) to determine their relationship to foam stability. The π_5 showed a distinct maximum near the pI of BSA and decreased rapidly below pH 5 and above pH 6.0 whereas π_{24} was relatively insensitive to pH between 4 and 6.0. As discussed below the π_5 behavior may be more relevant to foam formation and stability.

Surface pressure is a function of both concentration and conformation of proteins at the air-water interface. Films with high surface pressures are formed when protein concentration at the surface is high. At pH values away from the pI, protein molecules carry a net charge and consequently there are two opposing phenomena affecting surface pressures. Intermolecular electrostatic repulsion decreases the rate of adsorption due to electrical potential set up at the interface by the charged molecules already adsorbed (primary effect) (MacRitchie, 1978). The second effect results in an increase in π reflecting intramolecular repulsion which expands the molecules in the interface. The latter phenomenon may involve rearrangement of protein at the interface which may take hours (Graham and Phillips, 1979a). Therefore, the rate of surface pressure change depends primarily on the rate of adsorption. However, when proteins are allowed to equilibrate, the secondary effect together with other thermodynamically favorable effects, i.e., hydrophobic interactions, partly offset the primary effect so that the equilibrium surface pressures are comparable at different pH values. Similar behavior was reported by Graham and Philips (1979b) for bovine serum albumin and lysozyme.

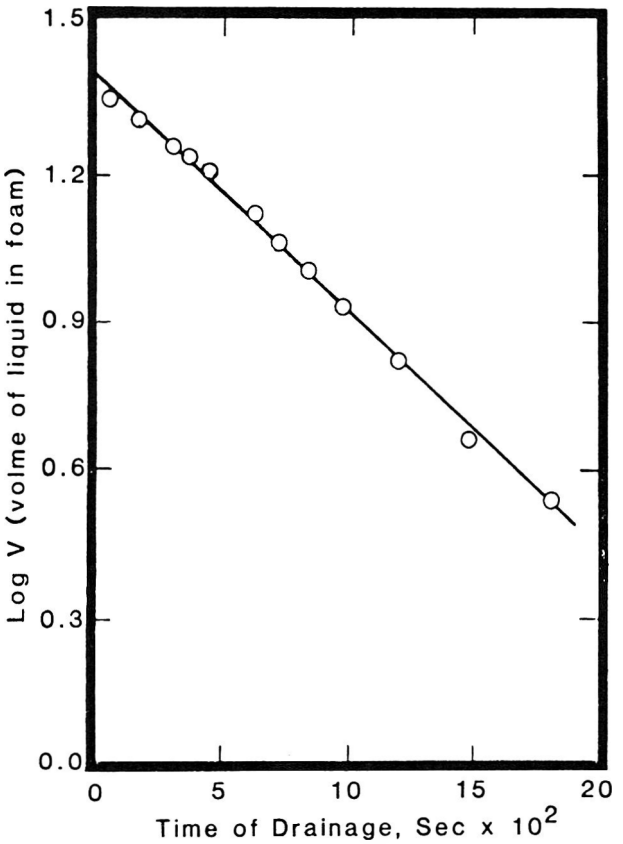

Fig. 2—First order plot showing rate of drainage of liquid from foams made with bovine serum albumin (0.4% in phosphate buffer, 10 mM, pH 7.0 containing 0.1M NaCl).

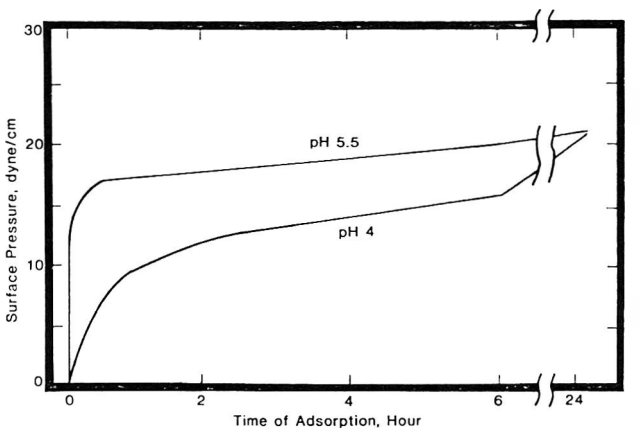

Fig. 3—Surface pressure development of bovine serum albumin at two pH values. Protein 5 × 10^{-3}% in citrate buffer (10 mM).

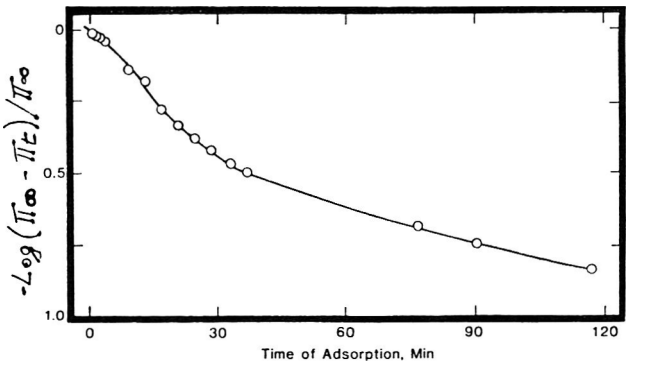

Fig. 4—Rate of surface pressure development at progressive times (ageing of film). BSA concentration 5 × 10^{-3}% in citrate buffer (10 mM, pH 4.0).

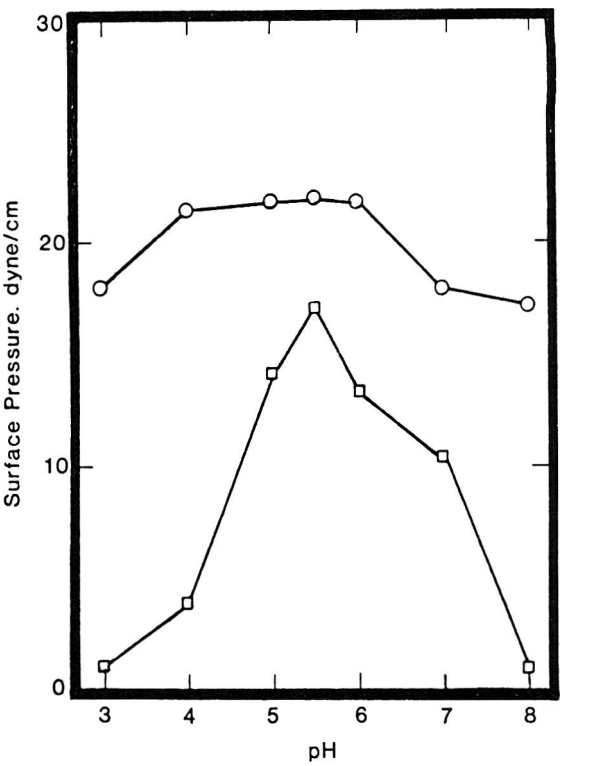

Fig. 5—Effect of pH on surface pressure of bovine serum albumin films after 5 min (□) and at equilibrium (o). Protein concentration 5 × 10^{-3}%, in 10 mM citrate (pH 3–5.5) and phosphate (pH 5.7–8) buffer.

Surface viscosity

The rate of destabilization of a foam reflects mostly the rheological properties (surface viscosity and film elasticity) of films (Graham and Phillips, 1976). The surface viscosity of protein films is implicated in stabilizing foams against drainage due to gravitation and hydrostatic pressure by imparting cohesion, strength and preventing marginal regeneration (Bikerman, 1973). Surface elasticity also reduces drainage resulting from rupture of lamellae by increasing the activation energy of growing a hole leading to rupture of the film in the nucleation process (de Vries, 1958). High values of elasticity of adsorbed layers tend to slow drainage of water from the film and thereby enhance stability (Graham and Phillips, 1976). Therefore, surface viscosity and elasticity of BSA films were determined.

By measuring surface viscosity (percent torque after 30 min) of BSA films at a range of pH values using rotor speeds of 6, 12, 30, and 60 rpm, the surface yield stress, (which reflects the strength and intermolecular cohesion in the film), was calculated for each pH (Fig. 6A). The maximum f_s occurred between pH 5 and 6 in the region of the isoelectric point of BSA and decreased rapidly especially above pH 6.O and modestly below pH 5.O. Buckingham and Reid (1974) reported that the surface viscosity of bovine serum albumin and cytoplasmic proteins attained maxima close to their respective isolectric points (pI).

Absolute values for surface yield stress were difficult to

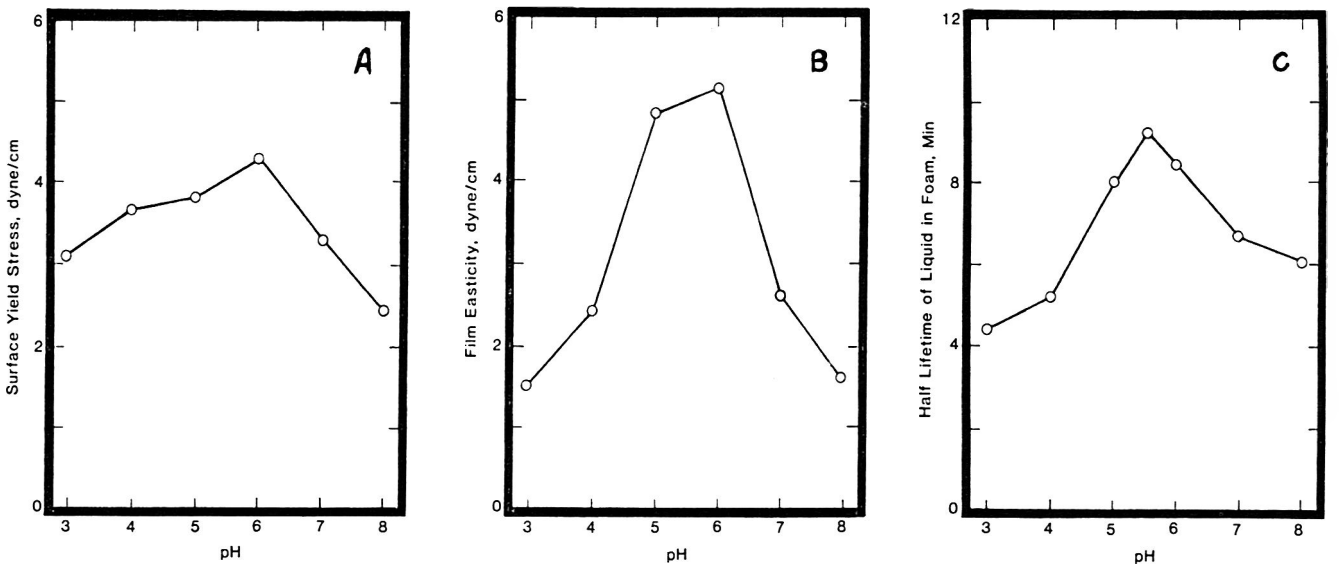

Fig. 6—Effect of pH on (A) surface yield stress of films of bovine serum albumin; (B) elasticity, of bovine serum albumin films; and (C) the half-life of liquid (stability) of foams made from bovine serum albumin. Foams made from BSA solutions 0.1% in citrate (pH 3-5.5) and phosphate (pH 5.7-8) buffers.

compare with the published data because of the limited data and the different protein concentrations used in determining the surface yield stresses. The values obtained in this study ranged from 2.4 – 4.3 dyne/cm, which were higher than the reported value of 0.01 dyne/cm for bovine serum albumin (Buckingham, 1971); however, a protein concentration of 0.005% was used compared to 0.1% employed herein.

Buckingham (1974) observed that η_s and f_s values taken during 10–30 min were relevant to foam strength but that f_s was more closely correlated to foam strength than η_s. Cumper (1953) reported tha bubble stability passed through a maximum as η_s increased.

Surface viscosity influences the ability of the surface film to dissipate disturbances and in foams it retards drainage of the lamellae. The surface yield stress is related to the viscoelasticity of the film and reflects the force required to overcome the elastic forces in the film. This depends very much on the number and extent of protein:protein interactions occurring between protein components in the film. High surface yield stress reflects a higher degree of inter- and intra-molecular interactions between polypeptides at the interface, which is in agreement with the formation of a condensed film at the interface near the pI. Apparently, this is due to the minimum electrostatic repulsion and optimum protein:protein interactions via hydrophobic interactions and hydrogen bonds, which help form a condensed film at the interface (July, 1972).

Film elasticity

Film elasticity (ϵ) determined at various pH values using tensiolaminometry revealed a marked maximum at pH 4.5–6 (Fig. 6B). Graham and Phillips (1980) reported that the dilatational modulus of bovine serum albumin at the oil-water interface was maximal near its pI. High film elasticity reflects a higher degree of inter- and intra-molecular interactions which result in the formation of stronger more cohesive films, that are more resistant to collapse. This is consistent with the fact that more condensed films are formed at the interface near the pI. Film elasticity modulus is defined by $\epsilon = -A\, d\, \pi/d \ln A$ where π is the surface pressure, and A is the area of the film. Therefore, the elasticity of a film is a measure of the resistance of a film to change in film area.

High film elasticity values are associated with a film which has a strong cohesive structure at the surface so that, when compressed, the molecules on the surface resist or are slow to change conformation and consequently exert greater surface pressure differential. If the molecules on the surface change their conformation rapidly or collapse during compression due to brittle characteristics of the molecules on the surface or if the molecules are not packed densely on the surface, they do not exert such a large surface pressure differential (Graham and Phillips, 1980). These authors showed a relationship between the facility of proteins to change conformation and elasticity. For example, β-casein with low relaxation time of about 10^{-8} sec had a low modulus while bovine serum albumin with high relaxation times possessed a high elastic modulus (Graham and Phillips, 1980). The rapid change of conformation was ascribed to the very flexible characteristics of the β-casein molecules whereas BSA with bulky tertiary structure was more resistant to change upon compression. The extent of molecular packing affects elasticity; that is, as surface is packed more tightly, elasticity increases up to a point beyond which further packing decreases elasticity due to collapse of the molecules at the surface (Graham and Phillips, 1980).

Foam stability

As discussed above, rapid reduction of surface pressure and strong rheological properties of films appeared to be directly correlated with an increase in foam stability. Since rapid reduction of surface tension and maxima in rheological properties occur near the pI, it is expected that foam stability should be maximal near the pI of bovine serum albumin which forms reasonably good foams (Graham and Phillips, 1976). Therefore, the foam stability as a function of pH was determined.

Plots of the log of drainage rates were linear (Fig. 2) at the various pH values studied and the half lifetimes of the foams were calculated from these data (Fig. 6C). The drainage stability of BSA foams showed a maximum in the isoelectric pH range indicating that the extensive protein:protein interactions which occurred in the films separating the foam lamella and which were responsible for the maxima in π, n_s, f_s and ϵ were important in foam stabilization. The data do not distinguish between the relative importance of the rate of π increase or viscoelastic properties of films and foam stability. Both may be important determinants. Obviously, rapid development is

critical in foam formation, eg., in food foams formation takes 2–5 min. Thus the values of π_5 are more relevant to foam formation than π at 24 hr. Our data were consistent with this because π_5 and foam stability showed a similar response to pH whereas the π_{24} was relatively insensitive to pH. In addition to rapid π increase, rapid maximization of η_s, f_s and ϵ were desirable in forming stable foams. These are favored where extensive protein:protein interaction facilely occurs and thus as shown by our data and others (Graham and Phillips, 1976, 1980; Waniska and Kinsella, 1985; Halling, 1981) were optimum near the isoelectric pH. They are markedly affected by all factors which influence protein:protein interactions, e.g., ionic strength, size, conformational and surface characteristics of the proteins (Halling, 1981; Kinsella, 1981; Cumper, 1953).

This study demonstrated some relationships between select physical characteristics of BSA protein films and stability of foam. The general validity of these relationships are being evaluated in other food proteins (β-lactoglobulin, soy 11S, ribulose-bis-phosphate carboxylase) (Waniska and Kinsella, 1985; Kim and Kinsella, 1985; Barbeau and Kinsella, 1985).

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Selective Concentration of Bovine Immunoglobulins and α-Lactalbumin from Acid Whey using $FeCl_3$

T. KANEKO, B. T. WU, and S. NAKAI

ABSTRACT

Immunoglobulins and α-lactalbumin of acid whey were concentrated in supernatant and precipitate when $FeCl_3$ was added at pH 4.2 and 2.8, respectively. Optimized conditions of pH 4.2 were preferable because of higher retention of immunochemical activity of immunoglobulins. In acid whey treated with 7.5 mM $FeCl_3$ at pH 4.2 and 4°C, 90% of β-lactoglobulin coprecipitated with serum albumin while 70% of immunoglobulins (92% immunochemically active IgG) and 95% of α-lactalbumin were retained in the supernatant. More than 98% of added iron was subsequently eliminated as precipitate by holding the treated whey at pH 8–9 and 4°C, without losing immunochemical activity of immunoglobulin G, in addition to retained activity of immunoglobulins A and M.

INTRODUCTION

WHEN FOOD is designed, not only texture and nutrients but also, to some extent, protection from diseases must be considered. This should be emphasized especially for artificial infant formula.

The resistance of breast-fed infants to diseases is generally ascribed to immunoglobulins (Igs), lactoferrin (Lf), lysozyme (Ly), leucocytes, lactoperoxidase, the bifidus factor and interferon (Packard, 1982). Among them, a synergistic bactericidal activity of secretory immunoglobulin A and Lf is an important specific immune defence mechanism in the intestinal tract of infants (Gindrat et al., 1972; Rogers and Synge, 1978; Stephens et al., 1980). Since these specific and nonspecific immune factors are contained much more in colostrum than in milk at any other lactation periods (Ogra and Ogra, 1978), infants should be fed mother's colostrum at the early infantile stage in particular. In the formulation of modern artificial infant milks, priority thus should not be restricted to the compositional and nutritional imitation of mother's milk, but at least some of the immunological properties of human milk should be incorporated.

Although human colostral milk is considered to be the most ideal source to supply Igs to infants, its industrial utilization is difficult. It has been reported by Hilpert et al. (1975) that bovine Igs from hyperimmune colostrum showed similar immunological properties and performances to those of human milk Igs both in vitro and in vivo, and that Igs from bovine colostrum can be added to infant formula. However, supply of the colostrum may be limited and unstable because of demand for it in calf-feeding.

From this point of view, bovine milk whey can be a potential source to provide Igs for infant formula. The direct utilization of whey proteins for infant formula, however, introduces an undesirable increase in β-lactoglobulin (β-Lg) which is absent or quite low in concentration in human milk and which is an allergenic milk protein (Lebenthal, 1975). Therefore, selective elimination of β-Lg with maximal retention of Igs, and α-lactalbumin (α-La) is desirable.

The separation of α-La and β-Lg in cottage cheese whey by a $FeCl_3$ precipitation method was reported by Kuwata et al. (1985). In this paper, the same method was studied in an attempt to concentrate selectively Igs in acid whey by reducing β-Lg to the maximum extent.

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MATERIALS & METHODS

ACID WHEY was prepared from raw milk obtained from the University Animal Science Farm. The raw milk was centrifuged at 4,000 × *g* for 30 min at 10°C to remove fat. Acid whey was then prepared from the skimmed milk by adding 50% lactic acid solution to pH 4.6 at 25°C and centrifuging at 10,000 × *g* for 15 min to remove casein precipitate.

β-Lg and α-La were purchased from Sigma Chemical Company (St. Louis, MO) and bovine serum albumin (BSA) was from Chemical Dynamic Corporation (South Plainfield, NJ). Crude immunoglobulin G (IgG) from bovine colostral whey was prepared by ammonium sulfate precipitation according to the method of Fey et al. (1976). Rabbit antibovine whole serum, IgA and M antisera and goat antibovine IgG antiserum were purchased from Miles Laboratories (Elkhart, IN).

Optimization procedure

The mapping super-simplex optimization (MSO) of Nakai et al. (1984) was carried out to find the most suitable conditions for the $FeCl_3$ treatment of whey which would give the maximum separation efficiency of Igs. A pocket size computer with 18 KB RAM, Sharp PC-1500 (Sharp Corp., Osaka, Japan), was used for computation of the MSO program.

Two optimizations were carried out for maximizing the recovery of Igs in the supernatant ("supernatant study"), and in the precipitate ("precipitate study"). The experimental conditions (factors) were varied according to MSO within the following ranges: (1) the supernatant study: pH 3.0–5.0; $FeCl_3$ concentration 2.0–8.0 mM; and temperature 10–40°C; (2) the precipitate study: pH 2.5–4.0; $FeCl_3$ concentration 2.0–8.0 mM; and temperature 10–40°C.

$FeCl_3$ treatment of acid whey

An aliquot of 1M $FeCl_3$ solution was added to 50 mL of pH adjusted whey while maintaining the pH by dropwise addition of 3N NaOH solution. The temperature was adjusted and the reaction mixture was held for 2 hr, then centrifuged at 10,000 × *g* for 15 min. The precipitate was dispersed in 5 mL 1M Tris-HCl buffer pH 7.0 and made up to 50 mL volume after further pH adjustment to 7.0, while the supernatant was neutralized to pH 7.0 with 3N NaOH solution. Experiments were carried out in duplicate and the pair of experiments was repeated when their difference was greater than 5% of their averge.

pH stability profile of IgG

To 1.8 mL pH adjusted acid whey, 0.2 mL 2% crude IgG preparation in 10 mM Tris-HCl buffer pH 7.0 was added. The mixture was incubated at either 10 or 30°C for 2 hr, and neutralized by adding 3 mL 1.5M Tris-HCl buffer pH 7.0. The immunochemical activity of IgG was determined by radial immunodiffusion method. Stability of IgG was expressed as percentage of the IgG content of the sample of pH 7.0 kept at 4°C for 2 hr.

Elimination and determination of iron

Ferric IgG concentrate was adjusted to pH 7–10 with 3N NaOH solution, held at 4°C for 4 hr, and centrifuged at 10,000 × *g* for 15 min. The supernatant was neutralized to pH 7.0, followed by volume adjustment. Iron content of both pH-treated and -untreated Igs concentrates was determined by atomic absorption spectrophotometry (model 560, Perkin-Elmer, Norwalk, CN).

Dodecyl sulfate-polyacrylamide gel electrophoresis

The method of Laemmli (1970) was used after modifications. Polyacrylamide gel electrophoresis in the presence of 0.2% sodium dodecyl sulfate (SDS-PAGE) was performed in a slab type vertical gel system using the Atto SJ 1060 SDH Electrophoresis unit (Atto Co., Tokyo, Japan).

A whole gel was composed of separating gel (lower gel) of 0.2 cm thick, 11 cm long, and 13.5 cm wide, and stacking gel (upper gel). Ten and 3% polyacrylamide gels were used as the separating and stacking gels, respectively, of which the ratio of acrylamide to N,N'-methylene-bis-acrylamide was 25. Polymerization of both gels was catalyzed by 0.02% ammonium persulfate.

One mL whey solution was treated with 5% SDS and 0.2 mM 2-mercaptoethanol in boiling water for 1.5 min, followed by the addition of 200 mg sucrose and 50 μL 0.05% bromophenol blue tracking dye solution. Twenty five μL of the treated whey solution was applied to the sample slot after the sample slots and upper electrode chamber were filled with Tris-glycine electrode buffer (3g Tris + 14.4g glycine + 1g SDS in 1L, pH 8.3).

Electrophoresis was performed at room temperature with a constant voltage of 90 volts until the tracking dye marker migrated to 1 cm from the gel bottom, in approximately 4.5 hr. After electrophoresis the gel was immersed in 0.25% coomassie brilliant blue R-250 dye solution for 1.5 hr with gentle stirring of the dye solution. The gel was rinsed with water, transferred to a diffusion destainer (model 172A, Bio Rad Laboratories, Richmond, CA), and destained vertically for 18–20 hr with a circulation of destaining solution (a mixture of 10% acetic acid and 7.5% methanol) through a cartridge of activated carbon.

Evaluation of separation efficiency

Peak areas of Igs and other whey proteins on the electrophoretograms were analyzed using the Kontes fiber optic scanner (model K-494800, Kontes Scientific Instruments, Vineland, NJ) together with the Varicord variable response recorder (model 42B, Photovolt Corp., New York, NY).

Separation efficiency (SE) was expressed as the "Igs to β-Lg ratio" calculated from the peak area of Igs (PA_{Igs}) and β-Lg ($PA_{\beta\text{-}Lg}$) on the densitometric patterns:

$$SE = PA_{Igs}/(PA_{Igs} + PA_{\beta\text{-}Lg}).$$

PA_{Igs} was estimated by multiplying the heavy chain peak area by a coefficient of 1.4 because the determination of light chain peak area was difficult due to overlapping with other minor protein components. The coefficient 1.4 was derived from analysis of IgG standard purchased from Sigma Chemical Company.

For quantitative analysis, the variation of staining and destaining conditions during electrophoresis was standardized using an internal standard of ovalbumin (ICN Pharmaceutical Inc., Cleveland, OH). Five μL 0.1% ovalbumin solution treated with SDS and 2-mercaptoethanol in the same way as described above was added to each whey sample solution and analyzed simultaneously. The mean value of ovalbumin peak areas was measured for every run and compared with the one obtained from samples for standard curves drawing. The ratio of ovalbumin values thus obtained was used as a correction factor.

Immunochemical analyses

Immunoelectrophoresis and immunodiffusion were carried out according to the method of Williams and Chase (1971) with modifications. Nine mL of 1% agarose in 0.05M barbital acetate buffer pH 8.3 was gelatinized over Gelbond film (0.02 × 7.5 × 10 cm, FMC Corporation, Marine Colloid Division, Rockland, MA). Three μL whey sample was applied to a punched sample well with a diameter of 2 mm and immunoelectrophoresis was performed at room temperature for 45 min with a constant voltage of 60 volts. Sixty μL antibody was added to the trough and diffusion was performed overnight at room temperature. Immunodiffusion was also carried out overnight at room temperature, in which 3 μL sample or antibody was applied to the respective well. After deproteinization by shaking in 0.3M, 0.15M NaCl solutions, and then water each for 1 day, the gel was air-dried, then stained with amido black 10B dye solution.

Immunochemical quantitative analysis of IgG was carried out by radial immunodiffusion (R.I.D.) with R.I.D. kit (Miles Laboratories). Whey samples were dialyzed against 20 mM sodium-phosphate buffer pH 7.0 for 2 days and freeze-dried. The powders were dissolved in 0.05M barbital acetate buffer pH 8.3 to give a concentration 10 times that of the original whey for Igs concentrate and original whey, and 20 times concentration for β-Lg concentrate.

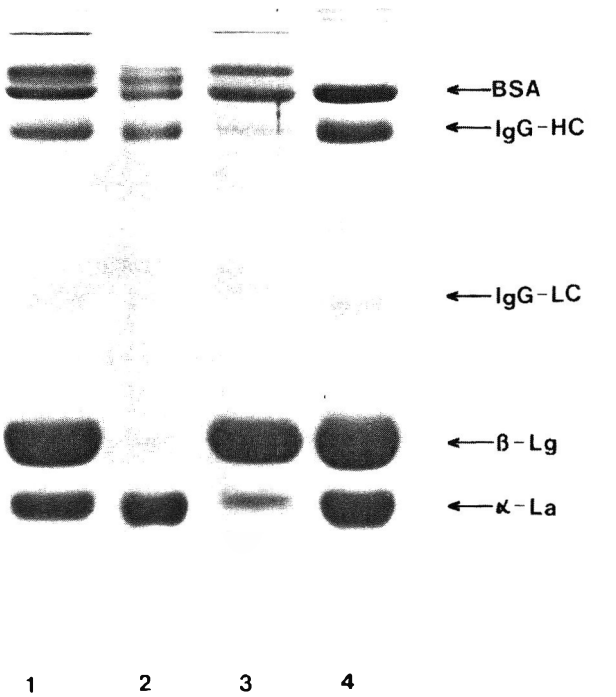

Fig. 1—Comparison of whey protein component by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (1) control acid whey; (2) precipitate; (3) supernatant, obtained after treatment with 5.3 mM $FeCl_3$ at pH 2.8 and 24°C; and (4) standard proteins. (BSA = bovine serum albumin; IgG-HC and -LC = immunoglobulin G heavy and light chains, respectively; β-Lg = β-lactoglobulin; α-La = α-lactalbumin).

RESULTS & DISCUSSION

Optimum conditions for separation of Igs and β-Lg

By incubating whey with 5.3 mM $FeCl_3$ at pH 2.8 and 24°C for 2 hr, 70% and 80% retentions of Igs and α-La, respectively, in the precipitate with 95% elimination of β-Lg in the supernatant were achieved (Fig. 1). These conditions are similar to those obtained by Kuwata et al. (1985). However, R.I.D. showed less than 25% recovery of the immunochemical activity in the precipitate. More than 60% of the activity was lost during the low pH treatment.

In the supernatant study, on the other hand, the mapping was executed with respect to pH and $FeCl_3$ concentration only after 27 experiments (Fig. 2a and b) because the reaction temperature was constant at 10°C after four experiments. From each response surface pattern, the locations of optimum pH and $FeCl_3$ concentration were estimated at 3.85 and 7.0 mM, respectively, which are indicated with a "T" in each Figure.

Additional experiments (changing pH and $FeCl_3$ concentration) were carried out for further improvement of the separation efficiency, SE; eight simultaneous shift experiments in which each factor level was simultaneously varied according to the MSO program by using the target values estimated above and the reaction temperature lower than 10°C. The responses are plotted against pH in Fig. 3a. The highest SE was obtained

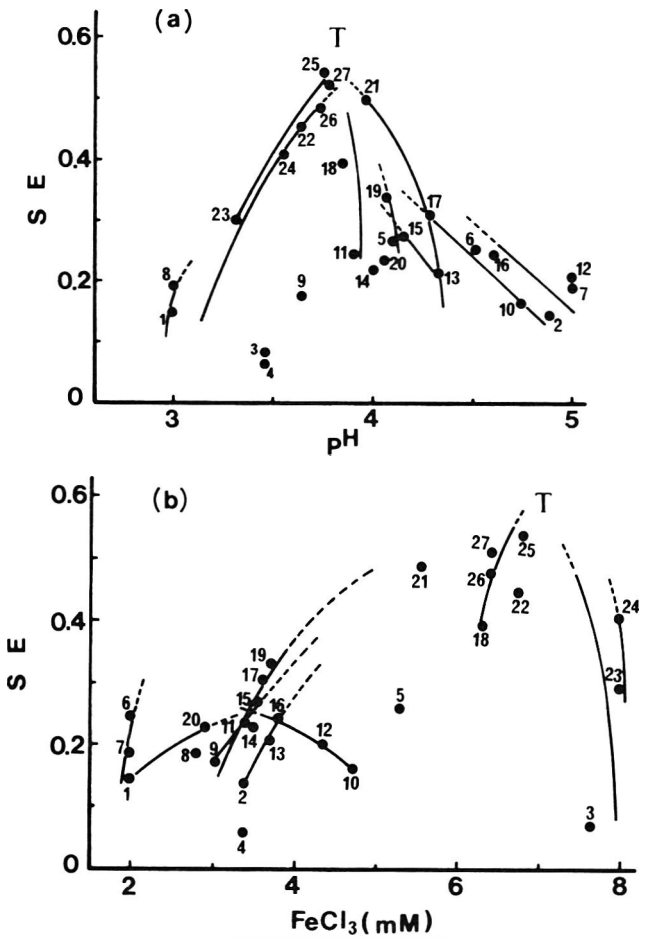

Fig. 2—Approximate response surface patterns for pH (a) $FeCl_3$ concentration (b) obtained by mapping data from the simplex optimization. Numbers beside each point correspond to the vertex number in the simplex optimization. "T" indicates the target value of each factor for the subsequent simultaneous shift procedure.

when whey was treated with 7.5 mM $FeCl_3$ at pH 4.2 and 4°C.

The percentage recovery of Igs and α-La in the supernatant as well as the percentage elimination of β-Lg are shown in Fig. 3b. Higher recovery of both Igs and α-La was obtained by increasing pH to 5, whereas the elimination of β-Lg appeared to be maximum at pH 4.2 and decreased rapidly above pH 4.5. At pH 5.0, most of the four major whey proteins including BSA were restored in the supernatant.

The highest SE with the maximal removal of β-Lg was achieved when whey was incubated with 7.0–7.5 mM $FeCl_3$ at pH 4.0–4.3. Since not only the efficiency of separation but also the recovery of Igs are important in the real operation, a 'separation recovery product value' of Igs (SRP) was calculated by multiplying SE by the percentage recovery of Igs quantitated from electrophoretogram. The highest SRP was 0.46 when 7.5 mM $FeCl_3$ was incubated with whey at pH 4.2 and 4°C for 2 hr (Fig. 3c). Under these conditions, 90% of β-Lg was precipitated and 70% of Igs and 95% of α-La were recovered in the supernatant (Fib. 3b). These optimal conditions are almost the same as the optimum conditions of Kuwata et al. (1985).

The electrophoretic patterns of the supernatant and precipitate of this optimized preparation are compared along with the untreated whey in Fig. 4, where the majority of Igs and α-La were in the supernatant while the major portion of β-Lg and BSA were in the precipitate. Recovery of Igs in the supernatant was evident immunochemically (Fig. 5a), where precipitated

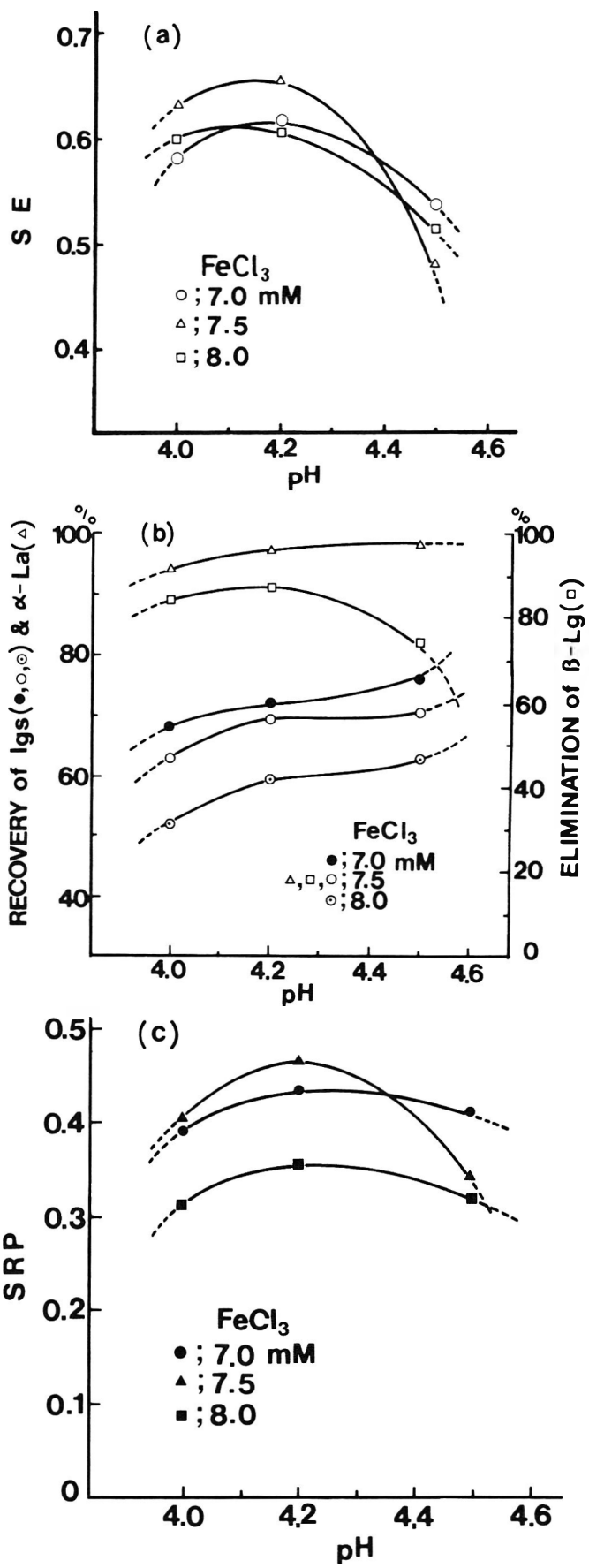

Fig. 3—Effects of pH and $FeCl_3$ concentration on the separation efficiency of immunoglobulins, SE, at 4°C (a), recoveries of immunoglobulins, Igs, and α-lactalbumin, and elimination of β-lactoglobulin, (b), and the separation recovery products (SRP) value of Igs (c).

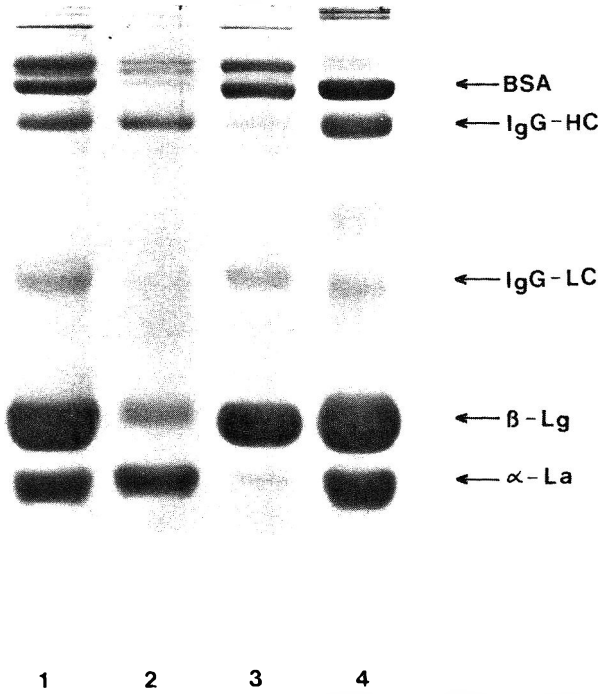

Fig. 4—Comparison of whey protein component by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (1) control acid whey; (2) supernatant; (3) precipitate, prepared at the optimum separation conditions (7.5 mM $FeCl_3$, pH 4.2, 4°C); and (4) standard proteins. For abbreviations see Fig. 1.

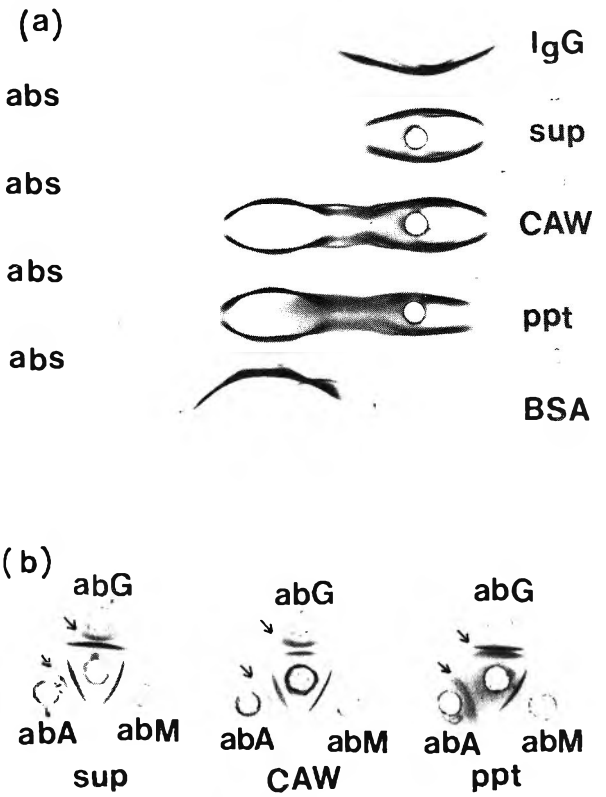

Fig. 5—Immunoelectrophoresis (a) and immunodiffusion (b) of whey preparations. [IgG = crude immunoglobulin G preparation; sup and ppt = supernatant and precipitate, respectively, obtained at the optimum separation conditions (7.5 mM $FeCl_3$, pH 4.2, 4°C); CAW = control acid whey; BSA = bovine serum albumin; abs = rabbit antibovine whole serum antiserum; abG = goat anti-bovine IgG; abA and M = rabbit anti-bovine IgA and M antisera, respectively. Precipitin lines indicated with an arrow are of BSA.]

BSA was observed. Approximately 92% of Igs in the supernatant was determined to be IgG by means of R.I.D.

All three major bovine immunoglobulin classes, IgG, A, and M, are identified in the control acid whey and the supernatant while no IgA is in the precipitate (Fig. 5b). The characteristic rection of $FeCl_3$ with IgA is similar to that of $ZnCl_2$ reported by Butler and Maxwell (1972).

pH stability profile of IgG

It was observed during the precipitate study that low pH treatments deteriorated the immunochemical activity of Igs. To confirm this possibility, the effect of acidic conditions on the immunochemical property of Igs was investigated by using a crude IgG preparation.

IgG was relatively stable at pH 3.5–7.0 (Fig. 6). At pH below 3.0, the immunochemical activity of IgG was more stable at 10°C than at 30°C. This result explains the loss of immunochemical activity of IgG recovered in the precipitate, as the optimal pH and temperature for the treatment have been 2.8 and 24°C, respectively.

It is concluded that the supernatant method (optimal treatment pH 4.0–4.3) is preferable to the precipitate method as conditions for the selective separation of Igs from whey. The conditions of the supernatant method are similar to those that Kuwata et al. (1985) have considered inappropriate due to coprecipitation of the majority of Igs with β-Lg, thus losing the immunological components in precipitates. This contradiction with regard to Igs behavior is probably derived from different electrophoresis techniques. In contrast to the smear, i.e., a broad and obscure electrophoretic trail of Igs by alkaline discontinuous-polyacrylamide gel electrophoresis (disc-PAGE), the SDS-PAGE produced clear densitometric peaks.

A rapid quantification of whey proteins by disc-PAGE was reported by Ng-Kwai-Hang and Kroeker (1984) and relatively sharp peaks were shown for Igs. However, IgM is probably not analyzed by this method because of difficulty in penetration into the separation gel due to its large molecular size. SDS-PAGE of mercaptoethanol-reduced samples is considered to be a superior quantitative analytical method for the total Igs content (IgA, G and M may all have been dissociated to heavy and light chains) in whey as well as for other major whey proteins.

Iron elimination

The iron content in the ferric Ig-La concentrate was 460 mg/g IgG. The direct utilization of this preparation into infant formula without reducing the iron level will result in the presence of great excess of iron in the formula. The iron level recommended for 0–6 month old infants by NAS/NRC (1979) is maximum 10 mg iron/13.2g protein/570–870 Kcal/day. In addition, excess iron may saturate the chelating capacity of Lf resulting in loss of the antimicrobial effect (Bullen et al., 1972; Reiter, 1983). Therefore, elimination of the excess iron is required.

Block and Zweig (1954) and Kuwata et al. (1985) reported chromatographic removal of iron from ferric whey protein using a cation exchange resin. Amberlite IR-112 revealed the best performance among various cation-exchange resins. Another chromatographic method by using carboxymethyl cellulose was reported by Sato et al. (1981) for the preparation of iron-free blood globin. In either method, pH lower than 2 was required for complete iron removal from iron-bound protein. However, the immunochemical activity of IgG was not determined in the iron-free Ig-La concentrate after treatment with Amberlite IR-112 at pH 1.5 by Kuwata et al. (1985). The loss of immunochemical activity at low pH is probably due to a structural transformation on the immunoglobulin molecule as indicated in the circular dichroism studies by Jirgenson (1973).

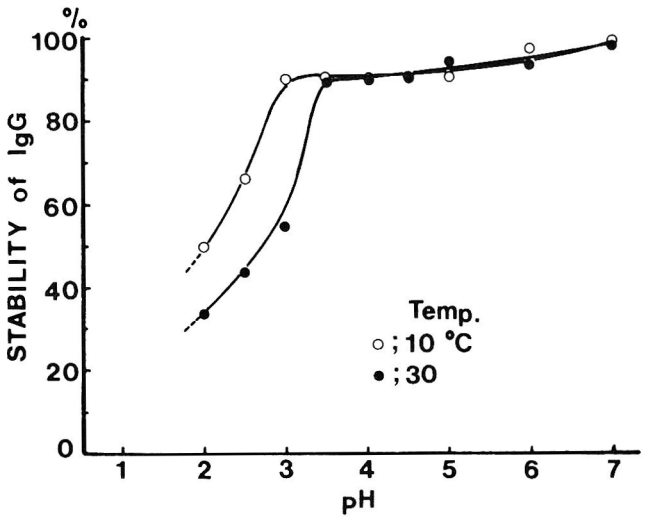

Fig. 6—Effect of pH on the immunochemical stability of immunoglobulin G at 10 and 30°C.

Table 1—Elimination of iron from the ferric immunoglobulins concentrate as a function of pH

pH	Fe mg/g IgG	Efficiency of Fe elimination (%)
4.2	460	0
7.0	42	90.9
8.0	7.2	98.4
8.5	5.2	98.9
9.0	4.4	99.0
9.5	4.0	99.1
10.0	3.6	99.2

Although the ion exchange for iron removal from iron-bound proteins has been shown to be useful, this method is not recommended due to a loss of immunochemical activity of immunoglobulins.

As a milder approach, low solubility of iron ions in the form of hydroxide was utilized for iron removal. When pH of the ferric Ig-La concentrate was adjusted to 8.0–10.0 more than 98% of the added iron was eliminated by centrifugation (Table 1), probably as insoluble complexes of hydroxide and phosphate. This high efficiency of iron removal by mild alkaline treatment (pH 8–9) implies that the iron in the ferric Ig-La concentrate is not bound to the proteins under these conditions.

The effect of mild alkaline treatments on the protein composition and immunochemical activity of IgG was studied. In the relatively large amount of yellowish precipitates, probably calcium citrate phosphate complex in addition to ferric hydroxide, only a trace of protein was electrophoretically detected (Fig. 7a). Whereas, no distinctive compositional change of protein was observed in the low-iron supernatants, which were colorless and clear. High retention of the immunochemical activity of these fractions during the mild alkaline pH treatment is shown in Fig. 7b which has been confirmed as 100% retention of the immunochemical activity of IgG by R.I.D.

When infant formula is fortified with Igs using this concentrate at the level of 36 mg/1.06g protein/100 mL which is equal to IgA level in 3–4 wk postpartum human milk (Kuvaeva et al., 1979), the resulting iron level is 0.14 mg/100 mL. The converted iron level per 13.2g protein is 1.8 mg, which is within the maximum iron level recommended by NAS/NRC. The excessive amount of iron in the Ig-La concentrate was thus efficiently reduced by a mild alkaline treatment.

Proposal of new infant formula

Table 2 compares the protein composition of the new infant formula to that of human and cow's milk and a current whey-

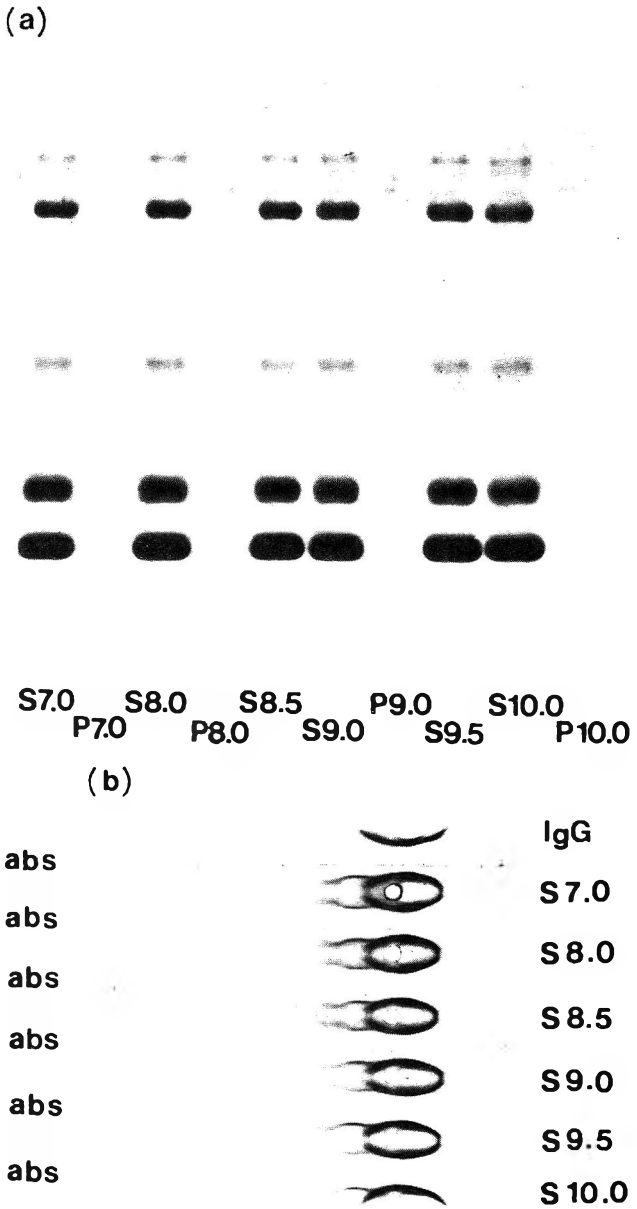

Fig. 7—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (a) and immunoelectrophoresis (b) of low-iron immunoglobulins and α-lactalbumin concentrate. (S and P 7.0–10.0 = supernatant and precipitate, respectively, obtained after treatment for iron elimination at pH 7.0–10.0; IgG = crude immunoglobulin G preparation; abs = rabbit anti-bovine whole serum antiserum.)

based formula. In the current whey-based infant formula, cow's milk has been substituted by several types of modified cheese wheys such as demineralized whey and whey protein concentrate, and its whey/casein protein ratio (W/C) is increased from about 20/80 of cow's milk to about 60/40 of human milk. However, as mentioned previously, β-Lg is the major whey protein in this type of humanized formula while α-La and lactoferrin are the predominant whey protein in human milk.

If β-Lg is eliminated from cow's whey with complete retention of Igs and α-La, the result can be as shown in the new infant formula (Table 2), in which Lf and Ly are fortified as nonspecific defence factors in addition to the Ig-La concentrate. Lysozyme can be separated from egg white as proposed by Friend et al. (1983). Based on an antimicrobial activity of ovotransferrin similar to that of lactoferrin from human colostrum, therapeutic administration of ovotransferrin to infants fed

Table 2—Protein composition of human and cow's milks and whey-based and proposed β-lactoglobulin (β-Lg) free infant formulae

Protein	Human[a] Total, %	Cow[a] Total, %	Whey-based formula Total, %	β-Lg free formula Total, %
Total	100	100	100	100
Caseins	35	79	40[b]	40
Total whey	65	21	60[b]	60
α-lactalbumin	17	3.5	10[c]	12[c]
β-lactoglobulin	--	9.0	26[c]	--
lactoferrin	17	--	--	17
serum albumin	6	1.0	3[c]	3[c]
lysozyme	6	--	--	6
immunoglobulins	11	3.0	8[c]	9[c]
others	8	4.5	13[c]	13[c]

[a] Gurr (1981).

[b] Friend et al. (1983).

[c] Whey protein composition is calculated based on the data of Gurr (1981).

cows' milk was suggested (Valenti et al. 1983). Although the complete elimination of β-Lg with a high recovery of Igs may be difficult to achieve using ferric precipitation method, our final Ig-La concentrate will give a similar profile to this proposed new formula.

The new infant formula may be advantageous immunologically, as the binding capability of IgG to Staphylococcae protein A is well recognized and the antiviral activity of IgG has been reported by Skvaril (1983).

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Comparison of Sensory and Instrumental Texture Profile Techniques for the Evaluation of Beef and Beef-Soy Loaves

P.L. BRADY, F. K. McKEITH, and M. E. HUNECKE

ABSTRACT

Relationships among sensory texture profile parameters, among values for textural parameters obtained through different methods of interpreting instrumental texture profile analysis (TPA) curves obtained with an Instron, and between sensory and instrumental TPA values were examined for beef and beef-soy loaves. The only singificant relationship among sensory parameters was between cohesiveness and springiness. Although a number of significant relationships existed between the various methods of interpreting instrumental parameters, a number of these were associated with secondary parameters having common primary parameters. A strong relationship was found between sensory springiness and adjusted downstroke cohesiveness. Sensory cohesiveness was related both to this instrumental parameter and to downstroke cohesiveness. Chewiness values calculated for these two methods for obtaining cohesiveness values showed strong negative associations with sensory fracturability scores.

INTRODUCTION

BECAUSE TEXTURE is a sensory property of foods that results from a multifaceted group of physical components (Bourne, 1982), evaluation of this quality attribute has frequently involved human panelists profiling the texture by assessing the mechanical, geometric and moisture-fat characteristics of the product, the degree of each of these present and the order of their appearance during the mastication process (Brandt et al., 1963). In such a texture profile procedure the trained panel functions as a calibrated instrument supplying a reproducible, unbiased description of the product's texture.

The disadvantages of expense and time associated with sensory texture profiling have led to investigations of instrumental procedures to provide descriptions of the textural attributes of a product. Instrumental texture profile analysis (TPA) can be extremely useful in evaluating the textural quality of foods; however, since texture is by definition a sensory characteristic (Szczesniak, 1963), the usefulness of any instrumental procedure is limited by its relationship to sensory assessment. A number of researchers have attempted to correlate sensory and instrumental TPA techniques and have shown varying levels of association (Bouton et al., 1971, 1975; Cross et al., 1980). The present study was designed to investigate the relationship of sensory evaluation of the textural parameters of beef and beef-soy loaves to values obatined through various methods of interpreting Instron TPA curves.

MATERIALS & METHODS

Sample preparation

Ground beef loaves were prepared by the Meat Science Laboratory, University of Illinois, Urbana, IL. Prior to any additions, the meat contained 25% fat. The ground beef was divided into three aliquots and salt was added to each to a 1% level. Unflavored, caramel-colored textured soy protein (ADM TVP u-218, minced 180) hydrated at a level of 2:1, water:soy, was added to aliquots to yield loaves containing 0, 15, or 30% soy. Four 1135-g loaves were prepared for each soy treatment. Pans and loaves were wrapped with foil and placed in a freezer at −30°C for 12 days. They were then moved to a −20°C freezer for storage until used, 3–14 days.

Loaves were assigned to days for heating such that two of the three treatment goups were represented on a given day. Forty-eight hours prior to heating, frozen loaves were placed in a refrigerator (4°C) to thaw. Loaves were heated in a preheated conventional electric oven at 177°C until an alcohol immersion thermometer inserted into the center of the loaf indicated an internal temperature of 70°C. They were allowed to cool, loosely covered with foil, for 30 min.

The crust was removed from the ends of each loaf and discarded; then the loaves were cut crosswise into 1.3-cm slices. Cylindrical cores, 2.5 cm in diameter, were removed from the slices and sealed in plastic wrap until used. Cores were randomly assigned for either sensory or instrumental testing.

Sensory evaluation

The texture of the beef and beef-soy loaves was evaluated by a 10-member trained sensory panel. The panel consisted of graduate students and staff from the Depts. of Foods & Nutrition and Food Science, Univ. of Illinois. All panelists were female ranging in age from 20 to 50 years.

Panel training consisted of eight, 1-hr sessions. In the first, the purpose of the project was explained and the panelists were familiarized with texture terminology (Civille and Szczesniak, 1973). The next two sessions were devoted to applying these concepts to the evaluation of a variety of food products. During the fourth session, panelists used the concepts learned in earlier sessions to develop a ballot for meat and to evaluate several meat products. The final four

Table 1—Interpretation methods for texture profile parameters from Instron curves

Textural parameter	Method of curve interpretation
Hardness	Maximum force of first compression cycle (kg) (Bourne, 1968)
Cohesiveness	Total = area curve 2/area curve 1 (Friedman et al., 1963)
	Downstroke = downstroke area curve 2/downstroke area curve 1 (Bourne, 1968)
	Adjusted Downstroke = (downstroke area curve 2 - upstroke area curve 2)/ (downstroke area curve 1 - upstroke area curve 1) (Peleg, 1976)
Springiness	Width between Curves (Friedman et al., 1963)
	Width Downstroke curve 2 (Bourne, 1968)
	Width Downstroke Ratio = width downstroke curve 2/ width downstroke curve 1
Gumminess	Hardness × Cohesiveness: (calculated for each hardness × cohesiveness combination)
Chewiness	Gumminess × Springiness: (calculated for each gumminess × springiness combination)

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Table 2—Correlation coefficients among instrumental TPA parameters of beef and beef-soy loaves

Instron TPA parameters	GuC	GuB	GuA	SpC	SpB	SpA	CoC	CoB	CoA	H
Hardness (H)	0.095	0.476	0.703*	0.274	−0.380	−0.215	−0.387	−0.408	−0.512	1.00
Cohesiveness (Co)										
A. Total	0.315	0.327	0.243	0.689*	0.140	−0.011	0.544	0.807**	1.000	
B. Downstroke	0.780**	0.591*	0.228	−0.383	0.243	−0.325	0.921**	1.000		
C. Adjusted	0.868**	0.527	0.043	−0.172	0.262	−0.389	1.000			
Springiness (Sp)										
A. Between Curves	−0.555	−0.537	−0.259	−0.290	−0.136	1.000				
B. Downstroke 2	0.123	−0.131	−0.337	0.445	1.000					
C. Downstrokes Ratio	0.029	−0.131	−0.236	1.000						
Gumminess (Gu)										
A. GuA	0.403	0.833**	1.000							
B. GuB	0.825**	1.000								
C. GuC	1.000									

n = 12.
* $p < 0.05$.
** $p < 0.01$.

Table 3—Correlation coefficients among instrumental TPA parameters of beef and beef-soy loaves

Instron TPA parameters	ChCC	ChCB	ChCA	ChBC	ChBB	ChBA	ChAC	ChAB	ChAA
Hardness (H)	0.168	−0.033	0.014	0.560	0.193	0.357	0.851**	0.397	0.467
Cohesiveness (Co)									
A. Total	0.110	0.311	0.378	−0.074	0.350	0.373	−0.222	0.327	0.210
B. Downstroke	0.631*	0.771**	0.785**	0.349	0.660*	0.450	−0.040	0.373	−0.055
C. Adjusted	0.770**	0.862**	0.855**	0.421	0.629*	0.336	−0.075	0.203	−0.281
Springiness (Sp)									
A. Between Curves	−0.582	−0.534	−0.258	−0.560	−0.483	0.191	−0.382	−0.294	−0.505
B. Downstroke 2	0.229	0.453	0.078	0.150	0.499	−0.232	−0.041	0.419	−0.404
C. Downstrokes Ratio	0.310	0.188	−0.082	0.432	0.168	−0.349	0.385	0.075	−0.446
Gumminess (Gu)									
A. GuA	0.314	0.243	0.365	0.593*	0.516	0.735**	0.782**	0.707*	0.699*
B. GuB	0.748**	0.693*	0.744**	0.823**	0.783**	0.712**	0.707*	0.687*	0.330
C. GuC	0.956**	0.939**	0.944**	0.792**	0.809**	0.541	0.399	0.453	−0.083
Chewiness (Ch)									
A. ChAA	−0.179	−0.209	0.098	0.096	0.084	0.775*	0.413	0.411	1.000
B. ChAB	0.448	0.550	0.401	0.670*	0.859**	0.555	0.723*	1.000	
C. ChAC	0.496	0.348	0.308	0.846**	0.598*	0.507	1.000		
D. ChBA	0.440	0.411	0.703**	0.526	0.539	1.000			
E. ChBB	0.811**	0.899**	0.743**	0.825**	1.000				
F. ChBC	0.880**	0.767**	0.696*	1.000					
G. ChCA	0.883**	0.877**	1.000						
H. ChCB	0.938**	1.000							
I. ChCC	1.000								

n = 12.
* $p < 0.05$.
** $p < 0.01$.

sessions were devoted to refining the ballot for beef and beef-soy products.

The final ballot consisted of eight parameters each evaluated on a 15-cm line scale: hardness, cohesiveness, springiness, adhesiveness, fracturability, chewiness, gumminess, and mealiness (Szczesniak, 1963). For all parameters, except mealiness, the lines were anchored 1 cm from the left end with the term slight and 1 cm from the right end with the term extreme. Anchors for mealiness were low, on the left, and high, on the right. Responses of panelists were numercially interpreted by measuring the distances, in cm, from the beginning of the scale to the slash mark made by the panelist.

At each test session, the order of sample presentation was randomized among the panelists. Samples were coded with 3-digit random numbers and served at room temperature under green incandescent light. The panelists were seated in individual booths and received two cores from each treatment, served on white plates. They were instructed to rinse their mouths with apple juice before testing and between samples.

Instrumental testing

Cores were compressed to 75% of their original height using a 5.8 cm diameter plunger attached to an Instron, Model 1132. A 500-kg load cell was used with a range setting of 5 and crosshead and chart speeds of 20 cm/min. Each core was compressed twice to give a "two-bite" work-force comrpession curve. Seven samples were tested for each replication. Textural parameters were derived from the curves as described in Table 1.

Statistical analysis

A completely random design with four replications was used. Correlation coefficients were calculated to determine relationships among sensory parameters, among instrumental parameters and between sensory and instrumental parameters (Sokal and Rohlf, 1969).

RESULTS & DISCUSSION

THE ONLY SIGNIFICANT relationship among sensory texture profile parameters was between cohesiveness and springiness ($r = 0.794$, $p < 0.01$). This may be related to the fact that both of these parameters tend to reflect the strength and resilience of the internal bonds of a product.

Many of the relationships which existed among instrumental TPA parameters or the various methods for interpreting them were associated with secondary parameters having common primary parameters (Tables 2 and 3). The relationship between gumminess A (calculated as hardness × total cohesiveness) and gumminess B (hardness × downstroke cohesiveness) probably resulted from both samples having the common hardness factor and the strong correlation which existed between cohesiveness values calculated by the two methods.

Sensory springiness scores correlated with three instrumental TPA parameters (Table 4): adjusted downstroke cohesiveness, chewiness H (the product of hardness, adjusted downstroke cohesiveness, and downstroke 2 springiness) and chewiness I

Table 4—Correlation coefficients between instrumental TPA parameters and sensory texture profile parameters of beef and beef-soy loaves

Instron TPA parameters	Sensory texture profile parameters							
	Springy	Hard	Cohesive	Fracture	Gummy	Chewy	Adhesive	Mealy
Hardness (H)	−0.208	−0.136	−0.373	−0.301	0.000	0.016	−0.468	0.572
Cohesiveness (Co)								
A. Total	0.221	0.320	0.475	−0.245	−0.148	−0.063	−0.113	−0.319
B. Downstroke	0.556	0.027	0.757**	−0.531	0.070	0.054	−0.222	−0.269
C. Adjusted	0.592*	−0.209	0.744**	−0.557	0.168	0.066	−0.182	−0.221
Springiness (Sp)								
A. Between Curves	−0.356	0.570	−0.292	0.435	0.270	−0.034	0.115	−0.469
B. Downstroke 2	0.323	0.393	0.227	0.378	−0.107	−0.376	−0.088	−0.024
C. Downstroke Ratio	0.247	−0.169	0.052	0.210	0.167	0.055	0.140	0.314
Gumminess (Gu)								
A. GuA	−0.051	0.076	0.000	−0.579*	−0.069	−0.010	−0.592*	0.329
B. GuB	0.346	−0.169	0.383	−0.807**	0.075	0.010	−0.544	0.239
C. GuC	0.536	−0.294	0.680	−0.745**	0.215	0.124	−0.393	0.000
Chewiness (Ch)								
A. ChAA	−0.284	0.508	−0.223	−0.181	0.121	−0.028	−0.426	−0.038
B. ChAB	0.177	0.394	0.140	−0.251	−0.139	−0.331	−0.631*	0.248
C. ChAC	0.092	−0.037	0.000	−0.395	0.076	−0.028	−0.450	0.473
D. ChBA	0.141	0.290	0.294	−0.583*	0.323	0.102	−0.564	−0.145
E. ChBB	0.483	0.133	0.492	−0.464	0.044	−0.178	−0.568	0.125
F. ChBC	0.433	−0.203	0.406	−0.597*	0.233	0.068	−0.430	0.301
G. ChCA	0.509	−0.126	0.707*	0.702*	0.338	0.171	−0.401	−0.208
H. ChCB	0.615*	−0.126	0.680*	−0.537	0.163	;0.024	−0.392	−0.019
I. ChCC	0.661*	−0.320	0.637*	−0.664*	0.306	0.159	−0.312	0.074

n = 12.
* $p < 0.05$.
** $p < 0.01$.

(hardness × adjusted downstroke cohesiveness × downstroke ratio springiness). The cohesiveness ratio obtained when the upstroke area was subtracted from the downstroke area seemed to be well associated with sensory springiness.

Sensory cohesiveness was highly correlated with both downstroke and adjusted downstroke cohesiveness. The latter relationship was further supported by the correlations of sensory cohesiveness with chewiness G, H and I parameters, all of which had adjusted downstroke cohesiveness as a factor in their calculation.

Instrumental chewiness values obtained from combinations of hardness with either downstroke cohesivness or adjusted downstroke cohesiveness showed significant negative correlations with sensory fracturability scores. Instrumental chewiness was a force encompassing measurement as was sensory fracturability. Since samples which fractured in the mouth with low levels of force were not judged to be chewy, this negative relationship was expected.

In conclusion, some relationships existed between sensory texture profile parameters and instrumental TPA values for beef and beef-soy loaves. Texture profiling is useful in providing a picture of the parameters present within a food and the intensities of each; however, further research is needed to determine how values obtained from instrumental TPA relate to sensory responses to texture.

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Heat Gelation Properties and Protein Extractability of Beef Myofibrils

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ABSTRACT

At a heating rate of 1°C/min suspensions (pH 6.0) of isolated beef myofibrils were found to start forming gels at 43–56°C, as detected by dynamic rheological measurements. The increase in gel storage modulus levelled off at temperatures >65°C. At medium to high (0.3–0.6M) concentrations of sodium chloride, addition of pyrophosphate (plus magnesium chloride) had the following effects: (1) both the protein extractability of non-heated myofibrils and the storage moduli of heat-induced gels were markedly increased; (2) the apparent activation energy for gel formation was decreased. Increasing concentrations of sodium chloride, up to 0.5–0.6M, increased the protein concentration of the liquid phase of the gels.

INTRODUCTION

WATER-HOLDING and binding (gelling) properties are the important factors that determine the quality of comminuted meat products. These properties are closely interrelated. Additives such as salt and various polyphosphates are commonly used to improve water-holding and binding properties.

Salt (sodium chloride, alternatively potassium chloride) is well known to aid in the solubilization of myosin; at low salt concentrations (<0.4M) myosin filaments prevail. Yasui et al. (1964a, b) reported that polyphosphates influenced the solubility of myosin B (actomyosin). They also reported that actomyosin was dissociated into myosin and actin by the addition of pyrophosphate to the solution and suggested that this dissociation may be the factor that influences the improvement of binding properties the most directly. Siegel and Schmidt (1979) confirmed that the increased binding obtained when adding polyphosphates was due to increased solubility of myosin derived from dissociation of actomyosin. That binding is dependent on the presence of dissolved myosin coincides with other results (Fukazawa et al., 1961; Samejima and Yasui, 1978). Hamm (1970) pointed out that phosphates also act by increasing pH and ionic strength as well as by binding to meat proteins.

The mechanisms of gel formation of myosin or of myosin in the presence of actin, upon heating, have become more clear through recent extensive work (Yasui et al., 1979, 1980; Ishioroshi et al., 1979, 1980, 1981; Samejima et al., 1981, 1982, 1984; Acton et al., 1981). From these results, the characteristics of myosin and actomyosin gel formation correspond well with the binding properties observed for comminuted meat products. Nevertheless, the binding properties pertaining to meat products constitute a complex phenomenon involving more than one factor (Schnell et al., 1970). It must be expected that studies of systems more complex than pure protein solutions/suspensions will provide further understanding of the binding properties.

In the present work myofibrils prepared from beef were the subject for study. The objective was to acquire more of the fundamental knowledge needed to understand and, as the ultimate goal, predict binding in comminuted meat products by characterizing myofibril properties with respect to gel formability and protein extractability.

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MATERIALS & METHODS

Preparation of myofibrils

Beef (cow) loin (Longissimus dorsi) was obtained from a local abattoir within 2 hr after slaughter. The meat was immediately (pre rigor) frozen at −30°C and stored for about 3 months at the same temperature. Frozen beef was chosen as an appropriate raw material since, in the majority of cases in Norway, comminuted meat products are made from frozen materials. Thawing took place at 5°C; muscle contraction was not deemed a problem since sarcomere length has been shown to have no major effect on protein extraction or swelling of myofibrils (Offer and Trinick, 1983). The procedure for myofibril isolation has been described by Harbitz et al. (1982) and involves a final centrifugation step in which a sucrose density gradient is used. The sucrose-containing myofibril fractions were diluted with an equal volume of borate buffer (pH 7.1; 25 mM NaCl; 5 mM EDTA) and centrifuged (Beckman JA-20 rotor, 4000 rpm, 20 min); the myofibrils were then washed by resuspension in the same buffer and re-centrifuged. All preparation steps were carried out at 5°C.

Gel formation; measurements of gel strength

To facilitate comparison with results obtained for myosin (Yasui et al., 1979), actomyosin (Yasui et al., 1980) and myofibrils from rabbit (Samejima et al., 1983) pH 6.0 was selected. The myofibrils were equilibrated at pH 6.0 by washing twice in a 0.04M phosphate buffer containing 0.1M sodium chloride. The firm pellet of myofibrils obtained was then suspended in the same buffer, containing various concentrations of sodium chloride, with or without 5 mM pyrophosphate and 5 mM $MgCl_2$. (The presence of Mg^{2+} is necessary for pyrophosphate to act as a dissociating agent of the actomyosin system). After adjusting the volume to give standard concentration (10 mg protein/mL), 2.5 mL were placed in the temperature controlled cell of a Bohlin Rheometer System (Lund, Sweden) and heated from 25–72°C at a rate of 1°C/min. Gel strength was evaluated in terms of dynamic shear stress response to small amplitude oscillations at 1 min intervals. The dynamic measurements were made at a fixed frequency of 1 Hz and strain of 0.06, the oscillations going on for 0.13 min at the times of measurement only.

Determination of protein extractability and protein concentration in the liquid phase of gels

The relative protein extractability of myofibril preparations with or without 5 mM pyrophosphate and $MgCl_2$ in the various salt concentrations (0.1–0.6M NaCl) at pH 6.0 was determined by measuring the absorbance at 280 nm of the respective supernatants: samples were centrifuged at 40,000 rpm for 1 hr (Beckman Ti-50 rotor). Centrifugation of gels at 20,000 rpm for 30 min (Beckman Ja-20 rotor) yielded small pellets and clear supernatants; the optical density of the latter constituted a relative measure of the protein concentration in the liquid phase of the gel.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), was carried out essentially in accordance with Porzio and Pearson (1977).

Protein concentrations were determined by the biuret method (Gornall et al., 1949).

Reproducibility Several replicates, starting with Longissimus dorsi

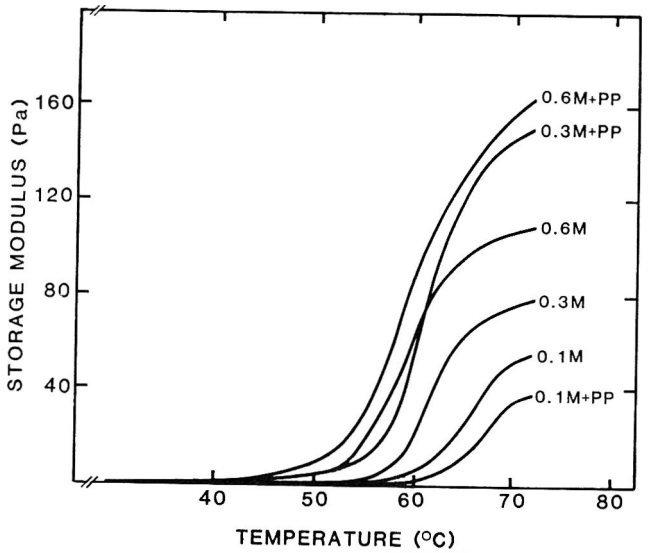

Fig. 1—Effect of salt concentration (molarity of NaCl) and presence of pyrophosphate (5 mM) and $MgCl_2$ (5 mM), indicated by PP in the figure, on the development of gel strength, expressed as storage modulus, in suspensions of myofibrils (10 mg/mL) at pH 6.0.

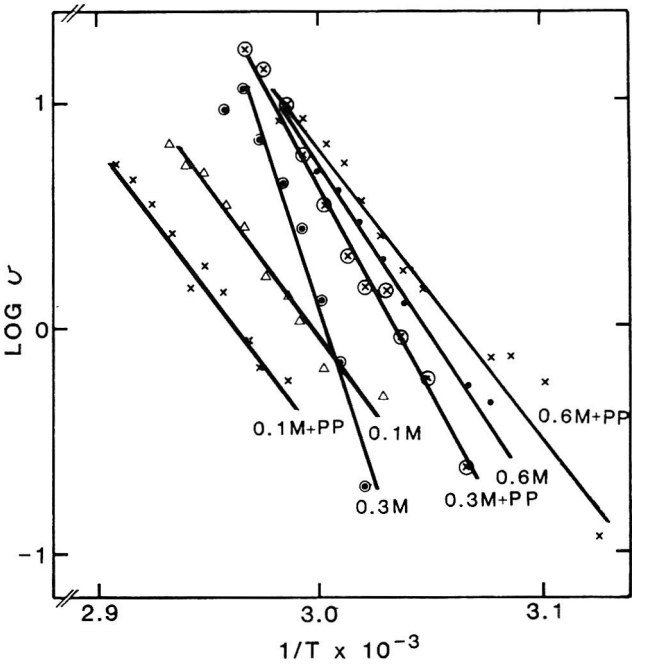

Fig. 2—Arrhenius plots derived from the curves given in Fig. 1. The corresponding apparent heats of activation, derived from the slopes of these lines, are given in Table 1.

from cows of similar age, all yielded storage moduli within ± 10% of the mean value at 72°C. The rheometer reproducibility is excellent; variations of ± 2.5% on reproducing thermograms of the same myofibril preparation are almost entirely ascribed to inaccurate protein determinations.

RESULTS

THE EFFECT of heating on myofibril preparations is shown in Fig. 1. Gel formation was first detected at temperatures of about 43°, 53°, and 56°C, respectively, when myofibrils were heated in salt concentrations of 0.6, 0.3, and 0.1M NaCl. Clearly, the gel strength of the heated myofibril samples decreased in the order 0.6, 0.3, and 0.1M NaCl. Addition of pyrophosphate dramatically increased the storage modulus of the 0.6M and 0.3M NaCl systems and also lowered the temperature of initial gel formation. However, the positive effect of pyrophosphate on gel strength was not observed in the case of 0.1M NaCl. Supplementary experiments including higher temperatures confirmed the sigmoid shape of the storage modulus versus temperature curves (results not shown).

Arrhenius plots, obtained from the results of Fig. 1, are shown in Fig. 2. The apparent heat of activation $[(E_a)_{app}]$ for gel formation was calculated from the slopes of Fig. 2, using the Arrhenius equation. As seen in Table 1, $(E_a)_{app}$ in 0.3M NaCl was markedly higher than those observed in the case of 0.6M and 0.1M NaCl. In the presence of 5 mM pyrophosphate, $(E_a)_{app}$ was lowered in the 0.6M and 0.3M NaCl systems, while making no difference when 0.1M NaCl was used.

Fig. 3 shows that the extractability of proteins from non-heated myofibrils increases with increasing salt concentrations. If pyrophosphate were added to these systems, the extractability was drastically promoted at salt concentrations above 0.2M NaCl.

Fig. 4 shows, in analogy, how salt affects the final protein concentration of the liquid phase of heat-induced gels, as determined by heating myofibrils in various salt concentrations and centrifuging the resulting gels. Evidently, the amount of protein which was not included in the gel structure, directly or indirectly, increased with salt concentration. It should be noted that, at higher salt concentrations, pyrophosphate decreased the protein concentration of the liquid phase, even though pyrophosphate initially brought much more protein into solution (Fig. 3).

Table 1—Estimated apparent heats of activation for gel formation of myofibril suspensions (10 mg/mL, pH 6.0); calculated from the results given in Fig. 2

Conc of NaCl (M)	Presence of pyrophosphate and $MgCl_2$ (both 5 mM)	Activation energy (kJ/mole)
0.6	–	297
0.6	+	247
0.3	–	515
0.3	+	339
0.1	–	268
0.1	+	272

The SDS gels of the supernatant before and after heating myofibril samples are shown in Fig. 5. [Protein assignments in accordance with Porzio and Pearson (1977).] There was no protein band in the supernatant of unheated samples in 0.1M NaCl, even in the presence of pyrophosphate (result not shown), but it is seen that troponin-T (Tn-T) and tropomyosin (TM) as well as small amounts of, presumably, myosin light chains (LCs) and troponin-I (Tn-I) were solubilized upon heating in 0.1M NaCl (b, c). On the other hand, a larger amount of myosin was extracted from the myofibril preparation in the presence of pyrophosphate in the 0.3 and 0.6M NaCl systems (e, i). But the electrophoretogram also showed that actin, Tn-T,TM, Tn-I and -C were also extracted on addition of pyrophosphate, especially in 0.6M NaCl (e, i). The proteins in the liquid phase of the heated samples were almost the same regardless of salt concentration (c, g, k). A slight decrease may be seen, however, in the intensity of the protein bands corresponding to LCs, Tn-I and -C when pyrophosphate was present. As far as the 0.6M NaCl condition was concerned, this qualitative observation in Fig. 5 corresponded to the analytical data of Fig. 4. However, further quantitative studies are needed.

In Fig. 6 final gel strengths from Fig. 1 have been correlated with the protein extractibility of the initial myofibril suspensions (Fig. 3). However, data for the 0.1M NaCl condition were not used due to uncertainties in the low absorbance readings.

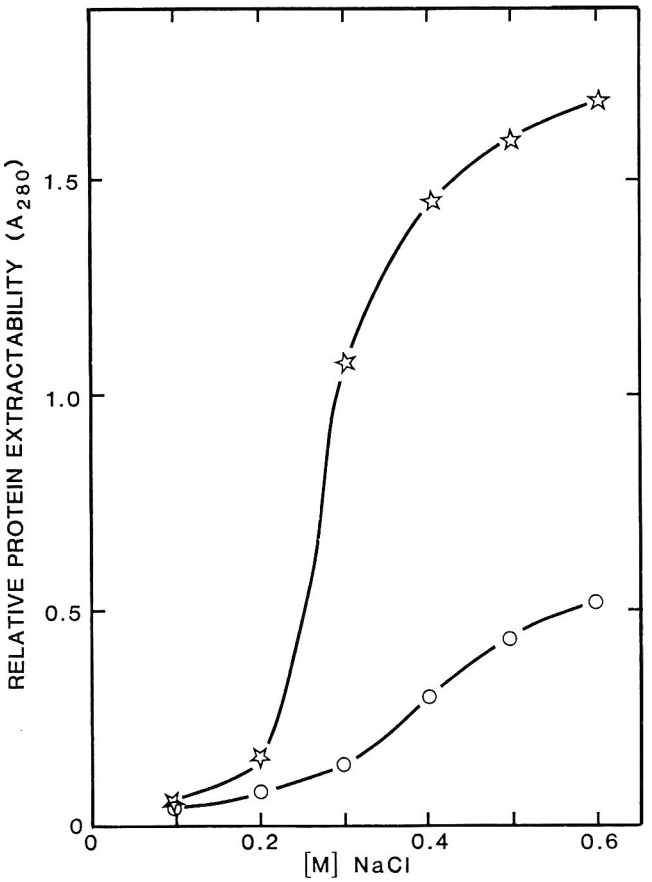

Fig. 3—Effect of salt concentration and presence of pyrophosphate (5 mM) and $MgCl_2$ (5 mM) (starred curve) on the protein extractability of nonheated myofibrils.

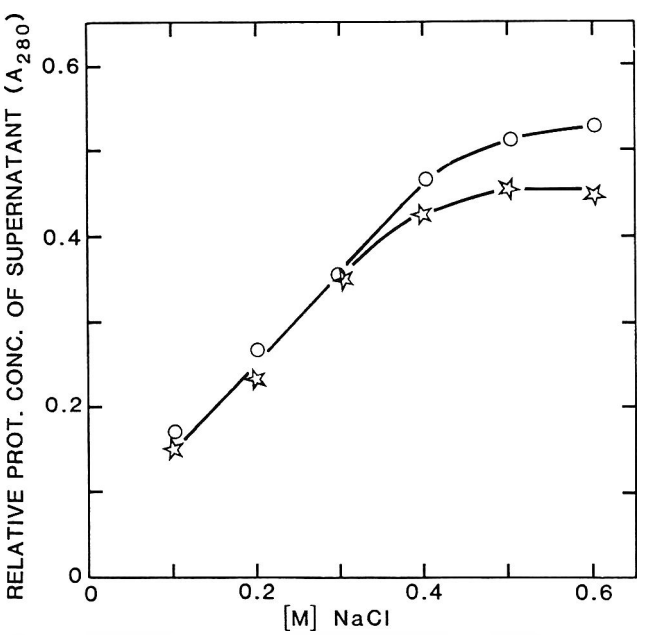

Fig. 4—Identical with Fig. 3 except pertaining to the protein concentration in the liquid phase, obtained by centrifugation, of myofibril gels heated to 72°C.

DISCUSSION

YASUI et al. (1979, 1980) investigated the heat-induced gelation of rabbit myosin and actomyosin in model systems. They reported that gel rigidity started to develop at about 40°C and reached a maximum level at about 60°C. Samejima et al. (1981) found a similar result for the gelation of myosin and its subfragments, as well as for rabbit myofibrils (unpublished data). Acton et al. (1981) showed that bovine actomyosin gels at temperatures as low as 30°C when held for 30 min.

The higher temperatures at which gelation of myofibrils was found to occur in this study (Fig. 1) should be viewed in the light of: (1) our myofibril samples were heated at a constant rate of 1°C/min from 25–72°C, i.e., the curves of Fig. 1 are not equilibrium curves; (2) the detection of gelation onset is a question of instrument sensitivity; in order to include measurements on the relatively strong gels at enhanced temperatures we did not opt for maximum sensitivity at the lower temperatures in this investigation; (3) the properties of the specific preparations studied may also be of consequence, for example, Asghar et al. (1984) reported marked differences in the heat-induced gelation behavior of myosin prepared from red and white muscle.

The positive correlation between protein extractability and binding properties has been established in fundamental studies as well as in work of direct relevance for meat processing (Acton and Saffle, 1969; Acton, 1972; Randall and Voisey, 1977; Siegel and Schmidt, 1979; Miller et al., 1980; Morita et al., 1983). Our results, Fig. 1 and 3, again confirm the importance of maximizing the concentration of dissolved protein prior to heat treatment. As shown in Fig. 6, gel strength — based on the storage moduli at 72°C, Fig. 1 — appears to be a linear function of the concentration of dissolved protein in the solution heated (from Fig. 3) in the lower range of dissolved protein concentrations. Extrapolation of the curve suggests that maximal extraction of protein will increase gel strength about 2.5-fold compared to a hypothetical zero-concentration situation (i.e., nonextracted myofibrils suspended in pure buffer).

Gel strength appears to level off towards a maximum value in Fig. 6. This is probably related to extraction of higher amounts of proteins such as Tn-T, TM and possibly Tn-I and -C in the 0.6M NaCl plus pyrophosphate condition than in the corresponding 0.3M NaCl case (Fig. 5; e,i). Earlier results (Fukazawa et al., 1961; Samejima et al., 1982; Yasui et al., 1982) have made it clear that the presence of TM and Tn does not enhance the strength of myosin gels.

Mahon (1961) demonstrated a synergistic effect on water retention of the addition of salt and phosphates, as was also later reported for binding strength in meat products (Siegel and Schmidt, 1979; Theno et al., 1978; Moore et al., 1976). Recently, however, Trout and Schmidt (1984) concluded that effects from salt level, as well as phosphate type and concentration, on binding in beef rolls could be accounted for in terms of changes in ionic strength and pH. Since the thermograms in Fig. 1 were all obtained at pH 6.0, there is no doubt that our results for the strength (storage modulus) of heat-induced myofibril gels confirm a synergistic effect. We suggest that our results do not agree with those of Trout and Schmidt (1984) because, as they point out, measurement of tensile strength in their system puts two elements of the total strength to the test: the strength of homogenate adhesion to the surfaces of meat particles as well as the strength of the homogenate itself. One would expect the observed tensile strength for the restructured beef rolls to depend primarily on the weaker of the two. Thus, if adhesion constitutes "the weak link in the chain," changes in homogenate strength may pass more or less unnoticed. It is also conceivable that positive effects on adhesion may have adverse effects on homogenate strength or vice versa. Our myofibril suspensions/gels correspond to homogenates free of meat particles, and observed effects lend themselves more easily to interpretation.

Pyrophosphate decreased the activation energies of the gel-forming interactions when myofibril samples were heated in 0.3 and 0.6M NaCl (Fig. 2 and Table 1). (Note: These are *apparent* activation energies since the rate curves used (Fig.

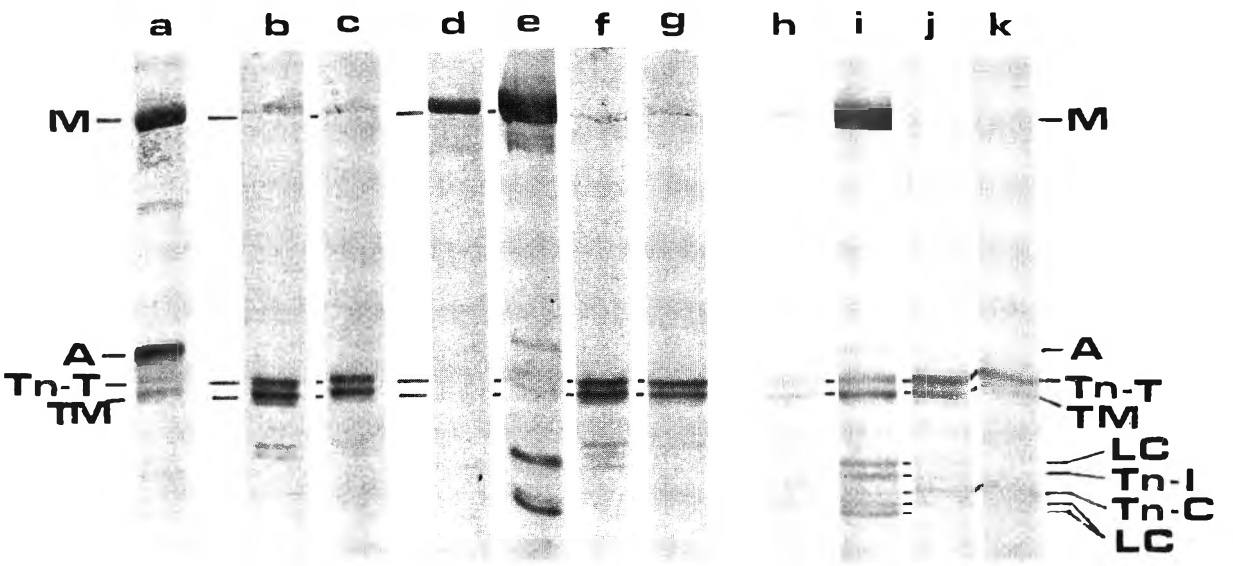

Fig. 5—Results from SDS-PAGE of myofibrils and of supernatants obtained by centrifugation of myofibril suspensions before and after heat treatment: M = myosin heavy chains; A = actin; Tn-T = troponin-T; Tn-I = troponin I; Tn-C = troponin C; TM = tropomyosin; LC = myosin light chains.

(a) Myofibril sample [control, native (non heated)];

*(b), (c) 0.1M NaCl: (b) Heated; (c) Heated with PP**

(d), (e), (f), (g) 0.3M NaCl: (d) Native (nonheated); (e) Native with PP; (f) Heated; (g) Heated with PP

(h), (i), (j), (k) 0.6M NaCl: (h) Native; (i) Native with PP; (j) Heated; (k) Heated with PP

**PP = pyrophosphate (5mM) + $MgCl_2$ (5 mM)*

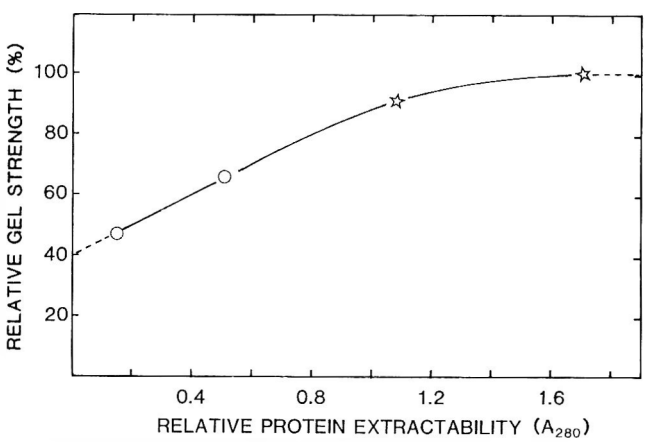

Fig. 6—Dependence of gel strength, measured as storage modulus after heating to 72°C (Fig. 1), on the protein extractability (Fig. 3) of myofibrils in the suspensions from which the gels were made by heating. Data for the 0.1M (+PP) conditions were not included.

1) are not genuine equilibrium curves). However, it had no effect in the 0.1M NaCl case, presumably because the ionic strength remained too low for any myosin to be dissolved. It is not clear from the present study why the activation energy in 0.3M NaCl was so high compared to those of the 0.1 and 0.6M NaCl situations. Possibly, special characteristics of the structure of myofibrils in 0.3M NaCl are reflected; Offer and Trinick (1983) have reported that the salt concentration has marked effects on myofibril structure around 0.4M. Further studies are needed to gain better understanding of this observation. The apparent heat of activation determined for the 0.6M NaCl condition was about 2.5-fold higher than that reported by Ziegler and Acton (1984) for aggregation of actomyosin. Clearly, a discrepancy was to be expected since myofibrils differ from actomyosin. However, it should also be borne in mind that (irreversible) aggregation/gelation depends on preceding denaturation. Estimated apparent activation energies will, therefore, depend on the relative importance of these two processes for the macroscopic measurements carried out. At 1/20th of our protein concentration, Ziegler and Acton's (1984) turbidimetric observations were probably more affected by the aggregation process than were our rheological thermograms.

In general, the cooking loss of meat products on heat treatment decreases with increasing salt concentration. For example, Neer and Mandigo (1977) reported that the cooking yield and water-holding capacity of a flaked, cured pork product increased with increasing salt levels. The results of Fig. 4 suggest, however, that the concentration of protein in the liquid released after heat treatment increases with increasing salt concentrations.

Finally, it should be pointed out that it is necessary to exercise care when relating results from experiments such as these to the behavior of complete meat systems. Nonetheless, the properties of meat batters are no more than a composite of the constituents' properties, including how the constituents interact. Characterizing myofibrils, therefore, implies partial characterization of comminuted meat systems.

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Effect of Subprimal Fabrication and Packaging Methods on Palatability and Retail Caselife of Loin Steaks from Lean Beef

M. F. MILLER, G. W. DAVIS, and C.B. RAMSEY

ABSTRACT

Eighty subprimals were fabricated from 20 pairs of beef strip loins from lean (fat thickness, 0.2–0.4 cm) US Standard and US Good carcasses. Three vacuum packaged (VP) and one dry aged treatments were randomly assigned to short loin sections of each carcass. Steaks from VP intact and VP not-reassembled subprimals were rated highest in overall appearance and eating quality. Dry aging reduced retail caselife, produced greater shrinkage and trim losses and lower overall satisfaction ratings by panelists. Precutting subprimals into steaks followed by VP aging tends to decrease retail caselife (especially if steaks are reassembled) and decrease juiciness and tenderness.

INTRODUCTION

TECHNOLOGY currently exists to centrally cut and vacuum package (VP) fresh beef into case-ready retail cuts. The advantages of centrally cutting and packaging steaks and shipping steaks to retailers as a case-ready product, becomes more evident as economic pressures on the meat industry mount. Jaye et al. (1962) and Seideman et al. (1976) reported VP of meats prolong the shelf life of retail cuts as compared with those packaged in oxygen-permeable film. Studies comparing intact and various methods to assemble precut steaks for VP aging have not bee reported. More lean surface area is exposed when steaks are precut from intact subprimals. Although a need exists to reduce labor costs in fabrication and distribution of case-ready retail beef, a greater area of exposed lean may increase shrinkage, reduce juiciness scores, reduce tenderness ratings (due to the lubrication effect) and reduce retail caselife. Lean meat was selected for this study because many consumers prefer convenient to store and prepare lean beef, which is likely to be less juicy and less tender (Tatum et al., 1982) and have a higher moisture content than higher quality beef. Thus, the primary objective was to study the effect of precutting VP-aged lean steaks on retail caselife and palatability traits. Because a topic of recent discussion has been flavor differences between VP and dry-aged beef (Bauer, 1983), a second objective was to study flavor of cooked steaks from dry- and VP-aged subprimals.

MATERIALS & METHODS

Selection of carcasses/cuts.

Eighty subprimals were fabricated from 20 pairs of beef strip loins. The strip loins were from two groups of lean (fat thickness, 0.2–0.4 cm) heifer (n = 10) carcasses varying in grade from U.S. Standard to U.S. Good. After selection, strip loins, short cut (IMPS No. 179) were removed 48 hr postmortem and shipped at 2°C to the Texas Tech University Meats Laboratory.

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Fabrication and packaging.

Three VP subprimal fabrication treatments were randomly assigned to one-third of a short loin from each carcass. A dry aging control treatment was assigned to the other short loin from each carcass. Paired strip loins were stored (14 days at 1–3°C) after fabrication as follows: VP treatment A - intact subprimals (one-third of one strip loin); treatment B - precut steaks (2.54 cm) from subprimals and reassembled; and, VP treatment C - precut steaks from subprimals and not reassembled (stored flat). Product for treatments A, B and C were placed in low oxygen-barrier bags (OTR = 0.06 cc/sq cm/24 hr), packaged using a Multivac AG 500 VP machine, and stored at 1–3°C. All strip loin steaks were weighed prior to packaging and following storage to determine weight loss. Two 2.54 cm steaks from each subprimal were wrapped in polyethylene coated freezer paper, stored at −20°C for subsequent sensory analysis.

Retail display

Following the aging period, the dry aged strips and treatment A subprimals were cut into 2.54 cm steaks. Steaks from each treatment were placed in plastic foam trays and overwrapped in a oxygen-permeable polyvinyl chloride (PVC) film (OTR = 6,500cc/m^2/24 hr) and displayed at 2–4°C. Steaks were displayed in a Tyler (model number DGC6) retail case under Sylvania Gro-Lux F404W fluorescent lighting at 80 ft-c following commercial time-patterns of lighting (14 hr on, 10 hr off).

Evaluation

All retail cuts were evaluated 15 min after being removed from the vacuum bags and after 1, 2, 3, and 4 days by a trained three-member panel for: odor (4 = off odor, 1 = extremely off odor); lean color (8 = extremely bright cherry red, 1 = extremely dark brown); surface discoloration (Jeremiah and Greer, 1982), (7 = 0% surface discoloration, 1 = 100% surface discoloration); and, overall appearance (8 = extremely desirable, 1 = extremely undesirable). Steaks were evaluated for off odor 15 min after removal (initial) from the vacuum packages and after four days of storage in the retail case.

Palatability

Two steaks from each strip loin of each treatment were removed from the freezer, following a 30-day storage period, thawed 24 hr at 2°C and broiled on Farberware Open-Hearth broilers. The internal temperature (end point, 70°C) was monitored by LN 040418 metal thermocouples and printed on a Leeds and Northrup Speedomax Recorder. The lateral half of each steak was cut into 1.9 × 1.3 × 1.3 cm pieces and served warm to an eight-member sensory panel trained according to Cross et al. (1978). Each member independently evaluated each sample for juiciness (8 = extremely juicy, 1 = extremely dry), tenderness (8 = extremely tender, 1 = extremely tough), flavor intensity (8 = extremely intense flavor, 1 = extremely bland flavor) and overall satisfaction (8 = extremely desirable, 1 = extremely undesirable). The medial half of each steak was cooled to room temperature (25°C) and cored (1.27 cm cores) parallel to the muscle fiber orientation for Warner-Bratzler shear force determinations. Each steak was weighed frozen, thawed and after cooking to determine thaw and cooking losses.

Statistical analysis

Retail display traits, sensory panel ratings, thaw and cooking losses and shear force determinations were analyzed using a random block design (Steel and Torrie, 1980) with a split plot arrangement (Snedecor and Cochran, 1967). Packaging/precutting treatment (n = 4) and days (n = 5) were the main effects. When the main effect was significant across treatments or days, means were separated using Bonfer-

ronis test (Kirk, 1982). The predetermined level of probability was 5% for all analyses and will be used throughout this discussion.

RESULTS & DISCUSSION

IN DATA not shown in tabular form, no differences existed among the three VP treatments for percentage weight loss during aging, retail display, thawing or cooking. However, the dry aged subprimals had about 17% higher losses during aging than all VP aging treatments, which was due to extensive trim losses from dry aging of beef under aerobic refrigerated conditions. This finding is in agreement with Hodges et al. (1974).

A significant treatment × day interaction for odor (Table 1) occurred. Steaks from dry aged intact subprimals had a slight to moderate off odor at initial and day 4 evaluations. The other treatments were rated higher intially, but lower at day 4. Means for odor scores at the intial day of retail display (same day as cuts were removed from aging treatment) were higher for cuts from VP treatments than for steaks from dry aged strips (Table 1). A greater amount of surface spoilage of beef stored aerobically (Brown and Hoffman, 1972) may explain this finding. Aging beef under anaerobic conditions resulted in no difference among VP treatments (Table 1) in odor scores on the initial day of retail display agreeing with Bowling et al. (1977). However, steaks from VP intact subprimals were rated higher for odor than all other treatments following 4 days of retail display.

A significant day × treatment interaction occurred for lean color score (Table 1). Steaks in treatments B and D became brown before steaks in treatments A and C. A comparison of the two precutting treatments (B and C) indicated steaks VP and placed flat in an oxygen impermeable bag possessed a brighter, more appealing cherry red color after 1–4 days in a retail case than precut-reassembled steaks (Table 1). Steaks from treatments B and D were slightly brown after 2 days of retail caselife, which was exceeded by 1 day for steaks in treatments A and C. These data indicate subprimals aged intact will produce steaks with a brighter cherry red lean color after 3 days than precut VP steaks or steaks from dry aged subprimals.

Mean surface discoloration scores indicate all treatments contained 25% or less surface discoloration after 2 days retail display with treatments A and C being rated higher than steaks from treatment B (Table 2). The increased surface discoloration which occurred in the VP reassembled treatments may be due to the increased surface contamination from increased handling. Steaks from treatments B and D had 26–50% surface discoloration and were rated lower than treatments A and C after 3 days retail display. Hood and Riordan (1973) reported consumers discriminated against steaks that contained greater than 25% surface discoloration. Steaks from packaging treatments developed 51–75% surface discoloration at the end of 4 days retail display (Table 2). Steaks from treatments A and C were rated not different from initial through day 4 of retail display and were acceptable through day 3. All treatments produced steaks which were 51–99% discolored at day 4.

A significant day × treatment interaction for overall appearance was observed (Table 3). Staks from subprimals which were either dry aged (D) or precut (B and C) declined in overall appearance score at a faster rate than steaks from intact VP aged subprimals. Overall appearance scores (Table 3) from treatments A and C were higher than steaks from treatments B or D at days 2 and 3. This loss in desirability occurred as surface discoloration increased. Steaks from treatments A and C had an additional day of retail caselife as steaks from treatments B and D were rated "slightly undesirable" after day 3. Steaks from all packaging treatments were rated very undesirable at the end of 4 days of retail display which concurs with Davis et al. (1977). These results show VP will extend retail caselife by 1 day over dry aging except when steaks are reassembled. Berry et al. (1971) reported steaks from VP ribs had approximately 1 day of additiona retail caselife when compared to steaks from ribs protected with polyvinyl chloride film.

Broiled steaks from treatment A intact subprimals were rated higher than VP reassembled subprimals (treatment B) for juiciness and tenderness (Table 4). This trend was also evident for juiciness and tenderness ratings between treatments A and C. Although fabrication and distribution factors are advantages for case-ready beef steaks, reduced eating quality (Table 4) and reduced retail caselife (Table 3) of VP precut steaks (treat-

Table 1—Means of lean odor[a] and color[b] scores for strip steaks during retail display from four primal cut packaging/precutting treatments

Day of retail display	Treatments: A VP[c] intact	B VP reassembled	C VP not reassembled	D DA[d] intact	Rank order (highest to lowest) across treatments[e]
			Odor		
Initial	3.6[f]	3.7[f]	3.7[f]	2.5[f]	B C A D
4	2.7[g]	2.0[g]	2.4[g]	2.3[f]	A C D B
			Color		
Initial	6.8[f]	6.5[f]	6.5[f]	6.7[f]	A D B C
1	6.8[f]	6.1[g]	6.4[f]	6.4[g]	A D C B
2	6.5[g]	5.5[h]	6.1[g]	5.7[h]	A C D B
3	5.6[h]	4.5[i]	5.3[h]	4.9[i]	A C D B
4	3.4[i]	2.7[j]	3.4[i]	3.2[j]	A C D B

[a] Means based on 4-point scale (4 = no off odor, 1 = extreme off odor).
[b] Means based on an 8-point scale (8 = extremely bright cherry red, 1 = extremely dark brown).
[c] VP = vacuum packaged.
[d] DA = dry aging.
[e] Means in the same row underscored by a common line do not differ (P>0.05).
[f,g,h,i,j] Means in the same column within color or odor bearing a common superscript do not differ (P>0.05).

Table 2—Means of surface discoloration[a] score for strip steaks during retail display from four primal cut packaging/precutting treatments

Day of retail display	Treatments: A VP[b] intact	B VP reassembled	C VP not reassembled	D DA[c] intact	Rank order (highest to lowest) across treatments[d]
Initial	7.0[d]	7.0[d]	6.9[d]	7.0[d]	A B D C
1	6.9[d]	6.7[e]	6.7[d]	6.3[e]	A B C D
2	6.4[e]	5.6[g]	6.2[e]	5.8[g]	A C D B
3	5.5[g]	4.5[h]	5.2[g]	4.7[h]	A C D B
4	3.0[h]	2.7[i]	3.2[h]	2.8[i]	C A D B

[a] Means based on a 7-point scale (7 = no surface discoloration, 1 = complete surface discoloration).
[b] VP = vacuum packaged.
[c] DA = dry aging.
[d] Means in the same row underscored by a common line do not differ (P>0.05).
[e,f,g,h,i] Means in the same column bearing a common superscript letter do not differ (P>0.05).

Table 3—Means of overall appearance[a] scores for strip steaks during retail display from four primal cut packaging/precutting treatments

Day of retail display	Treatments: A VP[b] intact	B VP reassembled	C VP not reassembled	D DA[c] intact	Rank order (highest to lowest) across treatments[d]
Initial	7.3[e]	7.3[e]	7.1[e]	7.3[e]	A B D C
1	7.2[e]	6.6[f]	6.8[f]	6.4[f]	A C B D
2	6.7[f]	5.7[g]	6.3[g]	5.9[g]	A C D B
3	5.7[g]	4.4[h]	5.1[i]	4.4[h]	A C D B
4	2.5[h]	2.3[i]	2.9[i]	2.3[i]	C A D B

[a] Means based on an 8-point scale (8 = extremely desirable, 1 = extremely undesirable).
[b] VP = vacuum packaged.
[c] DA = dry aging.
[d] Means in the same row underscored by a common line do not differ (P>0.05).
[e,f,g,h,i] Means in the same column bearing a common superscript letter do not differ (P>0.05).

Table 4—Means of sensory panel scores and shear force value for broiled strip steaks from four packaging/precutting treatments

	Treatments			
Trait	A VP[a] intact	B VP reassembled	C VP not reassembled	D DA[b] intact
Juiciness[c]	6.2[e]	5.5[f]	5.7[ef]	5.9[ef]
Tenderness[c]	6.3[e]	5.6[f]	5.9[ef]	6.2[ef]
Flavor[c]	6.0[e]	5.7[e]	5.7[e]	6.7[f]
Overall satisfaction[c]	5.9[e]	5.4[ef]	5.9[e]	4.7[f]
Shear force value[d]	7.2[e]	7.9[e]	7.3[e]	7.1[e]

[a] VP = vacuum packaged.

[b] DA = dry aging.

[c] Means based on 8-point rating scales (8 = extremely juicy, tender, intense flavor, desirable overall; 1 = extremely dry, tough, bland flavor, undesirable overall).

[d] Force in kg required to shear a 1.3 cm core of cooked longissimus dorsi muscle.

[e,f] Means in the same row bearing a common superscript do not differ (P>0.05).

ments B and C) provide some product problems. These limitations require further study before precutting of steaks from intact subprimals becomes a common practice as a means of reducing labor costs. Low quality, lean beef steaks may have more of a tendency to be less juicy and less tender when precut from intact subprimals than steaks from higher quality grading beef.

Broiled steaks from treatment D subprimals were rated higher than all VP aged treatments for flavor intensity. Ingram (1962) reported the organisms causing aerobic spoilage, through proteolysis, produce amino compounds which are the principle cause of off-odors and off-flavors. No differences among VP aged treatments were found for overall satisfaction; however, the dry aged treatment D was rated lower than either treatments A or C. No significant differences existed among all four treatments for shear force value of cooked strip steaks (Table 4).

SUMMARY & CONCLUSIONS

THE CONCLUSIONS were: 1. Precutting subprimals into steaks followed by VP aging tends to decrease retail caselife (especially if steaks are reassembled) and decrease juiciness and tenderness. 2. A comparison of the two precutting treatments (B and C) indicated steaks VP and placed flat in an oxygen impermeable bag possessed a brighter, more appealing cherry red color with less surface discoloration, one additional day of retail caselife but no advantage in eating quality over steaks precut, VP and reassembled. 3. Dry aged subprimals had much higher trim and shrink losses (17.8%) during aging then all VP treatments.

Although greater labor efficiency may be an advantage for centralized precutting of steaks from subprimals, results from this study indicate no shrinkage or palatability advantages exist, and some disadvantages (juiciness, tenderness, caselife) exist for use of this distribution system. Based on these results a precut not assembled (laid flat in oxygen impermeable bags) VP method of centralized handling of subprimals would be recommended over a precut, reassembled VP handling method.

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Denaturation Kinetics of Myofibrillar Proteins in Bovine Muscle

J. R. WAGNER and M. C. AÑON

ABSTRACT

Differential scanning calorimetry was used to monitor the thermal denaturation kinetics of myofibrillar proteins in bovine muscle at pH 5.6. The activation energy, pre-exponential factor, rate constant and mean life time for each transition were calculated by means of a dynamic method. The kinetic values and the proposed reaction order (n = 1) were confirmed applying an isothermal method as a test. The position of the endothermal peaks proved to be pH dependent. Evidences suggest that peak III is a result of actin denaturation and possibly other thin filament proteins. Peaks I and II would represent the thermal transition of thick filaments. The number of bonds involved in each transition were estimated.

INTRODUCTION

HAMM (1975, 1977) and Martens et al. (1982) have related changes in the quality factors of meat during heat treatment with denaturation of muscle proteins, especially myofibrillar ones. Some researchers studied the heat-induced denaturation of isolated proteins from muscle, especially myosin and its fragments (Burke et al., 1973; Goodno et al., 1976; Samejima et al., 1976). Previously, Pelletier and Ouellet (1961) studied the thermal inactivation kinetics of myosin isolated from rabbit muscle by loss of ATPase activity.

Differential scanning calorimetry (DSC) offers a direct method to study the thermal transition of muscle proteins "in situ" (Wright et al., 1977; Stabursvik and Martens, 1980). Duswalt (1974) described in detail the different methods to obtain kinetic data and resolution of complex systems by DSC. Applying one of these methods (isothermal method). Martens et al. (1982) determined the thermal denaturation kinetics of actin in whole rabbit muscle.

To clarify the nature of the meat heating process and provide thermodynamic and kinetic data to determine the effects of different environmental factors on the thermolability of myofibrillar proteins, the thermal denaturation kinetics of myofibrillar proteins in bovine muscle was studied by DSC using a dynamic method.

MATERIALS & METHODS

Bovine meat

Experiments were performed with 48 hr portmorten semitendinosus muscle (pH 5.6) from cows classified A or B by the National Meat Board of Argentina. Samples were stored at 4°C before being used.

pH adjustment and sarcoplasmic proteins and connective tissue removal

Small pieces of muscle (weight ca. 30 mg) were cut and treated with modified Ringer's solution (0.15M NaCl, 3 mM KCl, and 3 mM $MgCl_2$) and then magnetic stirred for 4 hr at 4°C. During this period the pH was adjusted to the desired value four times with 1N NaOH or 1N HCl. After overnight storage at 4°C the final pH value was measured. The connective tissue was carefully removed with a scalpel (Stabursvik and Martens, 1980).

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Differential scanning calorimetry

DSC studies were performed in a Du Pont Model 910 System attached to a Hewlett Packard 7046 B recorder. The temperature calibrations were performed according to ASTM Norm E 474/80 using the Indium and ice melting thermograms. The samples (12–24 mg wet weight) were placed in DSC hermetic aluminum pans, assuring a good contact between the sample and the capsule bottom. After DSC analysis the capsules were punctured and the dry matter weight determined by drying at 105°C overnight (dry matter weight, 3–6 mg). As reference, an hermetic capsule with 15 μL distilled water or an empty double one was used. The endotherm areas were measured with a Morphomat 34 Zeiss (1% error) image analyzer.

Calculation of thermal denaturation kinetic constants

Two methods were used:

(a) **Dynamic method** (to calculate the kinetic constants). DSC thermograms were run from 10–110°C with different heating rates β, according to ANSI-ASTM E 698/79 Norm (β = 1–20°C min^{-1}). The peak temperatures (T_{max}) were determined in each run.

(b) **Isothermal method** (to confirm the values obtained by the dynamic method). Samples in hermetic pans were immersed in a water bath at different temperatures for a period of time equivalent to the mean life time ($t_{1/2}$) for each transition. The $t_{1/2}$ values were previously calculated by the dynamic method. The pans were then heated in the DSC equipment from 10–110°C (β = 10°C min^{-1}). The area of the remainder DSC endotherm was measured. Control samples without isothermal treatment were run under the same heating conditions. Experiments were performed at pH 5.6.

RESULTS & DISCUSSION

DSC thermograms of protein denaturation in bovine muscle

The thermograms of whole muscle (a) and muscle depleted of sarcoplasmic proteins and connective tissue (b) show three peaks (Fig. 1). According to Wright et al. (1977) and Stabursvik and Martens (1979, 1980), the first and second peaks correspond to myosin and the third (III) to actin. In the case of whole muscle the second peak (II) reflects also the sarcoplasmic proteins and connective tissue contribution (Fig. 1, thermograms (c) and (d)).

In thermograms of bovine muscle depleted of sarcoplasmic proteins and connective tissue, from pH 5.4 through pH 6.2, the main difference observed between thermograms was in the myosin thermal transition (Fig. 2). At pH 5.4 two well defined peaks appeared at T_{max} 57.5°C (peak I) and 62.8°C (peak II). At higher values of pH these peaks came closer to each other, collapsing at pH 6.2 with a T_{max} = 60.3°C. The third peak (peak III), ascribed to actin, presented a single transition along the assayed pH range: at pH 5.4 T_{max} = 73.9°C and at pH 6.2 T_{max} = 72.6°C. The peaks are ascribed almost exclusively to myosin and actin since they are the myofibril's major proteins. However, minor proteins (e.g. tropomyosin, troponin and actinins) have a denaturation heat which would contribute to the total endotherm. The T_{max} vs the pH values are plotted in Fig. 3. In the case of peak I the T_{max} value increased with pH; for peak II it decreased until it reached the same value as peak I near pH 6.0. For peak III, T_{max} had an almost linear and decreasing dependency with pH.

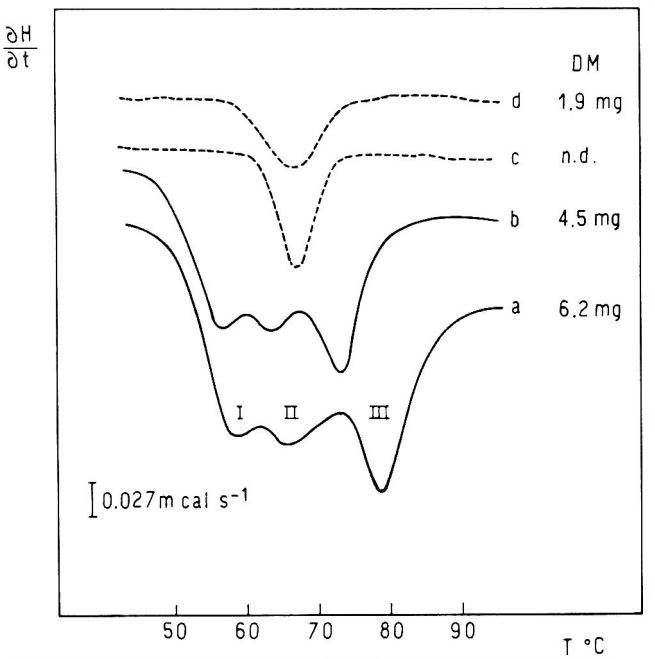

Fig. 1—DSC thermograms of: (a) Whole muscle pH 5.6; (b) Muscle depleted of sarcoplasmic proteins and connective tissue pH 5.6; (c) Connective tissue; and (d) Sarcoplasmic proteins. Heating rate β = 10°C min⁻¹. DM = dry matter.

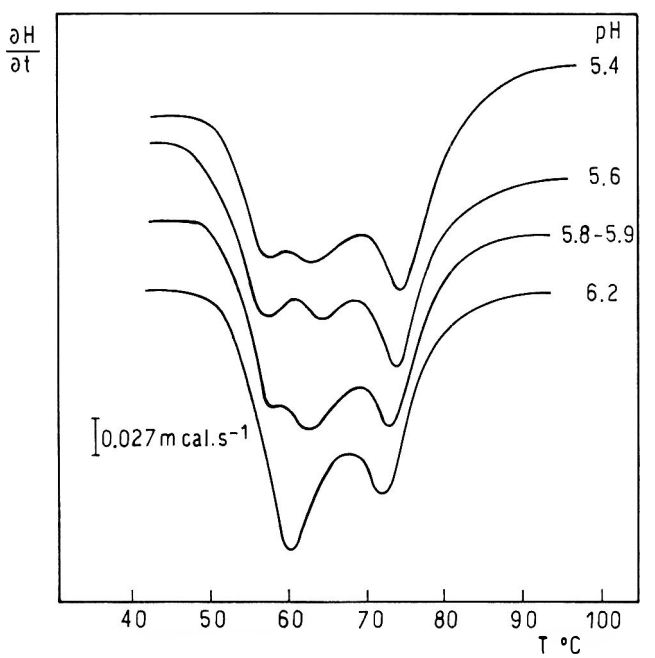

Fig. 2—DSC thermograms of bovine muscle depleted of sarcoplasmic proteins and connective tissue at different pH values. Heating rate β = 10°C min⁻¹. Dry matter: 3—5 mg.

Goodno et al. (1976) observed an increase of the melting temperature of myosin and its fragments with pH. This was explained in basis of a proton transfer mechanism along the thermal denaturation process. Our results suggest that peak I proteins would accept protons. Peak II and III proteins would act as proton-donor compounds due to the decrease of T_{max} with pH.

Levy and Benaglia hypothesis (1950) assumed that proteins exist in different ionization states for each pH value, with a different concomitant denaturation rate constant for each of the

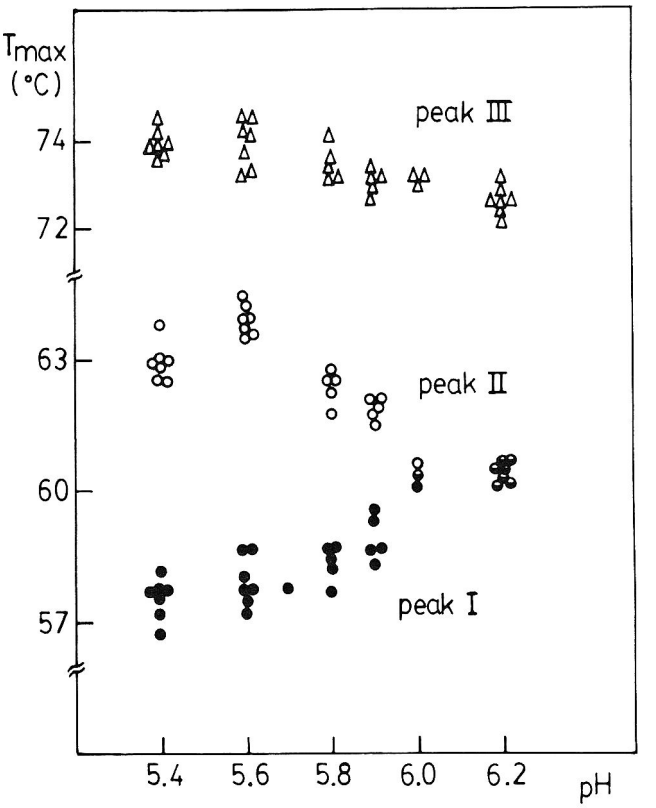

Fig. 3—T_{max} of peaks I, II and III corresponding to the DSC thermograms of bovine muscle depleted of sarcoplasmic proteins and connective tissue at different pH values. Heating rate β = 10°C min⁻¹.

states. According to this hypothesis there is a pH value (pH min) associated with a minimum denaturation rate constant (k_d). For pH values below this pH min, protein denaturation process involves capture of protons and above pH min a proton release takes place. Considering the relation between T_{max} and k_d, that arises from the combination of Eq. (1) and Arrhenius eq. (T_{max} increased when k_d decreased), it is possible to correlate the T_{max} values with Levy and Benaglia hypothesis. For peak I a minimum k_d was observed at pH 6.0–6.5, so below 6.0 there was a proton uptake and above pH 6.5 (according to results obtained by Stabursvik and Martens, 1979) only a proton release. For peaks II and III in the pH range studied there was only an increment in the values of k_d.

Thermal denaturation kinetics

Dynamic method. When plotting $-\ln(\beta/T^2_{max})$ vs $1/T_{max}$ (Fig. 4) a straight line should be obtained with a slope proportional to the activation energy, E_a, according to the following equation (Ozawa, 1970):

$$\ln(\beta/T^2_{max}) = \ln\left(\frac{ZR}{E_a}\right) - \frac{E_a}{RT_{max}} \qquad (1)$$

where β is the heating rate (K min^{-1}); T_{max} the peak temperature (K); Z the pre-exponential factor of Arrhenius equation (min^{-1}), and R the gas constant (cal mol^{-1} K^{-1}). In this way E_a, Z and rate constant value (k_d) for each peak were calculated. If a denaturation kinetic of order one with respect to time is assumed, the mean life time $t_{1/2}$ can be obtained. A least square minimum linear regression was applied to $-\ln(\beta/T^2_{max})$ vs $10^3/T_{max}$, selecting $-\ln(\beta/T^2_{max})$ as the independent variable (Pravisani et al., 1984). Table 1 shows the calculated values of activation energies and pre-exponential factors for each transition with the corresponding rate constants and mean life times at different temperatures.

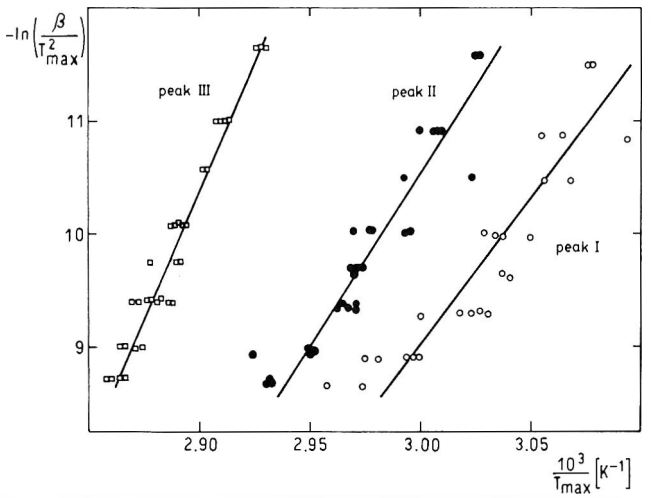

Fig. 4—Calculation of the activation energies (E_a) for the myofibrillar protein thermal denaturation according to the dynamic method. Slopes were used to calculate the corresponding E_a value for each transition.

Our calculated activation energy values are similar to those reported by other authors. Pelletier and Ouellet (1961) reported for heat-inactivated ATPase activity of rabbit muscle myosin an E_a = 56 kcal mol^{-1}, which is similar to those corresponding to peak I denaturation. It could be assumed that this transition corresponds to the myosin head (HMM-S1), which is in agreement with the results obtained by Wright and Wilding (1984). Martens et al.(1982) reported a value of 83.7 kcal mol^{-1} for actin in rabbit whole muscle at pH 5.4 which is also similar to the value found by us.

These results would allow us to suppose that actin denatures with approximately the same activation energy in whole muscle, myofibrils and isolated proteins. Myosin would behave in the similar manner. Thus, denaturation rate (thermal lability) would be affected by the pre-exponential factor which depends on the T_{max} and β. Based on this assumption a nondenaturated fraction was calculated through isothermal heatings at different temperatures for whole muscle and muscle depleted of sarcoplasmic proteins and connective tissue (Table 2). A higher thermal resistance of whole muscle proteins, especially of actin, was observed.

It would be also possible to calculate a nondenaturated fraction of myosin and actin from any meat systems knowing T_{max} of each peak and β.

Isothermal method. Since myofibrils thermograms have more than one peak, it is not possible to apply the isothermal method to obtain the kinetic constants (Duswalt, 1974). Nevertheless, the isothermal method was used to confirm the values found by the dynamic method and solve the complex endotherm by estimation of the total area of each individual transition.

Table 2 shows the partial disappearance of peak II when isotherm heating was applied for a period of time equivalent to peak I $t_{1/2}$. On the contrary, when isotherm heating was performed for a period corresponding to peak II $t_{1/2}$, peak I was not completely denatured. So, the following equation system can be solved:

$$\text{(i) } I + II = M \qquad (2)$$

$$\text{(ii) } I(1 - X_1) + II(1 - X_2) + III(1 - X_3) = Y$$

where I = peak I area; II = peak II area; M = peak (I + II) area; III = peak III area; Y = endotherm total area; X_1, X_2 and X_3 are the respective denatured fractions of I, II and III. Fig. 5 shows the endotherm corresponding to isothermal pretreated samples. The total area Y for each t value was measured on each endotherm (Yexp) and the values (1 - X) were theoretically calculated applying the expression ln (1 - X) = $-k_d t$. The correspondence between Y_{exp} and the theoretical Y (error 3%) indicates that both the kinetic values obtained and the reaction order proposed are correct. The areas III and (I + II) were determined from those endotherms in which myosin was completely denatured (15 min at 57°C). Areas I and II were estimated by solving Eq. 2i, ii and then they were adjusted by iteration until the experimental (1 - X) and theoretical values became similar.

Thus, the total area fractions of each transition were:

M = 0.52 Y and III = 0.48 Y

I = 0.45 M and II = 0.55 M

Estimation of △Hd of the individual transitions

Taking into account the fraction of the total area of each peak and the corresponding percentages of dry matter weight, individual △Hd can be calculated. Myofibrils are constituted by 25% actin, 58% myosin and 17% minor proteins (Ockerman, 1977 - which was also confirmed by us; data not shown). According to these percentages:

$$\Delta H_{total} = 0.58\Delta H_{myosin} + 0.25\Delta H_{actin} + 0.17\Delta H_{minor\ proteins}$$

Since minor proteins have denaturation heats associated to their thermal transitions and no additional peaks appear, it is expected that these heats would contribute to one of the two main transitions. Analyzing only the two extreme possibilities, it follows that either (a) minor proteins contribute to myosin peaks (peak I and II), and actin is the only protein that takes part in peak III. $\Delta H_{actin\ den.}$ would take the value 9 cal g^{-1} and $\Delta H_{myosin+minor\ proteins)den.}$ = 3.3 cal g^{-1} or (b) minor proteins contribute to peak III giving: $\Delta H_{(actin\ +\ minor\ proteins)\ den.}$ = 5.4 cal g^{-1}, and $\Delta H_{myosin\ den.}$ = 4.2 cal g^{-1}.

Wright et al. (1977) have reported enthalphy values of denaturation heat of isolated myosin and actin that are similar with the second possibility (b). So, peak III would include thin filament denaturation and peak I and II as a whole to the thick filament denaturation.

Estimation of thermodynamic functions

The rate constant of a chemical reaction can be expressed in terms of thermodynamic functions. Taking into account that protein denaturation is an unimolecular reaction, entropy of activation, △S*, can be calculated, once Arrhenius pre-exponential values Z are known, using the Eyring relation (Laid-

Table 1—Denaturation kinetic constants of myosin (Peak I and II), and actin (Peak III)[a]

Peak	T_{max} (°C)	$E_a \pm \sigma$ (kcal · mol^{-1})	Z (min^{-1})		Temperature (°C) 47	53	57	63	67
I	57.8 ± 0.6	54.5 ± 6.52	2.4 × 10^{36}	k_d(min^{-1})	1.53 × 10^{-1}	7.40 × 10^{-1}			
				$t_{1/2}$ (min)	4.50	0.94			
II	63.9 ± 1.0	61.0 ± 5.85	9.5 × 10^{39}	k_d	2.21 × 10^{-2}	1.29 × 10^{-1}	4.03 × 10^{-1}	2.12	
				$t_{1/2}$	31.40	5.38	1.72	0.33	
III	74.2 ± 0.4	90.6 ± 6.82	3.9 × 10^{57}	k_d	---	---	4.34 × 10^{-3}	5.08 × 10^{2}	2.51 × 10^{-1}
				$t_{1/2}$			160	13.6	2.75

[a] T_{max} = Peak temperature; E_a = Activation energy; Z = Pre-exponential factor of Arrhenius' equation; k_d = Rate constant; $t_{1/2}$ = mean life time; ± = standard deviation

Table 2—Percentage values of nondenatured fraction of each thermogram peak after heating at different temperatures for 5 min

Sample	Peak	Temperature (°C) 47	53	57	63	67	73	77
Muscle depleted of	I	47%	2.5%	0%				
sarcoplasmic proteins and	II	89.5%	52.5%	13.3%	0%			
connective tissue-pH 5.6	III	100%	100%	97.8%	77.6%	28.4%	0%	
Whole muscle	I	57%	6.6%	0%				
pH 5.6	II	95.5%	76.5%	43.2%	1.2%	0%		
	III	100%	100%	99.8%	97.8%	89.6%	32.6%	0.6%

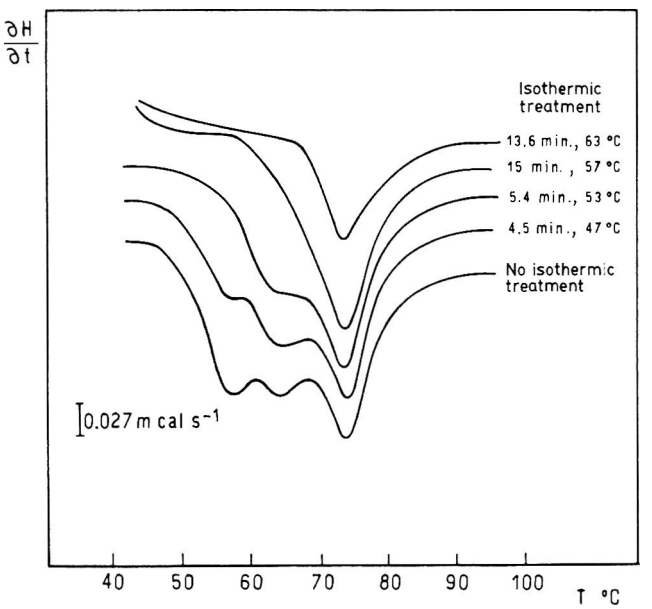

Fig. 5—DSC thermograms of muscle depleted of sarcoplasmic proteins and connective tissue at pH 5.6, with and without previous isothemal treatment at different temperatures and periods of time.

Table 3—$\triangle S^*$ and $\triangle G^*$ values for thermal transitions of the myofibrillar proteins in bovine muscle free of sarcoplasmic proteins and connective tissue pH 5.6[a]

Peak		$\triangle S^*$ (kcal mol^{-1}K^{-1})	$\triangle H^*$ (kcal mol^{-1})	$\triangle G^*$ (kcal mol^{-1})
I	at T_{max}	0.098	53.84	21.35
	at 60°C	0.098	53.83	21.14
II	at T_{max}	0.115	60.33	21.66
	at 60°C	0.115	60.33	22.11
III	at T_{max}	0.196	89.90	21.92
	at 60°C	0.196	89.93	24.70

[a] T_{max} = peak temperature; $\triangle S^*$ = Activation entropy; $\triangle H^*$ = Activation enthalpy; $\triangle G^*$ = Activation free energy.

ler and Bunting, 1973). $\triangle G^*$ and $\triangle H^*$ were estimated using the usual thermodynamic relations.

Table 3 shows the thermodynamic function values for transitions I, II and III, calculated at the corresponding T_{max} temperature for each peak and at 60°C. These large activation enthalpies and entropies are normal for enzyme inactivation and protein denaturation. $\triangle S^*$ and $\triangle H^*$ are practically independent of temperature and they verify the linear relation found by Multon (1970) valid for denaturation of proteins in aqueous and solid medium:

$$\triangle S^* = 0.0030\ \triangle H^* - 0.075 \quad (3)$$

Table 3 also shows that $\triangle H^*$ and $\triangle S^*$ increase from transition I to III, which could be due to an increase of the number of bonds broken during the denaturation process ($\triangle H^*$) and to an increase of the molecular disorder of the protein and of its immediate surrounding ($\triangle S^*$), to achieve the activated state. The breaking process of several low-energy bonds, possibly accompanied by dehydration, might correspond to the structural changes mentioned.

$\triangle G^*$ remains practically constant which would indicate that the probability to reach the denatured state is the same in the three transitions at the corresponding T_{max}.

According to Eyring and Stearn (1939) thermal protein denaturation involves the rupture of one disulphide bond (which contributes with a $\triangle H^* = 25$ kcal mol^{-1} and a negligible $\triangle S^*$, and the rupture of n hydrogen bonds ($\triangle H^* = 4$ kcal mol^{-1} and $\triangle S^* = 0.012$ kcal mol^{-1} K^{-1}) per protein molecule. Thus, the following equations can be expressed:

$$n = \frac{\triangle S^*}{0.012} \text{ and } n = \frac{\triangle H^* - 25}{4} \quad (4)$$

Applying these equations the calculated n values are: $n_I \simeq 8$; $n_{II} \simeq 10$; and $n_{III} \simeq 16$.

Although this hypothesis offers a good explanation for the lineal relation (3) between $\triangle H^*$ and $\triangle S^*$, we must take into account that available data indicate that none of the myofibrillar proteins has disulfide bonds in their native states (Buttkus, 1970; Hamm, 1977; Matthews et al., 1980). So, if only the rupture of hydrogen bonds is involved in the denaturation process the number of broken bonds could be higher: $n_I \simeq 13$; $n_{II} \simeq 15$; $n_{III} \simeq 22$ (data calculated with respect to $\triangle H^*$ value).

However, it is possible that the rupture of other kind of bonds (hydrophobic and low energy bonds) and reaction of capture and release of protons are included in the $\triangle H^*$ and $\triangle S^*$ values.

CONCLUSIONS

THE RESULTS suggest that the thick filament formed almost completely of myosin (peak I and II), is more sensitive to heat than the thin filament formed by actin and minor proteins (peak III). Denaturation of peak I occurs more easily at pH 5.4–5.6 than at higher pH values. The opposite effect is observed in the denaturation of peak II and peak III.

The kinetic constants of the three thermal transitions of the complex endotherm were calculated applying the dynamic method. The following values of the activation energy were obtained: 54.5, 61.0, and 90.6 kcal mol^{-1} for the transitions I, II, and III, respectively. Applying an isothermal method, the kinetic values found and the reaction order one for each reaction were confirmed.

It was also possible to solve the complex endotherm and estimate the contribution of each transition to the endotherm. Consequently, the thick filaments would contribute the 52% and the thin filaments the remainder of the total area.

The thermodynamic function values associated with each transition were calculated, and the number of bonds broken during denaturation were approximately determined.

The results obtained are useful to clarify the nature of the meat heating process and to determine the effect of different environmental factors on the thermolability of myofibrillar proteins. It would also be possible to calculate a nondenaturated fraction of myosin and actin from any meat system knowing T_{max} and the heating rate used.

—Continued on page 1563

Color, Pigment and Iron Content of Meat Loaves with Blood, Blood Emulsion, or Mechanically Deboned Meat Added

INGER M. OELLINGRATH and ERIK SLINDE

ABSTRACT

The hemoglobin, myoglobin, and iron content in slaughter-house blood, a blood emulsion, mechanically deboned meat (MDM) and meat minces with these raw materials added, were determined. Blood and blood emulsion were added in the range 0–5%. MDM was added as a substitute for ground beef. The pigment and iron content in the minces were compared with the surface color of heated meat loaves produced. Minces containing blood yielded lighter meat loaves upon heating (*i.e.* a higher lightness L* was observed) than did minces containing blood emulsion or MDM, even if the amounts of extractable hemoglobin and myoglobin in the minces were equal. At equal concentrations of iron, minces with blood and MDM gave lighter meat loaves than did minces to which blood emulsion had been added.

INTRODUCTION

IN COUNTRIES where meat constitutes a substantial part of the diet, hemoglobin and myoglobin are important contributors of absorbable dietary iron. Heme iron is generally more available to the body than inorganic iron, since the availability of nonheme iron is affected by promoting and inhibiting components in the diet (Conrad *et al.*, 1967). According to current knowledge, heme iron is absorbed selectively as the intact iron porphyrin complex by a high affinity intestinal heme receptor, depending on the body's need of iron (Gräsbeck *et al.*, 1982). Slaughterhouse blood is rich in heme iron, and utilization of more blood for human consumption should be a nutritional advantage.

Traditional blood products are dark in color, since blood contains about 14% hemoglobin (Warris and Rhodes, 1977). They also have a characteristic taste. The dark color is the main sensory quality problem when blood is added to meat products (Mielnik and Slinde, 1983). Studies have shown that small amounts of blood (2–3.5%) may be added to wiener sausages without causing significant changes in appearance or taste (Eskeland and Slinde, 1981; Slinde and Martens, 1982).

In order to reduce the problems of dark color, blood may be added to meat products as a decolorized emulsion. Russian researchers first developed a method for emulsifying blood in a mixture of melted fat and casein (Zayas and Zyrina, 1975). Wismer-Pedersen (1978, 1979) found that homogenization by high pressure (150–300 kg/cm^2) increased the degree of dispersion, thereby decreasing the dark color. Consequently more blood could be added when blood emulsions were used in a recipe.

Mechanically deboned meat (MDM) is another slaughterhouse product containing high amounts of hemoglobin. The hemoglobin content depends on the amount of marrow released from the bones during the deboning process (Field *et al.*, 1980). MDM may be used in meat products as a substitute for ground beef. MDM has a consistency much like that of a blood emulsion.

Utilization of blood in any form in meat products requires color optimalization of the recipes (Mielnik and Slinde, 1983).

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To be able to make an objective estimate of the final product color, one has to know more about the concentration of total pigment (*i.e.* hemoglobin and myoglobin) present in the raw materials (Slinde *et al.*, 1982).

The purpose of this study was to produce meat loaves containing MDM and small amounts of blood and blood emulsion, and determine the effect of the pigment and iron content of the raw materials on the color of the heated products.

MATERIALS & METHODS

Blood emulsion, mechanically deboned meat (MDM), and whole blood

The blood emulsion was delivered frozen from Nutridan Engineering A/S (Denmark). It had been produced, as described by Wismer-Pedersen (1979), by emulsification at high pressure using melted pork fat (42%), sodium caseinate (6%), red blood cells (27%) and water (25%). MDM from beef bones and frozen citrated beef blood were also used in the production of meat loaves.

Production of meat loaves

One series of meat loaves was produced according to the basic recipe (100 kg): 20 kg ground beef (18.4% protein, 15% fat, 66% water), 19 kg ground beef (17.3% protein, 21% fat, 61.5% water), 12 kg pork fat trimmings (6.8% protein, 68.5% fat, 24% water), 0.5 kg soy isolate, 1.7 kg salt, 4 kg potato starch, 0.3 kg seasonings, 4.25 kg skim milk powder, and 38.25 kg water. To this amount was added 1, 2, 3, 4, or 5 kg blood or blood emulsion. Three series containing MDM were produced: (a) Basic recipe and 2 kg MDM added; (b) in the basic recipe 10 kg of the ground beef (15% fat) were replaced by MDM, and further 2 kg MDM were added; (c) in the basic recipe 20 kg of the ground beef (15% fat) were replaced by MDM, and further 2 kg MDM were added.

When blood was used, it was thawed at room temperature, added to the dilution water to induce hypoosmotic lysis, and then added to the mince in the chopper. When frozen blood emulsion or MDM was used these raw materials were cut into small pieces and added to the mince in the chopper. The recipe ingredients were chopped, portioned (1 kg) in aluminum forms, and heated in a cooking chamber to a core temperature of 78–79°C. The heating time was approximately 2 hr. The meat loaves were cooled at 5°C overnight, and then vacuum packed and frozen at −40°C.

Determination of hemoglobin and myoglobin by high performance liquid chromatography (HPLC)

Horse skeletal myoglobin and bovine hemoglobin were purified on a Sephadex G-100 molecular sieve column using 50 mM potassium phosphate, pH 6.9. Purified myoglobin and hemoglobin were dissolved in buffer A, containing 0.05M sodium phosphate, 0.01M KCN, and 0,1M Na_2SO_4, pH 7.22 (Schroeder and Huisman, 1980). Maximum absorbance of hemoglobin and myoglobin dissolved in buffer A was found by scanning the Soret region, using a Shimadzu UV-300 spectrophotometer (Shimadzu Sesakusho Ltd. Kyoto, Japan). The amount of pigment was calculated using the millimolar extinction coefficients for horse cyanohemoglobin at 419 nm ($\epsilon_{mM} = 124.0$) and horse skeletal cyanomyoglobin at 422 nm ($\epsilon_{mM} = 116.0$) (Antonini and Brunori, 1971).

Hemoglobin and myoglobin were determined in their cyano ferric form, essentially as described by Slinde *et al.* (1982). After filtration through micro filters, 10 μL were injected into the HPLC, Spectra Physics model 748 with a Bio-Sil TSK-SW (300 × 7.5 or 600 × 7.5 mm) molecular sieve column and a Bio-Sil TSK guard column (75 × 7.5

mm) from Bio-Rad Laboratories, California. Buffer A was used as the mobile phase at a flow rate of 1.0 mL/min. Eluent absorbance was measured at 420 nm, and peak areas were integrated with a Spectra Physics integrator, model 4270.

To 10g blood, blood emulsion, MDM or mince were added 30 mL buffer A. The samples were homogenized in a Colworth Stomacher 400 for one minute and centrifuged in a Beckman J2-21M centrifuge (rotor JA 21, approximately 10^6 g_{av}·min, 2°C). Small samples of the supernatants were filtered through micro filters and 10 µL were injected into the HPLC. When 10 samples of ground beef (15% fat) were extracted, the standard deviation was 7% of the total mean; (1.0 mg hemoglobin/g wet weight + 5.9 mg myoglobin/g wet weight) ± 0.5.

Determination of total pigment content in the extracts

The total pigment content (hemoglobin + myoglobin) was also measured by scanning the Soret region, using the Shimadzu spectrophotometer. The amount was calculated from the absorbance maximum, using the extinction coefficient of horse skeletal cyanomyoglobin at 422 nm (ϵ_{mM} = 116.0) It was assumed that hemoglobin contributed as four myoglobin molecules. When 10 samples of ground beef (15% fat) were extracted, the standard deviation was 4%; 6.2 mg total pigment (hemoglobin + myoglobin)/g wet weight ± 0.2.

Determination of total iron

Blood, blood emulsion, MDM, and meat loaves were freeze-dried, ashed (500°C, 2 hr), evaporated to dryness in the presence of HNO_3/HCl (1:2), heated to 500°C for 20–30 min, evaporated again to dryness in the presence of 5 mL HCl, solubilized by heating in HCl/H_2O (1:2), and diluted to 100 mL. If necessary, the samples were microfiltered or centrifuged. The amount of iron was then determined by atomic absorption spectrometry. When 10 samples of ground beef (15% fat) were analyzed, the standard deviation was 6%; 0.023 mg iron/g wet weight ± 0.001.

Color measurements

Reflection spectra of the thawed sliced surface of meat loaves were recorded as described by Slinde *et al.* (1982), using the Shimadzu UV-300 spectrophotometer with an integrating sphere attachment. Magnesium oxide was used as white reference. Both sample and reference were covered by a 1 mm thick glass plate to protect the integrating sphere. Measurements on three different cuts of each meat loaf were made. The reflectance spectra were recorded in the visible region (380–760 nm). All color parameters were calculated for C-light, using the CIE 1976 L*, a*, b* color system (Hunter, 1975).

RESULTS

Separation of hemoglobin and myoglobin by HPLC molecular sieve chromatography

The chromatogram of a Bio-Sil-test protein solution (Fig. 1A) shows that the column separates proteins according to their molecular weights. In a MDM extract the retention time (RT) of myoglobin was found to be 23.5 min (Fig. 1B). Hemoglobin showed a longer retention time than expected, 21.4 min for a molecular weight of 64,500 daltons. This was more than for ovalbumin (RT = 20.4), which has a molecular weight of 44,000 daltons. Although the separation of hemoglobin and myoglobin was not complete, the two proteins could be satisfactorily determined in a mixture. The standard curves for bovine cyanohemoglobin and horse skeletal cyanomyoglobin when dissolved in buffer A showed good linearity (Fig. 2).

Content of iron, hemoglobin, myoglobin and total pigment in the raw materials and in the meat minces

The analysis of the raw materials and the calculated amounts of iron in hemoglobin and myoglobin (Table 1) indicate that all the iron in blood and approximately 70% of the iron in the blood emulsion were associated with extractable hemoglobin as heme iron. MDM contained approximately 55% iron not bound as extractable hemoglobin or myoglobin. In the ground beef used, most of the iron was extractable heme iron.

The iron and pigment contents determined (Table 2) show

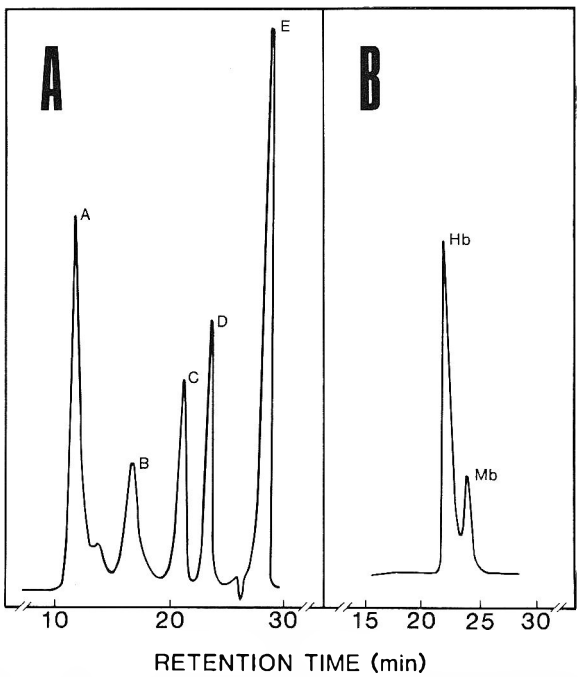

*Fig. 1—(**A**) HPLC chromatogram of a Bio-Sil-TEST protein solution containing the proteins bovine tyroglobulin MW 670000 daltons, retention time, RT = 11.3 min (A); bovine IgG, MW 158000 daltons, RT = 16.4 min (B); chicken ovalbumin, MW 44000 daltons, RT = 20.4 min (C); horse skeletal myoglobin, MW 18500 daltons, RT = 23.5 min (D); cyanocobalamin, MW 1350 daltons, RT = 27, 2 min (E). Injected amount: 10 µL. Detection: Absorbance at 254 nm. (**B**) HPLC chromatogram for hemoglobin, RT = 21.4, and myoglobin, RT = 23.5, extracted of 10g mechanically deboned meat with 30 mL sodium phosphate, Na_2SO_4, KCN buffer, pH 7.22. Injected amount: 10 µL. Detection: Absorbance at 420 nm.*

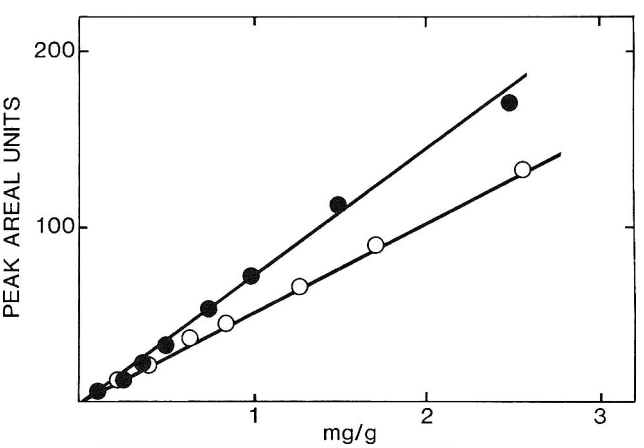

Fig. 2—Standard curves for hemoglobin (●) and myoglobin (○) dissolved in sodium phosphate, Na_2SO_4, KCN buffer, pH 7.22; determined by HPLC with a Bio-Sil TSK-SW (600 × 7.5 mm) and a guard column Bio-Sil TSK (75 × 7.5 mm). Injected amount: 10 µL.

that the values for total pigment measured by HPLC are in agreement with those measured spectrophotometrically except when small amounts of hemoglobin are present.

The relation between percent blood or blood emulsion added and iron content in the minces are shown in Fig. 3. Use of blood gave an average increase in iron content of 3.9 mg Fe/kg wet weight for each percent added. This is in agreement with results obtained by Eskeland and Slinde (1981). The average increase was 2.2 mg Fe/kg wet weight per percent blood

Table 1—Amounts of hemoglobin, myoglobin and iron (mg/g wet weight) in raw materials used, determined by HPLC and atomic absorption spectrometry[a]

Raw material	Hemoglobin	Myoglobin	Iron	Theoretically calc amount of iron in hemoglobin and myoglobin
Blood	125	–	0.44	0.43
Blood emulsion	54	–	0.28	0.19
Mechanically deboned meat	10.2	3.6	0.104	0.046
Ground beef (15% fat)	1.0	5.6	0.023	0.020
Ground beef (21% fat)	0.9	4.0	0.017	0.015

[a] The mean of three measurements are given; standard deviation 7% for total pigment (hemoglobin + myoglobin) and 6% for iron. Molecular weights of 64,500 daltons for hemoglobin and 18,500 daltons for myoglobin were used to calculate the theoretical amount of iron.

Table 2—Analysis of meat minces[a]

Mince	Iron	HPLC Hemoglobin	HPLC Myoglobin	Spectrophotometrically detm total pigment (Hemoglobin + Myoglobin)
Basic recipe	0.010	0.2	2.7	2.3
Basic recipe 2% blood added	0.021	2.7	2.7	5.4
Basic recipe 2% blood emulsion added	0.012	1.6	2.8	4.3
Basic recipe 2% MDM added	0.011	0.5	2.8	2.7
Basic recipe 2% MDM added 10 kg ground beef (15% fat) replaced by MDM	0.016	1.3	2.1	3.4
Basic recipe 2% MDM added 20 kg ground beef (15% fat) replaced by MDM	0.023	2.1	2.0	4.5

[a] Amount of iron (mg/g wet weight) determined by atomic absorption spectrometry. Hemoglobin and myoglobin (mg/g wet weight) determined by HPLC. Total pigment measured spectrophotometrically at approximately 420 nm by scanning the Soret peak. The mean of two measurements are given for each sample; standard deviation 7% for total pigment (hemoglobin and myoglobin) using HPLC, and 4.0% using the spectrophotometer.

emulsion added, thus on an iron basis about 3.5% blood emulsion equals 2% blood when added to the basic recipe.

Surface color of the heated meat loaves

It was shown by Mielnik and Slinde (1983) that addition of blood to sausages (up to 7 kg to a 100 kg batter) gave a steady decrease in reflectance at all wavelengths. The spectra in this work were similar (Fig. 4), and they were used to calculated L*, a*, b* values.

The L* (lightness) values of the meat loaves (Table 3) decreased and the a* (redness-greenness) values increased when blood, blood emulsion or MDM was added. The b* (blueness-yellowness) values varied slightly. The lightness, L*, was the main cause of change in product appearance. The darkening of the meat loaves, represented by L*, can be correlated with the amount of hemoglobin and myoglobin that could be extracted from the minces (Fig. 5A). Meat loaves containing blood showed less decrease in lightness than did products to which the same amount of pigment had been added as blood emulsion or MDM. Among the meat loaves containing blood, blood emulsion or MDM with equal amounts of total iron, those with blood emulsion were the darkest (Fig. 5B).

DISCUSSION

AN ACCEPTABLE COLOR of blood-containing products is necessary, in order to increase the content of heme iron in the

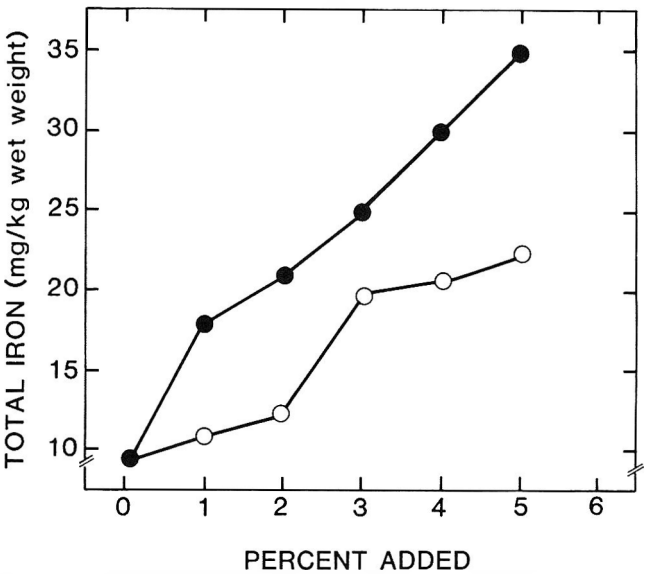

Fig. 3—Amount of iron (mg/kg wet weight) determined by atomic absorption spectrometry of freeze dried samples from meat loaves were 0–5% blood (●) or blood emulsion (○) had been added to the basic recipe.

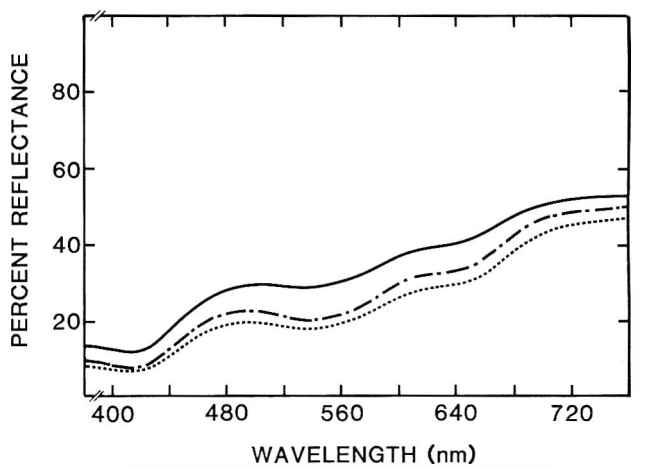

Fig. 4—Percent reflectance of the surfaces of meat loaves. From top to bottom: basic recipe, 2% blood emulsion, 2% blood. Each spectrum represents an average of three measurements.

diet. The main contributors to meat product color are myoglobin and hemoglobin, and a quantification of these compounds in the raw materials and minces was carried out. The high retention time of hemoglobin observed by HPLC molecular sieve chromatography (Fig. 1A and B), is probably due to specific interactions between the protein and the column material. Interactions between proteins and column material are usually influenced by pH and ionic strength; however, these parameters were varied without significant effects on RT. Thus, an affinity probably existed between the silica resin and hemoglobin. After prolonged use of the column, hemoglobin and myoglobin eluted as one peak, and the column is therefore not suitable for routine measurements of hemoglobin and myoglobin.

During this work we have found that the commercial hemoglobin and myoglobin batches were more or less polymerized, from 0–25%. This was found by HPLC, where the polymerized forms were eluted in front of the monomeric species. The polymerization affected both the absorbance spectra and the chromatographic behavior of the proteins on the HPLC col-

*Table 3—Color parameters calculated in the CIE 1976 L*a*b* system for surfaces of heated meat loaves with blood, blood emulsion or MDM added*[a]

Products	L*	a*	b*
Basic recipe	63.2 ± 0.1	2.4 ± 0.3	16.4 ± 0.3
Basic recipe 2% blood added	53.2 ± 1.3	5.8 ± 0.3	15.5 ± 0.5
Basic recipe 2% blood emulsion added	56.2 ± 0.05	5.9 ± 0.1	15.2 ± 0.5
Basic recipe 2% MDM added	59.9 ± 0.1	4.2 ± 0.2	15.5 ± 0.3
Basic recipe 2% MDM added 10 kg ground beef (15% fat) replaced by MDM	56.4 ± 0.2	5.8 ± 0.3	15.3 ± 0.2
Basic recipe 2% MDM added 20 kg ground beef (15% fat) replaced by MDM	53.6 ± 0.5	6.4 ± 0.8	15.8 ± 1.3

[a] The mean of three measurements and the standard deviation are given. The amount of extractable pigment in the minces are given in Table 2.

umn, giving an overestimation of hemoglobin and myoglobin in the extracts. For this reason, standards of both hemoglobin and myoglobin should be purified on a molecular sieve column, such as Sephadex G-100.

Due to autoxidation, hemoglobin and myoglobin preparations were obtained in the ferric form. Extraction with KCN was chosen since the cyanide ferric derivative is very stable (Antonini and Brunori, 1971). However, a slight decrease in concentration was observed during storage. Therefore, measurements were performed immediately.

The hemoglobin content in blood (Table 1) was in agreement with that determined by Warris and Rhodes (1977), and the amount of iron was within the range of iron contents determined for blood samples from different animals (Rauen, 1964). Approximately 70% of the iron in the blood emulsion was detected as extractable hemoglobin, indicating that some denaturation of hemoglobin took place during the emulsification process. The remaining iron may exist as nonextractable heme or as nonheme iron.

The pigment and iron level of MDM vary with the age of the slaughtered animal, the specific bones used, and the pressure applied in the bone separator (Field *et al.*, 1980). Hemoglobin from marrow is partially separated from the bone substance during the separating process. Since only approximately 45% of the iron was found as extractable hemoglobin and myoglobin, the additional iron probably came from the iron stores in bones (Field *et al.*, 1980), or from hemoglobin and myoglobin denatured during the separation process.

The iron and pigment contents determined (Table 1) are in the range expected (Cook and Monsen, 1976; Warris and Rhodes, 1977). However, the amount of iron bound as heme iron (Table 1) in the ground beefs used, approximately 85%, are higher than the 60% heme iron found by Cook and Monsen (1976) in beef. Horse skeletal myoglobin was used as standard with its corresponding extinction coefficient (Antonini and Brunori, 1971). If the extinction coefficient for bovine myoglobin was known, it may have altered the results. On the other hand, Cook and Monsen (1976) determined iron using the bathophenanthroline method (Torrance and Bothwell, 1968). In this procedure, nonheme iron is determined after heating the tissue for 65°C for 20 hours in acid solution. Such a procedure may remove the iron from the heme group (Fuhrhop and Smith, 1975). When the HPLC and absorbance measurements yielded somewhat different amounts of hemoglobin and myoglobin (Table 2) this may also be due to the extinction coefficient used.

The reflectance spectra in Fig. 4 show direct measurements of meat loaf surfaces. The lightness, L* (Table 3) is the most interesting color parameter, because the decrease in lightness caused most of the visible changes in surface appearance. Equal amounts of extractable pigment from minces with different raw materials (Fig. 5A) did not give the same final surface lightness. In MDM the marrow part contains denatured hemoglobin which is nonextractable, but still contributes to the product darkening. In the blood emulsion hemoglobin and heme are covered by fat and protein (Wismer-Pedersen, 1978, 1979). The relatively dark blood emulsion products (Fig. 5A) may be explained by emulsion breakage during heating, exposing the non-extractable heme pigment. Thus, the lightness could not be described by extractable hemoglobin and myoglobin alone, but was also a result of the raw materials used.

Product lightness was not linearly related to the total iron content in the minces (Fig. 5B). Due to the iron stores in bones (Field *et al.*, 1980) MDM contains inorganic iron in addition to the color-associated heme iron. The blood emulsion probably contains more heme iron than that associated with extractable hemoglobin. The relatively light surfaces of blood meat loaves may be due to some heme destruction during heating (Schriker and Miller, 1983).

Color optimalization of hemoglobin enriched meat products requires further research on the total heme content in the raw

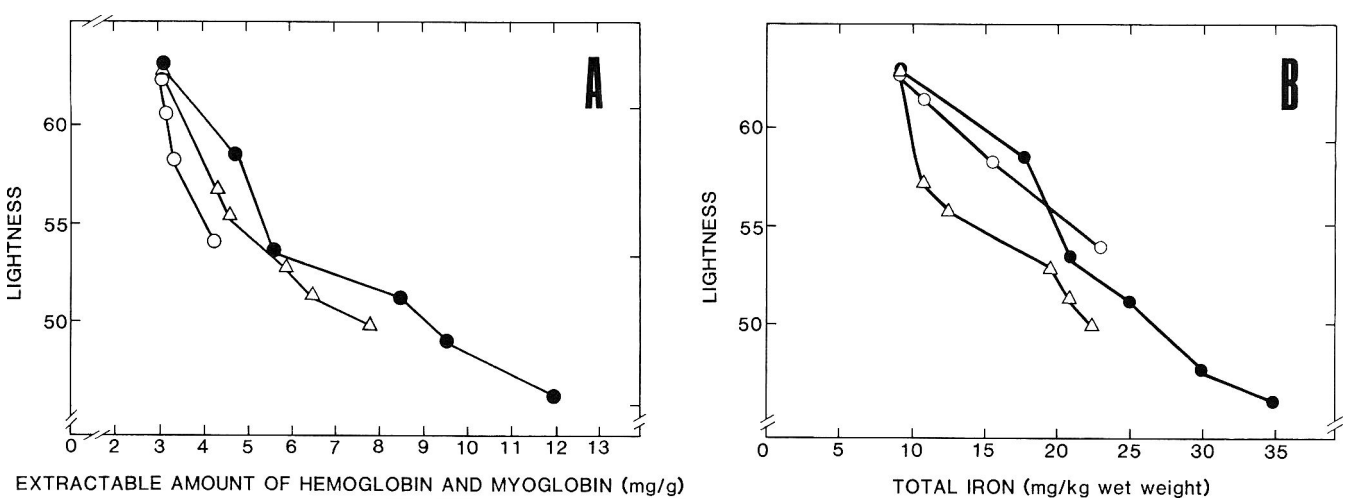

Fig. 5—(A) Relation between total pigment in minces, measured by HPLC, and lightness L; (B) relation between iron and lightness L* of surfaces of meat loaves to which increasing amounts of blood, 0–5% (●), blood emulsion, 0–5% (△), or MDM, (○) had been added.*

materials and the amount of heme destroyed during heating. The final heme iron content is also of nutritional interest, since the availability is reduced when iron splits off from heme. Slinde and Martens (1982) found that products with 2–3.5% blood yielded acceptable color. In the present study, products with 3% blood and products with 4% blood emulsion showed comparable color (Fig. 5A). Thus, an addition of 3.5–5.0% blood emulsion should be possible without causing negative changes to product appearance. This is in agreement with the recommendations of the manufacturer.

In practice, blood emulsion may be more attractive to handle than blood because it is delivered in frozen blocks and has an appearance much like MDM.

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Sensory Characteristics, Shear Values and Cooking Properties of Ground Beef Patties Extended with Iron- and Zinc-Fortified Soy Isolate, Concentrate or Flour

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ABSTRACT

Ground beef patties made from 100% beef or beef extended with 20% rehydrated soy isolate, concentrate, or flour, with or without iron and zinc fortification, were evaluated for sensory, shear and cooking properties. With the exception of patties formulated with soy isolate, soy-added patties were rated as more tender ($P<0.05$) than all-beef patties. Soy isolate imparted textural characteristics to patties that were more similar to those of all-beef patties than to those of soy concentrate or soy flour extended patties. Patties made with soy flour had the highest cooking yields. Also, patties extended with soy flour had lower incidences of rancid flavor, but higher incidences of soy flavor compared with all other beef-soy formulations. Iron and zinc fortification produced a higher frequency of rancid flavor when used with soy isolate and concentrate.

INTRODUCTION

SOY PROTEINS provide an economical and nutritional method of extending ground beef products. The U.S. Department of Defense purchases both ground beef patties and bulk ground beef which contain 20% rehydrated soy concentrate. Local school districts, under the USDA school lunch program, also purchase ground beef extended with soy protein. The concentrate form of soy protein is used in government-purchased ground beef primarily because of its bland flavor characteristics, nutritional quality, and economic value. Currently, the government pays about 10% less per pound for ground beef extended with 20% soy than for 100% ground beef (Duncan, 1984).

Many studies have compared sensory and cooking properties of all-beef patties and patties extended with one or two types of soy protein (Kotula et al., 1976; Smith et al., 1976; Bowers and Engler, 1975). Ziprin et al. (1981) compared the sensory and cooking characteristics of all-beef patties and beef patties extended with 10% soy protein (flour, concentrate and isolate). They found that ground beef patties extended with soy protein did not differ significantly from all-beef patties in texture, flavor or juiciness. However, the addition of soy protein to ground beef produced patties having a higher ($P<0.05$) cooking yield than all-beef patties.

Recently, several investigators reported that human consumption of soy-extended ground beef resulted in decreased absorption of nonheme iron from the diet (Cook et al., 1981; Hallberg and Rossender, 1982). These studies indicated the possibility of a nutritional risk to populations consuming soy-extended ground beef. Further studies were needed to confirm or refute the evidence of lower nonheme iron absorption.

The overall study was initiated to examine iron and zinc utilization in humans consuming ground beef extended with 20% rehydrated soy proteins. Refining processes may affect nutritional values and thus, the various forms of soy protein (flour, concentrate and isolate) were examined. Furthermore, iron and zinc fortification of the soy proteins was included in the event that fortification becomes commonplace as a result of the decreased iron absorption that was previously observed. Results of studies dealing with iron and zinc utilization in humans consuming ground beef extended with soy proteins will appear in another paper. This paper contains information derived through trained sensory evaluations and objective measurements concerning the eating quality and cooking properties of ground beef patties extended with 20% textured soy flour, concentrate, or isolate with or without iron and zinc fortification. This information is vital if results of nutritional analyses indicate a change in soy type or fortification is necessary.

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MATERIALS & METHODS

Processing and storage

Seven ground beef formulations were manufactured at a commercial meat processing establishment. One formulation was 100% ground beef while six ground beef-soy formulations were manufactured in the ratio of 80% beef and 20% rehydrated soy protein. The six formulations were: soy isolate (PP-220, Ralston Purina Company, St. Louis, MO); soy isolate fortified with iron and zinc; soy concentrate (Procon 2060, A.E. Staley Manufacturing Company, Decatur, IL); soy concentrate fortified with iron and zinc; textured soy flour (TVP, Archer-Daniels Midland Company, Decatur, IL); textured soy flour with iron and zinc.

Protein values for dry soy products were 53.5%, 69.5%, and 87.1% for textured soy flour, concentrate and isolate, respectively. Rehydration ratios were dependent upon these protein values in the dry soy product. To achieve a 19% protein value in all formulations, textured soy flour was rehydrated on the basis of 1 part flour to 1.8 parts water, soy concentrate received 1 part concentrate to 2.6 parts water, and soy isolate contained 1 part of isolate to 3.5 parts water. The soy and water were mixed for 5 min. The rehydrated soy was held for 5–10 min prior to mixing with the beef. Iron and zinc fortification was accomplished by the addition of 60 mg iron (ferrous fumarate) and 25 mg zinc (zinc oxide) per 100g of soy protein. Only the soy flour was texturized.

The beef materials used in all formulations consisted of 90% lean and 10% fat cow meat, and 50% lean and 50% fat U.S. Choice trimmings. The final fat content was adjusted to 22.0%. The cow lean and U.S. Choice fat materials were separately ground through a 1.9 cm plate. In the case of soy-extended formulations, the soy was added to the lean and these two materials were mixed for 4 min. The fat materials were then added followed by an additional 4 min of mixing. In the all-beef formulation, the lean and fat components were mixed together for 4 min. Each formulation was then ground to a final particle size of 0.3 cm prior to processing into patties (115g) by means of a Hollymatic (Model 200) patty machine (Hollymatic Corp., Countryside, IL). Patties were stacked (eight high), placed in plastic bags and sealed with tape. Patties were frozen (−29°C) in boxes at the processing plant. The frozen product was shipped to a local cold storage warehouse and stored 4 months (−29°C) prior to analyses.

Cooking methods

Frozen patties were broiled (275°C) on electric broiling units (Farberware 450A, Walter Kidde and Co., Inc., New York, NY). Total cooking time for all treatments was 17 min. Patties were cooked 5 min per side and then between 10 and 17 min, turned as needed to avoid excessive browning. Patties were cooked to a temperature endpoint equivalent to well done, which was determined using an eight-

point photographic scale (1 = extremely well done; 8 = extremely rare). Frozen and cooked patty weights were recorded for patty yield determinations. Patty diameter and thickness measurements were determined for purposes of detecting configurational changes in patties as a result of cooking.

Sensory analyses

A ten-member descriptive attribute panel, trained according to the procedures of Cross et al. (1978a), evaluated patties for tenderness, juiciness, connective tissue amount, ground beef flavor intensity, presence of other flavors and their intensities, and mouthcoating of fat. Eight-point structured scales were used (8 = extremely tender, juicy, intense in flavor, no connective tissue and no mouthcoating; 1 = extremely tough, dry or bland in flavor, abundant in connective tissue and abundant in mouthcoating). Panelists received two pieces (one-ninth of a patty per piece) per sample. Warm apple juice and unsalted melba toast were eaten between samples. Panelists participated in two sessions/meeting with either four or five samples given per session. Each formulation containing soy was evaluated four times while all-beef patties were evaluated seven times in a total of seven sessions.

Textural properties of cooked patties were assessed by a ten-member texture profile panel trained according to procedures of Civille and Szczesniak (1973). Procedures, terms and textural characteristics used by the panel for the cooked patties are presented in Table 1. Panelists used a 15 cm unstructured scale to quantitate their evaluations. Low numbers reflect low degrees of a textural characteristic while high numbers reflect high degrees of a textural characteristic. Panelists met twice a week and evaluated three treatments per day. Approximately 1 hr was required to complete the evaluations for a treatment in a given day.

Table 1—Characteristics, terms and procedures used by the texture profile panel to evaluate all-beef and ground beef-soy patties

I. Visual
   A. Distortion — degree to which an uncut full patty has warped.
II. Partial compression — place warm precut 2.54 cm^2 section in mouth and using molars against cooked surfaces, gently compress 4-5 times, wait a count of 2 for each press and evaluate for:
   A. Springiness — degree to which sample springs back and/or deforms.
III. Surface — place 1/4 of patty into mouth, rub across lips and tongue and evaluate for:
   A. Abrasiveness — degree to which surface feels harsh.
   B. Moistness — degree to which sample feels wet/oily, not dry.
   C. Debris — the amount of loose material on the surface.
IV. First bite — bite off 2.54 cm^2 from another 1/4 patty with incisors and evaluate for:
   A. Cohesiveness — degree to which sample deforms before shearing.
   B. Hardness — amount of force required to bite through sample.
   C. Density — degree of compaction across the area where incisors are biting.
   D. Moisture release — amount of juice released as teeth apply pressure.
V. Mastication — take a warm 2.54 cm^2 section and align in mouth as for partial compression for first bite. Turn the sample 90 degrees and take a second bite. Continue chewing and evaluate for:
   A. Crust — amount of crust detected after first two chews.
   B. Juiciness — amount of juice released following 10 chews. After juiciness, move bulk of mass to center of mouth, using tongue as feeler and evaluate for:
   C. Cohesiveness of mass — degree to which particles stick together following 15 chews.
   D. Coarseness of mass — degree to which irregular particles can be detected following 15 chews.
   E. Moistness — degree to which mass feels wet following 25 chews.
   F. Hard particles — amount of material present that is similar to the hardness of bone.
VI. End of mastication
   A. Gristle — amount of rubbery particles present.
   B. Webbed tissue — amount of webbed-like connective tissue present.
   C. Number of chews — total number of chews required to make sample capable of being swallowed.
VII. After swallow
   A. Toothpack — amount of sample material remaining in and around teeth.
   B. Mouthcoating — amount of oily residue left on surfaces of mouth.

Instron measurements

Eight patties/formulation were cooked and cooled to room temperature (25°C). Each patty was cut into four 3.0 cm square pieces prior to measuring shear force with a Universal Instron Machine (Model 1122, Instron Corp., Canton, MA), equipped with a straight edge shear blade. Newtons were calculated from shear force values and cooked patty measurements.

Statistical analyses

Analysis of variance procedures (Snedecor and Cochran, 1972) were used to test the effect of soy formulation. When an analysis of variance revealed a significant ($P<0.05$) effect, the mean separation technique of Duncan (1955) was employed.

RESULTS & DISCUSSION

INITIAL AND FINAL tenderness scores were higher ($P<0.05$) for patties extended with soy concentrate and textured soy flour than for all-beef patties and patties extended with soy isolate. Compared to patties extended with soy isolate, the higher tenderness values for patties made with textured soy flour and soy concentrate may be related to the higher percentage of soy, on a dry weight basis, necessary to rehydrate these two formulations to a 19% protein value. Patties formulated with soy isolate were similar to all-beef patties in tenderness and required the least amount of soy protein (dry weight) in the rehydrated formulation (Table 2). Ziprin et al. (1981) found patties formulated with 10% textured soy flour to have slightly more desirable texture characteristics than all-beef patties or patties formulated with 10% soy isolate or concentrate. Generally, other investigators have reported that the addition of soy protein to ground beef, regardless of type, improves patty tenderness (Huffman and Powell, 1970; Kotula et al., 1976).

The perception of tenderness in ground beef patties is affected by the connective tissue levels in the raw materials (Cross et al., 1978b). Detectable levels of connective tissue were lower ($P<0.05$) in patties extended with soy concentrate and soy flour than in all-beef or soy isolate patties (Table 2). The substitution of soy protein for muscle would dilute the quantity of connective tissue in soy extended products and account for the lower levels of detectable connective tissue. Obviously, patties extended with soy isolate did not have higher levels of connective tissue than patties extended with the other two types of soy. Thus, the use of soy isolate must create a type of binding during processing and cooking and a type of product breakdown during chewing that is more similar to what is found in all-beef patties than for soy concentrate or flour-added patties. With the exception of ground beef patties formulated with fortified soy concentrate, the texture profile panelists found higher ($P<0.05$) levels of webbed tissue (string-like connective tissue) in all-beef patties than in patties formulated with soy protein. Shear force values were lower ($P<0.05$) for all of the ground beef-soy formulations compared to the all-beef patties. Again, this was probably due to the substitution of soy protein for muscle. When shear force was expressed as Newtons, values were also higher ($P<0.05$) for all-beef than for soy-added patties.

The only differences found by the descriptive attribute panel for juiciness were the higher values obtained in the patties formulated with iron and zinc fortified soy flour when compared to all other formulations, with the exception of patties made with nonfortified soy flour and patties made just from beef (Table 3). A similar trend was found by the texture profile panel for first-bite moisture release. Nonsignificant differences in ground beef patty juiciness have been reported by other investigators who have compared all-beef and ground beef-soy extended patties (Bowers and Engler, 1975; Ziprin et al., 1981; Seideman et al., 1977; Smith et al., 1976). The texture profile panel found nonsignificant ($P>0.05$) differences due to formulation for all the other textural characteri·tics.

Ground beef flavor intensity scores were unaffected by patty formulation (Table 4). Patties from all treatments were con-

Table 2—Tenderness, detectable connective tissue, webbed tissue, shear force and Newton mean values and standard deviations for all-beef and beef-soy patty formulations

Formulation	Dry soy in the rehydrated soy that was added, %	Initial tenderness[a]	Final tenderness[a]	Connective tissue amount[a]	Webbed tissue[b]	Shear force, kg	Newton, kg/cm²
All-beef ground beef	0.0	4.7 ± 0.8[d]	5.1 ± 0.9[d]	5.1 ± 0.9[e]	4.0 ± 2.1[c]	11.5 ± 1.2[c]	43.6 ± 3.5[c]
Ground beef with soy isolate	22.2	4.8 ± 0.5[d]	5.3 ± 0.6[d]	5.5 ± 0.8[d]	2.2 ± 1.2[d]	7.8 ± 2.5[de]	29.7 ± 4.7[de]
Ground beef with soy isolate, Fe and Zn fortified	22.2	5.0 ± 0.9[d]	5.4 ± 0.7[d]	5.6 ± 0.7[d]	2.7 ± 2.4[d]	8.7 ± 1.9[d]	31.4 ± 4.3[d]
Ground beef with soy concentrate	27.8	5.7 ± 0.6[c]	6.1 ± 0.7[c]	6.1 ± 0.7[c]	2.2 ± 2.1[d]	7.5 ± 1.4[de]	28.6 ± 3.8[de]
Ground beef with soy concentrate, Fe and Zn fortified	27.8	5.7 ± 0.5[c]	6.1 ± 0.6[c]	6.0 ± 0.7[c]	3.2 ± 2.7[cd]	7.8 ± 1.7[de]	28.3 ± 4.2[de]
Ground beef with textured soy flour	35.7	5.6 ± 0.7[c]	6.0 ± 0.5[c]	6.1 ± 0.6[c]	2.0 ± 1.8[d]	7.8 ± 1.5[de]	31.6 ± 4.6[d]
Ground beef with textured soy flour, Fe and Zn fortified	35.7	5.8 ± 0.8[c]	6.2 ± 0.5[c]	6.3 ± 0.6[c]	2.2 ± 2.3[d]	6.9 ± 1.0[e]	25.5 ± 3.7[e]

[a] Values based on eight-point structured scales where 8 = extremely tender and no connective tissue; 1 = extremely tough and abundant in connective tissue.
[b] Values based on 15 cm unstructured scale. High numbers indicate a high amount of webbed tissue, low numbers indicate a low amount of webbed tissue.
[cde] Means within a column with different superscripts are significantly different ($P<0.05$).

Table 3—Juiciness and moisture release mean values and standard deviations for all-beef and ground-beef-soy patties

Formulation	Dry soy in the rehydrated soy that was added, %	Juiciness[a]	Moisture Release[b]
All beef ground beef	0.0	4.8 ± 0.7[cd]	4.9 ± 1.8[c]
Ground beef with soy isolate	22.2	4.5 ± 0.6[de]	3.5 ± 1.8[d]
Ground beef with soy isolate, Fe and Zn fortified	22.2	4.5 ± 0.5[de]	3.9 ± 1.6[d]
Ground beef with soy concentrate	27.8	4.3 ± 0.6[e]	4.3 ± 2.3[cd]
Ground beef with soy concentrate, Fe and Zn fortified	27.8	4.4 ± 0.5[de]	3.8 ± 1.7[d]
Ground beef with textured soy flour	35.7	4.7 ± 0.7[cde]	5.0 ± 1.9[c]
Ground beef with textured soy flour, Fe and Zn fortified	35.7	4.9 ± 0.6[c]	4.3 ± 1.6[cd]

[a] Values are based on eight-point scales where 8 = extremely juicy; 1 = extremely dry.
[b] Values obtained from the use of a 15 cm unstructured line where 0 is the absence of an attribute and 15 is the extensive presence of an attribute. Definitions for texture profile attributes are given in Table 1.
[cde] Means within a column with different superscripts differ significantly ($P<0.05$). When no superscripts are present, Analysis of Variance was nonsignificant ($P>0.05$) for that attribute.

Table 4—Ground beef flavor intensity and frequency of other detected flavors

Formulation	Dry soy in the rehydrated soy that was added, %	Ground beef flavor intensity[a]	Flavor note and percent occurrence within formulations[b]							
			Sour	Bitter	Metallic	Sweet	Rancid	Fishy	Soy	Other
All-beef ground beef	0.0	4.1 ± 0.9	4.5	1.8	13.5	5.4	49.5	13.5	2.7	9.0
Ground beef with soy isolate	22.2	4.1 ± 0.5	13.8	3.4	6.9	8.6	39.7	3.4	5.2	19.0
Ground beef with soy isolate, Fe and Zn fortified	22.2	4.1 ± 0.8	3.6	5.4	10.7	1.8	51.8	5.4	5.4	16.1
Ground beef with soy concentrate	27.8	4.2 ± 0.9	7.0	1.7	24.6	8.8	15.9	0.0	17.5	24.6
Ground beef with soy concentrate, Fe and Zn fortified	27.8	4.0 ± 0.9	7.9	3.2	17.5	7.9	27.0	0.0	17.5	19.0
Ground beef with textured soy flour	35.7	4.0 ± 0.8	2.6	7.7	10.2	23.1	16.6	1.3	21.8	16.6
Ground beef with textured soy flour, Fe and Zn fortified	35.7	3.9 ± 0.9	1.4	4.1	9.6	30.1	16.4	0.0	23.3	15.1

[a] Mean values and standard deviations based on an eight-point structured scale where 8 = extremely intense in flavor and 1 = extremely bland in flavor.
[b] Values represent percent occurrence of a flavor in relation to all the other flavor notes identified within a formulation.

sidered as "slightly bland" in ground beef flavor. However, the presence of other detectable flavors may account for the lack of differences in ground beef flavor among the formulations. The frequency of other flavors, expressed as percentages within a formulation, are given in Table 4. Patties were stored 4 months prior to analyses in order to appraise any antioxidant properties inherent to soy, as well as any prooxidant effects of the iron and zinc fortification. Rancid was a frequently identified flavor in all-beef patties. This was also the case for patties extended with soy isolate, especially when fortified with iron and zinc. For patties processed with soy concentrate, iron and zinc fortification also elevated the incidence of rancid flavor. However, for patties processed with soy flour, regardless of fortification, the incidence of rancid flavor was lowest. The lower incidence of rancid flavor in these patties is attributable, in part, to the antioxidant properties inherent especially to soy flour (Hayes et al., 1977). However, in previous studies (Ziprin et al., 1981; Kotula et al., 1976) soy flour has not been

Table 5—Mean values and standard deviations for cooking properties of all-beef and ground beef-soy patties

Formulation	Dry soy in the rehydrated soy that was added, %	Yield, %	Change in patty diameter from raw to cooked, %	Degree of doneness score[a]
All-beef ground beef	0.0	55.5 ± 1.8^{bc}	−24.1 ± 3.3^{bc}	2.5 ± 0.6^{b}
Ground beef with soy isolate	22.2	56.3 ± 2.8^{c}	−22.3 ± 2.1^{cd}	2.4 ± 0.9^{bc}
Ground beef with soy isolate, Fe and Zn fortified	22.2	54.7 ± 1.9^{b}	−25.2 ± 2.3^{b}	2.3 ± 0.3^{bcd}
Ground beef with soy concentrate	27.8	60.9 ± 1.5^{d}	−19.4 ± 1.3^{e}	2.1 ± 0.5^{cd}
Ground beef with soy concentrate, Fe and Zn fortified	27.8	61.2 ± 1.7^{d}	−22.0 ± 2.0^{cde}	2.1 ± 0.3^{cd}
Ground beef with textured soy flour	35.7	63.2 ± 1.8^{e}	−20.9 ± 1.9^{de}	2.1 ± 0.2^{cd}
Ground beef with textured soy flour, Fe and Zn fortified	35.7	62.4 ± 1.4^{e}	−23.7 ± 2.1^{bcd}	2.1 ± 0.1^{d}

[a] Values based on an eight-point photographic scale where 8 = extremely rare and 1 = extremely well done.
^{bcde} Means within a column with different superscripts differ significantly ($P<0.05$).

found to differ in antioxidant properties from other forms of soy. Furthermore, characteristic soy-like flavors were most frequently reported in patties processed with soy flour, and they may have masked the presence of rancid flavors. Traditionally, the carry-over flavor of soy has limited the use of soy flour in processed meats (Rakosky, 1974). A higher incidence of "sweet" flavor was also reported in patties containing soy flour and was probably attributable to its carbohydrate content. Patties made with nonfortified soy isolate had the highest incidence of "sour" flavor.

Mean cooking yields of patties by formulation are presented in Table 5. Patties processed with soy flour produced the highest cooking yields compared with all other formulations. Patties processed with soy isolate produced yields similar to all-beef patties which were the lowest, while patties made with soy concentrate produced cooking yields lower than textured soy flour but higher than soy isolate. Fortification with iron and zinc decreased ($P<0.05$) yields when used in patties extended with soy isolate. Other investigators have shown that the addition of soy protein increases ground beef cooked patty yields (Ray et al., 1981; Smith et al., 1976; Bowers and Engler, 1975; Miles et al., 1984). The higher cooking yields are attributable to the moisture binding properties associated with soy. In particular, soy flour, because of fewer steps in the refinement process, has an affinity for moisture greater than that observed in patties extended with soy concentrate or isolate (Rakosky, 1974; Wolf, 1970). Furthermore, in our study, cooking yields were directly related to the amount of dry soy and added water in the ground beef-soy formulation (Table 5). The greater quantity of soy (and correspondingly less water) increased moisture retention and decreased total cooking losses. Fortification of soy protein appeared to increase patty shrink, especially for patties extended with soy isolate.

Degree of doneness values reflect a slightly less well done appearance in the all-beef patties and patties made with soy isolate compared to the other formulations (Table 5). In considering this finding with that of yield, it is possible that with a constant broiling time, patties made with soy concentrate or flour cook at a faster rate than all-beef patties or those processed with soy isolate.

CONCLUSIONS

RESULTS of sensory and instrumental analyses consistently indicated that soy isolate imparted textural characteristics to ground beef-soy patties which were more similar to all-beef patties than to other beef-soy formulations. Cooking properties (yield, degree of doneness, percent shrink) of patties processed with soy isolate were also similar to all-beef patties.

The higher cooking yield of patties processed with soy flour, compared with all other formulations, may provide important implications for those in the hotel, restaurant and institutional trade where cooking yields are extremely important. However, the higher incidence of "soy" and "sweet" flavors in these patties may be objectionable to consumers. Soy flour usage in patties produced antioxidant properties as evidenced by reduced presence of rancid flavors. Iron and zinc fortification elevated the occurrence of rancid flavors in patties formulated with fortified soy isolate.

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Rapid Chilling and Mechanical Portioning as a Fresh Pork Processing System

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ABSTRACT

Several rapid chilling techniques were applied after a variable delay time in an effort to link hot pork processing and mechanical portioning. Boneless pork loins (n = 80) were removed (1, 3 or 5 hr post-stunning) and assigned to one of three rapid chilling treatments: brine chilling, CO_2 gas or CO_2 snow. These were compared to the traditional method of chilling the carcasses 24 hr prior to blast freezing of excised loins. Loins were chilled until crust frozen, then tempered and mechanically portioned. Muscle color, pH, and sarcomere lengths were measured. Cooked chops were evaluated by taste panel and Warner-Bratzer shear force. There were no major differences in these parameters between rapid chilling methods when a delay time of at least 3 hr was used.

INTRODUCTION

RECENTLY, the application of brine chilling to hot-processed pork has been investigated as a means of linking hot pork processing and mechanical portioning (Frye et al., 1985.) Such a system would allow for hot boning and rapid processing of pork, thereby improving processing efficiency and hastening the movement of pork through the plant. Mandigo and coworkers (Hinnergardt et al., 1973; Mandigo and Henrickson, 1966a, b; Mandigo et al., 1977, 1979) have demonstrated that hot pork processing can be conducted without reduction in wholesale cut yields. However, care must be taken at the time of rapid chilling to prevent muscle toughening (Frye et al., 1985; Galloway and Goll, 1967; Hinnergardt et al., 1973; Weiner et al., 1966).

Miller et al. (1984), Kastner and Russell (1975) and McCrae et al. (1971) have demonstrated that delaying the onset of chilling in hot boned pork, beef and lamb, respectively, results in acceptable tenderness. This delay allows glycolysis to continue, depleting the ability of the muscle to regenerate ATP and thus reducing problems with muscle shortening and toughening. Following this logic, this research was conducted to determine appropriate delay times prior to rapid chilling of hot boned pork for mechanical portioning. In addition, alternative rapid chilling techniques were compared.

MATERIALS & METHODS

Experimental design

Loins were removed from conventionally slaughtered, market weight hogs (n = 40) and randomly assigned to one of 10 treatments (each treatment having eight loins). The 10 treatments consisted of a control and three different methods of chilling applied after one of three delay times. After a delay time of either 1, 3 or 5 hr post-stunning, loins were removed from carcasses held in a cooler (0–2°C) and rapidly chilled by brine chilling, exposure to carbon dioxide in a cabinet or by packing in carbon dioxide snow (dry ice). Loins from the control treatment were removed 24 hr post-stunning and chilled in a blast freezer.

The rapid chilling treatments consisted of either an 88° salt brine that was at −23°C (BC)—the loins for this rapid chilling treatment were placed in unsealed vacuum bags and then secured between two wire racks to prevent flotation; a CO_2 gas cabinet (CC) that was operated at −94°C; or packing in CO_2 snow (CS) which functioned at −68°C. The loins for the CC and CS treatments were protectively wrapped in polyvinylchloride film prior to chilling. All combinations of delay times and rapid chilling types were compared to a control. Loins used as a control were removed after a 24 hr chill period in a 0–2°C cooler, and then blast frozen at −30°C to an internal temperature of −2°C. Thermocouples were inserted into the center of all loins to monitor the decreasing temperatures. Loins were kept in rapid chilling treatments until an internal temperature of −2°C was reached. The loins were then removed and tempered for 60 min in a −2°C cooler. After tempering, they were pressed into an oval shape (die no. 210) using 2.76 MPa of pressure in a Bettcher Model 70 hydraulic press (Bettcher Ind., Inc., Vermilion, OH). Pressed loins were portioned into 2.9 cm thick boneless pork chops with a Model 81 Bettcher Power Cleaver (Bettcher Ind., Inc., Vermilion, OH). Chops were vacuum packaged and frozen at −30°C for later analysis.

Chill time

The chill time was measured as the total time (min) required for the loins to be kept in the rapid chilling treatment until the target internal temperature of −2°C was achieved.

Muscle pH

The pH of the loins was monitored during three different phases: before application of the rapid chilling treatment (pre-chill pH), after cleaving (post-chill pH), and after thawing for 24 hr at 0–2°C (ultimate pH). Triplicate readings of pre-chill muscle pH were obtained with a Broadly-James Model 10511 (Broadly-James Corp., Santa Ana, CA) hand-held pH meter. The puncture electrode was inserted into center portions of the loins. Post-chill pH samples were taken from thinly sliced portions of the center regions of the pressed loins. These samples were quickly frozen and stored in a −60°C freezer. At a later date (within 1 wk), these samples were powdered and 10g samples of each were combined with 100 mL of 0.005M (pH 7.0) iodoacetate (used to arrest glycolysis) and homogenized for 1 min in a 1L Waring Blendor jar. The pH was measured with an Orion Model 801A (Orion Research, Cambridge, MA) research pH meter. The ultimate pH of thawed chops was obtained similarly to post-chill pH. However, the thawed meat samples were mixed with 100 mL of water instead of the iodoacetate solution.

Sarcomere length

The measurement of sarcomere lengths involved the use of a Zeiss (Carl Zeiss, Germany) light microscope and measuring ten sets of ten sarcomeres for each treatment with an ocular micrometer. The muscle samples were prepared by taking 3g of frozen meat pieces and blending them with about 35 mL of 0.25M sucrose at high speed in a Waring Blendor for 30 sec. Small drops of each homogenate were placed on glass slides, covered with a cover slip and observed under the oil immersion lens (1000 x).

Chop appearance

Prior to cooking, each thawed chop was evaluated on the Hunterlab colorimeter (Hunter Assoc. Laboratory, Inc., Fairfax, VA), in duplicate, for "L", "a_L" and "b_L" values. The "L" value measures lightness to darkness, 0 (black) to 100 (white); the "a_L" value defines redness (when positive) and greenness (when negative); and the "b_L" value represents the degree of yellowness (when positive) and blueness (when negative). The thawed chops were evaluated in their vacuum

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package. The Hunterlab colorimeter was standardized to a pink standard plate (C2-6071).

Cooking procedures

Chops were thawed in a 0–2°C cooler for 24 hr, oven roasted at 177°C to an internal temperature of 35°C, turned and then removed after reaching 70°C. Internal temperatures were measured by copper-constantan thermocouples inserted into the centers of the chops. Thaw loss, cook loss and cook times were recorded. Thaw loss was determined on all chops by the difference between the weight of the thawed, vacuum packaged chop (with its free exudative moisture-purge) and the unpackaged chop (after removing the average weight of the packaging material). Cooking time was recorded as the total time (in min) required for the internal temperature to reach 70°C. Cooking yields were determined on all chops by weighing before and immediately after cooking.

Sensory evaluations

An eight member, trained (Cross et al., 1978) sensory panel evaluated the parameters of tenderness and juiciness on a 15 cm unstructured line scale. Sensory panelists rated samples by placing a horizontal mark across the 15 cm line. The distance of these marks from the origin of the line was then measured: 0 = tough or dry, 15 = tender or juicy.

Textural measurements

A Warner-Bratzler shear force (WBS) attachment to an Instron Universal Testing Machine (Instron Corp., Canton, MA) was used to measure chop tenderness. After cooking and then cooling to room temperature, the chops were trimmed and 12.5 mm diameter cores were taken. A 500 kg load cell and a 50 kg range were used with a crosshead speed of 250 mm/min. Peak forces are expressed as kg of force required to shear the sample core. Area under the shear curve represents the total amount of work required to shear the core samples and is reported in square cm.

Statistical analyses

This experimental was analyzed by using a 4 delay time by 4 rapid chilling treatment, incomplete factorial. Mean comparisons were analyzed using Duncan's new multiple range test according to Steel and Torrie (1980). Statistical analyses were conducted using the statistical analysis system of Barr et al. (1979).

RESULTS & DISCUSSION

INITIAL pH VALUES of loin muscles taken prior to rapid chilling application are shown in Table 1. As expected, as time before rapid chilling application increased, pH generally decreased. Initial muscle pH values were obtained to monitor rigor development before loins were exposed to rapid chilling. When beef muscle pH values are above 6.0 prior to chilling, susceptibility to cold shortening is increased (Hamm, 1982). This situation was evident for all loins rapidly chilled at the 1 hr delay time.

The time required for loins to remain in the rapid chilling methods until the internal target temperature of −2°C was reached is shown in Table 1. Delay time did not alter the time required to chill the loins for any of the rapid chilling treatments. No major differences between rapid chilling methods were noted. Loins of the control treatment, however, required more time to reach the internal target temperature. It was hypothesized that the differences in temperature between the rapid chilling methods would cause different rates of chilling. However, no significant differences in rapid chilling rates were observed in this study.

Post-chill muscle pH values were taken from loins immediately after tempering, pressing and cleaving and are given in Table 2. Loins delayed 1 hr prior to rapid chilling had the highest pH values, while loins in the 3 and 5 hr delay times had generally equivalent values. No major differences were observed between rapid chilling methods. Muscle pH was obtained after rapid chilling to determine if these rapid chilling methods retard postmortem glycolysis and create conditions conducive to cold shortening and/or thaw rigor. It appears that rapid chilling of pork loins within 1 hr post-stunning does maintain higher muscle pH values. Studies of beef and lamb have shown that postmortem glycolysis proceeds at an accelerated rate as the internal muscle temperature approaches its freezing point (Bodwell et al., 1966; Newbold and Harris, 1972; Behnke et al., 1973). However, pork muscles may require lower temperatures before this phenomenon can be observed (Hamm, 1982). No direct evidence for this phenomenon was observed with these data. The high pH values observed with loins rapidly chilled in the 1 hr delay time suggest that at least part of the loin was frozen before rigor onset, and severe muscular contraction may have been a problem (Hamm, 1982).

Sarcomere lengths, obtained from frozen muscle, are shown in Table 2. Sarcomeres of loins from the 1 hr delay treatment had the shortest lengths. No difference between the BC and CC groups was observed, while it appeared that the CS rapid chilling treatment had a less severe effect on sarcomere shortening. Loins of the control group had the longest sarcomere lengths. The combination of high muscle pH (indicating absence of rigor development) of loins from the 1 hr delay time (Table 2) and their short sarcomere lengths indicate that cold shortening or thaw rigor may have toughened these samples.

Ultimate pH values taken from thawed muscles are given in Table 2. No differences in muscle pH were observed between delay times, rapid chilling methods or the control. These results correspond to previous work with rapid chilling of pork loins (Frye et al., 1985). Rapidly chilled loins delayed 1 or 3 hr had higher post-chill pH values than the control, yet no differences were detected in ultimate pH. This would suggest that postmortem metabolism was completed during slow thawing (Hamm, 1982). Thus, slow thawing may lessen some of the tenderness problems brought on by thaw rigor. Also, it is apparent that measurement of ultimate pH of slowly thawed

Table 1—Initial pH prior to rapid chilling and time required for boneless loins to reach −2°C

Chill Treatment[a]	Initial pH				Chilling time (min)			
	Delay time (hr)				Delay time (hr)			
	1	3	5	Control[b]	1	3	5	Control[b]
BC	6.58^{cx} (0.32)	5.87^{dx} (0.48)	5.80^{dx} (0.46)	5.53^{e} (0.06)	100.88^{cy} (16.56)	114.88^{cy} (33.76)	113.65^{cy} (12.98)	168.25^{d} (0.50)
CC	6.49^{cx} (0.48)	6.07^{dx} (0.49)	5.89^{dx} (0.32)	5.53^{e} (0.06)	91.72^{cy} (31.90)	84.75^{cy} (31.00)	79.62^{cz} (16.22)	168.25^{d} (0.50)
CS	6.50^{cx} (0.49)	5.78^{efx} (0.53)	6.01^{dex} (0.35)	5.53^{f} (0.06)	95.38^{cy} (24.56)	98.00^{cy} (42.61)	88.29^{cyz} (15.01)	168.25^{d} (0.50)
Control[b]	5.53^{y} (0.06)	5.53^{y} (0.06)	5.53^{y} (0.06)		168.25^{x} (0.50)	168.25^{x} (0.50)	168.25^{x} (0.50)	

[a] Boneless loins rapidly chilled in either a brine chill (BC), carbon dioxide gas cabinet (CC), or carbon dioxide snow (CS).

[b] Control loin muscles were removed at 24 hr postmortem, then chilled in a blast freezer.

[cdef] Means in the same row for each trait bearing different superscripts are significantly different ($P<0.05$).

[xy] Means in the same column bearing different superscripts are significantly different ($P<0.05$).

Parenthetical values indicate standard deviations.

Table 2—Post-chill pH values and sarcomere lengths after rapid chilling, tempering, pressing and cleaving, and ultimate pH of thawed muscle

Chill Treatment[a]	Post-chill pH: Delay time (hr) 1	3	5	Control[b]	Sarcomere length (μm): Delay time (hr) 1	3	5	Control[b]	Ultimate pH: Delay time (hr) 1	3	5	Control[b]
BC	6.38^{cx} (0.21)	5.86^{dx} (0.31)	5.86^{dy} (0.28)	5.58^{e} (0.10)	1.17^{cz} (0.18)	1.70^{dy} (0.26)	1.79^{dy} (0.21)	1.98^{e} (0.19)	5.55^{cx} (0.15)	5.48^{cx} (0.15)	5.52^{cx} (0.10)	5.51^{c} (0.05)
CC	6.23^{cx} (0.20)	5.98^{dx} (0.24)	5.88^{dy} (0.30)	5.58^{e} (0.10)	1.26^{cz} (0.24)	1.70^{dy} (0.25)	1.72^{dy} (0.25)	1.98^{e} (0.19)	5.52^{cx} (0.16)	5.54^{cx} (0.08)	5.50^{cx} (0.22)	5.51^{c} (0.05)
CS	6.30^{cx} (0.25)	5.96^{dx} (0.35)	6.13^{dx} (0.15)	5.58^{f} (0.10)	1.38^{cy} (0.33)	1.88^{dx} (0.17)	1.46^{cz} (0.35)	1.98^{d} (0.19)	5.51^{cx} (0.24)	5.42^{cx} (0.24)	5.57^{cx} (0.11)	5.51^{c} (0.05)
Control[b]	5.58^{y} (0.10)	5.58^{y} (0.10)	5.58^{z} (0.10)		1.98^{x} (0.19)	1.98^{x} (0.19)	1.98^{x} (0.19)		5.51^{x} (0.05)	5.51^{x} (0.05)	5.51^{x} (0.05)	

[a] Boneless loins rapidly chilled in either a brine chill (BC), carbon dioxide gas cabinet (CC) or carbon dioxide snow (CS).
[b] Control loin muscles were removed at 24 hr postmortem, then chilled in a blast freezer.
[cdef] Means in the same row for each trait bearing different superscripts are significantly different ($P<0.05$).
[xyz] Means in the same column bearing different superscripts are significantly different ($P<0.05$).
Parenthetical values indicate standard deviations.

pork muscle does not indicate the chilling history of the chop prior to freezing.

Warner-Bratzler shear force and area under the shear curve values are shown in Table 3. More force and total work were required to shear core samples from the 1 hr delay time than for the other delay times and the control, although tenderness of chops from loins delayed at least 3 hr prior to rapid chilling was generally equal to control chops. Similar results were reported by Miller et al. (1984). No major differences in shear force were detected between rapid chilling methods.

The relationship between shear force values and tenderness in this research was similar to that reported in previous studies. Marsh and co-workers (Marsh, 1977, 1981; Marsh and Carse, 1974; Marsh and Leet, 1966; Marsh et al., 1974) found no major differences in tenderness of beef muscles when shortening was less that 20% (as found in the 3 hr delay times of all rapid chilling methods and the 5 hr delay time of the BC and CC methods), while severe tenderness damage was noted when shortening was between 35–40% (as found with many samples in the 1 hr delay time for all rapid chilling methods). The decline in toughness beyond this sarcomere shortening range in beef muscle may be due to Z-line penetration by myosin filaments (Marsh and Carse, 1974). However, further microscopy is needed to investigate this shortening phenomenon with pork myofibers.

Sensory panel tenderness scores are also given in Table 3. As the delay time prior to each rapid chilling method increased, a trend for more tender chops resulted. However, the differences were not consistent enough to be statistically significant. It seems that the 5 hr-CS treatment provided the greatest tenderness.

Sensory panel juiciness scores are given in Table 4. The greatest juiciness ratings were with the 1 hr-BC and the 5 hr-CS treatments. Past studies with pork and beef have found that muscles processed before rigor onset were generally juicier than post-rigor muscle (Berry et al., 1981; Cross et al., 1979; Frye et al., 1985; Mandigo and Henrickson, 1966a), while Schmidt and Gilbert (1970) and Marriott et al. (1980) found no benefit to juiciness. However, the differences reported here do not seem to be of practical significance. No differences were found between treatments for thaw loss, cook loss or cook times (data not shown).

Chop appearance, as measured by the Hunter "L", "a_L" and "b_L" scores, was not influenced by delay time or rapid chilling method (data not shown). Borchert and Briskey (1964) have suggested that the condition of pale, soft and exudative pork (PSE) could be prevented if the pork muscles were chilled quickly enough. Since PSE was not observed in this study, any changes in color were inconsistent and of little value. For situations where PSE is present, however, the rapid chilling methods examined here could prove quite beneficial.

Problems with technique and strict quality control exist with brine chilling. As fresh pork products cannot have any salt in them, great efforts were made to keep the brine solution from direct contact with the surface of the loins. In an industrial use, cold brine could be sprayed onto the wrapped meat; however, problems may occur when salt comes into contact with the meat. Perhaps the use of a glycol chill spray would be beneficial, as it would remove the necessity of protectively wrapping the muscles prior to chilling.

In summary, severe chilling within 1 hr postmortem resulted in high shear force values and short sarcomere lengths; a delay time of at least 3 hr is needed to prevent detrimental changes

Table 3—Tenderness characteristics of chops rapidly chilled under controlled conditions

Chill Treatment[a]	Shear force (kg): Delay time (hr) 1	3	5	Control[c]	Area under shear curve (cm²): Delay time (hr) 1	3	5	Control[c]	Tenderness ratings[b]: Delay time (hr) 1	3	5	Control[c]
BC	4.21^{dx} (1.76)	3.73^{exy} (1.01)	3.49^{e} (0.69)	3.42^{e} (0.98)	172.58^{dx} (60.07)	159.25^{dx} (62.40)	144.48^{fx} (29.50)	149.28^{ef} (43.17)	8.13^{dex} (4.24)	9.15^{dx} (3.64)	7.41^{ez} (3.40)	8.20^{d} (3.74)
CC	3.94^{dx} (1.00)	3.75^{dex} (1.02)	3.60^{ex} (0.90)	3.42^{f} (0.98)	165.61^{dx} (47.27)	160.65^{dx} (43.59)	158.10^{dex} (35.93)	149.28^{e} (43.17)	8.06^{dx} (3.26)	8.16^{dx} (3.76)	9.34^{dxy} (3.03)	8.20^{d} (3.74)
CS	4.16^{dx} (1.06)	3.55^{exy} (1.04)	3.15^{fy} (0.81)	3.43^{ef} (0.98)	173.86^{dx} (48.94)	150.68^{ex} (43.77)	130.62^{fy} (32.08)	149.28^{e} (43.17)	9.11^{dx} (3.28)	9.51^{dx} (3.42)	10.15^{dx} (2.69)	8.20^{e} (3.74)
Control[c]	3.42^{y} (0.98)	3.42^{y} (0.98)	3.42^{xy} (0.98)		149.28^{y} (43.17)	148.28^{x} (43.17)	149.28^{x} (43.17)		8.20^{x} (3.74)	8.20^{x} (3.74)	8.20^{yz} (3.74)	

[a] Boneless loins rapidly chilled in either a brine chill (BC), carbon dioxide gas cabinet (CC), or carbon dioxide snow (CS).
[b] Evaluated by an eight member, trained sensory panel on a 15 cm unstructured line scale (0 = tough, 15 = tender).
[c] Control loin muscles were removed at 24 hr postmortem, then chilled in a blast freezer.
[def] Means in the same row for each trait bearing different superscripts are significantly different ($P<0.05$).
[xyz] Means in the same column bearing different superscripts are significantly different ($P<0.05$).
Parenthetical values indicate standard deviations.

Table 4—Sensory juiciness ratings of chops rapidly chilled under controlled conditions

Chill treatments[a]	Delay time (hr) 1	3	5	Control[b]
BC	10.26[cx] (2.57)	8.35[dx] (3.18)	9.03[dy] (3.32)	7.84[d] (3.51)
CC	8.85[cy] (2.58)	8.87[cx] (3.23)	8.61[cy] (3.43)	7.84[c] (3.51)
CS	8.41[dy] (3.44)	8.59[dx] (3.83)	10.48[cx] (2.42)	7.84[d] (3.51)
Control[b]	7.84[y] (3.51)	7.84[x] (3.51)	7.84[y] (3.51)	

* Evaluated by an eight member trained sensory panel on a 15 cm unstructured line scale (0 = dry, 15 = juicy).

[a] Boneless loins rapidly chilled in either a brine chill (BC), carbon dioxide gas cabinet (CC), or carbon dioxide snow (CS).

[b] Control loin muscles were removed at 24 hr postmortem, then chilled in a blast freezer.

[cd] Means in the same row bearing different superscripts are significantly different ($P<0.05$).

[xy] Means in the same column bearing different superscripts are significantly different ($P<0.05$).

Parenthetical values indicate standard deviations.

to product parameters. No major differences existed among rapid chilling methods.

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Prediction of Bind Value Constants of Sausage Ingredients from Protein or Moisture Content

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ABSTRACT

Ingredients used in comminuted meat products were divided into four classes: Class I—striated, skeletal muscle meats; Class II — striated, nonskeletal muscle meat; Class III — organ and smooth muscle meats; and Class IV — nonmeat proteins. Within this classification scheme, bind value constants developed by different workers were subjected to regression anaylsis using protein or moisture as the independent variable. Linear or multiple regression equations with high correlation coefficients were obtained for Class I and Class III meats indicating reliable predictive value of moisture or protein content. These equations should prove useful for esimating bind value constants for meat ingredients in these classes for which such constants have not been established by experimental procedures.

INTRODUCTION

AN UNDERSTANDING of the structure and function of the protein matrix formed during the manufacture of comminuted meat products has been sought for many years. Hansen (1960) suggested that the formation of an emulsion occurs during comminution with the soluble proteins and water as the continuous phase and fat as the discontinuous phase. Brown (1972) postulated that, rather than an emulsion, a protein-salt-water matrix is formed and is responsible for the stability of comminuted meat products. Current theory of comminuted meat systems recognizes the gelation of salt-soluble proteins as critical to their characteristic structure. The concept of these products as emulsions led to the investigation of the emulsifying capacities and emulsion stabilities of meat and meat proteins in various model systems (Hansen, 1960; Swift et al., 1961; Carpenter and Saffle, 1964; Hegarty et al., 1963; Saffle and Galbreath, 1964; Borton et al., 1968; Inklaar and Fortuin, 1969; Tsai et al., 1972). From these and other studies, many of the factors affecting the emulsifying capacity and stability of meats were determined. Protein concentration, comminution time, temperature, rate of oil addition, initial quantity of oil added and mixing rate affect emulsifying capacity as determined by Hansen (1960) and Swift et al. (1961). In addition, the stability of meat "emulsions" is influenced by the water-holding capacity of the meats, mechanical treatment, heat treatment, and levels of meat, fat, water, salt and nonmeat additives in the emulsion (Schut, 1976). Preblending, freezing and the use of prerigor meat affect emulsifying capacities and emulsion stability (Swift and Sulzbacher, 1963; Trautman, 1964; Acton and Saffle, 1969) as well.

Although many factors influence the functional properties of meat proteins in comminuted meat systems, singular numerical values, known as bind value constants, have been assigned to different meat types to estimate their functional performance. Least cost formulation of emulsion-type meat products has been guided for many years by established bind value constants for various meat ingredients. These constants were developed by several workers (Saffle, 1966; Anderson and Clifton, 1967; Kramlich et al., 1973; Porteus, 1979) based on emulsifying capacity, emulsion stability, soluble protein and/or protein content of the meat ingredients. Bind value constants have proven very useful in the formulatin of sausages in spite of the wide variety of processing conditions under which they are applied.

Though bind value constants have aided considerably in least cost production of emulsion-type sausages, these values are subject to a number of limitations. Brown (1972) suggested that approximately 30% of the bind value constants developed by Saffle (1966) are in error as to emulsion stability. In addition, the constants were determined for each meat ingredient with specific protein, water and fat compositions. A sausage manufacturer often has a particular ingredient available but its composition is not the same as that associated with the bind value "constant." In this case, either the obviously incorrect bind value constant is used or an attempt to estimate a more correct bind value constant is made. Another limitation of established bind value systems is that they do not include any values for a number of meat ingredients and contain no estimates of the functional contribution of nonmeat protein additives. Some ingredients commonly used in sausages such as mechanically deboned chicken and turkey, soy flours and concentrates and nonfat dry milk have not been assigned bind value constants.

The influence of protein content on the emulsifying capacity of meats has been considered in several studies. Acton and Saffle (1972) found a curvilinear relationship between emulsifying capacity (ml oil emulsified/g meat) and protein concentration of meats. Gillett et al. (1977), however, found that emulsification values (mL oil/25 mL protein extract) closely followed protein concentration for nine meat sources and suggested that determination of binding ability of meat may be unnecessary when soluble protein is known. The purpose of this work was to use existing bind value systems to determine equations that will predict the bind value constants of different types of meat from the protein or moisture content of these meats. In addition, the functionality of nonmeat ingredients was also investigated.

MATERIALS & METHODS

RAW MATERIALS for comminuted meat products were divided into four classes: Class I — striated, skeletal muscle meats; Class II — striated, nonskeletal muscle meats; Class III — organ and smooth muscle meats; Class IV — nonmeat protein additives (Table 1). Meat ingredients used to develop the respective bind value constant systems of Saffle (1966), Anderson and Clifton (1967), and Porteus (1979) were classified according to this scheme. The nonmeat proteins investigated by Comer (1979) comprised Class IV.

Within each of the three bind value systems, Class I meats were subjected to linear and polynomial regression using Minitab (Ryan, 1976) to find best fit equations with protein or moisture as the independent variable and bind value constant as the response variable. Linear and/or polynomial regression was also applied to Class II and Class III meats for the Saffle (1966) and Porteus (1979) systems. The Anderson and Clifton (1967) system was not subjected to regression analysis for these classes because the bind value constants in this system are not continuous values, and a very limited number of meats fall within these two classes. Emulsifying capacities (mL oil emulsified/g nonprotein additive) were calculated for Class IV, the nonmeat proteins, and were also subjected to regression analysis (emulsifying

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Table 1—Classes of sausage ingredients

Class I	*Class II*
Straited, skeletal muscle meats	Straited, nonskeletal muscle meats
Bull meat, 50/50 pork trimmings, mechanically deboned chicken, backfat, cheek meat, boneless picnic	Tongues, hearts, diaphragms, tongue trimmings
Class III	Class IV
Organ and smooth muscle meats	Nonmeat protein additives
Liver, tripe, brains, pork, stomachs, spleens, thymus	Soy flour, soy concentrate, wheat flour, sodium caseinate, milk powder

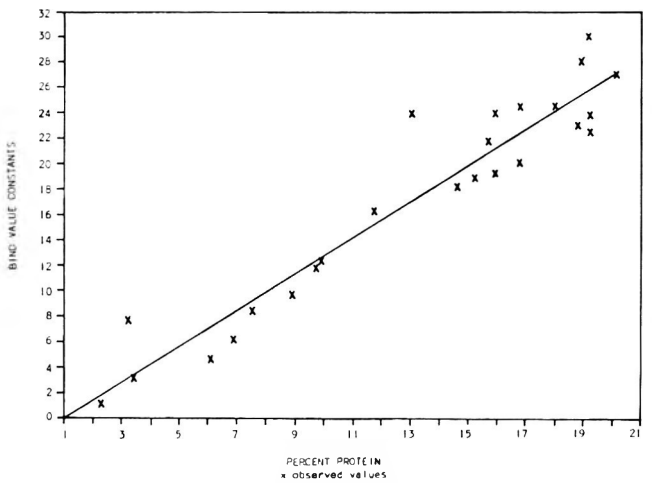

Fig. 1—Bind value constants of Class I meats based on protein. Saffle system.

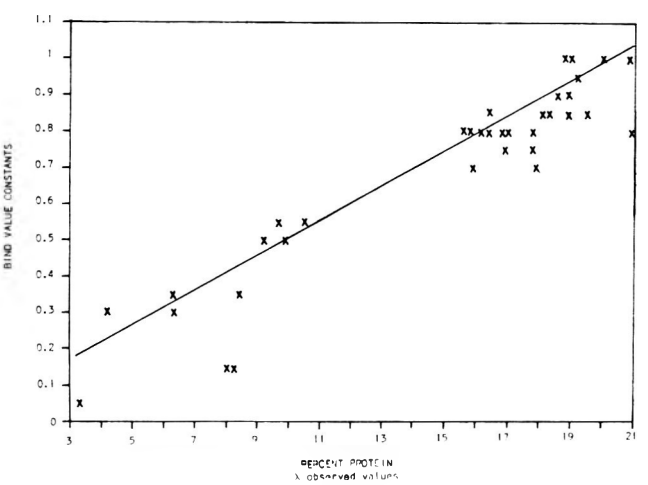

Fig. 2—Bind value constants of Class I meats based on protein. Anderson system.

capacity on total protein). Some bind value constants in each system were not included in the analysis due to discrepancies between the relative bind value constant reported in that system and those reported in other systems. In addition, 1% of the total protein for each meat item in the Porteus (1979) system was subtracted from the total protein. This was done because the sum of the moisture, fat, and protein in this system was often greater than 100% and the protein appeared to be approximately 1% higher than values usually reported for the meat items studied.

RESULTS & DISCUSSION

RELATIONSHIPS between bind value constants and protein for Class I meats in the Saffle (1966), Anderson and Clifton (1967), and Porteus (1979) systems are illustrated in Fig. 1, 2, and 3. The relationship between protein and the bind value constants was linear for the Saffle (1966) and Anderson and

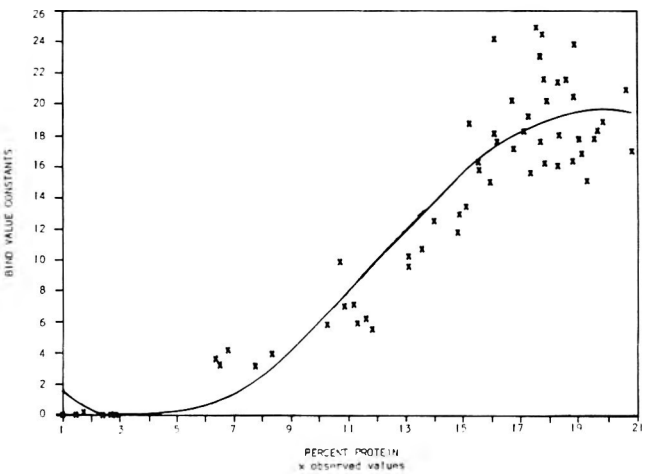

Fig. 3—Bind value constants of Class I meats based on protein. Porteus system.

Clifton (1967) systems and curvilinear for the data from the Porteus (1979) system. The regression equations, standard deviations, ranges, R^2 values, F values, and number of observations for each class under different systems for bind values as predicted by protein appear in Table 2. The R^2 values of Class I meats were 94.5, 89.0, and 87.9% for the Saffle, Anderson, and Porteus systems respectively, indicating highly significant ability of protein to predict bind values ($p < 0.001$). Gillett (1977) reported that oil volume emulsified (mL oil/25 mL protein extract) closely followed soluble protein concentration (mg/mL) regardless of protein source when studying the emulsifying capacity of nine different meat sources. This indicates that the correlation between soluble protein and total protein for Class I meats should be very high. The meat sources within this class must contain protein of a very homogeneous nature in terms of emulsifying capacity since emulsifying capacities and stabilities are the basis for establishing bind value constants. The protein within this class is derived from striated, skeletal muscle tissue which contains relatively constant concentrations of actin, myosin, actomyosin and other proteins; therefore, it is not surprising that soluble protein closely relates to total protein.

The close linear relationship between protein and bind values found for the Saffle and Anderson systems was not the best model for the Porteus system. The polynomial equation in Table 2 relating protein to bind value was a better fit for this system due to the greater range of bind value constants for Class I meats with higher protein. In fact, when compared to the other two systems, the Porteus system appeared to underestimate the binding ability of these higher protein meats which may explain why the linear fit of protein to bind values was not the best fit for this system.

Natural logarithmic transformations of both protein and bind value constants also showed a linear relationship between the two variables by regression analysis. The Porteus system had the highest R^2 value (92.0%) when logarithmetric transformation of the data was applied.

Table 3 shows the regression equations, standard deviations,

Table 2—Regression equations, standard deviations, ranges, F values, and R^2 values of Class I, II and III meats for bind values predicted by percent protein

Class	System	Regression equation	R^2	Std. dev. BVC[a]	BVCs range[a]	n	F Value
Class I	Saffle	Y[b] = −1.676 + 1.42264X[c]	94.5	2.12	0.01 to 30.01	26	409.97***
	Anderson-Clifton	Y = 0.0245 + 0.0484X	89.0	0.09	0 to 1.0	36	274.06***
	Porteus	Y = 3.475 − 2.276X + 0.35418X² − 0.010004X³	87.9	2.66	0.02 to 24.88	59	133.22***
Class II	Saffle	Y = 0.65436 + 0.79821X	78.2	2.44	7.03 to 15.95	4	7.18
	Porteus	Y = −5.94 + 1.1732X	62.6	2.07	7.98 to 13.94	4	3.35
Class III	Saffle	Y = 12.53 − 1.876X + 0.06967X²	76.7	0.59	0.01 to 2.12	6	6.68*
	Porteus	Y = 2.54 − 0.4913X + 0.02746X² − 0.000361X³	97.5	0.14	0.08 to 1.25	6	25.90**

[a] BVC is bind value constant
[b] Y = bind value constant
[c] X = percent protein
* $p < 0.10$
** $p < 0.05$
*** $p < 0.001$

Table 3—Regression equations, standard deviations, ranges and R^2 values of Class I, II and III meats for bind values predicted by percent moisture

Class	System	Regression equation	R^2	Std. Dev. BVC[a]	BVC Range	n	F Value
Class I	Saffle	−1.176 + 0.37369X	90.8	2.74	0.01 to 30.01	26	237.29***
	Anderson-Clifton	−0.01798 + 0.013000 9X	87.4	0.09	0 to 1.0	36	235.43***
	Porteus	8.609 − 1.1264X + 0.037603X² − 0.0002766X³	86.8	2.78	0.02 to 24.88	59	120.36***
Class II	Saffle	1.795 + 0.17658X	76.2	2.55	7.03 to 15.95	4	6.42
	Porteus	−8.65 + 0.3218X	57.9	2.20	7.98 to 13.94	4	2.72
Class III	Saffle	7.156 − 0.08365X	60.8	0.72	0.01 to 2.5	7	7.76**
	Porteus	−7.907 + 0.2796X − 0.002211X²	50.0	0.50	0.08 to 1.25	6	1.50

[a] BVC is bind value constant
[b] Y = bind value constant
[c] X = percent moisture
** $p < 0.05$
*** $p < 0.001$

ranges, R^2 values, F values, and number of observations for bind values as predicted by moisture for Classes I, II and III using the different bind value systems. Moisture and protein within Class I are very highly correlated, thus, the R^2 values of the regression equations closely follow those with protein as the predictor variable. Subjective estimation of bind value constants for meats of different composition from those for which constants have been experimentally determined can be avoided by use of the predictive equations established in this work. A bind value constant for mechanically deboned chicken from the Saffle system fit the regression equations for this system quite well (standardized residual = −0.11). This indicates that the regression equations developed may be successfully used to assign bind values to Class I meats for which bind value constants do not presently exist, including poultry meat.

Class II meats, striated, nonskeletal muscle meats, consisted of diaphragms, hearts, tongues and tongue trimmings. Because this class contained a very limited number of observations, good prediction equations relating protein or moisture to bind value were difficult to obtain. Tables 2 and 3 contain the regression equations for Class II meats as well as standard deviations, ranges, R^2 values, F values and the number of observations. Though R^2 values for this class are not significant, the standard deviations for each system are comparable to those of Class I meats. Difficulty in obtaining good prediction equations for this class was also limited by the discrepancies prevalent in the relative bind value constants assigned to these meats within and between the different systems. Beef and pork hearts are particularly difficult to characterize in terms of their binding capabilities based on information in the literature. The Saffle (1966) and Anderson and Clifton (1967) systems assigned intermediate bind value constants to beef and pork hearts. The Porteus (1979) system gives beef hearts a very low bind constant and pork hearts a very high bind constant. In the bind value system reported by Kramlich et al. (1973), fresh beef and pork hearts were given an intermediate bind value and frozen pork hearts assigned a very low bind value. Borton et al. (1968) found that heart muscle has good emulsifying capacity, but low emulsion stability which may explain the differences in the relative binding abilities reported under different systems. Hearts were not used in obtaining the regression equations for Class II meats, only diaphragm, tongue and tongue trimmings were utilized. It appears that the binding capabilities of Class II meats are still poorly defined and warrant further investigation.

Class III meats, organ and smooth muscle meats, were best defined by curvilinear relationships between protein and bind value constants (Tables 2 and 3). Moisture content was not as good a predictor variable as was protein. The correlation between protein and moisture for the various organs and smooth muscles within this class is evidently not very high. The greater variety of proteins present in different organs and smooth muscle as compared to striated, skeletal muscle might be expected to be associated with greater variation in percent moisture.

Meat proteins are recognized as performing two functions in the formation of a sausage emulsion. One function is to "emulsify" fat and the other is to bind water (Kramlich, 1971). Hamm (1973), however, suggests that it is the ability of muscle proteins to swell and bind water that results in a mechanical fixing of fat particles in the protein filament structure. The bind value constants for organ and smooth muscle meats are quite low and appear to be related to the ability of the meat to bind additional water. In the Saffle system, the bind value constants assigned to these Class III meats are more closely associated with the added water value for these meats than the

Table 4—Regression equation, standard deviation, R^2 value, and F value of Class IV ingredients for emulsifying capacities predicted by percent protein.

Regression equation	Std. dev.	R^2	F Value
$Y = -8.9769 + 5.5495X - 0.155IX^2 + 0.001278X^3$	23.01	94.1%	10.7*

* $p < 0.10$

percent protein or moisture content. Since the added water value is an indication of the capacity of a meat item to bind moisture in the final product, the very small bind value contribution of Class III meats probably results from water binding, not fat "fixing." Striated muscle meats, on the other hand, mechanically "fix" fat as well as bind water.

Class IV ingredients are the nonmeat proteins. Many nonmeat proteins have been evaluated for their performance in comminuted meat systems but bind values for these products have not been established. Present bind value systems are based largely on estimations of the emulsifying capacities of meat ingredients but emulsifying capacity is known to be quite limited in predicting the binding properties of nonmeat proteins in comminuted meat products. Smith et al. (1973) reported that solubility and emulsifying capacity of several nonmeat proteins had little effect on their abilities in stabilizing frankfurters. The limited value of using emulsifying capacities to predict the functionality of fillers is demonstrated by the work of Comer (1979) with six different fillers. Comer determined the emulsifying capacity of a soy concentrate, soy flour, sodium caseinate, potato starch, wheat flour and milk powder. When a multiple regression of emulsifying capacity (mL oil emulsified/g nonmeat protein) on total protein of these products was performed, an equation with a 94% correlation coefficient was obtained (Table 4). The functional contribution of these nonmeat proteins does not, however, follow the emulsifying capacity of the proteins. Johnson (1970) investigated soy sodium proteinate (approximately 90% protein) and sodium caseinate (approximately 90% protein) in heated water dispersions of the proteins and in frankfurters. The soy sodium proteinate produced a firmer gel in the water dispersion and produced less fat capping frankfurters. Johnson (1970) concluded that both of these nonmeat proteins may serve as emulsifying agents, but the soy sodium proteinate contributes much more to the gel structure of frankfurters than does sodium caseinate. Kinsella (1979) points out that the important functional properties of soy proteins in comminuted meat products involve water-holding, emulsion stabilization, and gellability. Clearly, if bind value constants for the nonmeat proteins are to be determined, emulsifying capacities of these proteins will play a minor role in their establishment.

CONCLUSIONS

THE REGRESSION EQUATIONS determined for Class I meats (striated, skeletal muscle meats) should prove useful in estimating bind value constants based on the moisture or protein content of these sausage ingredients. These equations should be particularly helpful in assigning bind value constants to Class I meats for which no bind value constants presently exist. Bind value constants for Class III Meats (organ and smooth muscle meats) within the Porteus system may also be predicted by protein content using the appropriate regression equation.

The classification system for sausage ingredients presented here may be a valuable aid in the development of a new means of estimating functional contributions of different meat and nonmeat ingredients in comminuted meat systems. The concept of using emulsifying capacities and/or emulsion stabilities to estimate functional performance (bind value constants) of meat ingredients has been used for the last twenty years. New concepts in estimating the functional contribution of meat and nonmeat items, those which emphasize water holding capacity and the gelation phenomenon associated with comminuted meat systems, are needed. If such concepts are applied and result in better defining ingredients used in sausage products, significant improvement in least cost formulation may be achieved.

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Weight Loss and Sensory Attributes of Temperature Conditioned and Electrically Stimulated Hot Processed Pork

J. O. REAGAN and K. O. HONIKEL

ABSTRACT

The effects of different electrical stimulation (ES) and conditioning systems on various attributes of postmortem pork muscle were investigated, using three hot boning systems (HB/11°C—5 hr; HB; 17°C—3 hr; HB/ES— 1hr) and two cold boning systems (CB/controls; CB/ES—24 hr). After each treatment, the cuts were vacuum packaged and chilled to 3°C. The loin, top ham, bottom ham and knuckle of each were evaluated. Only the knuckle was not affected by processing treatment. The three hot boning systems produced cuts with significantly lower purge values in two-thirds of the cuts. CB/ES—24 hr resulted in high levels of purge, high cook losses and low sensory ratings for juiciness. Hot processed cuts were either equal to or superior to the conventionally processed cuts in every trait evaluated.

INTRODUCTION

RAPID CHANGES in the world economy, coupled with the continuing rise in energy costs have created an acute awareness for new technology in the industrial world. The meat industry has felt the pressures of higher interest rates and energy costs in lower profit margins. A renewed interest in hot processing systems has been expressed by the meat industry as hot processing offers the possible advantages of greater processing efficiency, decreased inventory turnover time and reduced energy costs.

The revival of hot processing in the U.S. began several years ago with the development of whole hog sausage operations where packer sows were deboned immediately after slaughter and processed in bulk or link-type breakfast sausage. The move to hot processing in the production fresh meat cuts and cured meat items has been slow due to the problems associated with the production of these products (Reagan, 1983, 1984). Major problems such as decreased tenderness, cut distortion, increased levels of purge and the development of alternative chilling systems must be overcome if hot processing is to become a reality in the pork industry (Locker, 1960; West, 1984; Reagan, 1984; Honikel et al., 1981).

The purpose of this study was to determine the effects of electrical stimulation (ES) and conditioning treatments on various physical and sensory characteristics of postmortem pork muscle.

MATERIALS & METHODS

FIFTY-FOUR market weight hogs (100–110kg) were slaughtered using conventional methods, split in half and the sides were randomly assigned to one of five different postmortem treatments. These treatments were: (a) HB/11°/5 hr–muscle excision within 30 min postmortem, place in plastic bag, condition in 11°C water bath for 5 hr, vacuum package, then chill in glycol chiller; (b) HB/17°/3 hr–condition sides at 17°C for 3 hr, excise muscles, vacuum package, chill in glycol chiller; (c) HB/ES/1 hr–electrically stimulate (ES) within 10 min postmortem (pulse stimulation, 30 sec at 550V< 1.5A, 2 sec on, 1 sec off), excise muscles within 1 hr postmortem, vacuum package, condition at 0°C for 30 min, then chill in glycol chiller; (d) CB/ES/24 hr–ES same as previous treatment, chill sides at 7°C for 24 hr, excise muscles, vacuum package and chill in glycol chiller; (e) CB/control–chill sides at 7°C for 24 hr, excise muscles, vacuum package and chill in glycol chiller.

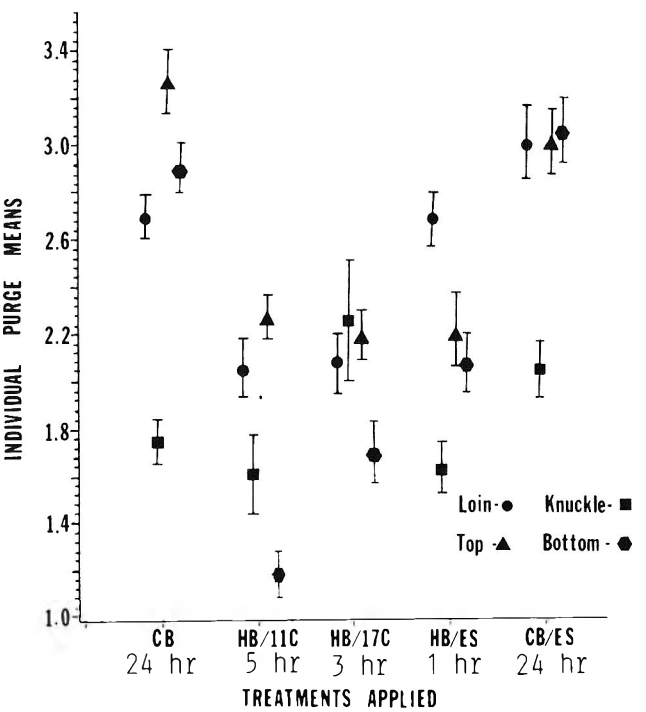

Fig. 1—Mean values (± 1 standard deviation) for percentage purge for individual cuts within processing treatments.

The four cuts obtained from each side were the loin, knuckle (ham) and top and bottom ham muscles.

Following excision, these cuts were placed in Barrier Bags® (Cryovac) and vacuum packaged using a Multivac AG500 with a vacuum setting of 5.5 which provided a vacuum of 760 Torre. The bags were then heat shrunk (3 sec at 90°C water bath) before chilling. All cuts were chilled in a glycol chiller at −3°C until an internal temperature of +3°C was attained. Cuts from conventionally chilled carcasses (CB/controls) were glycol chilled 1–1½ hr while those from hot processed carcasses were glycol chilled 2–2½ hr. Following the glycol chilling, the cuts were placed in cardboard boxes and stored at 0°C for 21 days.

At the end of each storage period, values for percentage purge (natural juices which often appear in vacuum packaged meat) were obtained for each cut and the boneless loins were placed in a −30°C freezer for approximately one month prior to cutting into chops, 2.54 cm thick. Chops from each loin were analyzed for protein, fat and moisture (AOAC, 1980) and subjected to shear force, thaw loss and cook loss determinations. Prior to shear determinations and sensory evaluations, the chops were thawed at 0°C for 24 hr before cooking on open-faced Farberware grills to an internal temperature of 68°C. Sensory chops were sliced into 1.25 cm cubes and served as warm as possible to the panel. Loin chops were also evaluated by an eight member trained sensory panel for flavor, juiciness, tenderness and desirability using eight point rating scales (8 = extremely acceptable, juicy and tender; 1 = extremely unacceptable, dry and tough).

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Table 1—Mean values for muscle pH and internal temperature (°C) at the time of vacuum packaging of primal and subprimal pork cuts from carcasses subjected to different postmortem treatments

		Loin		Ham subprimals					
				Knuckle		Top		Bottom	
Treatment	n	T	pH	T	pH	T	pH	T	pH
HB/11°C-5 h	18	12.9[c]	5.8[b]	12.0[c]	6.1[c]	12.4[c]	6.0[d]	12.6[c]	6.0[c]
HB/17°C-3 h	18	21.7[b]	5.6[ac]	26.8[b]	5.7[a]	25.9[b]	5.7[ab]	29.8[b]	5.7[ab]
CB/Controls	36	10.5[d]	5.5[a]	11.1[d]	5.7[a]	11.6[d]	5.6[a]	10.9[d]	5.7[a]
HB/ES-1 h	18	30.9[a]	5.7[b]	31.6[a]	5.9[b]	32.5[a]	5.8[bc]	33.4[a]	5.8[b]
CB/ES-24 h	18	10.0[d]	5.6[ab]	11.1[d]	5.8[ab]	11.1[d]	5.7[bc]	11.6[d]	5.7[ab]

[a,b,c,d] Mean values in the same column bearing unlike superscripts differ significantly ($P < 0.05$).

Table 2—Mean values for percentage purge of primal and subprimal pork cuts from carcasses subjected to different postmortem treatments

			Ham subprimals		
Treatment	n	Loin	Knuckle	Top	Bottom
HB/11°C—5 hr	18	2.1[a]	1.6[a]	2.3[b]	1.2[c]
HB/17°C—3 hr	18	2.1[a]	2.3[a]	2.1[b]	1.7[bc]
CB/Control	36	2.7[ab]	2.0[a]	3.3[a]	2.8[a]
HB/ES—1 hr	18	2.7[ab]	1.6[a]	2.2[b]	2.1[b]
CB/ES—24 hr	18	3.0[b]	2.1[a]	3.0[ab]	3.1[a]

[a,b,c] Mean values in the same column bearing unlike superscripts differ significantly ($P < 0.05$).

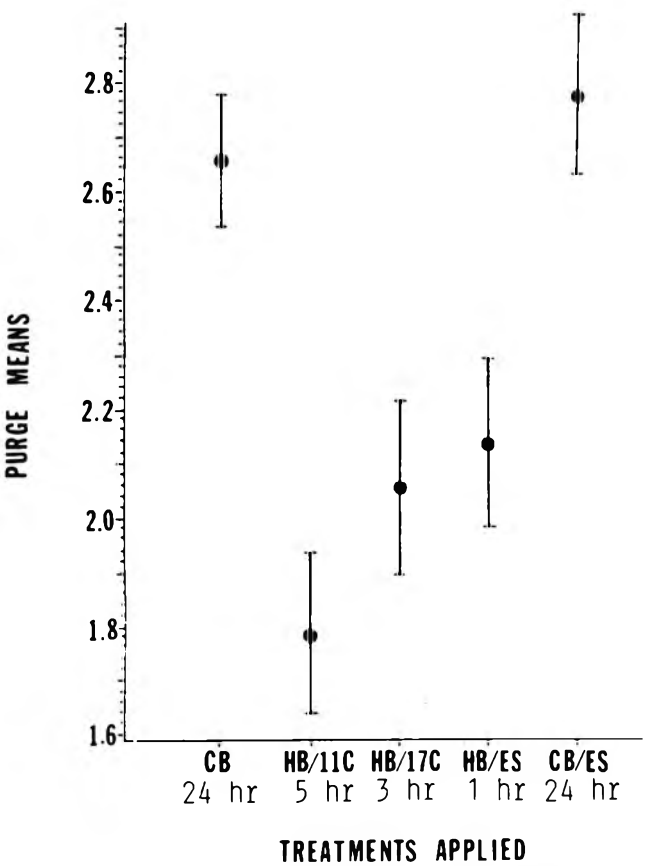

Fig. 2—Overall mean values (± 1 standard deviation) for percentage purge within processing treatments.

pH and Temperature

Muscle pH and temperature were obtained at the time of vacuum packaging and upon completion of each storage period. Values for pH were determined using a Corning Model 610A portable pH-meter with an Ingold Model 616 puncture type electrode. Muscle temperature was measured using a CYSI, Model 42SC Telethermometer with a No. 418 probe.

Table 3—Mean values for sensory panel ratings of loin chops obtained from carcases subjected to different postmortem treatments

Treartment	n	Juiciness[a]	Flavor[a]	Tenderness[a]	Overall desirability[a]
HB/11°C—5 hr	18	5.5[b]	5.5[b]	5.2[b]	5.5[b]
HB/17°C—3 hr	18	5.3[bc]	5.8[b]	5.1[b]	5.5[b]
CB/Control	36	5.5[b]	5.8[b]	5.5[b]	5.6[b]
HB/ES—1 hr	18	5.1[bc]	5.9[b]	5.2[b]	5.4[b]
CB/ES—24 hr	18	4.9[c]	5.6[b]	5.3[b]	5.2[b]

[a] Means based on 8-point rating scale (8 = extremely juicy, desirable or tender; 1 = extremely dry, undesirable, tough).

[b,c] Mean values in the same column bearing unlike superscripts differ significantly ($P < 0.05$).

Table 4—Mean values for various physical properties of pork loin chops obtained from carcasses subjected to different postmortem treatments

Treatment	n	Thaw loss (%)	Cook loss (%)	Total loss[a] (%)	Shear values (kg)
HB/11°C—5 hr	18	8.7[b]	23.1[b]	33.9[b]	2.7[b]
HB/17°C—3 hr	18	7.8[b]	22.5[b]	32.4[b]	2.6[b]
CB/Control	36	8.2[b]	23.9[b]	34.6[b]	2.8[b]
HB/ES—1 hr	18	6.9[c]	26.2[b]	35.7[b]	2.8[b]
CB/ES—24 hr	18	6.6[c]	25.9[b]	35.5[b]	2.7[b]

[a] Total loss = thaw loss + cook loss + purge loss.

[b,c] Mean values in the same column bearing unlike superscripts differ significantly ($P < 0.05$)

Shear force determination

Shear force values were obtained from two cores (1.27 cm in diameter) from each of the two chops from each loin. Peak shear force (kg) was measured using an Instron Universal Testing Instrument (Model #1009) with a Warner-Bratzler shear attachment. Values reported are the average of four independent measurements.

Statistical analysis

Analysis of variance was performed according to the Statistical Analysis System of Barr and Goodnight (1972). Duncan's Multiple Range Test (Steel and Torrie, 1960) was used to test for differences between treatments. Significant differences were accepted at the 5% level of probability.

RESULTS & DISCUSSION

MEAN VALUES for internal muscle temperature and muscle pH at the time of vacuum packaging stratified by postmortem treatment are reported in Table 1. In general, cuts from the HB/11°C—5 hr treatment exhibited the highest numerical pH values and in three of the four cuts observed, these values were significantly higher. Muscle pH values associated with the HB/17°C—3 hr, CB/controls and CB/ES—24 hr treatments were similar in all four cuts. The HB/ES — 1 hr treatment appears to be an effective method of rapidly lowering the muscle pH as indicated by the low pH values for all muscles within 1 hr postmortem ($pH < 6.0$).

Mean values for percentage purge of primal and subprimal cuts are reported in Table 2. Of the four cuts evaluated, three were significantly influenced by postmortem treatment. Cuts from the HB/11°C—5 hr, HB/17°C—3 hr and HB/ES—1 hr treatments exhibited mean purge values which were signifi-

cantly lower than the control samples in two of the three cuts influenced by postmortem treatment. These findings indicate that vacuum packaged pork cuts with minmal amounts of purge can be produced by hot boning systems. Honikel et al. (1984) reported that the development of rigor in porcine muscle occurs at pH 5.9. The latter study implies that muscles could be rapidly chilled at this point of the rigor process without the occurrence of excess muscle shortening which has been related to higher levels of purge in vacuum packaged meat. It seems feasible that the hot boning systems in the present study could have been even more effective if earlier boning times would have been used as most pH values were lower than 5.9 (Table 1) at the time of deboning.

In general, the CB/ES–24 hr system produced either the highest, or next to the highest purge level in each of the four cuts. The higher levels of purge for this treatment would be expected since the ES treatment lowered the muscle pH very rapidly within 1 hr postmortem (Table 1). Reagan (1983) and Honikel et al. (1984) reported that a rapid drop in muscle pH coupled with a high internal temperature would result in elevated purge losses. A comparison of the HB/ES—1 hr and CB/ES–24 hr treatments shows the importance of the relationship of temperature and pH to percentage purge in vacuum packaged pork. The HB/ES—1 hr cuts were chilled to 3°C within 4 hr postmortem while the CB/ES—24 hr cuts were slow chilled to ca. 11°C within 24 hr. then rapidly chilled to 3°C. The rapid chilling of the HB/ES—1 hr cuts produced mean purge values which were 0.3 to 1.0% lower than the CB/ES—24 hr cuts.

These findings suggest that pork quality may be improved by increasing the rate at which carcasses are chilled. Rapid chilling would especially be of importance to packers that produce high levels of low quality (PSE) pork carcasses. A similar suggestion was made by Borchert and Briskey (1964).

Variations in percentage purge among the different cuts within postmortem treatments are presented in Fig. 1. No single postmortem treatment consistently produced the lowest values for percentage purge in every cut evaluated which implies that cuts react differently to the various treatments. Of the systems evaluated, the CB/control system exhibited the greatest variation in purge level among cuts while the HB/17°C—3 hr exhibited the least amount of variation. There were distinct differences in overall purge between hot boned cuts (HB/11°C—5 hr, HB/17°C—3 hr, HB/ES—1 hr) and cold boned cuts (CB/control, CB/ES—24 hr) (Fig. 2). Hot boning systems produced cuts with lesser amounts of purge in the package which would imply that from an appearance standpoint, wholesale cuts produced by these systems would be of greater merchandising value in the retail case.

Mean values for sensory traits of loin chops from loins subjected to different postmortem treatments are presented in Table 3. Loin chops produced by the CB/ES–24 hr treatment were rated as being less juicy ($P < 0.05$) than chops from the CB/control and HB/11°C—5 hr treatments. Reagan et al. (1984) also reported that the combination of ES and cold boning resulted in lower sensory ratings for juiciness in the M. longissimus. Flavor, tenderness and desirability were not affected by postmortem treatment in the present study. The mean values (Table 3) indicate (with the exception of the CB/ES—24 hr treatment) that the postmortem treatments produced loin chops which were acceptable (ratings > 5.0) in juiciness, flavor, tenderness and overall desirability.

Means for percentage thaw, cook and total loss are reported in Table 4. HB/ES—1 hr and CB/ES—24 hr chops exhibited significantly lower thaw loss values. However, no significant differences were observed for cook loss or total loss due to postmortem treatment. Honikel et al. (1981, 1984) showed that cooking loss is dependent upon final pH. Since the mean values for the M. longissimus ranged from 5.6–5.8, no significant differences would be expected. Shear values reported in Table 4 indicate no significant differences due to postmortem treatments which is in agreement with the sensory panel tenderness ratings.

CONCLUSIONS

HOT BONING SYSTEMS (HB/11°C—5 hr. HB/17°C—3 hr. HB/ES—1 hr) produced cuts which exhibited lower levels of purge than control treatment samples in two of the three cuts affected by postmortem treatment. Electrical stimulation coupled with slow chilling (CB/ES–24 hr) produced vacuum packaged wholesale cuts with high levels of purge and retail cuts which exhibited high cooking losses and low sensory ratings for juiciness. Overall, cuts from the hot processed carcasses employing either conditioning or ES/rapid chill were either equal to or superior to cuts from conventionally processed systems in every trait evaluated.

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Improved Cooking Yields of Meat Batters Formulated with Potassium Sorbate and Reduced Levels of NaCl

JOHN N. SOFOS

ABSTRACT

The influence of varying NaCl levels (0.8, 1.2, 1.6, 2.0, and 2.4%) on the cooking yield and composition of comminuted meat (beef-pork) products (frankfurters and cans) was tested in the absence and presence of 0.26% potassium sorbate. Parameters measured included frankfurter smokehouse yields and consumer cook losses; product losses in cans; and, moisture and fat contents of the cooked products. Reducing the NaCl level resulted in increased product losses during cooking. Potassium sorbate decreased these losses. Cooking yields at most NaCl levels ($\leq$ 1.6%) tested were improved ($P<0.01$) with the addition of potassium sorbate. The positive influence of sorbate on product binding could not be attributed to pH or ionic strength changes, but potential mechanisms of sorbate action are discussed.

INTRODUCTION

SODIUM CHLORIDE in meat products serves as a flavoring and antimicrobial agent and also influences functionality of muscle proteins (Sofos, 1984). Manufacture of comminuted meat products involves mechanical action on the meat in the presence of NaCl which extracts and solubilizes myofibrillar proteins. These proteins surround fat and other ingredients and form a matrix which stabilizes product structure and results in acceptable yield and quality (Schmidt et al., 1981). Presence of insufficient amounts of NaCl results in reduced protein extraction which decreases water holding and fat binding and gives products of unacceptable yield, texture and flavor. The preservative capacity of these products is also decreased (Hauschild, 1982; Sofos, 1983a, b, 1984; Madril, 1984; Madril and Sofos, 1984; Whiting, 1984a, b; Whiting et al., 1984; Trout and Schmidt, 1984).

Consumption of NaCl, however, is considered a major source of Na^+ in the human diet. Since Na^+ intakes have been implicated in the development of human hypertension, various health and regulatory authorities have indicated that there is a need to reduce NaCl levels in processed foods. Amounts of NaCl used in the processing of various foods, however, should be reduced without compromising their quality, stability, shelf-life and safety.

Potassium sorbate and sorbic acid are well established antimicrobial agents used throughout the world for the preservation of various products including foods (Lueck, 1976, 1980; Sofos et al., 1979; Sofos and Busta, 1981, 1983). In general, sorbates have been accepted as effective inhibitors of yeasts and molds. In recent years, however, sorbates have been found effective against various bacteria of importance to foods. The level of 0.26% potassium sorbate has been proposed as an antimicrobial alternative to presently used levels of nitrite in cured meats (Sofos and Busta, 1981; Robach and Sofos, 1982). Certain studies have also shown that the antimicrobial activity of sorbates is enhanced by other food additives, including NaCl, polyphosphates, acids and antioxidants (Robach and Stateler, 1980; Davidson et al., 1981; Restaino et al., 1981; Nelson et al., 1983). Sofos (1985) also observed improved antimicrobial activity of low NaCl fonnulations combined with potassium sorbate in uncured poultry products.

The recent study by Sofos (1985) with uncured poultry products also indicated that potassium sorbate (0.26%) significantly reduced cooking losses (% wt) of uncured turkey products formulated with 0.65% NaCl. This unexpected observation was the first indication that sorbate may be influencing the functional properties of meat products, in addition to its effects as an antimicrobial agent.

Thus, the objective of this study was to determine any potential influence of potassium sorbate in improving yields during thermal processing of comminuted meat products formulated with varying levels of NaCl.

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MATERIALS & METHODS

Ingredients

Fresh (<5 days postmortem), lean (5% fat) bull meat and fresh pork trimmings (55% fat) obtained from local processors were ground twice through a 0.95 cm plate and refrigerated overnight (2°C). Each treatment was formulated with approximately equal amounts of meat from each source in order to achieve 30% fat in the total formulation. The pH of the raw meats was 5.50–5.65 for the bull meat and 6.05–6.20 for the pork trimmings.

Other common ingredients in each treatment were water (8%), ice (8%), dextrose (0.5%), corn syrup solids (0.5%), white pepper (0.25%), nutmeg (0.0625%), sodium erythorbate (0.03%) and sodium nitrite (0.01%).

Treatments

Five levels (0.8, 1.2, 1.6, 2.0 and 2.4%) of granular NaCl (Diamond Crystal Salt Company, St. Clair, MI) were tested in the absence and presence of 0.26% potassium sorbate (Monsanto Company, St. Louis, MO). Amounts of NaCl and potassium sorbate added were based on the total meat weight of the batch (5 kg). The experiment was replicated three times.

Processing

All the ingredients, with the exception of the pork trimmings, were first mixed and chopped in a Meissner model VE bowl chopper (RMF Steel, Kansas City, MO) at intermediate bowl speed and 4,000 rpm blade (six blades) speed for a constant time of 20 bowl revolutions. The product temperature after this chopping was 2–4°C. The pork trimmings were then added and the mixture was comminuted at high bowl speed and 4,000 rpm blade speed to a constant end-point temperature of 12.8°C. Bowl revolutions for this chopping were in the range of 48–55.

After chopping, part of each batter was extruded with an E-Z Pak water powered piston stuffer into 24 mm diameter cellulose casings (Union Carbide, Chicago, IL). Another part of the raw batter was extruded into five (per treatment) cans (208 × 108) provided by the American Can Company (Chicago, IL). The frankfurters were heat processed and smoked to a final internal temperature of 70°C in a Vortron smokehouse (Sofos, 1983a). The cans were sealed with a Dixie can closing machine and heat processed to an internal temperature of 70°C in an open air, agitated and thermostatically controlled retort (Dixie Equipment Company, Athens, GA) serving as a water bath at 75°C. The internal temperature of the cans was monitored with

thermocouples (O. F. Ecklund, Inc., Cape Coral, FL) placed in the center of two cans prepared in a manner similar to treatment cans. After thermal processing, cans and frankfurters were cooled with tap water and stored (4°C) overnight until tested on the following day.

Testing

Yield. Weight losses during thermal processing of frankfurters were determined by weighing the raw batters in the casings before thermal processing and the cooked frankfurters after thermal processing. Cooked frankfurter weight was determined after peeling and removal of the separated fat and gel from the surface of the frankfurters. The peeled casings were also washed, dried and weighed together with the peeled frankfurters, because the initial raw weight included the weight of the casings. Frankfurter weight after thermal processing was expressed as a percentage of the raw batter and reported as percent yield. This procedure was followed to avoid misleading results, especially in the low binding treatments. A major portion of the fat and other material that separates during thermal processing of the batters remains entrapped on the frankfurter surface in the form of fat caps and jelly pockets.

Product yields in cans were expressed as percent of raw batter weight. In addition, the volumes of separated material and fat in the cans were measured and expressed as milliliters of total (including fat) material separated and milliliters of fat released per 100g of raw batter. The weight of the raw batter in each can was known (95g). For the determination of losses the cans (4 per treatment) were warmed up by immersing in 50°C water for 10 min to melt separated fat and other material inside the can. Each can was then opened and its contents poured on a funnel which was placed in a graduated cylinder or centrifuge tube. The cooked products was left on the funnel to drain and cool to room temperature (23°C) for 15 min. During this time the warm fat rose to the top of the remaining material in the graduated cylinder. The drained product was then weighed and the volumes of total separated material and fat were measured.

Consumer cook loss. Weight losses of frankfurters during simulated consumer cooking were determined by immersing samples of approximately 100g (4 per treatment) in boiling water for 2 min. The heated frankfurters were then placed on paper towels to drain and cool to room temperature for 15 min, weighed and the difference in weight was expressed as percent consumer cook loss. This test was performed after the separated fat was removed from the surface of the frankfurters. Without removal of surface fat and jelly pockets, the consumer cook test might have shown more dramatic reductions in product quality with low NaCl levels. The separated material was intentionally removed, however, because the products would not be sold with separated fat on their surface. In addition, peeling results in accidental removal of some of the separated material and the amount removed can be variable among samples and treatments.

Fat, moisture and pH. Moisture and fat of cooked frankfurters and canned product were analyzed according to standard AOAC (1975) procedures. Raw meat, batter, and cooked product (4 samples per treatment) pH values were determined on a blend of 10g of sample and 90 mL of distilled water with a Corning pH meter.

Statistical analysis

The experimental design was a complete 5 (salt levels) × 2 (sorbate levels) factorial with three replicates. The data were analyzed by analysis of variance and when the F value was significant the least significant difference was determined for means (Snedecor and Cochran, 1971).

RESULTS

STATISTICAL ANALYSIS of the various cook yield data indicated highly significant ($P<0.01$) NaCl, sorbate and NaCl × sorbate interaction effects. In certain instances, the replicate effect (meat source) was also significant ($P<0.05$).

Effect of NaCl

Reduction of the NaCl level, by 0.4% increments, from 2.4% to 0.8%, resulted in ($P<0.01$) lower cook yields (Fig. 1-5). Product losses in frankfurters and canned batters increased ($P<0.01$) with decreasing NaCl levels. The losses were especially high (exceeded 25% of the initial raw weight) when the NaCl level was reduced to ≤1.2%. These results are in agreement with previous findings (Sofos, 1983a; Whiting, 1984a, 1984b) and confirm conclusions that NaCl levels below 1.5–2.0% (depending on product pH) result in comminuted meat products of low binding capacity and reduced yields.

Effect of sorbate

Frankfurter and canned batter yields and consumer cook losses clearly indicated that potassium sorbate reduced losses during thermal processing (Fig. 1–5). This conclusion is supported by all data collected, including frankfurter smokehouse yields, consumer cook losses, canned product yields, and total material separated and fat released during thermal processing of canned batters. Sorbate improved yields significantly in all tests performed at the 1.2% and 1.6% NaCl levels. In certain instances, the positive effect of sorbate on yield was also significant at the 2.0% and 0.8% NaCl levels (Fig. 1–5).

The influence of sorbate in improving cooking yields can be of economic significance at both regular and reduced NaCl levels. Frankfurter yields in the absence of sorbate were 66.4%, 68.7%, 81.9%, 85.2%, and 88.6% for the 0.8%, 1.2%, 1.6%, 2.0%, and 2.4% NaCl levels, respectively. With potassium sorbate in the formulation the corresponding yields were 67.3%, 76.4%, 86.8%, 89.1%, and 91.1%.

The improvement in yield with potassium sorbate was even more dramatic in batters cooked in cans. The cooking yields for the no-sorbate treatments from low to high NaCl level were 65.6%, 70.8%, 85.4%, 88.9% and 91.8%. Potassium sorbate increased these yields to 68.8%, 82.2%, 91.0%, 92.4%, and 94.7%, respectively. Thus, potassium sorbate may improve cooking yields of comminuted meat products formulated with regular NaCl levels, and it can be valuable in decreasing cooking losses of reduced NaCl products. Furthermore, sorbate improves microbial shelf-life of various meat products (Sofos and Busta, 1981). These properties make sorbate a potentially multifunctional ingredient, similar to NaCl and sodium nitrite.

Table 1—Moisture and fat of cooked comminuted frankfurters and cooked/drained cans formulated with varying NaCl levels in the presence and absence of potassium sorbate (0.26%)

	NaCl (%)										
	Sorbate (0%)					Sorbate (0.26%)					LSD[a]
Parameter	0.8	1.2	1.6	2.0	2.4	0.8	1.2	1.6	2.0	2.4	(<0.01)
					Frankfurters						
Moisture (%)	56.72	51.03	51.90	53.27	53.79	54.46	53.15	53.62	53.99	54.39	1.53
Fat (%)	20.09	26.00	28.07	28.89	29.14	23.63	27.98	28.83	29.19	29.38	1.15
Total	76.81	77.03	79.97	82.16	82.93	78.09	81.13	82.45	83.18	83.77	
					Canned Frankfurter Batters						
Moisture (%)	59.41	57.48	56.64	57.87	58.16	58.99	57.51	56.99	58.32	58.46	1.08
Fat (%)	17.82	22.73	25.12	25.19	25.45	20.44	24.88	25.42	25.56	25.74	1.55
Total	77.23	78.21	81.76	83.06	83.61	79.43	82.39	82.41	83.88	84.20	

[a] LSD, least significant difference (three replicate experiments).

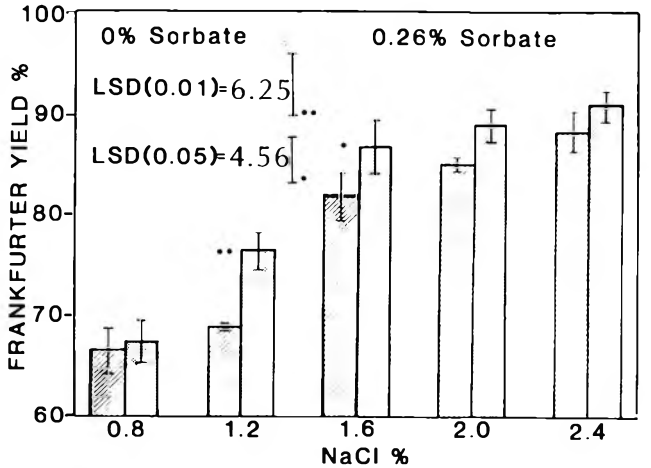

Fig. 1—Yield of frankfurters cooked to 70°C in the smokehouse (LSD, least significant difference; asterisks indicate statistical significance within a pair of treatments; bars indicate averages of three replicates ± SEM).

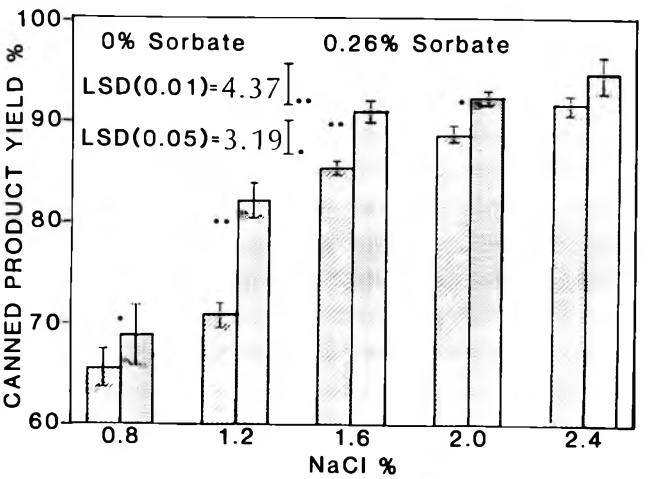

Fig. 3.—Yield of meat batters cooked to 70°C in 208 × 108 cans (LSD, least significant difference; asterisks indicate statistical significance within a pair of treatments; bars indicate averages of three replicates ± SEM).

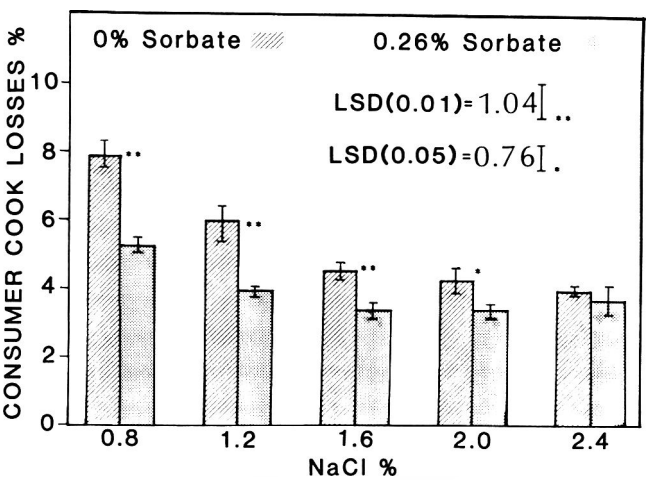

Fig. 2—Weight losses of frankfurters during simulated consumer-type boiling (LSD, least significant difference; asterisks indicate statistical significance within a pair of treatments; bars indicate averages of three replicates ± SEM).

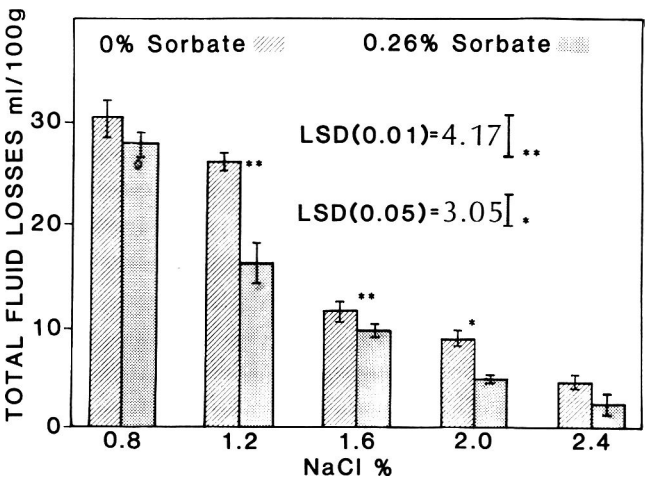

Fig. 4—Total material separated from meat batters cooked to 70°C in 208 × 108 cans (LSD, least significant difference; asterisks indicate statistical significance within a pair of treatments; bars indicate averages of three replicates ± SEM).

Effect on moisture, fat and pH

Fat and moisture contents (Table 1) reflect the binding stability of the various treatments. The reduced binding with lower NaCl levels was reflected by the increased fat losses during thermal processing. The higher ($P<0.01$) fat levels in low NaCl treatments (≤1.2% NaCl), formulated with sorbate, compared to treatments with the same NaCl levels, but without sorbate, confirmed the conclusion that sorbate improved binding. Moisture decreased as NaCl was reduced to 1.2% from 2.4% and the frankfurter batters became less stable. At 0.8% NaCl, however, greater losses of product solids resulted in higher moistures and lower fats.

Raw batters were formulated to contain 30% fat and as the results demonstrated stable frankfurter treatments (2.4% NaCl) were very close to 30% fat. The lower fat levels (Table 1) in the canned products, even at 2.4% NaCl, compared to frankfurters, reflect less overall losses in cans due to lack of product dehydration during thermal processing.

Raw batter pH values were in the range 5.80–5.88, but in cooked products, the pH values were increased by 0.13–0.22 in frankfurters (5.98–6.06) and by 0.18–0.25 in the canned (6.01–6.11) product (Table 2). Even though raw batter pH tended to be slightly lower with lesser amounts of NaCl and slightly higher with potassium sorbate in the formulation, these pH differences were very small (≤0.08 pH units). Although not proven, it is doubtful that these pH differences were influential in product binding. Potassium sorbate was tested instead of sorbic acid to avoid major effects on product pH which might influence binding (Sofos, 1981).

DISCUSSION

AS FAR AS could be determined, this is the first publication indicating that potassium sorbate can improve cooking yields of processed meat products. Previous work with sorbate in meat products has examined its inhibitory properties against spoilage and pathogenic microorganisms (Robach and Sofos, 1982). This experiment was repeated three times and results from all three replicates clearly demonstrated that potassium sorbate reduced losses during thermal processing of comminuted meat products. The results confirm the previous observation of improved yield with potassium sorbate in a low NaCl turkey product, which was the reason for initiating this study (Sofos, 1985).

Since the mechanism through which sorbate may improve

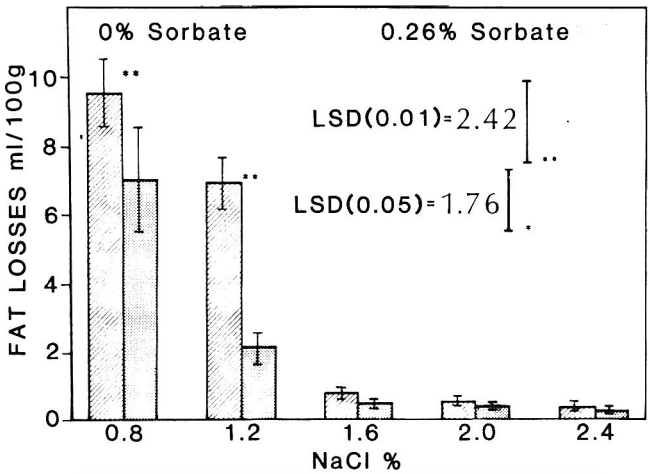

Fig. 5—Fat separated from meat batters cooked to 70°C in 208 × 108 cans (LSD, least significant difference; asterisks indicate statistical significance within a pair of treatments; bars indicate averages of three replicates ± SEM).

yields in meat products is unknown, a discussion of possible modes of action appears pertinent. The main factors that influence the binding ability of muscle proteins in processed meat products are pH, ionic strength and the addition of salts such as NaCl and polyphosphates, which also influence pH and ionic strength. The influence of pH and certain ions is due to changes in the electrical charges of the muscle proteins (Hamm, 1960, 1970).

Hamm (1970) also reported that the effect of polyphosphates on meat hydration is due to at least three factors including their effect on pH, ionic strength and some specific effects of phosphates which are due to interactions between the phosphate anion and myofibrillar proteins. These specific phosphate effects may include dissociation of actomyosin to actin and myosin; sequestering of protein-bound alkaline-earth ions (e.g., calcium, magnesium); and, a so-called specific polyphosphate effect through binding to meat proteins (Hamm, 1970). There are, however, some reservations relative to the importance of these effects in practical situations (Hamm, 1970; Trout, 1984; Trout and Schmidt, 1984). Trout (1984) found that ionic strength explained 53.5–59.4% of the variation in cook yield of beef rolls; pH accounted for a 24.7–30.5% of the variation; and polyphosphates explained 4.7–8.9%. It was suggested that this specific phosphate effect was likely due to the phosphate molecule affecting protein conformation and functionality (Trout, 1984).

The most influential factors in improving water-holding capacity and binding of muscle proteins are ionic strength and pH (Hamm, 1960, 1970; Trout and Schmidt, 1984). As the data of Table 2 indicate, however, sorbate resulted in only minor changes in product pH. The differences in raw batter pH between sorbate and no-sorbate treatments appeared to be too small (≤0.08) to explain the higher cook yields in treatments containing sorbate. Additional research, however, is necessary to test this assumption.

The maximum potential contribution of 0.26% potassium sorbate (which has a pKa value of 4.75) to the total ionic strength of each treatment could be only 0.017 (ionic strength calculations are based on the total formulation). The ionic strength contributions by the various NaCl levels (0.8–2.4%) were much higher (0.12–0.40). In the absence of potassium sorbate, treatments with 0.8, 1.2, 1.6, 2.0, and 2.4% NaCl had added ionic strengths of 0.12, 0.19, 0.26, 0.33, and 0.40, respectively. With potassium sorbate in the formulation these ionic strengths could become 0.14, 0.21, 0.28, 0.35, and 0.42, respectively. It is unknown whether such small increases in ionic strength by sorbate can cause major improvements in cook yield. Additional studies are needed to test whether changes in ionic strength by 0.02 will influence cook yield.

The influence of potassium sorbate on product binding may be due to the sorbate anion rather than to the addition of potassium cations. This assumption is based on results of other workers indicating that the swelling effect of NaCl in meat is primarily due to the chloride ion (Hamm and Grau, 1958; Hamm, 1960, 1970). In addition, it has been reported that the chloride salt of Na^+ is more effective in increasing hydration of muscle homogenates than the chloride salt of K^+ (Hamm, 1960). Furthermore, the amount of K^+ added with potassium sorbate was only minor (0.07%) compared to the amount of added Na^+. The amounts of Na^+ and $Na^+ + K^+$ added to each treatment as NaCl and NaCl + potassium sorbate were 0.31, 0.47, 0.62, 0.78, and 0.94% Na^+, and 0.38, 0.54, 0.69, 0.85, and 1.01% $Na^+ + K^+$, respectively. These differences are certainly small. Additional research is needed to determine whether such small increases in Na^+ and K^+ could be influential in cook yield.

Thus, it appears that any improvement of cook yield by the influence of potassium sorbate on pH, ionic strength and presence of potassium ions could be minimal. It is, therefore, likely that the improved yields observed may be due to presence of sorbate anions and their involvement in various reactions with meat proteins and other components of the product. Interactions of the sorbate ion with protein bound cations may be a reason for the results reported. This would be similar to the effect suggested by Hamm (1970) as a possible explanation for the influence of salts of weak acids on meat swelling. It was postulated that alkaline-earth ions may interlink certain protein structures by formation of intermolecular cross-linkages between peptide chains and thus, decrease swelling and water holding capacity of these proteins. The theory was used by Hamm (1960, 1970) to explain the observation that sodium salts of weak acids increased hydration of muscle homogenates in the order of lactate < tartrate < monophosphate < cyclotriphosphate < citrate < disphosphate < oxalate. Gluconate, glutamate, acetate and carbonate were almost ineffective (Hamm and Grau, 1958). Even though there is no clear evidence for the above mechanism proposed by Hamm (1960, 1970), the possibility exists that sorbate may be involved in such reactions.

Other potential explanations include possible reactions of sorbate with muscle proteins and rearrangement of their structure or dissociation of actomyosin to actin and myosin. Such reactions have been suggested as possible influences of certain polyphosphates in improving binding (Hamm, 1970; Siegel and Schmidt, 1979; Trout, 1984). Whether sorbate may have reacted with fat and improved its retention is also unknown.

Table 2—Influence of varying NaCl levels and potassium sorbate (0.26%) on the pH of raw batters, frankfurters and canned comminuted meat product

	NaCl (%)										
	Sorbate (0%)					Sorbate (0.26%)					LSD[a]
Product	0.8	1.2	1.6	2.0	2.4	0.8	1.2	1.6	2.0	2.4	(<0.01)
Raw batter	5.80	5.82	5.84	5.83	5.83	5.85	5.87	5.87	5.88	5.86	0.05
Frankfurters	5.98	5.99	6.00	6.01	6.05	6.01	6.01	6.03	6.04	6.06	0.05
Canned product	6.01	6.01	6.06	6.08	6.08	6.05	6.05	6.09	6.11	6.10	0.08

[a] LSD, least significant difference (three replicate experiments).

Also, interactions of sorbate with other non-meat ingredients in the formulation cannot be ruled out.

Since the influence of potassium sorbate on pH, ionic strength and addition of K^+ was only minor, any reaction of the sorbate ion with muscle proteins and/or other ingredients through its carboxyl group, double bonds or methyl group could be involved in improving cooking yields. Additional studies are necessary, however, to test the above proposed mechanisms and to determine the exact influence of sorbate in improving yields of processed meat products. Studies are also needed to determine similar effects with meat products of various types and composition, different sorbate types (e.g., sorbic acid) and concentrations, different pH levels, absence of NaCl, and in combination with other salts, including various polyphosphates.

Overall, the results have shown that potassium sorbate improved the cooking yields of comminuted meat products formulated with various levels of NaCl. Improved cooking yields at presently used NaCl levels can be of economic significance. Sorbate could also be valuable in facilitating reduction of NaCl levels through its influence on cooking yield and by its well established antimicrobial activity.

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Moisture Isotherms for Uncooked Meat Emulsions of Different Composition

G. S. MITTAL and W. R. USBORNE

ABSTRACT

The equilibrium moisture content of meat emulsions was obtained at different temperatures and fat-protein ratios. Various models were modified after considering the model constants as functions of product temperature and fat-protein ratio. These models were then fitted to predict the major portion of the isotherms. All models except one provided an adequate fit to the experimental data. The heat of desorption/sorption decreases with increase in moisture content and approaches a constant value.

INTRODUCTION

THE EQUILIBRIUM moisture content (EMC) of a material is important because of its relationship to storage, handling, and processing. The state of water in foods has a direct effect on their quality and stability through its effects on chemical and enzymatic reactions. For this reason, control and determination of water activity have been an area of concentrated research.

The hygroscopic isotherms of grains and other biological materials have been studied by many investigators, but little information is available on the moisture isotherms of meat emulsion products at different process temperature and product composition. Igbeka et al. (1976) determined the moisture isotherms of a cooked commercial bologna between 5 and 55°C. These moisture isotherms were sigmoidal in shape and were described by the Henderson equation with two pairs of constants. The heat of sorption decreased with the increase in moisture content upto a moisture content of 40% dry basis, and remained approximately constant with further increase in moisture content.

The objectives of this investigation were (1) to determine the equilibrium moisture content of meat emulsion at different temperatures and relative humidities and product composition, and (2) to find the suitability of existing models for predicting equilibrium moisture content as a function of temperature, relative humidity, and product composition.

Modelling

Table 1 comprises various original equilibrium moisture content models employed by various investigators to describe the water activity of cereal, vegetables, fruits, and other biological materials. These models were found most suitable, based on R^2 value, to describe the water activity of meat emulsions at different temperatures and compositions, out of many available models.

Henderson (1952) developed an empirical equation and found it to represent conventional equilibrium moisture data. Starting with a thermodynamic procedure, Chung and Pfost (1967) obtained heat and free energy changes of adsorption and desorption of corn hulls and its products. They also developed a general isotherm equation by adopting the framework of potential theory. Ferro-Fontan et al. (1982) developed a sorption equation, where A_{17} is a parameter which accounted for the 'structure' of sorbed water. When $A_{17} = 1$, this equation reduces to the Halsey's equation (Halsey, 1948). Many investigators (Iglesias and Chirife, 1976) showed that Halsey's equation described reasonably well, based on R^2 value, the sorption behavior of a large variety of foods. The equation of Ferro-Fontan et al. (1982) was able to describe the sorption isotherm of 18 different foods (oilseeds, starchy foods, proteins and others) in an extensive range of a_w (0.10–0.95) with only 2–4% average error in the predicted moisture contents (Chirife et al., 1983).

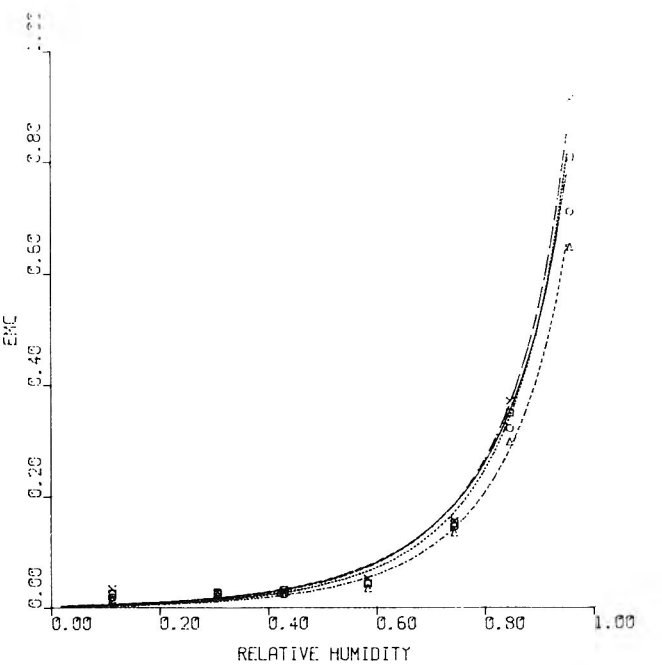

Fig. 1—Predicted and experimental values of the meat emulsion (EMC) at various temperatures and a FP of 2.56; △ ; 85°C; ○ ; 75°C; □ ; 65°C; and X; 55°C.

Konstance et al. (1983) developed an empirical expression to describe the moisture-a_w relationship, by the simple curvilinear regression analysis, of bacon products. The equation showed a good fit (R^2-0.91–0.98) for all bacon isotherms for $0.1 < a_w < 0.95$.

Harkins and Jura (1944) proposed an EMC equation based on the potential theory, i.e. the concept of a potential field existing above the surfaces of a solid. This equation has predicted grain moisture equilibrium isotherm satisfactory at RH values above 30% (Gustafson, 1972). Similarly, Smith (1947) developed the equation assuming the existance of two principal classes of sorbed moisture in polymers—bound and condensed moistures. The relationship between the bound moisture and the relative humidity was modelled by the Langmuir equation and the condensed moisture by the BET equation.

Boquet et al. (1978) applied the Oswin (1946) equation and several other isotherm equations including the Halsey and the Henderson equation on six different types of starch foods. They reported the Oswin (1946) equation to be the most suitable among the equations investigated.

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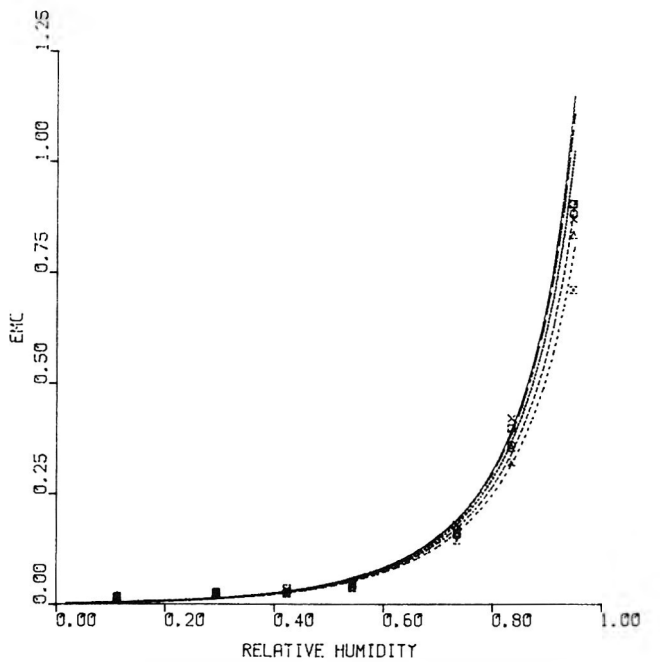

Fig. 2—Predicted and experimental values of the meat emulsion (EMC) at various fat-protein ratios and a temperature of 75°C: Σ; 2.56; △; 2.16; ○; 1.68; □; 1.30; and X; 1.10 fat protein ratios.

Chen (1971) developed a sorption isotherm equation based on the steady state of the drying equation. The analysis was limited to situations where diffusion is the principal mode of mass transport and where no chemical reaction occurs. Later on Chen and Clayton (1971) developed equation #10 on the basis of Chen (1971) equation, and modified the Hendersen equation into equation #9 after incorporating a temperature factor.

MATERIALS & METHODS

THE COMBINATIONS of four temperatures (55°, 65°, 75° and 85°C); five fat-protein ratios (1.10, 1.30, 1.68, 2.16 and 2.56), seven relative humidity values; and two replications were used in these experiments. Reported results are the average of these replications. However, actual data were used in statistical and regression analyses.

Fresh emulsions were prepared before starting each experiment in the laboratory using a small Hobart Chopper (Don Mills, Ontario; Model 84142). The formulation included nonfat dry milk (3%), salt (2.5%), sucrose (0.5%), corn syrup solids (2%), spices (black pepper, coriander, nutmeg, garlic powder and mace) and curing agents (sodium nitrite and erythorbate). Protein, fat and water were added according to the desired fat-protein ratio (FP). Most of the fat was taken from pork and protein from the lean beef. Meat was stored in the freezer at −17°C before use in making the emulsions.

Each 3–4g sample was placed in a 2 mm thick layer across the bottom of an aluminum foil basket, the basket was suspended into a wide mouth jar, which was put in a constant temperature cabinet controlled to within ±0.5°C. Under the above conditions an equilibrium period of 5–18 days was sufficient to establish moisture equilibrium. Each sample was weighed occasionally, the interval between weighing being short initially, and longer as the product approached a constant weight. For the purpose of this experiment, equilibrium was defined to have been reached when the weight change over three weighings, at one day intervals, was less than ±0.1g. All determinations of moisture content involved a standard oven drying method.

Wide mouths, 500 cm^3, glass jars were used as humidity control chambers. Weighing was done without removing the sample from the humidity chamber to obtain more accurate weights. During weighing, the jar temperature was maintained by keeping it in constant temperature environment. Furthermore, the atmosphere within the chamber

Table 1—Various equilibrium moisture content models

Reference	Basis & Assumptions	RH range	Model
1. Harkins & Jura (1944)	-concept of potential field above the surfaces of a solid -two dimensional gas theory	>0.30	$\ln(RH) = A_1 - A_2/m$
2. Smith (1947)	-existence of two principle classes of sorbed moisture in polymers: bound and condensed -combination of BET and Langmuir equations	0.50–0.95	$mw = A_3 - A_4 \ln(1 - RH)$
3. Henderson (1952)	-quantity of water is a function of the surface area upon which it is absorbed -partially empirical	0–1.0	$1 - RH = \exp(A_5 m^{A_6})$
4. Chung and Pfost (1967)	-potential theory with modified assumptions -free energy decrease exponetically with increase in moisture content -free energy also varies with temperature	0–1.0	$\ln(RH) = \frac{-A_7}{RT_a} \exp(-A_8 m)$
5. Halsey (1948)	-condensation of multilayers -potential energy of a molecule is proportional to the inverse rth power of its distance from the surface	0.1–0.8	$RH = \exp(-A_9/(R.T_a)m^{A_{10}})$
6. Oswin (1946)	-mathematical series expansion for S-shaped curves	0–1.0	$m = A_{11}(RH/(1-RH))^{A_{12}}$
7. Konstance et al. (1983)	-empirical	0.10–0.95	$mw = A_{13}\exp(A_{14}RH)$
8. Ferro-Fontan et al. (1982)	-Clausius-Clayperson eq. is applicable -net isosteric heat of sorption a function of moisture content -accounts for the structure of sorbed water	0.10–0.95	$\ln(a_{15}/RH) = A_{16}m^{-A_{17}}$
9. Chen and Clayton (1971)I	-modified Henderson eq. -empirical	0–1.0	$RH = 1 - \exp(-A_{18}T_a^{A_{19}}m^{A_{20}})$
10. Chen and Clayton (1971)II	-empirical for hydroscopic materials	0–1.0	$RH = \exp(-A_{21}\cdot T_a^{A_{22}}\cdot\exp(A_{23}\cdot m\cdot T_a^{A_{24}}))$
11. Chen (1971)	-diffusion is the principal mode of mass transport -no chemical reaction	0–1.0	$RH = \exp(A_{25} + A_{26}\exp(A_{27}m))$

Table 2—Moisture isotherm models for meat emulsions

1. Harkins & Jura (1944)
$$\ln(RH) = -3.4263E-3\,T - [-3.1495E-5\,T + 6.8218E-4\,\ln(T) + 3.8832E-5\,\ln(FP)]m^{-1}$$
2. Smith (1947)
$$mw = -1.1544E-2\,\ln(T) - [-4.2806E-3\,FP^2 + 0.0439\,\ln(T)]\,\ln(1 - RH)$$
3. Henderson (1952)
$$1 - RH = \exp[-(6.1807E-2\,FP^2 + 0.8549\,\ln(T))\,m^{0.1481\,\ln(T)}]$$
4. Chung & Pfost (1967)
$$\ln(RH) = -\frac{(-35.5953\,T + 3.6283E3\,\ln(T))}{RT_a}\exp[-(0.6122\,T + 0.6564\,FP^2)m]$$
5. Halsey (1948)
$$RH = \exp\left[\left(\frac{-(-3.4120\,FP^2 + 44.3074\,\ln(T))}{RT_a}\right)\cdot\left(m(8.2982E-3\,T - 0.3308\,\ln(T))\right)\right]$$
6. Oswin (1946)
$$m = 0.0189\,\ln(T)\,[RH/(1 - RH)]^{(-0.0196FP^2 + 0.2144\,\ln(T))}$$
7. Konstance et al. (1983)
$$mw = (-9.2260E-5\,T + 2.2169E-3\,\ln(T))\exp[(-3.5249E-2\,FP^2 + 1.3036\,\ln(T))\,RH]$$
8. Ferro-Fontan et al. (1982)
$$\ln\left[\frac{0.1787\,\ln(T)}{RH}\right] = [3.6391E-6\,T^2 + 0.0146\,\ln(FP)]\,m^{(-0.2199\,\ln(T))}$$
9. Chen & Clayton (1971)I
$$RH = 1 - \exp[-(0.0458\,T + 0.2699\,FP)\,T_a^{5.2109E-3\,\ln(T)}\,m^{0.1538\,\ln(T)}]$$
10. Chen & Clayton (1971)II
$$RH = \exp\left[-3657.687\,\ln(T).T_a^{-0.3806\,\ln(T)}\exp[-0.1270\,\ln(T).m.T_a^{(0.0158FP + 0.1074\,\ln(T))}]\right]$$
11. Chen (1971)
$$RH = \exp[-0.04034\,\ln(T) - 2.1682FP.\exp\{(-5.1140E-3\,T^2 - 39.016\,\ln(FP))\,m\}]$$

Table 3—Values of the coefficient of determination and mean sum of square of residuals for various EMC models

Model	R^2	Mean sum of square of residuals
Harkins and Jura (1944)	0.925	0.0823
Smith (1947)	0.970	0.0015
Henderson (1952)	0.977	0.0093
Chung and Pfost (1967)	0.921	0.0866
Halsey (1948)	0.986	0.0055
Oswin (1946)	0.941	0.0100
Konstance et al. (1983)	0.992	0.0004
Ferro-Fontan et al. (1982)	0.810	0.2083
Chen & Clayton (1971)I	0.979	0.0085
Chen & Clayton (1971)II	0.933	0.0274
Chen (1971)	0.981	0.0075

was not disturbed appreciably during the weighing of the samples. A hole, 4.8 mm in diam., was drilled in the original lid of each jar to facilitate the weighing process. A metal wire with two hooks was used to suspend the specimen basket. To cover the hole a stropper (4 cm diam metal plate) was attached with the wire.

The cover and attached sample basket were raised slightly and attached to the beam of an analytical balance for weighing. An aluminum foil basket, 4.5 cm in diam, was suspended with the help of a 3.2 mm diam metal wire and plastic thread. The sample baskets and suspending wires were coated with a high vacuum grease to prevent corrosion. The grease was found not to desorb or absorb moisture under the conditions used in the experiments.

Saturated salt solutions were used for relative humidity control. The following seven salts were: lithium chloride (11.2%), magnesium chloride (28.8 to 30.8%), potassium carbonate (42.1 to 42.9%), sodium nitrite (52.5 to 58.4%), sodium chloride (73.1 to 74.5%), potassium chromate (82.9 to 84.6%) and potassium sulphate (94.3 to 95.6%). The values in brackets are relative humidities between 55–85.°C (Hall, 1957). These salt solutions provided seven data points for each temperature and FP and were selected to obtain sufficient EMC data between 0 and 100% relative humidity. Approximately 200 mL of solution with excess salt was maintained within each humidity control chamber.

All the models in Table 1 were fitted to the moisture isotherm data using Nonlinear Regression Analysis (NLIN) of the Statistical Analysis System (SAS) (Barr et al., 1979). This method utilizes Gauss-Newton method to find the least squares estimates of the model parameters.

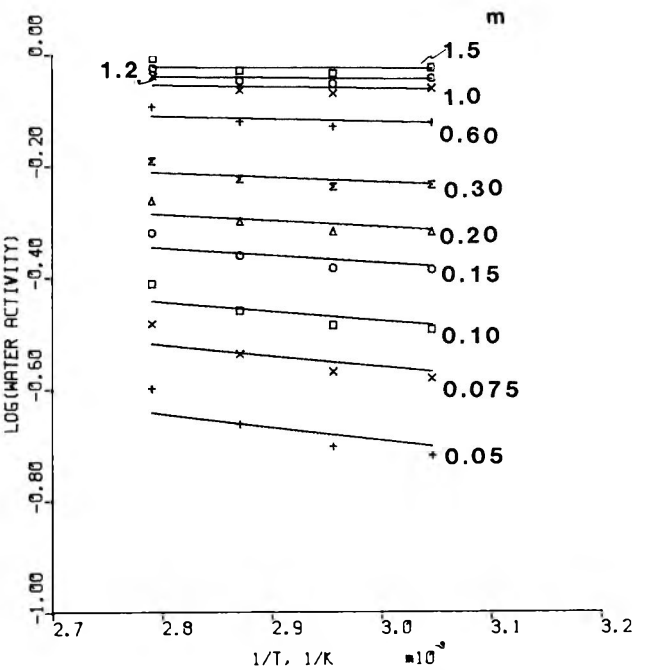

Fig. 3—Log$_e$ (a_w) versus 1/T_a plot at various meat emulsion moisture contents and FP of 1.303.

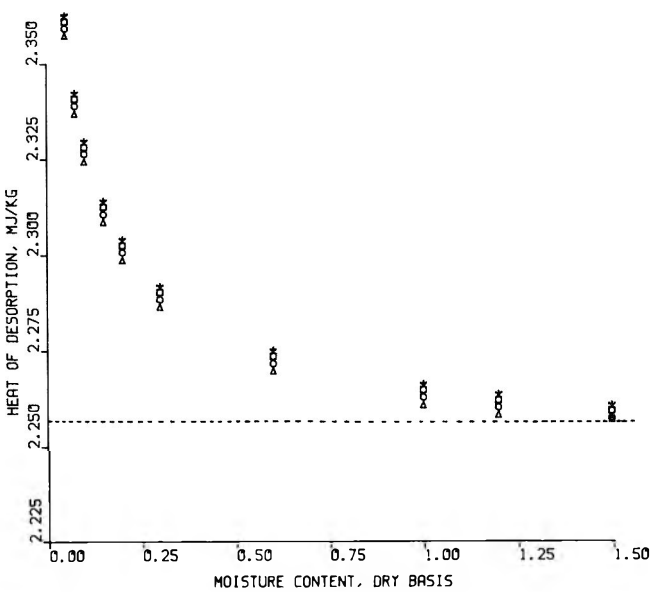

Fig. 4—Total heat of sorption or desorption at various moisture contents and fat-protein ratios of meat emulsion: △ ; 2.56; ○ ; 2.16; □ ; 1.68; X; 1.30; and + ; 1.10 fat protein ratios.

RESULTS & DISCUSSION

THE EQUILIBRIUM moisture data are shown in the form of isotherms in Fig. 1 and 2. These are signoid in shape and belong to the type III isotherm according to the classification of Brunauer (1954). The type III, which is uncommon in food materials, signifies that isotherms are approaching $a_w = 1$ asymptotically. There was very little chemisorption or monolayer adsorption i.e. the meat emulsion can absorb very small

amounts of water at monolayer level and there seems to be no capillary condensation phenomena. Most of the water was absorbed in multilayers. The EMC at the low RH (0.40) were relatively constant, resulting in an almost horizontal line. The slope of the line at this range increased with decrease in temperature. The behaviour of the isotherm in this RH range can be interpreted to mean that the product EMC is most stable in this range. An increase in the environmental RH in this range will only have a slight effect on the storage stability of the product, due to the fact that EMC will stay in one type of zone of moisture content. There was a consistent shift to the right due to increase in temperature at a given moisture content (Fig. 1). This shift plays an important role in the stability of stored food at different temperatures and a given RH. At all temperatures, the stability of the product became critical at RH above 70%. This was due to the increase in rate of reaction in the product as a result of microbial and enzymatic activities.

The various coefficients in the models (Table 1) were found to be the functions of product temperature and composition (FP). Suitable regression models (Table 2) for these coefficients were formulated using stepwise regression analysis. The model parameters were selected from T, FP, T^2, FP^2, ln(T), and ln(FP). The coefficient of determination (R^2) values vary between 0.810 and 0.992, and the values of the mean sum of squares of residuals vary from 0.0004–0.2083 (Table 3). On the basis of these two parameters the Konstance et al. (1983) model seems to be the best among all the 11 models evaluated. All other models, except the model of Ferro-Fontan et al. (1982), also provide an adequate fit to the experimental data.

Fig. 1, for different temperatures, and Fig. 2, for various fat-protein ratios, are the plots of observed and predicted values of EMC using the model of Konstance et al. (1983). The differences in the isotherms due to changes in composition and temperature were highly significant at 95% confidence level using Duncan's multiple range test (Barr et al., 1979).

The moisture isotherms for meat emulsions at different temperatures (Fig. 1) showed that, typically, the quantity of sorbed water at a given relative humidity increased as temperature decreased. Temperature effects are more apparent at RH values between 0.4 and 0.9.

Generally for the isotherms studied, at constant RH, the quantity of water sorbed increased with decreasing fat content (Fig. 2). Since fat is hydrophobic, it offers resistance to the diffusion of moisture and absorbs less moisture.

In this study, the meat emulsion used was at an initial moisture content of 87–104%, dry basis. It is, therefore, possible that at high relative humidity levels, the meat emulsion will sorb moisture and at low relative humidity levels, it will desorb water. This hysteresis effect has been neglected in this study. To study moisture loss or gain during meat emulsion thermal processing, the moisture desorption data are required at low relative humidity, and moisture adsorption data are required at higher relative humidity. Therefore, these models will be useful in calculating moisture loss or gain by the meat emulsion products at various stages of cooking.

The heat of desorption or sorption, Q_s, was determined by using the Clausius-Clapeyron equation. This equation describes the effects of temperature on vapour pressure ratio or water activity. The differential form is given as (Daniels and Alberty 1975):

$$\frac{d(\log a_w)}{d(1/T_a)} = \frac{-Q_s}{2.303R}$$

A semilog plot of a_w versus $1/T_a$ at different moisture levels and fat-protein ratio produced a linear relation (Fig. 3). This is in confirmation with the Clausius-Clapeyron relationship. The slope of each line was calculated using General Linear Regression Analysis Procedure of the SAS (Barr et al., 1979). The Q_s was then calculated from the slope. The total heat of desorption/sorption is given by $\Delta H = Q_s + \Delta H_v$, where ΔH_v is the enthalpy of vaporization at atmosphereic pressure. Since log P versus $1/T_a$ was a straight line between 40° and 90°C, ΔH_v was constant and equal to 2257 kJ/kg. Fig. 4 shows a plot of the ΔH versus moisture content for different fat-protein ratios. The ΔH decreased with increased moisture content, and approached a constant value of 2257 kJ/kg at moisture content > 130% dry basis. This is in agreement with the findings of Chung and Pfost (1967) for corn and corn products. The ΔH also decreased with the increase in fat-protein ratio. Fig. 4 indicates that a large amount of energy must be supplied to desorb water at low moisture content from meat emulsions. This energy decreased rapdily until a moisture content of 90% dry basis was reached. An almost constant energy, equivalent to that required to evaporate pure water is needed to desorb moisture from meat emulsion above a moisture content of 140% db.

SYMBOLS

A1 - A27	Constants
a_w	Water activity
EMC	Equilibrium moisture content, dry basis
FP	Fat-protein ratio
m	Equilibrium moisture content, dry basis
mw	Equilibrium moisture content, wet basis
P	Pressure, kPa
Q_s	Heat of desorption or sorption, kJ/kg
R	Gas Constant [1.978 g-cal/(g.mole.K)]
R^2	Coefficient of determination
RH	Relative humidity, decimal
T	Temperatire, °C
T_a	Absolute temperature, K
ΔH	Total heat of desorption or sorption, kJ/kg
ΔH_v	Enthalpy of evaporation of pure water, kJ/kg

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Storage of Morwong (*Nemadactylus macropterus* Bloch and Schneider) in Combinations of Polyphosphate, Potassium Sorbate and Carbon Dioxide at 4°C

JO A. STATHAM, H. ALLAN BREMNER, and ANTHONY R. QUARMBY

ABSTRACT

Combinations of potassium sorbate and polyphosphate, in conjunction with vacuum or modified atmosphere packaging, were assessed for their preservative effects on morwong fillets (pH 6.75) stored at 4°C. Vacuum packaged and/or frozen fillets were used as comparisons for microbiological and taste panel analysis. A combination of potassium sorbate, polyphosphate and 100% CO_2 was the most effective packaging regime. Potassium sorbate on vacuum packaged fish was more effective than a 100% CO_2 atmosphere alone. Polyphosphate had no apparent additional effect on fillets stored under vacuum with or without potassium sorbate.

INTRODUCTION

A SATISFACTORY METHOD for extending the shelf-life of chilled fish and shellfish has long been sought, to ensure quality, continuity of supply and minimal wastage. Preservatives such as sorbic acid and its potassium salt have met with varying success. The reported effects of potassium sorbate dips range from being minimal (Fey and Regenstein, 1982) to extending the shelf-life of vacuum packed scallops from 4–5 days to 28 days at 4°C (Bremner and Statham, 1983). The use of modified atmospheres incorporating high proportions of CO_2 has also had success, with shelf-life extension ranging from 50-100% (Statham, 1984). A combination of these two treatments may give rise to additive or synergistic effects, providing greater assurance of product quality and stability.

Used in conjunction with atmospheres containing 60% CO_2, potassium sorbate dips or potassium sorbate incorporated into ice were effective in extending the shelf-life of red hake and salmon to 4 wk at 1°C (Regenstein, 1982; Fey and Regenstein, 1982). Pathogens such as *Salmonella enteriditis* (Elliot and Gray, 1981) and *Staphylococcus aureus* (Elliot et al., 1982) are inhibited to a greater extent in media containing potassium sorbate and incubated in CO_2 enriched atmospheres than by either treatment alone.

Fish packaged in atmospheres containing high levels of CO_2 (>60%) have been reported to suffer from excessive drip loss (Mills and Tiffney, 1982). Nitrogen may be used as an inert filler to lower the proportion of CO_2. Oxygen may also be added if the product is likely to undergo color changes or bleaching (Cann, 1984). However pre-mixed gases are not always readily available, therefore the use of 100% CO_2 in conjunction with polyphosphate dips to prevent drip may be a more viable alternative. Statham and Bremner (1985) discuss the importance of high partial pressures of CO_2 in MAP fish. Polyphosphate dips could be used to overcome this problem. There is some evidence that polyphosphates may themselves have an inhibitory effect on the bacterial flora of flesh foods (Elliot et al., 1964; Van Wazer, 1971); however, increases in shelf-life, when they occur, are in the order of 1–2 days (Spencer and Smith, 1962). Some suppression of toxin production by *Clostridium botulinum* in beef/pork frankfurter emulsions may occur as a result of synergism between sodium acid pyrophosphate, potassium sorbate and sodium nitrite (Wagner and Busta, 1983).

The purpose of this experiment was to assess the efficacy of a potassium sorbate dip, when used in conjunction with a 100% CO_2 atmosphere, to extend the chill-storage life of morwong (*Nemadactylus macropterus* Bloch and Schneider). Potassium sorbate, CO_2 and polyphosphate treatments were also used individually to allow the estimation of additive or synergistic effects of various combinations of treatments.

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MATERIALS & METHODS

FRESH MORWONG FILLETS (pH 6.75) were bought from the Melbourne fish market the day after catching and were air freighted in polyethylene bags, surrounded by ice, to Hobart the same evening. On arrival at the Tasmanian Food Research Unit (TFRU) the fillets were skinned by hand and stored in a 0 ± 0.5°C cool room overnight. Morwong is a medium to low-priced white fish which is representative of the perch family, having a composition of 19% protein, 1% fat, 78% moisture and 1.5% ash (unpublished results from this laboratory). These fish are widely distributed through Australian and New Zealand waters and are available for most of the year. At present the stocks are under-exploited in some areas (Wankowski, 1984).

Preparation of dip solutions

A 10% polyphosphate dip solution was prepared containing 5.3% sodium tripolyphosphate and 4.7% sodium pyrophosphate (Albright and Wilson (Australia) Limited) to give a pH of 6.2 in the final solution. A 1.2% potassium sorbate solution was prepared and the pH adjusted to 6.2 using hydrochloric acid. This concentration had previously been found to give a residual level of near 0.1% (w/w) potassium sorbate on the fillets. A composite polyphosphate/potassium sorbate dip with the solutes at the above concentrations was prepared and its pH adjusted to 6.2 using hydrochloric acid.

In all cases the fillets were immersed in the dips for 1 min as recommended by manufacturers of polyphosphates for the treatment of fish (Albright and Wilson (Australia) Ltd., Technical Bulletin - 'Mera' polyphosphate for treating seafood) and were allowed to drain for 5 min before packaging. Preliminary experiments showed that polyphosphate did not affect the rate or amount of sorbate uptake, that sorbate uptake had reached a maximum after 1 min in the dip and that 2 days after dipping surface levels were still higher than at the center of fillets 25 mm in thickness.

Packaging

In this experiment seven different treatments were employed, as shown in Table 1. Fillets were packed three to a bag (approximate total weight 240g). Twenty-eight packs were prepared for each treatment, except for treatment F, where extra packs were prepared. Cryovac U gauge barrier bags (W.R. Grace Pty. Ltd., Melbourne), a laminate of EVA/PVDC/EVA having a nominal OTR and CO_2TR of 3.5 and 12 mL m^{-2} $24hr^{-1}$ atm^{-1} at 3.5°C and 75% RH were used. Packaging was carried out using a Boss, Regina 2/100 Germany, vacuum packaging machine equipped for gas flushing. Fillets were packaged side by side with the minimum degree of overlap. In those treatments which were vacuum packed (V, F, SP, P and S) the film was in firm contact with the fillets. In those packs flushed with CO_2 (SPC and C) the gas initially held the film away from the upper surface of the fillet; however, as CO_2 was absorbed by the flesh the bags deflated. By 24

Table 1—Packaging treatments

Treatment	Code	Atmosphere	Dip	Storage temp (°C)
Vacuum	V	vacuum	nil	4
Frozen	F	vacuum	nil	−18
CO_2	C	100% CO_2	nil	4
Polyphosphate	P	vacuum	10% polyphosphate	4
Sorbate	S	vacuum	1.2% potassium sorbate	4
Sorbate + polyphosphate	SP	vacuum	1.2% potassium sorbate + 10% polyphosphate	4
Sorbate + polyphosphate + CO_2	SPC	100% CO_2	1.2% potassium sorbate + 10% polyphosphate	4

hr after packaging the film was in contact with the surface but not as tightly as in the vacuum packaged treatments.

Four packs from treatments V, F, SP and SPC and three packs from treatments C, P, and S were assessed after 0, 3, 6, 10, 13, 17, and 20 days in storage.

Sorbate analysis

Seventeen sorbate-treated fillets were frozen (−18°C) for analysis. After 4 months' storage the fillets were thawed, diced then extracted and the sorbate levels determined by U.V. spectrophotometry (AOAC, 1980). Previous experiments done in this laboratory indicate that potassium sorbate is stable on fish flesh for at least 10 months at a temperature of −18°C (Bremner, 1984, unpublished data).

Microbiology

Sampling. Treatments V, P, S, and SP were sampled immediately after packaging. Those samples subjected to the same dips but flushed with CO_2 or vacuum packaged were taken as being the same at that stage (i.e. SP = SPC, V = C). At subsequent sampling times all chill-stored treatments were sampled. Three packs from each treatment were selected at random and one fillet from each pack was sampled. From these fillets a 16 cm^2 surface sample was excised using a sterile template and scalpel. Following homogenization in a Colworth Stomacher, serial decimal dilutions were prepared in sterile saline and spread plated on to Tryptone Soya Agar (Oxoid). The medium was enriched by the addition of 0.2% yeast extract (Sigma), 0.2% glucose (M and B) and 0.5% NaCl to encourage growth of fastidious bacteria, particularly lactobacilli. Plates were incubated at 22°C for 3–5 days.

Identification of isolates. Twenty colonies were picked at random from plates from treatment V at time 0 and after 6 days, treatments SP and C after 10 days and treatment SPC after 20 days. The isolates taken from untreated fish at time 0 were considered to be representative of the initial flora, while the isolates taken later in storage were considered to represent the spoilage flora of each treatment. Gram negative isolates were identified using the scheme of Hendrie and Shewan (1979). Gram positive isolates were identified according to *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974).

pH, raw odor and appearance

The surface pH was measured at four positions on each fillet from each pack using an Orion surface pH electrode. Internal pH of three fillets from each treatment was measured at four positions on the cut surface directly below the area sampled for microbiological analysis. The appearance and odor of the raw fish was recorded by two people experienced in judging fish quality, and the types and intensities of odors were used to assess the suitability of the fish for taste panel analysis. The amount of drip produced in each pack was measured volumetrically, if present in sufficient quantity.

Profile panel

Frozen fish was used by the taste panel as reference "fresh fish" samples and as substitutes for treatments that were withdrawn from the tasting sessions as they became unacceptable. Two fillets from the four packs from treatments V, SP, SPC, and F, plus reference samples, were halved and placed in individual, previously coded, plastic bags. All bags were strung on a rod in the correct taste order for each panelist and the bags were suspended in a waterbath set at 84°C. The fish was cooked for 20 min to a center temperature of 75°C. When cooked the fish was removed from the bags and placed in heated, coded glass jars and served to the taste panel.

The profile panel consisted of 16 staff members who were unaware of the details of the experiment but were experienced in assessing seafoods and in the profile technique. One taster was absent at the first and fifth sessions and two tasters were absent at the seventh session. At each sampling time the 16 tasters were divided into two sittings of eight tasters and each taster was asked to taste an identified reference sample, then four coded samples, one from each of treatments F, V, SP, and SPC. The five samples were served at one time and the panelists were asked to taste them according to the order marked on their profile sheets. The 24 possible taste orderings were randomly allocated to the 16 tasters at the first taste session. The order of tasting was then varied cyclically within a session over the remaining six sessions so as to minimize taste order effects. Samples from treatments V and SP became unsuitable for tasting by the 10th and 13th days of storage, respectively, and were replaced by frozen fillets (equivalent to treatment F) to maintain sample numbers at all subsequent taste sessions.

The panelists were asked to assess the fish by the odor and flavor profiles used previously in this laboratory (Quarmby et al. 1982; Bremner and Statham, 1983; Statham and Bremner, 1983) with the following differences in methodology. The panelists were in isolated taste booths and they marked their score sheets independently with no round table discussion. Instead of the 6-point structured scale used previously, a 10-point unstructured scale (0 = absent, 9 = strong) was used for scoring the intensity of descriptors listed on the profiles. Scoring for the attributes of odor, off odor, flavor and off flavor was obligatory. Texture was assessed by scoring the initial characteristics, wetness, firmness, springiness, and the secondary characteristics, toughness, succulence and fibrousness (Howgate, 1977) on this same scale. The panelists were also asked to score for odor, flavor and overall acceptability of the samples on the 7-point facial 'Smiley Scale' (Bremner and Statham, 1983).

Profile data analysis

The scores for the attributes which panelists were obliged to score, viz. fish odor, off odor, fish flavor and off flavor were subjected to analysis of variance. For the remaining 'free choice' attributes the total panel score or total panel response was transformed to a proportion of the total possible panel response, i.e. if all panelists had responded and scored 9 for that attribute. These data are plotted as integer values on a 0 to 100 scale. This technique can thus be used to compare the responses from panels of different sizes. When an attribute at any one session was scored by only one panelist, then this datum has been deleted from the data set.

RESULTS

Sorbate levels

The mean potassium sorbate level was 0.08%. Morwong fillets of the size range used in this experiment (80–120g) have an area-to-weight ratio from approx. 0.45 to 0.55 g/cm^2. This means that for a 100-g fillet, potassium sorbate was applied at an average level of 0.4 mg/cm^2.

pH changes

Internal and surface pH values of the fillets were not significantly different ($p<0.05$) and were therefore combined to give the means plotted in Fig. 1. One fillet from each of two packs in treatment SPC sampled at 20 days had pH values significantly lower than the others in that treatment measured at the same time (mean pH 5.2 compared to 6.4). These values were considered to be unrepresentative and were therefore not included in the final means.

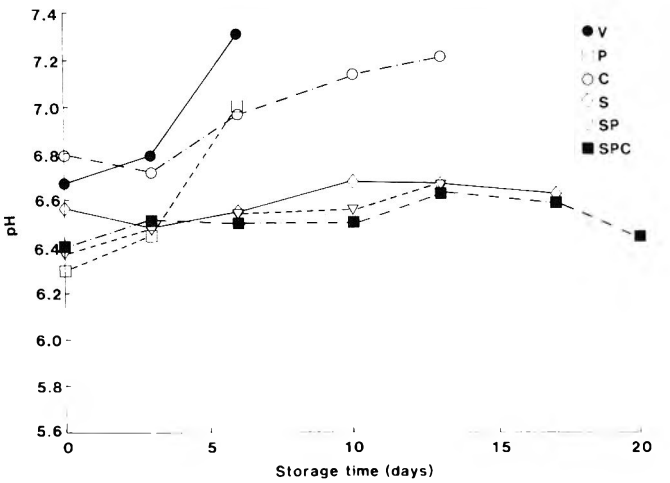

Fig. 1—pH of morwong fillets stored at 4°C. (See Table 1 for Code).

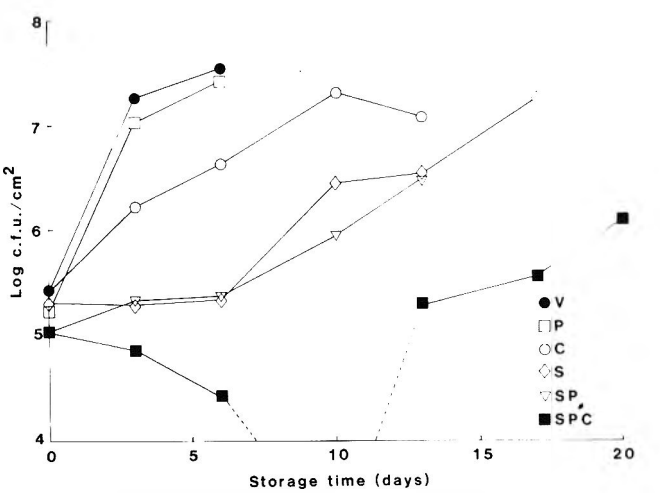

Fig. 2—Estimated bacterial numbers on morwong fillets stored at 4°C. (See Table 1 for code.)

The pH of fish in all packs on day 0 was between 6.8 and 6.2. Dipping in polyphosphate lowered the pH by near 0.4 units. The pH of fish from treatments V, C, and P increased rapidly as spoilage progressed, while those of fish in treatment S remained constant during the storage period.

Bacterial numbers

Changes in the estimated numbers of bacteria after each treatment are shown in Fig. 2. Rapid increases in bacterial numbers occured in treatments P and V, reaching around 5×10^7 cfu/cm^2 after 6 days. Bacterial numbers in these treatments did not differ significantly ($p<0.05$), showing that polyphosphate alone had no significant effect on the bacterial flora. Fish stored in carbon dioxide showed a slower increase in bacterial numbers, reaching around 4×10^7 cfu/cm^2 after 10 days. Treatments S and SP gave a lag period of 6 days, followed by a rise to around 5×10^6 cfu/cm^2 after 13 days. The bacterial numbers in these two treatments differed significantly ($p<0.05$) only initially and at 10 days. Polyphosphate therefore had little or no effect on the bacterial flora when used in conjunction with potassium sorbate. Treatment SPC had an initial bactericidal effect, with bacterial numbers being reduced to below the minimum level of detection (1000 cfu/g) during the first 10 days. By 13 days bacterial numbers had risen to around 2.5×10^5 cfu/cm^2 and continued to rise slowly during storage, reaching 10^6 cfu//cm^2 at the end of the storage period.

Bacterial flora

The bacterial flora of untreated morwong was composed of *Moraxella* species (60%) and *Alteromonas putrefaciens* (30%) (Table 2). *Lactobacillus* were present at low levels (5%). After 6 days in vacuum packs the proportion of *A. putrefaciens* increased to 45%. Fifty percent of the colonies selected showed only limited growth and could not be cultured for isolation and identification. *Vibrio/Aeromonas* species were present at a low level (5%). Addition of 100% CO_2 caused the development of a flora composed mainly of *Vibrio/Aeromonas* species. *Brochothrix thermosphacta* comprised 65–75% of the flora in treatments SP and SPC. The pH (6.4) and the low glucose levels in fish flesh may have given *B. thermosphacta* a competitive advantage over the *Lactobacillus* species.

Appearance and odor of raw fish

The only noticeable changes in the appearance of the fish in any of the treatments was a slight opacity of fish in treatment SPC after 3 days. This changed to a bleached appearance later in storage. No significant volumes of drip were noticed in any treatment. The changes in the odor of raw fish are listed in Table 3.

Profile panels

The profile results for the seven sessions at which fresh/frozen fillets were tasted have been consolidated (Fig. 3a, 4a). The major attributes contributing to the odor profile of the fresh/frozen morwong were sweet, shellfish, seaweedy and metallic with traces of starchy, boiled clothes, cardboard, wet feathers, musty, rubbery, pungent and putrid. For flavor the attributes of sweet, cardboard, chicken, salty and buttery were important which, with the exception of cardboardy, could be considered as desirable attributes. Odor intensity scores for all the treatments were uninformative and have been deleted from the profile results. The fish in treatment V deteriorated rapidly, undesirable off odors and off flavors were evident within 6 days of storage (Fig. 3b, 4b). The ammoniacal odor and flavor, the sour pungent acrid odors and sour, bitter, sulfide flavors that developed are typical for fish spoiled by *A. putrefaciens*. Fillets in treatment SP spoilt less rapidly than those in treatment V, off odors and off flavors increased, the desirable buttery flavor declined and astringent soapy flavors formed (Fig. 3c, 4c). There were few consistent changes in the odor profiles for the fish in the treatment SPC but pungent, acrid and putrid notes were quite noticeable after 20 days storage (Fig. 3d, 3e). The off flavor intensity increased and the desirable flavor characteristics such as sweet, buttery, chicken and broiled potato decreased while rancid, sour and bitter notes increased (Fig. 4d, 4e). Some of these changes may in part be due to autolysis and it seems unlikely that the rancid note would have arisen from oxidation but is more probably a result of lipolysis.

Texture

Very few significant textural changes were detected in or between treatments F, SP and SPC. Treatment V showed the most marked changes, becoming more tender, less succulent, less firm, less springy and less fibrous (Fig. 5). These changes may have resulted from the effect of increasing pH (Fig. 1) on protein structure (Love et al., 1979) or from bacterial proteolysis (Shewan, 1974). The texture of treatments SPC and F remained similar throughout the 20 days of storage.

Odor, flavor and overall acceptability

The results for odor and flavor correlated highly with those for overall acceptability and only the latter has been plotted (Fig. 6). After 6 days in storage the scores for treatment V were significantly lower ($p<0.05$) than scores for the frozen

Table 2—Changes in bacterial flora on morwong stored at 4 C Genera present, % of total

Storage time (Days)	Treatment	*Moraxella*	*A. putrefaciens*	*Vibrio/ Aeromonas*	*Enterobacteriaceae*	*Pseudomonas*	*Lactobacillus*	*B. thermosphacta*	Unidentified
0	V-Vacuum packaged	60	30	—	—	—	5	—	5
6	V-Vacuum packaged	—	45	5	—	—	—	—	50 no growth after isolation
13	SP-Polyphosphate + K-sorbate dip, vacuum packaged	5	—	5	5	—	10	75	—
	C-Packaged in 100% CO_2	—	—	90	—	10	—	—	—
20	SPC-Polyphosphate + K-sorbate dip, packaged in 100% CO_2	—	—	5	5	—	20	65	5

Table 3—Odor of raw fish
Storage time (days) at 4°C

Treatment*	3	6	10	13	17	20
V	slightly sour, fishy	pungent, rotting seaweed	—	—	—	—
C	fresh	sour	sour	sour, sickly		
P	fresh	sour, unacceptable	—	—	—	—
S	fresh	bland, slightly floury	very slightly spoiled	slightly stale, musty	smelly	—
SP	shellfish, sweet scallops	fresh seaweed, slightly marinated	slightly spoiled	sour	—	—
SPC	shellfish, mussels, seaweed	fresh seaweed	seaweed, fishy	fresh shellfish	slightly fishy	seaweed, not spoiled

* See Table 1 for treatment codes.

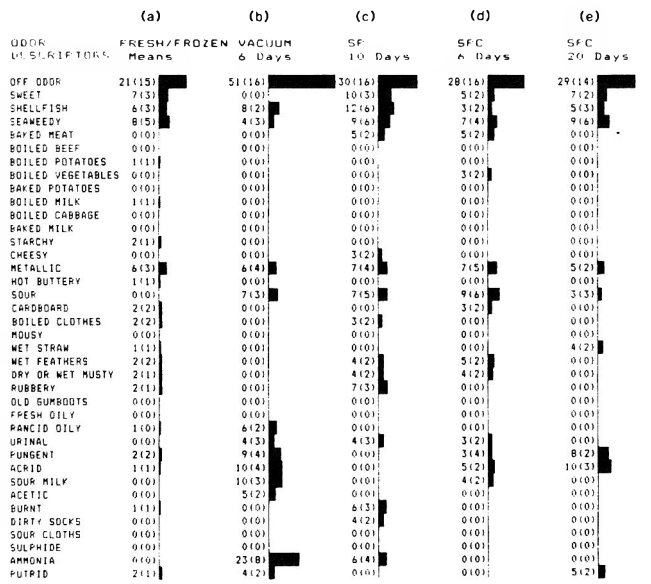

Fig. 3—Odor profiles of stored morwong: (a) fresh/frozen, (b) treatment V after 6 days, (c) treatment SP after 10 days, (d) treatment SPC after 6 days, (e) treatment SPC after 20 days. Total panel scores (0–100 scale) for each descriptor are shown, followed in parentheses by the number of panelists who marked that particular descriptor. In (a) the mean of all the sessions is shown to the nearest integer value.

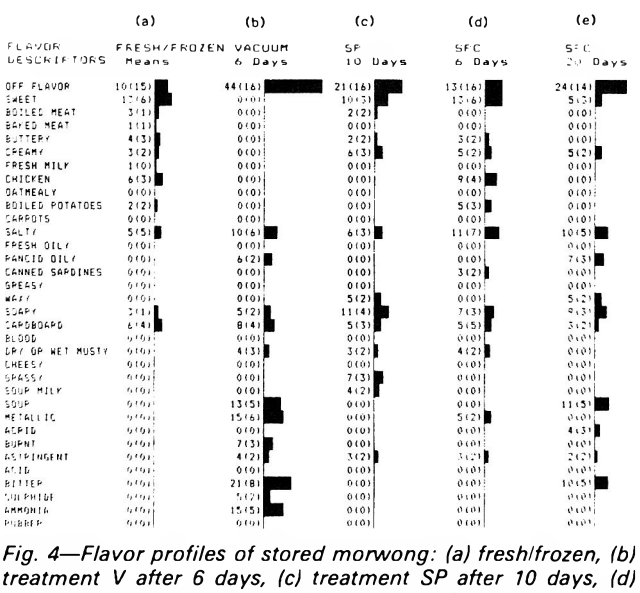

Fig. 4—Flavor profiles of stored morwong: (a) fresh/frozen, (b) treatment V after 6 days, (c) treatment SP after 10 days, (d) treatment SPC after 6 days, (e) treatment SPC after 20 days. Total panel scores (0–100 scale) for each descriptor are shown, followed in parentheses by the number of panelists who marked that particular descriptor. In (a) the mean of all the sessions is shown to the nearest integer value.

material. Flavor and overall acceptability scores for treatment SP were significantly lower than those for the frozen material after 10 days storage. Fish in treatment SPC remained as acceptable as the frozen fish for 13 days after which scores were significantly lower.

It could be suggested that the fish at the start of the experiment was of low quality even though it was supposedly chilled and obtained as soon as practical after catching. Experience

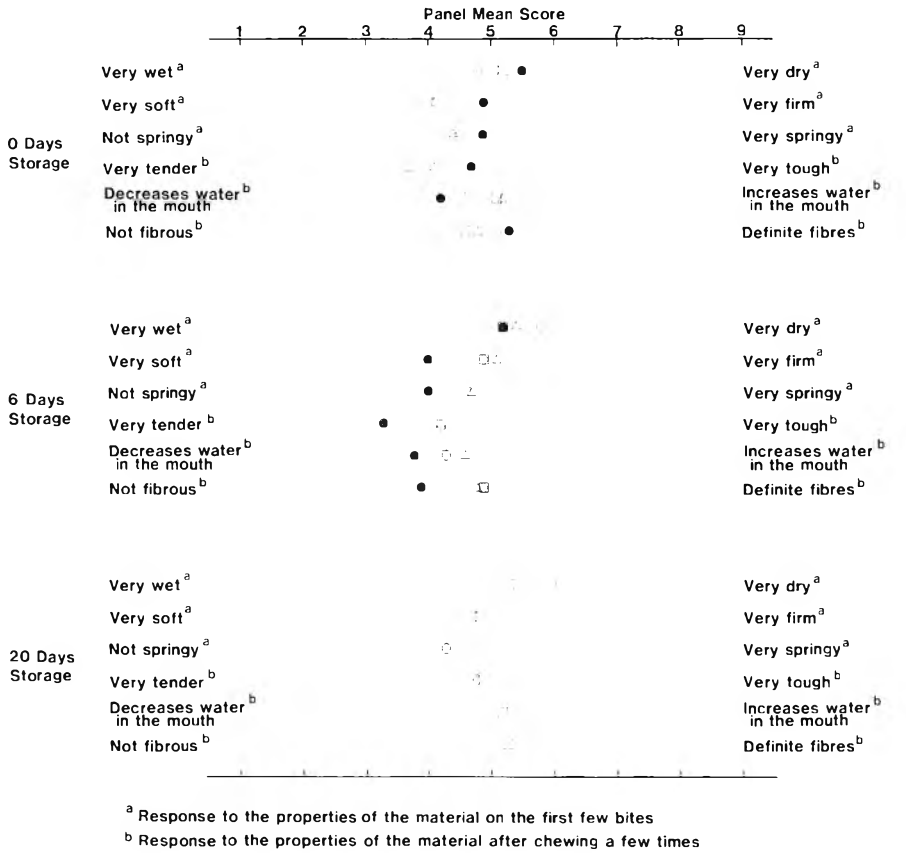

Fig. 5—Texture profiles of morwong stored for 0, 6 and 20 days; △ = SP, □ = SPC, ◇ = F, ● = V. (See Table 1 for Code).

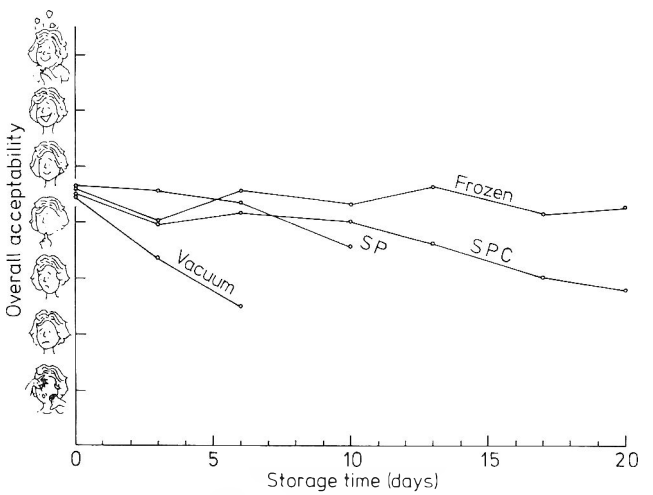

Fig. 6—Acceptability of stored morwong.

with these profile panels using this scoring method has shown that fairly low ratings are invariably given to plain cooked fish. The results for the frozen fish indicate the panel was consistent in its judgement.

DISCUSSION

THE COMBINED ACTION of CO_2 and sorbate (with polyphosphate) had an inhibitory effect on the bacteria (Fig. 2). Nevertheless, the fish still deteriorated steadily as indicated by increases in off odor and off flavor, decreased acceptability and, to a lesser extent, by the profile results (Fig. 3,4). The surviving bacterial flora was dominated by *Lactobacillus* and *B. thermosphacta*; genera which are regarded as having a low spoilage potential. Low numbers of *Vibrio/Aeromonas* also survived and it is likely that these organisms were a cause of the steady deterioration. *Vibrio* are well known to be spoilage organisms on other seafoods.

In addition, it is also possible that other spoilage organisms could have been present in numbers too low to be detected by the methods employed here which entail using high dilutions to enumerate 'total plate counts' on a non-specific medium. When the majority of the normal spoilage organisms have been suppressed and the shelf-life has been extended then even relatively low numbers of surviving spoilage organisms have the time and opportunity to produce significant amounts of deleterious byproducts which accumulate in the pack and migrate into the flesh. To enumerate low numbers of spoilage organisms would require the enormous workload involved with sequential sampling using a wide range of dilutions and a wide variety of selective media. Other mechanisms that may also have time to express their influence are degradation due to inherent autolytic or catheptic processes and bacterial enzymes released from cells no longer viable. When bacteria are killed by processes that do not denature their enzymes then the enzymes can remain active even though cell numbers are in decline.

Any or all of these mechanisms may account for the steady deterioration of the fish in treatment SPC. All of them place emphasis on the conventional wisdom of the need to obtain and package fish in as fresh a condition as possible. Given these circumstances the treatment of fillets with potassium sorbate, CO_2 and polyphosphate effectively extended the shelf-life of morwong fillets. Processors and regulatory authorities should determine if such treatment is safe and worthwhile on a commercial basis.

SUMMARY

THE VARIOUS COMBINATIONS of potassium sorbate, polyphosphate and CO_2 had markedly different effects on the keeping quality of morwong. The efficacy of the treatments used was: V = P <C <S = SP <SPC as indicated by changes

—Continued on page 1587

Proximate and Amino Acid Composition of the Roe and Muscle of Selected Marine Species

MARIKO IWASAKI and ROKURO HARADA

ABSTRACT

Crude protein, total lipid, water, cholesterol, and amino acid composition of the roe of eighteen Pacific marine species were determined and compared with those of muscle from the same marine species. Crude protein and total lipid were higher in roe than in muscle, while water was lower. Cholesterol in roe was generally tenfold higher than that of muscle but only around one-fourth that of hen egg yolk. Glutamic acid, leucine, and aspartic acid were generally found to be the major components of roe. Leucine and proline content were significantly greater in roe than in muscle. On the whole, roe was well balanced with the essential amino acid and had a good E/NE ratio and may be considered a food source of high-quality protein.

INTRODUCTION

OVER THE PAST several years, utilization of marine resources for human consumption has increased worldwide, primarily due to an escalated awareness of health and a growing concern for nutrition. Consequently, in the United States it is becoming increasingly important to explore the so-called underutilized species of fish and shellfish. In Japan, on the other hand, marine resources are utilized conventionally on a wide scale. Furthermore, Japanese eat not only fish muscle, but also roe, because of the roe's desirable taste. Roe is one of the most valuable food products from fishery sources.

In some fish, such as flatfish, mackerel, small poulp, and crab, etc., the boiled and seasoned roe may be eaten along with the flesh. In some fish the roe is removed and sold commercially, often as delicacies. In Japan separated mullet roe is known as "karasumi," salmon roe as "sujiko" or "ikura," herring roe as "kazunoko," cod roe as "tarako," and sea urchin roe is called "uni." Karasumi and uni are regarded as expensive delicacies. The separated roe may be salted and broiled for eating.

Due to a need for nutrient data on seafood, the composition data for 1000 species of fish are now available (Kritchevsky et al., 1967; Sidwell et al., Sweeney and Weihrauch, 1976). Although much information is still lacking on roe, recently Lu et al. (1979) and Kaitaranta et al. (1980) reported the chemical and nutrient composition of the roe of salted mullet, Baltic herring, and rainbow trout.

The purposes of the present study were: (1) to determine the proximate composition, the cholesterol, and amino acid composition of the roe and muscle of 18 Pacific marine species; (2) to determine whether there are any significant chemical and nutritive differences between the roe and muscle of corresponding marine species.

MATERIALS & METHODS

Fish and roe

Eighteen marine species with their roe were purchased from local markets between November, 1984 and February, 1985: Japanese bluefish (*Scombrops boops*); sea bream (*Pagrus major*); mackerel, Pacific (*Scomber japonicus*); mullet (*Mugil cephalus*); sardine (*Sardinops melanosticta*); herring, Pacific (*Clupea pallasi*); salmon, chum (*Oncorhynchus keta*); smellt, shishamo (*Spirinchus lanceolatus*); cod, Pacific (*Gadus morhua macrocephalus*), cod, sketo (*Theragra chalcogramma*); anglerfish (*Lophius litulon*); flounder, Pacific (*Paralichthys olivaceus*); flatfish, Pacific (*Limanda herzensteini*); flatfish, nameta (*Microstomus achne*); squid (*Doryteuthis bleekeri*); small poulp (*Octopus ocellatus*); crab (*Portunus trituberculatus*); and sea urchin (*Hemicentrotus pulcherrimus*). For all analysis the detached, raw, and nonprocessed roe and muscle were used.

Proximate analyses

Prior to analyses the detached roe and muscle were ground to a fine homogeneous consistency, and each type was analyzed in triplicate. Water was determined by freeze drying; crude protein (N × 6.25) by the micro-Kjeldahl method according to the AOAC (1980) procedure. Total lipid was determined by solvent extraction (Bligh and Dyer, 1959). Cholesterol was analyzed by the gas chromatographic method according to a modified procedure of Kovacs et al. (1977).

Amino acid analyses

Well homogenized, freeze-dried roe or muscle was hydrolyzed in 6N HCl containing 1% thioglycollic acid under vacuum at 110°C for 24 hr. The solution of hydrolysate was injected into a Hitachi 835 automatic amino acid analyzer equipped with an integrator calculator.

RESULTS & DISCUSSION

Proximate composition

The proximate composition of the roe and muscle from various species is shown in Table 1. Protein of crab roe (30.2%) was highest among the roe of species examined. The protein of roe was generally higher than that of the corresponding fish muscle.

The lipid of roe was generally higher than that of the corresponding fish muscle. The lipid contents ranged from a low of 3.0% for herring roe to a high of 19.8% for mullet roe.

The cholesterol contents in the roe of marine fish species is also shown in Table 1. The average cholesterol level in the roe of 14 species of finfish was almost tenfold that of corresponding finfish muscle. However, in crustacean and molluscs cholesterol was at relatively higher levels in roe than in muscle, but the difference was not large (cf. Sweeney and Weihrauch, 1976). Cholesterol content in the roe of marine fish species was about one-fourth that of hen egg yolk, which is known to be about 1000 ~ 1500 mg/100g (Sikka and Johari, 1979).

Amino acid content and protein quality

The results of the amino acid analyses of various roe and muscle are presented in Tables 2 and 3. The most abundant amino acid, glutamic acid, averaged 13.46 g/16g N in the roe of 14 species of finfish, followed by leucine or aspartic acid. Methionine and histidine showed up in low concentrations in finfish roe. The most abundant amino acid in finfish muscle was also glutamic acid, but was usually followed, in decreasing order, by aspartic acid and lysine. Leucine, proline, tyrosine, valine, alanine, and serine (especially leucine and proline) present in relatively higher concentration in the roe than in

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Table 1—Proximate composition of the roe and muscle of marine species.

	Water g/100g		Protein g/100g		Lipid g/100g		Cholesterol mg/100g	
	R[a]	Mu	R	Mu	R	Mu	R	Mu
	M SD	M SD	M SD	M SD	M SD	M SD	M SD	M SD
Bluefish	70.1 ± 0.9	76.9 ± 1.0	19.2 ± 0.3	18.9 ± 0.5	9.3 ± 0.8	2.9 ± 0.5	348 ± 12.5	34 ± 2.9
Sea bream	73.4 ± 0.7	75.9 ± 1.4	20.3 ± 0.4	19.2 ± 0.9	4.9 ± 0.5	3.6 ± 0.6	366 ± 14.6	35 ± 3.0
Mackerel	66.6 ± 1.0	71.5 ± 0.7	25.3 ± 0.7	21.5 ± 0.3	6.8 ± 0.7	5.1 ± 0.6	416 ± 7.1	53 ± 2.4
Mullet	50.4 ± 0.9	69.9 ± 1.2	28.7 ± 1.0	18.8 ± 0.4	19.8 ± 0.8	9.4 ± 1.1	486 ± 6.8	47 ± 1.8
Sardine	68.7 ± 0.3	78.0 ± 0.2	24.4 ± 0.6	16.9 ± 0.6	6.0 ± 0.5	3.8 ± 0.5	395 ± 6.1	39 ± 1.4
Herring	77.0 ± 0.7	77.4 ± 0.8	18.7 ± 0.4	18.4 ± 0.6	3.0 ± 0.6	2.8 ± 0.5	305 ± 5.8	32 ± 0.4
Salmon, chum	57.6 ± 1.1	75.7 ± 1.3	27.0 ± 0.3	18.9 ± 0.6	14.1 ± 0.6	4.1 ± 0.5	450 ± 8.6	40 ± 1.3
Smelt, shishamo	61.4 ± 0.3	79.6 ± 0.4	24.1 ± 0.6	13.4 ± 0.5	13.2 ± 0.7	5.8 ± 0.6	556 ± 16.3	68 ± 4.0
Cod, Pacific	67.9 ± 0.4	80.3 ± 0.3	26.5 ± 0.5	17.3 ± 0.3	4.3 ± 0.3	1.2 ± 0.1	304 ± 5.9	30 ± 0.8
Cod, sketo	67.4 ± 0.8	80.0 ± 0.6	25.8 ± 0.9	17.8 ± 0.3	5.2 ± 0.4	1.0 ± 0.1	315 ± 4.1	37 ± 0.9
Anglerfish	82.1 ± 0.8	83.2 ± 1.2	11.5 ± 0.2	13.1 ± 0.8	5.3 ± 0.4	2.5 ± 0.6	312 ± 11.8	32 ± 2.2
Flounder, Pacific	70.2 ± 0.7	77.2 ± 0.6	21.2 ± 0.8	19.9 ± 0.7	7.3 ± 0.5	1.6 ± 0.2	543 ± 9.4	43 ± 0.2
Flatfish, Pacific	68.4 ± 0.3	80.4 ± 0.5	27.2 ± 0.1	16.1 ± 0.3	3.3 ± 0.2	2.4 ± 0.2	414 ± 7.8	48 ± 0.4
Flatfish, nameta	68.3 ± 0.6	79.0 ± 0.6	27.1 ± 0.1	17.9 ± 0.7	3.5 ± 0.4	2.1 ± 0.3	424 ± 9.8	41 ± 0.8
Squid	70.0 ± 0.4	81.0 ± 0.2	23.4 ± 0.7	16.6 ± 0.5	5.1 ± 0.6	1.1 ± 0.1	374 ± 4.7	252 ± 5.8
Small poulp	77.1 ± 0.3	82.2 ± 0.7	17.3 ± 0.1	14.8 ± 0.3	4.4 ± 0.3	2.4 ± 0.4	186 ± 8.6	87 ± 2.4
Crab	55.4 ± 0.6	76.7 ± 1.1	30.2 ± 0.5	18.2 ± 0.7	13.0 ± 0.8	3.9 ± 1.2	494 ± 13.0	88 ± 1.8
Sea urchin	74.1 ± 1.1		16.3 ± 0.5		8.4 ± 0.7		310 ± 6.7	

[a] R = Roe; Mu = Muscle; M = Mean; SD = Standard deviation.

Table 2–1—Amino acid composition of the roe and muscle of finfish (g/16 g N)

	Bluefish		Sea bream		Mackerel		Mullet		Sardine		Herring		Salmon, chum	
	R[a]	Mu	R	Mu	R	Mu	R	Mu	R	Mu	R	Mu	R	Mu
Asp.	8.75	11.78	9.55	10.87	8.53	9.45	7.42	9.98	7.91	10.34	8.41	10.72	9.26	10.60
Thr.	4.87	4.58	4.67	4.42	4.29	4.14	4.39	4.15	5.05	4.35	5.55	4.35	4.31	4.58
Ser.	4.59	3.24	3.21	2.88	5.47	3.17	4.19	3.24	4.93	3.03	4.37	3.50	3.54	3.38
Glu.	13.25	16.53	13.82	15.57	12.38	13.77	13.53	14.64	13.23	16.10	12.97	16.71	12.46	16.04
Gly.	3.31	3.75	4.61	4.15	4.12	3.73	3.24	5.16	3.31	4.03	3.46	4.14	2.34	4.24
Ala.	7.03	5.77	6.13	6.10	7.31	5.49	7.29	5.94	7.66	5.76	8.11	6.36	7.74	6.34
Val.	6.26	5.21	6.04	5.76	5.82	5.37	6.29	5.10	6.87	5.48	7.17	5.49	7.37	5.83
Met.	3.20	3.51	2.82	3.43	2.77	3.07	2.53	2.99	2.96	3.33	2.90	3.58	3.28	3.65
Ileu.	5.28	4.86	4.87	5.24	5.00	4.73	5.25	4.58	6.03	4.89	6.19	4.82	6.15	5.16
Leu.	8.81	8.50	8.68	8.64	8.32	7.83	8.77	8.09	10.18	8.47	10.73	8.78	10.05	8.85
Tyr.	4.79	3.88	5.16	4.12	5.37	3.79	5.37	3.73	4.27	4.00	5.00	3.88	4.78	4.18
Phe.	4.80	4.49	4.96	4.59	4.74	4.15	4.90	4.17	4.23	4.41	4.53	4.60	5.61	4.66
Lys.	8.27	10.11	7.49	10.45	8.11	9.33	7.22	10.10	8.65	10.62	8.10	10.53	8.59	10.46
His.	2.87	2.76	2.57	3.08	3.53	7.67	2.68	2.81	3.01	4.15	2.57	2.51	3.19	2.22
Arg.	6.46	6.45	7.33	5.71	6.26	5.15	6.72	6.85	5.67	5.67	5.03	5.74	5.45	5.91
Pro.	5.78	3.12	5.64	2.97	4.31	2.50	8.34	4.11	4.36	2.89	5.42	2.70	6.10	3.13
E/NE	0.73	0.72	0.68	0.77	0.68	0.71	0.67	0.69	0.80	0.74	0.81	0.75	0.82	0.77

[a] R = Roe; Mu = Muscle.

Table 2–2—Amino acid composition of the roe and muscle of finfish (g/16 g N)

	Smelt, shishamo		Cod, Pacific		Cod, sketo		Anglerfish		Flounder, Pacific		Flatfish, Pacific		Flatfish, nameta	
	R[a]	Mu	R	Mu	R	Mu	R	Mu	R	Mu	R	Mu	R	Mu
Asp.	9.69	11.60	8.50	10.78	9.57	11.82	10.18	10.89	9.51	11.72	10.14	11.71	8.39	10.79
Thr.	5.24	4.64	4.62	4.32	4.86	4.34	3.61	4.04	4.57	4.67	5.03	4.62	4.05	4.46
Ser.	6.02	3.50	4.46	3.51	4.87	3.16	2.16	2.34	4.22	3.18	4.43	3.57	5.08	4.04
Glu.	12.22	16.92	14.23	16.60	14.04	16.79	13.62	16.76	13.75	16.61	14.31	16.64	14.65	16.54
Gly.	3.03	3.85	3.11	3.89	3.04	3.93	4.85	4.28	3.89	3.82	3.23	4.44	3.08	3.92
Ala.	7.24	6.10	7.48	5.91	7.44	6.12	5.90	5.51	6.55	5.92	6.98	5.89	7.14	5.79
Val.	6.39	5.28	6.47	5.21	6.37	5.19	6.05	5.20	6.15	5.22	6.50	5.21	6.11	5.67
Met.	3.08	3.56	2.80	3.46	2.70	3.53	3.40	3.71	2.72	3.42	2.33	3.42	2.36	3.49
Ileu.	5.59	4.74	6.07	4.78	5.94	4.80	5.09	4.94	5.07	4.92	6.02	4.82	5.82	5.12
Leu.	8.72	8.75	10.09	8.41	10.05	8.45	9.01	8.70	9.17	8.58	10.16	8.40	9.86	8.76
Tyr.	3.95	3.95	5.23	4.01	5.04	3.95	4.76	4.16	4.66	3.94	4.68	3.99	4.84	4.08
Phe.	4.08	4.42	4.45	4.27	4.43	4.47	5.19	4.37	4.55	4.39	4.64	4.33	4.63	4.50
Lys.	7.81	9.91	8.66	10.08	8.33	10.01	8.58	10.65	8.56	10.08	7.92	9.81	8.53	10.34
His.	3.11	2.42	2.63	2.53	2.47	2.52	2.73	2.38	2.64	2.61	2.52	2.32	2.78	2.66
Arg.	6.37	6.34	5.66	6.72	5.63	6.56	7.15	6.87	6.61	6.56	6.05	6.51	5.37	5.81
Pro.	4.90	3.12	5.42	2.86	5.91	2.94	4.38	2.95	4.99	3.10	5.55	3.11	7.08	2.69
E/NE	0.72	0.71	0.76	0.71	0.74	0.71	0.73	0.74	0.72	0.72	0.74	0.70	0.71	0.75

[a] R = Roe; Mu = Muscle.

muscle, while glutamic acid, lysine, and aspartic acid were in lower concentration in the roe than in muscle.

In crustacean and molluscan roe glutamic acid was also the major amino acid, along with leucine, aspartic acid, and lysine. Leucine was in relatively higher concentration in molluscan roe than in finfish roe. Glycine was found at an exceptionally high level (9.29 g/16g N) in the roe of the sea urchin as compared to the roe of the other species examined (2.2 ~ 4.9 g/16g N).

Our finding of proline in the roe of nonprocessed Pacific

Table 3—Amino acid composition of the roe and muscle of molluscs, crustacean, and echinoderm (g/16g N)

	Squid R[a]	Mu	Small pouple R	Mu	Crab R	Mu	Sea urchin R
Asp.	10.38	9.80	9.44	10.88	8.89	9.37	9.98
Thr.	6.04	3.96	5.65	4.34	4.89	3.84	3.95
Ser.	4.33	3.45	4.40	4.14	3.11	3.24	1.50
Glu.	13.53	15.59	16.29	17.00	14.59	14.76	11.63
Gly.	2.31	3.89	2.21	4.41	4.10	5.95	9.29
Ala.	4.03	6.15	3.23	5.48	4.75	6.39	4.56
Val.	7.35	4.29	7.03	4.60	6.81	4.56	5.52
Met.	2.93	3.16	2.82	3.20	3.33	2.80	2.94
Ileu.	8.27	4.63	7.10	5.00	5.78	4.35	4.42
Leu.	11.57	8.25	11.93	8.86	9.70	7.35	7.06
Tyr.	4.41	3.56	5.71	3.91	5.59	3.96	4.72
Phe.	3.65	4.07	3.85	4.38	4.65	4.18	4.63
Lys.	9.24	8.69	9.79	9.05	7.46	7.87	7.87
His.	2.35	2.05	2.86	2.42	3.24	2.47	2.60
Arg.	6.28	7.75	6.23	7.20	6.85	8.45	7.91
Pro.	4.42	7.29	3.76	2.80	4.80	4.56	4.08
E/NE	0.93	0.62	0.89	0.68	0.76	0.59	0.65

[a] R: Roe, Mu: Muscle

mullet (8.34 g/16g N) was comparable to 10.39% proline in the roe of salted Atlantic mullet as reported by Lu et al. (1979).

The ratio of essential and nonessential amino acid (E/NE) in roe protein is presented in Tables 2 and 3. The E/NE ratio in the roe of 14 species of finfish averaged 0.74 and was similar to that of corresponding finfish muscle of 0.73. However, the E/NE ratio in the roe protein of molluscs (ca. 0.9) was higher than that of muscle protein of corresponding species (ca. 0.6). It is evident from data generated that roe in general is well balanced with respect to the essential amino acids, has a favorable E/NE ratio, and may be considered as a food source of high-quality protein.

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in pH, raw odor and bacterial numbers and confirmed by taste panel results for treatments V, SP and SPC.

The use of polyphosphate, either by itself or in combination with sorbate, had no effect on bacterial growth.

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Effects of Added Egg White or Whey Protein Concentrate on Thermal Transitions in Rigidity of Croaker Surimi

J. C. BURGARELLA, T.C. LANIER, and D. D. HAMANN

ABSTRACT

Rigidities of surimi [refined minced fish (MF)] sols, alone and in combination with egg white (EW) and whey protein concentrate (WPC) were measured during constant-rate heating by a nondestructive technique. Each of the protein types tested showed measureable changes due to variation of the heating rate or NaCl concentration of the sol. Rigidity and differential scanning calorimetric (DSC) thermograms for mixtures resembled composites of the individual protein source profiles, indicating that no major changes occurred in thermal gelling properties of the component proteins due to mixing. Maximum rigidity values indicated more favorable gelation of MF/WPC than MF/EW combinations.

INTRODUCTION

THE INTRODUCTION of gel-type seafood products such as restructured or simulated shellfish meats to western markets has opened a broad and potentially profitable outlet for mechanically deboned fish meat in the form of surimi, a refined fish protein. This material generally possesses an ability to form strong gels when comminuted with salt and subjected to thermal processing, making it useful as the primary functional ingredient in the manufacturing of many restructured food products.

Nonmuscle proteins capable of forming heat-induced gels might conceivably be used in conjunction with fish muscle proteins in processed food items to lower costs or to modify the product texture in some desired manner. Several researchers have reported difficulty in predicting the functionality of admixed protein systems on the basis of properties manifested by the individual proteins (Yasumatsu et al., 1972; Hsü et al., 1977; Porteous and Quinn, 1979). Additionally, because interactions with nonprotein components of food formulations may have unanticipated effects on protein functionality (Kwasniewska et al., 1979), proteins which function well together in one product may not in another. Therefore a better understanding is needed of the protein interactions which may occur during gelation in mixed protein systems, and the net effects of such interactions on gel structure and texture (i.e. whether additive, synergistic, or antagonistic).

Egg white is a common ingredient of many surimi-based foods. Whey protein concentrates are now available commercially which have gelling properties similar to those of egg white. The present study was conducted to evaluate and compare the development of rigidity which occurs during thermal processing of minced fish (MF) proteins alone and in combination with egg white (EW) or whey protein concentrate (WPC), using a modified version of Thermal Scanning Rigidity Monitor (TSRM). Differential scanning calorimetry (DSC) of these proteins and their mixtures was conducted to supplement the rheological studies.

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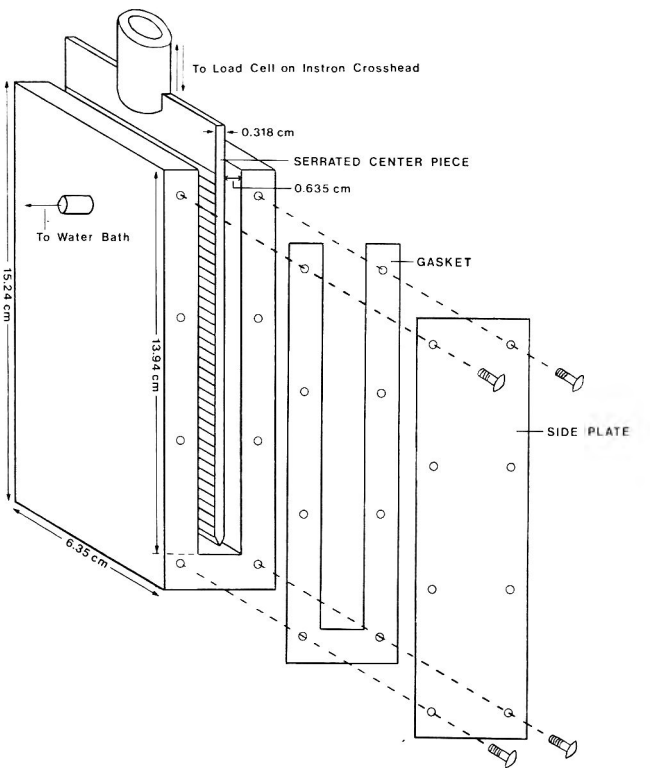

Fig. 1—Diagram of thermal scanning rigidity monitor used for this study.

MATERIALS & METHODS

FROZEN SURIMI prepared from Atlantic Croaker (*Micropogan undulatus*), with 4% sorbitol and 4% sucrose added as cryoprotective agents, was purchased from Nichibei Fisheries, Incorporated (Bayou LaBatre, AL). The surimi was cut into 800g blocks, vacuum packaged in oxygen impermeable bags, and stored at −20°C for the duration of the study. Spray dried egg white solids (type P-11) were obtained from Henningsen Foods, Incorporated (Omaha, NE). Whey protein concentrate (Alacen 882) was provided by New Zealand Milk Products, Incorporated (Petaluma, CA). All ingredients were represented by their respective manufacturer as being of the highest gelling quality available for that type product.

Sample preparation

Frozen surimi was tempered 45-60 min at room temperature and chopped with NaCl in a modified laboratory processor (Lanier et al., 1982) at high speed to a temperature of 2°C. Vacuum conditions were maintained while chopping to reduce air pocket formation in the minced fish (MF) pastes. Upon completion of chopping, the pastes were placed in plastic bags and packed in ice until loaded into the TSRM. Two samples from each batch were processed in succession. Loading of the first sample into the TSRM was accomplished within 20 min of chopping and within 2½ hr for the second.

The protein contents of the egg white (EW) and whey protein con-

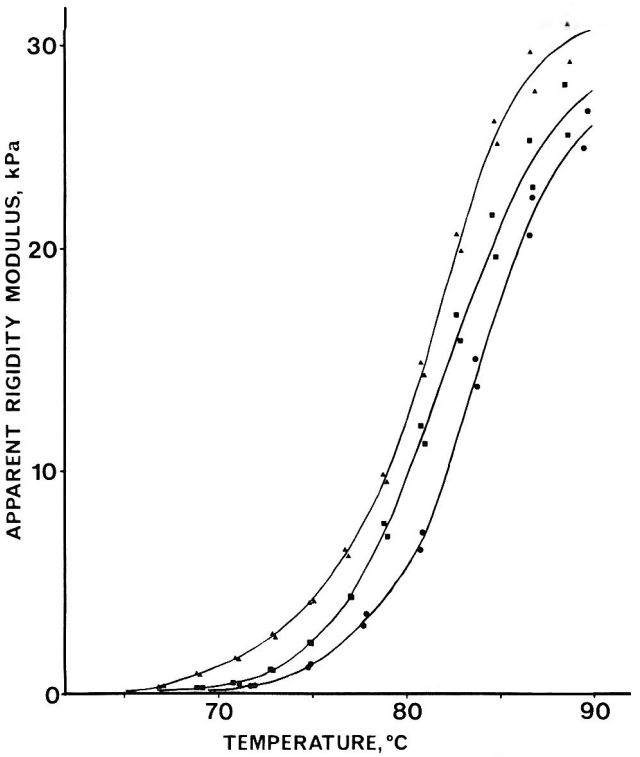

Fig. 2—TSRM thermograms of EW containing 2.0% NaCl heated at 0.5°C/min (▲), 1.0°C/min (■), and 1.5°C/min (●).

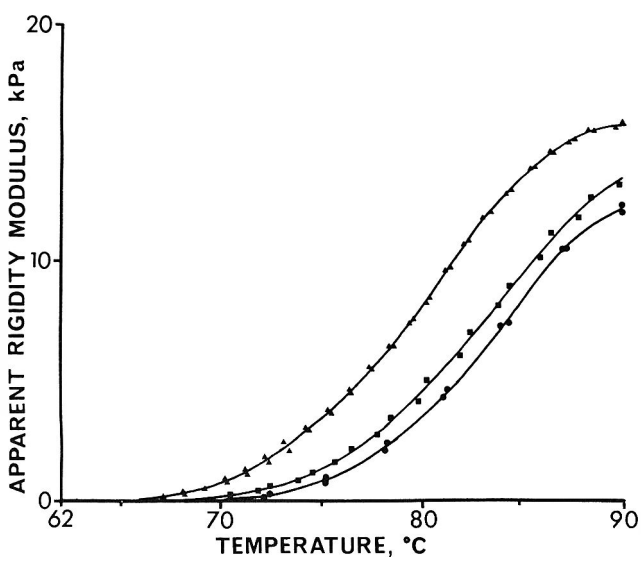

Fig. 3—TSRM thermograms of WPC containing 2.0% NaCl heated at 0.5°C/min (▲), 1.0°C/min (■), and 1.5°C/min (●).

centrate (WPC) powders were determined by micro-Kjeldahl analysis (AOAC, 1980) and 12% protein (85–86% water) EW and WPC sols prepared by dispersing the powders in deionized water at room temperature. NaCl, when added, was dispersed in the dry powder prior to the addition of water. Two samples from each dispersion were processed sequentially in the TSRM under the appropriate conditions as defined by the experimental procedures.

Combination sols composed of MF and WPC or MF and EW admixed in varying ratios of 12% total protein were prepared with 2.0% NaCl added by weight. Tempered MF was chopped under vacuum together with NaCl until crumbly and the EW or WPC powders then added alternately with ice during chopping to yield a homogeneous mixture with an internal temperature of 3–5°C. Total chopping time at a temperature greater than 0°C was approximately 5 min.

"Control" gels consisted of the predominant protein of a mixture,

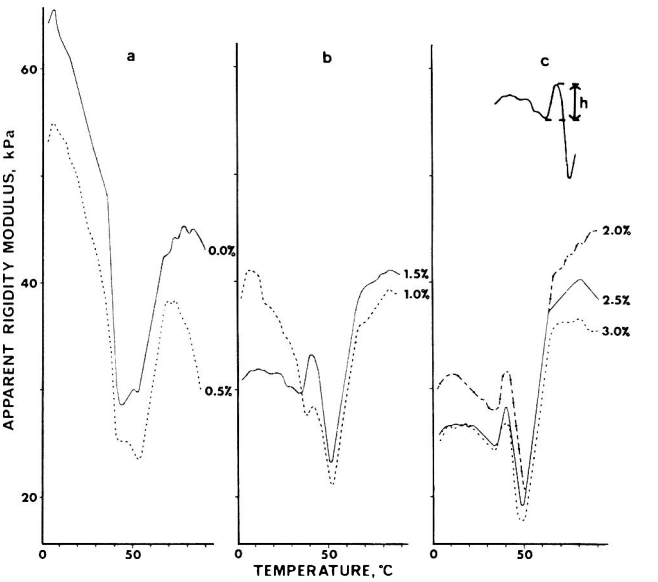

Fig. 4—TSRM thermograms of MF heated at 1.0°C/min, containing 0.0–0.5% NaCl (a), 1.0–1.5% NaCl (b), and 2.0–3.0% NaCl (c). Inset: Relative height (h) of 40°C peak.

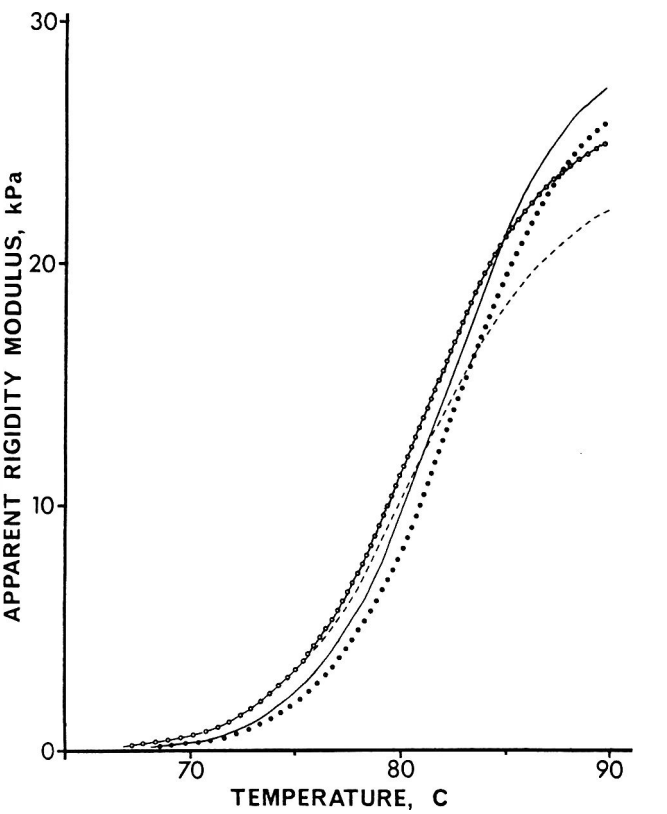

Fig. 5—TSRM thermograms of EW heated at 1.0°C/min, containing 0.0% NaCl (-----), 1.0% NaCl (○ – ○), 2.0% NaCl (—), and 3.0% NaCl (• • • •).

diluted by the amount of extra water which would have been present in the mixture. Thus, "controls" for those mixtures containing 80% or 60% MF consisted of NaCl and MF diluted with the same amount of ice as was added with the EW- or WPC-containing dispersions, but without the added dry protein. For gels containing 80% and 60% EW or WPC, the "controls" consisted of EW or WPC (at 12% protein) diluted with the same amount of water as was present in that amount of fish tissue which was added to the corresponding test mixtures. All samples containing MF were immediately placed in plastic bags and held on ice prior to use.

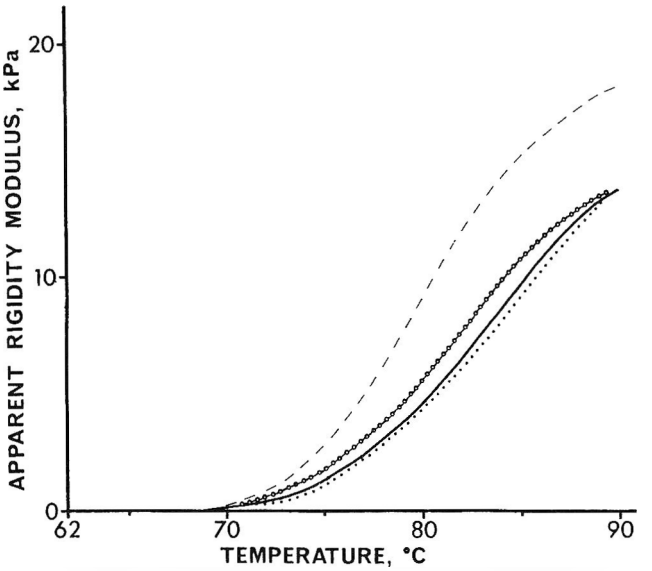

Fig. 6—TSRM thermograms of WPC heated at 1.0°C/min, containing 0.0% NaCl (-----), 1.0% NaCl (○ – ○), 2.0% NaCl (—), and 3.0% NaCl (● ● ● ●).

Modified TSRM

A thermal scanning rigidity monitor (TSRM) similar to that described by Montejano et al. (1983) was developed to continuously measure the rigidity modulus of protein sols during heating. Designed to accommodate both liquid and semi-solid samples, the modified TSRM (Fig. 1) consisted of two parts mounted on an Instron Machine (Model 1122). The brass serrated center piece attached to the Instron crosshead was positioned in the center of the U-shaped water-jacketed main body piece mounted to the base of the Instron. Semi-solid protein pastes were packed into the TSRM and the brass side plates screwed into place. Liquid sols were poured into the device with the sides already in position.

A programmable circulating water bath (Neslab, Inc.) connected to the TSRM served to regulate the temperature schedule. At 2-min intervals during heating the center blade was cycled in an up and down motion at a rate of 0.2 mm/min, bringing about small shear deformations in the sols (peak shear strains were approximately 0.003). Shear forces thus generated in the samples by the motion of the blade were recorded, and rigidity modulus calculated using the following equation:

$$G = (F \times t)/(2 \times l \times w \times d)$$

where G = rigidity modulus, Pa; l = sample length, 0.1394m; w = sample width, 0.0635m; t = sample thickness, 0.00635m; d = peak-to-peak sample deformation, 4×10^{-5}m; F = peak-to-peak force, N.

Instron texture profile analysis (ITPA)

Compression force values were measured at 50% axial compression of cylindrical samples (length, 2.54 cm; diameter, 1.27 cm) according to the methods of Lanier et al. (1982). Such a measurement gives a rough estimate of sample rigidity, independent of failure properties, which should correlate to rigidity as measured in the TSRM. Gels for this measurement were heat processed in capped stainless steel tubes immersed in a water bath, either at 90°C for 10 min or by a two-step process involving first "setting" the protein at 40°C for 30 min followed by processing at 90°C for an additional 10 min.

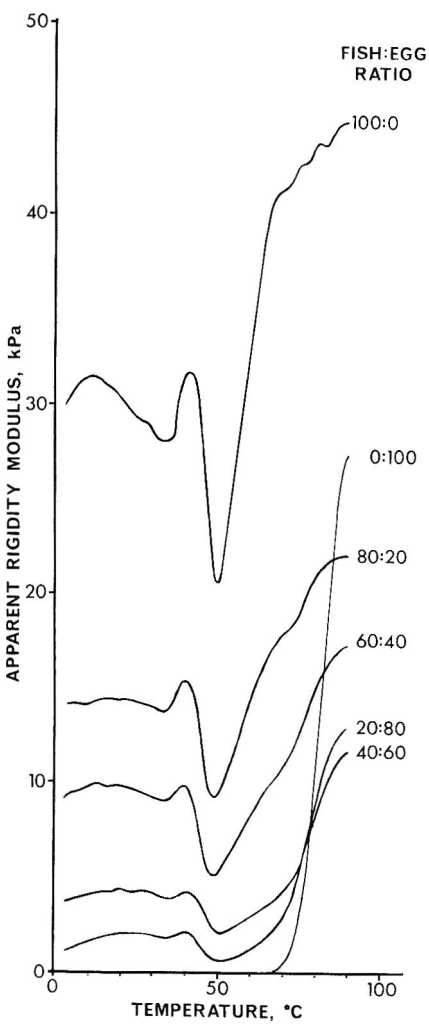

Fig. 7—TSRM thermograms of admixed MF/EW sols.

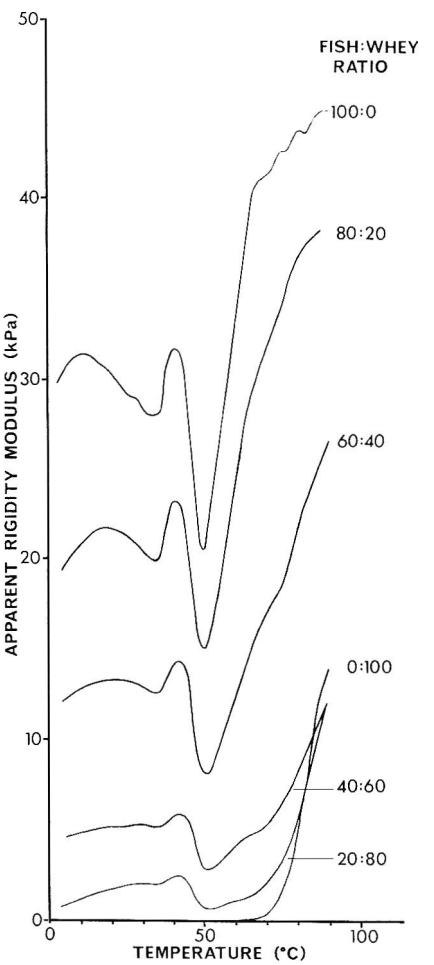

Fig. 8—TSRM thermograms of admixed MF/WPC sols.

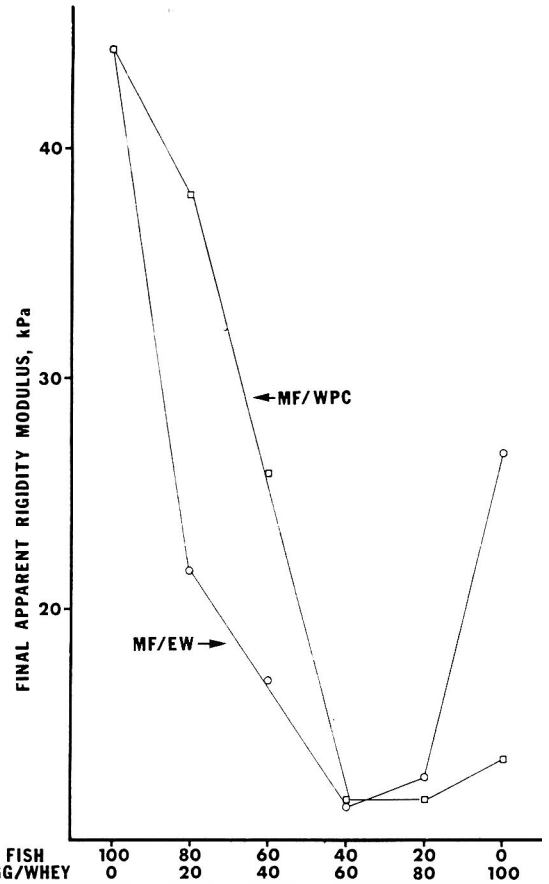

Fig. 9—Final apparent rigidity modulus at 90°C of admixed MF/EW (○) and MF/WPC (□) gels.

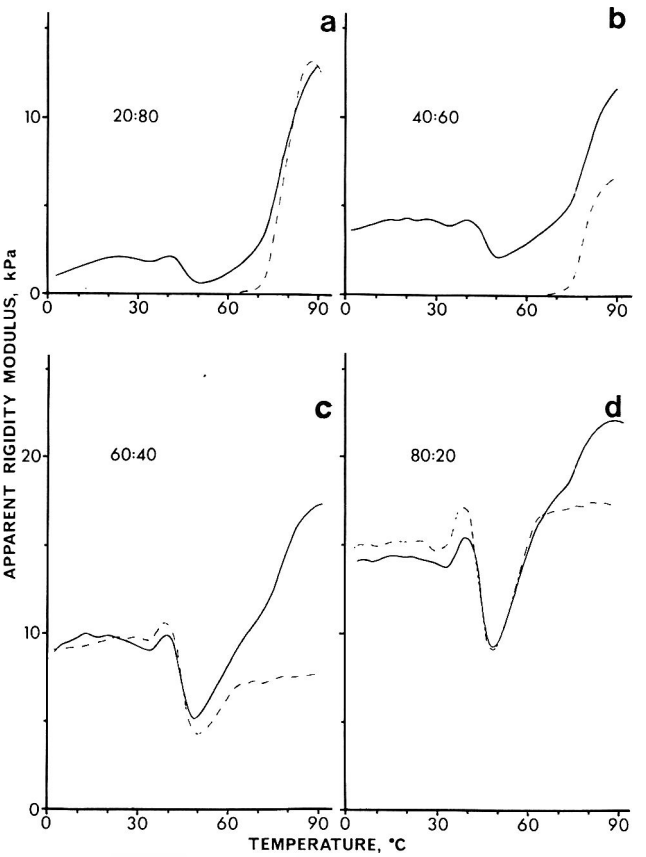

Fig. 10—TSRM thermograms of MF/EW combinations (—) and their respective "controls" (----): (a) EW, 9.6% protein, (b) EW, 7.2% protein, (c), MF, 7.2% protein, (d) MF, 9.6% protein.

Differential scanning calorimetry (DSC)

DSC thermograms of MF, EW, WPC and 1:1 mixtures of MF with EW or WPC sols were performed according to the method of Wu et al. (1985a).

Experimental

To evaluate the effects of NaCl concentration on rigidity development. MF, EW, and WPC sols containing 0.0 – 3.0% by weight NaCl were heated in the TSRM at a constant rate of 1°C/min. Because protein interactions are known to occur in MF sols at low temperatures (Deng et al., 1981; Lanier et al., 1982), all samples containing MF were processed from 2°C to 90°C. However, no change in rigidity modulus was detected in WPC or EW sols at temperatures below 60°C. Therefore, samples containing only EW or WPC were processed in the TSRM after they had been directly preheated to 50°C in a water bath.

To evaluate gel development as a function of the rate of temperature increase during processing, EW and WPC dispersions containing 2.0% NaCl were tested in the TSRM at heating rates of 0.5°, 1.0°, and 1.5°C/min. Effects of the rate of temperature increase on rigidity development in MF sols had previously been examined by others in this labortory (Montejano et al., 1983).

TSRM evlauations of the admixed protein sols were conducted under standardized processing conditions at a 1°C/min rate of temperature increase. At the completion of processing, all gels were subjectively evaluated for firmness by compressing between the fingers and for syneresis by visual assessment of the amount of liquid released.

RESULTS

THE PURE SHEAR CONDITIONS reported by Montejano et al. (1983) were not possible in the modified TSRM because of the addition of side plates and a bottom to the device. Bottom effects were particularly important, resulting in the introduction of compressive forces generated by the movement of the blade pushing the sample against the bottom of the modified TSRM. The effects of these forces were reflected in results obtained by processing identical MF sols in the modified and unmodified TSRMs (not shown). These data indicated that rigidity modulus as measured in the modified TSRM is greater than that measured in the unmodified TSRM of Montejano et al. (1983). Qualitatively, however, the modified TSRM (hereafter referred to simply as "TSRM") revealed rheological transitions of the same type and at the same temperatures as the TSRM of Montejano et al. (1983), as is evident in the TSRM scans of MF in this paper.

Heating rate effects

As the heating rate was increased, the characteristic rigidity thermograms for EW and WPC shifted to higher temperatures and the final rigidity modulus of each gel decreased (Fig. 2 and 3) in a manner similar to that reported for MF pastes by Montejano et al. (1983). Temperature shifts in these thermograms were more pronounced than previously reported for MF samples (Montejano et al., 1983).

NaCl concentration effects

The thermal gelation of each of the protein systems investigated was found to be sensitive to NaCl concentration (Fig. 4-6). Among these the development of rigidity modulus in the myofibrillar MF proteins was the most dependent on NaCl concentration, within the range studied.

The initial rigidity modulus of MF pastes (Fig. 4) substantially decreased as the NaCl content of the samples increased from 0.0 to 1.0%, and continued to drop, although less dramatically, as the amount of added NaCl was increased to 3.0%. Additionally, the position of the peak noted at 51°C in the MF sol containing no added NaCl shifted downward to 40°C as the salt concentration was raised to 3.0% NaCl. The relative

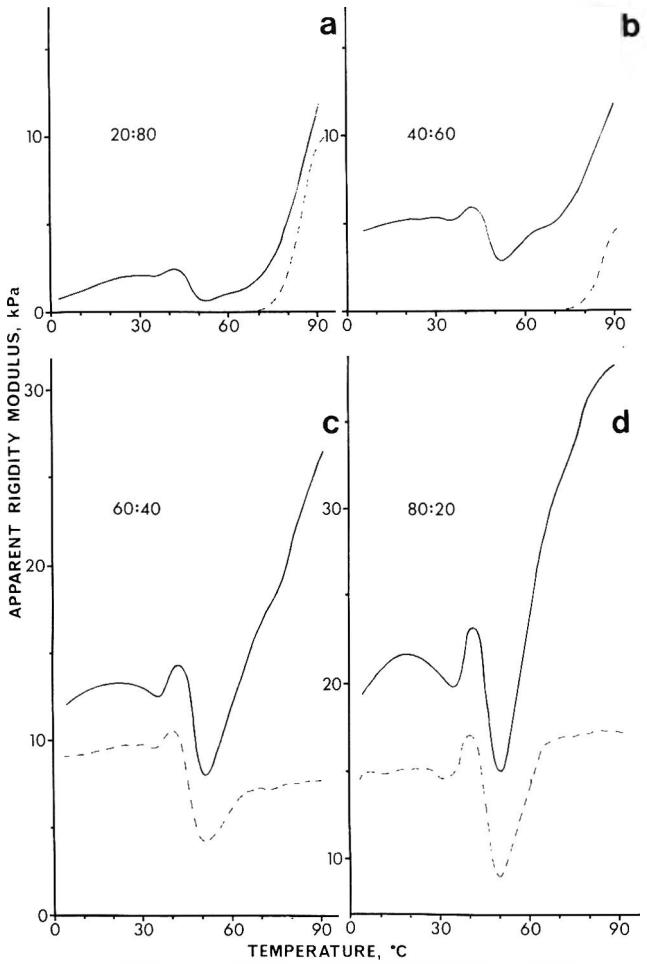

Fig. 11—TSRM thermograms of MF/WPC combinations (—) and their respective "controls" (----) (a) EW, 9.6% protein, (b) EW, 7.2% protein, (c), MF, 7.2% protein, (d) MF, 9.6% protein.

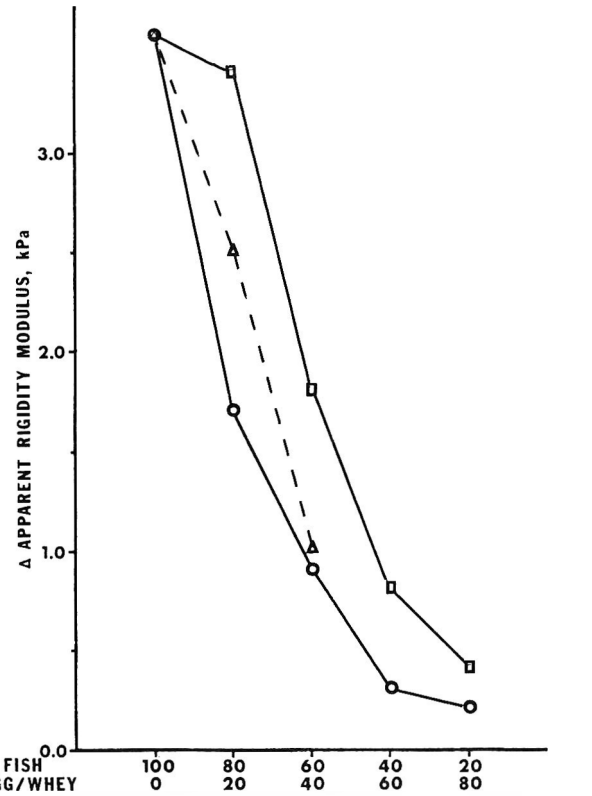

Fig. 12—Relative prominence of 40°C peak in MF/EW (○), MF/WPC (□), and "control" (MF only) sols (△).

height (h) of this peak (Fig. 4, insert) reached a maximum at 1.5–2.5% NaCl and decreased at 3.0% NaCl.

Addition of NaCl appeared to have less effect on the rigidity thermograms of EW than on those of WPC (Fig. 5 and 6). A slight delay in the onset of rigidity development, followed by a somewhat more rapid rate of rigidity increase as compared to the control, was noted in EW sols with increasing salt concentration (Fig. 5). The most rigid EW gel was produced with the addition of 2.0% NaCl to the gel. In the WPC samples the transition from sol to gel began at approximately 70°C regardless of the NaCl content of the gel (Fig. 6). In contrast to EW, both the rate of rigidity development and the final rigidity of the sol were substantially lower in WPC samples containing 1.0–3.0% added NaCl than in the control.

Mixing of proteins

At the completion of heating, the 100% MF and 100% EW gels were more rigid than any of the admixed MF/EW combination gels (Fig. 7, 9). Although WPC alone formed a gel only half as rigid at 90°C as did EW,both the 80% MF/20% WPC and 60% MF/40% WPC gels were at least twice as rigid as the MF/EW samples at the same combination levels (Fig. 8, 9). Additionally, the extent of reduction in the height of the 40°C peak of MF with increasing dilution by WPC was approximately half that noted in MF/EW sols at the same combination levels (Fig. 12).

The characteristic shape of the MF rigidity thermogram, with the dominant transitions at 40° and 50°C, was evident in the thermograms of the admixed MF/EW and MF/WPC sols; however, a general shifting of the curves to lower rigidity values and a flattening of the transition peaks at temperatures below 45°C was noted as the relative proportion of MF decreased. Final rigidity at 90°C in these combination gels reached a minimum at the 40% MF/60% EW level.

Comparison of rigidity thermograms for MF/EW and MF/WPC gels to those of the "control" gels (Fig. 10 and 11) indicates that, at the 20% level, MF proteins did not significantly contribute to the final rigidity of either MF/EW or MF/WPC gels since the "control" gels (no MF) were about as rigid as the combination gels. The final rigidity of each 40% MF combination gel was greater than that of its corresponding "control" (no MF) (Fig. 10b and 11b), indicating MF contributed to the final rigidity at 90°C in these gels.

In the mixed protein sols containing 60% and 80% MF, EW and WPC had different effects on the resultant rigidity thermograms. EW appeared to interfere slightly with the gelation of the MF proteins in the admixed MF/EW sols at temperatures below 50°C as evidenced by the greater rigidity of the "control" (no EW) in comparison to the MF/EW combination samples (Fig. 10c, d). Above 60°C the rigidity modulus of the control gel remained relatively constant, indicating that increases in rigidity of the admixed MF/EW gels above this temperature required the presence of EW proteins.

Conversely, the presence of WPC appeared to enhance the rigidity of the combination sols below 50°C (Fig. 11c,d). Above 65°C, rigidity development in the admixed protein sols again appeared to require the presence of WPC since the rigidity of the "control" gel did not change substantially above this temperature.

The effects of increasing EW and WPC concentrations on the 40°C MF peak of combination sols are shown in Fig. 12. In the 80%/20% and 60%/40% combination gels the relative height of this peak was substantially greater than that of the corresponding diluted "control" (MF only) for the WPC mixture, but less than that of the "control" for EW combinations.

DSC thermograms for EW, WPC and their combinations in

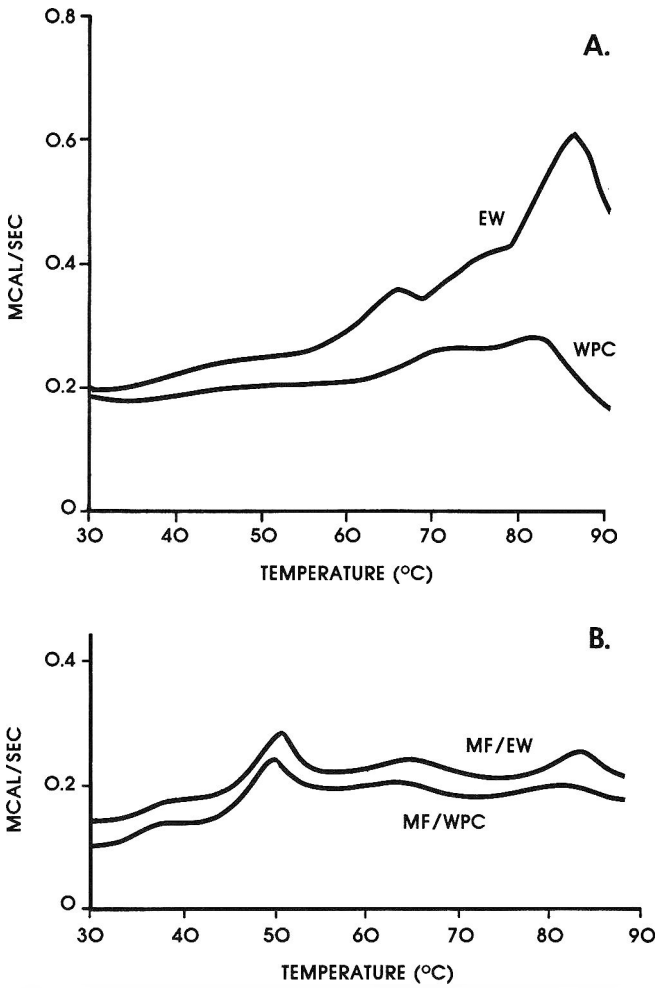

Fig. 13—DSC thermograms of EW and WPC proteins (a) alone and (b) in combination (1:1) with MF. Peaks are endothermic, heating rate was 10°C/min.

a 1:1 ratio with MF are shown in Fig. 13. Both EW and WPC exhibit two distinct endothermic peaks; WPC at 71° and 82°C, and EW at 65° and 86°C, with a third weak transition at a temperature roughly midway between these two. The first transition seems to correspond approximately with the initial development of gel rigidity (Fig. 5, 6). Upon mixing with MF, only a slight shift to lower temperatures of these two major peaks occurred. The peaks at 37° and 51°C correspond to transitions in the MF proteins as has been previously documented (Wu et al., 1985a).

The experiment represented by the data of Fig. 14 was an attempt to determine if simultaneous gelation of fibrillar and globular proteins could be achieved by rapid heating such that better interaction of the two protein types could occur in forming a gel matrix. However, compression force values measured at 50% deformation of the sample thickness, a measurement closely related to the rigidity measurement of the TSRM (Lanier et al., 1982), were not increased as a result of the one stage heating treatment for protein combination gels.

DISCUSSION

RIGIDITY THERMOGRAMS revealed that the gelation of MF sols was characterized by three distinct thermal transitions while that of EW and WPC involved single transitions (Fig. 2, 3, 4). As previously described by Montejano et al. (1983), the three transitions detected with the TSRM for MF sols likely relate to the onset of paste thickening due to protein-protein interactions at 10–15°C, "setting" of the paste into a translucent, cohesive gel near 40°C, and thermal gelation of the proteins into a firm opaque gel at temperatures above 50°C. The single transition identified in the rigidity thermograms of the two globular proteins similarly corresponds to the development of an opaque, firm gel by these sols.

Comparison of the final rigidity modulus of MF, EW, and WPC gels produced under the same processing conditions revealed that MF formed more rigid gels than EW, which in turn formed more rigid gels than WPC. However, because sample deformations during the measurement of rigidity modulus were minute (strains were less than 0.003), reported rigidity values cannot be interpreted as indicative of the gross physical properties at greater deformation (particularly the failure strengths) of these gels. When gel samples were manually manipulated, EW and WPC gels appeared to be more brittle (less cohesive) than the MF gels. EW and WPC gels also demonstrated a tendency to exude water when compressed while the MF samples showed no such tendency.

Heating rate effects

The shift of the rigidity thermograms to higher temperatures, as well as the decrease in final rigidity, noted in the three protein systems with increasing heating rate may both be attributed to the decrease in total processing time which is inherent with a more rapid heating rate. The longer heating time associated with a slow heating rate is thought to allow proteins to attain a greater degree of "order" (Acton and Dick, 1984) which is favorable to creating a fine, cohesive gel structure (Lanier et al., 1982).

Since heat transfer is not instantaneous through a sample, a small temperature gradient exists across the sample which may have also had a minor effect on the temperature shifts noted in the rigidity profiles. To avoid interfering with the measurement of rigidity modulus in the TSRM, sol temperature was monitored by thermocouples placed in the sol adjacent to the two heated surfaces of the TSRM. Hence, sol temperature reported was slightly greater than sol temperature at the blade where the rigidity measurement was actually made. At faster heating rates this gradient would have been steeper, thus resulting in a slight shift in the rigidity thermogram along the temperature axis.

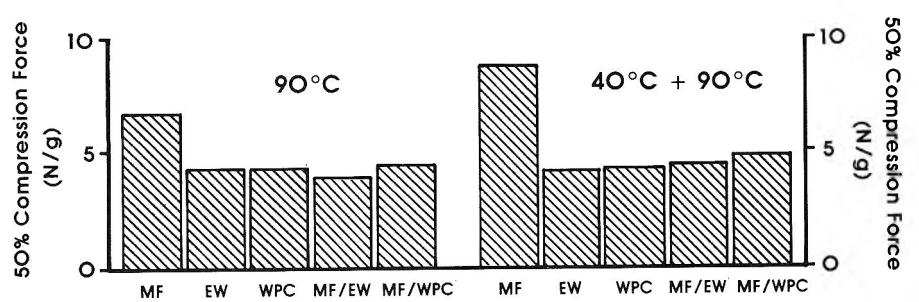

Fig. 14—Compression force measured at 50% deformation of cooked, cooled gels prepared from MF, EW, WPC, and combinations (1:1) thereof. Processing was by single (90°C) or two-step (40°/90°C) heating.

NaCl effects

Addition of NaCl to the protein sols affected the absolute rigidity of the samples as well as the transition temperatures revealed by the TSRM thermograms. In the MF pastes, the decrease in initial rigidity modulus modulus observed with rising NaCl concentration suggests the formation of more fluid and less particulate pastes due to the increasing solubilization of the salt-soluble myofibrillar proteins and dissolution of the myofibrils. The shift of the prominent middle peak to lower temperatures may reflect a NaCl-induced destabilization of the myofibrillar protein structure (Quinn et al., 1980). A similar shift in the thermograms of MF sols toward lower transition temperatures with increasing ionic strength has been reported by others in this laboratory using differential scanning calorimetry (Wu et al., 1985a). The increase in the relative height of this middle peak with increasing salt concentration may reflect a strengthening of the hydrophobic interactions thought to occur in MF sols in this temperature range (Lanier et al., 1982). Stronger hydrophobic interactions have been reported in rabbit myosin with increasing salt concentration, the strengthening effect being attributed to the decreased solubility of the nonpolar residues with the increasing polarity of the solvent (Josephs and Harrington, 1968).

Joly (1965) stated that various proteins were affected differently by neutral salts. The destabilizing effect of salt with respect to gelation temperature of the MF proteins is an effect common to muscle proteins (Quinn et al., 1980). Hermansson (1979) reported that NaCl improved the heat stability of soy proteins to coagulation but not of WPC; the present results indicated no destabilization of either EW or WPC with respect to gel initiation temperature.

Mixing proteins

Both TSRM (Fig. 7, 8) and DSC (Fig. 13) results indicate that MF, EW, and WPC exhibited little or no change in the temperatures at which transitions in rigidity development or energy uptake occurred as a result of their being admixed prior to heat treatment. These methods alone cannot yield information concerning the nature or degree of any direct interactions between protein types; therefore it is possible that interactions between protein types did occur, but did not affect the stability of the proteins with respect to the temperatures at which conformational changes or gel network development transpired.

TSRM data can, however, be useful in assessing the degree to which proteins cooperate in the gelation process to affect the gel rigidity. Final rigidities (90°C, Fig. 9) of all MF/EW mixtures were lower than the final rigidity of pure MF or EW. While two mixtures of MF/WPC (80/20, 60/60) had greater final rigidity than WPC alone, the plotted points for the final rigidities of the remaining mixtures lie well below an imaginary line connecting the points for pure MF and WPC. Such a line should represent the expected rigidity values of all combinations of MF and WPC if these proteins gelled in a truly additive manner. These data thus indicate that the two protein types in both cases do not seem to cooperate in gel formation; rather than one protein seems to "dilute" the other, or interfere with its gelation in some way.

It was considered that this may have been an artifact of the constant rate heating used in the TSRM, such that the WPC and EW proteins may have become reactive well above the temperatures at which gelation of MF proteins was essentially complete. However, the data of Fig. 14 indicate that rapid heating at a high temperature (90°C) was not effective in increasing the gel strength of the protein mixtures relative to that of the unmixed protein gels. These results indicate that a more cooperative gelation of the two protein types is not likely to be accomplished solely by manipulation of the heating schedule.

The negative interaction with respect to gel rigidity development was more pronounced in the case of MF/EW mixtures than for MF/WPC (Fig. 9). There was also a more marked reduction in the height of the 40°C peak of MF/EW as compared to WF/WPC gels (Fig. 12), which might indicate a reduced capacity of the MF proteins to form an orderly protein gel network (Acton and Dick, 1984; Lanier et al., 1982). Thus, there must be specific protein effects since WPC and EW have similar gelation temperatures but different effects on gel rigidity development when mixed with MF. This is supported by a report (Chang, 1982) that mixtures of WPC and EW produce a gel which is of poorer texture and lower water holding capacity than that produced by gelation of either protein alone.

Positive interactions with respect to rigidity development are detectable when the rigidity of mixtures is compared to that of the respective "control" samples (Fig. 10b and 11a). When MF proteins were present at 20% of the mixture, a concentration which in pure solution did not allow for detectable rigidity development, they did not contribute to the final rigidity of either admixed system. However, at the 40% MF level, the fish proteins in pure solution could be shown to develop rigidity, and the final rigidity of each 40/60 combination was greater than that of its respective "control". Likewise, rigidity increases at temperatures above 60°C for the 60/40 and 80/20 MF/EW and MF/WPC combinations (Fig. 10c, d and 11c, d) required the addition of the globular protein component. The latter increases cannot be solely attributed to gelation of the globular component since it was present at low concentrations in these mixtures. This positive interaction with respect to gel rigidity development is likely attributable to a "filler" effect of one protein on the other; that is, in each case the mixture has a higher solids/lower free moisture content than the "control" samples, such that one protein "fills" the interstitial spaces of the protein network formed by the other. This would be similar to the case of adding starch granules to MF sols to toughen the gel structure as it has been shown that the starch granules absorb water and swell within the protein gel matrix, but do not seem to directly interact with the proteins of the matrix (Suzuki, 1981; Wu et al., 1985b).

CONCLUSIONS

RESULTS of this study indicate that the TSRM was sensitive to rheological transitions which occurred in protein sols during heating. The effects of heating rate on transition temperature were likely due to differences in total processing time (total heat input) associated with different heating rates, while the addition of NaCl induced measurable but differing effects among protein systems. In admixed MF/EW and MF/WPC systems, the data suggest that the proteins gelled independently of one another. The admixing of fish (primarily fibrillar) with egg or whey (primarily globular) proteins seemed to result in a "dilution" of one protein sol by the other, such that gels prepared from mixtures were of a lesser rigidity than an additive relationship would predict.

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Gel Strength Development During Heating of Surimi in Combination with Egg White or Whey Protein Concentrate

J. C. BURGARELLA, T. C. LANIER, D. D. HAMANN, and M. C. WU

ABSTRACT

Gel strengths (work of penetration) of surimi (refined minced fish, MF) alone and in combination with egg white or whey protein concentrate were periodically measured by an annular pump during constant rate heating. Combination gels containing low percentages of MF were of a lesser strength than an additive relationship would have predicted. However, the strength of gels containing greater percentages of MF did relate to the gel strengths of the uncombined proteins.

INTRODUCTION

A PREVIOUS PAPER (Burgarella et al., 1985) described changes which occur in rigidity of fish, egg, and whey protein combinations during the thermally induced sol-gel transformation, as measured by a thermal scanning rigidity monitor (TSRM). While such a dynamic test for rheological properties is useful for nondestructive detection of rheological changes which occur in protein sols during heating, it employs only small sample deformations and therefore it reportedly would not be satisfactory for predicting some rheological properties only measurable at or near structural failure of fish gels (Hamann and Webb, 1979).

The purpose of the present work was to acquire measurements of the structural failure properties of gels prepared from fish, in combination with egg or whey proteins, which would be comparable to previously reported rigidity data for such mixtures obtained by TSRM (Burgarella et al., 1985).

MATERIALS & METHODS

THE MATERIALS used and sample preparation methods were the same as those reported by Burgarella et al. (1985). Briefly, frozen surimi (refined minced fish, MF), prepared commercially from Atlantic croaker (*Micropogan undulatus*), was tempered and chopped to a paste under vacuum with 2% added NaCl. Egg white (EW) and whey protein concentrate (WPC) sols were prepared from commercially obtained dry protein powders by dispersing the powder with 2% added NaCl in deionized water at room temperature. Combination sols were prepared by alternate addition of dry protein powder during chopping of the MF paste. All sols were prepared to have a protein concentration of 12% (moisture content 85–86%).

Annular pump

An annular pump apparatus similar to that described by Hickson et al. (1982) was employed to measure the work done in penetrating gel samples with a cylindrical strainless steel plunger (rounded tip, 5 mm in diameter) traveling at a rate of 100 mm/min into the samples. The force exerted as the plunger traveled a distance of 1.5 cm after establishing contact with the gel surface was recorded. Work of penetration was calculated from force-displacement curves.

Samples for the annular pump test were in capped culture tubes (1 cm i.d. × 7.5 cm) submerged in a water bath heated at a rate of 1°C/min temperature increase. Two tubes were removed from the bath at approximately 4°C intervals from 38°C to 90°C, immediately placed in a sample holder, and the test performed.

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RESULTS

WORK THERMOGRAMS for MF and the protein mixtures (Fig. 1, 2) are reported only for measurements made at 38°C and above. This was due to the insensitivity of the test to low temperature transitions in the MF sols prior to the onset of thermal gelation. Work values thus obtained for 100% MF sols increased with increasing temperature up to 65°C, then decreased, leveling off near 85°C (Fig. 1).

The initial gelling transition for both the 100% EW and the 100% WPC sols occurred near 65°C (Fig. 1, 2), approximately the same temperature at which gelation of these proteins was reported detectable using a TSRM (Burgarella et al., 1985). However, unlike the TSRM data, penetration data did not show maximum values at 90°C for both proteins. The maximum work value for EW, obtained near 85°C (Fig. 1), was approximately twice the maxiumum value for WPC, obtained at 90°C (Fig. 2). A similar relationship was reported between the max-

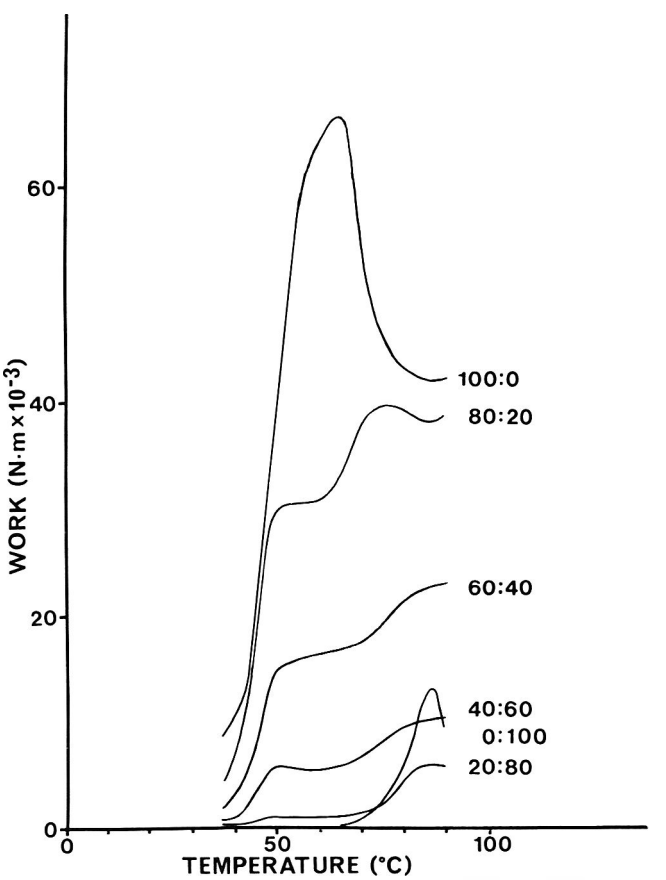

Fig. 1—Work required to penetrate minced fish/egg white gels in annular pump device during ramp heating (1°C/min). Relative proportions of minced fish: egg white are shown for each curve.

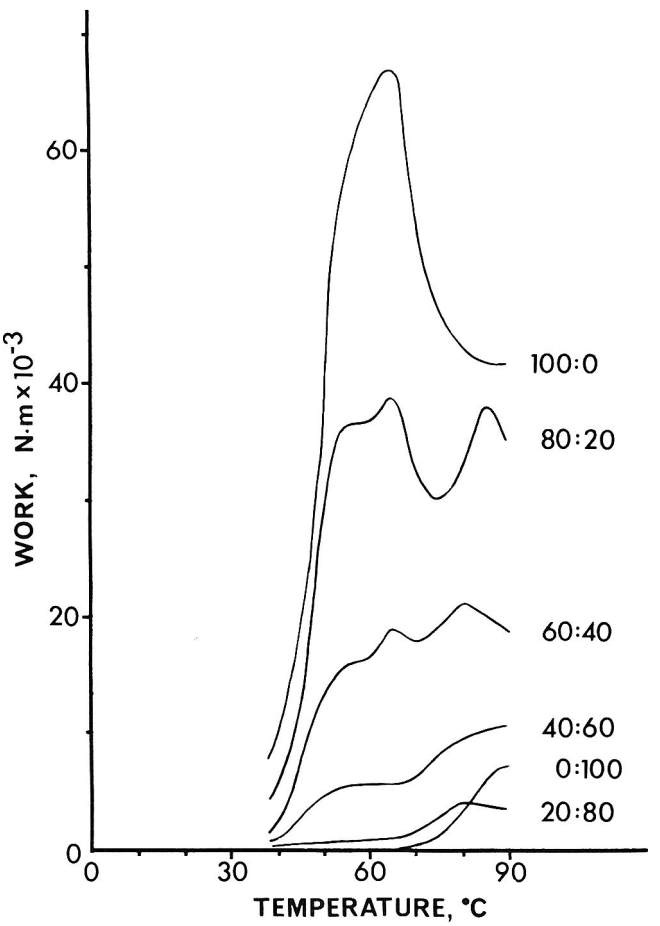

Fig. 2—Work required to penetrate minced fish/whey protein concentration gels in annular pump device during ramp heating (1°C/min). Relative proportions of minced fish:whey protein concentrate are shown for each curve.

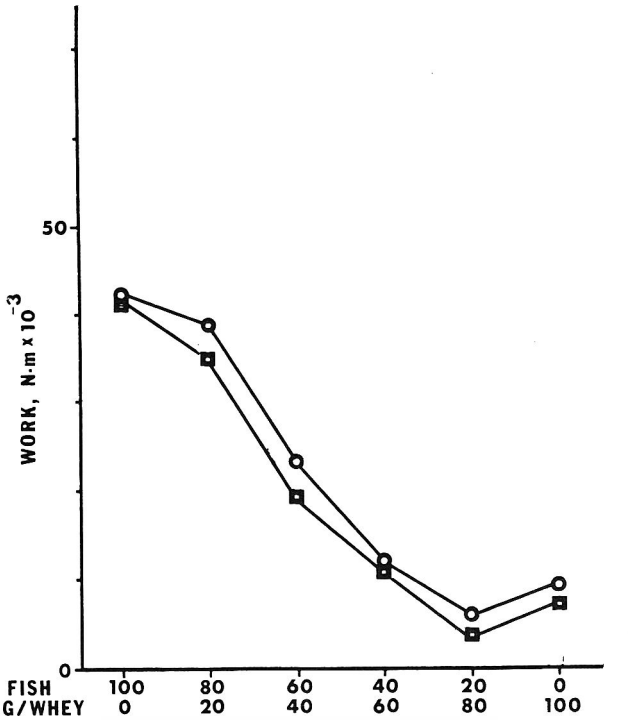

Fig. 3—Work required to penetrate minched fish/egg white (○) and minced fish/whey protein concentrate (□) gels upon heating to 90°C in the annular pump device. Relative proportions of minced fish to egg white or whey protein concentrate are shown along the ordinate.

imum rigidity values of EW and WPC gels measured by TSRM (Burgarella et al., 1985).

In the mixed protein gels (Fig. 1, 2), the temperature at which the globular protein component apparently began to gel was marked by an increase in the work required to penetrate the gel. This transition occurred at 60–65°C for the MF/EW samples and at 65–70°C for the MF/WPC samples. Following this transition, the work thermograms for MF/EW combinations generally rose to a single peak or plateau, while the work thermograms for MF/WPC combinations at 80/20 and 60/40 ratios subsequently showed two marked peaks.

DISCUSSION

THE ABSENCE of any drop or plateau in the work thermograms between 40°C and 60°C, as was so evident in TSRM thermograms of MF sols (Burgarella et al., 1985; Wu et al., 1985), suggests that the annular pump measurement is sensitive to cohesive properties (i.e., deformation to failure) of the material in addition to rigidity properties. While the rigidity of MF sols reportedly ceased to rise between 40°C and 50°C (Burgarella et al., 1985; Montejano et al., 1984a), the elasticity and cohesiveness are known to increase over the same temperature range (Lanier et al., 1982; Montejano et al., 1984a).

At temperatures above 50°C, the work thermograms for 100% MF sols resemble TSRM rigidity thermograms recently obtained by Wu et al. (1985) for surimi sols. The latter thermograms, obtained on samples of high (85%) moisture content, showed a marked decrease in the rigidity modulus at approximately the same temperature at which such a drop occurs in the present work thermogram for 100% MF sols (near 63°C, Fig. 1, 2). Kim (1984) also observed such a decline in the TSRM rigidity thermograms of surimi samples which had been subjected to extensive freeze-thaw denaturation. While no decline was noted by Burgarella et al. (1985) in the rigidity of MF sols at 63°C, a plateauing at this temperature was apparent. Montejano et al. (1984a) observed a slight decrease in the slope of TSRM rigidity thermograms for surimi at 63°C. It would seem from these reports that a decline in the rigidity thermogram near 63°C is most evident when the concentration of functional myofibrillar protein is low, thus yielding a weaker gel structure.

The evidence gathered to date would thus suggest that for proteins of Atlantic croaker, over the normal temperature range of heat processing (40–90°C), there are two main transitions in rigidity development and a single transition in cohesiveness development. With constant rate heating at 1°C/min, development of both cohesiveness and rigidity is substantially completed upon reaching a temperature of about 63°C, whereupon these rheological properties may decrease (presumably reversibly) with increasing temperature.

Work thermograms for 100% EW sols (Fig. 1) peaked near 85°C and declined at higher temperatures; whereas the TSRM thermograms for equivalent sols reported earlier by Burgarella et al. (1985) showed only a slight decrease in slope at this temperature. However, TSRM thermograms of native egg white, obtained from fresh egg and tested in the same instrument of Burgarella et al. (1985) by Montejano et al. (1984b), did show a distinct plateauing of the curve beginning at this temperature. The EW sols used in the present study by Burgarella et al. (1985) were of a higher protein content (12% vs 10% for native egg white), perhaps explaining their greater resistance to such a decrease in rigidity at this temperature. This difference in response may also be due to differences in functionality be-

tween native and spray-dried egg white. Just as a decrease in strength at 63°C was very apparent in the work thermograms of MF gels above, the decrease in integrity of EW gels at higher temperatures was more evident in thermograms obtained by the annular pump which entails higher strain values than does the TSRM.

Annular pump work thermograms of 20:80 and 40:60 MF/EW or MF/WPC mixtures (Fig. 1, 2) seem to show a single gelling transition for each protein type, beginning near 38°C for MF and near 65°C for EW and WPC. These may correspond to the temperatures of gel initiation for each protein type within the mixture since these temperatures are identical to those for gel initiation of these proteins as pure sols. Similarly, essentially no shifts were noted previously by Burgarella et al. (1985) in the DSC or TSRM transition temperatures of individual proteins upon their being mixed.

In the 80/20 MF/EW mixture, however (Fig. 1), the second marked rise in work of penetration occurred approximately 5°C earlier than the temperature at which pure EW sols begin to gel. Examination of the 80/20 and 60/40 MF/WPC mixtures (Fig. 2) also showed an increased slope at this temperature, about 5°C below the temperature at which gelation of pure WPC could be detected. A probable explanation is that the single large peak in the 100% MF work thermogram (Fig. 1, 2) is actually the result of two thermal transitions occuring in series. The presence of EW or WPC may weaken the MF gel structure in the combination gels so that two clear transitions are revealed in this region.

Note also that the extent of the rise in work of penetration values prior to the first plateau was less in MF/EW mixtures than in MF/WPC mixtures. A "setting" phenomenon is known to occur in MF proteins at these temperatures (40–50°C) which greatly increases the cohesive properties of the gel. Evidently, EW interferes with this initial rise in the work thermogram more than WPC: a similar effect of EW has been seen in the reduced rigidity values of MF/EW mixtures as compared to MF/WPC mixtures of the same MF content (Burgarella et al., 1985).

The increase in work of penetration beginning near 63°C for the 80:20 MF/EW mixture and near 75°C for the 80:20 MF/WPC mixture, which in both cases must be attributed, at least partially, to gelation of the globular component, are quite large considering the very low concentrations of EW or WPC in these mixtures. Similar effects on TSRM measurements occurred in mixtures at this ratio as reported earlier (Burgarella et al., 1985). This was attributed to a "filler effect" of one protein on the other such that, upon gelation of the globular component, the interstitial spaces of the already-formed MF gel network may be filled by solidified EW or WPC, forming a stronger structure than would be possible by gelation of either component alone at that concentration.

In the work thermogram of the MF/WPC mixture, 80/20 ratio (Fig. 2), a trough separate two peaks occuring at 65°C and 85°C. The 65°C peak may be attributed to MF while the 85°C peak may be caused by gelation of WPC, probably accentuated by the presence of a MF gel network. The trough is notably absent in the work thermogram of the MF/EW mixture. This would suggest that EW "filled" the MF gel structure more effectively, in terms of gel strength, over the 65–75°C range; the gelation of WPC seems to exert no noticeable effect below 75°C.

The overall decrease in work of penetration for mixed protein gels with increasing globular protein content (Fig. 1 and 2) paralleled a previously reported shift in rigidity thermograms of fish sols to lower values with increasing globular protein content (Burgarella et al., 1985). As with rigidity measurements, initial work values for some MF/globular protein mixtures were less than for the globular protein material alone (Fig. 3), indicating a "dilution" of one protein material by the other rather than an additive or positively synergistic interaction of the two protein types.

CONCLUSIONS

FINAL WORK VALUES (at 90°C) obtained from annular pump thermograms and of MF/EW and MF/MPC mixtures at lower levels suggested a "dilution" of one protein sol by the other, i.e., final gels prepared from mixtures low in MF content were of a lesser strength than an additve relationship would have predicted.

However, the final work values of mixture gels high in MF content suggest that MF/WPC, and to a lesser extent MF/EW, mixtures did approximate an additive relationship with respect to textural properties. This is of great practical importance since it is expected that EW and WPC would be used as low percentage gelling adjuncts or extenders, rather than as high percentage replacements, for MF proteins in commercial product applications (Chang, 1983).

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Effects of Salt and Tripolyphosphate on Some Quality Characteristics of Breaded Patties made from Catfish Trimmings

J.M. BURGIN, R.W. ROGERS, and G.R. AMMERMAN

ABSTRACT

Breaded patties formed from mechanically deboned and dewatered catfish mince were treated with 1% NaCl and 0.3% TPP (tripolyphosphate) alone and in combination. NaCl-TPP treated patties were firmer ($P < 0.05$) than the untreated controls and patties treated with NaCl or TPP alone as determined by Kramer shear press and trained panelists. Untrained panelists scored NaCl-TPP patties higher ($P < 0.05$) than NaCl-only and TPP-only patties for flavor and acceptance. Although the moisture/fat ratio was affected by treatment, cook yields of frozen patties were not. Treatment did, however, affect cook yields of fresh patties. Untreated mince averaged 75.2% moisture, 20.6% protein, 3.5% fat, 0.04% calcium, 0.08% phosphorus, 0.76% ash, 705,000/g total plate count and 2.82 mg/kg 2-thiobarbituric acid value.

INTRODUCTION

ANNUAL PRODUCTION of processed farm-raised catfish reached 81.5 million pounds in 1983 (Anonymous, 1984). An estimated 8.6 million pounds of frames and trimmings were generated by the filleting operation. Approximately 20% of this processing by-product can be recovered by deboning frames and trimmings and dewatering the resulting mince (McGilberry et al., 1984).

Although processing techniques for mechanically deboning fish to produce mince are well established in Asia and Europe, United States fish and seafood processors are only recently showing active interest in potential products from minced fish which also can be marketed without further processing. Minced fish can also be formed into acceptable patties and portions and a minimum of added ingredients and further processing. Extrusion, coextrusion and forming processes can be used to produce seafood and fish shapes. Fish mince can be added to meat to form beef-fish patties (King and Flick, 1972), franks and sausages (Miyauchi et al., 1979) or various ingredients can be added to the mince to enhance its acceptability (Mendelsohn, 1972; Morris and Dawson, 1979). Fish mince can also be used to improve the texture and flavor of dips, chowders, sauces, and sticks. Other alternatives are to market fish mince in frozen blocks or as surimi for further processing.

The objectives of this research were to evaluate the effects of added NaCl (sodium chloride) and TPP (tripolyphosphate) on texture, color and flavor characteristics of breaded patties made from MD (mechanically deboned) and dewatered catfish mince. The protein content and textural characteristics of the dewatered mince were sufficiently similar to ground beef to suggest that the mince might form patties without addition of traditional binders such as starches and alginates. Research by Ahmed et al. (1983), King et al. (1974), Shults et al. (1972) and Wierbicki (1983) indicated that 1% NaCl and 0.3% TPP would have maximum effect on texture without affecting flavor. Composition and microbiological characteristics of the mince were also evaluated to serve as a point of reference for this previously unreported raw material.

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MATERIALS & METHODS

Experimental design

The experimental design was a randomized complete block with four treatments replicated four times. A block design was chosen because of anticipated difficulties in obtaining samples and the resultant unequal days in storage before evaluation. The treatments were (1) untreated control; (2) addition of 1% NaCl; (3) addition of 0.3% TPP in the form of Lem-O-Fos (Stauffer Chemical Corporation, Westport, CT) and (4) addition of both 1% NaCl and 0.3% TPP.

Deboning

Four replications were collected from a cooperating catfish processing plant over a 60-day period, from July 27, 1983, to September 27, 1983. Preparation of the deboned mince prior to treatment was the same for each replication. Frames from the hand-filleting operation and belly flaps and other trimmings from Baader (Baader North America Corp., New Bedford, MA) automatic filleting machines were washed approximately 5 min in a Baader 653 washer and then conveyed to a Baader 694 deboner with a 3 mm mesh. The deboned mince was pressed in a Baader 523 dewaterer. Treatments were applied to the dewatered mince.

Samples of the mince for chemical analyses were collected by taking approximately 50g at selected locations from each batch of mince before treatment or patty formation. Samples were packaged in sealed polyethylene bags and stored at −18°C. Mince from the first three replications was analyzed on the 54th, 46th, and 41st day (± 7 days) after collection. Mince from the fourth replication was analyzed approximately 14 days after collection.

Patty formation

Plant equipment for patty formation consisted of a ribbon blender and a Koppens (Koppens Industries, Inc., Stone Mountain, GA) VM-400 patty former. The patties were formed from fresh mince and then conveyed through a Koppens EPRM-900 batter and breading machine and then to a CO_2 IQF tunnel. Batter and breading were proprietary commercial preparations (Giles Enterprises, Birmingham, AL). Dwell time in the tunnel was 7 min. Patties exiting the tunnel were frozen to −24°C. A spiral tunnel was used in the fourth replication with a dwell time of 10 min and an ending temperature of −24°C. The untreated control samples were made by putting the mince directly into the patty former rather than blending first, in keeping with probable commercial practice. Treated samples were prepared in a ribbon blender. Treatment additions were sprinkled in dry form over the mince during the first 30 sec of 3-min blending time.

Packaging

Formed patties were packaged in oxygen impermeable plastic bags and covered with CO_2 snow for transportation to Mississippi State University where they were held overnight in −18°C storage. They were then vacuum packaged in lots of 9 or 12 in oxygen impermeable Mylar packages on a Model B20000H Colmatic Maxivac (Colmatic Corporation, Long Island, NY) vacuum packaging machine and stored at −18°C. With the exception of samples analyzed for cook yields of unfrozen patties, all samples were kept in frozen storage before testing.

Cook yield

Cook yields of fresh and frozen samples were evaluated to determine whether treatment effects on cook yield were affected by freezing. Fresh, unfrozen patties were fried 3 min in vegetable oil at 190°C and drained 1 min. Frozen patties were treated the same except they were fried 5 min. Patties were weighed before and after cooking. Yield of cooked patties was expressed as a percent of uncooked patties. The mean of three determinations was used as the value of each observation.

Texture determination, instrumental

An Allo-Kramer Shear Press was used to measure resistance to shear in patties cooked after frozen storage (Food Technology Corp., Rockville, MD; Model TR-4 Integrating Texturecoder and FTC Model TR-2 Texturepress). All patties were cooked and drained in the same manner as for the cook yield study. Each patty was weighed, broken approximately in half, and placed horizontally, one-half on top of the other, in the compression cell. When the cutting blades were visible below the compression cell, the reading on the Texturecoder was taken. The Texturecoder was set at 30 in lb and readings were converted to g/cm of energy/g of sample. The mean of three determinations was used as the value of the observation.

Fat and moisture of cooked samples

Samples for chemical analyses were fried in the same manner as for the cook yield study and were drained for 1 min. Three samples were prepared from each treatment from each replication, packaged in twirl-bags and refrigerated overnight before being taken to the Mississippi State Chemical Laboratory for analysis of fat and moisture. The purpose of these analyses was to determine whether treatments affected moisture retention and, if so, whether a trained taste panel would detect any differences.

Chemical analyses of untreated sample

Chemical analyses were performed on untreated samples of the deboned and dewatered mince from each replication. AOAC (1980) procedures were followed in determining moisture, crude protein, crude fat, ash, phosphorus, and calcium. Total plate count was determined by the method of Sharf (1966) and TBA (2-thiobarbituric acid) by methods of Tarladgis et al. (1960).

Color

Color analysis was performed by using a Hunter Color Difference Meter Model 25 standardized to white disk No. 2165 ($L=92.7$, $a=-0.6$, $b=-0.2$). Samples were cooked in the same manner as for other evaluations, and the breading was sliced off the top. Color values were read while samples were still warm. Each reading was taken twice with an adjustment to standard between readings when required. The mean of three samples was used for statistical analysis.

Sensory evaluation

Sensory evaluations were conducted in two phases: (1) ranking by trained panelists and (2) hedonic judgments by untrained panelists. Although the degree of exactness required in screening tests and training for expert and trained panels has not been agreed upon, there is a concensus that trained descriptive or difference panelists should not be asked to evaluate attributes in terms of likes/dislikes or acceptability (Larmond, 1970; Peryam, 1957; ASTM, 1968; AMSA, 1978). A trained panel was used to provide sensory data on the saltiness, firmness, moistness and greasiness of the patties. An untrained panel was used to evaluate acceptance of flavor, texture, saltiness and general impression.

All panelists were volunteers from the Mississippi Cooperative Extension Services (MCES) staff. Preparation and taste testing of samples were done in the Home Economics Laboratory Kitchen at MCES. Screening tests were devised to determine whether a potential panelist could perceive differences in saltiness, texture, moisture and fat level. Panelists who passed the screening tests were taught by varying the qualities being evaluated and discussing the differences. With the exception of patties prepared for screening, patties were fried in vegetable oil at 190°C for 5 min, drained 1 min and kept warm in a 65°C conventional oven. Immediately before being served, patties were cut into quarters weighing approximately 14g. Each panelist received a quarter-patty from each treatment.

Each panel, both trained and untrained, consisted of 10 panelists and each set of treatments or block was evaluated at a separate time. A core of 15 panelists was selected and trained so that a panel of 10 could be more easily convened.

Statistical analysis

Statistical analysis of all data was performed using the methods of Steele and Torre (1980) for a randomized complete block. Sensory data were analyzed according to the methods described by Larmond (1970) before being subjected to analysis of variance. Means were separated by the Student-Newman-Keul method (Steele and Torre, 1980).

RESULTS & DISCUSSION

Patty texture

Patty texture was evaluated by the Kramer shear press, trained panelists (Fig. 1) and untrained panelists using an hedonic scale (Fig. 2). The addition of NaCl and TPP in combination resulted in firmer ($P < 0.05$) patties as measured by the Kramer shear press than either the untreated controls or the addition of NaCl or TPP alone. These results agree with reports

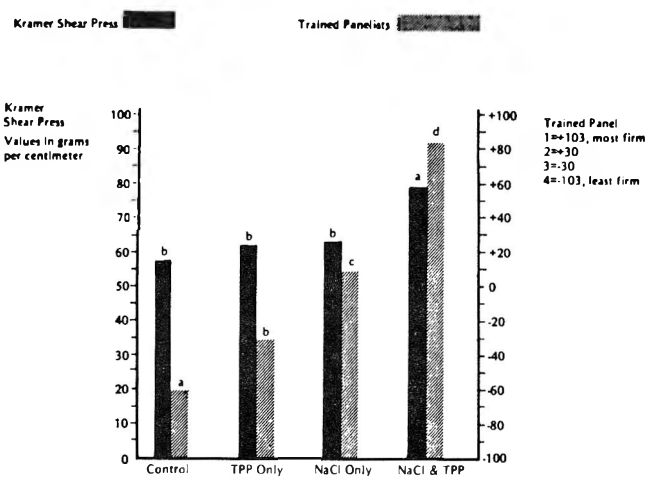

Fig. 1—Texture evaluations of four treatments of breaded patties made from deboned and dewatered catfish mince. Values are means of four replications. [a-d]Bars not marked by the same letter are different ($P < 0.05$).

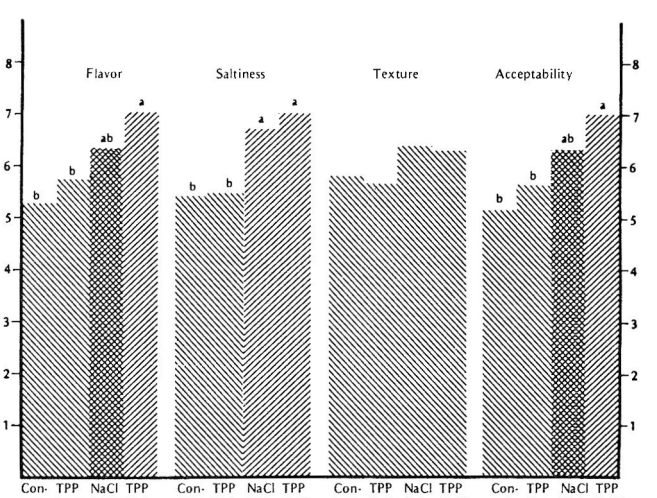

Fig. 2—Panel evaluation of four treatments of breaded patties made from deboned and dewatered catfish mince with panelists using an hedonic scale with 9 signifying most preferred and 1 signifying least preferred. Values are means of four replications. Means shaded differently are different ($P < 0.05$). When shading overlaps, means are not different.

by King et al. (1974), Baker et al. (1981), Ahmed et al. (1983) and Wierbicki (1983). Trained taste panelists ranked NaCl-TPP patties as firmest ($P < 0.05$); NaCl-only patties as second in firmness ($P < 0.05$); the untreated controls as the least firm ($P < 0.05$). Trained panelists distinguished differences in texture that were not found in the Kramer shear press data, although rheological and sensory data did support each other. Baker et al. (1981) also reported a correlation between experienced panelists' evaluation scores and Kramer shear press data.

Untrained panelists using the hedonic scale did not distinguish any differences ($P > 0.05$) in texture among treatments (Fig. 2). The mean score for texture was 6.06 points on a 9-point hedonic scale. The inability of untrained panelists to distinguish textural differences detectable by instruments and trained panelists has also been noted by Miyauchi et al. (1975), Morris and Dawson (1979), and Waters (1982). No textural differences related to days in storage (ranging from 7 to 68) or collection date were detected by any evaluation method used.

Saltiness

Evaluation by trained panelists did not indicate differences ($P > 0.05$) in the perception of saltiness related to the addition of TPP with NaCl. Untrained panelists preferred ($P < 0.05$) NaCl-TPP samples over TPP-only and untreated samples (Fig. 2) but did not score NaCl-only samples differently ($P > 0.05$) from all other samples. These results agreed with Ahmed et al. (1983) who reported that patties with 0.4% TPP and 0.7% NaCl were preferred over different levels of either ingredient.

Fat and moisture of cooked patties

The fat content of patties cooked after frozen storage was higher ($P < 0.05$) in untreated and TPP-only patties (Table 1). Average fat of NaCl-only and NaCl-TPP patties was 10.7%, while average fat of NaCl-treated patties was 13.6%. Samples treated with NaCl or TPP, alone or in combination, had higher moisture retention than the control ($P < 0.05$). Mean moisture of the cooked patties was 57.5% for all treatments except the control which was lower ($P < 0.05$) at 52.2%. The higher moisture of NaCl and NaCl-TPP patties could have resulted from increased water retention and is consistent with results of Suzuki (1981) and Lanier et al. (1982) who reported that addition of NaCl affected formation of protein gels in fish muscle. Increased moisture retention through the use of polyphosphates in ground beef has been noted by Shults et al. (1972), Jacoby and Berhold (1969), and Siegel and Schmidt (1979). There did not appear to be a meaningful relationship between fat and moisture in cooked samples (Table 1) and ranking by trained sensory panelists on the basis of greasiness and juiciness.

Samples from the third replication were lower ($P < 0.05$) in moisture than samples of any other replication (Table 4). This difference was probably not related to storage time, since other samples had longer or shorter storage times. However, input for the mincing operation was an uncontrollable factor and could have influenced the percent of moisture of the samples.

Table 1—Effect of treatments on moisture and fat in cooked, breaded fish mince patties

	Cooked patties	
Treatment	Moisture (%)	Fat (%)
Control	52.19[b]	15.04[a]
NaCl	57.80[a]	10.93[b]
TPP	57.05[a]	12.29[a]
NaCl, TPP	57.65[a]	10.63[b]

[a,b] Means not followed by the same letter within columns differ ($P < 0.05$).

Flavor and acceptability

Untrained panelists rated the flavor and acceptability of NaCl-TPP patties higher ($P < 0.05$) than TPP-treated and control samples (Fig. 2) but did not score NaCl-only samples differently ($P > 0.05$) from all other samples. These results agree with Ahmed et al. (1983) and Baker et al. (1981) who found that at certain levels NaCl and phosphates in combination increased acceptability of minced fish patties. Although ratings for NaCl-TPP samples were not significantly higher than NaCl-only samples, rating were numerically higher for both flavor and satisfaction scores. These results may indicate the subtle role texture plays in flavor perception. Although untrained panelists failed to perceive the texture difference, that difference could have enhanced the flavor of the NaCl-TPP patties.

Cook yields

The addition of NaCl and a combination of NaCl and TPP increased cook yield ($P < 0.05$) in unfrozen patties more than the addition of TPP (Table 2). Mean cook yield of NaCl and NaCl-TPP treated samples was 86.5%, compared to a mean of 81.5% for untreated and TPP only samples. Although the moisture/fat ratio was affected by the addition of NaCl, alone and in combination with TPP, cook yields of frozen patties were not affected by treatment. The mean cook yield of frozen patties was 80%. This difference in cook yield between frozen and unfrozen patties could have resulted from an increase in unbound water during frozen storage. The unbound water would be more readily evaporated during cooking. The higher cook yield from unfrozen patties could have cost benefits to processors of cooked products.

Color

Hunter L and a values were not affected by treatments (Table 3); however, samples stored at −18°C for 37 days were darker in color ($P < 0.05$) than samples stored 85, 90 and 98 days (Table 4). Samples stored at −18°C for 85 days were darker than samples stored 98 days. The steady increase in L values

Table 2—Effects of treatments on cook yield of unfrozen and frozen breaded fish mince patties

	Cook yield	
Treatment	Unfrozen (%)	Frozen[ns] (%)
Control	80[b]	79
NaCl	86[a]	83
TPP	83[b]	78
NaCl, TPP	87[a]	81

[a,b] Means not followed by the same letter within columns differ ($P < 0.05$).

Table 3—Effect of treatments on Hunter Colorimeter values for cooked, breaded fish mince patties

	Hunter Colorimeter Values		
Treatment	L[ns]	a[ns]	b
Control	64.87	−.16	8.97[b]
NaCl	63.31	−.15	8.07[a]
TPP	63.45	−.47	8.18[b]
NaCl, TPP	64.29	−.43	7.37[a]

[a,b] Means not followed by the same letter within columns differ ($P < 0.05$).

Table 4—Differences among blocks of breaded minced fish patties: Hunter L values, Hunter b values and moisture of cooked patties

Days in storage	Hunter values		% Moisture in cooked frozen patties
	L	b	
37	60.80[a]	9.02[a]	57.17[a]
85	63.63[b]	7.19[b]	58.30[a]
90	65.44[bc]	7.97[ab]	50.86[b]
98	66.04[c]	8.43[a]	58.39[a]

[a,b,c] Means not followed by the same letter within columns differ ($P < 0.05$).

over time may have resulted from oxidation which would occur more rapidly in minced fish than in whole or filleted fish. Samples stored 85 days were less yellow ($P < 0.05$) than samples stored 37 and 98 days (Table 4). These results agree with Baker et al. (1981) who reported differences between samples as well as between treatments. They suggested that these differences might be related to time of harvest. NaCl-TPP and treated samples were less yellow than TPP-treated and control samples, a difference that was possibly associated with lower fat in NaCl-treated patties.

Characteristics of the mince

Composition. The composition of the deboned mince after processing in the dewaterer averaged 75.2% moisture, 20.6% protein and 3.5% fat (Table 5). Deboned catfish mince that had not been dewatered averaged 69.6% moisture, 14.3% fat and 13.0% protein (Brooks, 1983). The percent of fat appeared to be greatly reduced by dewatering with a corresponding increase in the percentage of moisture and protein. This effect is not surprising since catfish lipids are liquified in the deboning process. The mince is more defatted than dewatered. Dewatered mince is also lower in fat and higher in protein than fillets from one-pound whole-dressed catfish which Yang (1983) reported to contain 76% moisture, 6% fat and 17% protein. The composition of MD whole dressed carp (*Cyprinus carpio*) was reported as 67% moisture and 15% fat (Rippen et al., 1982) which is similar to deboned, un-dewatered catfish mince. Dewatering catfish mince results in a composition that is similar to MD spot (*Leiostomus xanthurus*) (Waters, 1982) and whole edible portion of sucker (species not stated, USDA, 1963).

Calcium. Mean calcium levels were 0.04%, which is comparable to levels reported by Rippen et al. (1982) in MD carp and Morris and Dawson (1979) in MD sucker (*Catostomidae*) (Table 5). These levels are lower than allowed in MD products regulated by FDA (1978).

TBA. TBA values of the mince were within the range for MD fish at 0 to 2 month storage as reported by Lee and Toledo (1977) in MD mullet (*Mugil* spp.) and Dawson et al. (1978) in MD sucker (*Catostomus* spp.) (Table 5). Morris and Dawson (1979) had surprisingly low TBA values (less than 0.4) considering that fat was 2.3 – 5.9%. However, these researchers deboned whole dressed fish, not frames and trimmings. Some researchers have reported (Lee and Toledo, 1977) that lateral tissue, tissue along the visceral cavity, and bone marrow exudate appear to be the most susceptible to development of oxidative rancidity.

In addition to composition and origin of the mince, two other factors should be considered when comparing TBA values from independent studies: (1) TBA method used and (2) species of fish being analyzed. Morris and Dawson (1979) and Dawson et al. (1981) used the method of Tarladgis et al. (1960) on sucker (*Catostomus* spp.); Lee and Toledo (1977) used the method of Sinnhuber and Yu (1958) to analyze mullet (*Mugil* spp.). Either differences in species or differences in method could result in different TBA values.

Total plate count. TPC is within range of other species (Baker et al., 1981; Blackwood, 1974; Cann and Taylor, 1976) (Table 5). Higher microbiological counts of mince from frames as opposed to mince from fillets are to be expected according to Licciardello and Hill (1978). They reported that TPC ranged from 30,000 – 3,000,000/g in 208 commercial fish blocks of cod-frame mince. TPC of the catfish mince would be acceptable under the International Commission on the Microbiological Specification for Foods (1974).

Table 5—Composition and characteristics of washed, deboned and dewatered catfish mince

Factor	Average[a]	Range
Moisture %	75.2	73.4 - 76.6
Protein %	20.6	19.7 - 21.9
Fat %	3.5	3.0 - 4.4
TBA	2.82 mg/kg	1.84 - 4.39
Total plate count	705,000/g	200,000 - 980,000/g
Calcium %	0.04	0.03 - 0.06
Phosphorus %	0.08	0.04 - 0.1
Ash %	0.76	0.7 - 0.8

[a] Average of four samples.

Ash and phosphorus. Ash of the catfish mince was lower than that reported by Yang (1983) and Magee (1976) in catfish fillets (Table 5). This might indicate that a larger portion of inorganic materials is located in catfish fillets than along the frame and belly flap. Phosphorus was slightly higher than that reported for fillets by Magee (1976) and USDA (1963). Phosphorus content of untreated mince may have importance for the catfish processing industry in regulating the amount of phosphates added during processing.

DISCUSSION

These research results should be applicable to mince from any fish species if the ratios of fat, moisture and protein are similar. The muscle tissue bound well with the addition of NaCl and TPP in combination. Traditional binding agents, such as alginates and starches, were not necessary.

There were no off-flavor problems with the products stored for the times used in this study. However, some preliminary data from another study indicate that this type of product does have a limited shelf life. Although surimi-based analogs of high-cost seafood are increasingly accepted, formed products from fish mince cost less and are simpler technically to produce, so they can be priced competitively with well-established breaded fish products.

CONCLUSION

AN ACCEPTABLE breaded patty can be produced from MD and dewatered catfish mince recovered from fillet processing by-products by adding 1% NaCl to the mixture before forming the patties. Addition of 0.3% TPP increased firmness as measured by Kramer shear press and trained panelists, but untrained panelists did not detect any differences. Addition of NaCl in combination with TPP resulted in higher scores for flavor and acceptability. Addition of either NaCl or TPP increased the percentage of moisture in cooked patties, thereby decreasing the percentage of fat.

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Formation of Oligosaccharides During Hydrolysis of Lactose in Milk Using β-Galactosidase from *Bacillus circulans*

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ABSTRACT

The characteristics of β-galactosidase from *Bacillus circulans*, and its suitability for hydrolysis of milk lactose were compared with those of *Escherichia coli* and *Kluyveromyces lactis* enzymes. Enzyme activity of β-galactosidase from *B. circulans* was not affected by major cations and proteins present in skim milk, whereas milk proteins and sodium and calcium ions decreased the activity of the enzymes from *E. coli* and *K. lactis*. The enzyme from *B. circulans* produced less oligosaccharides than *E. coli* and *K. lactis* during hydrolysis of lactose in skim milk at different lactose concentrations. Oligosaccharides produced by the enzyme from *B. circulans* disappeared almost completely at the latter stage of hydrolysis, while a large amount of oligosaccharides were still detected even at 98% conversion of lactose with enzymes from *E. coli* and *K. lactis*.

INTRODUCTION

ALTHOUGH there have been many studies, hydrolysis of lactose in milk is still an important problem in nutritional improvement, particularly for people with low intestinal β-galactosidase activity (Holsinger, 1978; Finocchiaro *et al.*, 1980). A number of β-galactosidases from various sources have been tested for hydrolyzing lactose in milk, with their properties such as optimum temperatures, optimum pH's, *Km* values, and types of inhibition. However, only a few papers deal with the production of oligosaccharides by the transgalactosidation reaction during the hydrolysis of lactose in milk (Dahlqvist *et al.*, 1977; Burvall *et al.*, 1979; Greenberg and Mahoney, 1983). Ohmiya *et al.* (1977) studied oligosaccharides production during hydrolysis of lactose in skim milk with immobilized cells of *Lactobacillus bulgaricus*, *Escherichia coli*, and *Kluyveromyces lactis*. Burvall *et al.* (1979) studied the formation of oligosaccharides during hydrolysis of 5% lactose in milk with *K. lactis* β-galactosidase and found 5% oligosaccharide at its maximum. In the case of *Streptococcus thermophilus* β-galactosidase, a large quantity of oligosaccharide formation was observed with a maximum content of 25% of the total sugar (Greenberg and Mahoney, 1983). The oligosaccharides produced were allolactose and 6-*O*-β-D-galactopyranosyl-D-galactose.

In our previous study (Mozaffar *et al.*, 1984) two forms of β-galactosidase from *Bacillus circulans* were separated and purified. The two forms, β-galactosidase-1 and β-galactosidase-2, were similar in isoelectric points (4.5), and pH optimum (6.0), but considerably different in *Km* values, substrate specificity, and particularly in oligosaccharide-producing activity. β-Galactosidase-2 produced a large quantity of oligosaccharides (maximum content, 41.0% of total sugar), compared to β-galactosidase-1 (maximum oligosaccharide content, 6.0%) during hydrolysis of 4.56% lactose. The oligosaccharide produced by β-galactosidase-1 was decomposed rapidly to glucose and galactose, and disappeared completely at 80% conversion of lactose.

The objective of this study was to compare the ability of β-galactosidase-1 from *B. circulans* to hydrolyze milk lactose with β-galactosidases from *E. coli* and *K. lactis*, and to investigate the effect of milk constituents on the enzymatic activity and oligosaccharide formation.

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MATERIALS & METHODS

Materials

β-Galactosidase from *B. circulans*, partially purified by ultrafiltration, was supplied from Daiwa Kasei K. K (Osaka, Japan). β-Galactosidases from *E. coli* and *K. lactis* were obtained from Sigma Chemical Co. and Gist Brocades Co., respectively. Lactose was purchased from Wako Pure Chemical Industries Ltd. A Glucostat reagent kit and galactose ultraviolet test kit for the determination of D-glucose and D-galactose were obtained from Worthington Biochemical Corp. and Boehringer Mannheim GmbH, respectively. Sephadex G-25 Medium, Sephadex G-150, QAE-Sephadex A-50, and polybuffer exchanger (PBE 94) were products of Pharmacia Fine Chemicals (Sweden). All the other chemicals were of analytical grade, and purchased either from Wako Pure Chemical Industries, Ltd., or from Nakarai Chemicals, Ltd., Japan. Commercially available whole milk and skim milk powder (high heat treatment, lactose content in the total sugar: 98%) were used throughout the study.

Methods

Purification of β-galactosidase-1. β-Galactosidase-1 from *B. circulans* was purified using Sephadex G-150, and ion-exchange (QAE-Sephadex A-50) chromatography, chromatofocusing with polybuffer exchanger (PBE 94) and preparative polyacrylamide gel electrophoresis in a manner similar to that reported previously (Mozaffar *et al.*, 1984).

Separation of protein and low molecular weight fractions from skim milk. A protein fraction containing mainly casein and serum proteins and a low molecular weight fraction containing lactose and other low molecular weight ions were separated at room temperature by gel chromatography on a glass column (1.5 cm × 40.0 cm) packed with Sephadex G-25 Medium gel. Skim milk powder dissolved in distilled water (12.0%, w/v) was put onto a column and eluted with water. The protein and low molecular weight fractions eluted were collected separately. These fractions were concentrated on a rotary evaporator at 40°C, and their protein and lactose concentrations were adjusted to 4.4% and 5.7%, respectively. Then powdered lactose was dissolved in the protein fraction to a concentration of 5.7%. Milk protein and lactose content were determined by the biuret method (Gornall *et al.*, 1949) and phenol-sulfuric acid method (Dubois *et al.*, 1951), respectively.

Measurement of β-galactosidase activity on lactose in buffer solution. The activity of β-galactosidase-1 from *B. circulans* was measured using lactose (final concentration, 4.56%) as a substrate dissolved in the assay buffer (25 mM citric acid-potassium phosphate buffer, pH 6.6). When measuring the activity, 0.1 mL enzyme solution, appropriately diluted with the assay buffer, was mixed with 0.4 mL 5.7% (w/v) lactose solution and incubated at 30°C. After 10 min incubation, or at various times, 0.125 mL of the reaction mixture was removed and poured into 0.25 mL 0.375N ice cold NaOH solution to stop the reaction. After storage at 0°C for 1 hr., this mixture was adjusted to pH 6.0 with 0.625 mL of the solution of 0.25M acetic acid and 0.3M sodium phosphate buffer, pH 6.0. The amount of D-glucose produced was determined by Glucostat reagent (Nakanishi *et al.*, 1983). One unit of β-galactosidase activity was defined as the amount of enzyme producing 1 μmol D-glucose per minute at 30°C and pH 6.6. Activities for β-galactosidases from *E. coli* and *K. lactis*

were measured at 30°C and 25°C, respectively, using lactose (final concentration, 4.56%) dissolved in the assay buffer.

Measurement of enzyme activity on lactose present in the low molecular weight fraction separated from skim milk was done by the same method as that for pure lactose solution.

Measurement of β-galactosidase activity on lactose in skim milk. The activity on lactose in skim milk was measured using commercially available skim milk powder dissolved in distilled water with a final lactose content of 4.56% (w/v) at pH 6.6. Lactose content in skim milk was determined by measuring the amount of total saccharide as described previously. After 10 min incubation, or at various times, 0.15 mL reaction mixture was deproteinized with 0.3 mL 1.8% $Ba(OH)_2 \cdot 8H_2O$ and 0.3 mL 2% $ZnSO_4 \cdot 7H_2O$ (Nakanishi *et al.*, 1983). After separating by centrifugation, the supernatant was used for the analysis of glucose with Glucostat reagent. Activities of β-galactosidase from *B. circulans* and *E. coli* were measured at 30°C and of the enzyme from *K. lactis* at 25°C.

β-Galactosidase activity on lactose in whole milk and that dissolved in protein fraction separated from the skim milk were measured by the same method as for skim milk.

Examination of the effects of various cations on the β-galactosidase activity. The effect of individual cations on the activity was examined by adding the chlorides of sodium, potassium, magnesium, and calcium to lactose (final lactose concentration, 4.56%) dissolved in pH 6.6 buffers. The effects of sodium, potassium, magnesium, and calcium at low concentrations (up to 5 mM) on the enzyme activity were investigated in 25 mM citric acid-potassium phosphate buffer. When measuring the effect of calcium at high concentrations (10–50 mM) the 25 mM citric acid-potassium hydroxide buffer was used.

Hydrolysis of lactose in skim milk. Hydrolysis of lactose in skim milk with β-galactosidases from *B. circulans* and *E. coli* was done mostly at 30°C and that with the enzyme from *K. lactis* at 25°C. Skim milk powder, dissolved in distilled water, was combined with the enzyme solution appropriately diluted with the assay buffer in final lactose concentrations of 4.56%, 12.0%, and 20.0% (w/v). Hydrolysis of lactose using the enzymes from *B. circulans*, *E. coli*, and *K. lactis* was continued for 4, 6, and 6 hr with 4.56% lactose, and for 6, 24, and 10 hr with 12.0% lactose, respectively. In the case of hydrolysis of 20.0% lactose with *B. circulans* enzyme, the reaction was done for 12 hr. Hydrolysis of lactose (final concentration, 4.56%) in skim milk with β-galactosidases from *E. coli* and *K. lactis* was also done at 37°C for 4 hr. During the hydrolysis of lactose, the amounts of glucose, galactose, lactose, and oligosaccharides produced were measured at different times in small portions of the reaction mixture removed and deproteinized as described previously. This suspension was centrifuged and the amounts of glucose and galactose in the supernatant were determined with a Glucostat reagent and a galactose UV test kit, respectively. Lactose and other oligosaccharides were separated by paper chromatography on Whatman 3 MM paper as reported previously (Mozaffar *et al.*, 1984). A measured amount (5–20 μL) of the reaction mixtures (in duplicate) was put onto the paper and the saccharides were separated using four 24-hr developments in the solvent system of butanol:pyridine:water (6:4:3). From each duplicate sample, one portion was cut, stained by silver dip reagent (Trevelyan *et al.*, 1950), and the corresponding portion containing saccharides were cut in small pieces for determination of saccharide content. These pieces were suspended into 5 mL distilled water, incubated at 30°C under gentle shaking for 4 hr, and centrifuged. The total amount of saccharides in the supernatant was determined by the phenol-sulfuric acid method (Dubois *et al.*, 1951) with lactose as a standard. A blank portion of the chromatographed paper with no sample applied, was also eluted in water as before. Since the separation of each spot on the paper chromatogram was fairly good, and the recovery of standard materials was quite high, as shown later, the data were taken from only one run.

The recovery of saccharides from the paper was evaluated six times, using different amounts (20–500 μg) of glucose, galactose, lactose, and trisaccharide. The average percent recovery for each saccharide was higher than 95%, regardless of the amount used.

Preparation of standard oligosaccharides. A 4.56% lactose solution was treated with β-galactosidase from *B. circulans* partially purified by ultrafiltration, immobilized onto Duolite ES 762 and the reaction was stopped at 60% conversion of lactose. The lactose hydrolysate thus obtained was loaded on an activated charcoal column and the saccharides were eluted using aqueous 5, 10, 15, and 20% ethanol solutions as described by Nakanishi *et al.* (1983). These fractions were further purified by paper chromatography and their chemical compositions were determined by the enzymatic hydrolysis as reported (Mozaffar *et al.*, 1984). The major components for di-, and trisaccharides obtained were used as standard substances. These oligosaccharides were found to be composed of one molecule of glucose and one to two molecules of galactose.

Identification of oligosaccharides. Oligosaccharides produced by *B. circulans* enzyme were identified by comparing the R_L values with those for standard substances as described earlier. The R_L value is defined as a ratio of the distance travelled for the oligosaccharide to that for lactose on paper chromatography. For the oligosaccharides formed by the enzymes from *E. coli* and *K. lactis* only the R_L values are shown.

RESULTS

Activity of β-galactosidases on lactose in skim milk and whole milk

Initial rates of hydrolysis of lactose in skim milk and whole milk were measured with the β-galactosidases from *B. circulans*, *E. coli*, and *K. lactis*. As shown in Table 1, the lactose hydrolyzing activity of the enzyme from *B. circulans* is a little higher for skim milk and whole milk than that for a pure lactose solution. On the other hand, in the case of the enzymes from *E. coli* and *K. lactis*, the activity towards lactose in skim milk and whole milk decreased compared with that for a pure lactose solution. Particularly, in the case of the enzyme from *E. coli*, a very large decrease of 80% was observed.

The lactose hydrolyzing activity of the enzyme from *B. circulans* was unchanged with the low molecular weight fractions of skim milk and slightly higher with milk protein, compared to that on lactose in the buffer solution (Table 1). Enzyme activity of *E. coli* and *K. lactis* decreased in the presence of milk protein as well as with the low molecular weight fraction. The relative reduction of activity due to the addition of milk protein and with the low molecular weight fraction was 62% and 78%, for *E. coli*, and 29% and 37%, for *K. lactis*, respectively.

Effects of cations on the activity

Fig. 1 shows the effects of the salts of sodium, potassium, magnesium and calcium on the activity of β-galactosidases from *B. circulans*, *E. coli*, and *K. lactis*. The enzyme activity of *B. circulans* was increased slightly by the addition of sodium chloride, potassium chloride, magnesium chloride, and calcium chloride, but sodium chloride and calcium chloride considerably decreased the activity of *E. coli* and *K. lactis* enzymes. At 25 mM sodium chloride concentration, which exists in milk, the activities of the enzymes from *E. coli* and *K. lactis* decreased by 86% and 24%, respectively. At 5 mM calcium chloride concentration, the activities of these enzymes were reduced by 38% and 32%, respectively, and at 15 mM concentration, present in milk, the activities reduced by 47% and 86%, respectively, in 25 mM citric acid-potassium hy-

Table 1—Lactose-hydrolyzing activity of β-galactosidases from B. circulans, E. coli, and K. lactis in skim milk, whole milk, and protein and low molecular weight fractions of the skim milk

	Relative activity (%)				
Source	Lactose[a] in buffer	Skim milk[b]	Whole milk	Lactose with protein fraction[c]	Lactose with low molecular wt fraction[d]
B. circulans	100	118	118	120	105
E. coli	100	20	21	38	22
K. lactis	100	70	65	71	63

[a] Final lactose concentration, 4.56% in 25 mM citric acid-potassium phosphate buffer, pH 6.6.

[b] Final lactose concentration, 4.56% in water, pH 6.6

[c] Lactose dissolved in the protein fraction (final lactose concentration, 4.56% and protein concentration, 3.5%), pH 6.6

[d] Final lactose concentration, 4.56% with low molecular weight substances, pH 6.6. β-Galactosidase activity of the enzymes from *B. circulans*, *E. coli*, and *K. lactis* was 24.0, 34.0 and 77.0 units/min/mg, respectively. Activity for *B. circulans* or *E. coli* was determined at 30°C and that for *K. lactis* at 25°C.

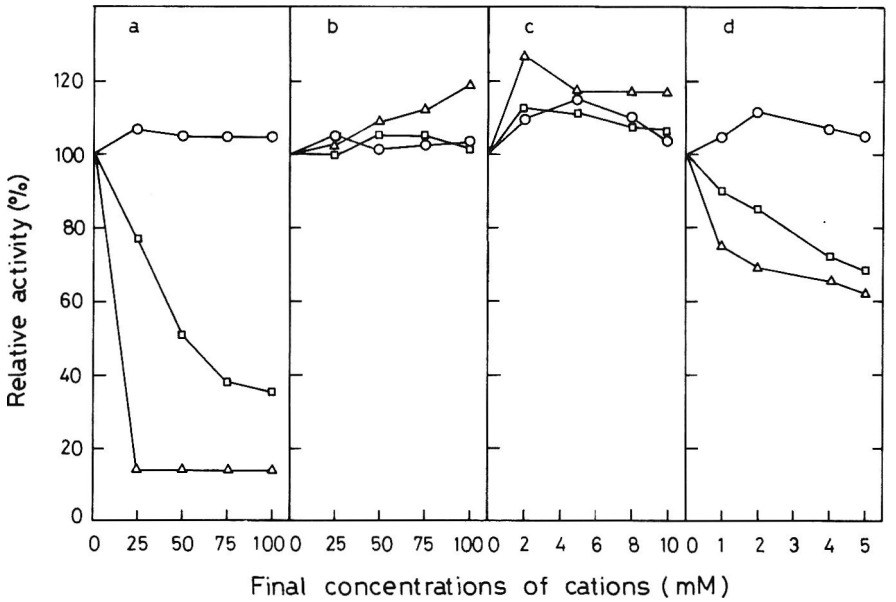

Fig. 1—Effects of (a) sodium, (b) potassium, (c) magnesium, and (d) calcium on the activity of β-galactosidase from B. circulans (– ○ –); E. coli (– △ –); K. lactis (– □ –) in 25 mM citric acid-potassium phosphate buffer, pH 6.6

droxide buffer, pH 6.6. Since chloride ions are common to all four salts used, cations such as sodium and calcium are the main factor for this decrease in the activity.

Hydrolysis of lactose in skim milk

Hydrolysis of lactose with a final lactose concentration of 4.56% in skim milk was done using β-galactosidases from *B. circulans* (1 unit/mL), *E. coli* (3 units/mL), both at 30°C and *K. lactis* (3 units/mL) at 25°C (Fig. 2). During the hydrolysis of lactose with β-galactosidase-1 from *B. circulans* (Fig. 2a) only a small amount of oligosaccharide was detected. The maximum amount of oligosaccharide (5.5% of total sugar) was produced at 39% conversion of lactose, and at 80% conversion no oligosaccharide was detected. The oligosaccharide produced was mainly a trisaccharide with one molecule of glucose and two molecules of galactose (Mozaffar *et al.*, 1984). On the other hand, β-galactosidase from *E. coli* produced a much larger amount of oligosaccharides than the enzyme from *B. circulans* during the course of the reaction. In the case of *K. lactis* enzyme, only a small amount of oligosaccharides was initially detected, and they accumulated in a large quantity at the latter stage of the reaction. As the reaction proceeded, a considerable amount of oligosaccharides with different R_L values on the paper chromatography was observed (Fig. 2b and 2c). Only the oligosaccharides with R_L value of 0.89, 0.78, and 0.58 were detected. Although the composition of these oligosaccharides were not determined in this study, they might be allolactose or other galactooligosaccharides (Huber *et al.*, 1976; Asp *et al.*, 1980; Nakanishi *et al.*, 1983). The total amount of oligosaccharide produced by β-galactosidase from *E. coli* reached a maximum value of 21% of the initial lactose concentration at about 80% conversion. At 98% conversion of lactose, 14% oligosaccharide still remains in the reaction mixture. In the case of the β-galactosidase from *K. lactis*, oligosaccharides were accumulated with a maximum value of 14% at 90% conversion. Almost the same amount remained at 98% conversion of lactose. The formation of oligosaccharides during the enzymic hydrolysis of lactose in milk (final lactose concentration, 5%) with β-galactosidase from *K. lactis* was also observed by Burvall *et al.* (1979). However, they reported less oligosaccharide production than in our study. This could be because formation of disaccharides other than lactose were not analyzed in their study. In the case of *E. coli* and *K. lactis*, a large amount of oligosaccharides remained in the reaction mixture even at 98% conversion of lactose, although three times more β-galactosidase units were used for the hydrolysis than with *B. circulans* (Fig. 2). The tendencies of oligosaccharides formation at 37°C with the enzymes from *E. coli* and *K. lactis* were similar to those observed at 30°C and 25°C, respectively, although the rate of reaction increased with temperature. The maximum oligosaccharide produced by the enzymes from *E. coli* and *K. lactis* were 22.5% and 20.0%, respectively. With the β-galactosidase from *E. coli*, 20.0% oligosaccharide was observed at 80% conversion. Fifteen percent still remained at 98% conversion. In the case of *K. lactis* enzyme, 20.0% oligosaccharide was detected at 90% conversion, and 10% at 98% conversion. However, in both case the oligosaccharides still remaining at 98% conversion of lactose, almost completely disappeared after 4 hr incubation at 37°C.

The concentrations of saccharides produced are plotted against the conversion of lactose in skim milk with a final lactose

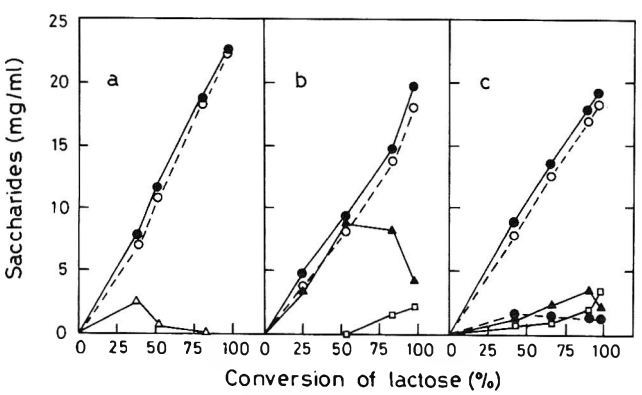

Fig. 2—Relationship between conversion of lactose in skim milk and saccharides produced with β-galactosidase from (a) B. circulans, (b) E. coli, and (c) K. lactis. Hydrolysis of lactose (final concentration, 4.56%) with β-galactosidase from B. circulans (final enzyme unit, 1 unit/mL), and E. coli (final enzyme unit, 3 units/mL) was done at 30°C and that with the enzyme from K. lactis (final enzyme unit, 3 units/mL) at 25°C. (a) – ● –, *glucose;* --- ○ ---, *galactose;* – △ –, *trisaccharide* ($R_L = 0.70$). (b) – ● –, *glucose;* --- ○ ---, *galactose;* – ▲ –, *oligosaccharide-1* ($R_L = 0.89$); – □ –, *oligosaccharide-2* ($R_L = 0.78$). (c) – ● –, *glucose;* --- ○ ---, *galactose;* – ▲ –, *oligosaccharide-1* ($R_L = 0.89$); – □ –, *oligosaccharide-2* ($R_L = 0.78$); --- ● ---, *oligosaccharide-3* ($R_L = 0.58$).

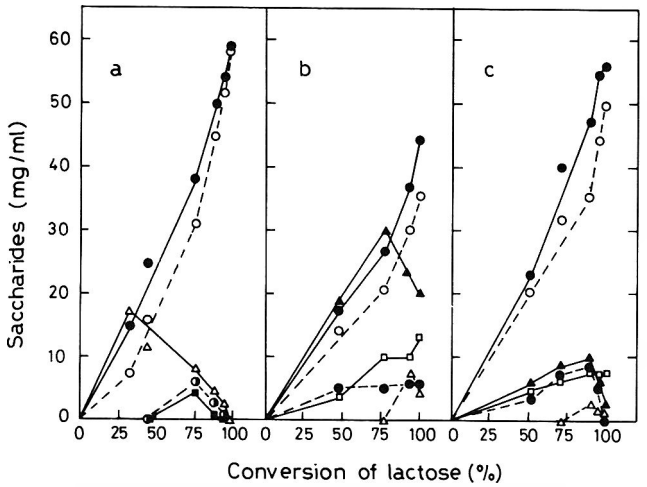

Fig. 3—Relationship between conversion of lactose in skim milk and saccharides produced with β-galactosidase from (a) B. circulans, (b) *E. coli, and (c) K. lactis. Hydrolysis of lactose (final concentration, 12.0%) with β-galactosidase from B. circulans (final enzyme unit, 5 units/mL), and E. coli (final enzyme unit, 7 units/mL) was done at 30°C and that with the enzyme from K. lactis (final enzyme unit, 7 units/mL) at 25°C. (a) – ● –, glucose; --- ○ ---, galactose; – △ –, trisaccharide (R_L = 0.70); --- ◑ ---, disaccharide-1 (R_L = 1.2); – ■ –, disaccharide-2 (R_L = 0.87). (b) – ● –, glucose; --- ○ ---, galactose; – ▲ –, oligosaccharide-1 (R_L = 0.89; – □ –, oligosaccharide-2 (R_L = 0.78); --- ● ---, oligosaccharide-3 (R_L = 0.58); --- △ ---, oligosaccharide-4 (R_L = 0.40). (c) Same as (b).*

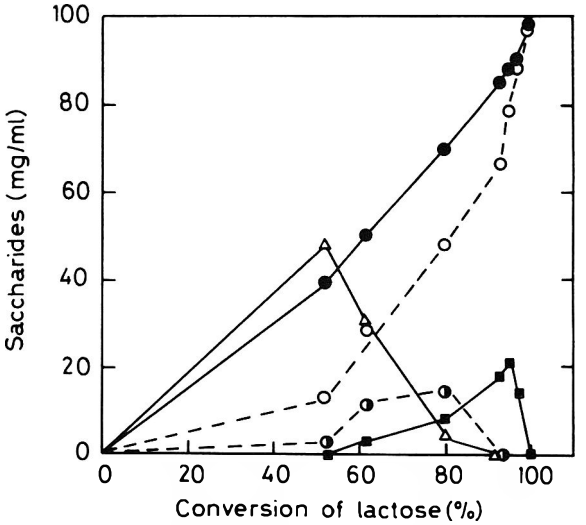

Fig. 4—Relationship between conversion of lactose in skim milk and saccharides produced with β-galactosidase from B. circulans. Hydrolysis of lactose (final concentration, 20.0%) with β-galactosidase from B. circulans (final enzyme unit, 6 units/mL) was done at 30°C. – ● –, glucose; --- ○ ---, galactose; – △ –, trisaccharide (R_L = 0.70); --- ◑ ---, disaccharide-1 (R_L = 1.2); – ■ –, disaccharide-2 (R_L = 0.87).

concentration of 12.0% for the three enzymes preparations (Fig. 3). With the β-galactosidase-1 from *B. circulans* (Fig. 3a) only trisaccharide was formed at the initial stage of reaction with a maximum yield of 14% at 33% conversion of lactose, and later it disappeared completely. When trisaccharide started to decrease, two disaccharides other than lactose with R_L values of 1.2 and 0.87 in paper chromatography appeared. These disaccharides were different from lactose, presumably in the type of linkage (Mozaffar *et al.*, 1984). They were gradually hydrolyzed and at 98% conversion all the oligosaccharides disappeared. β-Galactosidase from *E. coli* and *K. lactis* produced many more oligosaccharides (oligosaccharides 1–4) during the hydrolysis of 12.0% lactose in the skim milk, compared with the results of 4.56% lactose (Fig. 2). β-Galactosidase from *E. coli* produced oligosaccharide with a maximum yield of 38% in a conversion range between 75–90% (Fig. 3b). Even at 98% conversion, 35% oligosaccharide remained in the reaction mixture. Also, β-galactosidase from *K. lactis* formed oligosaccharide with a maximum amount of 23% at about 90% conversion (Fig. 3c). Sixteen percent of the oligosaccharide still remained at 98% conversion. With all three enzymes tested, oligosaccharide production was higher with 12.0% lactose in skim milk than with 4.56% lactose. Enhancement of transgalactosidation reaction with increasing lactose concentrations was shown by many investigators (Roberts and Pettinati, 1957; Burvall *et al.*, 1979).

Fig. 4 shows the plot of concentrations of saccharides produced against the conversion of lactose in skim milk with a final lactose concentration of 20% using β-galactosidase-1 from *B. circulans*. During the hydrolysis of lactose, trisaccharide was formed initially as a by-product. The trisaccharide produced reached its maximum (about 24% of the total sugar) at 52% conversion of lactose. As the trisaccharide content decreased, disaccharides other than lactose appeared, similar to the case with 12.0% lactose. However, these disaccharides gradually disappeared as the reaction proceeded. At 97% conversion of lactose only a small amount of disaccharide (7%), which had slightly lower mobility than lactose on paper chromatography, was detected. The oligosaccharide was finally hydrolyzed completely.

DISCUSSION

IN PREVIOUS WORKS, a number of β-galactosidases from various sources were studied for the purpose of hydrolyzing lactose in milk (Dahlqvist *et al.*, 1977; Finocchiaro *et al.*, 1980; Mahoney and Adamchuk, 1980). In this study, suitability of β-galactosidase-1 from *B. circulans* for hydrolysis of milk lactose was examined from the viewpoint of the effect of milk constituents on the enzymatic activity and oligosaccharide formation, in comparison with the enzymes from *E. coli* and *K. lactis*. The β-galactosidase-1 from *B. circulans* was affected neither by the milk proteins nor by the several major cations present in the milk. Thus, almost the same activity was obtained for the hydrolysis of lactose in skim milk, whole milk as well as in the buffer solution. On the other hand, the activities for the β-galactosidases from *E. coli* and *K. lactis* strongly decreased in the presence of sodium and calcium ions, with such concentrations as found in milk. The strong inhibition by sodium and calcium ions might be principally responsible for the reduction of the initial velocity for these enzymes in skim milk or whole milk. The decrease in activity caused by the addition of sodium and calcium ions were also reported in the case of *Kluyveromyces fragilis* (Mahoney and Adamchuk, 1980).

Only a slight increase of the activity was observed for both the enzymes from *E. coli* and *K. lactis* on the addition of magnesium ion. Reithel and Kim (1960) also observed, for the case of *E. coli* enzyme, that magnesium ion did not activate the hydrolysis of lactose. However, they found a strong activation of the hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG) by the magnesium ion. Such a strong activation for the hydrolysis of ONPG were also reported for *K. lactis* and *E. coli* enzymes (Biermann and Glantz, 1968; Tenu *et al.*, 1972).

The transgalactosidation activity of β-galactosidase was reported early in the 1950's (Wallenfels, 1951) and since then many investigators observed oligosaccharide formation from pure lactose solutions. The degree of transgalactosidation and the kinds of oligosaccharides produced were strongly dependent on the sources of the enzyme and substrate concentrations.

However, in the case of treatment of milk with β-galactosidase, only a few investigators (Ohmiya *et al.*, 1977; Burvall *et al.*, 1979; Greenberg and Mahoney, 1983) examined the oligosaccharide production. Ohmiya *et al.* (1977) showed qualitatively that fewer oligosaccharides were produced during hydrolysis of lactose in milk using immobilized cells of *L. bulgaricus*, *E. coli*, and *K. lactis*. Burvall *et al.* (1979) quantitatively studied the formation of oligosaccharides in the hydrolysis of 5% milk lactose with the β-galactosidase from *K. lactis*. Furthermore, they pointed out that oligosaccharides produced with the enzyme from *K. lactis* might cause intestinal discomfort, mainly in the form of flatulence (Burvall *et al.*, 1980). From this point of view, the oligosaccharide content in the milk treated with β-galactosidase should be reduced to a minimum.

Our previous study showed that one of the two β-galactosidases from *B. circulans*, β-galactosidase-1, produced much less oligosaccharide during the hydrolysis of lactose in buffer solution (Mozaffar *et al.*, 1984) and the oligosaccharide produced was rapidly hydrolyzed to glucose and galactose.

In this study, we used this enzyme for the hydrolysis of lactose in skim milk with different lactose concentrations, and the course of oligosaccharide production was compared with that for the enzymes from *E. coli* and *K. lactis*. The β-galactosidase from *B. circulans* showed less oligosaccharide producing activity in the hydrolysis of milk lactose with different lactose concentrations than that from *E. coli* and *K. lactis*. The amount of oligosaccharides produced by *B. circulans* enzyme reached its maximum at conversions of 30–60% and rapidly decomposed at the latter stage of the reaction. In the case of *E. coli* and *K. lactis* enzymes, the maximum oligosaccharide accumulation was observed in the conversion range between 80 and 90%.

Since a small amount of lactose in milk could enhance the absorption of calcium from the small intestine (Armbrecht and Wasserman, 1976) and stimulate the synthesis of several water soluble vitamins by intestinal microflora (Atkinson *et al.*, 1957), conversion of lactose is required to be kept around 80%. Thus, the enzyme from *B. circulans* might be particularly useful for production of low lactose milk with minimum oligosaccharide content.

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Energy Use for Continuous Thermal Processing of Milk

R.B. BIZIAK, K.R. SWARTZEL, and V.A. JONES

ABSTRACT

Energy use in a direct heat/vacuum cooling system was evaluated and compared to an indirect unit during start-up, sterilization, and milk processing. Product flow rates were 606–1325 L/hr. Process temperatures were 138 and 149°C. Energy for start-up was 1.7 to 2.9 × 10^5 kJ for the direct; 0.9 to 1.4 × 10^5 kJ, indirect. Energy for 30 min sterilization was 4.0 to 5.6 × 10^5 kJ for direct; 2.3 to 3.7 × 10^5 kJ, indirect. Product processing energy was 357.9–436.8 kJ/kg for indirect; 547.8–697.6 kJ/kg direct. Greater heat recovery potential and fewer pumps made the indirect system less energy intensive. Methods of energy conservation are suggested.

INTRODUCTION

CONTINOUS THERMAL PROCESSING and aseptic packaging are finding many uses in the food industry. Virtually any food that can be pumped can be processed and packaged in this manner. Benefits of continuous aseptic processing and packaging are reduced container costs (laminated paper or plastic containers vs cans or bottles) and reduced energy costs (eliminating refrigerated storage and distribution and greater potential for regenerative heating and cooling) (Department of Energy, 1979a,b).

Continuous thermal processing systems can be divided into two major types: direct and indirect systems. Direct systems use steam injectors or infusers to heat product by actual product-steam contact. A vacuum treatment is normally employed for partial cooling and water removal. Indirect systems separate heating/cooling media from product by a heat exchange surface. Plate, tubular, and scraped surface heat exchangers are examples of indirect systems.

Direct systems are often more complex than indirect systems. Direct systems require a culinary (food grade) steam supply. A vacuum chamber and aseptic homogenizer with appropriate instrumentation, controls, and pumps are required for products like milk. These factors make installation costs higher for direct systems (Wood, 1981). Limited heat recovery capacity and more required maintenance serve to make operating costs higher for direct systems (Burton, 1979). More rapid heating and cooling are attained in direct systems (less than 1 sec for each heat transfer operation with direct systems and greater than 10 sec for indirect systems). Product deposits can foul heat exchange surfaces and cause premature shut-down for clean-up with indirect heating.

Energy surveys for both systems were summarized by Hallström (1974). Direct systems used between 304 and 622 kJ/kg of steam energy at a milk flow rate of 1000 kg/hr (970 L/hr); while indirect systems were cited between 83.0 and 304 kJ/kg. Electrical energy for direct systems ranged between 32.4 and 54.0 kJ/kg while indirect systems consumed between 36.0 and 54.0 kJ/kg.

The University of Maryland conducted an energy study for a DASI (DASI Inc., Chevy Chase, MD.) steam infusion processing system (Department of Energy, 1979a,b). Flow rates of 1136 and 2271 L/hr were used with holding temperatures of 141° and 146°C with a 74°C preheat temperature. Energy use ranged between 671.3 and 946.9 kJ/kg. The sterilizer was the major user of energy (52–53%), preheating with heat recovery was next (14–19%), followed by product cooling, homogenization, and vacuum chamber (11–13%, 10–13%, and 7–9% of the total respectively). The study did not include aseptic packaging.

Energy use in a steam injection heating and aseptic packaging system was studied at North Carolina State University (Biziak, 1981). A product flow rate of 1010 L/hr with holding temperatures of 143° and 149°C, and preheat temperatures of 74° and 88°C were investigated. Energy inputs for product processing and packaging ranged between 919.9 and 1037.4 kJ/kg. Thermal regeneration was not utilized. Steam use for preheating, injection, and steam seals used 73% of the energy in each trial followed by energy for product cooling (13%), packaging (7%) and homogenization (3–4%).

A shell-and-tube indirect system was monitored for energy use at product flow rates of 606, 1022, and 1325 L/hr with holding temperatures of 138°, 143°, and 149°C (Biziak et al., 1982). Energy inputs for product processing ranged from 357.9–436.8 kJ/kg. Thermal regeneration contributed between 57.5 and 70.4% of the heat. Steam varied from 40–64% of the total energy depending on product flow rate and holding temperature.

This investigation was conducted to study the effect of product flowrate and processing temperature on energy utilization in a direct heating steam injection system and to compare energy requirements for direct and indirect systems operated under similar conditions (Bliziak et al., 1982).

MATERIALS & METHODS

Processing equipment

The flow chart of the steam injection processing system is shown in Fig. 1. Table 1 provides specifications for the electrical components. Product was pumped through two plate heat exchangers by the booster pump. The first plate heat exchanger was part of a thermal regeneration circuit (Biziak et al., 1982). Product temperature was raised to the specified preheat temperature in the second plate heat exchanger using hot water. The hot water was heated in a shell-and-tube heat exchanger by power plant steam and recirculated between the plate heat exchanger and the shell-and-tube heater by a centrifugal pump.

A pneumatically controlled variable speed, three piston, positive displacement pump forced product into the injector where culminary steam was injected into the product to give an almost instantaneous temperature rise. The incorporation of steam into the product with associated condensation leads to increased moisture in the product. A vacuum chamber was employed to remove the added moisture and to rapidly cool the product immediately following the holding tube and back pressure valve. Vapors were condensed using cooling-tower water and withdrawn by a vacuum pump. Pressure in the vacuum chamber was controlled automatically with an air bleed valve between the condensor and vacuum pump. A centrifugal discharge pump moved product from the vacuum chamber to the timing pump.

Further product cooling took place in the spiral tube (shell-and-tube) heat exchangers using cooling-tower water, and ice water as the media with a single remote manual homogenization valve. Product was homogenized between the regeneration and tower-water coolers. Heat recovered from the regeneration coolers was transferred to the regeneration circuit's plate heat exchanger. Steam seals were used on

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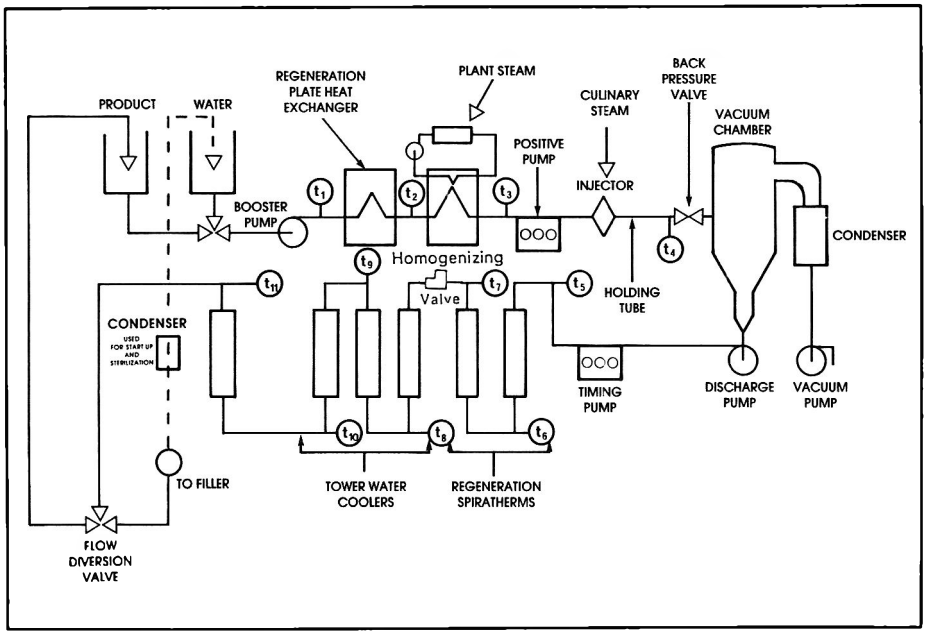

Fig. 1—Flow chart of direct processing unit with thermocouple locations.

Table 1—Electrical energy inputs

Equipment	HP[a]	Phase	Voltage	Full load current (Amps)
Product Booster Pump	2	3	230	5.2
Positive pump	2	3	208	9.2
Discharge pump	3	3	208	8.4
Preheater pump	3	3	208	8.0
Timing pump	20	3	208	60.0
Vacuum pump	5	3	208	14.0
Air compressor	10	3	460	12.4
Cooling tower	10[b]	3	460	13.4
Ice water pump	5	3	208	20.0
Regeneration pump	0.75	3	230	2.4

[a] Pump power rating is given in horsepower units by convention.

[b] Cooling tower contains two motors; one to operate the circulating pump and the other to operate the blower. HP value is the sum of the two motors. The same being true for the full load current value.

the back pressure valve, all vacuum chamber ports, discharge pump, flow diversion valve and homogenizer seals to prevent possible contamination due to a defective gasket.

Steam energy measurement

Culinary and power plant steam were the two sources of steam energy for the processing systems. Culinary steam was produced in a culinary steam generator, and used at the injector and steam seals associated with the vacuum chamber. Power plant steam was used to heat water for the second plate heat exchanger and to provide steam for aseptic seals at the timing pump and flow diversion valve.

Steam energy input was determined by measuring flow rate and quality as demonstrated by Biziak et al. (1982) Concentric bore orifice plates were mounted between flanges in 19.05 mm diameter pipeline for power plant steam and 31.75 mm diameter for culinary steam. The differential pressure generated by steam flow through the orifice plates was monitored using a differential pressure transmitter (Centry 470, Leeds & Northrup, North Wales, PA.). The steam pressure upstream of the orifice was measured using a pressure transmitter (Centry 471, Leeds & Northrup).

The orifice bore sizes for the culinary, preheater and Unitherm steam lines were 18.26, 15.69, and 13.25 mm respectively. Straight pipe lengths of at least 40 I.D. upstream and 25 I.D. downstream of the orifice were installed to facilitate normal flow distribution of steam.

Electric energy

Electrical energy data for the water cooling tower blower and pumps, air compressor and ice water circulating pump were determined in a previous study (Biziak, 1981). Near constant values from that study include for product processing, 9.6 kJ/kg for the air compressor, 19.9 kJ/kg for the cooling tower blower and pumps, 17.6 kJ/kg for the ice water pump, and 76.5 kJ/kg for the ice water for the ice water refrigeration. Total air compressor energy use during sterilization was near constant at 5.38 MJ.

All other electrical inputs were measured using watt transducers (PX-2000, F.W. Bell, Inc., Orlando, Fl.). The balanced load circuit described by Biziak et al. 1982 was used to monitor three phase power supplied to each motor. A D.C. mV signal was generated by the watt transducers proportional to the power being used by each motor.

System monitoring and operation

All temperatures were measured by type T (Copper-Constantan) thermocouples located as shown in Fig. 1.

A data logger (Model 9300, Monitor Labs, Inc., San Diego, CA.) recorded analog signals from thermocouples and transmitters. Because the large number of inputs exceeded the present input capacity of the data logger, some watt transducer measured electrical inputs were measured using a multipoint strip-chart recorder (Speedomax W Recorder with AZAR Module, Leeds & Northrup, North Wales, PA). Energy inputs to the systems were monitored during equipment start-up, sterilization, and product processing phases of operation. Inputs were recorded by the data logger and transmitted to a microcomputer (Apple II+, Apple Inc., Cupertino, CA) for storage on floppy disk. The data were edited using the microcomputer, and sent to a mainframe computer for further analysis.

Water was circulated in the product line during equipment start-up and sterilization. The start-up period was defined as the time period between initial equipment start-up and attaining equipment sterilization conditions. Equipment sterilization was the 30 min interval where 121°C or higher was recorded continuously at the aseptic filler. During this 30 minute interval the holding tube temperature was maintained at 150°C. Although it would have been more efficient to control temperature at the end of the filler at 121°C this was not done due to the mechanics of the system. Temperature controllers are not normally installed at the end of the filler line. Data were collected at 1 minute intervals for both start-up and sterilization periods. After equipment sterilization holding tube temperature was adjusted to the desired level and components downstream from the holding tube were cooled to processing conditions. Product flow was started and processing data were collected at 1 minute intervals after attaining steady state processing conditions. Duplicate trials were monitored for each set of processing conditions.

Whole milk (3.25% fat) was used during product processing at flow rates of 1022, 1173, and 1325 L/hr. Holding tube temperatures were 138° and 149°C. Preheat temperatures were 74° and 88°C. Homogenizing valve pressure was 0 MPa during equipment start-up and sterilization then increased to 20.68 MPa to homogenize milk during product processing. Steam pressure to the steam seals was 103 kPa at the homogenization valve. Steam pressure to the vacuum chamber steam seals was adjusted manually based upon visual observation of vapor discharge from steam seals.

The regeneration section was not operational during start-up and equipment sterilization. The recirculating water flow to the regeneration section was regulated at each product flow rate to give the highest possible heat recovery.

RESULTS & DISCUSSION

Equipment start-up and sterilization

For comparisons, data from a previous study were utilized (Biziak et al., 1982). In that study energy loads for a shell-and-tube indirect heating process system were examined during start-up, equipment sterilization, and product processing. Product flow rates were 606, 1022, and 1325 L/hr. Process temperatures were 138°, 143°, and 149°C. The regeneration section contributed 57.5–70.4% useable heat. Energy use remained relatively constant for varying start-up conditions and increased as a function of flow rate during equipment sterilization. Throughout the study all electrical and steam energy input valves were representative of actual energy delivered to the process and did not include a factor accounting for generation inefficiency in electric and steam facilities.

Steam heater inputs, and electrical inputs to the timing pump and chill water cooling system were directly proportional to the amount of product processed, and when expressed on an energy/mass product basis remained constant regardless of product flow rate. These energy inputs were classified as fixed per unit product (FP). Steam inputs to steam seals, regeneration, and chill water pumps; the air compressor; and cooling tower blower and pump were constant when expressed as energy/unit time. Inputs as these, being independent of product flow rate and proportional to the length of time a piece of equipment was operated, were classified as fixed per unit time (FT).

Total energy use for equipment start-up and sterilization for the direct system in this study and the indirect systems in the previous study is shown in Fig. 2. During both phases of equipment operation, the direct system used more energy than the indirect.

Start-up times ranged from 17 min at 1325 L/hr to 29 min at 757 L/hr for the steam injection system. They ranged from 7 min at 1325 L/hr to 21.5 min at 606 L/hr for the indirect system. Start-up time was always longer at lower flow rates due to the reduced rate of heat input into the system. For a given flow rate the direct system always had longer start-up times than the indirect. This is attributed to the size of the direct system (greater heat loss) compared to the indirect system. The larger surface area (i.e. vacuum chamber) of the direct system with greater heat losses retarded the achievement of equipment sterilization criteria (i.e. 121°C at the aseptic filler). Initial incoming water temperature to both systems for all flow rates was not significantly different with $\bar{x}$ = 23.2°C, σ = 2.4°C. However, due to recirculation during start-up and sterilization, the incoming water temperature steadily increased. To prevent flashing, a condenser was utilized between the filler and the water storage tank to reduce the water temperature below 100°C.

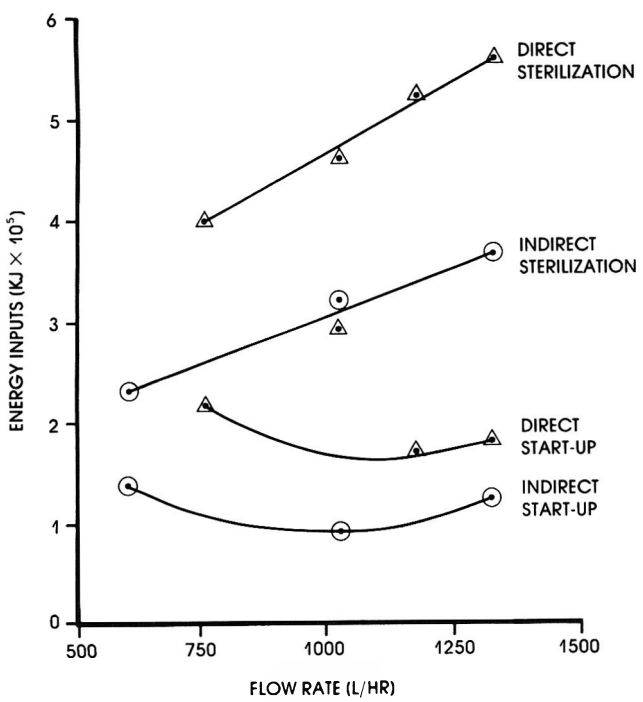

Fig. 2—Energy inputs for equipment start-up and sterilization.

Energy use during start-up for both systems (although slightly concave upward) was relatively constant with changing flow rates (for indirect heating, $\bar{x} = 1.15 \times 10^5$, $\sigma = 0.23 \times 10^5$ kJ; direct heating, $\bar{x} = 1.84 \times 10^5$, $\sigma = 0.32 \times 10^5$ kJ). Individual mean start-up data variability ranged from σ = 3.4% from mean values shown on Fig. 2 at the highest flow rate to σ = 14% at the lowest flow rate. The high variability at the low flow rate was attributed to unequal steam seal inputs for the duplicate runs.

For equipment sterilization the direct system at all flow rates required higher energy inputs than did indirect heating. Again, the direct system has a larger surface area (i.e. vacuum chamber) to heat than the indirect and, therefore, is subject to larger heat losses. These heat losses must be replaced by additional energy input. Had the temperature at the end of the filler been controlled at 121°C instead of the temperature at the end of the holding tube at 150°C, the overall energy for sterilization for both systems would have been reduced by as much as ca. 10%. However, the positive slope shown in Fig. 2 would still exist since more heat has to be made up at the higher flow rates due to temperature reduction of the sterilizing water at the condenser.

The coefficients of determination (R^2) were determined for sterilization data presented in Fig. 2. For the direct system R^2 = 0.91 with the intercept (a) = 187.37 MJ, standard error = 38.37; slope (b) = 0.278, standard error = 0.035. The indirect system had an R^2 = 0.98, a = 102.57 MJ, standard error = 12.12; b = 0.208, standard error 0.012. The student's t-test indicated the slopes for the two data sets are not significantly different. This infers that the rate at which energy use increases with flow rate is about the same for both systems. The energy use increases because more water has to be heated per unit time as flow rate is increased. For both systems steam use made up 93% of the total energy input during equipment sterilization. The electrical inputs to the booster, positive, discharge, preheater and timing pumps; cooling tower and air compressor made up the remaining 7% of the total energy.

Product processing

Product processing data are presented for the direct and indirect systems in Fig. 3. Data variability ranged from 2.1–6.9% of the mean values shown in Fig. 3 with no pattern as to flow rate on process temperatures. Energy use for direct processing exceeded that for indirect processing at all conditions. The most important reason for difference is the heat recovery (regeneration) available to either system. The indirect system recovered an average 68.8, 67.9, and 57.5% of the available heat at flow rates of 606, 1022, and 1325 L/hr, respectively. Heat recovery on the direct system is a function of the preheat temperature; therefore, an average 32.5 and 26.7% recovery was obtained at all flow rates for preheat temperatures of 88° and 74°C, respectively, due to higher vacuum chamber exit (regeneration entrance). Product processing energy in Fig. 3 represents both FT and FP inputs. Because FT inputs have a larger effect at low flow rates, energy use is high at those

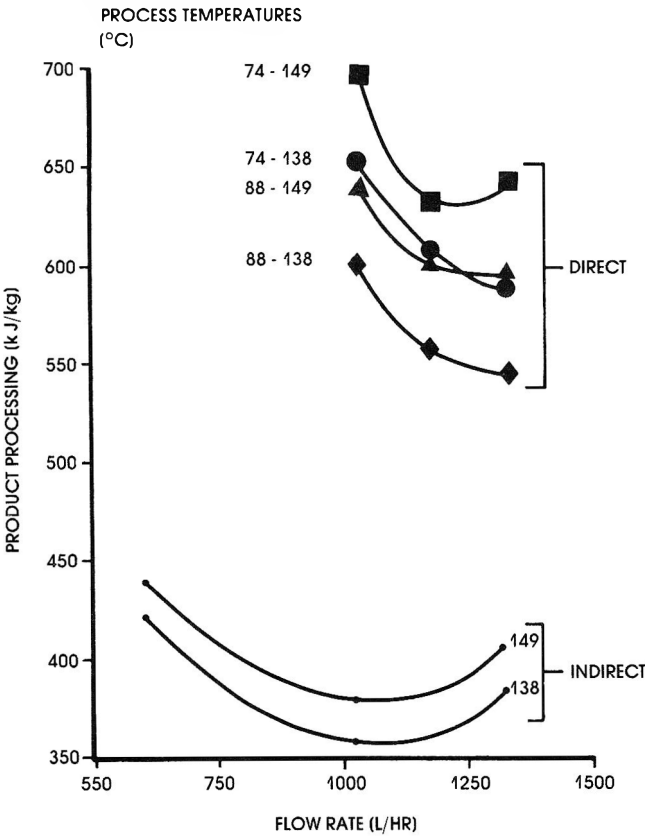

Fig. 3—Energy inputs for product processing.

flow rates. As the effect of FT inputs diminishes with flow rate energy use approaches a minimum. Steam heater inputs then increase the energy use due to decreased regeneation at higher flow rates.

Expansion cooling with a vacuum chamber in the direct system was an obstacle to additional heat recovery. Flash vapors in the vacuum chamber were condensed in a condenser using tower water; therefore, their useful heat content was lost from the system. Depending on preheat, holding and vacuum temperatures and the product flow rate, energy absorbed by the condensor ranged from 2.8–4.9 $\times$ 10^5kJ/hr (methods for recovering this energy are being reviewed). Flash cooling was controlled by the vacuum in the chamber. Product temperature in this study was controlled at 1–2°C lower than preheat temperature to prevent product dilution or concentration in the system monitored as determined by the method of Tsao (1981). Other direct heating systems may require lower product exit temperatures from the vacuum chamber (Tompkins, 1970) or higher (Perkin and Burton, 1970) depending on the heat loss associated with each individual system, and the degree of moisture in the steam supply.

Additional cooling occurred in the water jacketed leg of the vacuum chamber. The product was cooled slightly at this point to insure product temperature below the saturated liquid temperature at existing vacuum conditions. This reduced the possibility of cavitation and contamination by preventing product flashing in the discharge pump. This subcooling lowered product temperature to approximately 81° and 68°C for tests where preheat temperatures were set at 88° and 74°C, respectively. Product then entered the regenerators at these reduced temperatures.

The indirect system has hot sterile product at holding temperature (138°–149°C) entering the regeneration section, thus enhancing a much higher regeneration potential than the direct. For both systems, use of a product-product regeneration network would have increased recovery potential in all cases significantly.

Steam use for indirect heating averaged 41, 51, and 61% of the total energy for flow rates of 606, 1022, and 1325 L/hr, respectively, and ranged between 171 and 261 kJ/kg. Steam use at the injector ranged between 45–57% of the total for flow rates of 1022, 1172, and 1325 L/hr for the direct system (241–385.9 kJ/kg). At the same flow rates energy use for preheating the steam injection system used 26 and 20% of the total energy use for preheating temperatures 88° and 74°C, respectively, and ranged between 118 and 171 kJ/kg.

Heat input to the steam seals was a relatively constant valve; $\bar{x}$ = 6.68 $\times$ 10^4, σ = 0.46 $\times$ 10^4 kJ/hr for the indirect system; $\bar{x}$ = 2.15 $\times$ 10^4, σ = 0.18 $\times$ 10^4 kJ/hr for the direct system. The steam seals on the vacuum chamber were set manually to allow visual vapor discharge of steam from the seals. The steam seals on the homogenizer were set at specified pressures (ranging from 69–103 kPa). These different methods of establishing steam requirements at steam seals accounted for most of the discrepancy with these energy inputs. Numbers of steam seals and surface areas also influenced the energy consumption, but to a lesser extent. For the direct system, steam seals made up approximately 3% of the total at all flow rates. For the indirect system, they made up 25, 19, and 12% of the total at 606, 1022, and 1325 L/hr respectively.

Product cooling, after regeneration cooling, occured first in the tower water coolers. Final cooling to packaging temperature (ideally ambient temperature, and in this study set at 21°C) was accomplished in the chill water cooler.

Energy input to the cooling tower was accounted for by the electrical input to the cooling tower. The energy needed to produce chill water (temperature = 0.6°C) was calculated from the cooling load assuming a conservative Coefficient of Performance (C.O.P.) of 1 for the refrigeration system (Department of Energy, 1979a). For both systems product cooling loads accounted for approximately 10% of the total processing energy. An increase in C.O.P. will decrease the estimated energy input for chill water cooling.

Energy input per unit mass of product for both systems for the timing pump, (a high pressure, 3-piston pump that also supplied hemogenization pressure) was about the same ($\bar{x}$ = 28.1 kJ/kg direct, $\bar{x}$ = 28.8 kJ/kg indirect). This accounted for 5% of the direct energy use and 7% of the indirect.

Hallström cited steam energy use for a direct system between 304 and 622 kJ/kg. No value for heat recovery was reported. Other studies at similar flow rates with direct processing units (Department of Energy, 1979a; Biziak, 1981) cited values averaging 474 kJ/kg and ranging between 656 and 793 kJ/kg, respectively. This research concluded between 413 and 546 kJ/kg were used. All studies surveyed agree with Hallström's range of data except for Biziak (1981), where with no heat recovery the values were higher.

For individual systems, Hallström cited steam energy use in the range 83–304 kJ/kg. No details were available regarding heat recovery and process temperatures. The study on indirect processing by Biziak et al. (1982) placed steam energy use ranging between 248 and 270 kJ/kg at a similar flow rate.

From the data it is apparent that continuous thermal processing with indirect equipment is more energy efficient (as much as 30% better for product processing) due to greater thermal recovery potential and fewer pumps. Increasing regeneration potential and recovering heat energy from condenser cooling water, and other sources could help to even out the energy use between the two systems. Energy use for both systems could be reduced by incorporating product to product regeneration. Energy loads during sterilization could be reduced by installing a controller to control temperature at the filler. Energy loads could be further reduced if insulation were used especially on the vacuum chamber.

Research is continuing in the areas of increasing thermal

—Continued on page 1614

A Rheological Model for Milk Products

NAND KISHORE AWADHWAL & C.P. SINGH

ABSTRACT

A rheological model consisting of Maxwell units with yield elements is introduced. Mathematial analysis of the model for the case of loading at constant rate of deformation before and after yield and stress relaxation has been done. The model constants are determined by data superposition technique and an analytical method. The model can easily be adopted to suit the behavior of milk products which may or may not have yield value and may have any number of relaxation times. Results of tests on sweetened condensed milk, butter and Paneer are used to illustrate the model.

INTRODUCTION

KNOWLEDGE of rheological properties of milk products is essential for the design and operation of material handling and processing equipment used in the dairy industry. In the grading of several milk products, considerable importance is given to certain properties such as hardness, softness, viscosity, spreadibility and standing-up qualities because these properties determine the consistency of the product and influence its acceptability to consumers.

As with any other food product the rheological properties of milk products are also determined by their composition and structure. Many changes which occur in the properties of milk products under different environmental conditions are yet to be explained. Certain milk products, when subjected to deformation, exhibit yield and work softening, apparently due to break down of the structure. However, the structure may rebuild with time, when the product is allowed to set, and exhibit a thixotropic behavior (thickening), a characteristic of many food products. These products also show relaxation behavior which is strain as well as strain-rate dependent. To describe these phenomena, attempts have been made to modify the classical viscoelastic models consisting of springs and dashpots.

Diener and Heldman (1968) introduced a VMB (Viscous-Maxwell-Bingham) rheological model which consists of a dashpot containing a Saint Venant body in parallel and connected to a three-element fluid body to represent the small strain behavior of butter. Elliot and Ganz (1971) introduced a modified Bingham element, containing a Saint Venant element, to model materials like cheese spread. Chen and Rosenberg (1977) introduced a series-parallel model in which a yield element is introduced in the parallel branch for modelling food material such as American cheese. The concept of yield element is that no motion of the element takes place until a predetermined level of stress is reached, but once the motion takes place, the element itself offers no resistance to motion. Conceptually the yield element is similar to the Reiner's shear-pin mentioned by Sherman (1970) and the concept of fracture element reported by Peleg (1977).

THE PROPOSED MODEL

IN THIS PAPER, concept of a yield-Maxwell (Y-M) unit is introduced. A yield-Maxwell unit comprises a Maxwell unit containing a yield element in parallel to its dashpot element. The dashpot of the Y-M unit remains incapable of motion until the yield stress is reached. The proposed model is basically a generalized yield-Maxwell model. All the dashpots of the model remain immobilized until the applied stress reaches the yield value, which is equal to the sum of yield stresses of all the yield elements in the model. As soon as stress reaches the yield value, the yield elements unlock the dashpots and set them in motion. The model for milk products (Fig. 1) contains only two yield-Maxwell units because the products used for illustrations had only two relaxation times. The basic model can be adopted to suit the observed relaxation behavior of a variety of products (by arranging as many Y-M units as the number of observed relaxation time). In case the yield value of the material is negligible the basic model reduces to a generalized Maxwell model.

Model analysis

The proposed rheological model is shown in Fig. 1, in which: E_1, E_2 are elastic constants; η_1, η_2 are the dashpot constants; y_1, y_2 are the yield stresses of yield elements; $\sigma(t)$ is the imposed stress at any time t.

Loading at constant rate of deformation

Let a material described by the Y-M model be loaded at a constant strain rate ($\dot{\epsilon}$) and the time at which the yield stress is reached in the model be θ.

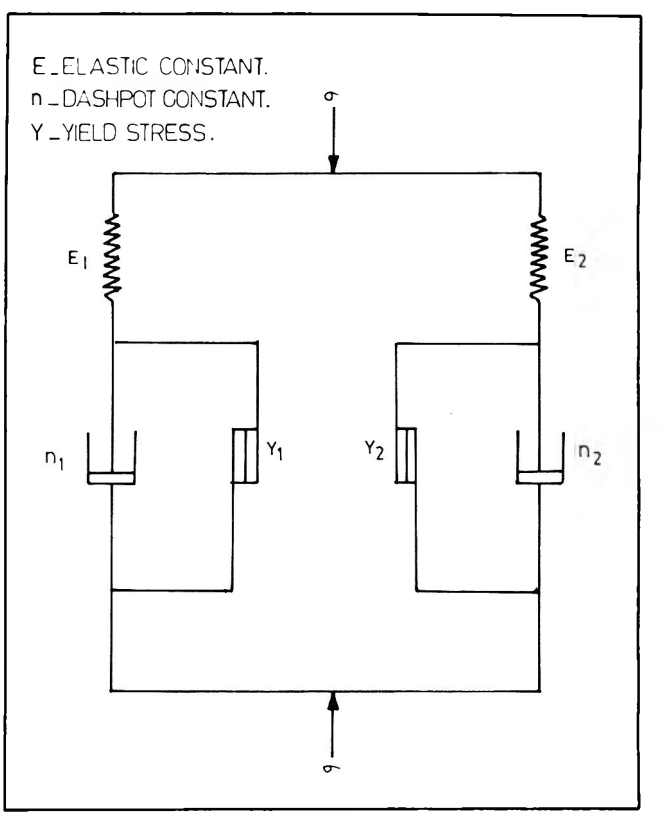

Fig. 1—Rheological model for milk products.

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Pre-yield response. For time $t<\theta$, the yield elements and hence the dashpots are incapable of motion, consequently only the elastic elements sustain the stress; therefore, the governing equation for the model can be written as:

$$\sigma(t) = \dot{\epsilon}t\,(E_1 + E_2) \qquad (1)$$

where the dot represents differentiation with respect to time.

Yield response. At time $t=\theta$, stress in the model reaches the yield value (Y) and the yield elements unlock. At this moment the state of stress is obtained by substituting θ for t in Eq.(1). It can be written as:

$$\sigma(\theta) = \dot{\epsilon}\theta\,(E_1 + E_2) \qquad (2)$$

The time for activation of yield element (θ) may be expressed from Eq.(2) as:

$$\theta = Y/\dot{\epsilon}\,(E_1 + E_2) \qquad (3)$$

For another strain rate $\dot{\epsilon}_1$, the above expression can be written as:

$$\theta_1 = Y/\dot{\epsilon}_1\,(E_1 + E_2)$$

Hence the ratio of time for activation of yield elements at two different strain rates can be expressed as:

$$\theta_1/\theta = \dot{\epsilon}/\dot{\epsilon}_1 \qquad (4)$$

Post yield response. If loading is carried beyond yield then the state of stress in the model at any time ($t>\theta$) is given by the sum of stresses in the two Maxwell units (Diener and Heldman, 1968) as:

$$\sigma(t)/\dot{\epsilon} = [\eta_1\{1-\exp(-(t-\theta)/T_1)\} + (\theta E_1)\exp(-(t-\theta)/T_1)] + [\eta_2\{1-\exp(-(t-\theta)/T_2)\} + (\theta E_2)\exp(-(t-\theta)/T_2)] \qquad (5)$$

where T_1 and T_2 denote the relaxation time η_1/E_1 and η_2/E_2,

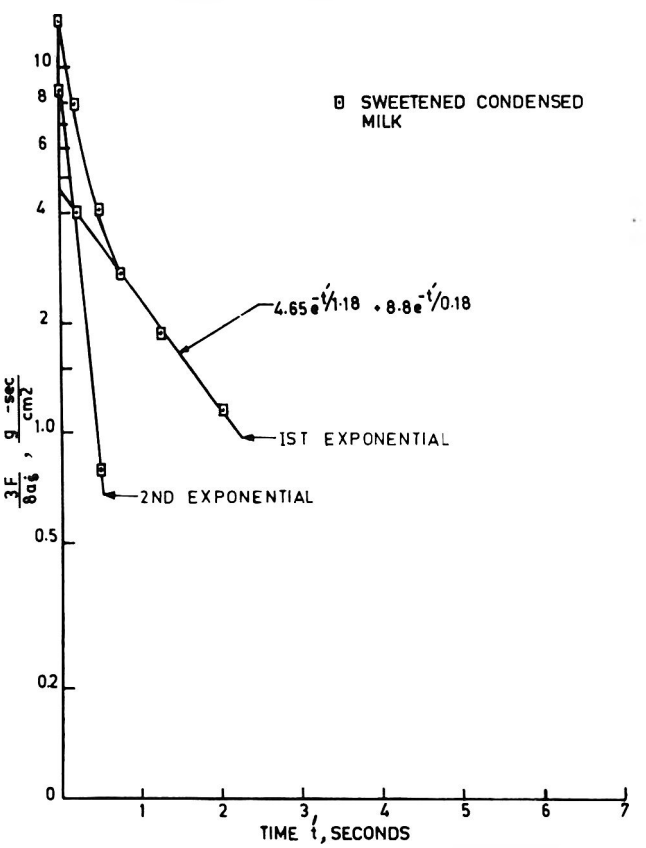

Fig. 2—Successive residual method for determination of relaxation time.

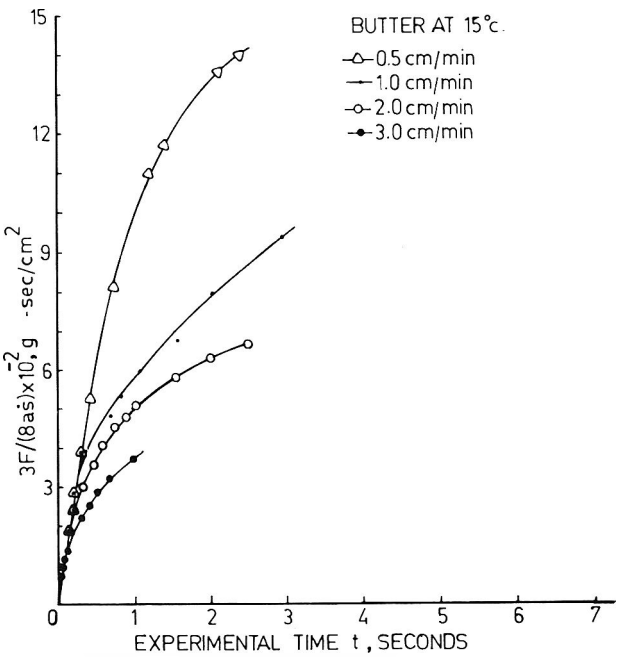

Fig. 3—Data superposition for loading response of butter.

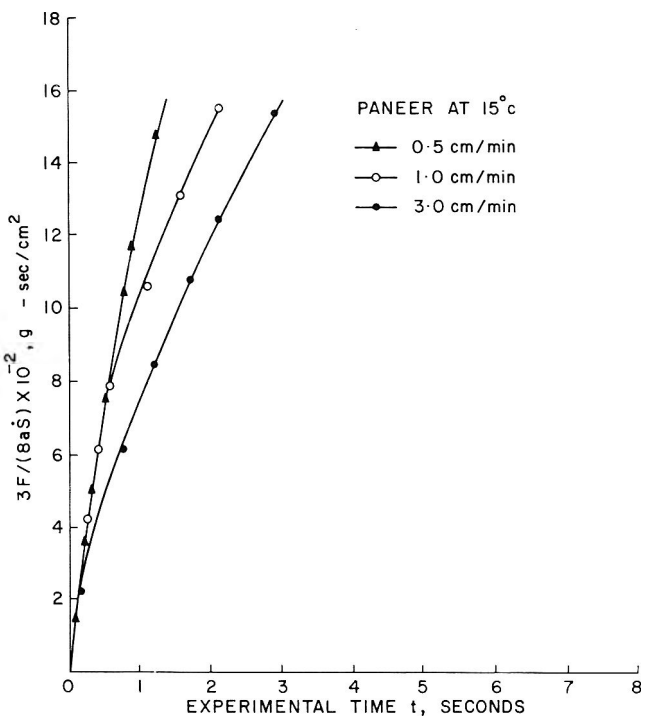

Fig. 4—Data superposition for loading response of paneer.

respectively. It can be noted that the value of stress at $t=\theta$, obtained Eq.(1) and Eq.(5) are one and the same.

Post yield stress relaxation

If loading is stopped at time t_0, such that $t_0>\theta$, and the strain is held constant at $\dot{\epsilon}t_0$, then the stress in the model at time t can be obtained by substituting t_0 for t in Eq.(5) and can be written as:

$$\sigma(t_0) = [\dot{\epsilon}\eta_1\{1-\exp(-(t_0-\theta)/T_1\} + (\dot{\epsilon}\theta E_1)\exp(-(t_0-\theta)/T_1)] + [\dot{\epsilon}\eta_2\{1-\exp(-(t_0-\theta)/T_2\} + (\dot{\epsilon}\theta E_2)\exp(-(t_0-\theta)/T_2)] \qquad (5a)$$

Table 1—Composition of milk products

Product	Component percent							
	Water	Fat	Protein	Lactose	Ash	Sucrose	Salt	Curd
Sweetened cond. milk	26.8	7.8	9.1	12.6	1.7	42	–	–
Paneer	51	30.3	14.2	2.44	2.06	–	–	–
Butter	15.9	80.4	–	–	–	–	1.8	1.9

Table 2—Model constants for the milk products

Product	Y_1	Y_2	Y	E_1	E_2	E_0	η_1	η_2	η
	(g/cm^2)			(g/cm^2)			($g\text{-}sec/cm^2$)		
Sweetened cond. milk	–	–	–	7.0	49.1	56.1	8.3	8.8	4.2
Butter	9.6	5.3	14.9	518.4	287.1	805.6	1.6×10^4	6.0×10^2	581.3
Paneer	30.7	16.1	46.8	754.6	441.2	1195.8	5.6×10^4	12.4×10^2	1218.2

Table 3—Predicted and observed average values of force on die for load deformation and relaxation test on paneer

Time[a] (sec)	Deformation (mm)	Force (g)	
		Predicted	Observed
00	00	00	00
0.12	0.2	31.6	30.4
0.24	0.4	62.7	60.8
0.36	0.6	93.3	101.3
0.48	0.8	123.5	128.5
0.60	1.0	153.2	153.3
1.38	1.0	139.4	140.0
2.58	1.0	123.9	121.7
3.78	1.0	113.3	112.8
9.78	1.0	90.8	88.8
15.58	1.0	82.2	80.1
21.78	1.0	75.5	76.0
42.78	1.0	57.4	59.2
81.78	1.0	34.1	35.6

[a] The observed values of force were read on lines marked on the strip chart hence the time is not in integers.

The magnitude of stress in the model at any time $t > t_0$, is given by the sum of stresses undergoing relaxation in the two Maxwell units of the model and can be expressed as:

$$\sigma(t) = [\dot{\epsilon}\eta_1\{1-\exp(-(t_0-\theta)/T_1)\} + (\dot{\epsilon}\theta E_1)\exp(-(t_0-\theta)/T_1)][\exp(-t'/T_1)] + [\dot{\epsilon}\eta_2\{1-\exp(-(t_0-\theta)/T_2)\} + (\dot{\epsilon}\theta E_2)\exp(-(t_0-\theta)/T_2)][\exp(-t'/T_2)] \quad (6)$$

where $t' = t - t_0$. This equation indicates that if relaxation is allowed for a long time then stress in the model will, ultimately, become zero.

The Boussinesq solution for a circular flat die on a half space given by Timoshenko and Goodier (1951) can be used for load deformation response of milk products (Diener and Heldman, 1968). For an isotropic, linear viscoelastic material, this equation can be written as:

$$F = 2\,a\,s\,E/(1-\nu^2) \quad (7)$$

where F is applied force, a is radius of the die, s is deformation, E is modulus of deformability, and ν is Poisson's ratio of the material being loaded. Taking $\nu = 0.5$, the above equation can be written as:

$$E = 3\,F/8\,a\,s \quad (7a)$$

In case the load deformation plot is not a straight line then the time dependent deformation modulus E(t) can be expressed as:

$$E(t) = \frac{3}{8a}\frac{dF}{ds}$$

or

$$\frac{3}{8a}dF = E(t)\frac{ds}{dt}dt \quad (8)$$

For constant rate of deformation ($\dot{s}$ = constant), Eq.(8) can be integrated to give:

$$3\,F/8\,a\,\dot{s} = \int E(t)\,dt \quad (9)$$

Diener and Heldman (1968) showed that the relaxation modulus E(t) can be determined from the slope of loading curve as:

$$\sigma(t) = \int E(t)\dot{\epsilon}dt \quad (10)$$

In case of constant strain rate ($\dot{\epsilon}$ = constant) Eq.(10) can be written as:

$$\sigma(t)/\dot{\epsilon} = \int E(t)dt \quad (11)$$

Comparing Eq.(9) and Eq.(11), we can write:

$$3\,F/8\,a\,\dot{s} = \sigma(t)/\dot{\epsilon} \quad (12)$$

Comparing Eq.(1) and Eq.(12) the equation governing the pre-yield response of the model can be written as:

$$3\,F/8\,a\,\dot{s} = t(E_1 + E_2) \quad (13)$$

Comparing Eq.(5) and Eq.(12) the post yield response can be expressed as:

$$3\,F/8\,a\dot{s} = [\eta_1\{1-\exp(-(t-\theta)/T_1)\} + (\theta E_1)\exp(-(t-\theta)/T_1)] + [\eta_2\{1-\exp(-(t-\theta)/T_2)\} + (\theta E_2)\exp(-(t-\theta)/T_2)] \quad (14)$$

The value of $\sigma(t)/\dot{\epsilon}$, obtained from Eq.(6), when substituted in Eq.(12) results in the following equation which expreses the post yield relaxation response of the model:

$$3\,F/8\,a\,\dot{s} = [\eta_1\{1-\exp(-(t_0-\theta)/T_1)\} + (\theta E_1)\exp(-t_0-\theta)/T_1)][\exp(-t'/T_1)] + [\eta_2\{1-\exp(-(t_0-\theta)/T_2)\} + (\theta E_2) \exp(-(t_0-\theta)/T_2)][\exp(-t'/T_2)] \quad (15)$$

Estimation of model parameters

The pre-yield response Eq.(13) is a function of time only whereas the post-yield response Eq.(14) is a function of time as well as deformation rate; therefore, on the time plot, the value of $3F/8a\dot{s}$, for different rates of deformation, should superpose to yield only. Hence, the yield value Y, and the yield time, θ, can be estimated, from the points where curves drawn at higher rates peel off from the master curve drawn at the slowest rate of deformation (Diener and Heldman, 1968). The ratio of yield stresses is equal to the ratio of the elastic constants and at the same time the yield value Y, is equal to summation of the yield stresses also; therefore, the yield stress can be estimated for each of the yield elements.

The initial deformation modulus E_0, is given by summation of the elastic constants and the normal viscosity η can be expressed as

$$\eta = 1/(1/\eta_1 + 1/\eta_2) \quad (16)$$

MATERIALS & METHODS

TO ILLUSTRATE the model, three commercially available milk products were studied: sweetened condensed milk, butter, and paneer (a product obtained from cow or buffalo milk by precipitation with sour milk, lactic acid or citric acid as per the Indian Standard Specification, IS:5162, 1962). The composition of these milk products is given in Table 1. Tests were conducted on the chosen milk products at 15°C using the Instron Universal Testing Machine (Model TM-M) to determine their force-deformation and force-relaxation behavior. The test samples of the milk products were 10 cm in diameter and 10 cm in height and were kept in containers of the same size. To obtain the force-deformation response of these milk products the samples were loaded with a flat circular die (15 mm diam) at five deformation rates of 0.5, 1, 2, 3, and 5 cm/min. A crosshead speed of 10 cm/min was used for all the relaxation tests and a deformation of 1 mm was maintained constant while the stresses relaxed.

Data superposition technique (Diener and Heldman, 1968) was applied to force-deformation curves to determine the yield values of the milk products. Successive residual method (Mohsenin, 1968) was applied to relaxation data of the milk products to determine their relaxation equations. (Fig. 2). The spring and dashpot constants were calculated from the relaxation equation parameters, using the analytical method presented by Chen (1971).

RESULTS & DISCUSSION

THE FORCE-DEFORMATION response at various loading rates showed good superposition (Fig. 3 and 4). The yield values at the loading rates of 1, 2, and 3 cm/min were 14.5, 15.6 and 14.6 g/cm^2, respectively, for butter and 43.7, 47.3, and 49.4 g/cm^2, respectively, for paneer. Thus yield value is nearly a constant as assumed for the model and has only a slight rate effect. The average yield value of butter calculated from the data reported by Diener and Heldman (1968) is 175.6 g/cm^2 which is higher than the average yield value obtained in this investigation (Table 2). The differences can be attributed to the variation in the physical properties of the butter used, due to difference in composition, processing, storage period and temperature at the time of conducting tests.

The data for condensed milk did not superpose indicating a negligible yield value. However, Sone (1972) reported that the yield value of condensed milk could range from 125–1000 dynes/cm^2 (i.e. 0.12–1.01 g/cm^2). The lack of agreement in the results reported here and elsewhere is due to the fact that the experimental set-up used in this study was not sensitive enough to detect such small forces as the yield value of the condensed milk. The average values of the model constants and the model parameters are given in Table 2.

Shearing viscosity of condensed milk was measured with a Hoppler viscometer to relate it to the normal viscosity obtained from this model. The measured shearing viscosity (1.39 g-sec/cm^2) was nearly one-third of the normal viscosity (4.2 g-sec/cm^2). This is in confirmation with the relationship, given by Reiner (1949) for the two. To verify the force deformation response of the model, the experimental stress relaxation curves of paneer samples were compared with those predicted from the model. The observed and the predicted force were found to be in good agreement (Table 3). The coefficient of variation of the data obtained from three repeated runs was below 10%, indicating reasonably good reproducibility of the experimental results.

The load deformation and relaxation behavior of a number of milk products can be represented by the proposed generalized Yield-Maxwell model. The model can be adopted to suit the behavior of products which may or may not have yield value and may have any number of relaxation times. The three model parameters, namely, yield value, initial deformation modulus, and normal viscosity, calculated from the model constants may be used as basic textural parameters for characterization of the milk products.

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regeneration potential, and in other areas of possible energy recovery.

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Thermodynamics of Water-Egg Powders Interaction

CHRISTOPHER C. LAI, SEYMOUR G. GILBERT and CHAIM H. MANNHEIM

ABSTRACT

Water sorption properties and differential thermodynamic parameters of two classes of egg powder were studied. Considerably higher water uptake was observed for whole egg with corn syrup and salt (CEP) than whole egg powder (WEP). Onset of water clustering began at a lower water activity for the WEP-water system than for the CEP-water system. The thermodynamic qualities were less negative for WEP than CEP. The presence of more sorptive sites with higher binding energies was suggested for CEP and WEP.

INTRODUCTION

WATER SORPTION PROPERTIES of dried eggs have been reported by several workers (Gane, 1943; Makower, 1945; Passy and Mannheim, 1982; Iglesias and Chirife, 1982). Egg white solids were found to have higher sorption capacity than whole egg powders under the same temperature and humidity conditions. It was suggested that this difference is due to the presence of fat in the yolk which acts as a water repellent. Gane (1943) showed that on a fat-free basis the protein and salts of egg yolk have almost the same water sorption isotherms as those of egg white.

Water vapor pressure of dried whole egg containing various levels of sucrose and corn syrup solids were studied by Davis and Kline (1965). At moisture levels commonly encountered in commercial whole egg solids (2–6%), additions of both carbohydrates were found to increase the equilibrium water vapor pressures of dried products. They suggested that the carbohydrates were found to increase the equilibrium water vapor pressures of dried products and suggested that the carbohydrates compete with water for the sorption sites on the proteins. In contrast, Lo et al. (1983) reported that sugars and salts reduced the water activity in the egg products.

Knowledge of the effects of composition and structure of eggs and additives on water distribution in the powder matrix are important for the selection of both optimum drying conditions for producing these products and the proper selection of barrier properties of packaging materials for the final products. The effect of adding corn syrup solids and salt on the water sorption and thermodynamic parameters of egg powders was the objective of this investigation.

MATERIALS & METHODS

Materials

Dried eggs (whole egg solids-standard, (WEP) and dried whole eggs with corn syrup and salt, (about 30% and 1% respectively in final product (basis) (CEP) were purchased from Henningsen Foods, Inc. (Omaha, NE). Samples were stored under refrigeration (4°C) until used. Proximate analyses were determined in accordance with methods of AOAC (1975) and are presented in Table 1.

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Table 1—Particle density and proximate analysis of egg powders

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Particle Density (g/cm^3)
WEP	4.74	49.40	32.43	3.95	1.20
CEP	5.26	29.70	21.40	3.53	0.99

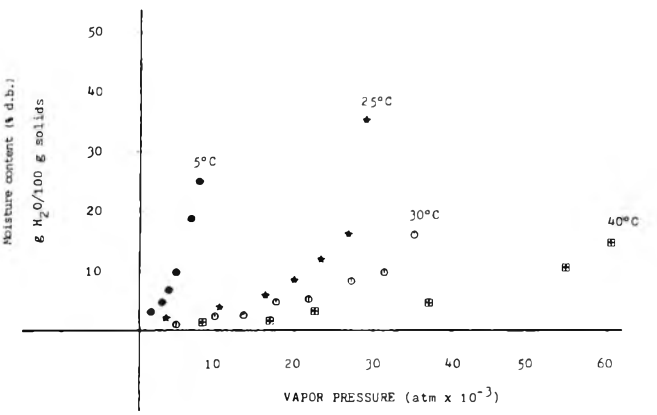

Fig. 1—Water sorption isotherms: Whole egg powder.

Methods

Water sorption isotherms at 5°, 25°, 30°, and 40°C for WEP and CEP were determined following the static procedure described by Labuza (1983). Moisture content of samples was determined by drying in a vacuum oven at 100°C for 5 hr at a vacuum of 760 mm Hg.

Water soluble protein was evaluated by a colorimetric procedure. Two-tenths gram of powder sample was mixed with 100 mL distilled water for 1 hr and centrifuged at 3,5000 prm for 20 min. The supernatant was filtered, and 1 mL filtrate was used for protein determination following the procedure of Miller (1959). The colored product was scanned from wavelengths of 300 – 800 nm using a Gilford UV-Vis Microprocessor-Controlled Spectophotometer System 2600. Two wavelengths, 325 nm and 689 nm, were found to give maximum absorbance. The 325 nm wavelength with slit width of 0.295 mm gave the best peak maximum and was used for quantification. The percent soluble solids was determined by drying an aliquot of the supernatant filtrate by heating to constant weight at 100°C for at least 5 hr in vacuum.

Particle densities were determined with a Beckman Air Comparison Pycnometer (Fullerton, CA).

Data analysis. All determinations were conducted in replicates. Statistical analysis including analysis of variance and least significant difference were performed according to Snedecor and Cochran (1967). Differences between isotherm curves were analyzed employing regression analysis using SAS programs (SAS, 1982).

RESULTS & DISCUSSION

THE WATER SORPTION ISOTHERMS of WEP and CEP are shown in Fig. 1 and 2 as functions of partial water vapor pressure (P_1) instead of a_w since different temperatures were compared. At 5° only a small change in pressure was accompanied by a sharp increase in water uptake while at the higher temperature the opposite occurred. Isotherms of WEP at 30°C and 40°C were in good agreement with those reported by Makower (1945).

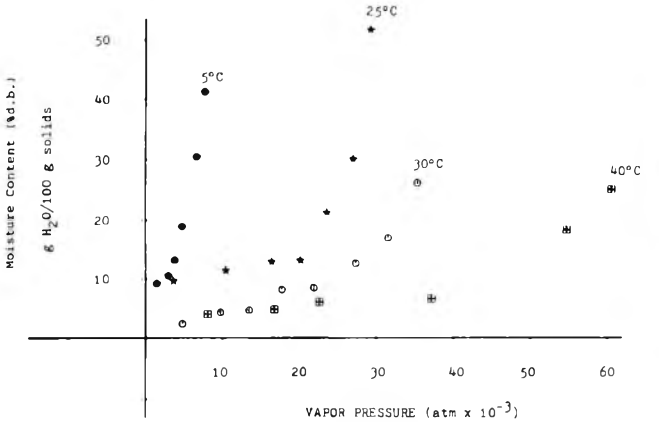

Fig. 2—Water sorption isotherms: Whole egg with corn syrup and salt.

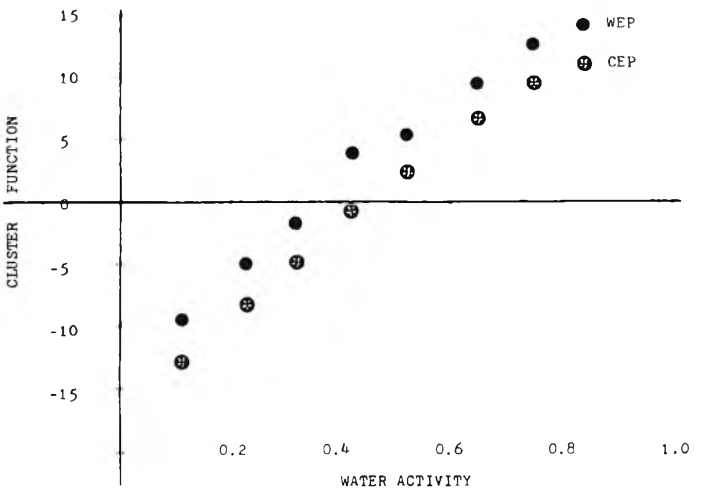

Fig. 3—Cluster function of egg powders at 30°C.

Table 2—Protein solubility of egg powders

Sample	λ_{max} (325nm)	Soluble Solids (%)	
WEP	2.60[a]	62	a
CEP	2.66[b]	100	b

[a,b] Values within a column with different letters differ significantly (P = 0.05).

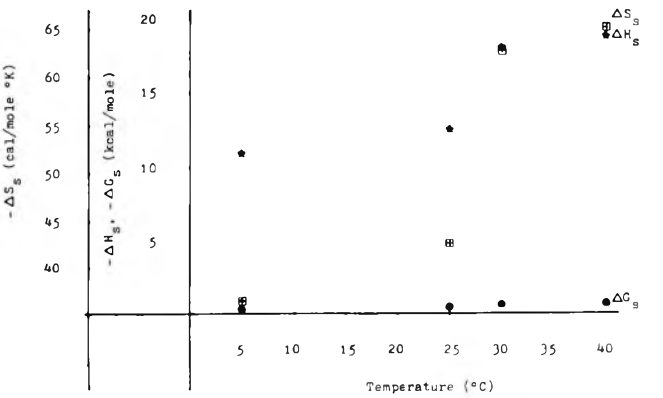

Fig. 4—Relationship between temperature and thermodynamic parameters of sorption at the onset of water clustering for whole egg with corn syrup and salt.

In contrast to Davis and Kline (1965), a lower vapor pressure was found for CEP than WEP at the same moisture content. The differences in amount of moisture uptake could be due to sample composition. Sodium chloride and sugars were reported to have lower water vapor pressure in foods (Lo et al., 1983). Differences in sorption could also be related to the degree of protein denaturation by the drying process used. Heat denaturation is known to affect the water sorption properties of proteins (Mellon et al., 1949; Altman and Benson, 1960; Tanaka, 1984). Heating or drying may alter the quaternary structure of the protein system by aggregation of individual molecules, which in turn reduced the availability of polar amino acid groups for binding water. Changes in the bonding sites of ovalbumin due to heat treatments were studied by Tanaka (1984) using inverse gas chromatography. Woodward and Cotterill (1983) reported that the addition of sugar and salt to whole eggs increased the thermal stability of the proteins.

Protein denaturation is generally associated with the loss of solubility. Table 2 shows the protective effect of additives on egg powders stability since the lower solubility of WEP compared to CEP supports the theory that there is less protein denaturation in CEP. Baxter and Hester (1985) also reported that protein solubility increased with sucrose in the heated gluten dispersions and suggested that sugar inhibited the heat coagulation of the protein.

For all temperatures increase in vapor pressure produced upwardly concave isotherms, particularly for CEP.

The degree and water sorption range of clustering of molecules at specific sites were determined with the sorption isotherm data (Hung et al., 1973). The plot of cluster function vs water activity at 30°C showed the tendency of water molecules to cluster within the sample matrix as the activity increased (Fig. 3). At low vapor pressures (a_w, 30°C) cluster function was strongly negative for water-WEP and water-CEP systems, indicating that water-egg powder contacts were favored. The tendency for water molecules to cluster was revealed by values of cluster function greater than -1.

The onset of clustering at 30°C began at a lower water activity for water-WEP system, indicative of less active site sorption on the WEP sample. There was less of a clustering effect as the temperature increased for both systems which could be explained by the increased fugacity of the water molecules at higher temperatures. Evidence of intermolecular bonding or clustering of water in the vapor phase has been reported by Carlon (1978) from measurements of the phase transition changes in the molecular absorption coefficiency of water in the infrared spectrum.

The differential thermodynamic quantities for water-egg powder interactions obtained from sorption data are presented in Tables 3 and 4. Free energy of mixing calculated from Gibb's equation (Gilbert, 1984) was negative in the sorption for the temperature range of 5 – 40° C. These negative values indicated that the mixing of water with the egg samples was an energetically favorable hydroscopic process. In all cases, the free energy values became less negative as the amount of water increased in accordance with the decrease in the driving force.

The enthalpy, calculated from Clausius-Clapeyron equation, became less negative with increasing amounts of water sorbed. The entropy, calculated from the Gibb's equation by difference, also showed the same trend. Much larger negative values were obtained from the enthalpies than from the free energies. The thermodynamic parameters for WEP were less negative than for CEP at the same conditions which further suggested that WEP had less active sites within a more random structure. On the other hand, the presence of corn syrup and salt contributed to more ordered structure and more effective sorptive mass based on more negative enthalpy and entropy values.

Data on enthalpy of sorption provided an indication of binding strength of water molecules to the egg powders. The "binding energy" ($-\Delta H_s$) values calculated from isotherms were much higher than those estimated by the B.E.T. equation (Brunauer et al., 1938) The values from B.E.T. were 11.6 and

Table 3—Calculated ΔH_s, ΔG_s and ΔS_s of whole egg powder[a]

Moisture (g/100g solids)	ΔH_s	5°C		25°C		30°C		40°C	
		ΔG_s	ΔS_s	ΔG_s	ΔS_s	ΔG_s	ΔS_s	ΔG_s	ΔS_s
3	−12.9	−0.9	−43.5	−0.8	−43.8	−0.7	−44.2	−0.7	−44.1
5	−12.5	−0.2	−42.9	−0.5	−43.2	−0.4	−43.4	−0.4	−43.4
10	−12.4	−0.3	−42.6	−0.2	−43.0	−0.2	−43.2	−0.2	−43.2
12	−12.1	−0.3	−42.5	−0.2	−41.8	−0.1	−41.9	−0.1	−41.9
15	−11.4	−0.2	−40.4	−0.1	−39.4	−0.1	−39.3	−0.1	−36.3

[a] ΔH_s (kcal/mole) = enthalpy; ΔG_s (kcal/mole) = Gibbs free energy; ΔS_s (cal/mole °K) = entropy

Table 4—Calculated ΔH_s, ΔG_s and ΔS_s of whole egg with corn syrup and salt[a]

Moisture (g/100g solids)	ΔH_s	5°C		25°C		30°C		40°C	
		ΔG_s	ΔS_s	ΔG_s	ΔS_s	ΔG_s	ΔS_s	ΔG_s	ΔS_s
5	−20.8	−1.8	−69.0	−2.0	−67.6	−0.7	−72.0	−0.8	−71.8
10	−14.6	−0.7	−48.9	−0.7	−49.0	−0.4	−50.3	−0.4	−50.3
15	−12.2	−0.4	−40.7	−0.4	−40.9	−0.2	−41.6	−0.2	−41.6
20	−10.9	−0.3	−36.1	−0.2	−36.3	−0.1	−36.7	−0.1	−36.7

[a] ΔH_s (kcal/mole) = enthalpy; ΔG_s (kcal/mole) = Gibbs free energy ΔS_s (cal/mole °K) = entropy.

12.0 Kcal/mole for WEP and CEP, respectively. Similar observations were reported by Almasi (1978). This difference has been explained by the existence of some active sites which have a higher affinity for sorption and thus display a more negative heat sorption; whereas in the B.E.T. assumption, all active sites in the first layer have a constant heat of sorption.

Randomization of the location of the water molecules was indicated by the increasing entropy of the matrix water increasing water uptake. This change appeared to be related to the formation of water clustering within the powder structure. The relationship between thermodynamic parameters at the onset of water clustering and temperature was evaluated. Slight changes in the thermodynamic quantities were found for the water-WEP system. On the other hand, values of ΔH_s and ΔS_s of the water-CEP system changed between 25° and 30° (FIg. 4). This corresponded to a large drop in moisture from 13.3% (A_w = 0.62, P_1 = 0.019 atm @ 25°C) to 7.0%. (a_w = 0.45, P_1 = 0.01 atm @ 30°C) dry basis, respectively. Again, this could be attributed to the more sorptive sites with binding energies for CEP than WEP.

CONCLUSIONS

THESE STUDIES have provided a quantitative comparison of the effect of additives of sugar and salt to whole egg solids on the water sorption and thermodynamic properties of the water-egg powder system. The structure of CEP protein appeared to be less denatured than WEP as evidenced by the experimental data. These findings further support the suggestion of the thermal stabilization effect of additives on egg proteins.

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Effect of Composition on the Flow Properties of Egg Powders

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ABSTRACT

Flow properties of two classes of egg powder were studied. Whole egg with corn syrup and salt (CEP) exhibited better flow properties than whole egg powder (WEP). Free-flowing characteristics of both powders decreased with increased temperature. Moisture content was found to have significant affect on the flowability of CEP, but not WEP, at level as low as in the (B.E.T.) monolayer region [Brunauer et al. (1938), JACS 60: 309]. Lipid, on the other hand, was shown to be the dominant factor in preventing easy flow of WEP. This was related to the presence of surface lipid that contributed to the cohesiveness between particles.

INTRODUCTION

FLOW PROPERTIES are of great concern to industries producing and utilizing powders. Pertinent reviews on the flow of powders were given by Peleg (1983), Sutton (1976), Carr (1976), and Sone (1972). Fat-containing egg solids are often difficult to handle in continuous blending operations because of caking and poor flowability (Forsythe et al., 1964). Passy and Mannheim (1982) noted that fat is the major component affecting flow properties of whole egg powder as it "glues" the powder particles and prevents easy flow. They observed that whole egg could not be easily sheared at regular pressures and that much higher loads had to be used for the compacted powder to rupture. There is little fundamental knowledge on the possible role of fat on the flow phenomena of powders and in particular of those with complex composition such as food powders.

Several methods have been introduced to improve the free-flowing characteristics of egg powders. Musher (1950) reported that the tendency of egg powders to stick together is decreased by saturating the egg powder with a fat solvent and then removing the solvent by volatilization. One way to overcome or minimize flow problems is to incorporate a flow conditioner into the powder. Several workers (Toney and Bergquist, 1983; Forsythe et al., 1964) reported that addition of silicoaluminate or silicon dioxide improved flowability of the spray-dried egg products.

When egg powders are dried with carbohydrates, their foaming and emulsification properties are better preserved along with other desirable physical characteristics. These powders exhibit better flowability, blend more easily with other ingredients, disperse and dissolve more rapidly in water (Toney and Bergquist, 1983). Forsythe et al. (1964) related the improved flow properties of egg yolk solids dried with carbohydrates to the changes in product composition, i.e., decrease in the level of fat.

The purpose of this work was to study the effect of egg lipid and codried carbohydrate and salt on flow properties of egg powders.

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MATERIALS & METHODS

Materials

The dried egg products previously described (Lai et al. 1985) were used in this study. These were whole egg powder (WEP) and whole egg dried with corn syrup and salt (CEP) obtained from Hensegsen Foods Inc., Ohama, NE.

Purified glass beads (Analabs, New Haven, CT) of uniform size in the range 100–120 mesh were used as inert host to study the influence of a lipid film on flow properties.

Sample preparations

Samples were conditioned in an environmental control chamber. For each condition, the samples were kept in trays inside the chamber and stirred every other day for about two weeks. Samples at the lowest moisture content were prepared by vacuum drying the samples for 6 hr at 18–20°C. Moisture content of all samples was determined by drying at 100°C for 5 hr in a vacuum oven to constant weight to obtain "bone dry" basis.

Egg powders, with lipids partially removed (WEP-LP and CEP-LP), were prepared by mechanical mixing the sample with methanol for 24 hr at room temperature (about 22°C) followed by filtration. This was repeated three more times with fresh solvent. Solvent was vacuum evaporated from the solids which were then spread on a pan and dried further in air. Residual lipid in the samples was determined by 6 hr Soxhlet extraction with methanol. About 7% of crude lipids were still found in these samples.

The mechanically extracted egg powders were further extracted with methanol (WEP-LE and CEP-LE) for 3 days, using a Soxhlet type extractor. Care was taken to ensure that the temperature inside the extractor chamber did not exceed 30°C by controlling the heating temperature and attaching an additional glass connector between the reservoir flask and the extractor. The solvent in the powder was removed by air drying 15 hr at 20°C.

Glass beads were coated with 1% whole egg lipid obtained from the first extraction. The glass beads were mixed with the egg lipid solution and dried in a rotary evaporator.

The apparatus used for tensile strength measurements was as described by Mannheim and Passy (1982). The measured load of force per unit area needed to split the sample powder bed is the tensile strength at the compact density.

The same modified tensile strength cell was employed to determine the compact density of the powders at different consolidating values. Compact density was defined as the net weight of the powder divided by the cell volume at a perpendicular compressive load. Loads used were 13.46, 30.16, 39.61, 59.90 g/cm^2 respectively.

The relationship between the compact density, ρ_c, and the applied normal compressive stress, σ_N, was expressed by Sone (1972) by the empirical equation:

$$\rho_c = A + B \log \sigma_N$$

where A and b are constants. The constant 'B' representing the change in compact density by the applied stress, is defined as 'compressibility'. This has been found to correlate with the cohesion of a variety of food powders and therefore could be a simple parameter to indicate changes in the flowability (Peleg, 1977).

Bulk density of the sample was determined by measuring the volume of a fixed weight freely poured into a graduate cylinder.

Scanning electron microscopy. A powder sample was affixed to an aluminum stub with copper tape, coated with gold-palladium. The powder was then examined with an ETEC Autoscan scanning electron microscope using a tilt angle at 40° at 20 kv of accelerating voltage.

All determinations were conducted in replicates at 2°, 23° and 30°C. Statistical analysis including analysis of variance and least significant difference were performed according to Snedecor and Cochran (1967).

RESULTS & DISCUSSION

EGG SOLIDS are finely divided particles which tend to aggregate and form soft agglomerates of nonuniform sizes. Scanning electron micrographs of WEP and CEP in Fig. 1 demonstrate that the particles are smooth and spherical in shape. They seemed to be soft and deformable, as evident by the dents on some surfaces of the particles, which were probably formed by collisions during spray drying. No morphological difference was seen on electron micrographs between regular and lipid samples.

Effect of temperature

Pilpel (1981) observed that most powders flow better at low temperatures, and the same was true for the egg powders studied. The values of bulk density, compressibility and tensile strength at three temperatures are listed in Table 1. The exposure to increased temperature showed a simultaneous decrease in bulk density and an increase in compressibility. This resulted from the ability of the cohesive powder bed to maintain open structure supported by the interparticle forces. These extremely weak structures collapsed under very small pressures giving rise to the measured compressibility.

Another factor that contributed to the increase in compressibility was the softening and plasticization of the particles at the higher temperatures (Dobbs et al., 1982). There was also a great increase of surface stickiness of the particles and consequently the powder cohesiveness was increased as indicated by the higher tensile strength. Surface stickiness is considered to be caused by the presence of lipids on the surface.

Lipid materials were shown to coalesce on the egg solid surface in the form of an oily film after being dried (Joslin and Proctor, 1954). Schultz et al. (1968) reported that there is a decreasing level of free lipid coalescing as the amount of sugar added to the egg increases. This explains the improved behavior observed from the CEP as compared to WEP. Further discussion on the effect of presence of surface lipid is given later.

The lowest tensile strength was found for WEP and for CEP at 2°C. At this temperature the particles were basically solid and fairly hard and hardly interacted resulting in high flowability.

Activation energy of particle bonding, EA, can be calculated from Arrhenius plot of the relationship between tensile strength and temperature at which it is measured (Malamataris and Pilpel, 1981). The bonding activation energies (E_a) for WEP and CEP are 8.81 Kcal/mole and 2.31 Kcal/mole, respectively. The lower bonding for CEP than WEP can be related to better flow properties of CEP. These values are of the order expected for physical or hydrogen bonding being one or two orders of magnitude less than E_a values characteristic of chemical reactions between the surfaces of the particles (Bohinski, 1976).

Effect of moisture

Moisture has long been recognized to be a major factor affecting the flow properties of powders. The water sorption properties of WEP and CEP were discussed by Lai et al. (1985). It was found that WEP was less hygroscopic than CEP due to its higher fat content and some protein denaturation. The greatest effect of moisture on the flow properties of CEP was found at the lowest moisture level (4.4%) (Fig. 2). As the moisture content increased the changes in flow properties decreased to a nearly constant value above 7% water. The low moisture level was in the region of B.E.T. monolayer capacity. The higher cohesiveness at the lowest water level indicated that the liquid water bridges formed near the contact points of the particles were not the cause of the low flowability in CEP, suggesting that hydrogen bonding between particles was responsible for the adhesion occurring in the early stages of humidity caking processing. Similar findings were reported by Kaiho et al. (1975) and Chikazawa et al. (1977).

Moisture was found to have almost no affect on the flow properties of WEP for the range studied (Fig. 2 and 3) This could be explained by the hypothesis that the higher concentration of lipids at the surface of WEP was the dominant factor in hindering flow. Other workers also attributed fat to be the major component affecting flow properties of fatty powders (Moreyra and Peleg, 1981; Passy and Mannheim, 1982).

When moisture is the detrimental factor in a packaged powder stability, the shelf life can be estimated by knowing the critical moisture level and storage condition of the product. In the case of CEP, the optimal moisture for free flowing would be in the region of monolayer value below 4.0% dry basis. This value also provides for maximum stability toward oxidative flavor deterioration (Salwin, 1963).

Effect of lipid removal

The solvent-removed methanol extract was a sticky viscous oil. The presence of this oil film on the egg solids may con-

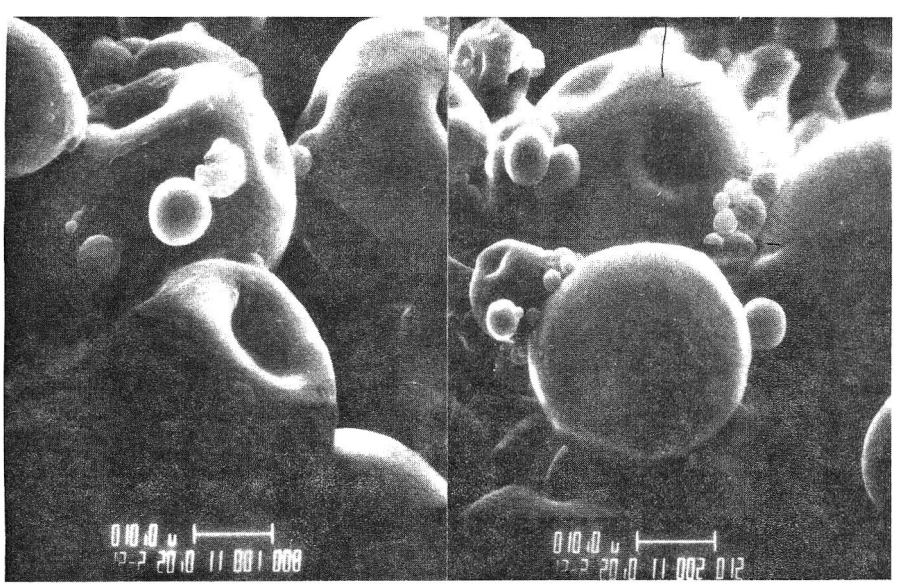

Fig. 1—Scanning electron micrographs of egg powders: Whole egg powder (left); Whole egg with corn syrup and salt (right).

Table 1—Effect of temperature on flow properties of egg powders (+ 14-28 mesh)

Sample	Temp. (°C)	Loose bulk density (g/cm³)	Compressibility	Tensile strength[g] (g/cm²)
WEP	2	0.27[c]	0.06[a]	2.19[a]
	23	0.21[b]	0.11[b]	8.02[b]
	30	0.20[a]	0.12[b]	8.94[c]
CEP	2	0.42[f]	0.06[d]	3.18[d]
	23	0.39[e]	0.07[d]	3.43[d]
	30	0.35[d]	0.10[e]	5.11[e]

[a-f] Within each sample and each property, values with different letters differ significantly (P = 0.05).
[g] Determined at a consolidation stress of 53 g/cm².

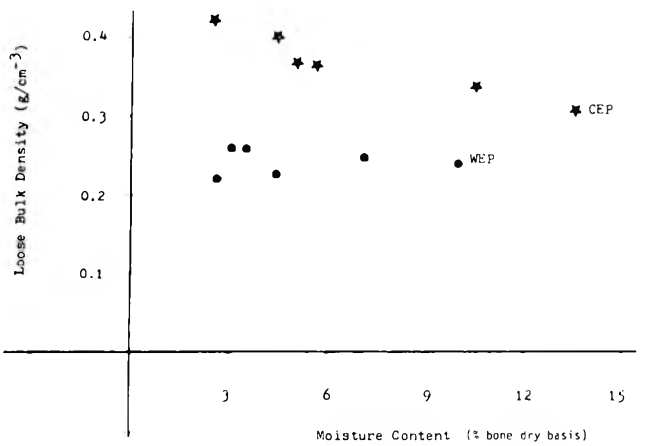

Fig. 2—Effect of moisture on loose bulk density of egg powders at 23°C.

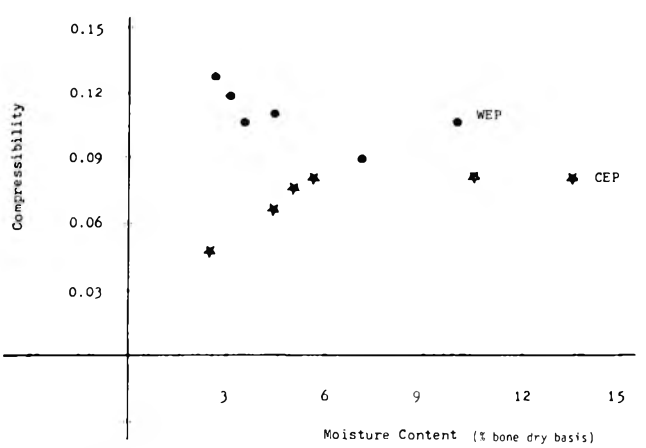

Fig. 3—Effect of moisture on compressibility of egg powders at 23°C.

Table 2—Effect of lipid removal on the flow properties of egg powders (+ 14-28 mesh) at 23°C

Sample	Moisture (% dry basis)	% Fat	Loose bulk density (g/cm³)	Compressibility	Tensile strength[g] (g/cm²)
WEP	4.37[a]	32.43[a]	0.21[a]	0.13[a]	8.02[b]
WEP-LP	4.33[a]	7.48[b]	0.34[c]	0.18[b]	8.78[b]
WEP-LE	4.42[a]	−0[c]	0.28[b]	0.12[a]	3.78[a]
CEP	4.91[d]	21.40[d]	0.39[d]	0.07[d]	3.43[d]
CEP-LP	5.11[e]	7.47[e]	0.42[e]	01.5[f]	5.27[e]
CEP-LE	5.65[f]	−0[f]	0.38[f]	0.12[e]	3.40[d]

[a-f] Within each sample and each property, values with different letters differ significantly (P = 0.05).
[g] Determined at a consolidation stress of 53G/cm².

Table 3—Effect of the presence of surface lipid on flow properties of glass beads (100-120 mesh) at 23°C

Sample (% lipid)	Moisture (% dry basis)	Loose bulk density (g/cm³)	Compressibility	Tensile strength[c] (g/cm²)
0	0.10[a]	1.39[a]	0.05[a]	1.96[a]
1.0	0.31[b]	1.20[b]	0.12[b]	6.37[b]

[a,b] Within each property, values with different letters differ significantly (P = 0.05).
[d] Determined at a consolidation stress of 53 g/cm².

tribute to the poor flow properties. Table 2 lists the effect of lipid removal on the flow properties of egg powders. Partial removal of lipid from WEP did not improve powder flowability. Instead the flow impediment increased probably because during the batch extraction fat globules within the particle were dissolved and released to the surface. A layer of the residual fat may thus have formed on the particle surface after the solvent was evaporated. This oil film was greatly reduced in the exhaustively extracted samples. As a result, the cohesiveness between particles was significantly decreased as shown by the tensile strength data. Neel and Hoseney (1984) also reported that defatted wheat flours are difficult to bridge and did not seem to possess any cohesive properties. The same phenomena were observed for the CEP with lipid removed. Similar flow parameters were obtained for CEP and CEP-LE suggesting a possible similarity in surface physical-chemical properties for both samples with little or no surface lipid present on CEP or CEP-LE.

Table 3 demonstrates the effect of the presence of surface lipid on the flow properties using a model powder. Considerable lower flow properties were found between the glass beads coated with 1% whole egg crude lipids compared to those without. The results confirmed the suggestion that surface lipid was a significant factor on powder flowability despite the many dissimilarities between glass beads and food powders such as solid density, shape and size distribution. Baker et al. (1980) also pointed out that flow difficulties of powdered shortenings may be caused in part by the presence of fat on the particle surface due to incomplete encapsulation.

Extracting lipid from egg solids with organic solvent could cause protein denaturation. Altman and Benson (1960) have reported the denaturating effect of ethyl alcohol on water-egg albumin interaction. The lipid removal procedure is not recommended for use to improve egg particles flowability if the product's functional properties still have to be preserved. Other alternatives such as maintaining the powder at low moisture content and temperature or incorporating the powder with the flow conditioner can be used.

CONCLUSIONS

WHOLE EGG co-dried with carbohydrates produced a powder with better flow properties powder than the standard whole egg solids in addition to other functional properties. Lower temperature improved the flowability of both powders. Surface lipid on WEP was the dominant factor contributing to the particle cohesiveness. While moisture was the major factor governing free-flowing characteristics of CEP, hydrogen bonding, not liquid bridge formation, was suggested as responsible for the cohesion in the caking of CEP at lower moisture.

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Physical State of the Dispersed Phases of Emulsions Prepared with Egg Yolk Low Density Lipoprotein and Bovine Serum Albumin

REIKO MIZUTANI and RYO NAKAMURA

ABSTRACT

Physical state of the dispersed phases of the emulsion prepared with egg yolk low density lipoprotein (LDL-emulsion) was compared with that of bovine serum albumin (BSA-emulsion). Results clearly showed that the mean globule size of LDL-emulsion was much smaller than that of BSA-emulsion and that stability of the LDL-emulsion during storage was much larger than the BSA-emulsion. These properties of LDL-emulsion appeared to be dependent on the high lipid-binding ability of LDL.

INTRODUCTION

IN PREVIOUS WORK (Mizutani and Nakamura, 1984) on the clarification of the characteristics of emulsifying properties of egg yolk low density lipoprotein (LDL), comparisons were made with egg lecithin and bovine serum albumin (BSA). The results showed that the emulsifying activity of LDL was much larger than that of egg lecithin or BSA although the creaming stability of LDL was almost the same as that of BSA. The stability of the emulsion prepared with LDL, however, differed from that of BSA emulsion. Although the stability of the BSA emulsion greatly decreased with addition of egg lecithin, that of the LDL emulsion was not affected. Furthermore, the stability of the BSA emulsion greatly decreased at a low protein concentration, but that of the protease-treated LDL emulsion having the same protein concentration as BSA was almost the same as that of the untreated LDL emulsion (Mizutani and Nakamura, 1985). Although these differences in the properties of emulsions prepared with LDL and BSA are very interesting, they can not be explained due to the lack of detailed knowledge about the LDL emulsion.

The purpose of this study was to obtain fundamental information about the physical state of the dispersed phases of LDL emulsions and to compare both viscosity and size distribution of fat globules of LDL emulsions with those of BSA emulsions.

MATERIALS & METHODS

Materials

LDL was prepared from fresh egg yolk of unfertilized White Leghorn hens according to the method of Raju and Mahadevan (1974). Briefly, egg yolk was diluted with an equal volume of distilled water, salted out with ammonium sulfate, applied on a DEAE-cellulose column and eluted with 0.05M Tris-HCl buffer, pH 8.2 containing 10^{-3}M EDTA. LDL solution was dialyzed against water containing 0.02% NaN_3 and concentrated by ultrafiltration. Since LDL is insoluble in water, measurements of emulsifying properties were made in the presence of 0.06M NaCl. Previous work (Mizutani and Nakamura, 1984) showed that the effect of NaCl on the emulsifying properties of LDL was relatively small. The protein component of LDL was assumed to be the main contributor to the emulsifying properties. The amount of LDL was determined by measuring the dry weight of salt- and lipid-free LDL in order to use the weight of active component of LDL. Both BSA (Fraction V) and peanut oil were purchased from Sigma Chemicals (St. Louis, MO) and Yoneyama Chemical Ind. Ltd. (Japan), respectively. BSA was dissolved in distilled water.

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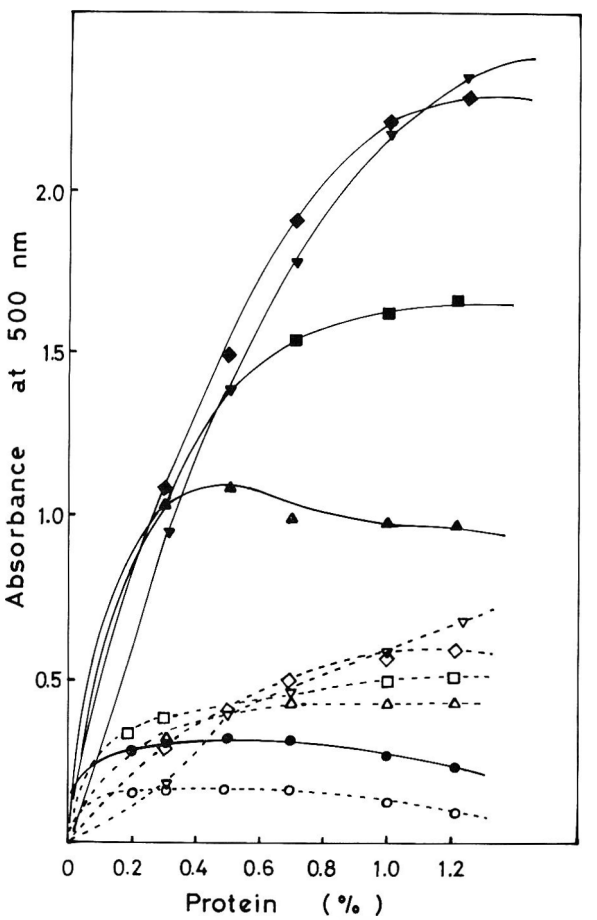

Fig. 1—Turbidity of emulsions as a function of protein concentration and oil volume fraction. Oil volume fractions were 1/4, (○, ●); 1/3, (△, ▲); 1/2, (□, ■); 2/3, (◇, ◆) and 3/4, (▽, ▼). — LDL, ---- BSA

Preparation of emulsion and measurement of turbidity

Emulsions were usually prepared as follows: a definite concentration of protein solution was mixed with measured amount of peanut oil and homogenized with an Ultra Disperser (Yamato Kagaku, Comp. Ltd., Tokyo, model LK-21) at speed setting 1 (10,000 rpm) for 60 sec.

Turbidity of the emulsion was measured by the technique of Pearce and Kinsella (1978) with a light modification as described previously (Mizutani and Nakamura, 1984), and the results were expressed as the optical density of emulsion diluted 1/500 at 500 nm.

Measurement of globule size

Emulsions were diluted about 20 times with 1% glycerol solution, and a drop of the diluted emulsion was placed on a glass slide with

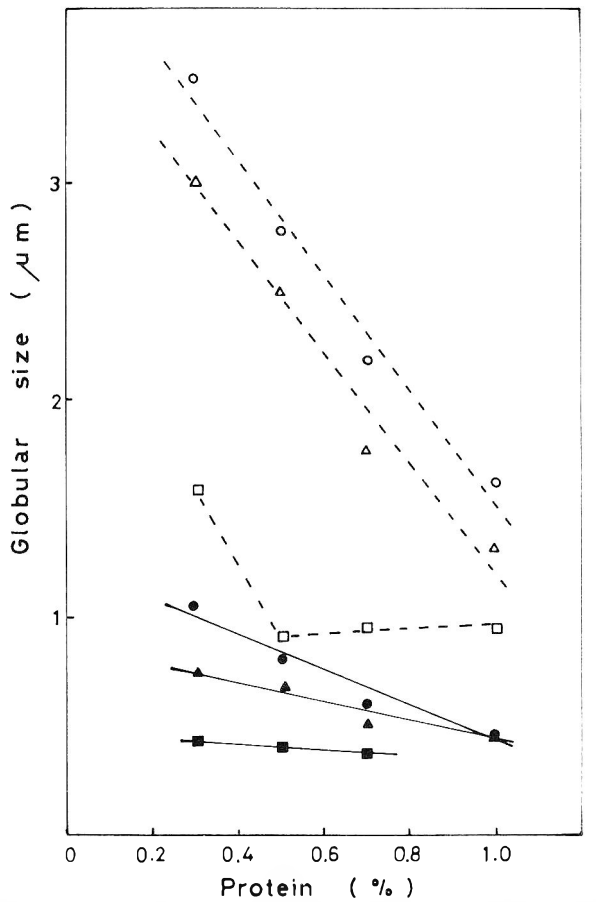

Fig. 2—Globular size of emulsions as a function of protein concentration and oil volume fraction. Oil volume fractions were 1/3, (□, ■); 2/3, (△, ▲) and 3/4, (○, ●). — LDL, ---- BSA

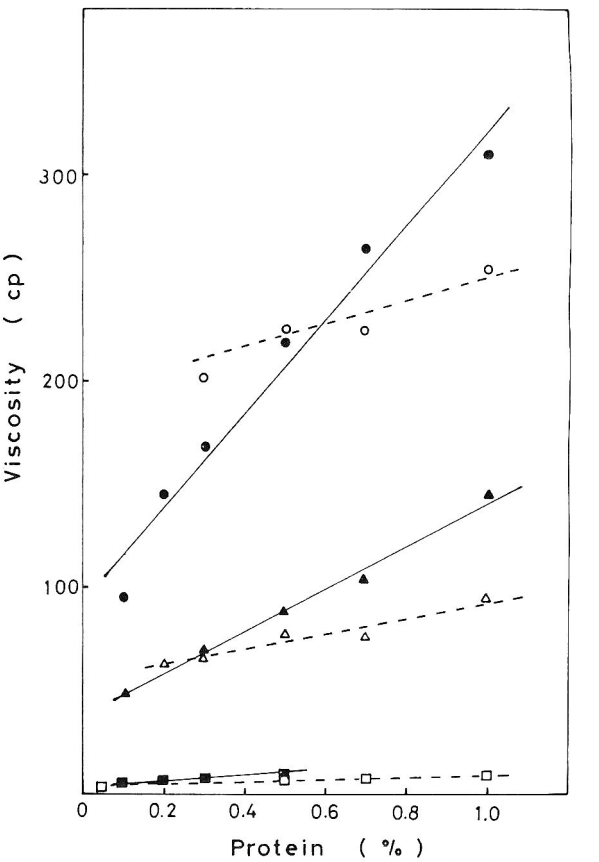

Fig. 3—Viscosity of emulsions as a function of protein concentration and oil volume fraction. Oil volume fractions were 1/3, (□, ■); 2/3, (△, ▲) and 3/4, (○, ●). — LDL, ---- BSA

a small depression in the center. The diameters of the dispersed globules were measured by using an optical microscope as described by Mita et al. (1973). The mean volume diameter of the globules, Dv, was calculated using the following relation:

$$Dv = \sqrt[3]{\frac{\Sigma n_i D_i^3}{\Sigma n_i}}$$

where n_i is the number of globules with diameter D_i.

Measurement of the amount of protein adsorbed to the oil globule surface

Emulsions were prepared with 0.1g of peanut oil and 3 mL of LDL or BSA solution containing various amounts of protein and centrifuged immediately at 1,000 × *g* for 30 min. An aliquot was taken carefully from the aqueous portion separated from the emulsion and its protein content was measured by the modified Lowry procedure (Markwell et al., 1978). The amount of protein adsorbed to the oil globule surface was calculated from the difference between the total amount of protein in the emulsion and that of the aqueous portion separated from the emulsion.

Viscosity measurement

Viscosity of the emulsion was measured at 20°C at a shear rate of 192 sec^{-1} with two types of cone plate viscometers (Tokyo Keiki Co., Ltd, Tokyo): one was type ELD for lower viscosity and the other was type EHD for higher viscosity. Measurements were performed ten times for each sample and the readings were averaged.

RESULTS & DISCUSSION

Dispersed state of emulsion

Turbidity of the emulsion prepared from LDL greatly increased with increased concentration of LDL (Fig. 1) A similar effect of concentration on the turbidity of the emulsion was also noted in the BSA emulsion although the extent of turbidity increase was much smaller. When the oil volume ratio was changed in the preparation of the emulsion, this turbidity difference between LDL and BSA emulsion became much greater. Even when the necessary time for homogenization was extended to 2 or 3 min, the relationship of turbidity between LDL and BSA emulsion was not changed. There is a simple relationship between the turbidity and the interfacial area of an emulsion (Kerker, 1969), so these results on the turbidity measurements of both emulsions seemed to show that the dispersed fat globules of the LDL emulsion were much smaller than those of the BSA emulsion.

To confirm the above assumption, the diameters of dispersed fat globules of both LDL and BSA emulsions were measured with an optical microscope. In this experiment since measurement of globule size is very time-consuming, tests were designed with emulsion systems having three oil-volume ratios, namely, $\phi = 1/3$, 2/3 and 3/4. The results are shown in Fig. 2. It is clearly seen in this figure that the mean globule diameters of all the LDL emulsions were much smaller than those of all the BSA emulsions and this difference of globule size was especially large when the protein concentration was small and oil volume ratio was high. Although the globule size of the BSA emulsion greatly decreased with the increased protein concentration, the decrease in the globule size of LDL emulsion was much smaller.

To obtain information about the properties of the emulsions, the viscosities of LDL emulsions at various conditions were compared with BSA emulsions. The results are shown in Fig. 3. The viscosity of the LDL emulsion was low at a low protein concentration and at a high oil volume ratio, but it rapidly increased with increased protein concentration. On the con-

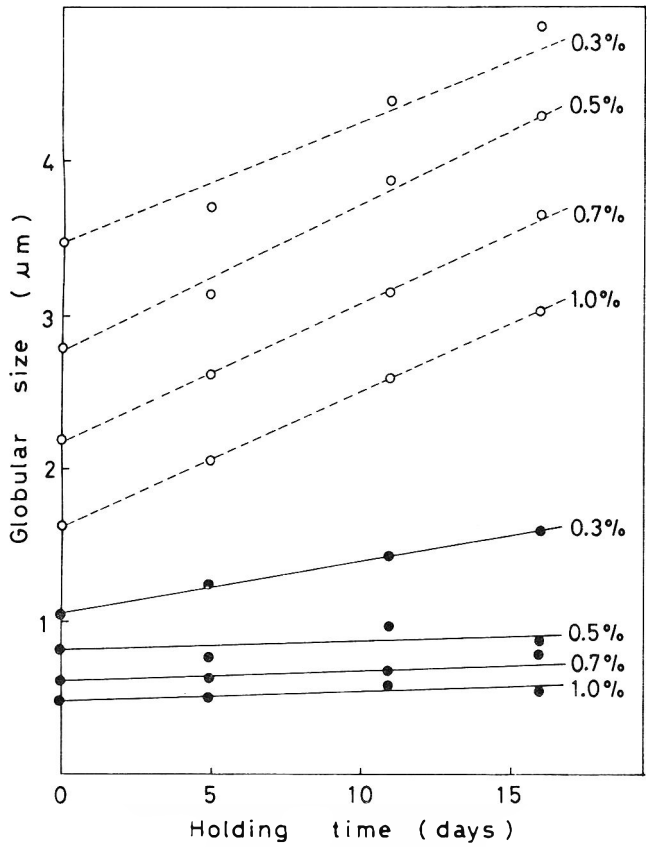

Fig. 4—Changes in the globular size of emulsions (ϕ = 2/3) during storage at 37°C. Protein concentration of — LDL and ---- BSA shown in percent.

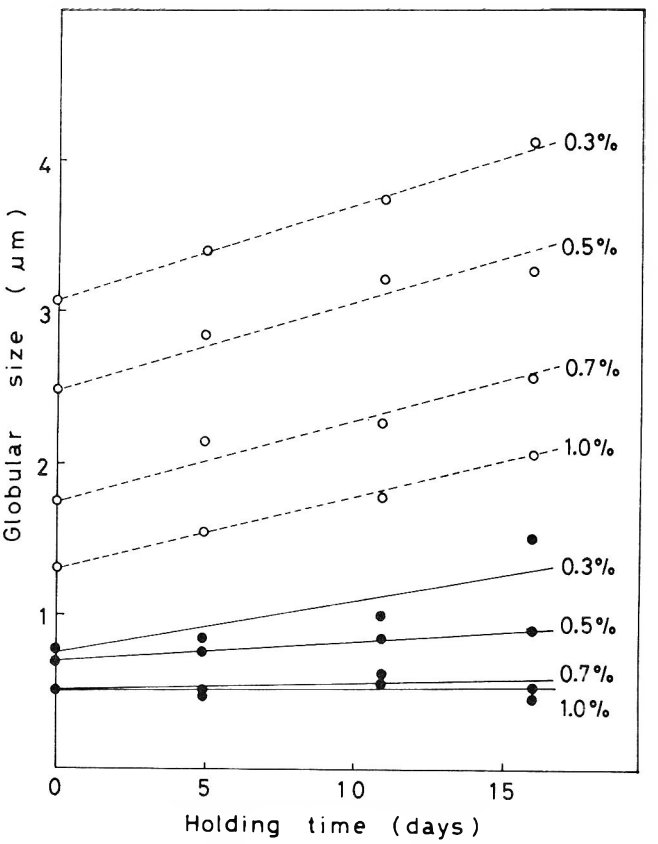

Fig. 5—Changes in the globular size of emulsions (ϕ = 3/4) during storage at 37°C. Protein concentration of — LDL and ---- BSA shown in percent.

trary, the viscosity of the BSA emulsion was high at a low protein concentration and gradually increased with increased protein concentration. These properties of both LDL and BSA emulsions may correlate well with both the size distribution and number of dispersed fat globules about these emulsions; at a low protein concentration such as 0.3% or below, the viscosity of BSA emulsion was larger than that of the LDL emulsion due to the large globule size of the BSA emulsion, but at a high protein concentration such as 0.7% or above, the viscosity of LDL emulsion was larger than that of BSA emulsion due to the rapid increase in the number of dispersed fat globules.

Stability of emulsion

To study the stability of emulsions, the diameters of dispersed fat globules were measured in the emulsions kept at 37°C for various times. The results (Fig. 4 and 5) show the emulsions with oil volume ratios of 2/3 and 3/4, respectively. When oil volume ratio was 1/3 or below, dispersed fat globules adhered to each other during holding at 37°C. It was very difficult to calculate the exact number of fat globules adhering to each other, and so data on the emulsions with a low oil volume ratio could not be obtained. In these two figures, the globule size of all the BSA emulsions increased gradually during holding at 37°C, but that of LDL emulsions did not change at all except at the 0.3% protein concentration. This difference noted in the size change between LDL and BSA emulsions may be caused by the difference in the initial globule size of both emulsions: mean globule size of all BSA emulsions was much larger than 1μ, but that of LDL emulsions was less than 1μ except for the 0.3% emulsion. Although the rate of globule size formation was almost the same for the BSA emulsion having the same oil volume ratio, that of an emulsion with higher oil volume ratio (ϕ = 3/4) was a little faster than that

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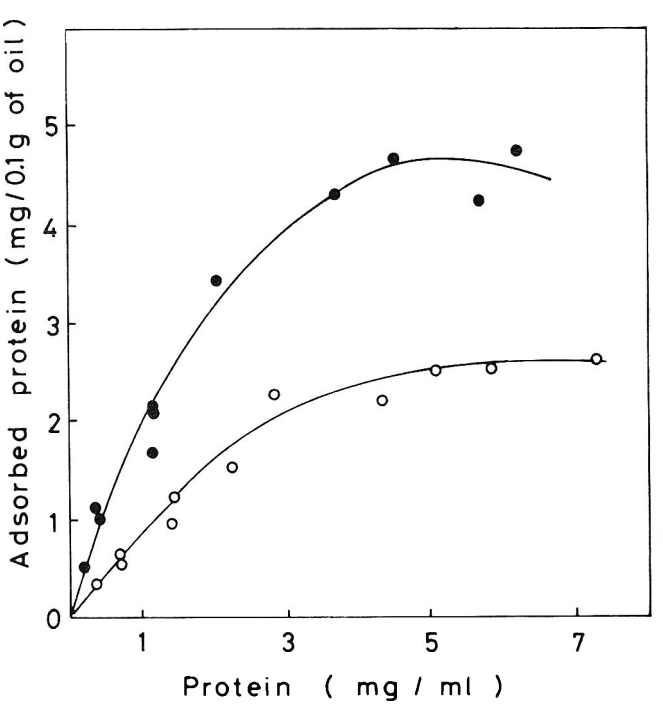

Fig. 6—Amount of protein adsorbed to oil as a function of protein concentration; – ● – LDL, – ○ – BSA

Preparation of Cooked Egg White, Egg Yolk, and Whole Egg Gels for Scanning Electron Microscopy

S.A. WOODWARD and O.J. COTTERILL

ABSTRACT

Cooked egg white, egg yolk, and whole egg gels, fixed with glutaraldehyde or unfixed, were frozen at −35°C or at −95°C and freeze-dried. Alternatively, unfrozen gels fixed with glutaraldehyde, osmium-thiocarbohydrazide-osmium, or osmium-tannic acid-uranyl acetate were dehydrated in ethanol and critical point-dried from carbon dioxide. Egg yolk and whole egg gels were defatted. Freeze-dried and critical point-dried gels were examined by scanning electron microscopy. Freezing and freeze-drying introduced artifacts due to ice crystal damage, with egg white gels distorted most, and egg yolk gels distorted least. Gels fixed only by glutaraldehyde shrank by 50% during critical point drying. Further fixation by osmium tetroxide and uranyl acetate stabilized gels against shrinkage. Removal of fat from egg yolk and whole egg was essential for observation of protein matrices.

INTRODUCTION

ONE IMPORTANT FUNCTIONAL PROPERTY of egg white (EW), egg yolk (EY), and whole egg (WE) is the ability to form gels upon heating. The physical attributes of the heat-formed gel, such as texture and water-binding ability, are highly dependent on the gel microstructure (Hermansson, 1982). It is therefore important to assess structural characteristics in order to understand the textural characteristics of gels. Scanning electron microscopy (SEM) is an excellent tool for evaluating gel microstructure because of the high resolution available. In preparation of samples for SEM, water must be removed due to the high vacuum conditions required in the electron microscope (Postek et al., 1980). For this reason, the sample must be chemically fixed or stabilized to withstand dehydration without changes in the gel structure.

Although scanning electron micrographs of egg gels have been published previously, relatively little attention has been focused on the method of preparation. Most micrographs of egg gels have been obtained from freeze-dried samples (EW gels: Kalab and Harwalker, 1973; Beveridge et al., 1980; Johnson and Zabik, 1981; WE gels: O'Brien et al., 1982). However, freezing and freeze-drying produce artifacts in most protein gel structures because of ice crystal formation (Hermansson and Buchheim, 1981; Davis and Gordon, 1984). Montejano et al. (1984) prepared EW gels for SEM using a method which combined fixation and freezing. Samples were fixed in glutaraldehyde, frozen and fractured in liquid nitrogen, thawed and further fixed in glutaraldehyde, dehydrated in ethanol, and critical point-dried. This procedure apparently minimized structural damage due to freezing; however, no comparisons were made to non-frozen samples. Beveridge and Ko (1984) recently published two micrographs of WE gels prepared for SEM by glutaraldehyde and osmium tetroxide (OsO_4) fixation, followed by ethanol dehydration and critical point drying. Cooked EY gels have not been studied previously by SEM.

The purpose of this study was to compare different techniques for the preparation of egg protein gels for SEM evaluation. The use of suitable SEM preparation methods should lead to more accurate information as to the microstructure of egg gels.

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MATERIALS & METHODS

Preparation of egg gels

Day-old eggs were obtained from Single Comb White Leghorns of a single strain. Albumen was separated from yolk, pooled, and blended in a Waring Blendor at low speed with a small Erlenmeyer flask held in the vortex to minimize incorporation of air. Yolks were rolled on wet cheesecloth to remove adhering albumen and chalazas. Yolk membranes were punctured and the yolk liquid was collected and stirred with a glass rod. WE was prepared by blending eggs at low speed as described for albumen. The pH of EW, EY and WE was adjusted to 9.0, 6.0, and 7.0, respectively, with 1.0M HCl or NaOH added dropwise. Gels were formed by heating liquid samples in 50 mL beakers at 85°C for 30 min, cooled to 20°C, and cut with a razor blade into 1.5 × 4 × 10 mm strips.

Freezing and freeze-drying

Gel strips (EW, EY and WE) were prepared by four treatments, comparing fixed and unfixed gels frozen at two temperatures. Samples (ca 10 strips per treatment) were fixed in 2% glutaraldehyde in 0.1M phosphate buffer for 2 hr. Both fixed and unfixed samples were then frozen on the cold plates of a RePP Model 15 laboratory freeze-dryer (VirTis Co., Inc., Gardiner, NY) at −35°C, and freeze-dried for 24 hr at a plate temperature of 24°C with the water vapor condenser at −60°C and a vacuum of 50 mTorr. A second set of fixed and unfixed strips was submerged in liquid hexane (−95°C) for a few seconds, immersed in liquid nitrogen (−196°C), and freeze-fractured (Rebhun, 1972). Fractured gel pieces were then freeze-dried in a TIS-U-DRY freeze-dryer (FTS Systems, Inc., Stone Ridge, NY) at a plate temperature of −55°C for 48 hr with the water vapor condenser at −95°C and a vacuum of 5 mTorr.

Freeze-dried EY and WE pieces were subsequently defatted in chloroform (Kalab, 1981), placed in absolute ethanol and critical point-dried with CO_2 as the transition fluid. Samples were mounted on copper tape and/or silver paint and sputter-coated with ca 500 Å gold. The samples frozen at −35°C were examined in a JEOL S1-SEM at 45° tilt and an accelerating voltage of 10 kV, while the samples frozen at −95°C were examined in a JEOL JSM-35 SEM at 0° tilt and 20 kV.

Fixation and critical point drying of unfrozen samples

EW and EY gels were treated by three different fixation methods: (1) glutaraldehyde, (2) glutaraldehyde followed by osmium-thiocarbohydrazide-osmium (OTO) post-fixation, and (3) glutaraldehyde followed by osmium-tannic acid-uranyl acetate (OTU) post-fixation. WE was treated only by the glutaraldehyde-OTU fixation procedure.

Glutaraldehyde fixation. Gel pieces were fixed for 3–4 hr at 20°C in 2% glutaraldehyde in 0.1M phosphate buffer, pH 6 (EY) or 7.5 (EW). EW samples were not processed above pH 7.5 to prevent glutaraldehyde polymerization (Hayat, 1970).

OTO procedure. The method of Postek and Tucker (1977) was followed. Fixed gel pieces were first cut to 0.5 × 1.5 × 4 mm to expose a fresh inner surface and post-fixed in 1% OsO_4 for 2 hr at 20°C in veronal acetate buffer, pH 6 (EY) or 7.5 (EW). Samples were rinsed three times in deionized water and placed in a filtered, saturated 1% thiocarbohydrazide solution for 30 min. After rinsing six times in deionized water, samples were placed in 2% OsO_4 in deionized water for 1 hr.

OTU procedure. This method, developed by Wollweber et al.

(1981), was designed to prevent shrinkage of specimens during subsequent critical point drying. Samples previously fixed in glutaraldehyde and post-fixed in OsO_4 were washed thoroughly in veronal acetate buffer and placed in 1% tannic acid in veronal acetate at pH 6 for 1 hr at 20°C. After three rinses with deionized water, samples were placed in 0.5% uranyl acetate in deionized water for 1 hr at 20°C and then rinsed again.

Samples from all three fixation treatments were stored overnight in deionized water at 4°C, and then dehydrated successively in 20, 40, 60, 80, 95, and three changes of 100% ethanol. EY and WE were defatted overnight in chloroform at 20°C and returned to ethanol. Samples were critical point-dried with CO_2 as the transition fluid, mounted, sputter-coated with ca 450 Å gold-palladium and examined in a JEOL JSM-35 SEM at 20 kV. An average of five samples from each treatment was observed in order to obtain representative micrographs.

RESULTS

Egg white gels

Freezing of EW gels produced extensive ice crystal damage. The EW gel frozen at −35°C (Fig. 1A) had pockets of 15–30 μm surrounded by thin protein walls. Spherically shaped structures 1–5 μm in diameter were attached to the network by protein strands. Pore openings from the gel frozen at −95°C (Fig. 1B and C) were 0.1–2.0 μm across, about 90% smaller than those in the more slowly frozen sample. The structure was a rather uniform network of interconnected protein strands.

Samples fixed only by glutaraldehyde shrank to about 50% of their original size during the critical point drying step. The gel structure (Fig. 1D) consisted of numerous small globules 0.1–0.2 μm in diameter, closely compacted together and interconnected by protein strands. Pore openings 0.1–0.2 μm across were present, but the overall structure was compact. The OTO-processed gel (Fig. 1E) had a more open structure, with globules and strands forming a weblike network. Pores ranged in size from 0.1–0.8 μm, and the strands were much finer than those present in freeze-dried EW gels. The OTU-processed gel (Fig. 1F) was similar in particle and pore size to the OTO-treated gel, with small particles interconnected by numerous strands.

Egg yolk gels

Slow-frozen EY gels, plain and defatted, are shown at low magnification in Fig. 2A and B, respectively. The lipid-containing sample consisted of spherically shaped particles 1–15 μm in diameter. Structural details were obscured by a viscous covering. The appearance of this material is probably related to freeze-induced gelation of yolk, which involves the complexing of lipoproteins into large aggregates (Powrie et al., 1963). Defatting the sample exposed a yolk sphere in a protein matrix (Fig. 2B). The irregular surface contained numerous voids and large cavities, suggesting extensive damage due to freezing. The surface of an EY gel frozen at −95°C before defatting (Fig. 2C and D) was covered by spherically shaped structures 0.3–2.0 μm in diameter, trapped in a fibrous matrix. These structures were tentatively identified as yolk granules based on their size and number (Chang et al., 1977). The void spaces were oriented diagonally across the micrograph, indicating limited ice crystal damage. The EY gel prepared by glutaraldehyde fixation (Fig. 2E) was similar in appearance to the freeze-dried gel (Fig. 2D). Both treatments allowed lipid removal, which exposed the underlying granules and supporting protein matrix. However, neither sample was free of arti-

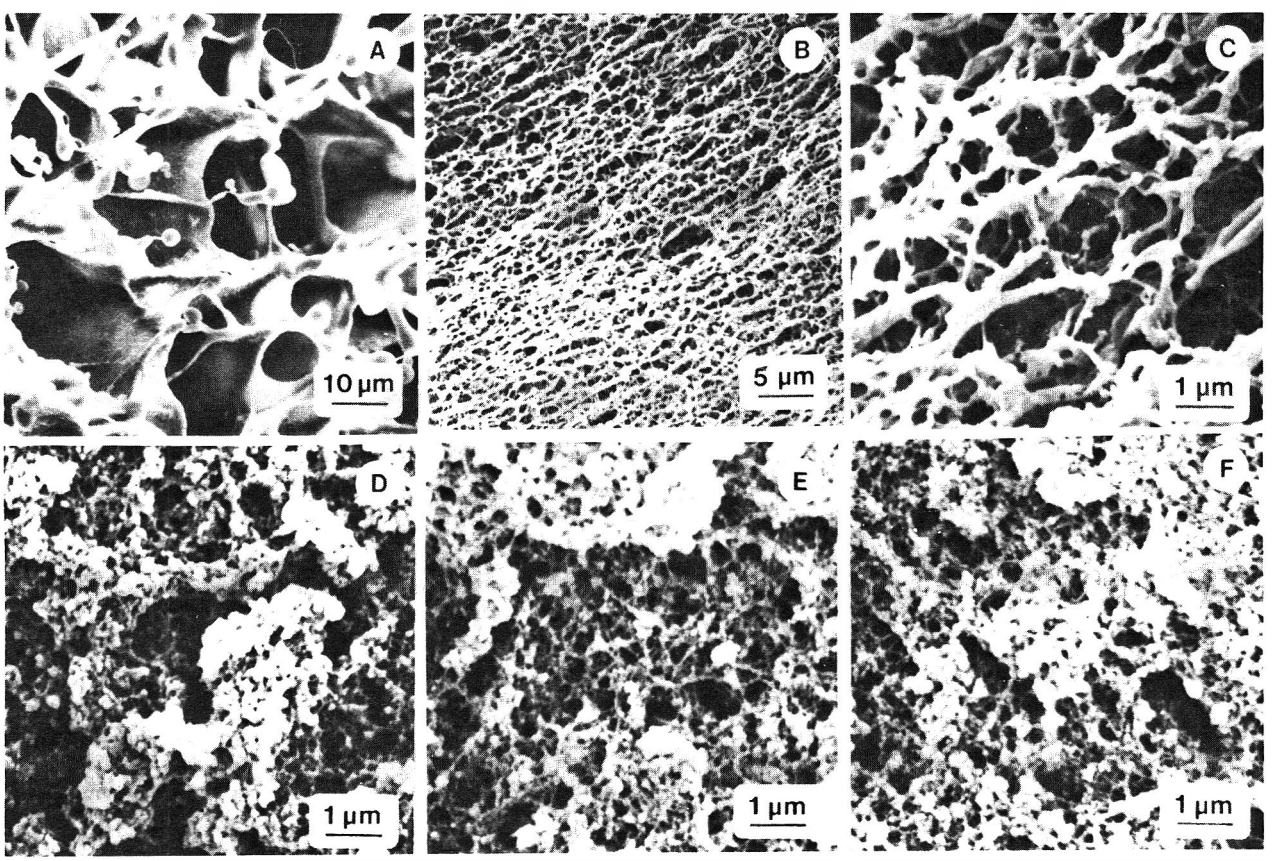

Fig. 1—Micrographs of cooked EW gels prepared for SEM by various methods. (A) Frozen at −35°C and freeze-dried; (B) Frozen in liquid hexane (−95°C), immersed in liquid nitrogen (−196°C), and freeze-dried; (C) Higher magnification of B; (D) Fixed in glutaraldehyde and critical point dried; (E) Fixed in glutaraldehyde followed by OTO post-fixation and critical point drying; (F) Fixed in glutaraldehyde followed by OTU post-fixation and critical point drying.

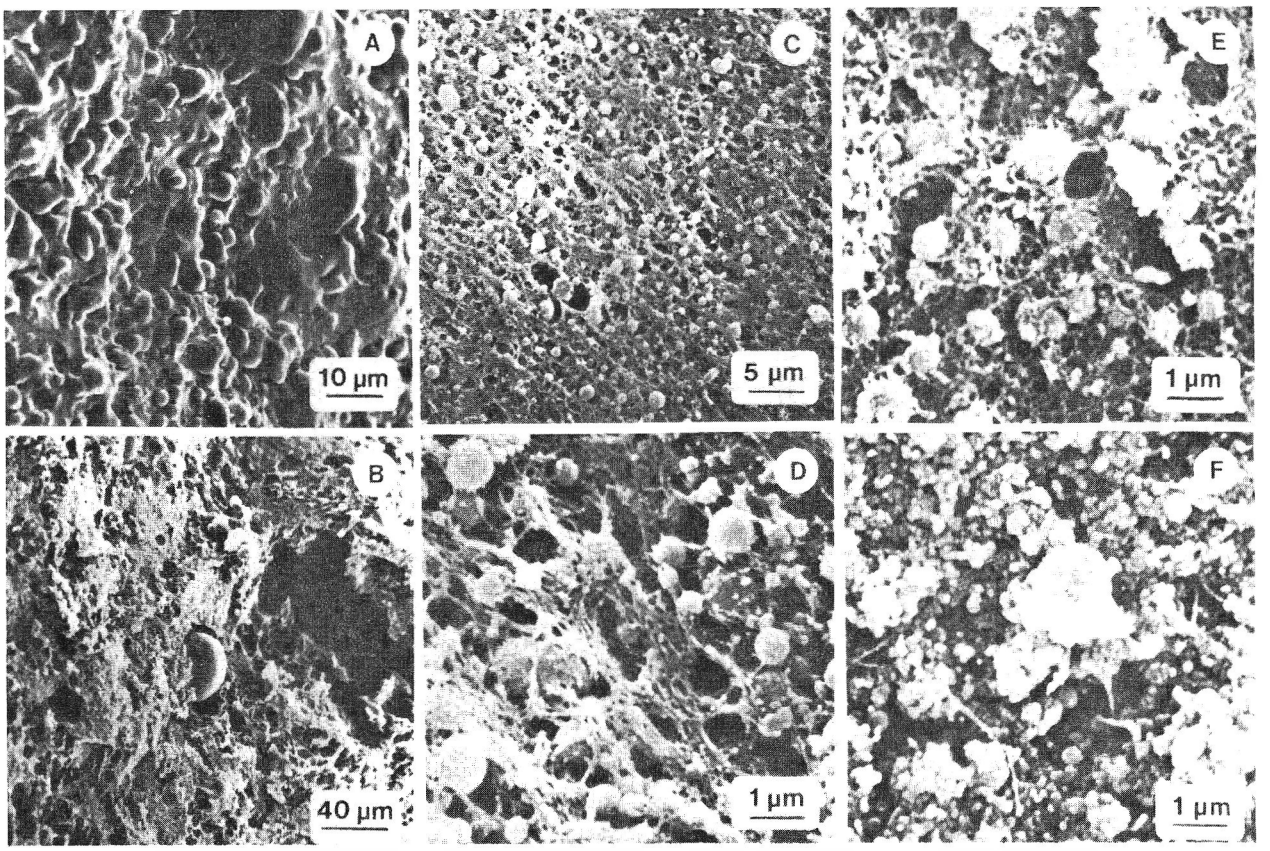

Fig. 2—Micrographs of cooked EY gels prepared for SEM by various methods. (A) Frozen at −35°C and freeze-dried; (B) Frozen at −35°C, freeze-dried, defatted and critical point dried; (C) Frozen in liquid hexane (−95°C), immersed in liquid nitrogen (−196°C), freeze-dried, defatted and critical point-dried; (D) Higher magnification of C; (E) Fixed in glutaraldehyde, defatted, and critical point-dried; (F) Fixed in glutaraldehyde, post-fixed by OTO, defatted and critical point-dried.

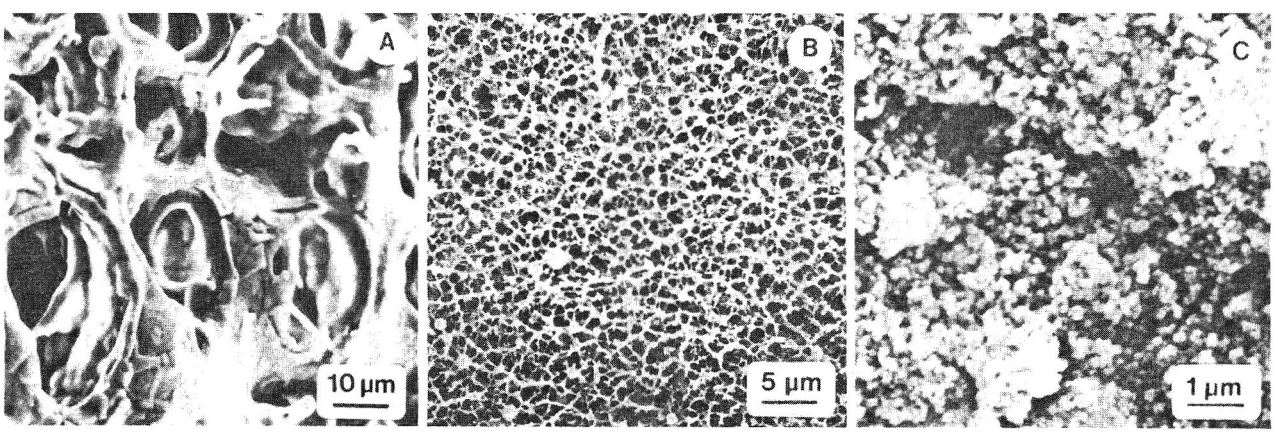

Fig. 3—Micrographs of cooked WE gels prepared for SEM by various methods. (A) Frozen at −35°C and freeze-dried; (B) Frozen in liquid hexane (−95°C), immersed in liquid nitrogen (−196°C), freeze-dried, defatted and critical point-dried; (C) Fixed by glutaraldehyde, post-fixed by OTU, defatted and critical point-dried.

facts, as the freeze-dried sample was distorted by ice crystal formation and the gel fixed in glutaraldehyde underwent some shrinkage during critical point drying. The EY gel prepared by the OTO treatment (Fig. 2F) had no pores but exhibited clusters of small globules from 0.1–0.4 μm in diameter. Based on their size, these globules may include low-density lipoproteins (LDL), clusters of LDL, and myelin figures, which are all present in yolk plasma (Chang et al., 1977; Garland and Powrie, 1978). Micrographs of EY gels prepared by the OTU procedure were similar to those of OTO-treated gels and therefore are not shown. Both the OTO and OTU treatments effectively prevented appreciable shrinkage of EY gels, but they also fixed some of the lipid-containing particles in the gels, obscuring the protein matrices.

Whole egg gels

The slow-frozen WE gel (Fig. 3A) had a coarse protein structure covered by an apparent lipid layer. Large pore open-

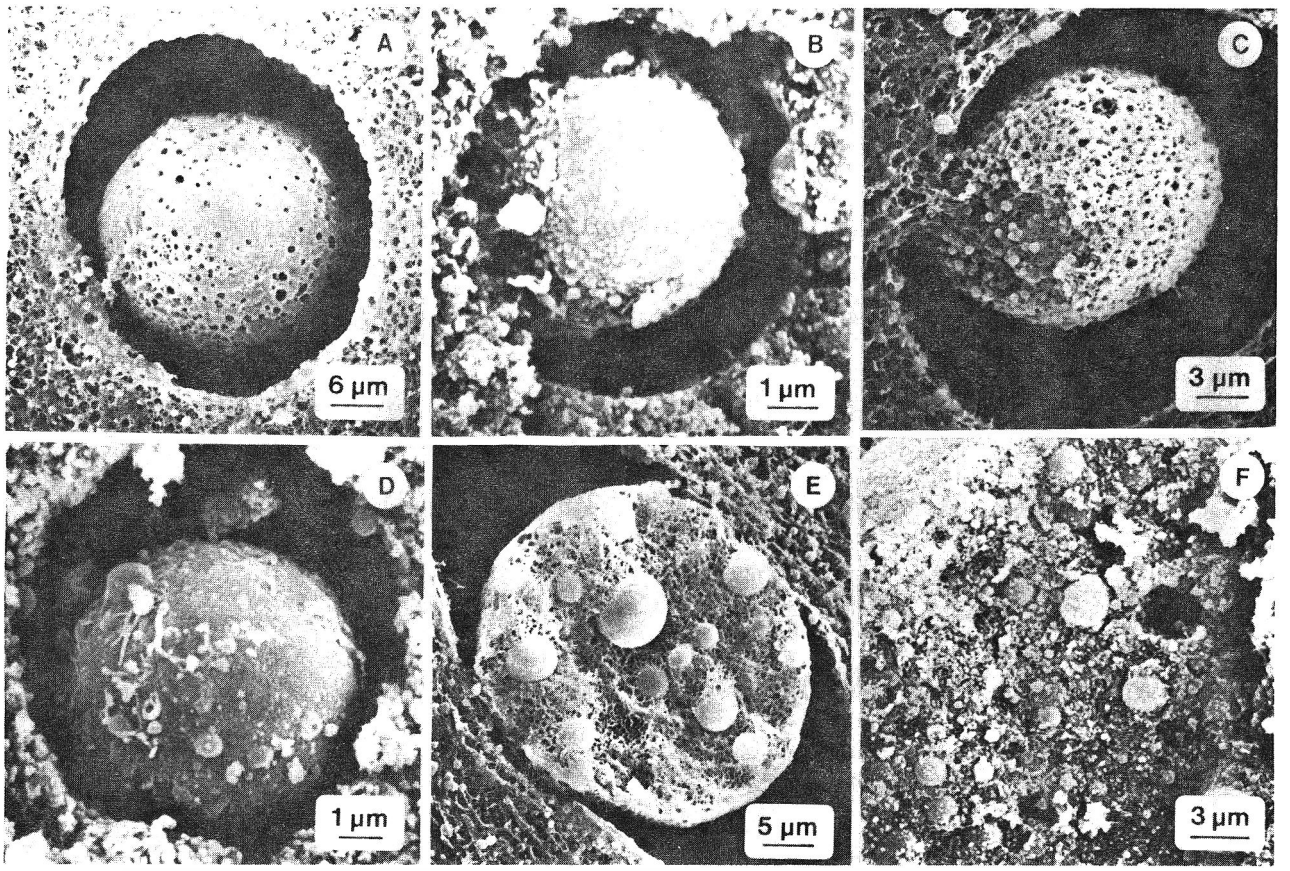

Fig. 4—Comparison of whole and fractured spheres from EY and WE prepared for SEM by freeze-drying or the OTU fixation procedure. (A) Sphere from EY gel frozen in liquid hexane, immersed in liquid nitrogen, freeze-dried, defatted and critical point-dried. (B) Sphere from OTU-processed, defatted EY gel. (C) Sphere from WE gel frozen in liquid hexane, immersed in liquid nitrogen, freeze-dried, defatted and critical point-dried. (D) Sphere from OTU-processed, defatted WE gel. (E) Interior of sphere from EY gel fractured in liquid nitrogen, freeze-dried, defatted and critical point-dried. (F) Interior of sphere from EY gel prepared by OTU fixation, defatted and critical point-dried.

ings of 10–20 μm across were present due to ice crystal damage. Freezing WE at −95°C resulted in an open network structure (Fig. 3B) with protein strands enclosing numerous ice crystal pockets that were 90% smaller than those in the more slowly frozen WE gel. The unfrozen gel processed by the OTU method (Fig. 3C) consisted of a uniform array of irregularly shaped globules from 0.05–0.2 μm in size. The surface was quite uneven and irregular and lacked the presence of pores or openings. The structure of this gel resembled that of EW gels prepared by the same fixation method.

Egg yolk and whole egg spheres

Whole and fractured spheres from EY and WE are shown in Fig. 4 in order to compare the effects of freeze-drying and the OTU treatment on the structural appearance of spheres. The spheres were lying in cavities probably formed by the removal of lipid and the shrinkage of spheres. Freeze-dried spheres (Fig. 4A, C and E) had surface pores where lipid had been removed, while OTU-treated spheres (Fig. 4B, D, and F) had no pores. A freeze-dried EY sphere 30 μm in diameter (Fig. 4A) had a fairly smooth surface interrupted by pores. The surface of the EY sphere prepared by OTU fixation (Fig. 4B) was covered by small particles 0.1–0.2 μm in size. A granule protruded from the adjacent cavity wall. The freeze-dried WE sphere (Fig. 4C) had a portion of its surface broken away. The interior of this 15 μm sphere was similar to its surface, with numerous 0.5 μm diameter granules connected by protein strands. The 7 μm WE sphere fixed by the OTU method (Fig. 4D) had a rather smooth surface interrupted by small particles and granules. The 35 μm EY sphere in Fig. 4E was fractured during liquid nitrogen freezing. It contained about a dozen smaller spheres ranging in size from 3–7 μm embedded in a protein matrix. The interior of the 40 μm EY sphere in Fig. 4F contained spherical particles and granules ranging from 0.1–3.0 μm in size. The protein matrix was not evident due to the presence of lipids or lipid-containing particles.

DISCUSSION

THE PREPARATION of egg protein gels for SEM by freeze-drying produced structural artifacts due to ice crystal formation. Damage was most severe in EW gels, less severe in WE gels and only slight in EY gels. The formation of numerous large ice crystals in EW and WE gels was related to their high water content of 88 and 75%, respectively (Cotterill and Glauert, 1979). EY gels, with high lipid levels and only 50% water, were damaged to a much lesser degree by freezing, as only minor crystal damage was detected. Ice crystal pockets were reduced in size but not eliminated by freezing in liquid hexane/liquid nitrogen. Fixation with glutaraldehyde prior to cryofreezing did not prevent artifact formation, but it made gel pieces somewhat brittle, as was reported by Hermansson and Buchheim (1981) in the preparation of soy protein gels.

Davis and Gordon (1984) stated that fast-freezing techniques produce artifacts in gel systems because of slow freezing rates. Hermansson and Buchheim (1981) explained that the networks formed in gels by ice crystallization can easily be mistaken for

the original gel structures. Most of the previously published SEM micrographs of egg gels were obtained by freeze-drying of samples, and the resulting structures contained evidence of ice crystal damage. Because such artifacts occur when egg protein gels are prepared for SEM by improper freezing techniques, alternative preparation methods are essential.

Freezing-induced gelation may have further affected the structure of EY and WE gels. Both raw EY and WE undergo increases in viscosity upon freezing, and the effects are especially pronounced at slow freezing rates (Cotterill, 1977). Hasiak et al. (1972), in a SEM study of raw EY microstructure, found that slow freezing gave EY a coarse network appearance. A similar coarseness was evident in the slow frozen EY and WE gels which had not been defatted in this study. The viscosity increase caused by freeze-induced gelation is based on the complexing of lipoproteins to form an insoluble gel (Powrie et al., 1963). It is possible that the lipoproteins in EY and WE gels would complex in a similar manner to produce the coarse structural appearance which was observed in this study.

Micrographs of egg gels prepared by fixation and critical point drying showed gel structures which were consistent with those of other heat-formed protein gels prepared by similar fixation procedures (Hermansson, 1979, 1982; Hermansson and Buchheim, 1981). The WE gel prepared in this study by the OTU method resembled the WE gel prepared by glutaraldehyde and OsO_4 fixation in the study of Beveridge and Ko (1984).

The structure of EW and EY gels fixed only in glutaraldehyde was distorted by shrinkage of gels during critical point drying, while the OTO and OTU procedures adequately prevented gel shrinkage. Wollweber et al. (1981) reported 45% shrinkage of cells fixed in both glutaraldehyde and OsO_4. Subsequent treatment in tannic acid and uranyl acetate limited shrinkage to 5%. The authors attributed the enhanced structural stability to the mordanting effect of tannic acid in binding OsO_4 and uranyl ions.

The presence of fat in gels tends to obscure protein matrices and may also cause charging artifacts in the electron microscope (Kalab, 1981). Lipid removal appeared to be most complete in EY and WE gels which were freeze-dried or fixed only in glutaraldehyde. Post-fixation of gels apparently prevented complete removal of fat by the binding of lipid-containing particles, such as LDLs, to the gel matrix by OsO_4. Although fixation by OTO and OTU obscured the protein matrices, these methods allow a comparison between the structure of lipid-free and lipid-containing gels.

The spheres of WE and EY compared favorably with the structures elucidated by Bellairs (1961). Both the freeze-drying and OTU procedures were useful in providing insight to the structure of spheres. The protein network was best seen in freeze-dried gels which were defatted. However, this network was undoubtedly distorted by the freezing process. Further efforts should be made to devise a procedure which would prevent the formation of artifacts due to freezing or shrinkage, while allowing the fat to be removed from lipid-containing gels.

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Preservation of Concentrated Cheese Whey by Combined Factors

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ABSTRACT

Concentrated sweet whey (about 100g solids/100g water), produced in the dairy industry as a preliminary step to spray drying, was stabilized against microbial spoilage by a combination of a slight reduction of water activity (a_w), lowered pH, and addition of potassium sorbate, thus avoiding the drying operation. The whey so obtained could be stored for up to 3 months at 30°C without bacterial or mold deterioration.

INTRODUCTION

WHEY is a by-product in the manufacture of cheese and approximately 9 kg of whey are produced for each kg of cheese. From figures available on cheese production one can readily calculate that very large amounts of whey are produced throughout the world. In many countries, however, only a small percentage of this whey is utilized or processed; the rest is merely disposed of by any available means. Whey is a highly nutritious product which can be well utilized when fed to animals, but only a small portion is used in liquid form for this purpose, because of transportation and contamination problems. Liquid whey is also concentrated by evaporation to about 50% solids and then spray-dried for human consumption. The increased demand for additional sources of food protein coupled with the need to reduce environmental pollution makes it urgent to find a satisfactory solution to the problem of surplus whey.

The purpose of the present work was to develop a simple process to achieve microbial stability of whey stored at ambient temperature. This process was based on the "hurdle" effect (Leistner et al., 1981) on which several food preservation processes were dependent. Combinations of reduced water activity (a_w), pH, and addition of permitted preservatives were the inherent hurdles studied to accomplish the desired microbial stability of whey.

MATERIALS & METHODS

Materials

Concentrated sweet whey, supplied by SANCOR Cooperativas Unidas Ltda., Buenos Aires, was obtained by concentrating liquid sweet whey (total solids 6–7%; lactose 4.6–4.8%; proteins 0.8–0.9%; fat 0.03%; ash 0.5%) in a falling film evaporator at 70°C until the solution contained about 50% solids. The water activity (a_w) of the concentrated whey was 0.940–0.945 and its pH ranged between 5.8–6.3; it was kept under refrigeration until used.

Glycerol and citric acid were from Mallinkrodt Chemical Works (St. Louis; MO); sodium chloride was from Merck (Darmstadt, West Germany); potassium sorbate was food grade.

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Sample preparation

Whey solutions of varying solids up to about 50% (for measuring a_w) were prepared by dilution of the concentrated product with distilled water. The microbial stability of concentrated whey solutions was studied at three levels of a_w (0.940; 0.920, and 0.900); various levels of pH (between 5.0 and 5.6); and in the presence of 0.1 or 0.2% potassium sorbate. The a_w of concentrated whey which was about 0.94–0.945 was further lowered by addition of the appropriate amounts of sodium chloride or glycerol. Citric acid was used to reduce pH to the desired value.

In order to study the microbial load of several samples and the evolution of the aerobic mesophilic plate count, 30 mL of sample were poured in moisture-proof pouches (Super Cryovac). The inoculated samples (*S. aureus* and yeast) were placed in sterile, hermetically sealed, glass flasks. Anaerobic growth was studied in samples placed in 18 mL test tubes. All the samples (pouches, flasks and test tubes) were stored at 30°C.

Determination of water activity

The water activity of the samples was determined using the "a_w-Wert Messer" manufactured by firma LUFFT Stuttgart, West Germany. To improve the reliability of the measurements, the instrument was operated following the procedure of Chirife and Ferro Fontán (1980). For this purpose, the instrument was carefully checked against different standard saturated salt solutions in the a_w range of interest to this study, and a calibration curve was obtained. The calibration curve was checked every 48 hr. Values for the standard saturated salt solutions were as follows: $BaCl_2$ 0.902; KNO_3 0.926; K_2SO_4 0.974 (Chirife et al., 1983). All a_w measurements were made at 25 ± 0.2°C in a forced convection constant temperature oven. All samples were equilibrated at 25°C for 24 hr before measurements; a_w determinations were made in duplicate, and the average was reported.

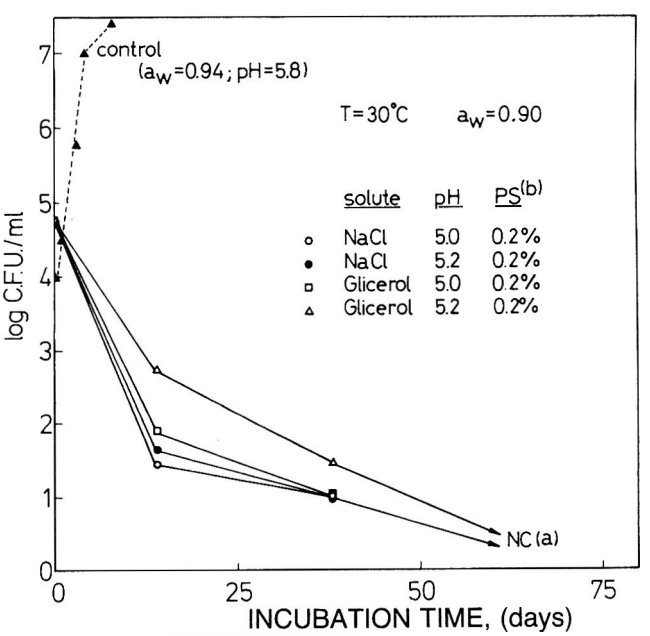

Fig. 1—Changes of aerobic mesophilic plate count of concentrated sweet whey adjusted to selected combinations of a_w, pH and potassium sorbate, during incubation at 30°C; (a) no counts at 69 days; (b) potassium sorbate.

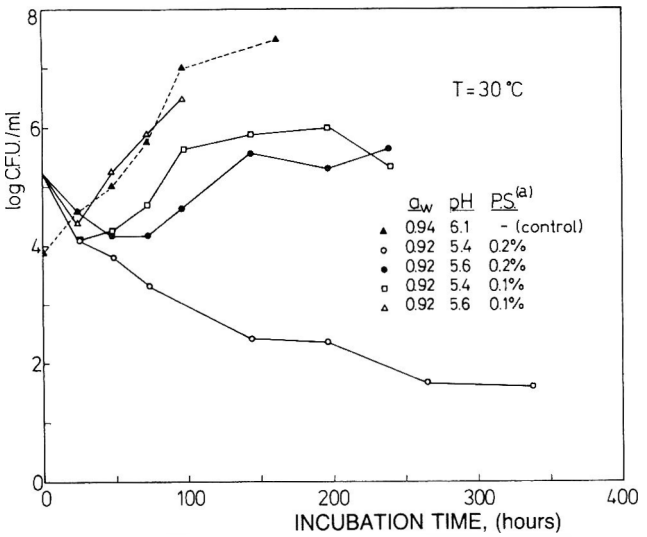

Fig. 2—Changes of aerobic mesophilic plate count of concentrated sweet whey adjusted to selected combinations of a_w, pH and potassium sorbate, during incubation at 30°C; (a) potassium sorbate.

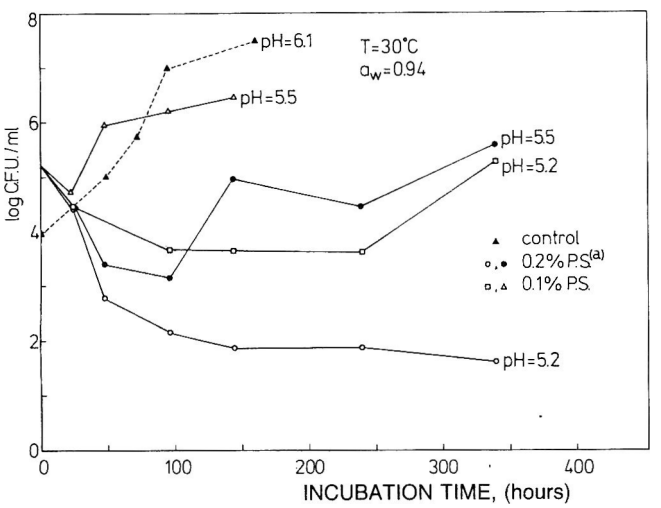

Fig. 3—Changes of aerobic mesophilic plate count of concentrated sweet whey (a_w = 0.94) adjusted to selected combinations of pH and potassium sorbate, during incubation at 30°C; (a) potassium sorbate.

Moisture and pH

Moisture of cheese whey was determined gravimetrically by drying in a forced convection oven at 100°C for 5 hr. The pH of the homogenized samples was determined with a glass electrode on a Metrohm pH Meter E 632.

Microorganisms

Staphylococcus aureus INM 82 was obtained from the Instituto Nacional de Microbiología "Dr. Carlos G. Malbrán," Buenos Aires. The molds and yeast utilized were isolated from spoiled commercial concentrated whey (a_w = 0.94; pH 6.1). They included *Geotrichum* sp; *Aspergillus flavus* and yeast designated W-84.

Inocula preparation and incubation of samples

The inocula of *S. aureus* were prepared by growing cells in nutrient broth at 37°C for 24 hr. Five hundred milliliters of whey samples were inoculated with 1 mL of the proper dilution of the culture to obtain an initial count between 10^4–10^6 CFU/mL. The inoculated samples were homogenized in a Sorvall Omni-Mixer, placed in sterile glass flasks and incubated at 30°C.

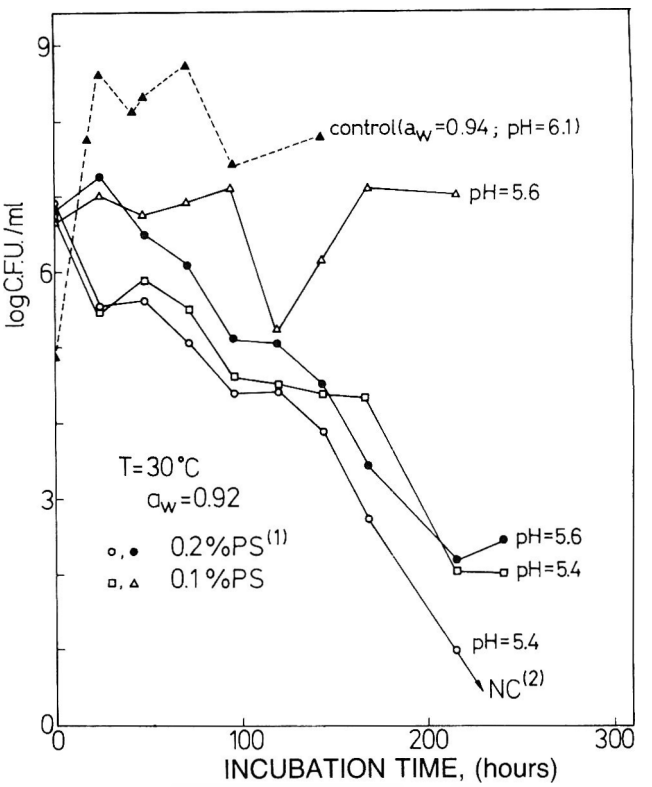

Fig. 4—Changes of counts of S. aureus INM-82 in concentrated sweet whey adjusted to selected combinations of a_w, pH and potassium sorbate, during incubation at 30°C; (1) potassium sorbate; (2) colony counts below 10 at 171 hr incubation.

The yeast inocula were prepared by growing cells in Sabouraud broth at 25°C for 24 hr. Five hundred milliliters of whey samples were inoculated with 1 mL of the proper dilution of the culture to obtain an initial count of about 10^4 CFU/mL. The inoculated samples were homogenized, placed in sterile glass flasks and incubated at 25°C.

Mold spores were washed from 7–10 days old slopes with 5 mL distilled water and a few drops of Tween 80 (Horner and Anagnostopoulos, 1973). Plates prepared for inoculation studies included Sabouraud media and commercial and modified concentrated whey samples. Inocula levels ranged between 10^3–10^5 CFU/mL. Each plate was inoculated at the center with 10 μL of suspension to give a circular inoculum of 3 mm diameter. The inoculated plates were incubated at 25°C. Occurrence of spoilage was defined as formation of a surface colony large enough to be visible. The diameter of such a colony was arbitrarily taken as 2 mm and the time required for its formation was defined as "rejection time." Results were calculated from the mean diameter of three replicate colonies.

Media and enumeration of micoorganisms

Samples were prepared by dilution of 1g whey plus 9 mL saline tryptone solution to give an initial dilution of 10^{-1}. Aerobic mesophilic plate counts were made on Standard Plate Count Agar (tryptone 5g, yeast extract 2.5g, glucose 1g, agar 15g, NaCl 0.5g, distilled water 1 L); *Micrococcaceae* on Chapman Agar (D-manitol 10g, agar 15g, NaCl 70g, nutritive broth 1L (minced meat 500g, distilled water 1 L, peptone 20g), phenol red 0.025g); *Staphylococcus aureus* on Baird-Parker Agar (E. Merck, Darmstadt, W. Germany); and *Enterobacteriaciae* on Violet Red Bile Glucose Agar (Oxoid Ltd., Basingstoke, Hampshire, England). Coliform counts were made by multiple tube technique (MPN), in tubes with Mac Conkey broth (peptone 20g, lactose 10g, NaCl 5g, sodium taurocolate 5g, distilled water 1 L, neutral red 1% 10 mL); and fecal streptococci in tubes with Rothe Broth (bacto tryptose 20g, glucose 5g, NaCl 5g, K_2HPO_4 2.7g, sodium azide 0.20g, distilled water 1 L) and tubes with Litsky Broth (Difco Lab., Detroit, MI). The Mac Conkey Broth substitutes for the Brillant Green Lactose Bile Broth (peptone 10g, lactose 10g, dehydrated bile 20g, brillant green 0.1% 13.3 mL, distilled water 1 L) (W.H.O., 1963). comparative studies conducted at the Instituto Nacional de Microbiología, "Dr. Carlos G. Malbrán," Buenos Aires,

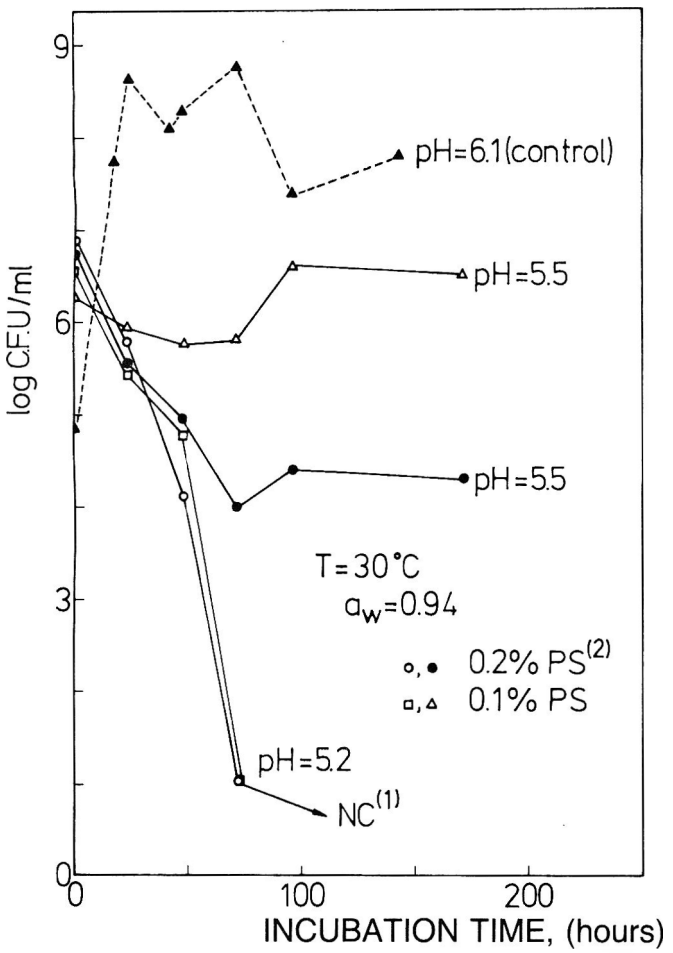

Fig. 5—Changes of counts of S. aureus INM-82 in concentrated sweet whey (a_w = 0.94) adjusted to selected combinations of pH and potassium sorbate, during incubation at 30°C; (1) colony counts < 10 at 241 hr incubation; (2) potassium sorbate.

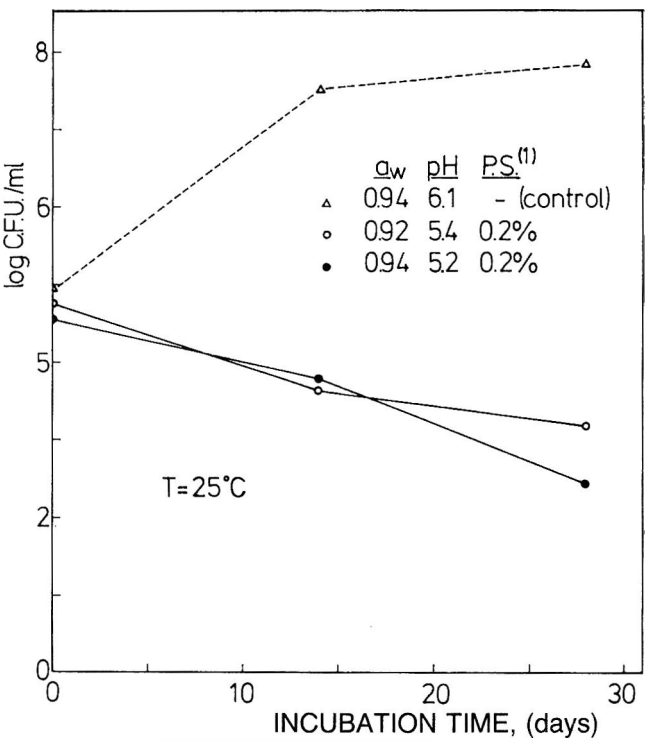

Fig. 6—Changes of counts of yeast INM-84 in concentrated sweet whey adjusted to selected combinations of a_w, pH and potassium sorbate, during incubation at 25°C; (1) potassium sorbate.

(unpublished results) demonstrated the equivalence of the Mac Conkey broth and the Brillant Green Lactose Bile Broth for the test of coliform MPN in different foods.

For anaerobic and facultative spore counts, samples and dilutions were heated at 70–75°C for 10 min and immediately immersed in cold water; then 1 mL portions of the dilutions were placed in screw-capped tubes with Brewer Anaerobic Agar (Difco Lab., Detroit, MI) medium at a temperature of 55°C, mixed and immersed in cold water. To study the sporulated aerobes, 1 mL portions from the tubes were incubated at 37°C for 48 hr. Molds and yeast were plated on Sabouraud-Agar medium (glucose 40g, peptone 10g, agar 20g, distilled water 1L) and O.G.A. medium (glucose 20g, yeast extract 5g, agar 20g, distilled water 1 L) at 25°C for 3–5 days.

RESULTS & DISCUSSION

Microbial stability

The microbial stability of concentrated sweet whey stored at 30°C was studied at various combinations of a_w (0.94–0.92–0.90), pH (5.0–5.2–5.4–5.6) and potassium sorbate level (0%–0.1%–0.2%). The amount of sodium chloride or glycerol needed to depress the a_w to 0.92 or 0.90 was calculated using Ross's (Ross, 1975) equation

$$(a_w) \text{ whey} = (a_w^\circ)_w \, (a_w^\circ)_s \qquad (1)$$

where $(a_w^\circ)_w$ is the water activity of the commercial concentrated whey ($\approx$ 0.94) and $(a_w^\circ)_s$ is the water activity of an aqueous solution of sodium chloride or glycerol at the same molality as in the water of whey. Values of $(a_w^\circ)_s$ (sodium chloride or glycerol) were obtained from the literature (Benmergui et al., 1979; Chirife et al., 1980). Predicted a_w values of solute-added whey were always found to be in very good agreement with measurement.

Table 1—"Rejection time" for Geotrichum *sp. and* Aspergillus flavus *inoculated in Sabouraud medium and commercial and modified whey concentrate samples incubated at 25°C*

Microorganism	Medium	Inoculum (spores/ml)	Rejection time (hours)
	Sabouraud	10^5	48
	Whey[b]	10^3	120
		10^4	120
		10^5	96
Geotrichum sp.	Whey[c]	10^3	(–)[a]
		10^4	(–)
		10^5	(–)
	Whey[d]	10^3	(–)
		10^4	(–)
		10^5	(–)
	Sabouraud	10^5	24
	Whey[b]	10^3	96
		10^4	96
		10^5	72
Aspergillus flavus	Whey[c]	10^3	(–)[a]
		10^4	(–)
		10^5	(–)
	Whey[d]	10^3	(–)
		10^4	(–)
		10^5	(–)

[a] After 3 months of incubation.
[b] Commercial concentrate; a_w = 0.94; pH 6.1.
[c] Modified commercial concentrate; a_w = 0.94; pH 5.2; 0.2% sorbate.
[d] Modified commercial concentrate; a_w = 0.92; pH 5.4; 0.2% sorbate.

The microbial load of ten different samples of commercial heat-concentrated ($a_w \equiv$ 0.94; pH 5.8–6.3) whey was determined. The aerobic mesophilic plate count averaged 10^4 CFU/mL; *Micrococcaceae*, *Staphylococcus aureus* and *Enterobacteriaceae* were below 10 CFU/mL; coliform, fecal streptococci and sporulated aerobes were around 25 CFU/mL; mold and yeast counts were negative; anaerobic spore counts averaged

about 10^2 CFU/mL. These results indicated that only the aerobic mesophilic plate count and to a lesser extent the anaerobic spore count were significant. This relatively low incoming microbial load of whey may be attributed to the heat treatment during the process of concentration by evaporation.

Fig. 1 shows the changes in the aerobic mesophilic plate count during incubation at 30°C of concentrated whey adjusted to several combinations of a_w (adding sodium chloride or glycerol), pH and sorbate. The number of counts in the commercial whey concentrate (a_w = 0.94; pH 5.8) increased rapidly during incubation, and sensory signs of microbial spoilage were evident. However, in the whey samples adjusted to a_w = 0.90 and pH 5.0–5.2 containing 0.2% sorbate, the aerobic mesophilic plate count declined throughout the incubation period leading to total loss of viable population at 69 days of incubation. Anaerobic spore counts also declined during incubation, with the count at or below 10 CFU/mL at 38 days incubation (data not shown). No counts of *Micrococcaceae, Staphylococcus aureus, Enterobacteriaceae*, coliform, fecal streptococci, aerobic spore, mold and yeast were recorded after 14 days of incubation in any of the whey samples having a_w = 0.90 (data not shown).

in view of these results, the evolution of aerobic mesophilic plate count was followed during incubation of whey concentrate having a_w 0.92 or 0.94. Sodium chloride was used to adjust a_w. The results are shown in Fig. 2 and 3. It can be seen that a combination of either a_w 0.92–pH 5.4–0.2% sorbate, or a_w 0.94–pH 5.2–0.2% sorbate, were equally effective in inhibiting the increase of the aerobic mesophilic plate count in whey incubated at 30°C.

Staphylococcus aureus can grow and produce enterotoxin in a variety of substrates and under a wide range of environmental conditions, such as a_w, pH and temperature (Tatini, 1973). Its ability to grow aerobically down to a_w 0.86 is well documented (Vaamode et al., 1982; Lotter and Leistner, 1978). For this reason it was used as a test organism to further study the microbial stability of modified whey concentrate. Fig. 4 and 5 show the behavior of *S. aureus* INM 82 inoculated in whey concentrate having a_w 0.92 or 0.94 and selected values of pH and sorbate addition; initial inoculum levels were above 10^4 CFU/mL and 10^6 CFU/mL. It can be seen that various combinations of hurdles (a_w–pH–sorbate) led to growth inhibition or were lethal to *S. aureus* INM 82. For example, in whey adjusted to a_w 0.92, pH 5.4 and 0.2% sorbate, *S. aureus* declined rapidly during incubation leading to almost total loss to viable population after 241 hr of incubation at 30°C. A similar behavior was observed in whey samples adjusted to a_w 0.94 and a slightly lower pH (5.2). In the presence of sorbate, a relatively slight reduction of pH strongly affected the behavior of *S. aureus*, since, in addition to the intrinsic effect of lowered pH on the growth of *S. aureus* at lower levels of pH, the activity of sorbate is increased (Parada et al., 1982; Davidson et al., 1981).

The results obtained on *S. aureus* growth inhibition are a good example of the aforementioned "hurdle effect;" although this microorganism can grow at a_w 0.86 when other environmental conditions are near optimal, it is totally inhibited in whey at a_w = 0.94, slightly reduced pH and added sorbate.

According to the results obtained, growth of bacteria in concentrated whey incubated at 30°C may be prevented by adjusting a_w, pH and sorbate to two combination of hurdles, namely, a_w 0.94–pH 5.2–0.2% sorbate or a_w 0.92–pH 5.4–0.2% sorbate. For this reason, only these two combinations were selected to study the growth of inoculated molds and yeast. Fig. 6 shows the behavior of the yeast W-84 in commercial and modified concentrated whey incubated at 25°C; initial inoculum levels were above 10^4 CFU/mL. The number of CFU of strain W-84 per milliliter declined throughout the incubation period in both samples of modified concentrated whey. In the commercial concentrated whey, however, yeast W-84 grew well reaching almost 10^8 CFU/mL after 28 days of incubation.

Table 1 shows the results of mold inoculation (*Geotrichum sp.* and *Aspergillus flavus*) in several samples incubated at 25°C. The "rejection time" in the Sabouraud plates or commercial whey concentrate (a_w = 0.94; pH 6.1) ranged between 24–120 hr; however, no surface colony formation of *Geotrichum sp.* or *Aspergillus flavus* was observed in any of the modified whey concentrate samples (a_w 0.94 or 0.92; pH 5.2 or 5.4; added sorbate) after 3 months of incubation at 25°C.

It may be concluded that microbial stability of concentrated cheese whey produced in the diary industry, (about 100g solids/100g water) may be achieved by a combination of a slight adjustment of a_w, lowered pH and addition of 0.2% sorbate. In this way, spray drying of the concentrated whey is avoided since the stabilized product may be stored for at least 3 months at 30°C without suffering microbial spoilage. Hopefully, this finding may open new possibilities for better utilization of this valuable by-product of the dairy industry.

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Nutritional Evaluation of Accelerated Ripened Cheddar Processed Cheese

VEENA TOMAR and A.O. DEODHAR

ABSTRACT

Accelerated ripened Cheddar cheese was prepared by blending two parts of shredded curd made from standardized cow's milk with one part of 5.2% NaCl solution and ripening at 30°C for 8 days. On a dry matter basis, protein and fat of accelerated ripened cheese were similar to that of conventionally ripened Cheddar cheese, while lactose and total ash were greater. Similar observation was made for processed cheese samples. No change in vitamin A or in riboflavin but a fourfold increase in folic acid was observed during accelerated ripening. Protein efficiency ratio, net protein utilization and digestibility coefficient by rat tests were slightly but significantly higher for conventionally ripened processed cheese. However, no difference in biological value was observed.

INTRODUCTION

CONVENTIONAL RIPENING of Cheddar cheese requires 3 to 12 months duration to acquire desired physicochemical and sensory characteristics. However, such prolonged curing adds subsequently to the cost of production, especially in terms of storage over long duration (Singh and Kalra, 1979). Several attempts have been made in recent years to accelerate the process of ripening. These include addition of certain proteolytic and lipolytic enzymes (Malkki and Mattson, 1979; Law and Wigmore, 1982), increased proportions of the starter culture (Monshikov, 1966), addition of certain trace materials (Hofi et al., 1973) and ripening at elevated temperatures (Govoryutkina et al., 1975). Such modifications exhibit certain limitations in that the ripening process may continue uncontrolled, resulting in over ripened product. This could be prevented by refrigerating the ripened product, which, in turn, adds to the cost of production. Of all these treatments, acceleration of ripening by curing the homogenous slurry of unpressed Cheddar curd with sterile sodium chloride solution at 30°C, as reported by Kristoffersen et al. (1967), appeared promising, because the desired consistency and flavor could be attained within an 8-day period and cold storage could be by-passed. The product thus obtained was reported to have highly acceptable flavor similar to conventionally ripened cheese (Singh and Kristoffersen, 1972, 1974). The processed Cheddar cheese made from it also had flavor and texture comparable to conventionally ripened processed cheese (Saluja, 1981).

Several biochemical reactions which profoundly influence the flavor and nutritional components in cheese are known to occur during ripening. While the duration of ripening, moisture level and incubation temperature would have considerable influence on these factors, little is known about nutritional characteristics of cheese obtained on accelerated ripening. This study was, therefore, designed to determine nutrient contents as well as protein quality of accelerated ripened Cheddar cheese and the processed cheese made from it in comparison to conventionally ripened Cheddar cheese.

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MATERIALS & METHODS

SAMPLES from five separate batches of experimental cheese were obtained from the Division of Dairy Technology of the Institute. The Cheddar curd was manufactured from cow's milk standardized to a casein/fat ratio of 0.7 by the standard procedure of Davis (1976). LF-40 (1%) was used as starter culture. In the case of accelerated ripened Cheddar cheese (Type A), after milling (at 0.5% lactic acid) and salting, the curd was left overnight in a cheese vat for completion of lactic acid fermentation. The following day, the curd was shredded in a cheese shredder. Two parts of shredded curd were mixed with one part of 5.2% sterile sodium chloride solution at 45°C and ground in a micropulverizer. The slurry was incubated at 30°C for the period of 8 days with agitation on alternate days to ensure proper aeration and to facilitate representative sampling for analysis at the beginning, the 4th and 8th day of ripening. The 9-month old conventionally ripened Cheddar cheese (Type B) was taken as control for comparison.

Processed cheese samples were prepared according to Kosikowsky (1966). In the case of accelerated ripened processed cheese (Type C), three parts of accelerated ripened cheese slurry were mixed with one part of 9-month old Cheddar cheese (on total solid basis) with sodium citrate (2.5%) as the emulsifier. In the case of conventionally ripened processed cheese (Type D), three parts of young cheese were mixed with two parts of 9-month old cheese. Aliquots of processed cheese samples were freeze-dried and taken for the preparation of diets in the feeding trials on experimental animals. The composition of the experimental diet is given in Table 1. Protein in the diet was 10%.

Proximate composition was determined according to the following procedures: protein and moisture (AOAC, 1980); total ash (ISI, 1968); fat (BSI, 1969); lactose (Acton, 1977); phosphorus (Ahovocova and Odavic, 1969); and calcium (Davies and White, 1962).

Vitamin A was estimated spectrophotometrically according to the method of Carr and Price (1926). Riboflavin and folic acid were determined microbiologically using *Lactobacillus casei* (ATCC 7469) and *Streptococcus faecalis* (ATCC 8043) respectively according to procedures described by Freed (1966).

Biological evaluation

Protein efficiency ratio (PER) was determined according to AOAC (1980), using 23-days old young male albino rats obtained from the stock colony of Small Animal House of the Institute. Net protein utilization (NPU) was determined according to Miller and Bender (1955) using female albino rats weighing between 50–55g. Biological value (BV) was determined according to the procedure described by Miller and Lachance (1977). In these experiments, animals were housed individually in cages made of anodized aluminum and given 10% protein diets and water *ad libitum* during the experimental period.

Table 1—Composition of test protein and protein free diets (g/kg)

Ingredients	Accelerated ripened processed cheese	Conventionally ripened processed cheese	Casein diet	Protein[b] free diet
Product/Casein	199.2[a]	191.9[a]	133.3	-
Sucrose	70.0	70.0	70.0	70.0
Groundnut oil	-	-	80.0	80.0
Cellulose	10.0	10.0	10.0	10.0
Vitamin mix[c]	10.0	10.0	10.0	10.0
Salt mix[c]	40.0	40.0	40.0	40.0
Corn starch	670.8	678.1	656.7	790.0

[a] Freeze-dried samples were used for the preparation of diet.
[b] Composition as given by Eggum and Jacobsen (1976).
[c] Composition as given by AOAC (1980).

Table 2—Proximate composition of accelerated ripened Cheddar cheese slurry and processed cheese made from it.[a]

Product	Protein (g)	Fat (g)	Lactose (g)	Total ash (g)	Calcium (mg)	Phosphorus (mg)
Cheddar cheese						
Type A	37.98[NS] ± 0.29	49.34[NS] ± 0.37	0.59** ± 0.10	8.45** ± 0.13	1801** ± 46.52	912** ± 5.46
Type B	40.65 ± 0.34	52.36 ± 0.24	0.24 ± 0.02	4.96 ± 0.29	1604 ± 51.68	961 ± 11.56
Processed cheese						
Type C	45.02[NS] ± 0.34	45.25[NS] ± 0.51	0.80** ± 0.01	6.61** ± 0.09	1507** ± 47.05	812** ± 11.36
Type D	45.50[NS] ± 0.29	47.87[NS] ± 0.37	0.49 ± 0.01	4.70 ± 0.13	1190 ± 46.30	602 ± 5.36

[a] Expressed on 100g solid basis. Mean of five observations ± S.E.
[NS] = not significant.
** = Significant at 1% level.

Table 3—Riboflavin, folic acid, and vitamin A contents in accelerated ripened Cheddar cheese Type (A) at different intervals during ripening.[a]

Vitamin	Type A			Type B
	0 day	4th day	8th day	
Riboflavin mg/100 g solids	0.58 ± 0.04	0.60 ± 0.03	0.60 ± 0.03	0.69 ± 0.03
Folic acid µg/100g solids	29.04 ± 1.71	88.30* ± 3.47	113.50* ± 2.27	28.96 ± 2.10
Vit. A I.U./100g solids	1530 ± 30.55	1563 ± 19.37	1581 ± 18.18	1737 ± 40.32

[a] Mean of five observations ± S.E. of mean.
* Significant at 1% level.

Table 4—Protein Efficiency Ratio (PER) for groups receiving experimental diets[a]

Diet based on	Average initial weight (g)	Average final weight (g)	Average protein intake in 28 days (g)	Average gain in body weight in 28 days (g)	Average PER	Adjusted PER
Type C	36.0	101.8	26.0	65.8	2.53 ± 0.02[NS]	2.42 ± 0.021
Type D	33.9	113.1	27.2	79.2	2.91 ± 0.04*	2.78 ± 0.038*
Casein	34.3	111.4	29.5	77.1	2.61 ± 0.09	2.5 ± 0.079

[a] Mean of eight observations ± S.E.
* Significant at 1% level.
[NS] not significant.

Table 5—Biological value for groups of rats receiving different experimental diets[a]

Diet based on	Average N intake (mg)	Average Fn-Fe[b] (mg)	Average Un-Ue[c] (mg)	Digestibility Coefficient (%)	Biological Value (%)
Type C	901.85	132.39	268.27	85.35 ± 0.41	65.23 ± 1.05
Type D (Control)	981.32	100.52	296.08	89.76* ± 0.22	66.38[NS] ± 0.72

[a] Mean of eight observations ± S.E.
[b] Fn = nitrogen in faeces of test group; Fe = nitrogen in faeces of protein free group.
[c] Un = nitrogen in urine of test group; Ue = nitrogen in urine of protein free group.
[NS] = not significant.
* = Significant at 1% level.

Table 6—Net protein Utilization (NPU) for groups of rats receiving different experimental diets[a]

Diet based	Average N-intake (mg)	Average N retained (mg)	NPU (%)
Type C	1328	1075	56.19 ± 0.384
Type D (control)	1342	1158	61.45 ± 1.266*

[a] Mean of eight observations ± S.E.
* Significant at 1% level.

Temperature in the animal was maintained at 23 ± 0.3°C. The influence of effect of different types of cheese samples was tested for statistical significance by F test.

RESULTS & DISCUSSION

DATA on the proximate composition of accelerated ripened Cheddar cheese slurry (Type A) and the processed cheese made from it (Type C) are given in Table 2. A comparison is made with the conventionally ripened Cheddar cheese (Type B) and the processed product made from it (Type D). It was observed that protein and fat were similar for both types of cheese, while lactose, total ash, calcium, and phosphorus were significantly higher ($p < 0.01$) in accelerated ripened Cheddar cheese. A similar observation was made for processed cheese samples. The increase in total ash was probably due to the use of saline in the preparation of Type A cheese. The relatively higher lactose level in accelerated ripened cheese (Type A and C) in comparison to conventionally ripened cheese (Type B and C) may be possibly due to its lesser utilization by starter culture because of the short duration of ripening.

Changes in vitamin content during accelerated ripening

Data on certain vitamin contents at different stages of accelerated ripening are given in Table 3. The values are presented on dry matter basis to overcome the influence of moisture on the trend of changes. There was practically no change in riboflavin at any state during 8 days of ripening. On the other hand, there was an almost fourfold increase in the folic acid levels. The increase was more pronounced during the first 4 days of ripening. Though little is known about changes during accelerated ripening, the trend observed for riboflavin was similar to that reported earlier by Dearden et al. (1945) who found no change in riboflavin during curing of cheese. The riboflavin content in 8 days accelerated cheese was found to be comparable to the level observed in the conventionally ripened cheese (Type B). Likewise, the trend for folic acid was similar to that reported by Nilson et al. (1965) who observed a sharp increase in vitamin content during the first week of conventional ripening of Cheddar cheese. It was further observed in the present study that folic acid in Type A cheese samples was markedly higher than in Type B samples. As regards vitamin A, contents were similar in Type A and Type B samples.

Biological evaluation of processed cheese

In view of proteolytic reactions reported during cheese ripening and a marked difference in the duration of ripening between conventional and accelerated ripened Cheddar cheese samples, feeding trials were conducted on growing albino rats to determine the protein quality of Type C cheese. Data were obtained on protein efficiency ratio (PER), biological value (BV) and net protein utilization value (NPU) and are presented in Table 4, 5, and 6, respectively.

Regarding PER, the value observed for the conventionally ripened cheese (Type D) based diet was significantly higher than that for diet based on accelerated ripened cheese (Type C). However, there was no significant difference between PER value for the casein group and the Type C group (Table 4).

Data on the nitrogen balance study carried out on experimental animals (Table 5) showed that the digestibility coefficient (DC) was slightly but significantly lower in the case of the Type C group when compared with the Type D group. However, there was no significant difference in respect to BV, though values were slightly lower for Type C.

Data on NPU are given in Table 6, which again showed slightly but significantly higher value for Type D in comparison to Type C.

From the data on biological evaluation experiments presented, it was observed that processed cheese made from conventionally ripened Cheddar cheese had distinct superiority over the one made with accelerated ripening process. This was evident from higher PER, NPU and DC values for Type D cheese. The superiority of Type D cheese cannot be ascribed to dietary factors other than protein, since all the dietary factors were the same in both diets. It is possible that better utilization of proteins in Type D cheese was due to more proteolytic changes resulting during prolonged ripening in the conventionally ripened cheese product in comparison to the accelerated ripened one. This was evident from the significantly higher DC for Type D cheese. Rasic et al. (1971) reported better nutritive value of proteins in yoghurt due to proteolytic degradation of milk proteins. However, the other possibility for a higher quantity of intracellular growth stimulating factor in type D cheese, secreted by the starter culture, also cannot be overlooked. In a recent study, Wong et al. (1983) reported secretion of such factor by *Streptococcus thermophillus* in yoghurt.

Despite such superiority, the overall protein quality for processed cheese made from accelerated ripened Cheddar cheese did not appear in any way alarmingly low, since values for various parameters of protein quality observed in this study were within the range reported by different workers for a variety of conventionally ripened cheese (Poznanski and Siudak, 1971; Deodhar and Dugal, 1981; Sehgal, 1982).

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Freeze Concentration of Pineapple Juice

R.J. BRADDOCK and J.E. MARCY

ABSTRACT

Juice, extracted from fresh Costa Rican pineapples using a commercial extractor, was adjusted by finishing and centrifugation to two pulp levels, 12% and 2% by volume. Physical, chemical and sensory differences were measured in heat stabilized single strength juices, evaporator concentrated, and freeze-concentrated juices. Results indicated no significant differences between samples for °Brix, % acid, total hexose, vitamin C, browning index, color, viscosity. Pulp content was lower in both freeze- and evaporator-concentrated samples. Significant ($P < 0.01$) flavor differences were detected between freeze- and evaporator-concentrated juices. Juices were ranked according to highest degree of fresh fruit taste as follows: (1) high pulp single strength, (2) freeze concentrate high pulp, and (3) evaporator concentrate high pulp. Flavor of reconstituted freeze-concentrated juice was comparable to single strength juice and preferable to evaporator concentrate.

INTRODUCTION

QUALITY AND FLAVOR of pineapple products, including juices and concentrates, are dependent upon ripeness, processing, and storage conditions. There have been studies to determine the composition of aroma and flavor constituents of pineapple and to identify differences between processed and fresh fruit and juices. Tressler and Joslyn (1961) reported that pineapple juice and concentrate flavors are susceptible to degradation during thermal processing and loss of volatiles during evaporation. Compositional analyses of essence from a juice evaporator identified significant concentrations of esters and lesser numbers of other oxygenated organic compounds (Flath and Forrey, 1970).

These reports show the complexity of defining flavor composition and emphasize the effects of processing which result in changing product flavor compared with fresh juice. Application of alternate processing methods could significantly improve juice and concentrate flavor quality. Particularly, use of freeze concentration, rather than evaporation could reduce thermal degradation. Freeze concentration is especially favorable in retaining very volatile aroma compounds and minimizing detrimental effects of thermal processing conditions on product quality (Thijssen and Van Oyen, 1977). Flavor advantages for such a process applied to orange juice concentration have been reported recently (Strobel, 1983).

The purpose of this study was to examine pilot scale processing parameters and product quality aspects of freeze concentration applied to fresh pineapple juice. Certain limitations as well as advantages of this process were considered and supported by experimental results. It was not the intent of this study to meet Federal Standards of Identity requirements for insoluble solids of canned pineapple juice prepared from concentrate.

Table 1—Parameters related to processing pineapple juice for freeze concentration

	High pulp	Low pulp
Pineapples (kg)	1,583	1,684
Extractor (kPa)	276	276
Finished juice (kg)	787	578
Finished pulp (kg)	57	50
Peel discarded (kg)	739	1,056
Juice recovery (%)	50	34
Peel, pulp refuse (%)	50	66
Finisher pressure (kPa)	414	414
Finisher screen (mm)	0.84	0.41
Finished juice (% pulp)	18-20	8-10
Finished juice (°Brix)	14.34	15.02
Finished juice (% acid)	0.71	0.63
Heat exchange (°C at 40 sec)	88	88
Flow rate (L/min)	3.8	3.8
Temperature out (°C)	27	27
Centrifuge (× *g*)	12,000	12,000
Feed rate (L/min)	19	15
Pulp out (%)	12-13	2-3
Cycle time (min)	7	5
Discharge time (sec)	2.5	3
Back pressure (kPa)	0	345

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MATERIALS & METHODS

Pineapple processing

Approximately 6.5 tons Costa Rican pineapples were received within 1 week after harvest at our pilot plant on March 8, 1984 and stored at 5°C. After preliminary tests on March 8 and 9, to determine equipment parameters, juice processing was performed the week of March 12, less than 2 weeks after harvest. Juice was extracted with a commercial extractor (Pine-o-mat, Brown International, Inc., Covina, CA). Approximately 1.6 ton batches of pineapples were used per run. The juice was finished with a screw finisher (Model 3900, Brown International), heat stabilized in a plate heat exchanger (Junior Paraflow, APV, Buffalo, NY) and centrifuged (Westfalia type SA 7-06-076, Centrico, Inc., Northvale, NJ) before concentration.

The experiment consisted of evaporation and freeze concentration of juice with two different pulp levels, 12% (termed high pulp) and 2% (low pulp). A flow schematic of the process is presented in Fig. 1 with conditions for typical high and low pulp runs listed in Table 1. The juice to be freeze-concentrated was heat stabilized for 40 sec at 88°C, cooled, centrifuged, and held at 2–4°C in a cold-wall tank. Juice for evaporation was heated to 60°C, centrifuged, and heat stabilized at 88°C in the preheater immediately prior to flashing in the first stage vacuum of the evaporator. Mass balances and flow rates were made by direct weight or volume determinations.

Juice concentrated by evaporation was processed in a pilot scale 225 kg/hr (water removal), 3-effect, tubular falling-film (commonly referred to as "Taste") evaporator (Gulf Machinery, Inc., Safety Harbor, FL). Freeze concentration was performed using pilot equipment (Model W-6, Grenco S.A., Hertogenbosch, Holland) operating at about 10 kg/hr water removal from single strength juice (14.5–15.0°Brix). Concentration continued until 50–55 kg of 40–42°Brix concentrate was obtained. Samples were canned in 176 ml (6 oz) enamel-lined metal cans and kept frozen at −17°C until needed. Other details of the operation of the freeze concentration unit may be obtained from Grenco (van Pelt, 1984; van Pelt and Swinkels, 1984).

Quality tests

Pineapple juice and concentrate samples were analyzed for total soluble solids (°Brix) by refractometer, titratable acidity (%, as citrate) and total hexose using an anthrone reagent (Stewart, 1975). Vitamin

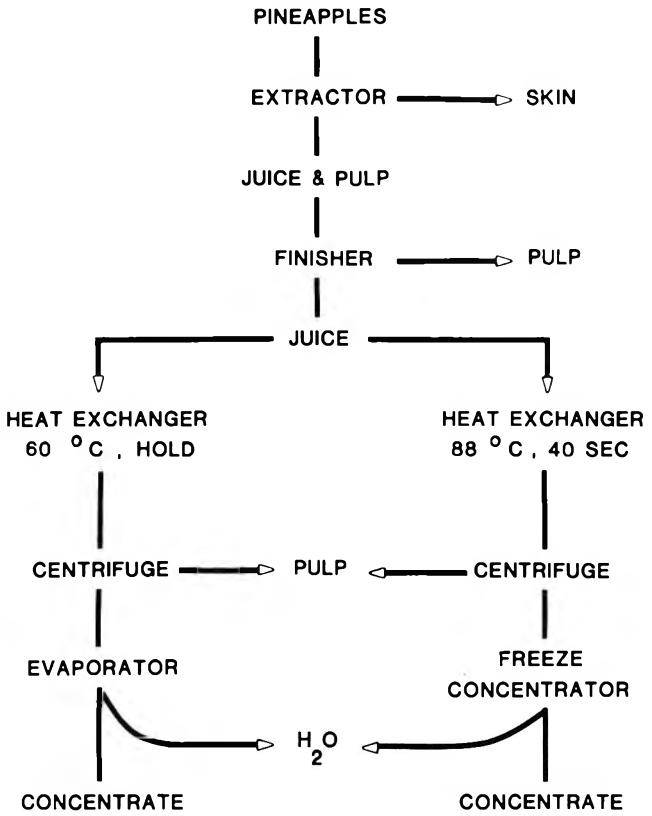

Fig. 1—Process schematic for preparation of pineapple concentrate.

C was measured in the juice using an indophenol dye titration (AOAC, 1980). Degree of browning (browning index) was estimated by measuring absorbance at 420 nm of the clear serum obtained from mixing 60% of 95% ethanol and 40% of single strength juice (Meydav et al., 1977). Viscosity was determined using a Brookfield viscometer (Brookfield, Stoughton, MA) with samples at 25°C. Spindle 1 at 60 rpm was used for single strength juices; while concentrate required spindle 2 at 60 rpm. Color of the single strength juices (x, y, z) was measured with a Hunter citrus colorimeter (Hunter Associates, Reston, VA). Pulp content was determined by first heating raw juice rapidly to 60°C, holding 1–2 min and cooling to 30°C in an ice bath. The juice (50 mL) then was centrifuged at 360 × *g* for 3 min in a clinical centrifuge. Percent pulp was calculated from the volume of sediment in the calibrated centrifuge tube. Previously heat stabilized juice was simply brought to 30°C and centrifuged.

Gas liquid chromatography

Sugars and organic acids were determined by gas-liquid chromatography (GLC) of the silylated derivatives using a modified procedure (AOAC, 1980). The concentrates were diluted to single strength (14.7 °Brix), 1 mL was brought to 10 mL volume with 95% ethanol and mixed; 0.1 mL saturated lead acetate was added and the mixture was centrifuged at 2000 × *g* to obtain a precipitate containing organic acids. The supernatant fluid was saved. The ppt was washed twice with 95% ethanol (10 mL), once with acetone, once with ether and dried to constant weight at 75°C. The silylating reagent (1 mL Tri Sil, Pierce Chemical Co., Rockford, IL) was added to the dry ppt, reacted for 30 min, and centrifuged to obtain a clear sample for GLC injection.

The supernatant (1 mL) containing sugars from the lead acetate ppt was evaporated to dryness under vacuum. Silylating reagent was added as before to the dry residue. This procedure prepared the sugar fractions for GLC analyses. GLC (Perkin Elmer Sigma 3B, Norwalk, CT) conditions were as follows: acids 0.5 μL injection; 0.25 mm × 30 m glass capillary column with SE-30 grade AA (Supelco, Bellefonte, PA); column oven was programmed from 90–220°C at 8°C/min with initial hold of 4 min and final hold time of 5 min; injector and detector temperatures were set at 275°C. Conditions for sugars were the same except oven temperature was 140–260°C at 8°C/min; triplicate injections of duplicate samples were made and the results averaged. Peak areas were automatically integrated and compared with 0.001 mg/mL standard solutions of acids and sugars.

Taste evaluations

Taste panel evaluations were performed by a 15 member panel experienced in tasting citrus juice. Most members had not previously evaluated pineapple juices. Concentrates were reconstituted to single strength (14.5°Brix) with distilled H_2O for analysis. Triangle test comparisons were made between pulp levels, concentration methods (evaporation vs freeze concentration), single strength heat-stabilized juice vs. freeze concentrate, single strength vs. evaporator concentrate (Larmond, 1967). Each test was performed with the same 15 panelists, once in the morning and once in the afternoon for a total of 30 responses per comparison. The same panel also ranked coded samples of the single strength juice, freeze and evaporator concentrates according to specified attributes. Attribute ranking (most = 1, least = 3) was used to determine if differences found in triangle tests were due to off-flavors produced or due to loss of desirable volatiles. A total of 30 responses was obtained when panelists were asked to rank the samples from most fresh fruit flavor to least. Ranking was also determined when panelists were asked to evaluate samples for most to least processed flavor.

Panelist's familiarity with fresh and processed juice flavor was established by presenting them with samples identified as being either fresh or heat-processed. The heat-processed standard was heated to 90°C for 15 min in a covered stainless steel cooking pot. Triangle tests and ranking were analyzed statistically using analysis of variance available through a computer service.

RESULTS & DISCUSSION

Processing

A reported advantage of modern freeze concentration systems is retention in the concentrate of components responsible for fresh fruit-like flavor (Thijssen and Van Oyen, 1977; Deshpande et al., 1982). To meet this fresh flavor requirement, it was necessary to extract juice from fresh pineapples in a manner as similar to commercial processing as possible. The results of processing conditions established in our pilot plant are presented in Table 1 and Fig. 1. These parameters were comparable to commercial processing conditions, with the major exception being lack of a two-stage commercial pressing of the pulp (Tressler and Joslyn, 1961). Results in Table 1 show that juice recovery is dependent on pulp content. As expected, the high pulp juice yield (50%) was much better than the low pulp (34%). Commercial juice yields have been reported to be about 50–55% of total ongoing fruit (Tressler and Joslyn, 1961).

Commercial pineapple juice contains about 20% pulp, which imparts body and characteristics consumers associate with pineapple to the product (Vrinten, 1984). Lower pulp contents (12 and 3% in our study) were necessary to efficiently freeze-concentrate the juice without significant loss of sugars, acids and flavors in the wash water (ice melt). At the two pulp levels studied, pulp was lost from the juice during freeze concentration (Table 2), going out in the water from the wash column. The reduction from single strength high pulp (SSHP, 12–13% pulp) to 4–5% pulp in the reconstituted freeze-concentrated high pulp (FCHP) product was considerable. A much smaller reduction also occurred for the low pulp freeze concentrate (FCLP) samples. This was also noted in the juice reconstituted from the evaporator concentrate and was probably due to particle size reduction during pumping. Neither high nor low pulp evaporator condensate nor the wash water from the low pulp freeze concentration tests had noticeable pulp.

Wash water from the FCHP had soluble solids from 0.6–0.8°Brix at product concentrations of 30–40°Brix. The °Brix of FCLP wash water was too low to read by refractometer. However, anthrone determinations indicated values in this fraction to be from 200–800 ppm total hexose during product concentration. In both FCHP and FCLP runs, concentration of soluble substances in the wash water increased with product concentration. This problem is dealt with during operation of

Table 2—Values of parameters for high and low pulp (HP, LP) single strength (SS), freeze- and evaporator-concentrated (FC, EC) pineapple juices[a]

	SSHP	SSLP	FCHP	FCLP	ECHP	ECLP
°Brix (SS)	14.5	15.0	14.7	14.7	14.7	14.7
Acid SS (%)	0.69	0.67	0.72	0.66	0.71	0.66
Ratio (Brix/Acid)	21.0	22.4	20.4	22.2	20.7	22.2
Hexose (g/100g)	11.3	12.6	13.1	11.4	12.7	11.8
Vit. C (mg/100 mL)	4.0	5.3	3.5	4.2	4.4	3.3
Browning index	0.08	0.07	0.08	0.07	0.08	0.07
Color (x)	13.3	11.2	12.1	11.2	13.6	11.5
(y)	14.4	12.2	13.1	12.0	14.7	12.4
(z)	7.7	6.5	7.0	6.6	7.8	6.7
Fiber (%)	12-13	2-3	4-5	1-2	10-11	1-2
Viscosity (mPas)	25.9	27.9	22.5	18.7	24.0	20.3
°Brix (conc)	--	--	40.5	41.2	40.5	41.9
Acid conc (%)	--	--	2.0	2.0	2.0	2.0
Viscosity conc (mPas)	--	--	91	93	85	85

[a] Values are means of three analyses each from three cans of sample.

commercial freeze concentrators (van Pelt, 1984) and is beyond the scope of this discussion. At any rate, for 200–800 ppm hexose, soluble solids losses for the FCLP samples were considered to be insignificant. The loss of solids during concentration of FCHP juice necessitated pulp reduction to 2–4% prior to freeze concentration.

Product quality

Juice properties. Comparison of commonly measured properties in Table 2 shows few differences among single strength, freeze or evaporator concentrated juices. Pulp content changes and a slight decrease in concentrate viscosities were noted. Also, color measurement of all samples on a single strength basis gave results indicating insignificant color changes as a consequence of processing treatment, such as might have resulted from the more severe heat treatment during evaporation.

Sugars and acids. Processing methods to produce concentrated product differed somewhat in the steps prior to concentration (Fig. 1). For example, to reduce foaming during centrifugation, heat treatment was necessary. Evaporator juice was held at 60°C and not cooled, while juice for freeze concentration was cooled from 88°C to ambient prior to centrifuging. Because of these differences, examination of the stability of individual sugars and acids during processing was justified. Values for total sugars (as hexose) for single strength samples or reconstituted concentrates (Table 2) agreed with the range published for pineapple juice (Li and Schuhmann, 1983; Tressler and Joslyn, 1961). These values (Table 2) averaged from 78–89% of the total soluble solids (°Brix) corrected for acid content. These results are similar to calculations estimating total sugars in pineapple by using the Brix reading minus three times the percent acidity (Singleton and Gortner, 1965).

Major sugars and acids are presented in Table 3. Previous reports identified fructose, glucose and sucrose as the primary sugars, with malic and citric, the primary organic acids (Gawler, 1962). In mature pineapple, the citric acid was about twice the malic acid concentration (Singleton and Gortner, 1965). In spite of small soluble solids losses in the wash waters from the freeze concentrator, there was little difference in the ratios of sugars or acids from the various pineapple juice processes (Table 3).

Taste evaluation

From samples taken off-line during the processing runs, it was apparent that the freeze concentrate retained more flavor than the evaporated product. This could be predicted, since vacuum steam evaporation would remove flavor substances. Attempts to recover the essence in our pilot evaporator were unsatisfactory, as this essence system was designed for citrus volatiles and did not function to produce a typical pineapple aroma.

Triangle tests. Comparison of high pulp and low pulp heat-stabilized, single strength juice gave: correct responses, 12 out of 29, no significant difference; FCLP vs. ECLP: correct responses, 23 out of 30, a significant ($P < 0.1$) difference between processes; FCHP vs. SSHP: correct responses, 17 out of 30, a significant ($P < 1.0$) difference between samples; ECHP vs. SSHP: correct responses, 26 out of 30, a significant ($P < 0.05$) difference between samples. In the triangle tests, the panel did not do as well comparing single strength (SS) juice with reconstituted freeze concentrate (FC) as when comparing SS or FC with evaporator concentrate.

Attribute ranking tests. Samples were ranked from most to least fresh fruit flavor as shown in Table 4. On two different occasions, the panelists ranked the samples with the single strength juice (SSHP) having the most fresh fruit flavor. Samples also were ranked from most to least processed flavor. On two occasions the panelists ranked the samples concentrated by evaporation (ECHP) as having the most processed flavor.

The intensity of processed flavor noted in evaporator concentrate may have been influenced by the 60°C treatment without cooling prior to centrifuging. Results of these tests indicated that flavor differences were due to both the absence of desirable flavor volatiles and the production of off-flavors in the evaporated product. One might consider that the flavor of the concentrate could be improved by addition of essence or juice volatiles. However, improving the flavor of evaporator concentrated pineapple juice by essence addition was not an ob-

Table 3—Sugars and acids in high and low pulp (HP, LP) single strength (SS), freeze and evaporator concentrated (FC, EC) pineapple juices

	g/100g SS juice[a]				
Sample	Fructose	Glucose	Sucrose	Malic acid	Citric acid
SSHP	1.4	1.5	8.4	0.23	0.46
SSLP	1.4	1.4	9.8	0.22	0.45
FCHP	1.2	1.7	10.2	0.29	0.42
FCLP	0.7	1.5	9.2	0.23	0.42
ECHP	1.5	1.7	9.4	0.26	0.45
ECLP	1.8	1.7	8.3	0.32	0.34

[a] Values are means of three analyses each from three cans of sample. Std. dev.: fructose (0.2), glucose (0.8), sucrose (0.8), malic acid (0.1), citric acid (0.1)

Table 4—Mean and standard deviations for ranking high pulp (HP) single strength (SS), freeze-concentrated (FC) and evaporator concentrated (EC) juice according to fresh and processed flavor[a]

Ranking	Sample	N	Mean	Std. Dev.
Fresh	SSHP	30	1.30	0.47
	FCHP	30	1.90	0.72
	ECHP	30	2.80	0.41
Processed	ECHP	30	1.36	0.59
	FCHP	30	1.94	0.71
	SSHP	30	2.68	0.58

[a] Means are significantly different ($P < 0.05$).
Pooled standard deviations were 0.54 (fresh flavor ranking) and 0.63 (processed flavor).

jective of this study, since this is being practiced commercially (Tressler and Joslyn, 1961). The taste tests showed that single strength juice was identified as having more fresh taste and less processed flavor than either the freeze or evaporator concentrated samples. It was noted that the flavor difference comparing SS and FC was less than that between SS and evaporator concentrate (Table 4).

Process comparisons. Freeze concentration processes are successful on a commercial scale for several liquid food products susceptible to flavor loss during concentration. Economics of this technique have been presented and compared with evaporation (van Pelt. 1984). Capital costs for a state-of-the-art process are now higher than for evaporation, but energy consumption is similar. However, improved flavor of concentrate would be a process advantage, which could conceivably offset higher capital costs of freeze concentration. Freeze concentration of pineapple juice should be feasible and preferable to evaporation if a fresh-flavored product, similar to single strength juice, is desired. It might be argued that evaporator concentrate with essence added would be comparable; however, it has been our experience with citrus, that evaporator essence does not match the aroma of fresh juice. This is partly true because essence manufacture involves heat treatment and distillation during the folding process. Results have been published giving profiles of some volatile flavor components comparing commercial concentrated orange juice with fresh and freeze-concentrated juices (Strobel, 1983). To prepare pineapple juice concentrate with either the pilot evaporator or freeze concentrator, finished juice pulp content (18% - 20%, Table 1) was reduced by centrifuging. Because of severe foaming, heating the juice prior to centrifuging was performed. For the evaporation process (Fig. 1), it was necessary that juice be exposed to heat for a longer time than for the freeze concentration process. This would also be true for commercial processes.

CONCLUSIONS

THESE RESULTS indicated that flavor of freeze-concentrated pineapple juice was comparable to single strength juice and preferable to evaporator concentrate. Freeze-concentrated pineapple juice also had other properties making it comparable to traditional product, the major exception being the limitation of lower pulp content. As a practical consideration, one would have to deal with decreased recovery when processing low pulp juice for freeze concentration. Pulp could be added to the juice after concentration or perhaps additional juice could be washed from the pulp as is done in processing other fruits.

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Detection of Enocyanin in Cranberry Juice Cocktail by Color and Pigment Profile

F. J. FRANCIS

ABSTRACT

Addition of enocyanin to cranberry juice cocktail can be determined by a four-step color and pigment profile. Enocyanin colorant is slightly more blue, therefore, a simple measurement of color will detect samples with 12% or less cranberry juice. Cranberry juice cocktail normally contains 25% cranberry juice. Anthocyanins and flavonoids, recoverable by a CG-50 ion exchange column, are lower in samples with 12% or less cranberry juice. Anthocyanin aglycones, found in grapes and not in cranberries, can be detected by paper chromatography in Formic reagent. Replacement of 50% of cranberry juice by a solution of enocyanin and citric acid, can be detected by paper chromatography of anthocyanins in 1% HCl in water.

INTRODUCTION

THERE IS CONSIDERABLE economic incentive to make cranberry juice cocktail with less than the expected amount of cranberry juice by addition of citric acid for acidity and colorants from grapes for color. The enocyanin colorants from grape skin extracts are excellent colorants for cranberry juice drinks but they do have a bluer hue. It should be possible to detect the addition of enocyanin to cranberry juice cocktail by the color. Methods to measure the color of cranberry juice cocktail are well developed (Francis and Servadio, 1963; Staples and Francis, 1968; Francis and Clydesdale, 1970; Starr and Francis, 1973).

The anthocyanins in grapes have been studied extensively in view of their importance in wines (Ribereau-Gayon, 1982). The enocyanin types of colorants produced from the skins of wine grapes (Markakis, 1982), reflect the anthocyanin composition of the grapes, even though they are highly polymerized. Cranberries have a relatively simple anthocyanin profile (Zapsalis and Francis, 1964; Fuleki and Francis, 1967). They have only six red pigments as compared with 17 for grapes. Therefore, it should be possible to detect the addition of enocyanin to cranberry juice cocktail by the pigment profile.

A logical approach to the detection of an adulterant to any food is to develop a chemical profile of both the adulterant and the product. For example, the organic acid profile of cranberry juice (Coppola et al., 1978; Fernandez-Flores et al., 1970) and the yellow flavonol pigments (Puski and Francis, 1967) of cranberries would be good candidates. Similarly, the phenolic acids (Chu et al., 1973) and the phenolic polymers (Wang et al., 1978) would be possibilities. Actually, any components would be profile candidates if a difference could be established between cranberry juice cocktail and enocyanin. All of these approaches will require considerable development work. This project was concerned with the development of a simple method, using a colorimetric and a pigment profile approach, to detect the addition of enocyanin to cranberry juice cocktail.

MATERIALS AND METHODS

Sources

Samples of commercial cranberry juice cocktail known to be authentic were obtained in the market as well as samples of commercial brands, suspected of being adulterated with a grape colorant preparation. Cranberry juice cocktail normally contains 25% cranberry juice with the remainder made up of sugar and water. Samples of commercial enocyanin (Enocianina from Italy) were obtained from an American distributor.

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Color measurement

Color was determined with a Gardner XL23 colorimeter with a transmission attachment. The instrument was standardized at L = 100, a = o and b = o using distilled water and 4 × 5 cm cells with a 2 cm light path. The data were reported as lightness (L) and Theta (θ). Theta is the angle formed by a line joining a point in a Hunter a b plot with the origin and the horizontal axis (Francis and Clydesdale, 1975). The function, Theta, is most easily calculated from Hunter data by calculating the angle whose cotangent is a/b.

Commercial samples of cranberry juice cocktail and Cran-Grape were measured immediately after opening, as well as samples diluted with a solution containing 0.8% citric acid and 0.2% enocyanin.

Anthocyanin and flavonoid content

The ion-exchange resin CG-50 (Rohm and Haas Co., 100-200 mesh) was slurried with distilled water and the fine particles decanted. The remainder of the resin was poured into a glass tube to make a column 10 cm long and 4 cm diameter. Excess water was removed by suction and 25 to 150 mL cranberry juice cocktail were added to the column. When the sample was almost absorbed on the column, 100 mL water were added. When the water was almost absorbed, 100 mL 0.1% HCl in methanol were added to elute the pigments from the column. When the pigments pass through the column under suction, there is an obvious break in the elution stream between the pigments that were not absorbed and those that were absorbed. The two fractions were collected. After the methanol was almost absorbed, 100 mL water were added to prepare the column for the next sample.

The two fractions were made to volume and absorbance measured on a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer. The pig-

Table 1—R_f values of cranberry and grape aglycones in formic reagent[a]

	Aglycone	R (×100)
Cranberries		
	Cyanidin (Cn)	22
	Peonidin (Pn)	30
Grapes		
	Delphinidin (Dp)	13
	Petunidin (Pt)	20
	Cyanidin (Cn)	22
	Malvidin (Mv)	27
	Peonidin (Pn)	30

[a] Separated by paper chromatography. Formic Reagent contains conc. formic acid:conc. HCl:water (5:3:1) (Harborne, 1967).

Table 2—R_f values of cranberry and selected grape anthocyanins in 1% HCl[a]

Cranberry pigments	R (×100)	Grape pigments	R (×100)
Cn-3-arab	5	Dp-3-glu	3
Cn-3-gal	7	Pt-3-glu	4
Cn-3-glu	7	Mv-3-glu	6
Pn-3-arab	9	Cn-3-glu	7
Pn-3-gal	9	Pn-3-glu	10
Pn-3-glu	10		

[a] Separated by paper chromatography (Harborne, 1967)

ment content was calculated by the methods of Fuleki and Francis (1967) and Lees and Francis (1971). The formulae are as follows:

$$\text{Anthocyanins} = \frac{1\%}{\text{E } 535 \text{ nm}}_{1 \text{ cm}} = 980$$

$$\text{Flavonoids} = \frac{1\%}{\text{E } 374 \text{ nm}}_{1 \text{ cm}} = 766$$

Pigment aglycones

The pigments recovered in methanol from a 50–200 mL sample of cocktail were treated with 2 mL conc HCl and boiled for 60 min. Sufficient water was added to maintain 30 mL of liquid in the flask. After cooling, the aglycones were extracted with 3 mL tertiary amyl alcohol. The amyl alcohol extract was evaporated to dryness, redissolved in 0.1% HCl in methanol and streaked on a 10 × 57 cm strip Whatman #3 chromatographic paper. The papers were developed in descending Formic reagent (5:3:1-conc formic acid: conc HCl:water) for 18 hours. Literature values (Harborne, 1967) for the aglycones (Table 1) indicate that delphinidin is the only grape aglycone likely to separate from the cranberry aglycones.

Recovery of grape pigments

A sample of enocyanin was passed through a CG-50 column and the absorbed pigments were eluted, streaked on Whatman No 3 paper, and developed in BAW (butanol:acetic acid: water-4:1:3). The chromatograms indicated at least seven pigments. When the enocyanin was combined with cranberry samples, the chromatograms were too complex for interpretation. Another solvent, 1% HCl in water was much more satisfactory for separation of the pigments of interest. Literature values (Harborne, 1967) for the monoglycoside pigments in cranberries and grapes are shown in Table 2. Two pigments in grapes would be expected to separate from the cranberry anthocyanins, but since delphinidin-3-glucoside (Dp-3-glu) occurs in grapes in greater quantities than petunidin-3-glucoside, it is logical to look for Dp-3-glu.

A sample of cranberry juice cocktail (200–600 mL) was purified on a CG-50 column. The eluted pigments were placed on a 7 × 57 cm strip of Whatman No 3 paper and developed for 6 days at 16°C in 1% HCl in water. This amount of pigment constitutes a gross chromatographic overload, but is necessary in order to have sufficient pigment for detection. The presence of delphinidin monoglucoside shows up as a distinct band above the massive bands of cyanidin and peonidin pigments contributed by both the cranberry juice and the enocyanin.

RESULTS & DISCUSSION

Color measurement

The addition of a colorant, such as enocyanin, to cranberry juice cocktail should result in a bluer color in the final product since the colorant has a bluer hue. Actually, the hues of the colorant and the cranberry juice are very close, but the differences are measurable.

The Theta values for authentic cranberry juice cocktail diluted with 0.8% citric acid solution (Curve 1, Fig. 1a) shows a shift towards the orange red area. Curve 2 for authentic cocktail diluted with 0.2% enocyanin in 0.8% citric acid shows lower Theta values. Curve 3, for a sample suspected of having added enocyanin, diluted with 0.2% enocyanin in 0.8% citric acid, shows much lower Theta values. Dilution without addition of colorant, is easily detected by the changes in lightness (Fig. 1b).

Table 3 shows data for commercial samples from four manufacturers. The Theta values indicate that samples D to H are authentic, whereas J-M have added colorant. Data for Cran-Grape are included since this is an obvious case of the addition of pigments from grapes.

A simple measurement of the color would serve as a quick and simple screening test for samples with 50% or less of the expected amount of cranberry juice. For grossly adulterated samples, this method would be foolproof.

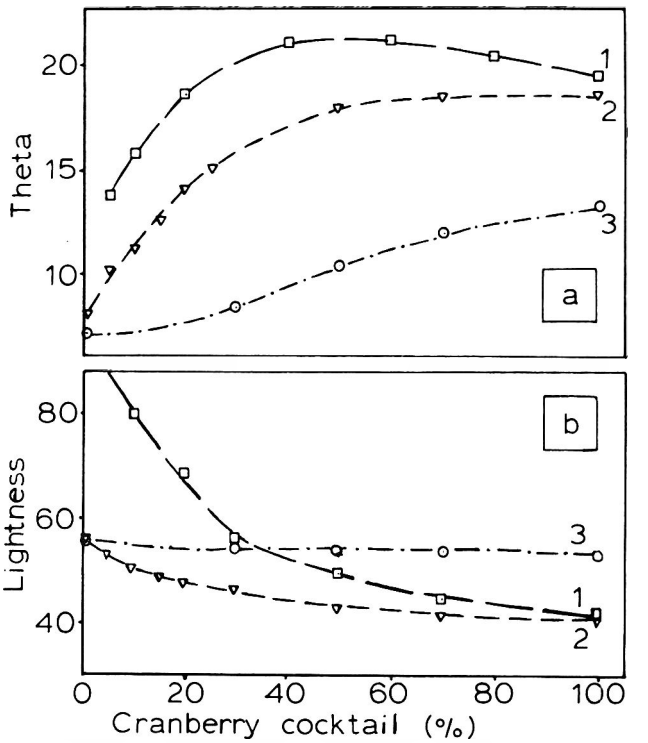

Fig. 1—Theta (a) and lightness (b) values for three dilutions of cranberry juice cocktail. Curve 1 refers to an authentic sample of cocktail (A) diluted with 0.8% citric acid in water (D). Curve 2 refers to cocktail A diluted with 0.2% enocyanin in 0.8% citric acid solution (C), Curve 3 refers to a commercial sample of cranberry juice cocktail (B), suspected of having added colorant, diluted with C.

Table 3—Color of commercial samples of cranberry juice cocktail

	Color			
Sample	L	a	b	θ
D	39.3	75.1	24.5	18.0
E	40.3	74.6	25.3	18.7
F	40.9	74.4	25.3	18.8
G	39.6	73.3	24.9	18.7
H	40.1	73.7	24.5	18.4
J	21.4	46.6	11.3	13.6
K	29.5	54.3	14.2	13.7
L	46.2	64.8	13.6	11.9
H	49.5	66.4	17.5	16.5
Cran-Grape	20.5	55.6	11.7	11.9

Anthocyanin and flavonoid content

The enocyanin colorants are more polymerized than the pigments found in cranberry juice cocktail due to the method of production of the colorant (Markakis, 1975). Therefore, cocktail with colorant added, would be expected to have less recoverable anthocyanin pigments. The colorant also contains less of the recoverable yellow flavonoid pigments. Both effects should show up in cocktail with added colorant. The resin column has a number of advantages for pigment recovery. First, it readily absorbs anthocyanins and flavonoids, whereas sugars, polymers, etc., pass through with the water wash. Second, the resin can be used over and over since previous research had shown that the resin retained its absorbent capacity even after 175 cycles (Chiriboga and Francis, 1970). Third, the pigment comes off the column in methanol which can be removed easily with minimum pigment degradation.

Table 4 provides data on the anthocyanin and flavonoid content of two samples of cocktail diluted with enocyanin in citric acid solution. There was a high recovery of both anthocyanins

+ 25% C

Table 4—Pigment content of cocktail with varying amounts of cranberry juice

Sample	Pigment content (mg/100 mL) Anthocyanins Absorbed (A)	Non-abs. (N)	Ratio A/N	Flavonoids Absorbed (A)	Non-abs. (N)	Ratio A/N
1. 100% A[a]	3.11	0.24	12.9	3.10	0.19	16.3
2. 75% A + 25% C	2.63	0.27	9.6	2.55	0.19	13.4
3. 50% A + 50% C	1.93	0.28	6.9	1.81	0.20	9.0
4. 25% A + 75% C	1.06	0.33	3.3	0.98	0.22	4.6
5. 100% C	0.15	0.50	0.3	0.06	0.30	0.2
6. 100% B	0.34	0.37	0.91	0.80	0.40	2.00
7. 75% B + 25% C	0.27	0.38	0.71	0.63	0.38	1.67
8. 50% B + 50% C	0.20	0.35	0.57	0.42	0.28	1.50
9. 25% B + 75% C	0.15	0.40	0.38	0.27	0.30	0.90
10. 100% C	0.07	0.50	0.14	0.08	0.37	0.21

[a] A is an authentic sample of cranberry juice cocktail. B is a sample suspected of adulteration. C is a 0.2% solution of enocyanin in 0.8% citric acid.

and flavonoids from cocktail sample A, whereas there was a very low recovery of both classes of pigment from sample B. Low quantities of both pigments are recoverable from enocyanin since it is the polymers which provide most of the colorant potential. The ratio of absorbed to nonabsorbed pigment is a good indication of the amount of undegraded pigment in the original cranberry juice. Table 5 shows the same data for nine commercial samples. The low pigment recoveries and low ratios of absorbed to nonabsorbed pigment provide good indications that samples J to M have added colorant.

The pigment data show good agreement with the color data. The addition of colorant can be detected in samples with 50% or less of the expected amount of cranberry juice.

Pigment aglycones

The color and pigment recovery analyses are limited in their ability to detect small quantities of enocyanin, so the obvious choice to increase the sensitivity is to detect the actual grape pigments. The simplest way to do this is to purify the pigment mixture, hydrolyze to free the aglycones and analyze for aglycones peculiar to grapes (Table 1). The methanol solution coming from the CG-50 column is ideal for hydrolysis since the methanol boils off, effecting a concentration, and an amyl alcohol extraction of the aglycones would accomplish another concentration.

Distinct bands for cyanidin and peonidin appeared in all samples. In addition, a band with the R_f value of delphinidin appeared in all samples suspected of enocyanin addition. The Cran-Grape sample yielded a particularly wide band, as did a sample of pure enocyanin.

A trace of the delphinidin band appeared in the authentic cranberry cocktail samples, albeit in much lower proportion. This was unexpected because delphinidin has not been found in cranberries. It may be that delphinidin is an artifact formed from a leucoanathocyanidin in the hydrolysis step, or that delphidin does actually occur in cranberries in very small amounts.

Table 5—Pigment content of commercial samples of cranberry juice cocktail

Sample	Pigment content (mg/100 mL) Anthocyanins Absorbed	Non-abs.	Ratio A/N	Flavonoids Absorbed	Non-abs.	Ratio A/N
D	3.55	0.31	11.5	3.52	0.30	11.7
E	3.48	0.27	12.9	3.53	0.23	15.3
F	3.31	0.27	12.3	3.26	0.30	10.9
G	2.48	0.42	5.9	2.95	0.26	11.3
H	2.56	0.39	6.5	3.43	0.24	14.3
J	0.39	0.46	0.86	7.16	0.57	2.0
K	0.76	0.72	1.06	1.03	0.54	1.9
L	0.30	0.54	0.55	0.61	0.23	2.6
M	0.80	0.42	1.90	0.76	0.21	3.6
Cran-Grape	2.89	2.06	1.40	1.98	1.84	1.1

Conventional paper chromatography in previous research (Zoumas and Francis, 1965; Fuleki and Francis, 1967) was not capable of detecting delphinidin in the proportion indicated here. Current research with HPLC methodology should solve this problem. Regardless, the presence of traces of delphinidin puts the analyst in the position of having to judge the importance of the trace pigments.

The detection of aglycones, foreign to cranberries, is a simple way to detect replacement of 25% or more of the expected amount of cranberry juice.

Recovery of grape pigments

The ambiguity of the analyses for aglycones suggested that possibly the pigments themselves could be detected. This is chromatographically much more difficult since the number of pigments is much larger and the separations are more difficult. Also the amounts of anthocyanins in enocyanin are very small. The samples of enocyanin used in this work derived about 90% of their colorant potential from polymers. This was determined by measuring the absorbance of a sample of enocyanin prior to placing it on the CG-50 column and measuring the absorbance of the absorbed pigment. Since, 0.2% of colorant is ample to color a synthetic cranberry juice cocktail, and the delphinidin, malvidin group may be 25% of the anthocyanin total, the analysis reduces to about 1 part in 200. However, this should be possible if the sample size is large enough.

The replacement of 25% or more of the expected amount of cranberry juice by enocyanin could easily be detected by the presence of the extraneous bands such as delphinidin monoglucoside. Bands from the enocyanin contribution were detected in all the commercial samples suspected of colorant addition. Attempts to refine the sensitivity of the method by increasing the sample size were not very promising. Apparently the limiting factor is the ability to separate a minor pigment on paper. The logical approach to this problem is to apply a more sensitive separation method, namely HPLC, and this research will be reported in another project.

DISCUSSION

THE FOUR STAGE PROCEDURE is a relatively simple method of screening samples of cranberry juice cocktail for adulteration with enocyanin. This approach was found to be effective for commercial samples purchased in the market place which were judged, by the code numbers, to be less than 3 months of age. For old samples, it is likely that the relationships would be different. Similarly, cranberry juice cocktail prepared from old, and/or degraded, cranberry juice concentrate, or old enocyanin, would show a different profile. It is anticipated that these situations would be evident from the color data and the relative amounts of the more stable flavonoid pigments but this will require more development. A higher priority for research would be to improve the sensitivity of the pigment recovery steps by HPLC and this will be reported in

—Continued on page 1661

Persimmon Pectinmethylesterase: Extraction and Variation during Ripening

MARCEL AWAD

ABSTRACT

The optimum conditions for the extraction of pectinmethylesterase (PME) from persimmon fruit (*Diospyros kaki* cv. Taubatè) were found to be: 1.6M NaCl + 1% (v/v) Triton X-100 + 5% (w/v) bovine serum albumin (BSA) for astringent fruit and 0.2M NaCl for nonastringent fruit. During ripening with ethephon, PME showed a close correlation with respiration up to the climacteric peak when fruits became soft and nonastringent. Untreated astringent fruits showed little variation in either respiration or PME activity.

INTRODUCTION

ANY STUDY in enzyme variation during ripening should involve extensive testing of extracting conditions as a result of the wide differences in physical and chemical conditions existing between green and ripe fruit. The astringent persimmon is a particularly interesting fruit due to the presence of large amounts of tannins which are confined to tannin cells and released to the extracting medium during homogenization and are capable of interfering with enzyme extraction and activity. The astringent persimmon, when treated with ethephon, loses its astringency and changes from a hard to a soft consistency (Awad and Amenomori, 1972). To our knowledge, there are no reports on the presence or variation of cell wall degrading enzymes in persimmon fruit during ripening.

Preliminary tests failed to show cellulase or polygalacturonase (PG) activity in ripe persimmon fruit but the presence of PME (EC 3.1.1.11) was easily detected. The purpose of this research was to establish the best conditions for extracting this enzyme and to determine its variation during ripening.

MATERIALS & METHODS

Fruit treatments

Fruits selected for ripening were treated the day after harvest with 50 µL of ethephon placed on the skin around and close to the calyx lobes.

Fruit respiration

The day after harvest single astringent fruits (cv. Taubatè, ave wt 170g) from Santa Martha Orchards (Bonfim Paulista, SP, Brazil) were placed in 2L glass jars at 25°C and flushed continuously with air (100 mL/min). At selected intervals, the outflow from the jars was directed to a Beckman 864 Infrared Gas Analyser for the determination of CO_2 evolution. The data obtained were used to draw the respiration curve of each fruit.

Fruit sampling

To study the variation in PME during ripening, every day a small conical sample was taken with a sharp knife from the equatorial region of the fruit stored in the glass jars. Holes were immediately closed with lanolin (Awad and Young, 1980). Respiration and PME values of control fruit were not significantly different from the sampled fruit. At the beginning and at the end of the experiments, soluble solids of the flesh were determined with a hand refractometer (Zeiss). To avoid the variability existing between fruits, samples, used to test the effect of a series of concentrations of a given compound, were taken from a single fruit.

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Extraction and assay of PME

After removal of the skin, the fruit sample (just over 1g), was weighed and placed in 10 mL of the cold (5°C) extracting solution and homogenized with a Polytron-type homogenizer (Superohm, Piracicaba, SP, Brazil) for 15 sec. After a 10 min desorption period (5°C), the mixture was centrifuged at 3,500 × g for 5 min (5°C). The supernatant below the surface layer was used for the PME assay which was a modification of the procedure of Rouse and Atkins (1955).

Two milliliters of the supernatant were added to 15 mL of a 0.5% (w/v) solution of Pectin NF (purified polygalacturonic acid methyl ester, Sunkist Growers, Inc., Ontario, CA) in 0.1M NaCl. This pectin concentration insured maximum enzyme activity. The mixture was brought rapidly to pH 7.5 with 1 N NaOH and the release of carboxyl groups by the action of PME on the substrate was followed with an automatic digital titrator (Metrohm, Herisau, Switzerland) using 0.1N NaOH at 25°C. PME units were expressed as µeq ester hydrolyzed/min/g fresh wt. Enzyme activity remained stable from 2° to 25°C for at least 30 min. Less than 30 min elapsed between sampling and the end of the assay. Boiled enzyme showed no activity.

RESULTS & DISCUSSION

AMONG THE COMPOUNDS tested to determine their influence on the extraction and prevention of PME inactivation by polyphenols in astringent persimmons, the following showed little or no effect: diethyldithiocarbamate, Na-salt (DIECA, 1 mM); basic anion exchange resin (Dowex-1,1%); nonionic polymeric adsorbent (Amberlite XAD-4, 1%); cross-linked insoluble PVP (Polyclar AT, 1%); Na-metabisulfite ($Na_2S_2O_5$, 2 mM); and 2-[N-morpholino] ethane sulfonic acid (MES, pH 6). The compounds that showed a positive influence on PME extraction from astringent fruit were: polyethylene glycol (Carbowax 20,000, 1%); ethylenediaminetetraacetic acid, Na-salt (EDTA, 2 mM); $CaCl_2$ (0.01M); NaCl; octyl phenoxy polyethoxyethanol (Triton X-100); and bovine serum albumin (BSA). PME displayed extreme sensitivity towards the last three.

The effect of NaCl on PME extraction is presented in Table 1. Maximum yield of PME was achieved with 1.6M NaCl in the presence of Triton X-100 for astringent fruit and with 0.2M NaCl for nonastringent fruit. PME from hard astringent fruit was much more sensitive to salt concentration in the extracting solution than PME from soft nonastringent fruit. Table 2 shows

Table 1—Effect of NaCl concentration in the extracting solution on the activity of PME from persimmon fruit

NaCl conc[a]	PME activity[b]	
	Astringent fruit	Nonastringent fruit
0	0.58	11.99
0.2M	0.71	14.68
0.4M	1.83	13.36
0.8M	3.63	14.58
1.6M	5.87	13.80
3.2M	5.79	13.34
4.8M	3.75	—

[a] 5% (v/v) Triton X-100 was added to the extracting solution used with astringent fruit.

[b] Assayed with 0.5% (w/v) pectin solution in 0.1M NaCl. Units are µeq ester/min/g fresh wt. Average of 2–3 fruits.

Table 2—Effect of Triton X-100 concentration in the extracting solution on the activity of PME from persimmon fruit

Triton X-100 conc (%)[a]	PME activity[b]	
	Astringent fruit	Nonastringent fruit
0	0.32	14.32
0.1	0.42	13.72
1.0	5.28	14.48
5.0	5.19	13.48

[a] 1.6M NaCl was added to the extracting solution used with astringent fruit. 0.2M NaCl was added when extracting nonastringent fruit.
[b] Assayed with 0.5% (w/v) pectin solution in 0.1M NaCl. Units are μeq ester/min/g fresh wt. Average of 2 fruits.

Table 3—Effect of bovine serum albumin concentration in the extracting solution on the activity of PME from persimmon fruit

BSA conc (%)[a]	PME activity[b]	
	Astringent fruit	Nonastringent fruit
0	6.84	15.86
1	9.23	14.59
5	11.30	12.87

[a] 1.6M NaCl + 1% (v/v) Triton X-100 were added to the extracting solution used with astringent fruit. 0.2M NaCl was added when extracting nonastringent fruit.
[b] Assayed with 0.5% (w/v) pectin solution in 0.1M NaCl. Units are μeq ester/min/g fresh wt. Average of 2-3 fruits.

that for astringent fruit more than a tenfold increase in PME activity was obtained with Triton X-100 concentrations between 0.1 and 1% (v/v). These results showed the strong influence of this nonionic surfactant in the release of the enzyme from astringent fruit. PME is commonly associated with cell wall components and extraction responds well to salt and detergents. Tables 1 and 2 show also that neither Triton X-100 nor NaCl used alone would result in high yields of PME from astringent fruit and that neither high NaCl nor high Triton X-100 had an adverse effect on PME extraction from nonastringent fruit.

When tissue samples from astringent and nonastringent fruits were homogenized (0.2M NaCl) and analyzed separately, PME activity in astringent fruit was low (0.36 units) whereas the PME activity of nonastringent fruit was very high (14.20 units). Homogenizing together equal weights from the same astringent and nonastringent fruits resulted in a total loss of activity, showing a very potent inhibition of the extract of astringent fruit on PME activity.

When equal weights of astringent and nonastringent fruit were homogenized together in the presence of 1.6M NaCl + 5% (v/v) Triton X-100, enzyme activity was fully preserved (10.72 units) and almost equaled the sum of the activities of separate samples from the same astringent (3.10 units) and nonastringent fruit (18.15 units) divided by two (10.63). These results showed that NaCl and Triton X-100 not only helped in the release of PME but also protected the enzyme from the effect of tannins in the astringent fruit.

BSA had little effect on PME extraction from astringent fruit in the presence of 0.2M NaCl (data not shown). BSA had a strong, positive effect on PME extraction from astringent fruit in the presence of high NaCl and Triton X-100 and a negative influence on the enzyme from nonastringent fruit in the presence of low NaCl (Table 3). The best BSA concentration for astringent fruit was 5%. BSA effects result from its high capacity for phenol and quinone binding.

The strong, positive effects of NaCl, Triton X-100 and BSA used together on PME extraction and protection from tannins present in astringent persimmon fruits confirmed that extensive testing of various extracting procedures was important before studying enzyme variation during fruit ripening. The best conditions obtained in this study were probably only an approximation of the ideal conditions.

The variation in PME activity, in relation to respiration, during ripening with ethephon applied one day after harvest, is shown in Fig. 1. BSA was omitted from the extracting solution because its positive effect on PME from astringent fruit was partially cancelled by its negative effect on PME from nonastringent fruit. The respiration curve was typical of a climacteric fruit. PME activity followed very closely the respiration curve up to the climacteric peak which occurred 2–3 days after the ethephon treatment. At the climacteric peak, the astringency of the fruit was nil, the flesh was soft, and the color of the skin (orange red) and the flesh (dark yellow) was typical of nonastringent, ripe fruit. Ethephon treatments resulted in the simultaneous occurrence of softening and loss of astringency. After the climacteric peak, we observed either a decrease, a small increase or no variation in PME activity depending on the fruit under study. Loss of astringency may or may not be linked to aerobic respiration. In this study, rapid loss of astringency was obtained under aerobic conditions and high respiration rates. The soluble solids of the fruit changed very little during ripening (data not shown).

Untreated astringent fruit showed little variation is respiration and PME activity and remained green and hard (Fig. 2). The role of the apparent increase in PME during persimmon fruit softening and loss of astringency is not known. PME is responsible for the de-esterification of pectin before PG starts the depolymerization of pectins resulting in fruit softening (Jansen and MacDonnell, 1945). The presence of PG in persimmon fruit was not detected. An increase in soluble pectin has been observed during the ripening of astringent persimmon fruit by Kitagawa (1963). According to Brady (1976), an increase in pectin solubility may result from an increase in methyl de-esterification of the polygalacturonates or from a decrease in chain length. He also suggests that limited action by PME may promote large changes in polygalacturonate solubility. If

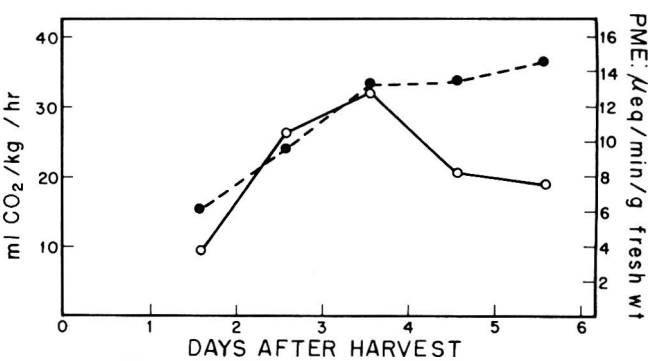

Fig. 1—Variation in PME (●- - -●) and CO_2 (○—○) in one persimmon fruit during astringency removal with ethephon applied one day after harvest. Extracted with 1.6M NaCl + 5% (v/v) Triton X-100. Assayed with 0.5% (w/v) pectin solution in 0.1M NaCl.

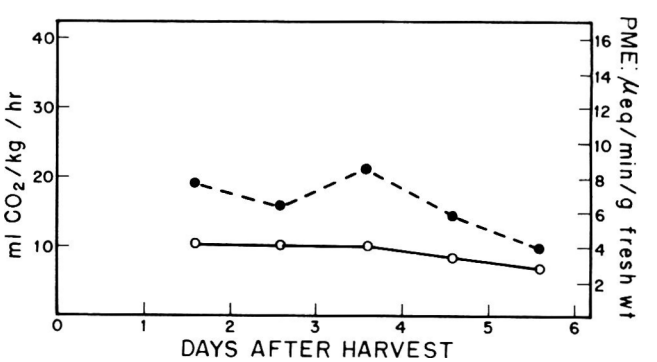

Fig. 2—Variation in PME (●- - -●) and CO_2 (○—○) in one untreated astringent persimmon fruit. Extracted with 1.6M NaCl + 5% (v/v) Triton X-100. Assayed with 0.5% (w/v) pectin solution in 0.1M NaCl.

this is true, the softening of the astringent persimmon fruit could be attributed to the increase in PME activity resulting from the ethephon treatment.

It has been suggested that the removal of astringency results from a combination of soluble tannin with soluble pectin rendering the tannin insoluble (Kitagawa, 1963). Gamma radiation increases water-soluble pectin and removes astringency from persimmon fruit (Kitagawa et al., 1962). Ethanol (Eaks, 1967) and acetaldehyde (Pesis and Ben-Arie, 1984), compounds of anaerobic respiration, remove the astringency of persimmon fruits. The addition of methanol induced a ripening response in persimmon fruit (Rakitin et al, 1957). Methanol, produced by the de-esterification of pectins by PME could possible contribute to the loss of astringency. Much research remains to be done to improve our understanding of the ripening of persimmon fruits.

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*FLOW PROPERTIES OF EGG POWDERS. . .*From page 1620

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*PHYSICAL STATE OF EMULSIONS. . .*From page 1623

of an emulsion with lower oil volume ratio ($\phi = 2/3$). This high value of oil volume ratio was very close to the maximum packing fraction of oil and so this emulsion might be a little unstable.

Amount of protein adsorbed to oil globule surface

With both LDL and BSA, the amounts of the protein adsorbed to the oil globule surface increased with protein concentration up to 5 mg protein/mL and remained unchanged with further increase of protein (Fig. 6). Maximum amounts of adsorbed protein, however, were very different between LDL and BSA, 4.8 mg/0.1g of oil for LDL and 2.5 mg/0.1g oil for BSA. The lipid binding ability of LDL was much greater than that of BSA. During the preparation of an emulsion, LDL seemed to rapidly bind to the surface of dispersed oil globules and form an emulsion of very small size with stability much greater than that of large size emulsion. LDL contained 86–89% lipid and was considered to be a large spherical molecule with a core of triglyceride and a surface layer of both phospholipids and protein (Powrie, 1977). The structure of LDL may correlate with its high lipid-binding property. Further studies should be done on the relationship between structure and function of LDL.

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Compositional Changes in Mango Fruit During Ripening at Different Storage Temperatures

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ABSTRACT

Chemical changes in Haden, Irwin, Kent, and Keitt mangos stored at 16–28°C and 85–90% RH were followed to determine the optimum storage and ripening conditions. Weight loss was slightly higher at 25° and 28°C than at 16–22°C. Breakdown in acidity during ripening was slower at 16°C. Vitamin C showed two basic trends; a general decrease as in Haden, Irwin, and Keitt or a steady increase as in Kent. Total and β-carotenoids were significantly higher at 22–28°C than at 16–20°C. No significant differences were observed with respect to carbohydrate and soluble solids content. However, sucrose increased spectacularly at all temperatures contributing most to the increase in sweetness. The pattern of chemical changes were strikingly similar in all the varieties. Temperatures of 20–22°C and 85–90% RH are recommended for storage and ripening of mangos to obtain sufficiently acceptable quality attributes.

INTRODUCTION

MANGO (*Mangifera indica* L.), is the most popular and the choicest commercial fruit produced in tropical regions. World production in 1982 was slightly more than 13.6 million metric tons with India as the largest producer with more than 8.5 million metric tons per annum. Mexico has an annual production around 663,000 metric tons and is among the top ten mango producing countries of the World (FAO, 1982).

Mangos grown in Mexico can be divided into three categories: (1) seedling mangos (monoembryonic); (2) manila mangos (polyembryonic); (3) cultivars (commercial varieties) from Florida, each corresponding to 35, 40 and 25%, respectively, of the total production. Mexicans generally prefer manila mango and therefore, all of it is consumed fresh within the country. The seedling mango, being confined to remote places of production, is consumed in the surrounding areas with 10–15% being absorbed by the processing industry. Florida varieties are preferred for export because of their attractive external color, uniformity in size, good quality and better resistance during handling and transport besides acceptance in foreign markets (Lakshminarayana, 1980).

Storage and ripening of mango are beset with a number of problems. Under tropical conditions fruits ripen within 6 to 7 days and become overripe and spoiled within 15 days after harvest. Since the beginning of the century low refrigeration temperatures have been employed to reduce the high perishability of the fresh fruit with little success. Mangos are highly susceptible to chilling injury when stored below 13°C. However, refrigerated storage of semiripe mangos of some selected varieties has met with some success. Even in such cases, the external and internal color, aroma and taste and flavor of the fruit have been found to be affected. These aspects have been recently reviewed by Lakshminarayana (1980). Therefore, storage and ripening of mangos continues to be a challenging problem and needs attention.

The objectives of this work were to study the effect of different storage temperatures on the chemical constituents and ripening characteristics of fruits of different varieties of mango grown in Mexico and to elucidate the optimum storage and ripening conditions by which successful storage and marketing of the fruit could be achieved.

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MATERIALS & METHODS

SPECIAL RIPENING CHAMBERS were designed to hold approximately 200 kg (500–600 fruits). Temperatures chosen were between 16–28°C and 85–90% relative humidity, uniformly, for all temperatures. The mango varieties studied were Haden, Irwin, Kent and Keitt. Haden and Irwin are early varieies; Kent and Keitt are late varieties. They have different genotypic characters and vary significantly with respect to form, size, external color, peel thickness and taste and flavor.

Five hundred fruits of each mango variety were harvested at physiological maturity (preclimacteric hard-green stage) from commercial orchards from two principle mango growing areas (Haden and Irwin from Cd. Valles and Kent and Keitt from Escuinapa). They were brought to the laboratory in Mexico City by road within 24 hr after harvest. On arrival, the fruits were classified for uniformity and culls removed. The rest were given a dip treatment in a cold solution of Benomyl at 250 ppm to reduce postharvest storage rots. Subsequently, the air-dried fruits were divided into six replicates of 75 fruits each, put in ventilated plastic bags and stored in different chambers with temperature and relative humidity as described above.

Six fruits picked at random, belonging to each one of the six replicates were marked to follow the daily physiological loss in weight. Six more fruits picked at random, one from each of the replicates, were divided into two sublots of three each, peeled, the pulp homogenated and used for chemical analysis using triplicate samples for each homogenate. Total acidity (% anhydrous citric acid), pH, total soluble solids (°Brix), starch (AOAC, 1980), true ascorbic acid (Robinson and Stotz, 1945), total and β-carotenoids (Anon., 1966) and sugars (Ting, 1956) were analysed daily or at shorter intervals depending on the variety and storage conditions. The values shown in Tables are averages of six estimations expressed on a fresh weight basis. Objective sensory evaluation tests were not conducted; however, the remarks made on the sensory quality are authors' opinion.

RESULTS & DISCUSSION

Physiological loss in weight

The varietal responses to storage temperatures, both after 2 and 10 days in storage, indicated no significant differences between 16–20°C. However, the weight loss noted in fruits at 25° and 28°C was higher than at 16°–22°C (Table 1)

Acidity and pH

Table 2 shows variations in acidity and pH values of mangos at ripe stage. The reduction in acidity and increase in pH was generally slower in fruits stored at 16°C as compared to higher temperatures, particularly at 25° and 28°C. There were no significant differences amongst varieties with regard to the general pattern of changes. A concomitant relationship between the acid content and pH values was noticeable to some extent at temperatures above 22°C in all varieties. However, there seemed to be no clear correlation between changes in acidity and pH values.

Table 1—Effect of storage temperature on the pattern of weight loss (%) in mangos during ripening

Variety	After 2 days in storage at (°C)							After 10 days in storage at (°C)						
	16	18	20	22	25	28	(S.E ±)	16	18	20	22	25	28	(S.E ±)
Haden	0.80	0.65	0.68	0.72	0.95	1.10	0.07	4.3	3.5	4.2	4.5	4.7	6.7	0.44
Irwin	0.85	0.55	0.55	0.80	0.85	1.20	0.10	3.8	3.3	3.5	3.7	4.3	5.9	0.39
Kent	0.72	0.55	0.60	0.62	0.75	0.80	0.04	2.5	2.0	2.3	3.6	4.5	5.2	0.53
Keitt	0.90	0.72	0.85	0.90	1.10	1.30	0.08	2.8	2.2	2.0	3.3	5.2	6.3	0.71

Table 2—Titratable acidity (% citric acid) and pH at ripe stage in mangos at different storage temperatures.

Variety	Acidity (Storage temp °C)							pH (Storage temp °C)						
	16	18	20	22	25	28	(S.E ±)	16	18	20	22	25	28	(S.E ±)
Haden	0.42	0.08	0.08	0.06	0.08	0.04	0.06	4.0	5.0	5.2	5.6	4.5	5.2	0.23
Irwin	0.21	0.18	0.20	0.07	0.10	0.05	0.04	4.3	4.6	4.4	5.8	4.5	5.2	0.24
Kent	0.18	0.12	0.20	0.18	0.23	0.12	0.02	4.5	5.0	4.7	4.7	5.0	5.2	0.11
Keitt	0.10	0.02	0.05	0.05	0.08	0.04	0.01	5.0	5.4	5.0	6.5	6.1	6.2	0.27

Ascorbic acid

Relative concentrations of ascorbic acid in all four varieties at ripe stage (Table 3) indicated a general loss during ripening at all temperatures varying from 16–23% in Haden, Irwin and Keitt, whereas, Kent showed twice as much quantity compared to the 3rd day's concentration, except at 28°C where it was at unity. Thus, the effect of storage temperatures on ascorbic acid changes was less prominent. It is well known that most fruits show reductions in vitamin C during postharvest ripening (Biale, 1960; Ramasarma and Banerjee, 1940). In banana, vitamin C increased slightly just after the respiration climacteric and then fell gradually. A 10% reduction has been reported in citrus fruits after harvest under reasonable conditions of handling and storage. In guava and mango, a general decrease during ripening has been observed (Hulme, 1971). The data on mango Haden, Irwin and Keitt are in conformity with the above findings. However, the data on Kent suggest a possible synthesis of vitamin C during ripening. This needs confirmation.

Carotenoids

The storage temperatures had a spectacular effect on the carotenoid composition of mango fruit. Both total and β-carotenoids increased with increase in storage temperature, higher quantities being noted in all varieties stored at temperatures from 22–28°C at the ripe stage than at 16–20°C (Table 4). In Kent and Keitt however, both carotene fractions (data not shown), after reaching maximum levels at ripe stage decreased slightly unlike Haden and Irwin where they remained steady. Modi and Reddy (1967) reported the participation of geraniol as an intermediate in carotenogenesis in mango. Later, Mattoo et al. (1968) observed an increase in the concentrations of mevalonic acid and geraniol before the onset of respiratory climacteric, reducing later, during the climacteric maximum. They also reported dephosphorylation of the intermediates during carotenogenesis by phosphatase enzyme. They did not however, relate these reactions with temperature. The phosphatase might have temperature optima above 20°C which to some extent might explain the low carotene formation observed in this study in fruits stored below 20°C. Therefore, it may be necessary to store the fruit above 20°C to obtain better pulp color (Lakshminarayana and Vázquez-Salinas, 1978; Thomas, 1975; Thomas and Janave, 1975).

Table 3—Ascorbic acid (mg/100g) of mangos at ripe stage at different temperatures relative to the concentration noted on the 3rd day after storage (%)

Variety	Storage temp (°C)						
	16	18	20	22	25	28	(S.E ±)
Haden	89.3	100.0	86.2	82.6	77.8	69.0	4.3
Irwin	86.1	148.3	87.2	109.4	65.8	75.0	12.2
Kent	242.8	253.3	278.6	176.2	194.7	100.0	26.5
Keitt	68.7	96.1	67.8	69.6	84.7	76.5	4.6

Carbohydrates and soluble solids

Table 5 shows the concentrations of sugars, soluble solids and starch in mangos at ripe stage. Glucose was always lower than fructose during the whole period of ripening. Sucrose, on the other hand, was significantly higher in all varieties at all temperatures registering values corresponding to more than 75% of total sugars, thus, contributing most for the increase in sweetness in mangos. No significant variations were noticeable with regard to total sugars, soluble solids and starch content. Thus, the pattern of changes with regard to carbohydrates and soluble solids in mangos was not seriously affected when fruit of different varieties were ripened at 16–28°C.

A significant amount of information is available on the changes in sugars in mangos during ripening. A simultaneous increase of glucose, fructose and sucrose has been reported by many workers (Krishnamurthy et al., 1971; Krishnamurthy and Subramanyam, 1970; Lakshminarayana, 1973, 1975; Lakshminarayana et al., 1970; Shashirekha and Patwardhan, 1976; Soule and Harding, 1956; Wahab and Khan, 1954) except for a stray case of disappearance of sucrose in Alphonso mango (Leley et al., 1943). Our results with Florida mango cultivars from Mexico indicate a gradual reduction in both glucose and fructose and a continuous increase of sucrose during ripening.

The mechanism of sucrose biosynthesis in mango is not fully elucidated. The increase of sucrose coincided with the disappearance of starch suggesting sucrose synthesis and its progressive accumulation was directly related to the hydrolyzed

Table 4—Maximum levels of carotenoids (μg/100g) produced by mangos during ripening at different storage temperatures

Variety	Total carotenoids (Storage temp°C)							β-carotenoids (Storage temp°C)						
	16	18	20	22	25	28	(S.E ±)	16	18	20	22	25	28	(S.E ±)
Haden	1725	3802	2622	6308	6187	6819	878.8	587	1112	582	3487	3123	3850	621.8
Irwin	1750	1827	1865	2603	2699	3231	246.8	1025	604	622	1087	1012	1250	107.1
Kent	3647	3850	4212	4797	4805	5457	278.2	1906	2675	2650	3195	3027	3090	193.0
Keitt	2595	3105	2618	5105	3895	5187	481.4	1087	1305	1105	2067	2055	2758	274.5

Table 5—Concentration of sugars (%), total soluble solids (°B) and starch (%) in mangos at ripe stage at different storage temperatures

	Storage temp (°C)																				
Variety	16	18	20	22	25	28	(S.E ±)	16	18	20	22	25	28	(S.E ±)	16	18	20	22	25	28	(S.E ±)
	Glucose							**Fructose**							**Sucrose**						
Haden	1.3	1.0	0.9	0.9	1.3	1.2	0.1	3.2	2.8	2.9	2.2	2.6	3.2	0.1	8.6	12.0	12.2	13.3	9.7	9.6	0.8
Irwin	1.7	1.4	1.0	1.0	1.0	0.9	0.1	4.3	5.2	4.6	4.3	3.6	4.5	0.2	7.7	8.2	9.4	11.4	9.3	11.5	0.6
Kent	1.4	0.9	1.0	1.3	1.3	1.3	0.1	4.8	3.8	4.2	4.5	3.4	3.5	0.2	12.8	15.9	15.5	15.0	12.3	12.4	0.7
Keitt	0.9	0.5	0.5	0.6	0.2	0.4	0.1	4.5	3.7	4.9	4.0	3.8	4.2	0.2	13.4	13.5	13.2	13.3	14.0	15.1	0.3
	Total sugars							**Total soluble solids**							**Starch**						
Haden	11.8	14.3	14.5	14.8	12.3	12.6	0.5	14.2	15.7	14.9	15.1	15.0	15.6	0.2	0.4	0.3	0.3	0.2	0.2	0.1	0.0
Irwin	12.4	13.4	13.5	15.0	12.6	15.2	0.2	14.0	14.6	16.1	17.3	17.4	18.3	0.7	0.3	0.3	0.1	0.2	0.2	0.2	0.0
Kent	17.1	18.6	18.6	18.7	15.3	15.4	0.4	20.6	21.3	20.0	20.1	21.1	21.7	0.3	0.4	0.4	0.2	0.1	0.1	0.1	0.1
Keitt	16.8	15.9	16.8	16.2	16.3	17.7	0.1	18.7	18.1	17.8	19.6	18.2	19.9	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.0

by-products of starch such as glucose. Terra et al. (1983) demonstrated starch to sucrose transformation via glucose-1-phosphate-UDP-glucose in bananas. It will be of interest to study whether a similar pattern exists in mango.

From the foregoing data on four mango varieties, it is clear that storage temperatures (16–28°C) had little influence on the overall compositional changes except on total and β-carotenoid content. Fruits ripened between 16–20°C showed slightly higher acidity and starch together with slightly lower pH, sucrose and a subdued taste and flavor and aroma, generally considered as effects of chilling. At temperatures between 22–28°C the reactions controlling these changes proceeded at a faster rate with the result fruits were sweeter with bright yellow pulp color and strong aroma. The moderate slow rate of ripening achieved at 16–22°C enhances the storage life to some extent which is beneficial to retailers, provided the moderate fruit quality is accepted by the consumer. Temperatures above 25°C are not desirable as the accelerated rate of ripening increase weight loss and decrease storage life, although, it may be ideal for obtaining excellent fruit quality. We recommend a temperature of 20–22°C and 85–90% relative humidity for storage and ripening where mangos obtain sufficiently acceptable quality attributes. In super markets it is a common practice to employ refrigeration for all fruits including mango. It may be necessary to build special facilities for mango to maintain temperatures around 20–22°C so that the consumer is assured of optimum fruit quality.

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Headspace Sulfur Dioxide and the Storage of Dried Apples

H. R. BOLIN, R. J. STEELE, and E. G. DAVIS

ABSTRACT

A gas chromatographic technique was used to determine sulfur dioxide and carbon dioxide in the headspace of packaged apples during storage. The headspace sulfur dioxide so determined decreased more rapidly and correlated better with nonenzymatic browning of the apples than free or total sulfur dioxide. The decrease in headspace sulfur dioxide, which was not accompanied by a comparable decrease in free sulfur dioxide, was attributed to the binding of sulfite to organic components of the apple. Free sulfur dioxide was found to measure not just the inorganic sulfite, which is commonly assumed, but also bound sulfite which was rapidly released under the acid conditions. Neither free nor total sulfur dioxide in a product could be quantitatively determined from headspace gas chromatographic techniques.

INTRODUCTION

SULFUR DIOXIDE is extensively used in the processing of dried fruits to retard enzymatic and nonenzymatic browning. Nonenzymatic browning, which occurs during storage, results from a number of chemical reactions, mainly between available amino groups, reducing sugars and/or organic acids, culminating in the formation of brown pigmented compounds. Sulfur dioxide retards the development of browning by blocking the reaction sequences that form the Maillard type compounds (McWeeny et al., 1974) as well as acting in a "bleaching" capacity by reducing the coloration of the products formed (McWeeny, 1981). The effectiveness of sulfur dioxide depends on its degree of dissociation in the food and on the presence of compounds that bind the preservative.

Wet chemical methods have been widely used to determine free and total sulfur dioxide during storage. The methods used however are of limited use because they are not specific to a single species of sulfite and they also severely disrupt the equilibria between the forms of sulfite. Free sulfur dioxide, which is defined as the amount of sulfite that can be immediately titrated when a sample is treated with acid, is assumed to be the inorganic sulfite available. Total sulfur dioxide is commonly defined as the amount of sulfite that can be immediately titrated after treating a sample with base and then acid. The bisulfite adduct of glucose, glucose sodium sulfonate, for example, has a low free sulfur dioxide level but has a total sulfur dioxide level of about 22% because the sulfite is released only under the alkaline conditions used to determine total sulfur dioxide. Legislaton on the maximum permitted levels of sulfur dioxide in foods is written in terms of total sulfur dioxide. Iodine titrations have been commonly used by industry for these determinations (AOAC, 1980).

Recently, Barnett and Davis (1983) described a gas chromatographic (GC) method to determine the concentration of sulfur dioxide in the headspace over liquids. The concentration of sulfur dioxide above a solution was found to be directly related to the concentration of molecular sulfur dioxide ($SO_2 \cdot H_2O$) in accordance with Henry's law. The molecular sulfur dioxide was directly related to free sulfur dioxide as measured by conventional techniques. The GC technique did not disturb the equilibrium of the system when gas samples were taken from flexible pouches. Conversely, free and total sulfur dioxide measurements do disturb the sulfite equilibria and require part of the sample to be destroyed. The GC technique, which is nondestructive, rapid and can also be used to determine carbon dioxide as well as sulfur dioxide, should be useful for testing sulfured dried fruit.

Furthermore the use of headspace sulfur dioxide can be thought of as being analogous to the use of an equilibrium relative humidity or water activity as an indicator of reaction potential and microbial stability. Its use in studies of nonenzymatic browning could provide a better understanding of sulfite/chromophore interactions than conventional free and total sulfur dioxide determination. Because legislation on permitted levels of sulfur dioxide in foods is based on measurements of total sulfur dioxide in the particular product, it was necessary to relate the headspace GC method to conventional methods.

This study was undertaken to examine the relationships between headspace, free and total sulfur dioxide as well as carbon dioxide evolution and chromophore development during storage of dried apples.

MATERIALS & METHODS

FRESH GRANNY SMITH APPLES were obtained from a wholesale market, peeled, cored and sliced into 10 mm thick rings of 60 mm maximum diameter. The pH of the apples was varied by soaking the rings for 30 min in juices which had been extracted from the same batch of apples and modified as follows: the natural pH of the apples, 3.4; a low pH. 2.9; and a high pH 3.9. Citric acid was added to the juice to achieve the lower pH and potassium hydroxide was added for the higher pH. About 350 mg L^{-1} of potassium metabisulfite were added to all the juices to prevent any enzymatic or microbial deterioration during the treatment.

The rings were dried in a hot-air dehydrator at 65°C for about 6 hr to lower the water activity (a_w) of the apple to 0.5. The dried rings were quartered, thoroughly mixed and 50g portions placed into flexible pouches. Dried apples of a_w 0.71 and varying SO_2 levels were produced by adding predetermined amounts of water containing varying amounts of sulfur dioxide to the pouches just before sealing in air. Two types of pouches were used. In the regular SO_2 study the pouches were made from a polyethylene metallized laminate of polyester, because this material has low permeability to sulfur dioxide, oxygen, and water vapor. For the CO_2 evolution study a laminate of polyvidylene chloride-coated Mylar, polyethylene, aluminum foil and polyethylene were chosen because this material has a low permeability to CO_2. The samples were stored at 36°C and removed periodically for analysis.

Headspace sulfur dioxide was determined at 25°C, according to the procedure of Barnett and Davis (1983). Headspace carbon dioxide was also determined in a series of experiments from the GC peak which occurred during the headspace analysis. Total sulfur dioxide was determined by iodometric titration (Ponting and Johnson, 1945). Free sulfur dioxide was determined by omitting the addition of alkali in the above methods and titrating with the iodine solution after the addition of 3 mL of 1M HCl to 50 mL of the filtrate.

Chromophore development was followed visually and by measuring the sample reflectance with a Hunter Color Difference meter (model D25D2M). Readings were made through a glass plate pressed onto a 40g sample contained in a 60 mm diameter white plastic dish 10 mm deep. A reading of 60 or less on the L scale corresponded to unacceptably brown apples. Water activity was determined with a Vaisala RH & T Indicator (model HMI 14) calibrated at the high range with saturated sodium chloride and at the low range with saturated lithium

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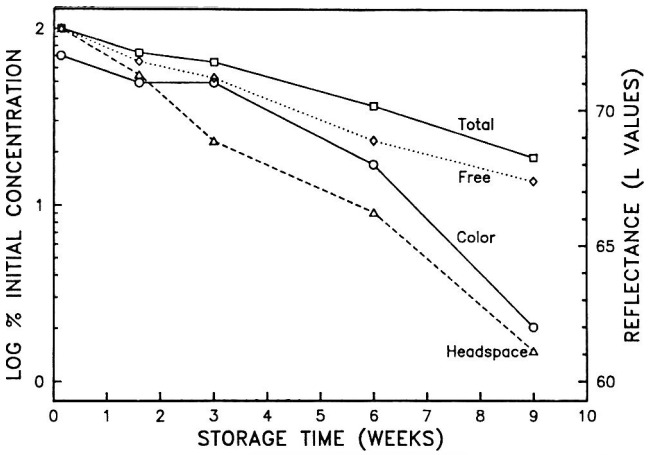


Fig. 1—Loss of the various forms of sulfur dioxide and darkening of pH 3.5 medium sulfured dried apples during storage.

Table 1—Correlation coefficients for darkening vs the logarithm of the sulfur dioxide concentration expressed as a percentage of the initial as determined by headspace and wet chemical analyses (n = 5)

	Sulfur dioxide			
	Titrametric			Treatment
pH	Total	Free	Headspace	level
2.88	0.9953	0.9760	0.9993	Low
3.49	0.9851	0.9535	0.9680	"
3.96	----	----	----	"
2.76	0.9736	0.9660	0.9835	Medium
3.45	0.9745	0.9360	0.9793	"
3.89	0.9686	0.9189	0.9711	"
2.75	0.9329	0.9550	0.9907	High
3.32	0.9051	0.9209	0.9396	"
3.87	0.9060	0.9014	0.9565	"

Table 2—Retention of sulfur dioxide in stored apples when darkened to L = 60

	Initial concentration			Amount retained		
	Headspace	Total	Free	Headspace	Total	Free
pH	(mg/L)	(mg/g)	(mg/g)	%	%	%
2.75	7.5	2.72	0.84	8	33	15
2.76	6.3	1.74	0.59	3	26	12
2.88	2.3	0.86	0.24	3	21	12
3.32	4.3	2.59	1.12	2	23	13
3.45	1.3	1.31	0.59	2	19	13
3.49	0.5	0.69	0.39	2	14	14
3.87	0.4	2.16	1.06	>1	20	6
3.89	0.1	0.81	0.45	>1	11	7*
3.96	0.1	0.42	0.22	>1	12*	15*

*Extrapolated to L = 60.

chloride. The pH was determined with a Ross electrode (Orion model 81-55SC) on a homogenized sample of dried apple and water in the ratio of one part apple to three parts water by mass.

RESULTS & DISCUSSION

SULFURING THE APPLES caused a small drop in pH which was proportional to the amount of sulfur dioxide absorbed. At pH 2.65, 3.44, and 3.98 the pH drop per 1000 ppm SO_2 was 0.03, 0.09, and 0.12 units, respectively, in the range 0.01-0.30 mg SO_2 per gram dried apple.

Headspace sulfur dioxide and shelf-life

Typical changes that occurred in the concentration of headspace, free and total sulfur dioxide during storage are shown in Fig. 1. The levels of total, free and headspace sulfur dioxide are expressed as a percentage of the original concentration on a logarithmic scale versus the storage time in weeks. Headspace sulfur dioxide decreased more rapidly than free sulfur

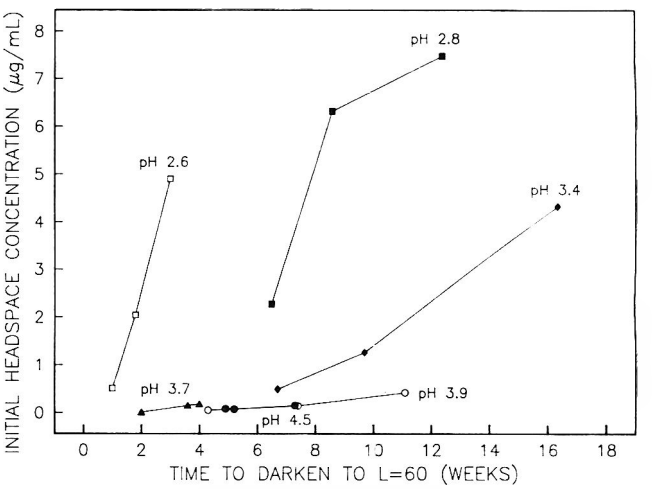


Fig. 2—Influence of apple pH and initial headspace sulfur dioxide on the rate of darkening.

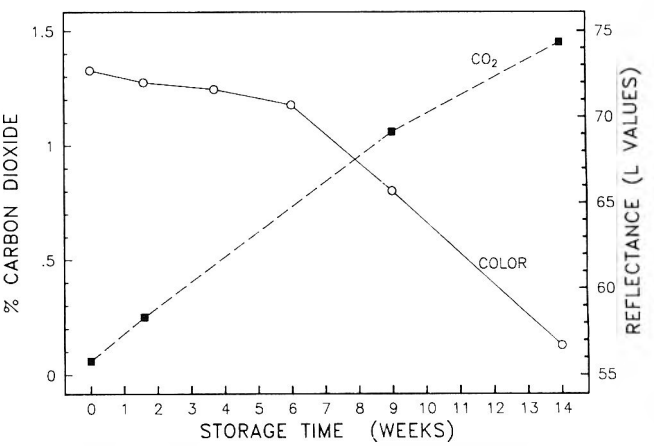


Fig. 3—Carbon dioxide evolution and darkening during storage of pH 2.8 high sulfured dried apples.

Table 3—Effect of low and high sulfuring levels of dried apples on carbon dioxide production during 9 wk storage at 36°C

pH	Original total sulfur dioxide (mg/g)	Carbon dioxide (%)
2.8	0.86	1.4
	2.72	1.0
3.4	0.69	1.5
	2.59	1.2
3.9	0.42	1.2
	2.16	0.6

dioxide, which decreased more rapidly than total sulfur dioxide.

Since the headspace concentration was the first to fall to low levels, often to less than 1% of the original, the GC technique could be used as a warning that the free and total sulfur dioxide values was also low and that nonenzymatic browning might occur if measures were not taken to raise the levels of sulfur dioxide.

The degree of browning (Fig. 1) correlated better with headspace sulfur dioxide concentration than either the free or the total sulfur dioxide. The correlation coefficients for L value vs the logarithm of the sulfur dioxide concentration for total, free or headspace techniques (Table 1) are, with one exception, higher for the headspace analysis than for the titrametric analysis of total or free sulfur dioxide. The GC technique was a better indicator of the extent of browning, especially at lower

Table 4—Effect of pH and storage time on the ratio of headspace to free sulfur dioxide (10^{-3}) in dried apples

pH	Theoretical[a]	Storage time (days) 1	11	21	42	63	84
2.75	3.75	8.91	10.93	11.23	8.86	6.38	2.75
2.76	3.67	10.71	8.41	8.74	6.17	2.33	1.67
2.88	2.85	9.46	5.31	3.13	1.40	----	----
3.32	1.09	3.87	2.61	2.17	2.17	1.98	1.21
3.45	0.81	2.14	1.75	1.32	0.73	0.17	----
3.49	0.74	1.26	0.77	0.46	0.17	----	----
3.87	0.31	0.40	0.43	0.38	0.28	0.08	----
3.89	0.30	0.31	0.23	0.17	----	----	----
3.96	0.26	0.23	0.08	----	----	----	----

[a] Based on the results in liquid product by Davis et al. (1983)

pH, than either of the other methods now commonly used in the industry. Surprisingly, free sulfur dioxide concentration had the lowest correlation with apple darkening.

Except for apples with pH above 3.8, the higher the levels of sulfur dioxide that were added to the apples the higher were the percentages of total sulfur dioxide remaining in the apples when they had darkened to L = 60. This pH interaction illustrates a limitation to using total sulfur dioxide as an indicator of the extent of darkening. Between 11-33% of the original total sulfur dioxide level remained when the apples had darkened to L = 60, which is consistent with the findings of earlier workers (Stadtman et al., 1946) who found that apricots retained about 35% of their original sulfur dioxide by the time they had darkened objectionably. Conversely, headspace sulfur dioxide in the darkened apples was always below 9% and usually below 3% of the original concentration (Table 2).

The shelf life, the time required for the reflectance of the apples (L value) to fall below 60, depended on the initial concentration of headspace sulfur dioxide (measured 24 hr after the pouches were sealed) and the pH of apples (Fig. 2). For all concentrations of headspace sulfur dioxide studied, the shelf life increased as the pH increased.

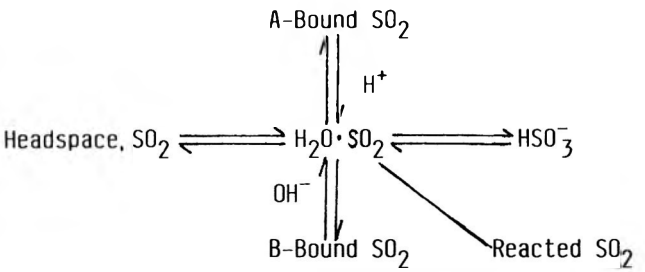
Headspace carbon dioxide and darkening

Carbon dioxide concentration in the headspace increased during storage (Fig. 3). As expected, the carbon dioxide concentration increased more rapidly in the low sulfured apples, because of their greater proclivity for darkening (Table 3). Davis et al. (1973), showed that carbon dioxide in foil containers increased during storage and was an indicator of deterioration of dried fruits. Our results agree with the results of their study and show the wide applicability of the GC method in studying nonenzymatic browning. Furthermore, where the reactions are sensitive to light, gas samples can be withdrawn from the headspace of a light-proof flexible container.

Headspace sulfur dioxide and free sulfur dioxide

Davis et al. (1983) found that free sulfur dioxide could be reliably estimated from the concentration of headspace sulfur dioxide and pH in dilute aqueous systems because the ratio of headspace to free sulfur dioxide was constant. In our study free sulfur dioxide could not be estimated from headspace sulfur dioxide because the ratio decreased during storage (Table 4). The possibility that Henry's law might not apply at the higher levels used in our study was investigated. The free sulfur dioxide levels based on the headspace concentration above citrate/phosphate buffers were estimated to within 7% of the levels found by titration up to 4 $mg.L^{-1}$, thus indicating applicability of Henry's law even at these higher levels.

The rapid fall in the headspace concentration during storage provided an insight into the mechanisms of sulfite loss in dried fruits and might explain, at least in part, why such a high concentration of total and free sulfur dioxide remained in the fruit when browning occurred. The ratio of headspace to free sulfur dioxide decreased because sulfur dioxide itself was progressively bound to compounds during storage. Some of the sulfur dioxide was bound irreversibly, being no longer measured by the usual analytical procedures, while some was bound reversibly. Some of the bound sulfite could be freed with acid and some with base as shown in the following scheme. Where



'A-Bound SO_2' was acid-reversible and was measured in the analyses of free and total sulfur dioxide, 'B-Bound SO_2' was base-reversible and was measured only by the analysis of total sulfur dioxide, and 'Reacted SO_2' was irrevesibly bound or reacted and could not be measured by any of the techniques used in this study. These results seemed to indicate that the free sulfur dioxide analysis not only measured inorganic sulfite, as commonly assumed, but it also measured some bound sulfite that was rapidly released with dilute acid. Amino acids are an example of compounds that could bind sulfur dioxide and cause a decrease in headspace concentration, but this portion may still be measured as available sulfur dioxide in the conventional analysis. Adams and Lipscomb (1949) found that the binding of sulfur dioxide to amine groups was rapidly reversed by the addition of acid. Since bound SO_2 is the difference between free and total sulfur dioxide, the poor correlation between free SO_2 and L value would indicate that bound sulfite would not have any retarding effect on nonenzymatic browning. This would be expected since only free sulfur dioxide is considered reactive enough to preferentially react with the "Maillard" intermediate compounds, such as 3-deoxy hexosulose, to retard the production of the pigmented products.

The ratio of headspace to free sulfur dioxide decreased as the pH of the apples increased in a similar manner to that found by Davis et al. (1983), except that ratio at the low pH in our study was about three times larger than the ratios expected for aqueous systems (Table 4). Henry's law and the equilibrium constants are valid only for dilute aqueous systems. Dried apple at a water activity of 0.71 does not have the same SO_2 equilibrium properties as a dilute aqueous system. Nevertheless 'pseudo' Henry's law and equilibrium constants, which could have been used to empirically estimate free sulfur dioxide, could have been calculated from our data if the ratio of free to headspace sulfur dioxide had not decreased during storage. Estimating total sulfur dioxide for legislative compliance was similarly not possible.

The GC technique was used successfully to monitor the sulfur dioxide and carbon dioxide levels in dried apples. While neither total nor free sulfur dioxide in apples could be quantitatively determined from the headspace results, the extent of nonenzymatic browning was more closely correlated to the

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Immobilized Condensed Tannins and Their Interaction with Proteins

HOON IL OH, JOHAN E. HOFF, and LAWRENCE A. HAFF

ABSTRACT

Condensed tannins from grapes immobilized on Sepharose 4B were found to have protein binding properties similar to tannins in free solution. Proteins bound to the gel at pH values below their individual isoelectric points and were eluted when the pH was increased. A good correlation was obtained between the isoelectric point and the elution pH for the following enzymes and proteins: alkaline phosphatase, ovalbumin, β-lactoglobulin, bovine serum albumin, α-amylase, γ-globulin, and cytochrome c. Trypsin represented an exception to the rule. α-Amylase retained enzymatic activity after elution from the gel.

INTRODUCTION

VEGETABLE TANNINS are widely distributed in higher plants and occur at significant levels in plant tissue utilized as human food or animal feed. They are polyphenolic substances of molecular weights in the range 500–3000 and have a characteristic ability to precipitate proteins (Gustavson, 1956). Most vegetable tannins in food belong to the class of condensed tannins or procyanidins which are structures formed by polymerization of flavan-3-ols or flavan-3,4-diols (Haslam, 1977).

Interaction between tannins and proteins in free solutions results in complexes which under suitable circumstances aggregate and form characteristic precipitates (Mejbaum-Katzenellenbogen, 1959; Calderon et al., 1968; Hagerman and Butler, 1978; Oh, 1978). The formation of hazes in beverages such as beer, wine, and fruit juices is believed to be due largely to the interaction of tannins with proteins. Tannins also manifest themselves in the oral cavity by formation of precipitates giving rise to the mouth sensation of astringency (Bate-Smith, 1954).

Condensed tannins are believed to remain unabsorbed by the digestive tract and to be wasted in the fecal matter. In passing through the digestive tract, however, their presence is probably associated with the formation of precipitates by interaction with food proteins and endogenous proteinaceous substances (enzymes, mucosal proteins). It is well known that tannins in the diet cause low growth rates and low protein utilization in rats and chickens (Maxson et al., 1973; Featherston and Rogler, 1975). The detrimental effects of tannins are believed to be brought about either they their inhibition of digestive enzymes (Tamir and Alumot, 1969, Davis and Hoseney, 1979), lower digestibility of food proteins when complexed with tannins (Feeny, 1969; Tamir and Alumot, 1970; Romero and Ryan, 1978), or by their adverse effect on the digestive tract (Vohra et al., 1966; Mitjavila et al., 1977).

These aspects of tannin-protein interactions requires that one studies the effect of pH and solute concentration as well as characteristics of individual proteins. The study of equilibria of initially homogeneous components in a system which becomes heterogeneous as a result of tannin-protein interaction is difficult analytically when the presence of one component (tannin) influences the analysis of the other (protein). However, the development of immobilized tannin gels (Armstong, 1976) has made it possible to study systematically the various factors that may influence the interaction phenomena mentioned earlier. When the immobilized tannin gel is operated in a column mode, it constitutes a convenient way of investigating association and dissociation phenomena of tannin-protein complexes, since the ligand can be physically separated from the adsorbent once dissociation has taken place.

The purpose of the present study is to examine the effect of pH on the association of various digestive enzymes and proteins with grape tannins (condensed tannins) and on the dissociation of the complexes formed in a system where one of the interacting component (tannin) is immobilized on Sepharose 4B.

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MATERIALS & METHODS

Materials

Source of grape tannins and their partial characterization were reported earlier (Oh and Hoff, 1979). Sepharose 4B was purchased from Pharmacia Fine Chemicals. Proteins, enzymes and their sources were as follows: bovine serum albumin (Fraction V), ovalbumin, γ-globulin, trypsin from hog pancreas (cryst.), α-amylase from porcine pancreas (2X cryst.), alkaline phosphatase from porcine intestinal mucosa, were all obtained from Sigma Chemical Co. β-Lactoglobulin and cytochrome c from horse heart were purchased from ICN Nutritional Biochemicals.

Preparation of immobilized tannins

Grape tannins were immobilized on Sepharose 4B (Pharmacia Fine Chemicals) via epoxy activation according to the procedure of Armstrong (1976). Washed and suction dried Sepharose (10g) was mixed with 10 mL 1,4-butane diglycidyl ether and 10 mL 0.6N sodium hydroxide containing 20 mg sodium borohydride. The reaction was terminated by washing the gel with large volumes of water (5L). Ten grams suction dried epoxy Sepharose and 250 mg grape tannins were mixed with 25 mL 0.5M carbonate buffer, pH 9.5 after deaeration, and reacted for 24 hr under a nitrogen blanket. The product was washed with water (500 mL) and subsequently treated with 25 mL 0.1N sodium hydroxide for 8 hr, washed with water again and finally four times with a sequence of 0.1M acetate buffer, pH 4 (500 mL), distilled water (250 mL) and 0.1M carbonate buffer, pH 9.5 (500 mL). The activated gel was finally suspended in 25 mL 0.1M universal buffer, pH 4 and stored at 5°C.

Chromatography

The tannin gel (3 mL) was packed into a Pharmacia K-9/15 column. Protein (5-10 mg, 1 mg/mL) was added at pH 4.0 in a 0.1M modified universal buffer (Johnson and Lindsey, 1939) consisting of citric acid, sodium monophosphate, Tris and sodium bicarbonate. After addition of the protein, the column was washed with 5–10 mL of the buffer and the protein eluted in a pH gradient produced by mixing the buffer with 0.1N NaOH in a gradient maker. The flow rate was maintained at 30 mL/hr. Three-mL fractions were collected, the pH measured and absorbance read at 280 nm. All operations were conducted at room temperature.

RESULTS & DISCUSSION

IT IS WELL KNOWN that tannins form complexes with proteins under suitable conditions in free solution. When tannin immobilized on Sepharose 4B were used in a column mode all the proteins investigated were quantitively bound to the column at pH 4.0 as evidenced by no further release of protein after washing the column.

Elution profiles of representative proteins obtained by separate experiments are shown in Fig. 1. When the pH of the eluting solvent was increased, the proteins were generally released from the gel at or near the isoelectric point of the individual proteins (Fig. 1, Table 1). Recovery of proteins varied from 55% for α-amylase to 100% for trypsin. The calculation of the protein recovery included only those fractions eluted below pH 11.0. In contrast to the results obtained using continuous pH gradient elution, bovine serum albumin was almost completely recovered from the tannin gel when a pH step gradient was used from pH 4.0 to 6.0.

Ovalbumin produced two peaks; the major peak eluted near the isoelectric point of the protein, and the minor peak at pH 8.3.

The major fraction of ovalbumin gave the sharpest peak (0.5 pH units), while trypsin had the largest peak width (3.0 pH units). No relationship between the peak widths and recovery of protein was indicated.

Binding and elution of α-amylase are shown in Fig. 2. A small amount of UV absorbing material failed to bind to the gel. This could represent denatured protein or impurities since assay of this material failed to show any enzymatic activity. On the other hand, fractions eluted in the vicinity of the isoelectric point were recovered in enzymatically active form. Similar observations were made by Armstrong (1976) who recovered 77% of the enzyme activity.

When the pH values of elution were plotted against their corresponding isoelectric points of protein, a close correlation ($r = 0.96$) was obtained (Fig. 3). Trypsin, which deviated sharply from this relationship, was excluded from the calculation. It is known (Maroux and Desnuelle, 1969) that trypsin easily undergoes autodigestion at temperatures used in these experiments. We have observed (Oh, 1978) that proteins are rendered more vulnerable to digestion by trypsin in the presence of tannins. Thus, the digestion of BSA increases up to 80% at pH 7.0 with a BSA-tannin ratio of 10:1. It is therefore reasonable to expect that trypsin could experience accelerated autodigestion when complexed with tannins, and we believe that this phenomenon explains its deviant behavior on the column. The overall results obtained from immobilized tannins parallel those from free solution interactions (Oh, 1978; Hagerman and Butler, 1978).

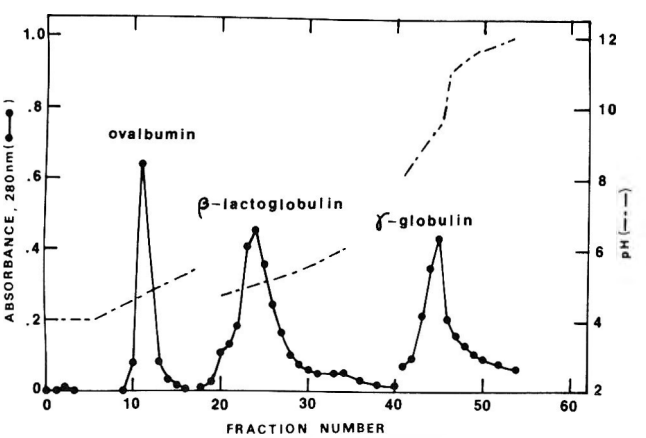

Fig. 1—Elution of protein from immobilized tannin gel, three separate experiments. Protein (10 mg, 1 mg/mL) was bound to the gel (3 mL) at pH 4.0 and eluted in a pH gradient following washing the column. The flow rate was maintained at 30 mL/Hr. Three mL fractions were collected, the pH measured, and absorbance read at 280 nm.

Tannins are believed to bind proteins primarily through the formation of multiple hydrogen bonds between the phenolic hydroxyl groups of tannins and the carbonyl functions of the peptide linkages of proteins (Gustavson, 1954; Cannon, 1955; Loomis and Battaile, 1966; Van Sumere et al, 1975). However, Goldstein and Swain (1965) suggested that condensed tannin-protein complexes may be stabilized by other interaction forces as well. More recent evidence (Oh et al., 1980) indicated that attachment of protein to the immobilized condensed tannin is to a significant extent hydrophobic, although the interactions are no doubt in part also the result of hydrogen bonding.

In contrast to hydrolyzable tannins, charged groups are absent in the condensed tannins (Haslam, 1966). Potentiometric titration of tannin-substituted Sepharose 4B with alkali revealed only insignificant acidity below pH 8.0 in the gel (data not shown). At this pH proanthocyanidins begin to dissociate by dissociation of the phenolic hydroxyl group with formation of phenoxide ions. Thus, the possible involvement of ionic forces in grape tannin (condensed tannin)-protein complexes at pH values below 8 can be disregarded. Near the isoelectric point of the protein positive and negative charges will cancel each other and a compact configuration is favored (Tanford, 1961). The release of protein from the tannin gel (Table 1) could thus be related to conformational changes of protein in the vicinity of the isoelectric point, which, in turn, will result in a decrease in available hydrophobic and other groups responsible for tannin binding on the surface of the protein.

Our experiments are not sufficiently extensive to determine whether the relationship between protein isoelectric point and the pH required for release of the complex with condensed tannins is generally valid. It is to be anticipated that relative concentrations of tannins and protein as well as their absolute concentrations will affect such relationships. We known (Armstrong, 1976) that high ionic strength (0.5M NaCl) significantly reduces the rate at which BSA interacts with tannins in forming complexes and drastically reduces the binding efficiency of immobilized tannins. It is further not established here whether it is possible to generalize with respect to tannins of different origin, although preliminary experiments with wattle and quebracho tannins give results similar to those of grape tannins (Armstrong, 1976).

Another question arises with respect to the validity of extending the characteristics of immobilized condensed tannins to those of tannins in free solution. The process of immobilization has derivatized at least one phenolic hydroxyl group per tannin molecule, and it might be inferred that this could interfere with the ability of the immobilized tannin to interact with

Table 1—pH of elution, peak widths and recovery of proteins from an immobilized tannin gel column

Protein	PI[a]	Elution pH	Recovery[b] (%)	Peak widths (pH units)
Bovine serum albumin	4.98, 5.07, 5.18	5.35	78	1.2
Ovalbumin	4.59, 4.71	4.8, (8.3[c])	68	0.5, (2.3[c])
β-lactoglobulin	5.1, 5.26, 5.34	5.0	96	1.3
γ-globulin	8.2	9.5	85	2.5
Cytochrome c	10.5	9.6 - 11.0	71	2.3
Alkaline phosphatase	4.4	4.4	81	1.8
Trypsin	10.0	6.4 - 7.1	100	3.0
α-amylase	5.2, 5.4	5.5	55	0.75

[a] Literature values.
[b] Total % protein recovered after elution.
[c] Minor peak.

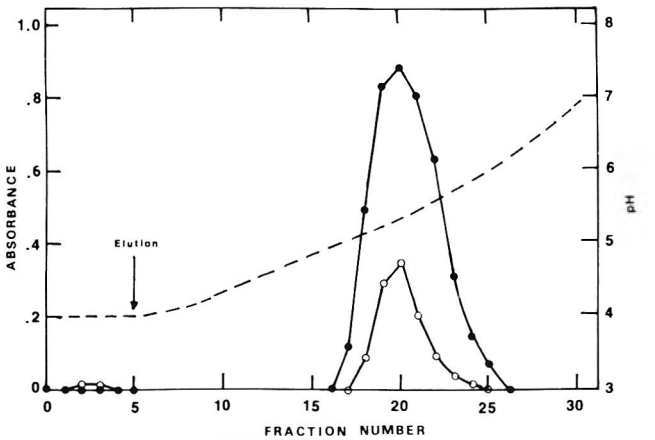

Fig. 2—Binding and elution of α-amylase from the tannin gel in a pH gradient. Five mg of α-amylase were added to the immobilized tannin gel at pH 4.0, washed with 10 mL universal buffer, pH 4.0, and eluted in a pH gradient (—). The flow rate was maintained at 30 mL/hr. Three mL fractions were collected and the pH was measured. An aliquot (25 μL) was assayed for enzymatic activity (●-●) with arbitrary units. Absorbance of eluate was measured at 280 nm (○-○).

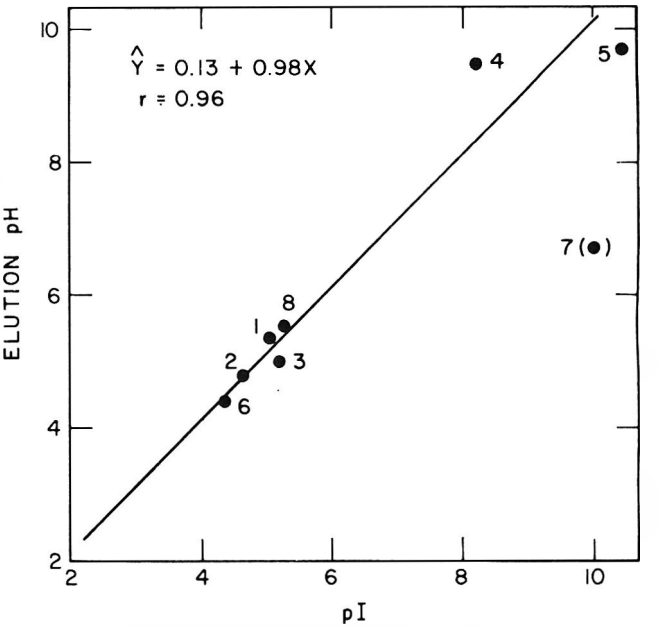

Fig. 3—Correlation between isoelectric points and elution pH values of proteins and digestive enzymes from immobilized tannin gel. Trypsin was excluded from the regression analysis. (1) bovine serum albumin; (2) ovalbumin; (3) β-lactoglobulin; (4) γ-globulin; (5) cytochrome c; (6) alkaline phosphatase; (7) trypsin; (8) α-amylase.

proteins. However, since tannin molecules being polymers with an average of 20 hydroxyl groups per molecule (degree of polymerization times four phenolic hydroxyl groups per monomer) it seems unlikely that derivatization would appreciably reduce that ability. As noted earlier, our experiments with tannins in free solution appear to verify this conclusion.

Our results differ to some extent from reports of Watanabe et al. (1979). But these workers used hydrolyzable tannins (Chinese gallotannin) which may contain acidic groups, and ionic bonding forces may have been important modes of the interaction in their experiments.

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Natural Occurrence of Undecaenes in Some Fruits and Vegetables

RALF G. BERGER, FRIEDRICH DRAWERT, HUBERT KOLLMANNSBERGER, and SIEGFRIED NITZ

ABSTRACT

1-(E,Z)-3,5-undecatriene and 1-(E,Z,Z)-3,5,8-undecatetraene were isolated under enzyme inhibition from edible parts of peach, passion fruit, and kiwi; the triene only was found in celery, parsley, mandarine, apple and pear. Capillary gas chromatography, sniffing-capillary gas chromatography, and combined "live"-capillary gas chromatography-mass spectrometry of the concentrated liquid-liquid extracts were used to establish their identity. Owing to their ultra-low odor detection thresholds and the proposed biogenesis, these trace compounds may be "character impacts" in a wide variety of food flavors of plant origin.

INTRODUCTION

ALICYCLIC and linear C_{11} hydrocarbons are constituents of brown algae species, where some of them act as androgamete attractants (Jaenicke, 1980) and are also formed in vegetative parts of higher plants like *Ferula galbaniflua, Ferula rubicaulis* (Chrétien-Bessière et al., 1967; Teisseire et al., 1967) and *Senecio isatideus* (Bohlmann et al., 1979). The odoriferous properties of 1-(E,Z)-3,5-undecatriene (I), a constituent of Galbanum essential oil, were already well known when Idstein and Schreier (1983) detected 1-(E,Z,Z,)-3,5,8-undecatetraene (II) ("finavarrene, a sex pheromone in the brown algae *Ascophyllum nodosum* (Müller et al., 1982)) in enzymatically liquefied mango fruit.

During a comprehensive study aimed at the odorous components in pineapple fruit, 1-(E-Z,)-3,5-undecatriene (I),1-(E,Z,Z)-3,5,8-undecatetraene and their (E,E) and (E,E,Z)-isomers, respectively, were identified (Berger et al., 1985). Sniffing-capillary gas chromatography showed both (I) and (II) to possess ultra-low odor detection thresholds in the range of some pico grams. In consequence, these compounds may markedly influence the over-all odor impression of plant flavors even at concentrations too low for clear FID response or conventional mass spectroscopy; a contribution to most flavors has to be taken for granted at concentrations above the FID detection threshold. Using sniffing-capillary gas chromatography and "live"-capillary gas chromatography-mass spectrometry a cross-section of well known or economically important fruits and vegetables were investigated to check the predicted widespread occurrence of C_{11} hydrocarbons like (I) and (II)(Boland and Mertes, 1985).

MATERIALS & METHODS

Materials

All plant materials were from local suppliers except parsley which was from our own cultivation. Fully ripe, completely healthy material was selected and included: Celery (*Apium graveolens* L., 200g peeled roots); parsley (*Petroselinum sativum* Hoffm., 300g leaves); carrot (*Daucus carota* L., 350g roots); mandarine (*Citrus reticulata* Blanco cv. Clementine, 1000g peeled fruits); apple (*Malus sylvestris* Mill. cv. Red Delicious, 1350g without core); pear (*Pyrus communis*L. cv. Bartlett, 1930g without core); strawberry (*Fragaria ananassa* L. cv. Senga Sengana, 2080g; peach (*Prunus persica* Batsch., 1000g without stone); passion fruit (*Passiflora edulis* Sims. cv. Maracuja, 440g peeled fruits) and kiwi fruit (*Actinidia chinensis* Planch., 1000g unpeeled).

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Preparation of samples

Plant material was cut into the the twofold amount of methanol (Burdick & Jackson, supplied by Fluka), homogenized, centrifugated, the supernatant diluted with water (MeOH conc. < 50% v/v) and neutralized ($NaHCO_3$, 5% w/v). The resulting solution was immediately extracted with pentane:methylene chloride (2:1), and the collected, Na_2SO_4-treated extracts concentrated by means of a Vigreux column and fractionated on silica gel, as previously described (Berger et al., 1983). Pulped passion fruits were processed in the same way but extracted with pentane:diethyl ether (1:1). Total extracts and first silica gel fractions, eluted with 100% pentane, were concentrated as above (Drawert et al., 1969) and used for further analysis.

Gas chromatography

A Carlo Erba Fractovap 2300 gas chromatograph, equipped with FID and an OV 1 25m × 0.3mm i.d. thick film (1 μm) bonded and crosslinked glass capillary column (G. Leupold, Freising-Weihenstephan) was used. Carrier gas was H_2 at 2 mL/min (50°C); injector and detector temperatures were 225°C; and the column was programmed from 40° to 210°C at 2°/min.

Sniffing gas chromatography

A Carlo Erba Fractovap 2350/AC gas chromatograph equipped with FID, variable effluent splitter (Siemens) and a CW 20 M 40m × 0.5mm i.d. SCOT glass capillary column (SGE) was used. Carrier gas was H_2 at 1 mL/min (65°C), injector and detector temperatures were 225°C, the column was programmed from 65° (6 min isothermal) to 190°C at 2°/min. Effluent split ratio was 1:6 (FID: Sniffing port, 65°C). Five persons, well acquainted with the odor of (I) and (II), tested the effluents. A complete description of the system was given in a previous paper (Nitz et al., 1984).

Combined gas chromatography-mass spectrometry

No. 1 A Finnigan 4021 (quadrupole) mass spectrometer was directly coupled with a Siemens Sichromat 2 double oven "live"-gas chromatograph, equipped with a packed OV 101, 1m × 2mm i.d. precolumn and a CW 20 M, 25m × 0.32mm i.d. glass capillary column. Carrier gas was He (70 cm/sec for the capillary column); injector temperature was 250°C; the precolumn was programmed from 70° to 250°C at 5°/min; the capillary column from 65° to 180°C at 2°/min. The ionization chamber operated at 70eV. Scan time was 0.5 sec for full spectra and 1 sec for multiple ion detection. This system permits independent temperature control for both columns and transfer of selected eluate cuts from the first into the second column. Repeated transfer of a cut into an intermediate trap allows enrichment of compounds in quantities sufficient for adequate mass spectrometric monitoring. A further increase in sensitivity was achieved by using the multiple ion detection mode (Fig. 1). Details on "live"-GLC (Müller and Goekeler, 1985) and coupled "live"-GLC-MS (Nitz, 1985) are described elsewhere.

No. 2 A Finnigan 1020 (quadrupole) mass spectrometer system with directly coupled gas chromatograph, equipped with a SE 54, 30 m × 0.32mm i.d. fused silica capillary column (J & W) was used. Carrier gas was He at 2 mL/min; injector and transfer line temperatures were 230°C; the column was programmed from 50 to 210°C at 2°/min; ionization chamber operated at 70 eV; scan time was 1 sec.

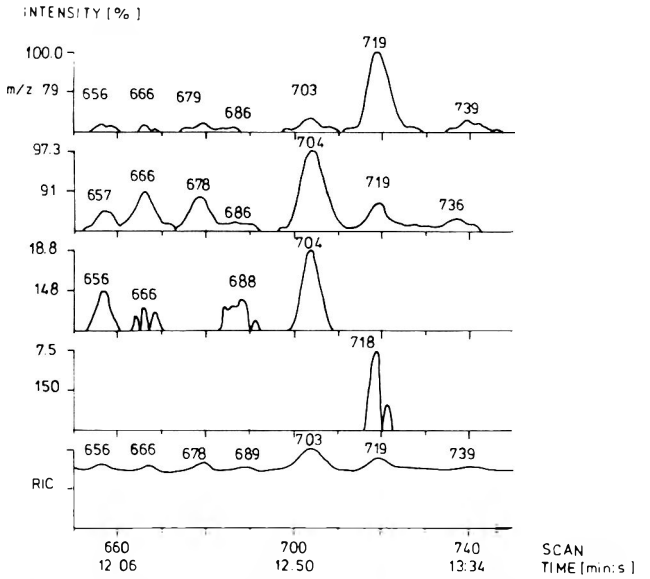

Fig. 1—Section of reconstructed ion current and mass chromatograms of the nonpolar fraction of apple volatiles.

RESULTS & DISCUSSION

IN SOLVENT EXTRACTS of a number of fruits and vegetables, traces (≤ 1 μg/kg) of 1-(E,Z)-3,5-undecatriene (I) and 1-(E,Z,Z,)-3,5,8-undecatetraene (II) were identified by comparison with retention times, sensory properties and mass spectra of authentic compounds (Table 1). Retention indices for (I), which showed a balsamic, pleasant odor with strong fruity undernotes, were $R_I = 1358$ (CW 20 M), 1175(SE 54), and 1166 (OV 1) under the given conditions. Mass spectral data were: m/z(%) 79(100),80(68),77(47),41(37),91(32), 39(27),93(22), 150(18). Data for (II), which showed an exotic-fruity odor, where $R_I = 1409$ (CW 20 M), 1177(SE 54), and 1167(OV 1); mass spectral data were: m/z(%) 79(100), 91(93), 41(48), 77(44), 39(31), 105(25), 65(19), 51(17), molecular ion 148(8).

To exclude the possibility of silica gel-catalyzed artifact formation total extracts and pentane fractions were compared; no significant sensory differences could be detected. In control samples consisting of all reagents but without plant material as well as in extracts from homogenized materials, which were allowed to stand for one day without enzyme inhibition, detection of both undecaenes failed. As with the mentioned *Ferula* species, other members of the *Apiaceae* family contained the triene (I) but not the tetraene (II)(Table 1). The amount was comparably high in celery (~1 μg/kg); nevertheless, the contribution to the over-all flavor remained unclear, since odor thresholds of the major constituents of the essential oil — namely the phthalides — are not known. In parsley the triene(I) was not well deparated from the large peak of 1,3,8-p-menthatriene (CW 20 M), but typical odor and difference spectra allowed identification. In carrot, another member of the *Apiaceae* family, neither (I) nor (II) could be detected.

The nonpolar fraction of citrus flavors contain numerous mono- and sesquiterpene hydrocarbons (van Straten and Maarse, 1983). During sniffing runs of extracts from mandarine, the main compounds limonene and traces of (I) gave the most pronounced sensory impressions on subsequent dilutions.

Apple, pear, strawberry, and peach are representatives of pome-, soft-, and stone fruits of the *Rosaceae* family, usually grown in temperate or subtropical climates. Again in one member of the family — the strawberry — identification of (I) or (II) failed (sniffing-GLC detn. limit ~5 ng/kg). The concentration of (I) in pentane fractions of apple and pear decreased markedly on prolonged storage, whereas in total extracts it remained constant, indicating a protective effect of other liquid-liquid extractable fruit constituents against degradation.

In most extracts compounds with molecular weights of 150 or 148 and mass spectra similar to those of (I) or (II), but with shorter retention times, were detected (Fig. 1). From reference compounds it seemed likely that linear undecaenes were accompanied by less odor active alicyclic isomers. In extracts from nectarine (*Prunus persica* Batsch. cv nectarina), triene (I) was located during sniffing runs, but mass spectrometric identification was not performed. Both (I) and (II) were found in extracts from peach, a fruit leading over to species with tropical habitats. For passion and kiwi fruits concentration ratios of (I) and (II) were calculated from the known intensities of m/z 150 and 148 and the fragment ion currents to be approximately 1:3 [(I):(II)]. A contribution to the typical flavors of both fruits has to be assumed due to the results of sniffing runs of subsequently diluted samples.

Jaenicke et al. (1974) and Moore (1976), studying algal undecaenes with pheromone activities, found structure elements common to the olefins and linoleic or linolenic acid (position of double bonds, E/Z geometries) and suggested the biosynthesis started with a lipoxygenase-analogous hydrogen abstraction from these polyunsaturated fatty acids or their β-oxidation catabolites. Recently, Boland and Mertes (1985) concluded from feeding experiments with Senecio cuttings that radical formation at the 1,4-pentadienyl segment (5-position) of dodecenoic acids is, in fact, the first step, followed by oxidation and fragmentation of the intermediate cation into an olefin and carbon dioxide. The same pathway may proceed in cells of edible fruits and may explain, why reported lipoxygenase activities of fruits and vegetables (Pinsky et al., 1971) and undecaene contents could not be correlated. In contrast to findings with Senecio (Boland and Mertes, 1985), a pool of unsaturated medium-chain fatty acids exists in ripening fruits as indicated by the presence of their esters in the spectrum of

—Continued on page 1667

Table 1—Natural occurrence of 1(E,Z)-3-5-undecatriene (I) and 1-(E,Z,Z)-3,5,8-undecatetraene (II) in fruits and vegetables

Source	I	II	typical odor and R_I[a]	"live" GLC-MS	GLC-MS No 2
Celery	+	–	+	–	+
Parsley	+	–	+	–	+
Carrot	–	–	–	–	–
Mandarine	+	–	+	+	–
Apple	+	–	+	+	–
Pear	+	–	+	+	–
Strawberry	–	–	–	–	–
Peach	+	+	+	+	–
Passion fruit	+	+	+	+	–
Kiwi	+	+	+	+	–
Pineapple[b]	+	+	+	–	+
Mango[c]	–	+			

[a] CW 20 M.
[b] Berger et al. (1985).
[c] Idstein and Schreier (1983).

Treatment of Potato Processing Wastewater with Coagulating and Polymeric Flocculating Agents

M. I. A. KARIM and W. A. SISTRUNK

ABSTRACT

Reduction of wastewater strength in abrasive-peeled, lye-peeled, and steam-peeled potato processing wastewater using 4 inorganic salts and 11 polymers was investigated. Treatment of wastewater with 150 mg/L $FeCl_3 \cdot 6H_20$ + 20 mg/L Purifloc Anionic Polymer #23 aided in coagulation and flocculation of suspended solids of effluent from abrasive-peeled potatoes including greater than 90% reduction in chemical oxygen demand (COD), total suspended solids (TSS) and turbidity. Treatment with 300 mg/L $CaCl_2$ + 25 mg/L Purifloc #23 reduced COD and TSS levels in wastewater from lye-peeled potato by 69% and 76%, respectively. In steam-peeled effluent, 350 mg/L $CaCl_2$ + 25 mg/L Nalco 7122 reduced TSS by 90% and COD by 60%. Waste strength of effluent from processing potatoes can be substantially reduced by treating with coagulating and flocculating agents before discharging into municipal or other treatment systems.

INTRODUCTION

FOOD PROCESSING INDUSTRIES normally discharge large volumes of wastewater that are characterized by high Chemical Oxygen Demand (COD) or Biological Oxygen Demand (BOD), large amounts of Total Suspended Solids (TSS) and various inorganic constituents including nitrogen, phosphorus, and potassium (Knapp, 1970; Pearson et al., 1972; Shannon et al., 1968; Smith, 1971). The high organic load in the processing wastewater creates a serious pollution problem and poses a threat to water quality when discharged to rivers and lakes.

With the establishment of the Federal Water Pollution Control Act Ammendments of 1972, PL92-500, most food industries are searching for methods to reduce pollution load and total effluent discharge. The costs for meeting standards for effluent discharge, either to a receiving water or to a municipal treatment plant are increasing.

Physical-chemical methods of treating wastewater are gaining more acceptance. They are used for treatment of municipal wastewater (Bishop et al., 1972; Leentvaar et al., 1978; Weber et al., 1970), and are reported to be effective in reducing suspended solids in pimiento (Bough and Shewflet, 1973), leafy green vegetables (Bough, 1974) and citrus packinghouse wastewater (Ismail, 1978). The techniques are also applicable to most food processing wastes which are often much more concentrated than municipal wastes. The physical-chemical methods normally employ the use of coagulating and flocculating agents such as various types of inorganic salts and polymers. These chemicals will coagulate and flocculate the suspended solids, and subsequently aggregate the particles to improve their settling and removal from the wastewater. In this way, significant reductions in total solids and COD can be achieved. Chemical coagulation normally involves the process of destabilization, aggregation and binding together of colloids. It is commonly accomplished by the addition of floc-forming substances such as lime, alum or aluminum sulfate ($Al_2 (SO_4)_3 \cdot 18 H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), or ferric chloride ($FeCl_3$), to coagulate suspended solids (National Canners Association, 1971). LaMer and Healy (1963) described the action of polymeric flocculating agents whereby the polymer destabilizes a colloidal suspension by adsorption of particles and subsequent formation of particle-polymer-particle bridges. This applies to anionic and nonionic polyelectrolytes that are used to coagulate negative colloids. Likewise, the cationic polymers which are positively charged can destablize a negative colloidal suspension by charge neutralization as well as bridge formation (O'Melia, 1972). During the past few years, various anionic, nonionic and cationic, water-soluble, synthetic and modified polymeric materials were available that have been used as a coagulant aid in conjunction with the conventional salts (Pressman, 1967).

Processing plants in the Northwest Arkansas region are faced with the problem of either reducing high COD, total solids and suspended solids in wastewater generated by peeling potatoes for processing or close the plant as a last resort. Thus the objective of this research is to evaluate the influence of certain inorganic salts and polymeric flocculating agents in reducing the turbidity, total suspended solids and COD levels in abrasive-peeled, lye-peeled, and steam-peeled potato processing wastewater.

MATERIALS & METHODS

Effluent samples

Composite samples of potato processing wastewater viz. abrasive-peeled, lye-peeled and steam-peeled wastewater were collected from Allen Canning Co. at Siloam Springs, Van Buren and Johnson, Arkansas. The samples were stored in the cold room (0 ± 1°C) and treated within a few days. During the storage period, no significant changes in the concentration of suspended solids or COD were observed.

Studies were designed as factorial experiments, including nonionic Hercofloc 827 (H827); anionic Hercofloc 1031 (H1031) and H1018, anionic Purifloc #23 (PA#23); cationic Magnifloc 2535CH (M2535CH) and M2540C, cationic Dubois GWP-25, cationic Nalco 7122 (N7122) and N7120, cationic Purifloc #43 (PC#43); and Varcofloc at 7 concentrations and 4 inorganic salts [$Al_2(SO_4)_3$(alum); $FeCl_3$(Ferric chloride); $Fe_2(SO_4)_3$ (ferric sulfate); and $CaCl_2$(calcium chloride)] at 7 concentrations. These polymers and inorganic salts were tested in all combinations at 3 pH levels (5, 7 and 9), except in lye-peeled effluent

Table 1—Effect of different polymers on reduction of turbidity of wastewater from abrasive-peeled potatoes at pH 5

Type of polymers	Turbidity value (NTU)[a]	Type of polymers	Turbidity value (NTU)
Control[b]	35.83	Dubois GWP-25	26.47
H 827	35.56	N 7122	29.94
H 1031	36.17	N 7120	31.50
H 1018	33.50	Varcofloc	37.14
M 2535CH	33.19	PC #43	32.58
M 2540C	33.61	PA #23	23.64
LSD 0.05[c]		0.36	

[a] Nephelometric Turbidity Units.
[b] Supernatant wastewater settled for 30 min.
[c] Least Significant Difference at 5% level.

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Table 2—Interaction of concentration of polymer and concentration of $FeCl_3$ on wastewater turbidity from abrasive-peeled potatoes at pH 5

Concentration of $FeCl_3$ (mg/l)	Concentration of polymer (mg/L) 5	10	20	40	60	80
	Turbidity (NTU)[a] Values					
0	86.75[b]	83.67	83.33	80.83	78.50	77.08
100	70.50	65.67	66.25	58.33	61.67	67.08
150	14.83	13.42	12.92	12.83	14.25	17.58
200	13.67	12.92	12.08	12.00	13.83	16.17
250	9.92	9.25	9.67	9.00	11.75	13.25
300	9.50	9.33	9.58	8.50	10.83	10.67
LSD 0.05p[c]			0.58			

[a] Nephelometric Turbidity Units.
[b] Means of 11 polymers and a control with 3 replications.
[c] Least Significant Difference at 5% level.

Table 3—Effect of concentration of polymer and $FeCl_3$ on reduction of wastewater turbidity from abrasive-peeled potatoes at pH 5

	Concentration $FeCl_3$ (mg/L) 0	100	150	200	250	300
	Turbidity (NTU)[a] Value					
Raw Wastewater	170					
Control[b]	90	75	16	14	10	10
Added PA #23 Polymer (mg/l)						
5	80	60	10	10	8	6
10	78	45	4*	4	4	4
20	75	40	4**	4	4	4
40	75	30	4	4	4	4
60	70	35	8	8	8	8
80	70	35	14	10	10	10
LSD 0.05[c]			2.8			

[a] Nephelometric Turbidity Units.
[b] Supernatant wastewater settled for 30 min.
[c] Least Significant Difference at 5% level.
* Small Flocs found in wastewater (not removable on 80-mesh screen).
** Large flocs found in wastewater (removable on 80-mesh screen).

where pH 11.3 (the original pH) was substituted for pH 9. Three replications of each combination were conducted. The pH of the wastewater was adjusted with either 5N NaOH or 5N H_2SO_4.

To test for coagulation and flocculation, the appropriate concentrations of polymer and inorganic salt were added to 100 and 200 mL samples of effluent in a beaker. The mixture was stirred for 3 min at approximately 100 rpm by using a six-position stirrer (Labline, Inc., Chicago, IL) at room temperature (25°C). The contents of the beakers were allowed to settle for 30 min before samples of the supernatant were drawn for turbidity measurement using a nephelometer by the procedure of Standard Methods (APHA, AWWA, WPCF, 1975). Also, the COD of the supernatant from fresh and treated samples was determined by the method of Mercer and Rose (1968) and TSS by Standard Methods (APHA, AWWA, WPCF, 1975).

The data were analyzed by the analysis of variance. Means of the main effects are separated by Duncan's Multiple Range test and the interactive effects by Least Significant Difference. Only a small part of the data are shown to illustrate the treatments that resulted in the greatest reduction in COD and TSS.

RESULTS & DISCUSSION

Abrasive-peeled potato wastewater

Many researchers (Bishop et al., 1972; Bough, 1974; Bough and Shewfelt, 1973) have used turbidity measurements as a means of measuring changes in TSS, COD and BOD present in wastewater. There was a high correlation between TSS, NTU and COD in each of the three effluents ($P<0.001$). The reduction in turbidity at pH 5.0, expressed as nephelometric turbidity units (NTU), was greater with $FeCl_3$, and $Fe_2(SO_4)_3$ than with $CaCl_2$ and $Al_2(SO_4)_3$ (Fig. 1). Using 300 mg/L $FeCl_3$, the NTU value of the wastewater was reduced from 200 to 12, representing a reduction in NTU of 94%, of the original raw

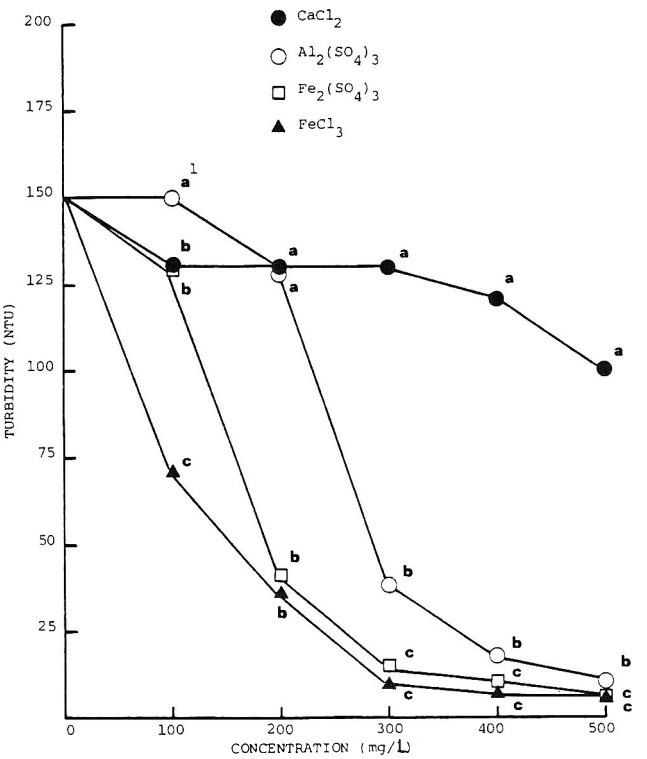

Fig. 1—Effect of concentrations of different inorganic salts on reduction of turbidity in abrasive-peeled potato wastewater at pH 5.0. Raw wastewater turbidity = 200 NTU. [1]Values followed by the same letter within concentrations do not differ signficiantly at the 5% level using Duncan's multiple range test.

wastewater. Sedimentation alone without adding chemicals reduced TSS only slightly.

In the wastewaters adjusted to pH 7 and to pH 9, greater reductions in NTU were observed with $FeCl_3$ than with the other inorganic salts (data not shown). In order to obtain larger floc by coagulation-flocculation of TSS, combinations of $FeCl_3$ and polymers were used. The polymer PA #23 reduced NTU more than the other polymers (Table 1). The interactive effects of polymers and concentration of $FeCl_3$ indicated that polymers alone in concentrations of 5–80 mg/L only reduced NTU slightly (Table 2). However, a large decrease in NTU was observed when 150 mg/L $FeCl_3$ was followed by 40 mg/L polymer. The greatest reduction occurred with the use of 300 mg/L $FeCl_3$ + 40 mg/L polymer, yet 5 mg/L polymer produced good results. Concentrations of polymers exceeding 40 mg/L in combination with $FeCl_3$ significantly ($P<0.05$) increased NTU values as a result of resuspension of solids, which is commonly associated with overdosage of polyelectrolytes (O'Melia, 1972).

The chemical treatment resulting in the most rapid separation of floc and reduction in turbidity of effluent from abrasive-peeled potatoes was 150 mg/L $FeCl_3$ + 10 mg/L PA #23 polymer (Table 3). However, a combination of 150 mg/L $FeCl_3$ + 20 mg/L PA #23 polymer not only reduced the NTU, but coagulated the suspended solids immediately into larger floc that settled rapidly and was removable on an 80-mesh screen.

Reduction of 91–99% of the TSS and 93–97% of the COD were achieved by treatment of effluent from abrasive-peeled potatoes with a combination of $FeCl_3$ and PA #23 polymer at pH 5,7 and 9, compared to the raw sample (Table 4). However, larger floc and faster settling of TSS that were removeable on an US Standard 80-mesh screen were found in the combination of $FeCl_3$ with 20 mg/L PA #23 polymer.

Steam-peeled potato wastewater

Calcium chloride was superior to $FeCl_3$, $Fe_2(SO_4)_3$, and $Al_2(SO_4)_3$ in the coagulation-flocculation of TSS at pH 5 as

Table 4—Main effects of coagulating and flocculating agents on turbidity (NTU), COD and total suspended solids (TSS) levels of abrasive-peeled potato processing wastewater at pH 5, 7, and 9

	pH 5					pH 7					pH 9				
Treatment	NTU	COD ppm	% COD reduction	TSS mg/L	% TSS reduction	NTU	COD ppm	% COD reduction	TSS mg/L	% TSS reduction	NTU	COD ppm	% COD reduction	TSS mg/L	% TSS reduction
Raw wastewater	170	2650		1720		170	2270		1650		170	2218		1650	
Control[a]	90	290	89	750	56	100	259	89	775	53	100	301	86	775	53
150 mg/L $FeCl_3 \cdot 6H_2O$	16	95	96	100	94	26	143	94	150	91	24	147	93	150	91
150 mg/L $FeCl_3 \cdot 6H_2O$ + 10 mg/L PA#23 polymer	4	85	97	20	99	8	116	95	90	95	6	121	95	25	99
150 mg/L $FeCl_3 \cdot 6H_2O$ + 20 mg/L PA #23 polymer	4	74	97	20	99	6	90	96	25	99	10	126	94	50	97
LSD 0.05[b]		39.8		23.4			45.4		22.3			63.2		20.1	
% CV[c]		3.4		2.5			4.3		2.3			6		2.1	

[a] Supernatant wastewater settled for 30 min.
[b] LSD .05 Least Significant Difference at 5% level.
[c] %CV Percentage Coefficient of Variation

Table 5—Main effects of coagulating and flocculating agents on turbidity (NTU), COD and total suspended solids (TSS) levels of steam-peeled potato processing wastewater at pH 5, 7, and 9

	pH 5					pH 7					pH 9				
Treatment	NTU	COD ppm	% COD reduction	TSS mg/L	% TSS reduction	NTU	COD ppm	% COD reduction	TSS mg/L	% TSS reduction	NTU	COD ppm	% COD reduction	TSS mg/L	% TSS reduction
Raw waste water	900	8230		4417		750	7220		4000		850	7325		4250	
Control[a]	140	4218	49	727	84	150	4547	37	803	80	140	4092	44	706	83
350 mg/L $CaCl_2$	90	3603	56	513	88	95	3620	50	576	86	65	3365	54	545	87
350 mg/L $CaCl_2$ + 25 mg/L N7122	65	3317	60	433	90	75	3708	49	600	85	85	3808	48	604	86
350 mg/L $CaCl_2$ + 25 mg/L PA #23	90	3750	54	493	89	70	3420	53	487	88	50	3202	56	408	90
LSD 0.05[b]		140		7.89			9.19		11.87			11.76		9.52	
% CV[c]		1.7		0.33			0.11		0.5			0.2		0.4	

[a] Supernatant wastewater settled for 30 min.
[b] LSD 0.05 Least Significant Difference at 5% level.
[c] % CV Percentage Coefficent of Variation.

Table 6—Effect of concentration of different inorganic salts on reduction of wastewater turbidity from lye-peeled potatoes at pH 11.3, 7, and 5

		Concentration of inorganic salt (mg/L) Applied						
pH	Inorganic salt	0	100	200	300	400	500	1000
		Turbidity (NTU)[a] Values						
11.3	Control[b]	130						
	$Al_2(SO_4)_3$		120a[c]	120a	140a	150a	150a	180b
	$FeCl_3$		120a	110b	120c	120c	130c	180b
	$Fe_2(SO_4)_3$		120a	120a	130b	130b	140b	190a
	$CaCl_2$		120a	120a	110d	95d	95d	180c
7.0	Control	220						
	$Al_2(SO_4)_3$		160b	160b	170a	170a	105a	85b
	$FeCl_3$		160b	160b	160b	110b	100b	85b
	$Fe_2(SO_4)_3$		170a	170a	170a	105c	100b	90a
	$CaCl_2$		120c	120c	120c	95d	95c	90a
5.0	Control	280						
	$Al_2(SO_4)_3$		200a	180a	160a	160a	160a	140a
	$FeCl_3$		180b	140b	100c	95c	100c	100c
	$Fe_2(SO_4)_3$		180b	130c	100c	90d	90d	85d
	$CaCl_2$		170c	130c	120b	100b	110b	110b

[a] Nephelometric Turbidity Units.
[b] Supernatant wastewater settled for 30 min.
[c] Means separated in columns within pH levels by Duncan's multiple range test, 5% level.

measured by NTU values (Fig. 2). In the effluents at pH 7 and pH 9, greater reductions in turbidity were also obtained using $CaCl_2$ than with the other inorganic salts (data not shown).

The interactive effects of different concentrations of polymers and $CaCl_2$ on turbidity of effluent from steam-peeled potatoes at pH 5 showed that the greatest reduction in NTU was achieved by applying 350 mg/L $CaCl_2$ + 25 mg/L N7122 polymer (Table 5). Maximum reductions in NTU of effluent adjusted to pH 7 and pH 9 were achieved with 350 mg/L $CaCl_2$ + 25 mg/L PA #23 polymer.

Table 7—Main effects of coagulating and flocculating agents on turbidity (NTU), COD and total suspended solids (TSS) levels of lye-peeled potato processing wastewater at pH 5, 7, and 11.3

	pH 5					pH 7					pH 11.3				
Treatment	NTU	COD mg/L	% COD reduction	TSS mg/L	% TSS reduction	NTU	COD mg/L	% COD reduction	TSS mg/L	% TSS reduction	NTU	COD mg/L	% COD reduction	TSS mg/L	% TSS reduction
Raw waste water	280	3710		1920		220	3650		1880		210	3600		1900	
Control[a]	210	1675	55	695	64	160	1650	55	665	65	130	1500	58	600	68
300 mg/L $CaCl_2$ + 25 mg/LPA # 23 polymer	150		ND			110		ND			70*	1113	69	450	76
300 mg/L $CaCl_2$ + 25 mg/L Dubois-GWP25	160		ND			120		ND			75	1346	63	480	75
350 mg/L $CaCl_2$ + 25 mg/L H1018	120		ND			90	1200	67	520	72	100		ND		
300 mg/L $FeCl_3$ + 25 mg/L H1018	70*	1100	70	475	75	160		ND			110		ND		
300 mg/L $Fe_2(SO_4)_3$ + 25 mg/L H1018	65*	1058	72	427	78	160		ND			120		ND		
LSD 0.05[b]		70.0		21.73			60.1		13.3			15.45		15.9	
% CV[c]		2		1.3			1.8		0.9			0.4		1.0	

[a] Supernatant wastewater settled for 30 min.
[b] LSD 0.05 Least Significant Difference at 5% level.
[c] % CV Percentage Coefficient of Variation
* Large flocs found in wastewater
ND No data available.

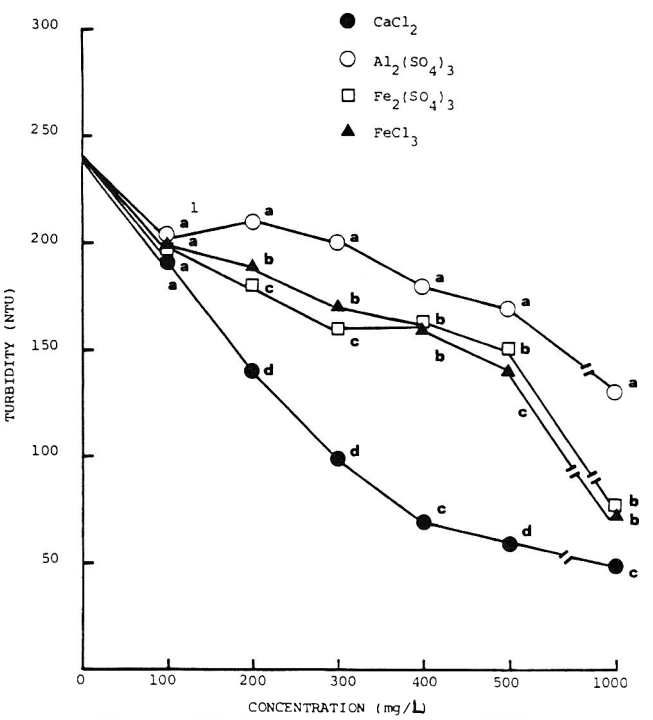

Fig. 2—Effect of concentrations of different inorganic salts on reduction of turbidity in steam-peeled potato wastewater at pH 5.0. Raw wastewater turbidity = 200 NTU. [1]Values followed by the same letter within concentrations do not differ signficantly at the 5% level using Duncan's multiple range test.

Treatment of raw effluent (pH 5) with 350 mg/L $CaCl_2$, 350 mg/L $CaCl_2$ + 25 mg/L N7122 polymer and 350 mg/L $CaCl_2$ + 25 mg/L PA #23 polymer reduced COD levels by 56, 60 and 54% and TSS by 88, 90 and 89%, respectively. With 350 mg/L $CaCl_2$ + 25 mg/L N7122 polymer, the TSS of raw wastewater was reduced 90% and COD 60%. The high COD level remaining in the treated effluent indicates that there was still a large proportion of dissolved solids, which might have been low molecular weight starch intermediate products, present in the water. Also, highly significant differences ($p<0.01$) were found in the COD and TSS levels of the treated effluents that were adjusted to pH 7 and pH 9 in comparison with the nontreated effluent.

Lye-peeled potato effluent

In the raw lye-peeled effluent (pH 11.3), the addition of the 300 mg/L $CaCl_2$ significantly reduced the turbidity of the water when compared with $Al_2(SO_4)_3$, $FeCl_3$ and $Fe_2(SO_4)_3$ (Table 6). In effluent adjusted to pH 7, treatments of 100 to 500 mg/l $CaCl_2$ were superior for reducing the NTU values in comparison with the other inorganic salts. The addition of $CaCl_2$ to the effluent from lye-peeled potatoes may produce $CaCO_3$ solids at moderate to high pH (9–11) as a result of bicarbonate or carbonate ions present in the wastewater. It may be possible for the organic colloids present to adhere to the $CaCo_3$ precipitates, causing settling out and reduction of TSS and NTU of the water.

Calcium ions, at moderate to low pH levels in wastewater, may also bind onto proteins and pectins, complexing these large molecules and promoting settling out from the supernatant. In effluent at pH 5,300 mg/L or more of either $FeCl_3$ or $Fe_2(SO_4)_3$ reduced the turbidity more than $Al_2(SO_4)_3$ or $CaCl_2$.

In the original effluent from lye-peeled potatoes (pH 11.3), treatments of 300 mg/L $CaCl_2$ + 25 mg/L PA #23 polymer and 300 mg/L $CaCl_2$ + 25 mg/L Dubois GWP-25 polymer reduced COD levels 69 and 63% and TSS 76 and 75%, respectively (Table 7). In the effluent adjusted to pH 7, treatment with 350 mg/L $CaCl_2$ + 25 mg/L H1018 polymer reduced COD by 67% and TSS by 72%. In the raw wastewater adjusted to pH 5, treatments of 300 mg/L either $FeCl_3$ or $Fe_2(SO_4)_3$ + 25 mg/L H1018 polymer reduced COD by 70–72%, and TSS by 75–78%.

SUMMARY & CONCLUSIONS

THE TSS AND COD LEVELS of potato processing wastewater were reduced by chemical treatments with certain inorganic salts and polymeric flocculating agents to form screenable

floc that was easily removed. The nature of the wastewater (abrasive-peeled, steam-peeled, and lye-peeled) when the pH was adjusted to different levels played an important role in determining the type and concentration of inorganic salts and polymers required in coagulation-flocculation of TSS and reduction of COD.

Wastewater strength from abrasive-peeled potatoes was greatly reduced by treatment with 150 mg/L $FeCl_3$ + 20 mg/L PA #23 polymer. In effluent from steam-peeled potatoes, $CaCl_2$ produced larger floc than either $FeCl_3$ or the other salts in combination with N7122 or PA #23 at pH 5. Reductions in TSS were 85 to 90% at all pH levels, but reductions in COD ranged from 48 to 60%, possibly due to the formation of starch degradation products by the high temperature steam. The use of $CaCl_2$ + PA #23 and H1018 resulted in the greatest reductions in TSS and COD in effluent from lye-peeled potatoes at pH 11.3 and pH 7; however, at pH 5, $FeCl_3$ and $Fe_2(SO_4)_3$ in combination with H1018 polymer gave the best results.

The data from these studies indicate that the treatment of effluent from potato processing with coagulating and polymeric flocculation does significantly reduce the TSS and COD. At the present time, municipal treatment plants are utilizing similar treatments, but without the knowledge of appropriate treatments to apply to wastewater of different composition, including pH, concentration, etc. These high strength wastes could be treated at the processing plant, thus eliminating erratic changes in effluent at the municipal plant. Biological treatment and utilization of the recovered biomass have been reported. (Karim and Sistrunk, 1985a, 1985b).

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*ENOCYANIN IN CRANBERRY JUICE COCKTAIL. . .*From page 1642

another paper. The present method will easily detect gross adulteration, defined as replacement of 50% or more of the normal amount of cranberry juice. However, in view of the above uncertainties, validation with a much larger number of samples would be highly desirable.

SUMMARY

A FOUR-STAGE METHOD to detect the addition of enocyanin to cranberry juice cocktail was developed and applied to a number of commercial samples. The method involves: (1) a color measurement with data expressed as Theta units; (2) recovery of anthocyanin and flavonoid pigments on a CG-50 column; (3) identification of grape anthocyanidins by paper chromatography in Formic reagent; and (4) identification of grape anthocyanins by paper chromatography in 1% HCl in water. All four procedures are relatively simple and sufficient to detect the replacement of 50% or more of the expected amount of cranberry juice by a solution of enocyanin and citric acid in water.

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Liquid Chromatographic Analysis of Sugars and Mannitol in Cabbage and Fermenting Sauerkraut

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ABSTRACT

High-performance liquid chromatography was used to measure concentrations of fructose, glucose and sucrose in several cultivars of cabbage as well as residual sugars and mannitol in sauerkraut. Cabbage cultivars contained from about 3.0–6.0% total sugars, and glucose was the most abundant sugar in entire cabbage heads. All sugars in a selected commercial tank of fermenting sauerkraut were depleted after 4 wk. However, total sugar concentrations to 0.8% were found in a variety of commercial sauerkraut samples. Mannitol accumulated early in fermentations, but concentrations in sauerkraut decreased during latter stages of holding (through 18 wk) before processing.

INTRODUCTION

SAUERKRAUT utilization by U.S. consumers has been declining, and this has been attributed to a variety of causes, including changing consumer preferences for foods, the high sodium content of sauerkraut, and unreliable or sometimes unpleasant flavor qualities. Additionally, incorporation of sauerkraut into contemporary marketing forms, such as polyethylene pouch-packaged raw sauerkraut for refrigerated distribution, frequently leads to problems with gas production or drops in pH because the product is not microbiologically stable. While a variety of factors can contribute to the microbiological instability of sauerkraut after it is removed from fermentation tanks (Pederson and Albury, 1969; Stamer and Stoyola, 1978; Fleming et al., 1983), the presence of residual sugar substrates has been viewed as the likely cause of secondary fermentations either by yeasts (Stamer and Stoyola, 1978) or *Lactobacillus sp.* (Chen et al., 1983). However, other fermentable substrates, such as mannitol and dextrans, also may be present in sauerkraut (Nelson and Beck, 1918; Fred et al., 1919; Pederson and Albury, 1969).

Fermentation of cabbage into sauerkraut utilizes the natural lactic acid-producing microflora, and relies on sodium chloride to control or direct the fermentation (Pederson and Albury, 1969). Initial fermentations are dominated by heterolactic microorganisms that yield a variety of metabolic products, including lactic acid, acetic acid, ethanol, carbon dioxide, mannitol, and dextran. Secondary stages of the fermentation rely on the selective growth of homofermentative lactobacillus that occurs under the influence of low pH conditions in the sauerkraut. Microbiological stabilization of several vegetables fermented by *Lactobacillus planterum* has been shown to be achieved by a pH of about 3.8 or below in combination with the existence of anaerobic conditions and the absence of fermentable sugars (Fleming et al., 1983).

Increased utilization of sauerkraut in contemporary foods and distribution systems will require dependable quality which will demand much greater controls over cabbage fermentations than have been achieved to this time. Since sugars and related fermentable substrates may play an influential role in the quality and stability of sauerkraut, this aspect requires investigation to provide a basis for processing adjustments needed to provide uniform and stable sauerkraut. Only limited data are available on the sugar content of cabbage (Peterson and Viljoen, 1925; Stamer et al., 1969; Lee et al., 1970; Martin-Villa et al., 1982), and these data are restricted to measurements of total reducing sugars (Peterson and Viljoen, 1925; Stamer et al., 1969) or limited selections of cultivars (Lee et al., 1970; Martin-Villa et al., 1982). Information about the rate of sugar depletion during sauerkraut fermentations and the composition of fermentable substrates does not appear to exist in the literature.

With the availability of powerful columns for the high performance liquid chromatographic (HPLC) separations of carbohydrates, the data from earlier, less-specific methods for sugars should now be reevaluated. Applications of HPLC to the analysis of sugars in vegetable materials have been reported by several groups of investigators (Conrad and Palmer, 1976; Martin-Villa et al., 1982; McBee and Maness, 1983; Wight and van Niekerk, 1983), and recently McFeeter et al. (1984) have described a method for the HPLC analysis of major substrates and products found in vegetable fermentations. This investigation was undertaken to extend the development of routine HPLC methods for the analysis of sugars and fermentation products in cabbage and sauerkraut, and to provide quantitative data on these substances for use in process adjustments to achieve sauerkraut with uniform and stable quality characteristics.

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MATERIALS AND METHODS

Samples

Heads of defined cultivars of cabbage were provided by Professor P. Williams and Paul Bosland (Dept. Plant Pathology, U. of Wisconsin, Madison), and were grown at the Southern Wisconsin Sauerkraut Cabbage Varietal Trial Plot Racine, WI. Additional heads of cabbage were purchased from local supermarkets. Cabbages were stored at 5°C in unsealed polyethylene bags until analyzed. Sauerkraut samples were obtained either directly from commercial fermentation tanks or processing lines of Wisconsin plants, or from local supermarkets. Sauerkraut samples from tanks (wood, 3.05m deep × 4.88m diameter; or fiberglass 3.87m deep × 3.57m length × 3.08m width) were composed of either Sanibel variety or a combination of Sanibel and Grand Slam varieties (top 2/5 tank, Sanibel; bottom 3/5 tank, Grand Slam, respectively). Samples (2.5 kg each) were obtained at five incremental depths from each tank. Commercially-packed sauerkraut contained in sealed polyethylene bags (454g each) and bulk raw sauer-

Table 1—Comparison of refluxing and blending methods for isolation of sugars from retail cabbage for HPLC analysis

	Concentrations (% wet wt)[a]				
Method	Sucrose	Glucose	Fructose	Total sugars	CV[b](%)
Sample 1					
Refluxing[c]	0.30	2.08	1.70	4.08	4.30
Blending	0.32	2.08	1.77	4.17	4.78
Sample 2					
Refluxing	0.58	2.06	1.83	4.47	6.05
Blending	0.55	2.01	1.84	4.40	4.39

[a] Values represent the mean of triplicate samples.
[b] CV %) = (Standard deviation Mean) × 100
[c] Conrad and Palmer (1976) method.

kraut samples (18 kg each) were stored a 2°C until analyzed within 2 wk.

Analysis of lactic acid and salt

Titratable acidities, expressed as % lactic acid, were determined by titrating 10 mL samples with standard (0.111N) NaOH using phenolphthalein as the indicator (AOAC., Sec. 22.060, 1975). Salt was determined by titrating 2 mL samples with 0.1 N silver nitrate using potassium chromate as the indicator (Pederson and Albury, 1969).

HPLC system

Analyses for fructose, glucose, sucrose and mannitol were carried out with procedures similar to those described by McFeeters et al. (1984), but with modifications to shorten analysis times and enhance resolution. A Sugar Pak column (300 mm × 6.5 mm i.d., Waters Associates, Milford, MA) was operated at 77 ± 3°C using a heated waterbath (Precision Scientific Co., Chicago, IL). A guard column was not used in this system to avoid sucrose inversion that could occur in the presence of calcium disodium EDTA contained in the mobile phase (Waters Assoc., 1982a).

The mobile phase, 50 mg/L calcium disodium EDTA (Geigy Industrial Chemicals, Ardsley, NY) in degassed, triple-deionized water was pumped through the system at a flow rate of 0.50 mL/min. Samples were introduced with a Rheodyne injection valve fitted with a 20 µL loop (Model 7125 Rheodyne, Inc. Cotati, CA). A differential refractometer detector (Model R401, Waters Associates, Milford, MA) was used at an attenuation of × 32, and data were processed with a computing integrator (Model SP 4100, Spectra Physics, San Jose, CA).

Sucrose (Mallinckrodt Chemical Works, St. Louis, MO), glucose, fructose (Aldrich Chemical Co., Milwaukee, WI), mannitol (Atlas Chemical Industries, Inc., Wimington, DE), and sorbitol (Aldrich Chemical Co., Inc., Milwaukee, WI) standards dissolved in 40% ethanol, were used for development of calibration curves to determine response factors. Peaks for sugars and mannitol were assigned by retention times that were located through spiking studies. Response factors for sugars and mannitol were determined by plotting the ratio of areas of the internal standard and the component of interest to the ratio of their concentrations. Concentrations chosen for evaluation ranged from 0.06–3.50% for sucrose, 0.12–4.23% for glucose, and 0.10–3.00% for fructose and mannitol, and covered the range encountered in samples. Response factors were linear over the ranges evaluated, and response factors for sucrose, glucose, fructose and mannitol were 0.94, 0.95, 0.90, and 1.00, respectively.

Isolation of sugars for HPLC analysis

Heads of cabbage were cored and the outer leaves were removed. Then, heads were rinsed with tap water, and wedges (2.5 cm thick at the outer surface) were cut perpendicular to the core of the heads to provide cross-sectional samples. Wedges were next shredded with a hand-held device constructed with 0.7 cm i.d. cutting holes, and then were thoroughly mixed.

Samples of shredded cabbage (10 g each) were weighed into screw-top test tubes, and 10 mL aqueous 80% ethanol containing 150 mg sorbitol (internal standard) were added. The tubes were then loosely capped, and heated in a waterbath (80–90°C) for 10 min. Tubes were then removed from the bath, and each sample was disintegrated with a Polytron homogenizer (Brinkman, Steinhofholde, Switzerland) for 1 min before capping and returning to the hot water bath for an additional 20 min. After adding 10 mL water to each sample, they were filtered through filter paper (Whatman, #4, 4 cm diameter) using a Buchner funnel. Liquid samples were further clarified by passing each through a 0.45 µm micropore filter (Acrodisc, Gelman Sciences Inc., Ann Arbor, MI). Cabbage samples used in method-comparison analyses were prepared based on the AOAC method (Sec. 3.002, 1975) and that of Conrad and Palmer (1976). Cabbage heads were shredded and 10g samples along with 150 mg of the internal standard maltose (Aldrich Chemical Co. Inc., Milwaukee, WI) were weighed into round-bottomed flasks, and refluxed in 80% ethanol (including the water contribution from the cabbage) for 1.5 hr. These samples were subjected to the same clean up procedures for HPLC as the previous cabbage samples.

Sauerkraut samples were prepared for HPLC analysis by similar procedures except that they were first neutralized with calcium carbonate powder (Mallinckrodt, St. Louis, MO) to a pH of 6.5 (±0.2) to prevent acid-catalyzed hydrolysis of sucrose during heating (Conrad and Palmer, 1976; Wight and Van Niekerk 1983). Extracts of sauerkraut were each filtered successively through an alumina Sep-Pak A cartridge (Waters Associates, Milford, MA) and a micropore filter (0.45 µm, Acrodisc) to further remove organic acids and pigments. Samples of sauerkraut fermentation brines were prepared for analysis by adding 10g brine to 150 mg sorbitol (internal standard), and then diluting to 20 ml with distilled water. These samples were then subjected to the same clean-up procedures as the sauerkraut samples.

Soluble solids (%) were determined for cabbage juices that were expressed from 40g samples of shredded cabbage using a hydrolic press (Carver, Summit, NJ) held at 10,000 psi for 30 sec. Expressed juices were analyzed with a hand-held refractometer (Kiruchi, Industrial and Mill Supply Co., Huntington Station, NY) that was equipped with scales to read 0–44% and 44–72% ranges of soluble solids.

RESULTS & DISCUSSION

Evaluation of the analytical method

McFeeters et al. (1984) reported the analysis of vegetable fermentation products with HPLC systems employing a combination of an Aminex HPX-87P column (Bio-Rad Labs, Richmond, CA) and a Radial-Pak C18 reverse phase column (Waters Assoc., Milford, MA). Ethanol and fructose coelute from Aminex columns but measurement of ethanol with Radial-Pak C18 reverse columns allowed estimation of fructose by difference. In this study the Sugar Pak column resolved both the sugars and the major fermentation products, and avoided problems encountered in the HPLC analysis of vegetables that have been noted by several investigators (Conrad and Palmer 1976; Bushway et al., 1984; McFeeters et al, 1984). Potential interference from fermentation-produced glycerol (Pederson and Albury, 1969) could occur because this compound coeluted with mannitol from the Sugar Pak column. However, parallel analysis of sauerkraut samples using a Bond-a-Pak carbohydrate column (Waters Associates, Milford, MA) which clearly separated glycerol from other fermentation-related constituents showed that only trace amounts were present. Thus, it was concluded that this limitation was of little consequence in the analysis of sauerkraut.

Typical separations of sugars and mannitol from fresh cabbage and sauerkraut with a Sugar Pak column are shown in Fig. 1A and B, respectively. All HPLC runs were completed within 20 min, and minimum detection concentrations for each sugar and mannitol were about 10 ppm. Sorbitol served as a suitable internal standard in these analyses, and it was stable to extraction procedures employed. Recoveries of sugars through the analysis were determined using the method of Dunmire and Otto (1981), and concentrations were selected for each substance that were typical of these encountered in corresponding sauerkraut and cabbage samples. Recoveries of sucrose, glucose, and fructose from cabbage through the analysis procedure were 92, 97, and 95%, respectively; while recoveries for glucose, fructose and mannitol in sauerkraut were 95, 97, and 98%, respectively. The coefficient of variation was less than 5% for each of the substances in both types of samples. Quantitative recovery of sugars and mannitol after passing through the alumina Sep-pak units was observed which was in agreement with earlier reports (Waters Associates, 1982b).

Traditional procedures for the isolation of sugars from plant materials have employed lengthy extractions with aqueous ethanol solutions (AOAC, Sec. 3.002, 1975; Conrad and Palmer, 1976; McBee and Maness, 1983; Wight and van Niekerk, 1983). Since one of the goals of this investigation was to substantially shorten the time to perform sugar analyses, the procedure employed a high-speed disintegrator to blend samples during an abbreviated heat treatment. Data in Table 1 for cabbage show that the blending procedure provided results comparable to the reflux method of Conrad and Palmer (1976), and the practice shortened the extraction time from about 1.5 to 0.5 hr. Similar observations on the suitability of high-speed

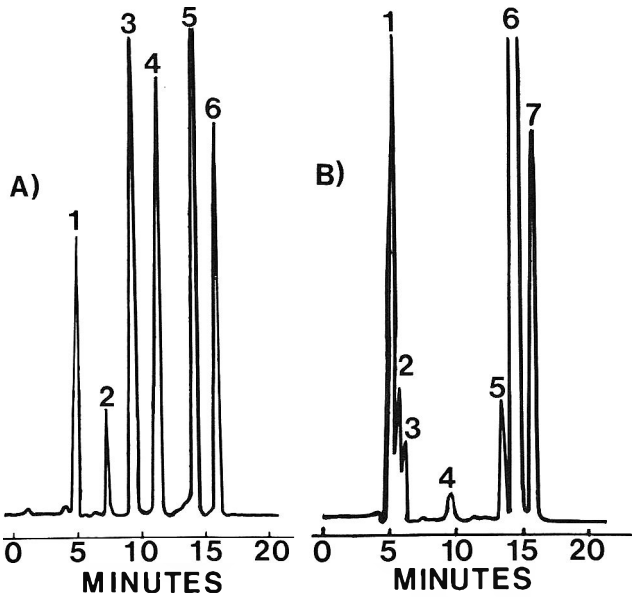

Fig. 1—HPLC separations obtained with a Sugar Pak column: (A) Cabbage: peak 1, unknown; 2. sucrose; 3, glucose; 4, fructose; 5, ethanol; 6, sorbitol (I.S.); (B) Sauerkraut: peak 1, sodium chloride; 2, unknown; 3, unknown; 4, glucose; 5, mannitol; 6, ethanol; 7, sorbitol (I.S.).

Table 2—Reproducibility of HPLC analysis of sugars and mannitol in cabbage and sauerkraut

Sugar	Concentration[a,b] (%, wet wt)	CV[c] (%)
Cabbage		
Glucose	2.35	0.65
Fructose	1.98	2.90
Sucrose	0.26	2.73
Sauerkraut		
Glucose	0.14	10.90
Fructose	0.04	14.40
Mannitol	0.47	16.70

[a] Means of 6 replicate analyses each for one lot of shredded and blended cabbage; and one lot of unblended sauerkraut.
[b] Mannitol not found in cabbage; sucrose not present in sauerkraut sample
[c] CV (%) = (Standard deviation/Mean) × 100

disintegrators for preparation of plant materials for sugar extractions have been made by Hurst et al. (1983) and McFeeters et al. (1984).

In studies to verify the reproducibility of the analytical procedure, replicate analyses (Table 2) gave a coefficient of variation of less than 3% for sugars in cabbage, and less than 15% for sauerkraut. The greater percentage variability for sauerkraut than for cabbage appeared to result from the low concentrations of sugars in sauerkraut.

While the data presented in this paper for the analysis of sauerkraut were developed with intact sauerkraut samples, earlier reports have indicated that analysis of either brine from fermented vegetables or the fermented vegetables for sugars gives comparable results (Peterson and Viljoen, 1925; McFeeters et al., 1984). Data shown in Table 3 for sauerkraut and brine samples obtained from tanks in this study generally supported earlier suggestions that comparable results can be expected from either type of sample. Some differences (<0.5%) in mannitol concentrations can be seen between brine and sauerkraut samples, and these probably reflect initial sampling variability as well as a component of analytical procedure variation. However, brine samples should provide reasonably reliable analytical results, and total analysis time would be reduced

Table 3—Comparison of sugar and mannitol concentrations in brine and intact sauerkraut

Weeks of fermentation	Samples[a]	Concentrations (%, wet wt) Sucrose	Glucose	Fructose	Mannitol	Total carbon source
2	Brine	0.0	1.9	0.2	2.2	4.3
	Sauerkraut	<0.05	1.9	0.2	—[b]	—[b]
3	Brine	0.0	0.1	0.1	2.1	2.3
	Sauerkraut	<0.05	0.1	0.1	1.9	2.1
4	Brine	0.0	<0.05	0.0	1.1	1.1
	Sauerkraut	<0.05	<0.05	0.1	1.0	1.1
5	Brine	0.0	0.0	0.0	1.1	1.1
	Sauerkraut	0.0	0.0	0.0	1.2	1.2
6	Brine	0.0	0.0	0.0	1.3	1.3
	Sauerkraut	0.0		0.0	1.2	1.2
7	Brine	0.0	0.0	0.0	1.1	1.1
	Sauerkraut	0.0	0.0	0.0	1.2	1.2
8	Brine	0.0	0.0	0.0	1.2	1.2
	Sauerkraut	0.0	0.0	0.0	1.1	1.1
9	Brine	0.0	0.0	0.0	1.1	1.1
	Sauerkraut	0.0	0.0	0.0	0.7	0.7
10	Brine	0.0	0.0	0.0	1.2	1.2
	Sauerkraut	0.0	0.0	0.0	0.9	0.9
11	Brine	0.0	0.0	0.0	1.0	1.0
	Sauerkraut	0.0	0.0	0.0	0.9	0.9
12	Brine	0.0	0.0	0.0	0.8	0.8
	Sauerkraut	0.0	0.0	0.0	0.8	0.8
13	Brine	0.0	0.0	0.0	1.1	1.1
	Sauerkraut	0.0	0.0	0.0	1.1	1.1
14	Brine	0.0	0.0	0.0	0.6	0.6
	Sauerkraut	0.0	0.0	0.0	0.8	0.8
15	Brine	0.0	0.0	0.0	0.7	0.7
	Sauerkraut	0.0	0.0	0.0	1.2	1.2
18	Brine	0.0	0.3	0.0	0.9	1.2
	Sauerkraut	0.0	0.1	0.0	1.2	1.3

[a] Samples taken from the thief hole of the tank located near top of tank; results of single analysis; CV for method of sauerkraut within 17% for all compounds.
[b] Data not available.

by the amount of time devoted to the blending and heating steps required for intact sauerkraut samples.

Analysis of cabbage for free sugars

Since variable initial concentrations of sugars in cabbage have been viewed as a potential cause of uneven fermentations as well as unexpected secondary fermentations, samples from a variety of cultivars of cabbage were analyzed for sugars (Table 4). Some cultivars were well established while others were in developmental stages, but representatives were distributed throughout a range of about 3–6% (wet weight) total sugars. These data reveal that a great deal of variation in the extent of acid development during fermentation can be expected among the various cultivars of cabbage, and this should be extremely important from a production standpoint. The high concentrations of total sugars found in many of the cabbage cultivars would be expected to lead to a growth-limiting pH before sugars were exhausted. Such residual sugars in sauerkraut would provide nutrients for secondary fermentations if suitable conditions were provided during subsequent post-processing handling and distribution of fresh sauerkraut. In addition, even though Maillard-type browning reaction rates are slow under acid conditions (Waller and Feather, 1983), the presence of residual reducing sugars and amino acids could contribute to browning discolorations in thermally processed sauerkraut.

Glucose concentrations in the cabbage samples were consistently elevated over fructose levels, and glucose was also the most abundant sugar present. Sucrose concentrations were quite low, and did not exceed 0.5% in any of the cross-sec-

Table 4—Concentrations of sugars in various cultivars of cabbage grown in Southern Wisconsin and in retail cabbages

Cabbage total cultivars[b]	Concentrations (%, wet wt)[a]			
	Sucrose	Glucose	Fructose	Sugars
55-694	0.2	1.7	1.4	3.3
King Cole	0.2	1.7	1.4	3.3
T-90	0.1	2.0	1.5	3.6
Kraut Packer	0.2	2.0	1.6	3.8
Falcon	0.2	2.0	1.6	3.8
Ocala	0.2	2.1	1.6	3.9
Roundup	0.2	2.1	1.7	4.0
Rio Verde	0.3	2.2	1.7	4.2
Sanibel	0.3	2.3	1.7	4.3
Condor	0.2	2.5	1.7	4.4
Bravo	0.3	2.5	1.8	4.6
T-90 (N. WI)	0.2	2.4	2.0	4.6
Little Rock	0.4	2.5	1.8	4.7
Grand Slam	0.3	2.5	2.0	4.8
Gourmet (S.E. WI)	0.5	2.7	1.8	5.0
Kraut King	0.3	2.7	2.2	5.2
Unknown (retail)	0.3	3.0	2.7	6.0
Unknown (retail)	0.3	3.3	2.4	6.0

[a] Means of analysis of two heads from each cultivar; CV for method for cabbage within 3% for all sugars.
[b] Grown in Southern Wisconsin trial plot unless otherwise noted.

Table 5—Concentrations of sugars in various locations of heads of cabbage

Cabbage culti-vars[a]	Location in head[b]	Concentrations (%, wet wt)			Total sug-ars
		Sucrose	Glucose	Fructose	
Unknown (retail)	Outer	0.2	2.8	2.2	5.2
	Middle	0.2	2.9	2.4	5.5
	Center	0.4	2.8	2.5	5.7
T-90	Outer	0.2	2.2	1.5	3.9
	Middle	0.2	2.2	1.8	4.2
	Center	0.3	1.9	1.9	4.1
Kraut King	Outer	0.2	1.2	1.1	2.5
	Middle	0.2	1.5	1.4	3.1
	Center	0.2	1.8	1.6	3.6
Little Rock	Outer	0.6	1.9	1.4	3.9
	Middle	0.3	2.4	1.7	4.4
	Center	1.5	2.2	1.8	5.5
	Core	3.5	1.4	1.0	5.8
Unknown (retail)	Outer	0.2	1.2	1.1	2.5
	Middle	0.2	1.5	1.4	3.1
	Center	0.2	1.8	1.6	3.6
	Core	3.2	1.1	1.0	5.3

[a] Means of duplicate analysis for each sample; CV for method is less than 3% for all sugars.
[b] Sections are outer, the exterior 2.5 cm; middle, the 2.5 cm beneath the outer layer; and center, the 2.5 cm surrounding the core.

Table 6—Comparisons of concentrations of sugars and solids in heads of cabbage from selected cultivars

Cabbage	Concentrations (%, wet wt)				Solids content (% wet wt)
	Sucrose	Glucose	Fructose	Total sug-ars	
	(----------by HPLC this study----------)				(Sol. solids)
Unknown (retail)	0.2	1.5	1.3	3.0	4.2
Unknown (retail)	0.4	1.2	1.1	2.7	4.5
Sanibel	0.5	1.3	1.3	3.1	4.5
Grand Slam	0.2	1.8	1.3	3.3	4.5
Kraut King	0.4	1.8	1.7	3.9	4.8
Bravo	0.3	2.5	1.8	4.6	5.8
Falcon	0.3	2.3	1.7	4.3	5.9
Rio Verde	0.7	2.3	2.0	5.0	6.8
Condor	0.1	2.4	1.9	4.4	6.9
Little Rock	0.3	2.1	1.8	4.2	7.5
	(------by paper chromatography[c]------)				(Total solids)[d]
Early Head	0.3	1.4	1.1	2.8	6.0
Glory 61	0.05	1.6	1.1	2.7	6.6
Special Golden Acre	0.2	1.7	1.4	3.3	7.4
Red	0.5	2.1	1.7	4.3	9.1

[a] Duplicate samples for HPLC and refractometer analysis taken from homogeneously shredded cabbage from selected heads; CV for method within 3% for all sugars.
[b] Determined by refractometer and expressed juice.
[c] Lee et al. (1970).
[d] Presumably by oven method.

Table 7—Concentrations of sugars and mannitol in commercial sauerkraut samples

Sauerkraut samples	Container type	Concentrations (%, wet wt)[a]				
		Sucrose	Glucose	Fructose	Mannitol	Total carbon source
1	poly bag	0.0	0.1	0.0	0.3	0.4
2	bulk (fresh)	0.0	0.0	.05	1.2	1.2
3	glass jar	0.0	0.5	0.0	1.2	1.7
4	bulk (fresh)	0.0	0.2	0.0	0.9	1.1
5	canned	0.0	0.8	0.0	0.6	1.4
6	canned	0.0	0.4	0.1	0.7	1.2
7	canned	0.0	0.4	0.0	1.1	1.5
8	canned	0.0	0.6	0.1	0.8	1.6
9	canned	0.0	0.4	0.0	0.9	1.3
10	canned	0.0	0.3	0.0	0.8	1.1
11	canned	0.0	0.1	0.1	1.0	1.2
12	glass jar	0.0	0.0	0.0	0.3	0.3
13	glass jar	0.0	0.0	0.0	1.1	1.1

[a] Means of duplicate analysis of each sample; CV for method for sauerkraut is less than 17% for all compounds.

tional wedge samples (Table 4). Some variation in sugar concentrations was found within individual heads of cabbage (Table 5), and higher sugar concentrations seemed to be associated with the core (stem) and more-recently formed leaves. The cores of each of two samples of cabbage analyzed (Table 5) contained quite high concentrations of sucrose (3.5 and 3.3%). Practices of including entire cabbage heads in some sauerkraut fermentations could elevate the amount of sugars, but the relative proportions of cores to leaves in cabbage heads would minimize these effects.

Overall, data for sugars in cabbage substantiate limited earlier reports concerning free sugars in cabbage (Peterson and Viljoen, 1925; Stamer et al., 1969; Lee et al., 1970; Martin-Villa et al., 1982), and lead to the conclusion that measurement of fermentable sugars would be useful in controlling sauerkraut fermentations and quality. However, measurement of total sugars in cabbage by HPLC analysis would be difficult to implement in a sauerkraut production setting. Thus, selected samples of cabbage were analyzed for free sugars by HPLC and soluble solids by refractometer, and the data were compared in Table 6. Generally, soluble solids concentrations appeared to reflect those of total sugars by HPLC although the soluble solids measurement included about 1.5–2.5% additional solids that probably are composed of ascorbic acid, amino acids, and possibly oligosaccharides. Earlier data reported by Lee et al. (1970) (Table 6) indicate that total solids measured for cabbage also appear to relate to those of measured free sugars. Based on these limited data, it would appear feasible to pursue the development of a valid association between solids measurements and HPLC sugar analysis data for use in predicting amounts of free sugars in cabbage that would be available for sauerkraut fermentations.

Residual glucose was found in a majority of the commercial sauerkraut samples analyzed (Table 7), and the concentrations were comparable with the reducing sugar levels found much earlier by Peterson and Viljoen (1925). Total carbon source ranged from 0.4–1.7% among the commercial samples, and most of this was contributed by mannitol. The rate of depletion of individual sugars and the accumulation of mannitol during the fermentation of a commercial tank of Sanibel cabbage is illustrated in Fig. 2. This cultivar of cabbage contained an intermediate concentration of total sugars (about 4%; Table 4), and depletion of all sugars occurred with 4 wk. Glucose persisted longer than sucrose or fructose, but was absent except

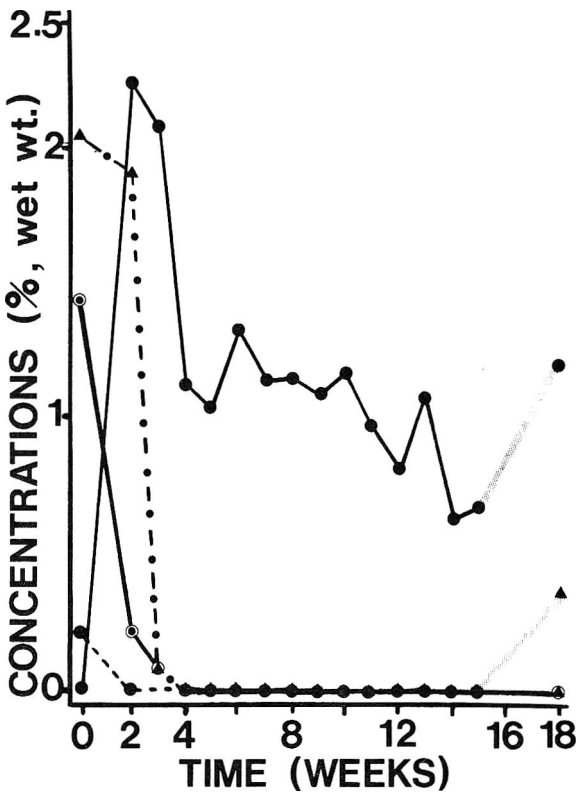

Fig. 2—Concentrations of free sugars and mannitol found in sauerkraut from a commercial tank through 18 wk of fermentation (Sanibel cultivar; 3.05m × 4.88m diameter wood tank; samples drawn from the top level of the tank): (●--●, Sucrose; ▲—●—▲, Glucose; ⊙———⊙, Fructose; ●———●, Mannitol).

in very late stages of sampling (18 wk). Peterson and Viljoen (1925) have reported that about 0.4% residual reducing sugars (glucose) remain in sauerkraut after the fermentation is complete and titratable acidities from 1.4–1.9% have been achieved.

Mannitol which can serve as a carbon source for microorganisms (Chen et al., 1983; Stinson, 1985) was produced rapidly presumably by *Lactobacillus sp.* in the fermentation (Fig. 2). The concentration of mannitol reached a maximum at 3 wk, and then declined slowly through 15 wk. Unexpectedly, at 18 wk both mannitol and glucose concentrations were elevated in the sauerkraut when it was analyzed, and at which time it was processed. Although the cause of the sudden increase in these compounds remained unresolved, it was speculated that some type of secondary metabolic activity had occurred. Slime (ropiness) or dextran is encountered sporadically in tanks of fermenting sauerkraut (Pederson, 1971), and it is usually attributed to growth of either *Leuconostoc mesenteroides* or *Leuconostoc dextranicum* early in the fermentation when sucrose is still present and the pH is only modestly low (Fruton and Simmonds, 1985; Buchanan and Gibbons, 1974). Dextran accumulations (slime) often disappear upon holding of sauerkraut, and hydrolysis could account for the unexpected appearance of glucose in the late stages of fermentations. Conversion of some of this nutrient to mannitol by viable *Lactobacillus sp.* (Pederson, 1971; Fleming et al., 1983) could also account for the simultaneous elevation of mannitol observed for the tank of sauerkraut (Fig. 2). Therefore, it would appear worthwhile to investigate this possible source of carbohydrate nutrient in finished, raw sauerkraut as a factor in unexpected secondary fermentations (Pederson and Albury, 1969; Stamer and Stoyola, 1978; Chen et al., 1983).

Two additional tanks of sauerkraut were sampled at various depths as they were emptied for processing (Table 8). In each of these tanks, fructose and sucrose were virtually absent at all depths, although very small amounts of each were encountered at mid-tank depths in one sample. Notable concentrations (to 0.6%, wet weight) of glucose were found at all depths in both tanks, as was mannitol (to 1.4%, wet weight). Some variation in concentrations of mannitol and glucose between the various layers was also observed, but the location in the tank did not appear to be a reliable indicator as to expected high or low concentrations of residual fermentable substrate. Data for a variety of commercially packed sauerkraut samples discussed earlier (Table 7) also showed that many contained considerable concentrations of glucose, but sucrose and fructose were virtually absent. Mannitol concentrations ranged from about 0.3–1.3% (wet weight), in the samples from the various layers of the tanks, and again contributed most heavily to the total fermentable carbon sources measured in sauerkraut.

Table 8—Concentrations of sugars and mannitol found in samples from various depths in commercial tanks of sauerkraut at the time of processing

Sample location in tank[a]	Concentrations (% wet wt)						
	Sucrose	Glucose	Fructose	Mannitol	Total carbon source	T.A.[b] (%)	Salt (%)
Fermentation tank 1 (0.6m increments[c]							
Top	0.0	0.05	0.0	0.7	0.7	2.9	2.8
2nd	0.0	<.05	0.0	0.8	0.8	2.9	2.7
3rd	<0.05	0.6	0.1	1.4	2.1	2.1	3.1
4th	<0.05	0.4	0.05	1.3	1.8	2.8	2.4
Bottom	0.0	0.1	0.0	1.1	1.2	2.8	2.4
Fermentation tank 2 (0.75m increments)[d]							
Top	0.0	0.5	0.0	1.3	1.8	2.0	1.3
2nd	0.0	0.3	0.0	0.8	1.1	2.0	1.5
3rd	0.0	0.4	0.0	0.9	1.3	1.8	1.4
4th	0.0	0.3	0.0	1.0	1.3	2.5	1.4
Bottom	0.0	0.3	0.0	0.9	1.2	2.2	1.8

[a] Means of duplicate analysis for each sample; CV for method for sauerkraut is less than 17% for all compounds.
[b] Titratable acidity expressed as % lactic acid.
[c] Prepared from a mixture of Sanibel and Grand Slam cultivars; fermented 5 wk.
[d] Prepared from T-90 cultivar; fermented 12 wk.

In summary, concentrations of individual sugars in cabbage and sauerkraut measured by liquid chromatographic procedures have been shown to vary widely among samples. The implications of these findings are that more predictable fermentations of cabbage and enhanced stability of sauerkraut could be achieved through control of fermentable carbon-based substrates. It would appear also that selection of cabbage cultivars for sugar content combined with attention to measured fermentable sugars in cabbage entering sauerkraut tanks would allow reasonable control of fermentations. Measurement of residual sugars in sauerkraut would allow selection of tanks essentially devoid of fermentable carbon substrates for use in market forms that are susceptible to secondary fermentation quality problems.

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*UNDECAENES IN FRUITS & VEGETABLES. . .*From page 1656

volatiles (e.g., apple, pear, pineapple (van Straten and Maarse, 1983)). Obviously, linear undecaenes and some alicyclic isomers are widespread olefins in the plant kingdom. Their occurrence and the triene (I):tetraene (II) ratios may correlate with climatic origin rather than with taxonomic relationship of fruits.

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Precooked Corn Flour for Venezuelan *Arepa* Preparation: Characterization of Particle Size Fractions

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ABSTRACT

Commercial precooked corn flour (used for Venezuelan *arepa* preparation) was sieved into fractions using a Ro-tap shaker. Particles 297 μm thick had the highest consistency. Particles 354 μm thick had the longest stability, their yellow color value (Hunter) was the smallest, and *arepas* made from them had the highest elasticity. Sedimented volumes were greater for particles 297 μm thick and coarser. Functional properties, evaluated by trained judges, were better for fractions with sizes between 297 and 420 μm. Regression analysis showed that particles between these sizes are the best suited for Venezuelan *arepa* preparation.

INTRODUCTION

IN VENEZUELA, *arepa* (traditional unleavened corn bread) has been an important part of the diet since ancient times. Earlier inhabitants of the country used to grind corn (called *erepa*) in hand milling stones, in order to prepare the corn bread from the dough so obtained (Cuevas et al., 1985; Padua and Padua, 1984). The dough could also be dried, and reconstituted with water during very long trips of nomad tribes (Coppens, 1980). In more recent times, several home processing practices have been developed to make dough and *arepas*. Presently, the use of precooked corn flour has replaced the traditional, time consuming methods for preparing corn dough. The main advantage of precooked flour is that by just adding water to it and hand mixing, a dough can be prepared within a few minutes. The *arepa* (prepared from precooked flour or in the traditional way) has to have a "typical" rigidity (as established by Venezuelan tradition), and a genuine color (white if white corn is used for dough or flour production).

Precooked corn flour is produced in Venezuela by steam and roll cooking; processing conditions affect the functional properties of the flour (Cuevas et al., 1985; Anderson et al., 1969a, 1969b). Padua and Padua (1984) studied the physical characteristics of Venezuelan *arepa* dough made from commercial precooked flour; they found that if the flour were milled in a pulverizing mill, a drastic change in the characteristics (viscosity, consistency and adhesiveness) of the dough was obtained. Cuevas et al. (1985) presented data on the properties of the coarse, medium and fine fractions of screened corn grits, flakes and flour (intermediate and final products in the commercial production of precooked corn flour); the data indicated that fine particles had a lower degree of gelatinization than the medium and coarse fractions. In pure starch gels, Eliasson and Bohlin (1982) found that particle size distribution influenced the rheological properties of the gels.

The current study was undertaken to evaluate the physical properties of the size fractions of commercial precooked corn flour, and the effect of flour particle size distribution on *arepa* dough, in order to identify (using functional properties evaluation by trained judges) the most suitable size for Venezuelan *arepa* preparation.

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MATERIALS & METHODS

VENEZUELAN commercial precooked corn flour (50g) was sieved into size fractions (32, 35, 42, 48, 60, 65, 80 mesh and pan in Tyler standard sieves, which corresponded to sieve openings of 500, 420, 354, 297, 250, 210 and 177 μm, according to Geankoplis, 1978). A Ro-tap testing machine was used long enough to obtain uniform and representative samples. Each fraction was analysed for several characteristics, which can be related to the suitability of the flour to form the dough for Venezuelan *arepa* preparation (Cuevas et al., 1985; Padua and Padua, 1984). Average chemical composition of the flour was (in percent): moisture, 12.47; protein (N × 6.25), 7.94; fat, 0.83; ash, 0.35; starch, 78.50 (all expressed in "as is" basis). Gelatinized starch, measured with glucoamylase and *o*-toluidine reagent (Chiang and Johnson, 1977) was 83.6 (percent of total starch). The *arepa* dough for all tests was prepared as follows (unless otherwise indicated): 40% flour (size fraction or whole) and 60% water were mixed in the farinograph at 23°C for 5 min at 40 rpm. The purpose of using the farinograph was to prepare the dough eliminating possible variations in hand making procedures; the relatively low speed was used to minimize any effect of mechanical mixing (other than preparing the dough).

Physical tests

Farinograph tests were carried out to measure peak consistency (in Brabender Units) and dough stability (in min), using the small (50g) bowl of a Brabender farinograph (model FA-MV100). Seventy five grams of a mixture formed with 60 parts by weight water and 40 parts of a flour size fraction or whole flour, was placed in the bowl and agitated at 63 rpm for 40 min (a long time was used to assure uniform dough formation because water pickup may not be uniform; Padua and Padua, 1984). The test was conducted at room temperature (23°C). The 60/40 ratio was selected for all samples and tests because it is very commonly used by housewives and cooks for *arepa* preparation in Venezuela, and hence has a practical implication (Cuevas et al., 1985). Furthermore, an acceptable dough, with good handling characteristics, is produced with 60% added water (Padua and Padua, 1984).

To measure elasticity, *arepas* were prepared following the method of Padua and Padua (1984): sixty nine grams dough was shaped into *arepas* (7 cm diameter and 1.7 cm thick), packed in polyester/polypropylene bags, cooked (in the bags) in boiling water for 6 min, and cooled in atmospheric air until they reached room temperature (23°C). Modulus of elasticity was determined in an Instron Universal Testing Machine (model 1132), fitted with the 454 kgf crosshead, which moved at 5.08 cm/min; chart speed, 30.48 cm/min. The samples were compressed with an acrylic plunger (68.51 cm^2 cross sectional area), and their elasticity was obtained in terms of the modulus of elasticity, as the ratio of stress to strain within the elastic range of the material (Mohsenin and Mittal, 1977).

Color of the *arepas* (prepared from size fractions or whole flour, as described for elasticity determination) was measured on a Hunter colorimeter (model D25D2), with a yellow standard (L = 78.2, a = 2.4, b = 21.7). Total color difference ΔE was calculated with respect to whole flour, using the equation $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$, where each term represents the difference in the color parameter between the fraction and whole flour (Bazúa et al., 1979).

Sedimentation of the flour fractions was obtained by a modification of the method of Pinckney et al. (1957) for wheat flour; the adapted method consists in weighing 14g flour (size fraction) and placing it into a 100 mL glass-stoppered graduated cylinder. Water at room temperature (25 °C) was added up to the 100 mL mark, and then the

flask was vigorously agitated, until the flour had been uniformly distributed and suspended; the cylinder was then left undisturbed, and sedimented volumes were recorded as a function of time. The data were plotted (volume vs time), and the initial slope of the curve was taken as the sedimentation rate (in volume/time).

Brookfield viscosity was determined by mixing the dough with water at 30 °C (70% water and 30% dough), to obtain a dough suspension. The viscosity was measured at room temperature (23 °C) with Brookfield model RVT using spindle No. 6 at speeds from 0.5–100 rpm; the readings were taken after 1 min of rotation at each speed. The apparent viscosity values were then plotted as viscosity vs. speed curves.

Chemical analyses

Protein, fat, ash, and moisture content of whole precooked flour were determined by the AOAC methods (1980). Total and gelatinized starch were measured according to the method of Chiang and Johnson (1977).

Functional properties of the flour

Three judges with previous experience in *arepa* preparation and sensory analysis were trained for the tests. The training given to the panel consisted in group sessions during which the judges prepared dough and arepas from whole flour (as are traditionally made in Venezuela from precooked flour), and evaluated the functional properties of the sample. Each of the three characteristics (mixability, adhesiveness, and moldability) were evaluated using a scale from one to five, where one was the worst and five the best score. It was not practical to have more than three expert judges because 200g of sample were needed for the test, but the amount of flour fraction obtained in each Ro-tap batch was small. However, the validity of the results was verified statistically. The flour samples were size fractions 420, 354, 297, and 250 + 210 μm, and whole flour, and were given to the judges randomly.

Dough was prepared from 40% flour (size fraction or whole) and 60% water (200g and 300g, respectively). Water was added to flour in a dish with one hand, while mixing with the other. When all water was added, the ingredients were mixed with both hands. The time required for obtaining a uniform and homogeneous dough (5 min) with whole flour was taken as reference. The difficulty or ease of dough formation and of water/flour mixing was evaluated during the mixing period, and taken as mixability. Dough adhesiveness was evaluated as the degree of sticking of the dough to the hands during mixing and once the dough was ready. Dough moldability was evaluated as the effort needed to shape a 100g *arepa* and the response of the dough to compacting and molding by hand.

Statistical evaluation

All physical tests were performed in duplicate, following completely randomized designs. Since some fractions were relatively small (in weight), and each Ro-tap batch consisted of 50g whole flour, large quantities of flour were screened for 30 min in order to obtain representative samples; the 50g used were taken randomly from 25 kg bags of commercial flour from a single production lot. The data from the physical and "sensory" tests were analyzed for the variance (Snedecor and Cochran, 1967). Regression equations were determined between each test and particle size (Draper and Smith, 1981). Using Lagrange's criterium (Himmelblau, 1970), a maximum or a minimum was found for the regression functions, and in those responses where a maximum or a minimum is desirable, the optimum particle size was calculated.

RESULTS & DISCUSSION

Particle size distribution

Particle size distribution is expressed as the mass (weight) fraction of a particular sample retained after sieving over a given screen (mesh), in relation to 50g whole flour. Most of the particles were between 297 and 420 μm (Fig 1), which correspond to particles retained by mesh 48 and mesh 35 screens, respectively. The semi-logarithmic coordinates were used to reduce data clustering (McCabe and Smith, 1976).

The results of Padua and Padua (1984) suggested that particle size affected the properties of the flour. However, those results were for particles obtained after the commercial flour was milled in a pilot plant Alpine mill, while the results reported herein are for particles of different sizes present in commercial precooked corn flour, as is obtained from the factory.

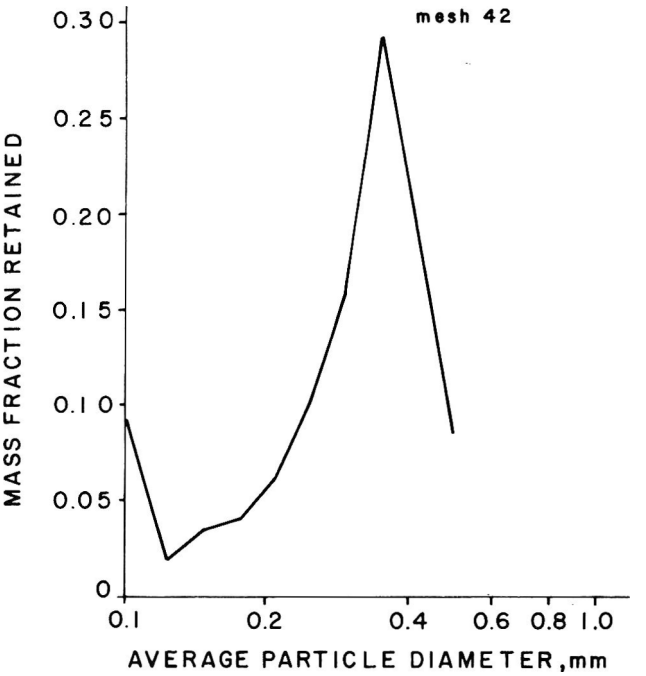

Fig. 1—Particle size distribution for a Venezuelan commercial precooked corn flour lot, in grams fraction/50grams flour. Particles of mesh 42 (354 μm) are the more highly concentrated; particles smaller than 177 μm correspond to mesh 100, 115 and the pan.

Table 1—Farinograph parameters for precooked corn flour size fractions

Average particle size (μm)	Tyler screen (mesh)	Peak consistency (Brabender Units)[a]	Stability (min)[b]
Whole flour	—	246	9.3
420	35	257 c	9.5 c
354	42	302 de	10.0 c
297	48	310 e	8.0 cd
250	60	305 de	5.5 de
210	65	300 de	3.0 ef
177	80	300 de	2.8 ef
<177	Pan	287 d	1.5 f

[a] Standard error of overall mean consistency = 4.31 B.U.; F = 2.62. Means followed by the same letter are not significantly different, P < 0.25 (in columns).

[b] Standard error of overall mean stability = 0.36 min; F = 14.43. Means followed by the same letter are not significantly different, P < 0.05 (in columns).

Physical tests

The farinograph consistency (Table 1) agrees fairly well with consistency reported by Cuevas and Puche (1984). A higher consistency was obtained for the fraction corresponding to mesh 48 (297 μm) (Table 1). Since *arepa* preparation includes a molding step, after the flour-water mixing step, it is important to have a dough with a high consistency. The farinograph stability is an indication of how long the dough stays at its peak consistency. From Table 1 it is clear that fraction mesh 42 (354 μm) had the longest stability among the fractions of precooked flour.

When the *arepa* was cooked in boiling water, a characteristic "cooked" texture developed, which may be related to the modulus of elasticity of the *arepa* (Padua and Padua, 1984). Particles 354 μm in size had the highest modulus of elasticity of all fractions of the flour (Table 2). These particles gave completely cooked *arepas*, which may be characterized as low-adhesiveness and high-elasticity products.

Table 2—Elasticity of arepas *prepared from precooked corn flour size fractions*

Average particle size (μm)	Tyler screen (mesh)	Modulus of elasticity (kgf/cm²)[a]
Whole flour	—	1.43
420	35	2.64 bc
354	42	2.93 b
297	48	2.72 c
250	60	2.55 d
210	65	1.45 d
177	80	1.30 de
<177	Pan	1.37 e

[a] Standard error of overall mean modulus of elasticity = 0.03 kfg/cm²; F = 70.0; means followed by the same letter are not significantly different, P < 0.05.

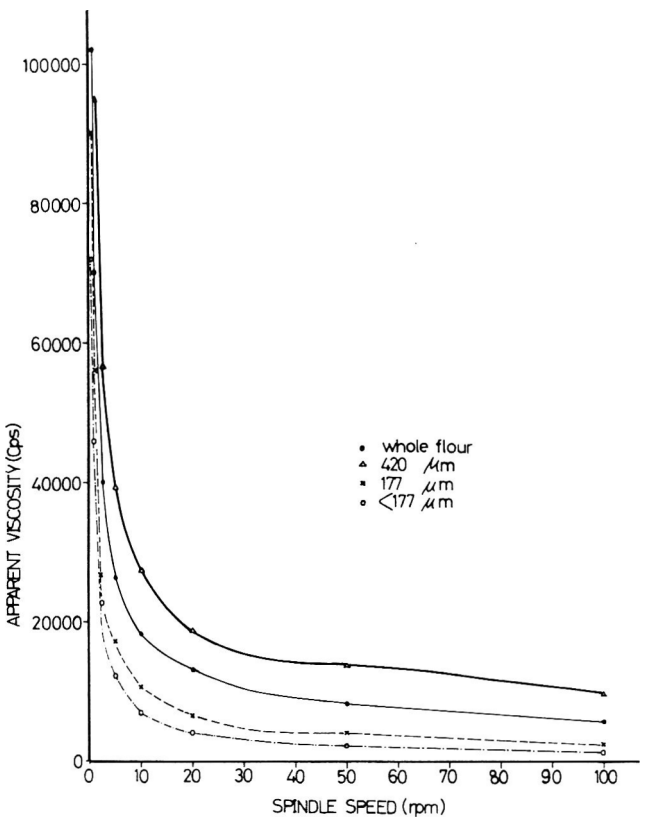

Fig. 2—Brookfield apparent viscosity of dough suspensions made from size fractions of precooked corn flour (spindle No. 6, 23 °C).

The Brookfield apparent viscosities were ploted as a function of rotational speed of the viscosimeter spindle (Fig 2). The dough suspensions behaved as non-Newtonian fluids, agreeing with the results reported for whole flour by Padua and Padua (1984) for several commercial lots. Those authors used a 25% dough suspension, while 30% suspensions were used in this study. Non-Newtonian behavior becomes accentuated as particle size of the flour fractions becomes smaller; therefore, it is expected that finer particles will form dough suspensions which are thinner or have lower apparent viscosities than those prepared with coarser particles.

Tables 1 and 2 and Fig. 2 show that a coarser flour fraction formed a more consistent dough and a more rigid *arepa*. It has been demonstrated (Cuevas et al., 1985) that during the industrial processing of corn to obtain precooked flour, the fine fractions (45% under mesh 80 and 94% under mesh 60) of milled intermediate and end products showed a lower degree of gelatinized starch than coarse fractions (84% over mesh 35). The degree of gelatinization of starch in corn and other cereals was due mainly to the intensity (time-temperature) of the heat treatment during steam and roll cooking, and to the moisture level in corn (Cuevas et al., 1985; Peplinski and Pfeifer, 1970; Anderson, 1982; Anderson et al., 1969a, 1969b; Powell, 1967; Whistler, 1970). Furthermore, the type of corn (horny or floury) affected the amount of fines produced at the flour plant (Cuevas et al., 1985; Manoharkumar et al., 1978; Kikuchi et al., 1982). Since the results in this study indicated that a relationship existed between particle size and physical properties of the flour, further investigation is needed to trace that relationship to corn type, processing conditions and/or other factors during production of commercial flour.

Sedimentation tests gave an idea of the behavior of the flour, under undisturbed conditions (being acted upon by gravity forces only), when put in contact with water. The time scale for settling for coarse particles was in seconds, while for small particles it was in minutes. These results suggested that since small particles will settle very slowly (10 min to 1 hr), in practical situations it is better to have a flour with large particles, because they are easier to handle and easier to mix with water.

According to Venezuelan tradition, *arepa* color should be traced back to corn color; boiled *arepas* from white corn are traditionally white. There was a tendency for the color of *arepas* to depart from the desired whiteness, as a function of particle size (Table 3). Since whiteness increased as parameter "L" increased (0 = black; 100 = white), it is evident that smaller particles formed *arepas* that were darker than those prepared from larger particles. The Hunter "a" value (greenishness) showed small values for large and small particles. In general, with respect to *arepas*, it is better to find values of "a" and "b" close to zero (small values in "a" and "b" indicate small departures from whiteness). The fraction with particle size of 354 μm had the smallest "b" value. As *arepas* were prepared from smaller particles, their color differed more from the color of *arepas* made from whole flour. It is, therefore, desirable to have a flour with large particles (around 354 to 420 μm) so that *arepas* have a clear white color. The values of modulus of elasticity (Table 2) and the values of total color difference ΔE (Table 3) indicate that large values of ΔE correspond to small values of elasticity, which means low degree of gelatinization and small particle size fractions. Again, a relationship is evident between particle size and functionality of the flour.

Functional properties of the flour

The practical implication of the physical tests was given by functionality evaluation of the flour fractions by trained judges. The fraction with particle size of 297 μm was "easier" to mix with water, and the 354 μm fraction was "easier" to form into an *arepa*, as shown in Table 4. All fractions were similar with respect to adhesiveness.

An analysis of variance of the data in Tables 1 to 4 revealed

Table 3—Color of arepas *prepared from precooked corn flour size fractions*

Average particle size (μm)	Hunter parameter[a]			
	"L"[b]	"a"[c]	"b"[d]	"ΔE"[e]
Whole flour	76.50	−2.50	12.50	--
420	73.75 f	−1.50 f	10.40 f	3.47 f
354	73.00 fg	−1.65 g	9.95 f	4.42 f
297	72.30 g	−2.65 f	11.15 f	4.45 f
250	72.20 g	−3.15 f	11.55 g	4.46 f
210	71.15 g	−2.65 f	12.25 gh	5.42 f
177	68.60 h	−1.55 g	12.35 gh	7.96 g
<177	64.60 i	−1.35 g	13.10 h	11.98 h

[a] Yellow standard: "L" = 78.2, "a" = 2.42, "b" = 21.7, "ΔE" = $(\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$; means followed by the same letter (in columns) are not significantly different, P < 0.05.
[b] Standard error of overall mean "L" = 0.22 "L" units; F = 32.06.
[c] Standard error of overall mean "a" = 0.09 "a" units; F = 8.85.
[d] Standard error of overal mean "b" = 0.15 "b" units; F = 9.39.
[e] Standard error of overall mean "ΔE" = 0.23 "ΔE" units; F = 25.50.

Table 4—Functional properties of dough and arepas, *prepared from precooked corn flour size fractions*[a]

Average particle size (μm)	Mixability with water[b]	Moldability[c]	Adhesiveness[d]
Whole flour	4.3	4.3	3.0
420	1.0 e	3.3 e	3.0 e
354	2.6 f	4.0 e	3.0 e
297	3.0 f	3.3 e	3.0 e
250 + 210	2.3 f	2.3 f	3.3 e

[a] Means followed by the same letter (in columns) are not significantly different, $P < 0.05$.

[b] 1 = very difficult to mix, 5 = very easy to mix; standard error of overall mean mixability = 0.15; F = 5.67.

[c] 1 = very difficult to mold, 5 = very easy to mold; standard error of overall mean moldability = 0.12; F = 13.87.

[d] 1 = very adhesive, 5 = nonadhesive; standard error of overall mean adhesiveness = 0.45; F = 0.11.

Table 5—Optimum particle diameter of precooked corn flour for physical and sensory responses[a]

Response	Optimum particle size (μm)	Tyler screen (mesh)
Stability	380	35-42
Hunter parameter "L"	362	35-42
Elasticity	368	35-42
Hunter parameter "b"	391	35-42
Brookfield apparent viscosity[b]	358	35-42
Mixability	303	42-48
Moldability	362	35-42

[a] Obtained from third degree polynomials : response = f(particle size).

[b] Smallest slope of logarithmic plots of apparent viscosity vs speed.

that it can be concluded (from the statistical point of view) that the size of the flour particles had a real effect on the physical and functional properties of the fractions, and hence of whole flour. The validity of using three expert judges in the functionality tests was confirmed by the analysis of variance (significant F values).

Among the responses studied in the physical and functionality tests, most of them (except sedimentation and "L" value) yielded values which may be fitted to polynomial equations. Lagrange's criterion (Himmelblau, 1970) was applied to third degree models (obtained by regression techniques; Draper and Smith, 1981), and the result was that most of the responses have a maximum (one has a minimum). The particle size for this value was calculated (Table 5). Corn processing to obtain precooked corn flour should be done in such a way that flour particle sizes lie between 420 and 297 μm (mesh 35 and mesh 48), the most desirable size being around 360 μm. This should give a flour with suitable characteristics for Venezuelan *arepa* preparation.

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concentration of sulfur dioxide in the headspace than either the free or total sulfur dioxide in the product.

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Baking Studies with Winged Bean (*Psophocarpus tetragonolobus* L.DC) Flour–Wheat Flour Blends

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ABSTRACT

Physical, rheological and baking properties of winged bean-wheat composite flours were studied and the acceptability of the bread was evaluated by sensory tests. The sponge-dough method (SPD) gave bread with significantly greater specific loaf volume ($P<0.05$) than the straight-dough method (STD). Incorporation of 1% sodium stearoyl 2-lactylate (SSL) significantly improved ($P<0.05$) the specific loaf volume at all concentrations of winged bean full-fat flour (WBFF) tested, except for 15 and 20% substitution, for both STD and SPD methods. Using the STD method, 5% to 8% substitution of WBFF without SSL and 10% substitution with SSL gave acceptable breads. Using the SPD method, WBFF substitution at 10% without SSL and 12% with SSL gave acceptable breads.

INTRODUCTION

THE WINGED BEAN (*Psophocarpus tetragonolobus* L.DC), so named because of the wing-like flanges of its pod, has received considerable international attention during the last decade due to its promising, but neglected, potential as a food source. Even though this plant has been cultivated for centuries as a garden crop in many Southeast Asian and African countries, it was brought to the limelight as a potentially useful crop only after an extensive survey was undertaken by the U.S. National Academy of Sciences in 1974 (U.S. National Academy of Sciences, 1975). Available information indicates that, like the soybean, winged bean seeds have a relatively high concentration of protein ranging from 32–43% and an oil concentration ranging from 15–22% (Ekpenyong and Borchers, 1978; Garcia and Palmer, 1980; Okezie and Martin, 1980; Sri Kantha and Hettiarachchy, 1981).

In most developing countries, climatic and soil conditions are not suitable for wheat cultivation. Such countries spend much money to import wheat flour for bread and other baked food items. Several third world countries have encouraged the setting up of programs to study the feasibility of alternate locally available composite flours as a substitute for wheat flour. The winged bean's natural habitat is in the humid tropics, which includes many developing and wheat-importing countries. Hence the use of winged bean flour as a substitute for wheat flour in baking seems desirable.

When compared to soy flour, few investigations have been reported on the use of winged bean flour in bread and other leavened and unleavened products. Gim and Lin (1978) reported that inclusion of 5% winged bean flour in a composite flour (75% wheat — 20% cassava — 5% winged bean) gave bread with acceptable specific loaf volume and sensory characteristics. Chang et al. (1979) studied the baking properties of winged bean flour and reported that a 10% enrichment of wheat flour with winged bean flour was feasible without any undesirable sensory effects. Okezie and Dobo (1980) found that, above 15% supplementation with winged bean defatted flour, dough stability, dough development time, extensibility and mixing tolerance of a composite flour (wheat-triticale-winged bean) were adversely affected.

The purpose of this study was to examine the physical, rheological, and baking properties of winged bean-wheat composite flours and to determine the acceptability of the resulting bread using sensory tests.

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MATERIALS AND METHODS

Preparation of composite flours

Mature dried winged bean (WB) seeds, obtained from the University Farm, Peradeniya, Sri Lanka, were soaked in a 1% sodium hydrogen carbonate and 1% citric acid solution, overnight at room temperature (27 ± 2°C). When the beans were soaked in 1% sodium hydrogen carbonate, they became softer which facilitated the easy removal of hulls. A 1% citric acid solution was added to reduce odor and to improve texture (Citroreksoko, 1981). The soaked beans were boiled in water for 20–30 min and dehulled (grain dehuller, The Baur Bros. Co., U.K.). The dehulled beans were blanched (100°C, 5 min), oven-dried (90°C, 2 hr), roasted (60°C, 5 min), ground in a micro pulverizer grinder (U.S. Filter Corp., New York) and sieved through an 80 mesh screen. The wheat flour (extraction rate 72% and protein content 12%, d.b.) used in this study was a blend of hard red spring and winter wheat milled by Prima Flour Milling Corp., Sri Lanka.

Blends containing 5, 8, 10, 12, 15, and 20% of winged bean full-fat flour (WBFF) and wheat flour (WF) were prepared by gradual mixing of WBFF into WF in an electric mixer (Artofex Mixer, Artofex Engineering Works Ltd., Middlesex, England).

Physical properties of single and composite flour

Particle size of WBFF and WF was determined according to the methods given in the Ceylon Standard of Specifications for wheat Flour (Anon., 1972a). Granularity was determined by evaluating: (a) percent as mass passing through a 180 μm sieve and (b) percent as mass retained on a 160 μm sieve. For color grading, a 300g sample of flour was made into a smooth slurry with 50 mL distilled water, transferred into the sample cell of a Color Grader (Kent-Jones and Martin Flour Color Grader, Henry Simon Ltd., England) and the grade was recorded.

Protein dispersibility index (PDI) was determined according to AACC method 46-24 (AACC, 1976). Protein content was determined by AACC method 46-11 (AACC, 1976).

Rheological evaluation

Farinograph characteristics and extensigraph properties of the WBFF-WF blends were studied using AACC constant flour methods 54-21 and 54-10, respectively (AACC, 1976). The pasting properties of WBFF-WF blends were investigated with the Brabender Visco-Amylograph (Mazurs et al., 1957). Flour (60g) was suspended in 350 mL distilled water using a Waring Blendor and blended for 10 min. The suspension was poured into the amylograph bowl, and the blendor rinsed with 100 mL buffer solution. The slurry was heated uniformly from 25° to 95°C at a rate of 1.5°C per min, held for 15 min at 95°C, and then cooled uniformly to 50°C. Pasting temperature, peak viscosity and peak temperature were obtained from the amylograph curve.

Baking quality

The WBFF-WF blends were baked using the straight-dough (STD) method and sponge-dough (SPD) method according to AACC methods 10-10 and 10-11, respectively (AACC, 1976). The baking for-

mulae were modified according to the Ceylon Standard Specifications for White Bread (Anon, 1972b) (Table 1). Only white pan bread was produced. In each baking five loaves of bread were made. The bakings were repeated twice. The amount of water used was estimated from the farinograph absorption and from the feel of the dough during mixing. Emplex (SSL) (Patco Products, Kansas City, MO) was used as dough conditioner.

Evaluation of bread characteristics

Loaf volume was measured by rape seed displacement after cooling the bread for 10 min. Specific loaf volume (mL/g) was then calculated from average loaf weight and volume. An experienced panel of twenty judges composed of research officers of the Ceylon Institute of Scientific and Industrial Research (CISIR), Colombo, Sri Lanka, performed an evaluation of the sensory characteristics and acceptability of the bread.

All evaluations were conducted in individual booths which were maintained at 21 ± 1°C and equipped with variable lighting facilities. Bread samples were sliced into pieces of uniform thickness (1 cm) and size (11 × 8 cm) weighing approximately 28g. The samples were coded, randomized and served on a black plastic tray with plain water, 18 hr after baking. Sufficient water was provided for mouth rinsing between the samples. Crust and crumb color, crumb texture, symmetry and appearance, flavor and acceptability were scored on a hedonic scale of dislike extremely (1) to like extremely (9) (Larmond, 1977). All sensory evaluations were conducted under low red illumination except for color and appearance which were judged under 200-watt incandescent white bulbs. Sensory tests were repeated twice. The order of presentation was randomized each time.

Statistical analysis

An analysis of variance was used to test the data (Steel and Torrie, 1960). Differences among means were compared by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Physical characteristics of WBFF and WF

Data on the physical characteristics of WBFF and WF are given in Table 2. The color grade for WF was 2–3, which indicated an extraction rate of 72–74% (Kent-Jones and Mitchell, 1962) and is in accordance with the Ceylon Standard Specifications for WF (1972). WBFF-WF blends showed a higher grade (4–8) for the 5–20% blends, while WBFF alone gave values greater than 10. This indicated that WBFF contained seed coat particles.

Only 88% of the WBFF passed through a 180 μm sieve as compared to 99% for WF (Table 2). According to the Ceylon Standards of Specifications for WF (Anon., 1972b), a minimum of 98% should pass through the 180 μm sieve in order to be used in bread making. Therefore WBFF had coarser particles than WF, and the blends of WBFF and WF would have varied particle sizes. This would limit the amount of substitution of WBFF for WF for acceptable bread.

WBFF also showed greater PDI value than WF (Table 2), indicating that WBFF contained more soluble proteins than WF, PDI values for WBFF have not been cited in the literature. However, 12–20% PDI indicates adequate heat treatment to retain the nutritional value of protein for soy flours (De, 1971).

Rheological properties of WBFF-WF blends

Table 3 shows the farinograph behavior of the dough made from WBFF-WF blends. Water absorption increased as the amount of WBFF increased. This could be due to the increased protein content of the flour mixture. The arrival time increased greatly beyond 12% WBFF substitution, probably due to the slow hydration of the coarser WBFF and to the longer time required to develop an optimum gluten network in the dough. Since dough stability was greater for 5%, 8% and 10% substitution with WBFF than for WF (0% WBFF substitution), these blends could be mixed for the optimum time without damaging the gluten structure. The stability time, however, decreased with 12%, 15%, and 20% substitution. The dough development time increased as the % WBFF increased. From these data it could be said that, with up to 10% WBFF substitution, the dough would give good-quality bread. Beyond 10%, although the protein content increased, the gluten content becomes diluted since WBFF contains no gluten.

The energy of the dough and the resistance of the dough to extension decreased with increasing amounts of WBFF substitution (Table 4). The extensibility of the dough decreased as WBFF substitution increased, while the proportional num-

Table 1—Baking formulae[a] for Straight-dough and Sponge-dough methods

Straight-dough method		Sponge-dough method	
Wheat flour	100%	Sponge:	
Salt	2%	Flour	60%
Shortening	1.5%	Water	variable
Sugar	2%	Yeast	2.5%
Yeast (compressed)	2.5%		
Non-fat dry milk	4%	Dough	
Water	variable	Flour	40%
Potassium bromate	15 ppm	Water	variable
Sodium stearoyl[a] 2-lactylate (when used)	1%	Salt	2%
		Sugar	2%
		Non-fat dry milk	4%
		Shortening	1.5%
		Potassium bromate	15 ppm
		Sodium stearoyl[a] 2-lactylate (when used)	1%

[a] Values are listed as percent of flour

Table 2—Physical characteristics of winged bean full-fat flour and wheat flour[a]

Flour	Particle size (% < 180 μm)	Color[b]	PDI[c] (%)
Winged bean full-fat flour	88.00	>10	12.5
Wheat flour	99.00	2-3	10.2

[a] Mean of two determinations.
[b] Kent-Jones and Martin Flour Color Grader Units.
[c] PDI = protein dispersibility index.

Table 3—Farinograph characteristics[a] of doughs with different amounts of substitution of wheat flour by winged bean full fat flour (WBFF)

Sub. with WBFF (%)	Water absorption (%)	Arrival time (min)	Development time (min)	Stability time (min)
0	55.7	1.1	1.8	5.1
5	58.5	1.6	4.3	6.0
8	60.3	1.9	4.0	5.5
10	61.5	2.0	6.0	5.2
12	63.5	2.0	6.0	4.9
15	63.8	4.5	6.8	4.7
20	67.0	6.0	9.0	4.3

[a] Mean of two determinations.

Table 4—Extensigram values[a] of doughs with different amounts of wheat flour substituted with winged bean full fat flour

Sub. with WBFF	Energy of dough (cm^2)	Resistance to extension (BU)[b]	Extensibility (mm)	Proportional[c] number
0	100	410	147	2.80
5	75	372	127	2.93
10	70	367	122	3.00
15	53	305	98	3.11
20	48	315	96	3.30

[a] Mean of two determinations, at 135 min rest.
[b] BU = Brabender Units.
[c] Proportional number = ratio of resistance/extensibility.

ber (ratio of resistance to extensibility) increased. An increase in the proportional number indicates that the resistance to stretching, compared with the extensibility, is too great and, on fermenting, the dough tends to be short (Kent-Jones and Mitchell, 1962). Ciacco and D'Appolonia (1978) also reported an increase in proportional number when cassava and yam flour was incorporated into WF.

The pasting temperature increased as the amount of WBFF substitution increased (Table 5). The decrease in peak viscosity observed with increased amounts of WBFF can be explained by a reduction in the total amount of starch in the blends. WBFF alone did not produce an amylograph curve. However, when starchy flours, such as cassava or rice were used, the peak viscosity of the blends increased with increasing amounts of the non-wheat flours (Olatungi and Akinrele, 1978).

Baking method

Using the STD method, loaf volume was found to decrease as the amount of WBFF increased from 5 to 20%. The same trend was observed using the SPD method, except for 5% WBFF substitution (Table 6). Loaf volumes obtained with the SPD method were consistently greater than those of the STD method in all cases.

The specific loaf volume also showed similar trends (Table 6). Using the STD method, the specific loaf volume obtained for the control (3.33 ± 0.01) was significantly greater than for 5 to 20% WBFF-substituted breads ($P<0.05$). However, with the SPD method, the control showed a specific loaf volume (3.55 ± 0.04) which was not significantly different ($P<0.05$) from that of 5% WBFF-substituted bread (3.60 ± 0.02). The specific loaf volumes for bread from the SPD method was significantly different in all cases when compared to the STD method. Thus, the SPD method is a better method of bread making compared to the STD method, for preparing WBFF-substituted bread. Reduction in loaf volume for soy full-fat flour-substituted bread has been reported (Tsen and Hoover, 1973).

Table 5—Amylograph values[a] for wheat flour containing different amounts of winged bean full-fat flour (WBFF)

Sub. with WBFF (%)	Pasting temp (°C)	Peak viscosity (BU)	Peak temp (°C)
0	54.0	500	84.0
5	57.0	470	86.3
8	60.0	440	83.0
10	63.0	430	86.0
12	64.5	425	94.0
15	66.8	420	96.4
20	69.0	345	98.3
100[b]	0	0	0

[a] 60-g dry solids/450 mL water; mean of two determinations.
[b] Winged bean full-fat flour alone.

Table 6—Effect of the method of bread making on loaf volume and specific loaf volume of bread produced from a winged bean full-fat flour-wheat flour composite

Sub. with WBFF	Loaf volume[a] (mL)		Specific loaf volume[b] (ml/g)	
(%)	STD[b]	SPD[c]	STD[b]	SPD[c]
0[d]	1450.5 ± 2.5	1545.3 ± 1.0	3.33 ± 0.01b[e]	3.55 ± 0.04a
5	1372.4 ± 1.5	1550.2 ± 2.4	3.13 ± 0.03c	3.60 ± 0.02a
8	1277.6 ± 2.2	1390.4 ± 1.6	2.91 ± 0.01d	3.15 ± 0.06c
10	1254.5 ± 1.4	1305.3 ± 0.9	2.80 ± 0.05e	2.95 ± 0.03d
12	1177.3 ± 2.1	1190.4 ± 1.5	2.70 ± 0.02f	2.80 ± 0.01e
15	1156.2 ± 1.7	1166.6 ± 2.8	2.66 ± 0.01f	2.72 ± 0.01e
20	935.3 ± 2.1	980.4 ± 3.0	2.16 ± 0.04h	2.30 ± 0.06g

[a] Each value is a mean ± S.D. of 10 determinations from duplicate bakings with five loaves in each baking.
[b] STD = Straight dough method.
[c] SPD = Sponge dough method.
[d] 100% wheat flour (control).
[e] Means within columns not followed by the same letters are significantly different from each other ($P<0.05$).

Effect of sodium stearoyl 2-lactylate (SSL)

Using the STD and SPD methods, SSL improved the specific loaf volumes significantly ($P<0.05$) except at 15% and 20% substitutions (Table 7). The specific loaf volume of 5% WBFF-substituted bread, using the SPD method with SSL, was significantly greater than the control in contrast to the STD method. The highest specific loaf volumes were obtained for 5% WBFF-substituted bread using the SPD method and SSL. Okezie and Dobo (1980) reported that the use of food emulsifiers improved loaf volumes of bread made with defatted winged bean flour, triticale flour and WF composite mixtures.

Sensory evaluation of breads

Straight-dough method with and without SSL. The crust and crumb color did not vary significantly ($P>0.05$) with or without SSL, except for crumb color with SSL at 5% WBFF substitution (Table 8). Crumb texture did not vary significantly ($P>0.05$) from 0–10% WBFF-substitution when SSL was not employed, but beyond 10%, texture scores were significantly lower ($P<0.05$). It was observed that bread made with 5% and 8% WBFF without SSL did not vary significantly ($P>0.05$) in all of the sensory characteristics evaluated. There was significant variation in acceptability ($P<0.05$) among WBFF substitution without SSL, except for the 5% and 8% levels. When SSL was not used, the control (0% WBFF-substitution) had a significantly ($P<0.05$) higher acceptability score compared to all WBFF-substitution. Except for the 20% WBFF substitution, the addition of SSL significantly improved ($P<0.05$) the

Table 7—Effect of SSL[a] on specific loaf volume (mL/g) of breads made from winged bean-wheat composite flours[b]

Winged bean flour (%)	Straight-dough method		Sponge-dough method	
	No SSL	1% SSL	No SSL	1% SSL
0	3.33 ± 0.01a[c]	—	3.55 ± 0.03b	—
5	3.13 ± 0.03bc	3.40 ± 0.02a	3.60 ± 0.02b	3.80 ± 0.04a
8	2.91 ± 0.01de	3.29 ± 0.04ab	3.15 ± 0.05d	3.41 ± 0.02c
10	2.70 ± 0.04ef	3.11 ± 0.02bc	2.95 ± 0.03e	3.10 ± 0.02d
12	2.68 ± 0.02f	3.05 ± 0.01cd	2.80 ± 0.01f	2.91 ± 0.03e
15	2.66 ± 0.02f	2.70 ± 0.03f	2.72 ± 0.01f	2.75 ± 0.05f
20	2.16 ± 0.04g	2.20 ± 0.05g	2.30 ± 0.06g	2.32 ± 0.05g

[a] SSL = Sodium stearoyl 2-lactylate.
[b] Mean ± S.D. of 10 determinations from duplicate bakings with five loaves in each baking.
[c] Means within columns not followed by the same letters are significantly different from each other ($P<0.05$).

Table 8—Sensory qualities of winged bean-wheat flour composite bread (straight-dough method with and without SSL[a])[c]

Winged bean flour (%)	SSL[a] (%)	Crust color	Crumb color	Crumb texture	Symmetry and appearance	Flavor	Acceptability
0	0	7.05a [b]	7.5ab	7.2b	7.5bc	7.2b	7.3b
5	0	6.80ab	7.2b	7.0b	7.0cd	6.9bc	6.9c
8	0	7.10a	7.1b	6.9b	6.8d	6.8cd	6.9c
10	0	6.80ab	6.9b	6.8b	6.5d	6.5de	6.6d
12	0	6.55ab	5.0c	5.1d	5.0e	5.0f	5.3e
15	0	6.05c	4.7c	4.9d	4.3e	4.2f	4.9f
20	0	4.60d	3.6d	3.0e	2.8f	2.8g	3.4g
5	1	7.20a	8.0a	8.3a	8.4a	7.7a	8.0a
8	1	7.10a	7.4ab	7.2b	7.8b	7.2b	7.3b
10	1	6.60abc	7.0b	7.0b	7.4bc	6.9bc	6.9c
12	1	6.50abc	5.1c	6.0c	6.7d	6.2e	6.2d
15	1	6.25bc	4.8c	5.8c	5.8e	4.8f	5.5e
20	1	4.60d	3.8d	2.9e	3.2f	3.0g	3.6g

[a] SSL = Sodium stearoyl 2-lactylate.
[b] Each value is a mean of 20 observations. Means within columns not followed by the same letters are significantly different from each other ($P<0.05$).
[c] Values are from a nine-point hedonic scale: 1-dislike extremely; 5-neither like nor dislike; 9-like extremely.

acceptability scores. When SSL was used, 5% WBFF-substituted bread gave an acceptability score of 8.0, which was significantly greater ($P<0.05$) than that of the control. From the sensory evaluation results (Table 8), it can be seen that acceptable bread was produced with up to 5% to 8% WBFF-substitution without SSL and with up to 10% WBFF substitution with SSL.

Sponge-dough method with and without SSL. As with the STD method, there were no significant differences ($P>0.05$) observed in the scores for crust color between treatments with and without SSL (Table 9). The 5% and 8% WBFF-substituted breads without SSL did not show any significant differences ($P>0.05$) in all of the sensory characteristics evaluated. Using the STD method, the addition of SSL did not give a significantly greater ($P>0.05$) acceptability score at the 20% WBFF-substitution compared to that with no SSL. However, in the SPD method, the addition of SSL gave greater acceptability scores in all cases. It was observed that acceptable bread could be produced using the SPD method with up to 10% WBFF-substitution without SSL and with up to 12% WBFF-substitution with SSL.

CONCLUSIONS

THE COLOR (creamy yellow) and the coarse texture of the WBFF would limit the amount of substitution for WF to make an acceptable bread. The slow hydration of the coarser WBFF resulted in the longer time required to develop an optimum dough. The extensigraph studies indicated that with increased WBFF-substitution, the dough tended to be short. The SPD method was found to be a better method compared to the STD method for preparing WBFF-substituted bread. It was observed that increased levels of WBFF were detrimental to bread quality. However, the addition of SSL improved bread quality at the lower levels of WBFF-substitution. The taste panelists' comments and the test results showed that using the STD method, 5% to 8% substitution of WBFF without SSL and up to 10% substitution with SSL gave acceptable breads. Using the SPD method, WBFF-substitution at 10% without SSL and 12% with SSL gave acceptable breads. The incorporation of 1% SSL may increase the cost of production of bread. However, since WBFF contains a high amount of protein compared to the WF, the improved nutritional value may assume importance. This study suggests that this underutilized legume may be used as a WF supplement in bread making as it would enable the industry to save WF and at the same time open up a new field of application for WBFF.

Table 9—Sensory qualities of winged bean-wheat flour composite bread (Sponge-dough method with and without SSL[a])[c]

Winged bean flour (%)	SSL[a] (%)	Crust color	Crumb color	Crumb texture	Symmetry and appearance	Flavor	Acceptability
0	0	7.2ab[b]	7.6bc	7.8b	8.0b	7.5bc	7.8c
5	0	6.9abcd	7.3cd	7.4bc	7.5c	7.2cd	7.4cd
8	0	6.7cd	7.1d	7.0cd	7.2cd	7.0de	7.0de
10	0	6.5d	6.9d	6.7de	6.9d	6.5f	6.7ef
12	0	6.5d	5.4e	5.2g	5.4f	5.3g	5.6g
15	0	5.9d	4.5g	4.3h	4.0g	3.9h	4.4h
20	0	4.2e	3.0i	2.5j	2.4i	2.2j	2.8j
5	1	7.3a	8.5a	8.9a	8.8a	8.5a	8.6a
8	1	7.1abc	7.8b	7.6b	8.2b	7.7b	7.9b
10	1	6.9abcd	7.2cd	7.1cd	7.6c	7.4bcd	7.2d
12	1	6.8bcd	5.1ef	6.4ef	7.2cd	6.7ef	6.5f
15	1	6.5d	4.8fg	6.2f	6.1e	5.4g	5.8g
20	1	4.4e	3.9h	3.0i	3.2h	3.3i	3.7i

[a] SSL = Sodium stearoyl 2-lactylate.

[b] Each value is a mean of 20 observations. Means within columns not followed by the same letters are significantly different from each other ($P<0.05$).

[c] Values are from a nine-point hedonic scale: 1-dislike extremely; 5-neither like nor dislike; 9-like extremely.

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Factors Affecting Water Uptake in Milled Rice

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ABSTRACT

Water uptake in milled rice kernels was measured as a function of soaking condition, pretreatment (steaming at 107°C for 15 min), and variety. Soaking conditions included water to rice ratio (280g water/g dry rice solids (drs) and 0.55g water/gdrs), temperature (50°C and 90°C), and presence of solutes (salt, sucrose and/or glyceryl monostearate). Time constants, determined from an empirical equation, indicated water uptake was reduced by limited water, low temperature, or the presence of solutes (salt and/or sugar). Water uptake was enhanced when glyceryl monostearate was present in 0.55g water/gdrs and by steaming prior to soaking with sucrose present in 0.55g water/gdrs. Steaming broadened the DSC endotherm but did not cause gelatinization.

INTRODUCTION

WATER DIFFUSION and starch gelatinization are two important physical changes occurring during rice processing. Water can be reversibly absorbed into starch granules at room temperature, which results in a small amount of swelling. As the temperature is increased to the gelatinization temperature, swelling becomes irreversible. Starch granules within the kernel become disrupted, release exudate, and lose their crystallinity. This disordering is termed gelatinization and has profound effects on the functional, organoleptic and nutritional properties of the starch.

Many studies have been directed to water absorption and starch gelatinization of cereal starches other than rice (Becker, 1960; Fan et al., 1963; Ayernor and Steinberg, 1977; Kulp and Lorenz, 1981) or have focused on the parboiling process in rice before milling (Bandopadhyay and Ghose, 1965; Indudhara Swamy et al., 1971; Bandyopadhyay and Roy, 1977, 1978; Bakshi and Singh, 1979, 1980).

Cooking of cereal grains can be influenced by several factors. These include: moisture content (Collison and Chilton, 1974; Priestley, 1975), time and temperature of soaking (Suzuki et al., 1976, 1977), variety of amylose content (Bhattacharya and Sowbhagya, 1971; Shanthy et al., 1980), type of solutes in the cook water (Rockland et al., 1977; Savage and Osman, 1978; Evans and Haisman, 1982), steam pretreatment (Peplinski and Pfeifer, 1970; Priestley, 1975), and kernel age or time after harvest (Indudhara Swamy et al., 1978; Jones and Boulter, 1983). Few researchers have studied *in situ* water absorption into milled rice.

It has been pointed out that one may not assume a constant mass diffusivity when modelling water diffusion in starch systems (Chittenden and Hustrulid, 1966). A finite element model that accounts for the dependence of concentration of diffusion during the soaking of rice has been described (Zhang et al., 1984). Because experimental moisture gradient data were not obtained in this study, an empirical model based on simplicity and fit was used to differentiate the effects of conditions studied.

The objectives of the present study were to characterize physical and chemical properties of various rice varieties, to study water uptake rates in these varieties under conditions of limited and unlimited water, and to determine the effect of sucrose, sodium chloride, glyceryl monostearate, steaming and temperature rates of water uptake in rice kernels.

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MATERIALS & METHODS

THREE AGED (greater than 4 months old) rices were used in this study: (1) an Arkansas/Louisiana medium grain mixture (PR), Nato and Mars varieties; (2) California short grain rice (CSG), SG variety; and (3) Texas long grain rice (TLG), Lemont variety. Rice was packaged in heat-sealed aluminum laminated pouches, under vacuum, and stored at 5°C until use.

Amylose content of the rice flour was determined by the amperometric method of BeMiller (1964).

The chemical solutes, and their concentrations in aqueous solution, used were: (1) commercial food grade fine granulated sucrose (Southern Minnesota Sugar Cooperative, Renville, MN) at 30.0% (w/w); (2) reagent grade sodium chloride crystals (MC&B Manufacturing Chemists, Norwood, OH) at 4.0% (w/w); (3) commercial food grade surfactant, Myvaplex 600 (90.0% monomer, 1.0% glycerol, 91.0% saturated and 1.0% unsaturated fatty acid ester. Congeal point 70.0°C. Clear point 78.0°C. From Eastment Chemical Products, Inc., Kingsport, TN) at 0.2% (w/w).

Water absorption procedures

Prior to water absorption studies, rice was placed in a single layer on cheesecloth and allowed to equilibrate to room humidity for 2 days (a time determined to be sufficient). Initial moisture content was determined by AOAC (1975) vacuum oven procedure (5 hr at 100°C), after grinding 30 sec in an Analytical Mill (Tekman Co., Cincinnati, OH).

Procedure for absorption studies with unlimited water

Approximately 2g rice samples were weighed to an accuracy of ±0.00001g (Mettler H51AR) and placed in wire baskets with metal lids on top. Rubber bands secured the lid. There was sufficient cross-sectional area of the basket so that the rice kernels did not touch each other. The samples were placed in a constant temperature water bath (±0.5°C), which had been equilibrated to the desired temperature, containing the desired liquid for absorption. The volume was held constant at one liter and the liquid was buffered at pH 6.2 (0.0075M phosphate buffer). Continuous agitation in the water bath was provided by an overhead stirrer. At specified times (±1 sec), baskets were removed from the water bath with tongs and the soaked rice emptied onto four double layers of cheesecloth. If solutes were present in the water, the sample was dipped three times in room temperature distilled water to remove surface solutes before blotting.

The kernels were moved with a metal spatula to a dry area on the cloth, spread into a single layer and lightly and quickly blotted with another four layers of cheesecloth four to six times to remove the shiny layer of water from the rice. After blotting, the rice was transferred onto a prepared, glassine weighing paper and weighed to an accuracy of ±0.0005 (Mettler P162). Final solids content of the sample was then determined by the AOAC vacuum oven procedure.

Procedure for absorption studies with limited water

Approximately 2g rice samples were placed in dry, separate glass stoppered vials and equilibrated to the temperature of the water bath. Distilled water, buffered with phosphate to pH 6.2 at the desired temperature and containing the desired concentration of solutes, was equilibrated to temperature in the water bath. When the solution reached bath temperature, a predetermined amount was added to the equilibrated rice with a pipette. The amount of solution added was calcu-

lated from the initial water content of the rice and a required final moisture content of 0.55g water/g dry rice solids (drs). Distilled water at room temperature was added to the vial at the end of soaking to remove surface solutes. The rice was quickly removed, blotted as described for water absorption in unlimited water and weighted to an accuracy of ±0.0005g (Mettler P162). The AOAC vacuum oven procedure (1975) was used to determine total solids to calculate solute gain assuming no solids lost during soaking. Moisture content was determined by weight difference. Moisture values were expressed as total grams water present per gram drs.

Steaming

Two gram samples of raw milled rice were placed in wire baskets and subjected to steam treatment at 107°C (225°F) for 15 min in a retort. Come-up time of sample temperature was about 20 sec.

Modelling

Data obtained by soaking in limited (0.55g H_2O/g drs) and unlimited water were analyzed using a one-parameter model:

$$F = \exp[-t/\tau]$$

where $F = (M_{max} - M_t)/(M_{max} - M_i)$ = unaccomplished moisture ratio; M_{max} = 0.55g H_2O/g drs (the maximum amount of water of interest in the experiment); M_i = initial moisture content (d.b.); M_t = moisture content at time t (d.b.); t = time (min); τ = time constant representing 63.2% of the change in F.

Gelatinization was studied using a DuPont 990 Thermal Analyzer equipped with a cell base and differential scanning calorimetry (DSC) cell (E.T. DuPont De Nemours & Co., Inc., Wilmington, DE). Endotherms were obtained using a 10/1 (w/w) water to starch ratio and a heating rate of 10°C/min over the temperature range from 10° to 130°C (Chungcharoen, 1983). Water was used in the reference pan. Indium, with a known heat of fusion of 6.79 mcal/mg, was used for calibration purposes.

RESULTS & DISCUSSION

CHARACTERISTICS of rice varieties in this study are shown in Table 1. Grain categories are based on length to width ratios. Gelatinization temperature and cooking characteristics are directly related to grain length (Halick and Kelly, 1959; Webb and Stermer, 1972). Short and medium grain rices show similar gelatinization temperatures while long grain rices are typically 5–7°C higher (Webb, 1975). Our studies confirm that observation.

CSG had the greatest bulk density due to its relatively more spherical shape that allows for closer packing (Table 1). Amylose content (Table 1) was highest for the long grain rice and lowest for the medium grain rice. Even within a cultivar, amylose content may vary as much as 6% (Juliano, 1972). Amylose content will vary depending on the temperature at harvest, with warmer harvest conditions producing higher amylose rices. Thus, a variety grown in Texas may be expected to have a higher amylose content than that same variety grown in California (Webb, 1983).

Swelling and water uptake capacity during cooking are generally greater in high amylose rices, which are less sticky following cooking (Burns, 1972). However, amylose content alone is not a good indicator of water uptake at 77°C (Webb, 1975).

Differences in the degree of stickiness and the ability to hold granular integrity were observed for the rices soaked at 90°C with 280g water/g drs. Excessive clumping occurred for the short grain rice (CSG) after 55 min, at which time approximately 17% solids had been lost. At the other extreme, TLG rice did not show excessive clumping in 5 hr of soaking, although after more than 2 hr the TLG kernels were too soft to blot without damage. At 2 hr TLG rice had lost approximately 10% solids. Plant rice exhibited characteristics between those of CSG and TLG. Plant rice could be blotted up to 95 min, at which time 13% solids were lost. Although stickiness has been inversely related to amylose content (Webb, 1975; Burns, 1972) these studies indicate stickiness was related to grain length. It is generally known that the surface of long grain rices are drier after cooking than those of short and medium grain rices. Batcher et al. (1957) found California rices to be more cohesive or sticky after 20 min cooking than those of the same varieties grown in Texas.

During soaking of rice in unlimited water, solids were continuously leached from the kernel (Fig. 1). At 50°C, in 20 min, both CSG and PR rices lost about 2% of their initial dry rice solids due to leaching. Solid loss increased slightly up to 90 min. At 90°C, the rate of solids loss was greatest for CSG and least for TLG. At 20 min, the percentage of solids loss for TLG, PR and CSG were 4.3, 5.4, and 8.4%, respectively.

The power function was used to model the data in Fig. 1.

$$y = t^{(a)}$$

where: y = % solids loss (d.b.); t = time (min); a = coefficient.

The rate of solids loss was significantly greater for CSG at 90°C than for PR and TLG at 90°C. At 50°C, there was no difference between rates of solids loss in CSG or PR samples. Total amylose content of the original starch was not a good indicator of solids loss. Others have found the amount of solids in the cooking water to be inversely related to the total amylose content of the native starch (Burns, 1972). In this study, however, solids loss was inversely related to grain length and gelatinization temperature. This would be expected because starch with lower gelatinization temperatures would absorb water more rapidly and dissolution would start sooner (Juliano, 1972).

Rices with higher gelatinization temperatures may have more hydrogen bonds and greater association between their starch molecules that allow the kernel to endure heat-moisture stresses better than rices with low gelatinization temperatures. TLG has the highest gelatinization temperature and the least amount of solids loss at 90°C.

Though the type of starch molecule leaching from the soaked kernels was not determined in this study, the amount of amylose leached has been found to be proportional to the amount of amylose in the native starch after 15 min of soaking at 100°C (Juliano, 1972). The initial starch molecule leached was amylose (Lindqvist, 1979; Miller et al., 1973) and leaching was necessary for gelatinization to occur (Lindqvist, 1979).

Water uptake

Time constants (τ) for each soaking condition were calculated and values are presented in Tables 2 and 3. The time constant represents the time for 63% of the total change to

Table 1—Selected characteristics of rice samples

Rice source	Variety	Length/long diameter	Grain category	Density (g/cm³)	Bulk density (g/cm³)	Amylose[a] content (starch basis)	Gelatinization temperature[b] Range (°C)
California Pearl (CSG)	S-6	1.58	Short	1.42	0.83	22.3 (medium)	64-71-79 (63-73-89)[c]
Louisiana/Arkansas Plant Rice (PR)	Nato & Mars	2.19	Medium	1.37	0.81	18.4 (low)	66-74-82 (66-78-91)[c]
Texas Long Grain (TLG)	Lemont	3.15	Long	1.43	0.80	25.9 (high)	74-79-86 (72-81-92)[c]

[a] Amperometric titration method.
[b] Initial, peak and conclusion temperature as determined by differential scanning calorimetry.
[c] Gelatinization temperature range for steamed rice.

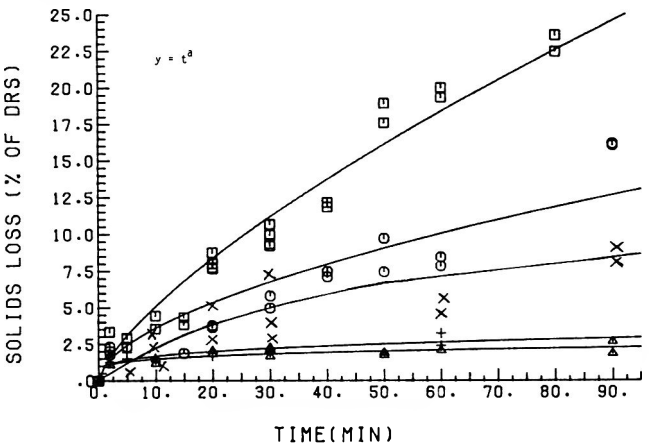

Fig. 1—Rice solids loss during soaking for milled rice at 50°C and 90°C. △ Plant Rice (PR), 50°C, a = 0.187; + California Short Grain (CSG), 50°C, a = 0.242; X Texas Long Grain (TLG), 90°C, a = 0.459; ○ Plant Rice (PR), 90°C, a = 0.565; □ California Short Grain (CSG), 90°C, a = 0.711.

occur. Thus, a greater time constant signifies a slower rate of water uptake.

Moisture content

Water uptake in the three varieties of rice with various solutes was measured as a function of moisture to dry rice solids at 90°C. When water was limited to 0.55g water/g drs, water uptake rates were smaller than those with unlimited water with the exception of TLG soaked in the presence of surfactant. The decreased rates were expected since the driving force for moisture absorption decreased as water was limited.

The difference in time constants between unlimited and limited water was consistently the least for TLG. TLG had both the slowest rate of water uptake, in limited and unlimited water conditions, and the smallest difference between the two. Only for TLG did the magnitude of difference between limited and unlimited water consistently decrease from surfactant to sodium chloride to sucrose soaking conditions. Generally, TLG was not as sensitive as CSG or PR to water to rice ratio or pretreatment conditions.

Amylose

Generally, water uptake rates for the three varieties were inversely related to their amylose content. This is contrary to the observation of Juliano (1972) that an increase in amylose content increased the capacity of the starch granules to absorb water and expand without collapsing because amylose had a greater capacity to hydrogen bond compared to amylopectin. In practice, researchers have found exceptions to Juliano's concept. Bhattacharya and Sowbhagya (1971) found no correlation between amylose content and water uptake in 20 varieties of rice.

Gelatinization temperature

TLG had a higher gelatinization temperature than PR or CSG which may have influenced the differences in water uptake rates between the rices. A higher gelatinization temperature is indicative of a more crystalline structure which is more resistant to water penetration and swelling as well as gelatinization. Halick and Kelly (1959) found the extent of water uptake to be negatively correlated with gelatinization temperature.

Soaking temperature

For all rices and soaking environments studied, water uptake rates at 90°C were significantly greater than water uptake rates at 50°C. From kinetic theory this would be expected, although in this study 90°C was above the gelatinization temperature for the rices and gelatinization can confound the effects of increasing temperature. As temperature increases, the rate of gelatinization increases.

Two aspects of gelatinization could slow diffusion: (1) immobilization of water by hydrogen bonding to starch and (2) plugging of microchannels by diffusing molecules of constituents of starch. Gelatinization also increases the overall water-holding capacity of starch by orienting the molecules to enhance accessibility of water to water-binding sites. Thus rates obtained from mass average data may reflect this greater water-holding capacity due to gelatinization.

Solutes

In unlimited water (280g water/gdrs) for each variety (Table 2), water uptake rates were larger for conditions of no solutes or presence of surfactant and progressively smaller in the presence of sodium chloride, sucrose or combinations of the two. In limited water (0.55g water/g drs) (Table 3) and in the absence of steaming, water uptake rates within a variety were significantly different at the 95% confidence interval and the order was: surfactant > no solutes > sodium chloride > sucrose.

Gray and Schoch (1962) reported a mixture of glyceryl monosterate and monopalmitate depressed both swelling power and solubility of corn, waxy sorghum and potato starch up to a temperature of 120°C. The authors suggested this effect was due to the formation of an amylose-surfactant complex. Above 120°C, the complex broke down and the swelling power and solubility increased, suggesting that the surfactant was now acting as a wetting agent. In our study, the surfactant-amylose complex should be stable at 90°C, though Gray and Schoch (1962) did show that a digestion time of preferably 3 hr at 50°C was necessary for an effective stearic acid-starch complex to form. This time for complex formation was longer than the extent of our experiment. The effect of the complex formation on water uptake was not investigated.

In unlimited water for all rice varieties, sodium chloride decreased water uptake rates as compared to soaking in water with no solute present (Table 2). In limited water, only the

Table 2—Time constants[a] of milled rice soaked at constant temperature in a water to rice ratio of 280g H_2O/g drs[b]

Temp (°C)	Rice type	Conditions[c] N.S.	Surf	Salt surf	Salt	Sugar surf	Sugar	Sugar salt	Sugar, salt surf
90	CSG	1.3	1.5	2.3	2.2	4.9	6.1	16.4	10.9
	PR	1.2	0.9	2.0	2.0	4.8	7.7	13.5	15.9
	TLG	1.8	2.9	–	5.4	–	23.8	34.5	–
50	CSG	4.1	–		5.8		19.6		
	PR	15.3	9.7		9.7		21.3		
	TG	20.0	–		–		–		

[a] Time constant is the time to effect 63.2% of maximum change; standard error is 10%.
[b] drs = dry rice solids.
[c] N.S. = No solutes present, samples were not steamed prior to soaking.
Surf = Surfactant glyceryl monostearate.

Table 3—Time constants[a] of milled rice soaked at constant temperature (90°C) in a water to rice ratio of 0.55g H_2O/g drs

		No steaming				Steaming	
		Conditions					
Rice type	Grain category	N.S.[b]	Surfactant	Salt	Sugar	N.S.[b]	Sugar
CSG	short	5.0	3.0	6.4	28.6	4.1	13.4
PR	medium	3.1	1.9	4.8	25.1	3.1	10.6
TLG	long	4.4	2.9	7.6	30.3	5.4	22.7

[a] Time constant is the time to effect 63.2% of maximum change; standard error is 10%.
[b] N.S. = no solutes present.

water uptake rate of TLG was decreased by the presence of sodium chloride.

The effect of sodium chloride on water uptake rate may be due to competition between water and sodium chloride in the starch. Salt has been shown to increase gelatinization temperature, possibly by binding with starch and protecting it against gelatinization (Oösten, 1982). This type of mechanism may also be acting to inhibit water migration.

In unlimited water, sucrose caused a significant decrease in water uptake rates in all three rices whether soaking in sucrose was compared to soaking with no solutes, or sucrose and surfactant was compared to surfactant alone, or sucrose and sodium chloride was compared to sodium chloride. Compared to no solutes, sucrose decreased water uptake two or three times more in TLG rice than in PR and CSG, respectively. For all three rices in limited water, water uptake rates in sucrose solution were decreased to about the same extent compared to those obtained with no solutes.

As with sodium chloride, sucrose may be competing with water for the starch. Sucrose can bind with both water and starch. In addition, at the starch-solution interface, water migration would result in an increase in sucrose concentration since water would diffuse into the kernel faster than the larger sucrose molecule. This would increase the viscosity and decrease water penetration from the solution into the kernel. Based on these studies with sodium chloride and sucrose, if they must be added to rice during soaking (cooking) procedures, they should be added as late in the process as possible.

Steaming

The effect of steaming rice prior to soaking in limited water was investigated. Steamed samples of CSG and PR rices exhibited increased water uptake rates during subsequent soaking in a sucrose solution (Table 3). However, when no solute was present in the soaking solution, steaming did not affect water uptake rates. Steaming affected the short and medium grain rices (CSG and PR) to a much greater extent than it affected the long grain rice (TLG).

Measurements by DSC showed no gelatinization occurred during steaming. Although the temperature was above gelatinization temperature, there was insufficient moisture in the rice (CSG 16–19% d.b.; PR 17–20% d.b.; TLG 19–22% d.b., as measured by oven drying after steaming). For gelatinization to begin, Wirakartakusumah (1981) reported that the moisture content must be at least 30% (d.b.). Although no gelatinization was observed, the gelatinization peak did broaden following steaming because of an increase in conclusion temperature (Table 1). This may be due to reordering of molecular structure within the starch granule, resulting in a greater range in degree of crystallinity within and between starch granules.

Donovan et al., (1983) treated starch for 16 hr at 100°C and a moisture content of 27% d.b. and found the endotherm widened, peak temperature increased and enthalpy decreased.

Solute gain was also affected by steaming. Sucrose absorption as well as water absorption increased during subsequent soaking in sucrose solution for both CSG and TLG rices. This may be due to an increase in porosity at the rice surface as a result of steaming.

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Binding of ^{14}C-Labeled Food Mutagens (IQ, MeIQ, MeIQx) by Dietary Fiber *In Vitro*

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ABSTRACT

Binding of three mutagens, known to occur in fried or broiled foods, by thirteen different types of dietary fiber was investigated *in vitro*. Nonspecific binding by other food polymers was minimized by using protease and amylase treatment. Water-insoluble fiber components were responsible for most of the binding capacity. Generally, a slightly larger proportion of 2-amino-3,4-dimethylimidazo [4,5-*f*]quinoline (MeIQ) than of 2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo] -4,5-*f*]quinoxaline (MeIQx) was bound. There was a significant correlation between Klason lignin content and binding of mutagens. Optimum pH for binding was between 4 and 6. Dietary fiber from sorghum had the highest binding capacity, which could be due to the presence of a large Klason lignin fraction.

INTRODUCTION

EPIDEMIOLOGICAL STUDIES indicate that between 35 and 56% of human cancers could be attributed to dietary habits (Shamberger, 1984). Foods are known to contain carcinogens of natural origin, as well as those produced microbiologically. In addition, cooking of food may produce several carcinogens, e.g., polycyclic aromatic amines and nitrosamines.

During the last decade, two new groups of carcinogenic heterocyclic amines have been demonstrated in fried beef, beef extracts, and broiled fish. The first group comprises compounds formed subsequent to pyrolysis of amino acids, such as 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*] indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) obtained from tryptophan (Sugimura et al., 1977), and 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1) and 2-aminodipyrido [1,2-*a*:3',2'-*d*]imidazole (Glu-P-2), obtained from glutamic acid (Yamamoto et al., 1978). The second group, probably resulting from Maillard reactions (Jägerstad et al., 1983; 1984), includes 2-amino-3-methylimidazo[4,5-*f*] quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) originally isolated from broiled dried sardines (Kasai et al., 1980a, b), and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), present in fried beef (Kasai et al., 1981) as well as in commercial beef extracts (Hargraves and Pariza, 1983; Turesky et al., 1983).

Studies of the tumor-inducing capacity of these heterocyclic amines are in progress. To date, tumors of the liver, small intestine, and colon in both rats and mice have been reported (Matzukura et al., 1981; Sugimura and Sato, 1983; Ohgaki et al., 1985a, b; Takayama et al., 1984a, b). The relationship of these heterocyclic amines in human cancer has not been established. This requires more extensive knowledge as to how these substances are absorbed, metabolized, and excreted and to what extent various food components affect these processes. Recently, uptake and excretion of ^{14}C-labeled IQ and MeIQ in rats was reported (Sjödin and Jägerstad, 1984). A major part of these two substances appeared in the bile, indicating enterohepatic circulation; approximately equal excretion of both IQ and MeIQ in the urine and feces were also found. The enterohepatic circulation, together with the tumor-inducing capacity of heterocyclic amines necessitates determination of the extent that dietary fiber is capable of interfering with the gastrointestinal fate of these compounds.

A high intake of dietary fiber has been associated with a decreased risk of developing colonic cancer in man (Burkitt, 1971; Cummings and Branch, 1982). Several possibilities on how fiber could protect against carcinogens have been discussed. One is an active binding of mutagenic and/or carcinogenic compounds to certain dietary fiber components, e.g., uronic acids (Smith-Barbaro et al., 1981). Another is the ability of the dietary fiber to shorten the transit time and thus reduce temporal contact and action of food carcinogens in the gastrointestinal tract, especially in the colon (Burkitt, 1971). Still another possibility is that dietary fiber might alter the microflora of the colon with consequences for the action of food carcinogens (Hill, 1975). Indeed, all of these possibilities might play a role when considering that dietary fiber constitutes a large group of complex carbohydrates with various solubilities, chemical compositions, and structural arrangements — all of which affect physiological behavior in different ways. Further, earlier methodological limitations for assessing fiber have posed a major obstacle for reliable evaluations of the relative importance of specific fiber components.

The purpose of the present *in vitro* study was to compare fiber preparations (from various sources and of different compositions) as regards their capacities to bind the three IQ compounds (IQ, MeIQ, MeIQx) and from the results to select the most interesting fiber preparations for further evaluation *in vivo*. All the dietary fibers were characterized regarding chemical composition. The binding capacity was investigated with enzymatic degradation of digestible food polymers, such as protein and starch.

MATERIALS AND METHODS

Food mutagens/carcinogens

IQ, MeIQ, and MeIQx, labeled with ^{14}C at position 2, were synthesized by the Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, Uppsala. The specific activity of the compounds was 2.03 mCi/mmol and the radiochemical purity was at least 96%. The compounds were dissolved in acetic acid buffer (pH 4.5–5.0) to a concentration of 20 μg/mL (except in the dose experiment).

Fiber preparations

Fiber preparations from wheat bran, carrot, and guar gum were obtained by methanol extraction and were the same as those used by Cummings et al. (1978). Whole-grain flours of hard winter wheat, rye, spring barley, corn, and four kinds of sorghum (mixed, Feterita, Dabar, and Argentinean) were obtained from the Carlsberg Research Center, Copenhagen, Denmark. Low methoxyl pectin was obtained from the Copenhagen Pectin Factory Ltd, Copenhagen, Denmark; the sugar-beet pulp preparation (Fibrex®), from the Swedish Sugar Company, Arlöv, Sweden; pellets (pelleted stock laboratory diet), from Ewos, Södertälje, Sweden; and Fiberform® (dephytinized and concentrated wheat-bran fiber), from Tricum AB, Höganäs, Sweden. Oat bran containing the husk only was a gift from AB Hässle, Mölndal,

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Table 1—Dietary fiber content (g/100g dry matter) and composition (%) in the preparations

Fiber preparations	Dietary fiber									
			Composition (%)							
	Total (g/100g)	Soluble (g/100g)	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Klason lignin
Guar gum	91	na[b]	1	2	1	58	34	3	1	0
Low methoxyl pectin	92	na	1	0.5	0.5	0.5	2	0.5	95	0
Carrot	72	24	3	9	1	2	13	31	40	1
Whole-rye flour	16	4	0	22	32	3	2	24	4	13
Whole-barley flour	19	4	0	12	21	3	1	41	4	18
Sugar-beet fiber	74	15	1	29	2	1	5	27	32	3
Whole-wheat flour	12	1.5	0	20	30	1	2	28	2	17
Oat bran	89	5	0	4	36	0	1	40	3	16
Whole-corn flour	9	0.5	0	20	26	1	4	27	6	16
Whole-sorghum flour[a]	9	0.5	0	13	11	2	1	37	8	28
Fiberform®	82	3	0	19	32	1	2	24	3	19
Pellets	21	na	1	17	25	2	4	31	8	12
Wheat bran	62	2	0	22	30	1	2	29	5	11

[a] Mixed sorghum flour
[b] Not analyzed

Sweden. All the fiber preparations were milled to a particle size less than 0.4 mm.

Chemical composition. Total dietary fiber (separated into water-soluble and water-insoluble components) was isolated using the method of Asp et al. (1983). The dietary fiber was further characterized regarding carbohydrate composition using gas-liquid chromatography for the neutral sugars and a decarboxylation method for the uronic acids as described by Theander and Åman (1979). The residue, insoluble in 12M H_2SO_4, was defined as Klason lignin (Theander and Åman, 1979). The amount of polyphenols as catechin equivalents was determined according to Price et al. (1978).

Binding of ^{14}C-labeled IQ, MeIQ, and MeIQx

Water-insoluble dietary fiber. Vials containing fiber preparation corresponding to 10 mg dietary fiber, 1 μg of IQ, MeIQ, or MeIQx, 2.5 mL 0.1M phosphate buffer (pH 6.0), and 10 μL thermostable amylase (Termamyl®, 120 L, Novo A/S, Bagsvaerd, Denmark) were incubated for 15 min at 90°C on a water bath to degrade the starch. After cooling, the pH was adjusted to 1.5 with HCl (2.0 mL 0.2N) containing pepsin (Merck, No. 7190, 5.0 mg/mL HCl). The suspension was incubated at 37°C for 60 min on a water bath with agitation. The pH was then adjusted to 6.8 with NaOH (0.5 mL 1N) and further digestion of protein and starch was effected by adding 2.0 mL pancreatin solution (Sigma, No. P-1750, 5.0 mg/mL phosphate buffer (pH 6.8)). The samples were shaken for an additional 60 min at 37°C and then adjusted to the final pH with acetic acid followed by continued shaking at 37°C for another 30 min. The final pH was 4.5 except when studying the effect of pH on binding capacity (final pH 2.8–8.0). After centrifugation at 4000 rpm for 10 min, the radioactivity remaining in the supernatant (I) was determined by liquid scintillation counting, which gave the amount of radioactivity *not bound* to water-insoluble fiber. By subtracting this value from the added radioactivity, the amount of radioactivity bound to water-insoluble fiber was obtained. All samples were run in triplicate.

Water-soluble dietary fiber. Two milliliters of the supernatant (I) were precipitated with 4 volumes of 95% ethanol for 1 hr. After centrifugation at 4000 rpm for 10 min, the radioactivity of the supernatant (II) was determined. The amount of bound mutagen to water-soluble fiber was calculated as the difference in radioactivity in the two supernatants, I and II.

Blank. In all the assays, blank controls were included in triplicate. The blank values were obtained by running the procedure for water-insoluble and water-soluble fibers without a fiber preparation but with the enzymes.

Radioassays

Liquid scintillation counting of all samples was performed in a Searle Mark III apparatus with scintillation fluid (Packard Instrument Co., Stockholm, Sweden). Efficiency was determined by external standard-channel ratios.

Statistic

Differences in mean values were analyzed by a two-way analysis of variance. Significant ($P < 0.05$) differences were further evaluated by the Bonferroni test. Correlation coefficients were used to evaluate associations between the components of the dietary fibers and their capacities to bind IQ, MeIQ and MeIQx, and a standard test was applied at a significance level of 5% (Draper and Smith, 1981).

RESULTS

Binding to nonfibrous components

The comparison of binding capacity of the various fiber preparations was based on the fact that each sample contained 10 mg dietary fiber. As seen in Table 1, the fiber preparations contained different proportions of dietary fiber. The composition of the dietary fibers also differed markedly (Table 1), which might have implications for their various capacities to bind the mutagenic compounds tested. We found that 100 mg pure corn starch and proteins (as casein and gluten) could bind between 22 and 50% of added IQ. To exclude such nonspecific bindings to food polymers other than fiber, protein and starch-hydrolyzing enzymes were included in the preparations of dietary fiber for the final binding assay. The total capacity of wheat bran, oat bran, pellets, and mixed sorghum flour to bind IQ was investigated in the presence and absence of hydrolyzing enzymes. The decline in binding capacity was significant ($p < 0.05$) only for sorghum, which bound approximately 20% less IQ after enzymatic treatment. Wheat bran and pellets, with a lower content of protein and starch, showed a small decrease in binding capacity. Finally, oat bran, with a very low protein and starch content (0.2% and < 1%, respectively), did not display any change in binding capacity.

Binding to dietary fiber

The binding of IQ, MeIQ, and MeIQx by the various fiber preparations is shown in Fig. 1. The thirteen fiber preparations are ranked in order of their content of water-insoluble fiber.

When corrections were made for the blank values, the binding capacity of the various fiber preparations was similar for IQ and MeIQx, whereas, in general, significantly ($P < 0.05$) more of the MeIQ was bound. As seen in Fig. 1, the binding was most pronounced for fiber preparations containing a high proportion of water-insoluble fiber components. The capacity of the water-soluble fiber fraction to bind IQ, MeIQ, and MeIQx was, except for low methoxyl pectin (8% of IQ; 5% of MeIQx), on the whole, negligible.

In all the investigated cereals except sorghum, the binding by water-insoluble fiber was 10–17% for IQ, 12–22% for MeIQ, and 8–17% for MeIQx. Pellets and Fiberform®, also containing cereal fibers, bound the mutagens in amounts comparable to those of the other cereals. Whole-grain sorghum flour exhibited a considerably higher capacity to bind the compounds than the other cereal fiber preparations, 52% of IQ, 52% of MeIQ, and 47% of MeIQx being bound by the water-insoluble

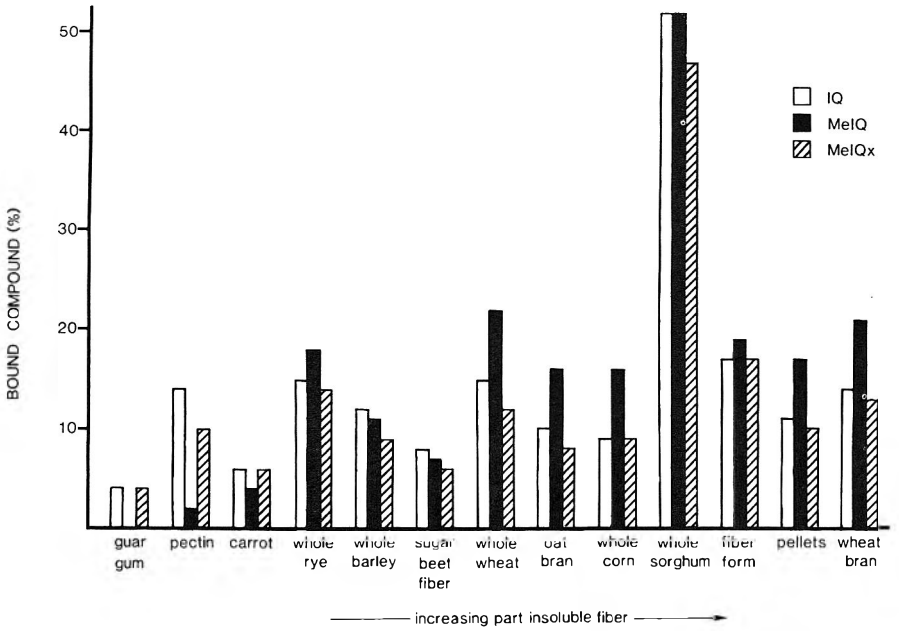

Fig. 1—Capacity of 10 mg dietary fiber in thriteen fiber preparations to bind IQ, MeIQ, and MeIQx. The fraction of water-insoluble dietary fiber is increasing from left to right.

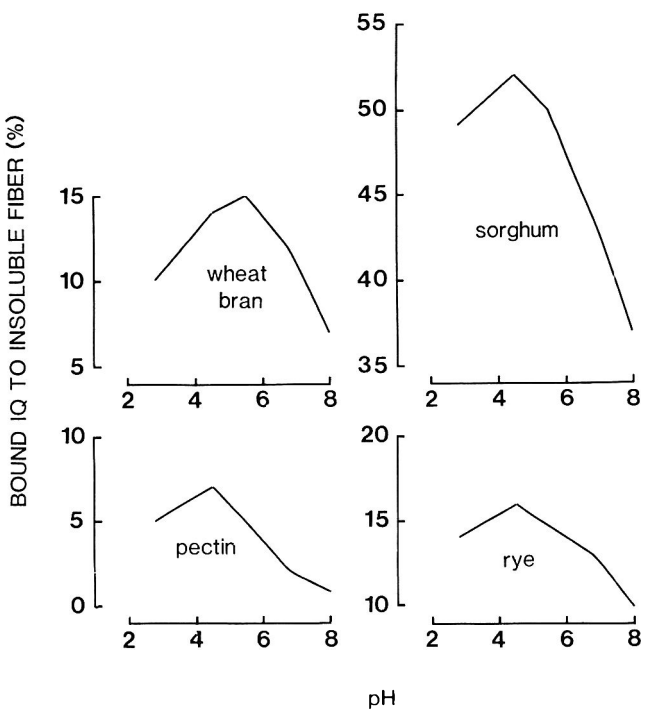

Fig. 2—The effect of pH on the capacity of the water-insoluble dietary fiber of wheat bran, low methoxyl pectin, whole-grain mixed sorghum flour and whole grain rye flour to bind IQ.

Table 2—Capacity of the water-insoluble fraction of 10 mg dietary fiber from mixed sorghum flour to bind different amounts of IQ

Added IQ (μg)	Bound IQ (%)
0.25	49
0.5	47
1.7	48
3.6	48
4.8	44
9.9	44

fiber fraction. The binding by sorghum seemed to be rather strong, and three repeated washings of the fiber fraction with 0.1M phosphate buffer released totally only about 10% of the bound IQ.

The binding of IQ by water-insoluble fiber in wheat bran, sorghum, rye, and low methoxyl pectin was affected by pH, displaying a maximum at pH 4.5–5.5 (Fig. 2).

In the experiment where 10 mg dietary fiber of mixed sorghum flour was incubated with different amounts of IQ, almost 50% was bound by the water-insoluble fiber fraction regardless of the amounts of IQ added (Table 2). For the highest amounts, five and ten times our usual dose, a slightly lower binding percentage was observed (44%).

Influence of chemical composition of dietary fiber on binding capacity

To evaluate the influence of the dietary fiber composition, tabulated in Table 1, on the capacity of the fiber preparations to bind IQ, MeIQ, and MeIQx, correlation studies were performed. The correlation coefficients for all thirteen fiber preparations are given in the upper part of Table 3, which shows that only the Klason lignin was significantly ($P < 0.01$) correlated with the binding capacity. Because the binding capacity of sorghum fiber differed appreciably from the other fiber types, the correlation coefficients were also calculated after excluding sorghum. These correlation coefficients still show a high significance ($P < 0.01$) for the Klason lignin (lower half of Table 3). But in addition, the correlation coefficients are now also high for xylose ($P < 0.001$). Weak significances are seen for galactose ($P < 0.05$, negative), uronic acids ($P < 0.05$, negative), rhamnose ($P < 0.05$, negative), and arabinose ($P < 0.05$, positive).

DISCUSSION

THE MECHANISMS by which the various dietary fibers bind the mutagens have not been elucidated. We found that the adhesion of IQ to water-insoluble fiber resisted repeated washings with buffer, suggesting a rather strong binding. A strong and pH-dependent binding suggests electrovalent bonds. An interesting finding was that despite the amounts of IQ added to a fixed amount of dietary fiber (10 mg sorghum fiber), about 48% was bound (Table 2). This clearly shows that at least

Table 3—Correlation coefficients between dietary fiber components and binding of IQ, MeIQ, and MeIQx to various fiber preparations

Mutagen	Dietary fiber components[a]							
	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Klason lignin
All fiber preparations (n = 13)								
IQ	−0.408	0.142	0.101	−0.225	−0.341	0.378	−0.248	0.743**
MeIQ	−0.521	0.285	0.336	−0.341	−0.466	0.510	−0.396	0.847***
MeIQx	−0.496	0.186	0.252	−0.240	−0.374	0.409	−0.356	0.816***
Sorghum excluded (n = 12)								
IQ	−0.654*	0.598*	0.834***	−0.483	−0.615*	0.408	−0.496	0.772**
MeIQ	−0.658*	0.627*	0.927***	−0.528	−0.644*	0.560	−0.601×	0.837***
MeIQx	−0.691*	0.517*	0.914***	−0.387	−0.532	0.397	−0.605×	0.832***

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

[a] See Table 1.

Table 4—Binding of IQ to sorghum fiber (10 mg) with various contents of polyphenols and to pure tannic acid

Sample	Bound IQ		Polyphenols (mg)	
	%	(SD)	Tannic acid	Condensed tannins[a]
Sorghum preparation				
Dabar	22.7	(2.4)		0
Feterita	51.0	(1.2)		0.34
Argentinean	44.8	(0.2)		3.22
Pure tannins				
Conc 1	5.9	(3.1)	0.24	
Conc 2	10.2	(1.0)	0.48	
Conc 3	25.2	(6.0)	0.96	

[a] Measured as catechin equivalents according to Price et al., 1978.

sorghum fiber has the capacity to bind more IQ than 0.1 μg per mg dietary fiber. That sorghum could not bind more than 50% of the IQ would suggest that IQ occurs in more than one form. To be bound to fiber by electrovalent bonds, the IQ molecules would have to be in a protonated state. However, the probability that only half of the IQ molecules are protonated at pH 4.5 seems small when considering that the pk_a of IQ is 6.6 (Kasai et al., 1981). Because IQ manifests its maximum binding at pH 4.5 and not at a lower pH leads us to believe that the binding mechanism is complex and cannot solely be accounted for in terms of electrovalent bonds.

Composition is another factor affecting the capacity of dietary fiber to bind IQ compounds. Statistical evaluation of the relationship between binding capacity and composition indicated that the proportion of Klason lignin was significantly associated with the binding capacity. As seen in Table 1, the large Klason lignin fraction of sorghum fiber is one of the main differences when compared with the other cereals. Klason lignin, however, contains not only lignin but also other compounds. Polyphenols are often found in appreciable amounts in the Klason lignin fraction. However, when testing three different whole-grain sorghum preparations containing different amounts of polyphenols, no relationship could be found between the amounts of polyphenols and the capacity to bind IQ (Table 4). That the polyphenols are not solely responsible for the high binding capacity in sorghum is well demonstrated by Argentinean sorghum flour. This sorghum binds less IQ than Feterita, which like the mixed sorghum flour contains a much smaller amount of polyphenols. On the other hand, an amount of pure tannic acid equal to the polyphenolic content present in mixed sorghum flour bound about 10% of added IQ. This binding also showed a dose-response relationship (Table 4). However, the tannins present in sorghum are condensed tannins, a more complex group of polyphenols, and their binding capacities might not be comparable to that of tannic acid. One must bear in mind that the polyphenols represent a large group of compounds and thus marked variations of the polyphenols between sorghums from different sources are to be expected.

Uronic acid is another component of the dietary fiber that has been reported to bind carcinogens (Smith-Barbaro et al., 1981). This carbohydrate occurs especially in the water-soluble fiber fraction (Table 1). Low methoxyl pectin fiber, for instance, consists almost exclusively of uronic acids (95%) and was the only fiber preparation that bound IQ and MeIQx in the water-soluble fiber fraction in significant amounts. The other fiber preparations rich in water-soluble fiber and containing half the amounts of uronic acid had negligible binding capacities in the water-soluble fiber fraction. Further, our correlation studies indicated a weakly significant ($p < 0.05$) but negative relationship between uronic acid content and binding capacity (Table 3). However, the binding capacity of water-soluble fiber fractions containing uronic acids reported here should perhaps be interpreted with caution, as the binding capacity of these fiber fractions was studied after precipitation with 95% ethanol, a procedure which might have interfered with the binding.

It should be emphasized that our data regarding the binding capacity of various fiber preparations represent true binding by the dietary fiber because the nonspecific binding by digestible materials, such as protein and starch, was minimized in our test procedure. This is important when comparing the results of the present study with others. In a recently published *in vitro* investigation, wheat bran was demonstrated to bind 40–50% of IQ (unlabeled), about three times the binding capacity found in our study (Barnes et al., 1983). No enzymatic treatment, less IQ per mg fiber (0.3 μg/5 mg fiber), and the very different techniques used in measuring the *in vitro* binding capacity might account for the differences in the results obtained between their study and ours. However, in both studies, the pH optimum of the binding capacity was found to be between 4.5 and 5.5.

The *in vitro* method used in the present investigation included several enzymatic steps performed in the pH interval between 1.5 and 6.8 with a final treatment at pH 4.5, where maximum binding occurred. *In vivo*, foods are exposed to a similar pH range, 1.5–6.8, during their passage through the gastrointestinal tract. In the proximal jejunum where the major part of absorption occurs, the pH is around 6.5, but approaches 8 in the ileum (Borgström et al., 1957).

The binding capacity of the insoluble dietary fiber investigated in the present study might seem low, ranging between 8 and 22% except for sorghum, which bound around 50%. This means, then, that each gram of insoluble fiber could bind between 8 and 22 μg and in sorghum as much as 50 μg per gram insoluble fiber at pH 4–6. Even at the highest physiological pH, the binding capacity would be considerable. At pH 8, the capacity of insoluble wheat bran fiber to bind the IQ compounds is 7 μg/g dietary fiber and for sorghum as high as 37 μg/g dietary fiber. Although available data of the daily intake of these food mutagens/carcinogens are scarce, an estimation of, at most, a few micrograms could be made (Knize et al., 1984). The daily intake of dietary fiber in Western diets is around 10–20g, which is approximately 3–6g at each meal (Arnbjörnsson et al., 1982). To what extent dietary fiber affects the gastrointestinal fate of these IQ compounds is therefore of considerable interest.

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Functional Properties of Canola Meals Produced by a Two-phase Solvent Extraction System

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ABSTRACT

The functional properties of glucosinolate-free canola meals were studied and compared with those of hexane-extracted canola and soybean meals. The glucosinolate-free meals were obtained by a two-phase solvent extraction of canola seed using 10% NH_3 in methanol or in methanol containing 5% water, and hexane as the second phase. The glucosinolate content decreased to trace levels. The crude protein content in the meal was comparable to that of soybean meal. The two-phase solvent extraction process gave canola meals with lower solubility properties but with very high fat absorption compared to both the corresponding laboratory-prepared, hexane-extracted meals and commercially produced soybean meal.

INTRODUCTION

RAPESEED ranks fifth among the world's oilseed crops, and in Canada it is second only to wheat in value and in area planted (Banfield, 1974). Presently, rapeseed is used for the production of edible oil and feed-grade meal. The meal protein is reasonably well balanced in amio acids, with a good level of sulfur-containing amino acids (Ohlson, 1978), and therefore, it may also be considered as a source of protein in human nutrition. However, its use is limited by the presence of glucosinolates at concentrations of up to 2% (Fenwick et al., 1982; Maheshwari et al., 1981). The products of the enzymatic hydrolysis of glucosinolates can be soluble in water and in oil and are goitrogenic, affecting both growth and reproduction of experimental animals (Hill, 1979; Butler et al., 1982). This problem was partially resolved by the development of low-glucosinolate cultivars of rapeseed, now renamed canola, which contain less than 3 mg of glucosinolates per gram of defatted seed. However, even at these glucosinolate levels canola meal cannot be used in food products.

The formation of toxic degradation products can be prevented by heat inactivation of myrosinase, the enzyme which hydrolyses glucosinolates. Such treatment affects the functional properties of rapeseed protein limiting its usefulness. The color of the meal is also adversely affected. However, the presence of glucosinolates in the meal is still the greatest stumbling block to its use in food. Even if the myrosinase is destroyed, the possibility remains of glucosinolate breakdown in the gastrointestinal tract to produce toxic compounds (Fenwick et al., 1982).

Recently in these laboratories a process was developed for removal of glucosinolates to trace levels. A two-phase solvent extraction system is used. One phase consists of a 10% solution of ammonia in methanol or in methanol containing 5% water. The second phase is hexane (Rubin et al., 1984; Diosady et al., 1985; Rubin et al., 1985).

The objective of the present study was to evaluate the functional properties of the protein meal obtained by the two-phase solvent extraction process and to compare it to conventionally prepared canola and soybean meals.

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MATERIALS & METHODS

SOYBEAN MEALS were obtained from Canadian Vegetable Oil Products Ltd. (Hamilton, Ontario) and commercial canola meal from CSP Foods Ltd. (Saskatoon, Saskatchewan). Hexane-extracted meals of Tower, Regent, Candle and Altex Cultivars were prepared by grinding the seeds in a Phillips coffee mill and extracting with hexane for 12 hr using a Soxhlet apparatus. The defatted meal was dried at 40°C in a vacuum oven.

The glucosinolate-free canola meals were prepared by the methanol-ammonia method developed in these Laboratories (Rubin et al., 1984, 1985; Diosady et al., 1985). Sixty grams of ground seed were mixed at low speed (approximately 2000 rpm) in a 4L Waring Blendor for 2 min with 400 mL of a 10% solution (w/w) of ammonia in methanol, or in methanol containing 5% water (v/v). After a quiescent period of 15 min, 400 mL hexane were added, and the mixture was again blended for 2 min. The meal containing residual fat was separated by vacuum filtration using Whatman No. 41 filter paper, rinsed three times with 100 mL methanol and dried at 40°C for 4 hr in a vacuum oven. The meal was further defatted with hexane using a Soxhlet apparatus and again dried at 40°C in a vacuum oven.

Proximate analyses were performed using AACC procedures (AACC, 1976). The total concentration of glucosinolates was measured by the method of Wetter and Youngs (1976). This is a rapid screening technique for glucosinolate determination. The sensitivity of this method is 0.2 mg glucosinolate/g. Below this level, the glucosinolate content of the meals is in doubt, and such meals will be referred to as glucosinolate-free. It is perhaps not entirely justified.

Nonprotein nitrogen was determined by the method of Bhatty and Finlayson (1973) with the following modification: 1g of meal was shaken for 1 hr at 20°C with 40 mL of 10% trichloroacetic acid (TCA) solution. The insoluble material was removed by filtration using Whatman No. 41 filter paper and rinsed three times with 15 mL of 10% TCA solution. The filtrate was made up to 100 mL with distilled water and an aliquot was taken for the determination of soluble nitrogen by the Kjelhdal method (AACC, 1976).

The pH values were determined using a 10% dispersion (w/v) of meal in distilled water free of CO_2.

The nitrogen solubility index (NSI) and protein dispersibility index (PDI) were determined by AACC methods (AACC, 1976). Both NSI and PDI are proposed by the AOCS (1976) and AACC (1976) as official methods for the characterization of plant products, although NSI is more widely used. In one experiment the effect of pH on the NSI was determined on Tower meals prepared by treatment with MeOH/NH_3, MeOH/NH_3/H_2O or by hexane extraction and, for comparison, on commercially prepared meal. The pH was adjusted by the addition 1% HCl or 1% NaOH solutions.

The water hydration capacity (WHC) was measured by the AACC method (AACC, 1976). Water absorption (WA) was determined by a combination of the methods developed by Sosulski (1962), AACC (1976) and Rutkowski and Kozlowska (1981). A two-gram sample was dispersed in 16 mL distilled water. The contents were mixed for 30 sec every 10 min using a glass rod, and after seven mixings were centrifuged at 2000 x *g* for 15 min. The supernatant was carefully decanted, then the tube was inverted and drained for 15 min and finally weighed. The water absorbed was expressed as the percentage increase of sample weight.

Fat absorption of meals was assayed by the method of Sosulski et al. (1976) by dispersing a 2 g sample in 12 mL of soybean oil in a 25 ml centrifuge tube.

The emulsifying activity was determined by the method of Yamasutsu et al. (1972). A 3.5g sample of meal was homogenized for 30

sec with 50 mL water using a Model A Polytron homogenizer (Brinkmann, Westbury, NY) at setting 6 (approximately 10,000 rpm). Soybean oil (25 mL) was added, and the mixture was again homogenized for 30 sec. A further 25 mL of soybean oil were added, and the mixture was homogenized for 90 sec. The emulsion so obtained was divided evenly into four 25 mL centrifuge tubes and centrifuged at 1100 x *g* for 5 min. Emulsifying activity was calculated by dividing the volume of the emulsified layer by the volume of emulsion before centrifugation × 100.

The emulsion stability was determined using the material prepared for the measurement of the emulsifying activity. It was heated for 15 min at 85°C according to the procedure of Inklaar and Fortuin (1969), cooled and divided evenly into four 25 mL centrifuge tubes. The emulsion stability was expressed as a percentage of the emulsifying activity remaining after heating.

Whippability and foam stability were determined by the method of Lin et al. (1974). Fifty milliliters of a 3% dispersion of meal in distilled water were homogenized using a Model A Polytron homogenizer at a setting of 6 (approximately 10,000 rpm). The mixture was immediately transferred into a 250 mL graduated cylinder and the foam volume noted. The volume increase expressed as a percentage was used as the whippability. Foam stability was expressed on the basis of 200 mL of a 3% dispersion as the volume of foam remaining after a 20, 40, 60, and 120 min quiescent period. The results of the above determinations were calculated according to the method of Lawhon and Cater (1971).

The functional property tests described above were carried out at the natural pH of each protein product. The results given in the tables and figures are mean values of four to six determinations.

RESULTS & DISCUSSION

CANOLA MEALS obtained by the two-phase solvent extraction method had about 25% more crude protein [N × 6.25] than the corresponding hexane-extracted meals (Table 1). The increase of crude protein from 37–42% to about 50% was the result of the solubility in the ammonia-methanol layer of 8–10% of solids containing carbohydrates, phospholipids and other constituents. Results shown in Table 1 suggest that the crude protein [N × 6.25] in the meals produced by the methanol-ammonia method is not affected by the addition of 5% water to the solvent. Moreover, the crude protein in glucosinolate-free canola meal was very close to that of soybean meal. The ash content of glucosinolate-free meals averaged about 18% higher than that of hexane-extracted meals (Table 1).

The glucosinolate-free canola meals contained about half the level of nonprotein nitrogen than the meals produced by hexane extraction (Table 1). The lowest concentration of nonprotein nitrogen was present in meals prepared by extraction with methanol-ammonia containing 5% water, close to that of soybean meal. The drop in the nonprotein nitrogen content was due to dissolution in the methanol layer. The removal of nonprotein nitrogen could be partially responsible for preventing the darking of glucosinolate-free meal during the extraction and desolventization. Rutkowski (1970) reported that nonprotein nitrogen was involved in the formation of melanoid compounds in rapeseed. However, the prevention of darkening was probably the result of the extraction of polyphenols by the methanol-ammonia solution (Naczk et al., 1985).

The two-phase solvent extraction using methanol-ammonia decreased the glucosinolates content in Regent, which has high glucosinolate, by 70%. For Tower, Candle and Altex the removal was 82–86% (Table 1).

In the presence of 5% water glucosinolate was reduced to trace levels for all of four cultivars. The process gave results close to hose reported by Sosulski et al. (1976) for meals obtained by diffusion extraction at 80°C, or by the procedure developed by in the Food Research Institute, Ottawa (FRI) (Eapen et al., 1968; Gorrill et al., 1974).

The meals prepared by methanol-ammonia method were sandy to light tan in color, depending on the cultivar used. Moreover, the products had an almost bland flavor probably due to the removal of polyphenols and some of nonprotein nitrogen compounds.

The pH of a 10% dispersion of meals in distilled water varied over a wide range. The glucosinolate-free canola meals generally had pH values that were more than one unit higher than hexane-extracted meals (Table 2).

Treatment of canola seed with a 10% solution of ammonia in methanol decreased the nitrogen solubility index (NSI) by a factor of 2 for Candle to 2.7 for Altex and Tower and the protein dispersibility index (PDI) even more (Table 2). Addition of 5% water increased these differences for both NSI and PDI. The loss of solubility of canola protein in water was the result of the formation of aggregates. Methanol removed the water surrounding the protein molecules, which facilitated the formation of protein aggregates insoluble in water. An almost linear relationship between NSI and PDI of canola meals with a high correlation coefficient r = 0.99 was found. The regression equation can be represented as follows: PDI = 1.86 (NSI) − 6.39.

The influence of pH on the NSI was determined. The shapes of the solubility curves for Tower meals in Fig. 1 show two minima, similar to those reported earlier for rapeseed meal (Gilberg and Tornell, 1976). The points of minimum solubility occurred at pH 4.8 and pH 7.0 for hexane-extracted meal. The glucosinolate-free meals had two wider minima in the acidic and alkaline pH ranges. The commercial canola meal had only one broad minimum in the acidic pH region as a result of the heat treatment of rapeseed during processing. Values of NSI for the commerical meal in this region are very close to the

Table 1—Chemical composition of canola and soybean meals

Meal		Crude protein % N × 6.25 dry basis	Ash % dry basis	Total glucosinolates mg/g	Nonprotein nitrogen [% of crude protein]
Tower	A[a]	39.0 ± 1.0	6.7 ± 0.1	1.72 ± 0.09	9.9 ± 0.3
	B	49.7 ± 1.0	8.0 ± 0.2	0.25 ± 0.05	4.8 ± 0.3
	C	49.5 ± 2.5	7.8 ± 0.2	0.05 ± 0.03	4.5 ± 0.3
Regent	A	42.4 ± 0.7	6.6 ± 0.1	2.65 ± 0.25	8.4 ± 0.5
	B	50.6 ± 1.6	7.5 ± 0.1	0.80 ± 0.05	6.4 ± 0.2
	C	50.3 ± 0.6	6.8 ± 0.2	0.10 ± 0.02	4.5 ± 0.3
Candle	A	36.8 ± 0.2	6.7 ± 0.2	0.90 ± 0.09	8.1 ± 0.4
	B	46.8 ± 1.3	8.4 ± 0.2	0.13 ± 0.04	5.1 ± 0.5
	C	45.2 ± 1.0	8.0 ± 0.1	trace	4.2 ± 0.3
Altex	A	39.1 ± 0.2	7.5 ± 0.2	0.91 ± 0.05	9.0 ± 0.3
	B	47.1 ± 0.5	9.0 ± 0.1	0.17 ± 0.02	5.3 ± 0.1
	C	46.4 ± 2.0	8.6 ± 0.1	0.07 ± 0.02	4.9 ± 0.3
Commercial canola meal		39.7 ± 1.3	7.5 ± 0.1	2.86 ± 0.06	8.6 ± 0.5
Soybean meal I		48.9 ± 1.1	6.2 ± 0.1	--	4.4 ± 0.3
Soybean meal II		55.3 ± 2.6	6.3 ± 0.2	--	3.4 ± 0.3

[a] A = extracted with hexane only. B = extracted with 10% NH_3 in methanol, and hexane; C = extracted with 10% NH_3 in methanol containing 5% water, and hexane.

Table 2—Functional properties of canola and soybean meals

Meal		Colour	pH	PDI [%]	NSI [%]	WHC [gH_2O /g]	WA [%]	Fat absorption [%]	Emulsifying activity [%]	Emulsion stability [%]
Tower	A[a]	Light, tan, sandy	5.97	43.1 ± 2.3	25.3 ± 0.3	3.32	369.8 ± 5	188 ± 10	63.7 ± 2.2	108 ± 5
	B	Light, sandy, speckled	7.11	10.0 ± 0.5	9.5 ± 0.1	3.56	390.4 ± 8	327 ± 8	61.9 ± 1.7	97 ± 6
	C	Light, greyish, sandy	7.45	8.4 ± 0.5	7.1 ± 0.3	3.46	363.3 ± 7	280 ± 3	60.8 ± 1.7	98 ± 6
Regent	A	Light, tan	6.00	36.2 ± 1.0	23.5 ± 0.4	3.07	342.9 ± 15	203 ± 8	63.4 ± 2.5	102 ± 5
	B	Light, cream	7.24	8.4 ± 0.4	10.0 ± 0.5	3.10	345.2 ± 8	332 ± 4	64.2 ± 1.8	95 ± 5
	C	Light, greyish, sandy	7.31	7.7 ± 0.2	8.3 ± 0.3	3.34	351.7 ± 11	301 ± 9	62.0 ± 1.5	97.5 ± 4
Candle	A	Yellowish	6.00	24.8 ± 0.5	17.5 ± 0.6	3.78	382.6 ± 12	190 ± 14	62.5 ± 1.7	106 ± 3
	B	Off-white	7.49	8.6 ± 0.1	7.8 ± 0.6	3.32	379 ± 12	327 ± 3	63.2 ± 2.3	101 ± 4
	C	Off-white	7.25	6.8 ± 0.2	5.3 ± 0.3	3.76	400 ± 13	314 ± 6	61.3 ± 0.6	101 ± 4
Altex	A	Light-beige	5.87	44.0 ± 0.6	27.6 ± 0.4	3.33	377.5 ± 12	219 ± 11	62.4 ± 2.9	93 ± 6
	B	Light, sandy	7.29	11.4 ± 0.3	10.4 ± 0.8	2.89	327.2 ± 3	271 ± 5	62.8 ± 1.4	98 ± 5
	C	Light, sandy	7.29	9.2 ± 0.4	8.4 ± 0.0	3.42	359.7 ± 10	271 ± 6	59.7 ± 2.6	100 ± 4
Commercial Canola meal		Dark brown	6.21	18.2 ± 1.0	13.7 ± 0.4	2.00	278.5 ± 7	134 ± 8	41.6* ± 2.5	70* ± 7
Soybean meal I		Light brown	6.60	36.4 ± 0.6	16.3 ± 0.5	2.38	311.2 ± 5	105 ± 4	50.0* ± 2.6	103* ± 7
Soybean meal II		Light brown	6.65	39.8 ± 0.2	15.5 ± 0.5	2.52	301.3 ± 6	96 ± 5	53.8* ± 3.6	99.3* ± 9

[a] A = extracted with hexane only; B = extracted with 10% NH_3 in MeOH and hexane; C = extracted with 10% NH_3 in MeOH containing 5% water and hexane; * = Oil is released.

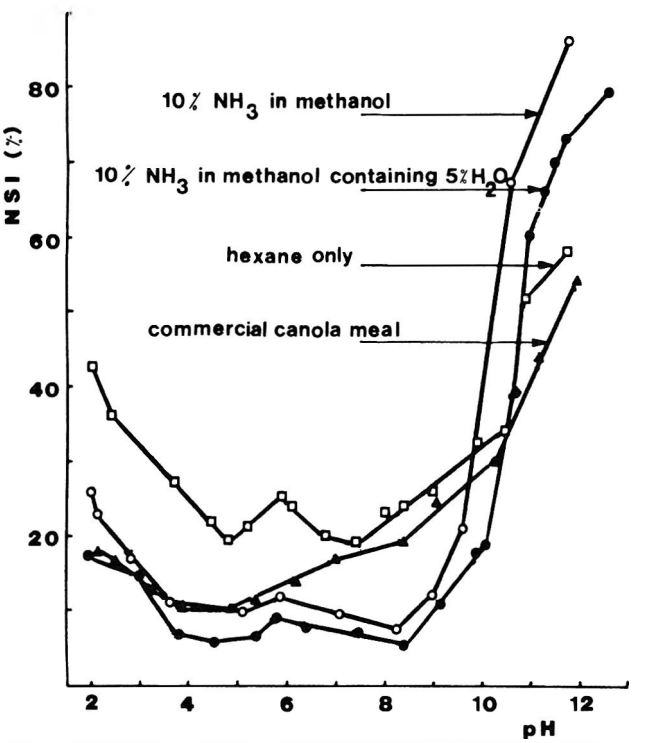

Fig. 1—Influence of pH on the nitrogen solubility index of canola meals.

values of NSI for meals treated with methanol-ammonia. At pH 10 to 11 the glucosinolate-free Tower meals had a high solubility, and the protein extracts were relatively light in color when compared with extracts of conventional meals. This is most probably due to the removal of polyphenols by the methanol-ammonia extraction (Naczk et al., 1985).

The water-binding properties of a protein determine its degree of interaction with water. These are usually reported in the literature as water absorption (WA). The Approved Methods of AACC (AACC, 1976) propose the use of WA as the standard method for soybean-protein products having water solubilities of up to 25%. However, for more soluble protein products the hydration capacity (WHC) is used (AACC, 1976). The above methods use different conditions for the determination of water-binding properties. A linear correlation between WA and WHC of canola meals with a correlation coefficient r = 0.96 was found. The regression equation can be represented as follows: WA = 62.54 [WHC] + 150.2.

Both laboratory-produced hexane-extracted canola meals and glucosinolate-free canola meal showed a high WA. The WA of commercial canola meal was 15-37% lower (Table 2). This is no doubt due to the exposure to high temperature during processing. Sosulski et al. (1976) reported that rapeseed-protein products produced by diffusion extraction and by the method developed by Food Research Institute (Ottawa) (Eapen et al., 1968; Gorrill et al., 1974)) had a high WA. Canola meals treated by the methanol-ammonia process did not moisten well on first contact with water but gave good absorption of water after vigorous mixing.

Canola meals, prepared by methanol-ammonia process, absorbed about three times more soybean oil than commercial soybean and canola meals. Moreover, the meals obtained by the methanol-ammonia treatment showed 23% (for Altex) to 74% (for Tower and Candle) higher fat absorption than laboratory-produced, hexane-extracted canola meals (Table 2). The mechanism of oil absorption, according to Kinsella (1976), relies mostly on the physical entrapment of oil by capillary attraction. However, in the case of glucosinolate-free canola meal the increase of fat absorption may also be explained by the presence of a high concentration of hydrophobic groups on the surface of the protein molecules, which have a great affinity for oil. Also, results obtained by Voutsinas and Nakai (1983) suggest that the hydrophobicity of proteins is likely to play a major role in fat absorption. However, the improvement of both fat absorption and water-binding properties of glucosinolate-free meals compared to hexane-extracted meals may result from the unfolding of protein which allows the presentation of appropriate hydrophobic and hydrophillic groups at an oil-water interface as suggested by Morr (1979) for whey-protein molecules.

The emulsifying properties of oilseed meals were investigated by measuring both the emulsifying activity and emulsion stability. These determinations were very closely correlated. However, comparison with other studies of plant proteins was difficult because of the nonstandard conditions used in the determinations. Almost all meals studied by us showed good emulsion activity (Table 2). The exceptions were commercial canola and soybean meals, which released some oil on centrifugation. Moreover, their emulsions had by visual observation a great deal lower viscosity than those prepared from Tower, Regent, Candle and Altex meals by the methanol-NH_3 process. Laboratory-produced, hexane-extracted canola meals had about

Table 3—Foaming properties of canola and soybean meals

Meal		Whippability as % volume increase on whipping	Foam stability* [volume of foam after whipping] mL				
			0.5 min	20 min	40 min	60 min	120 min
Tower	A[a]	123	360	257	224	211	136
	B	106	291	241	232	234	214
	C	80	228	187	179	173	161
Regent	A	118	342	245	228	214	178
	B	111	287	222	190	155	100
	C	60	190	146	142	135	130
Candle	A	82	251	162	139	106	68
	B	73	222	176	168	163	151
	C	63	181	143	137	126	113
Altex	A	115	323	235	220	212	182
	B	96	251	220	216	210	198
	C	92	243	219	217	214	203
Commercial canola meal		56	122	53	40	35	24
Soybean meal I		80	200	157	126	88	69
Soybean meal II		82	203	166	150	139	88

[a] *Expressed on the basis of 200 mL of 3% dispersion of meal. A = extracted with hexane only; B = extracted with 10% NH_3 in methanol and hexane; C = extracted with 10% NH_3 in methanol containing 5% water and hexane; The results are mean values of at least four replicates.

the same emulsifying activity as those produced by the two-phase solvent extraction process. Heating of the emulsions did not significantly affect the emulsion stability of the above meals except for commercially prepared canola meal (Table 3). According to Wolf and Cowan (1975), the fat absorption of protein meals is related to the formation and stabilization of emulsions.

Data on the foaming properties of meals are presented in Table 3. Significant differences in whippability were observed between different meals. The methanol-ammonia method generally decreased the whippability of meals, especially in the presence of 5% H_2O. The commercial canola meal had the lowest value. The two-phase solvent extraction process did not influence the foam stability. The stability of foams prepared from canola meal produced in the laboratory was higher than for those prepared from the soybean meals and particularly from the commercial canola meal.

CONCLUSIONS

THE PROCESS of extraction of canola seeds by 10% ammonia in methanol or in methanol containing 5% water gave meals with only trace levels of glucosinolates and with a protein content similar to that of soybean meal.

Methanol-ammonia extracted meals had a lower nitrogen solubility index, a similar water absorption, but a higher fat absorption than meals obtained by hexane extraction in the laboratory. Moreover, these meals had a much higher fat absorption capacity than commercial canola and soybean meals.

The nitrogen solubility index of laboratory-produced, hexane-extracted Tower meal showed minima at pH 4.8 and 7.0. The glucosinolate-free Tower meals also showed two, somewhat wider minima. However, commercial canola meal had only one significant minimum in the acidic pH region.

The two-phase solvent extraction process did not change the emulsifying properties of canola meals compared to hexane-extracted meal. The emulsifying activity of these meals was much better than that of commercial canola meal and better than that of the soybean meals.

The two-phase solvent extraction process decreased the whippability of canola meals, but the relative drop in foam stability was lowered. Moreover, all laboratory-prepared Canola meals except for Candle had better foaming properties than commercial soybean and canola meal.

The functional properties of glucosinolate-free canola meals suggested that they could be used as binders in meat products and as extenders for meat protein. Their detailed toxicological properties still remain to be evaluated. The functional properties of canola meals depended to some extent on the cultivar used.

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Effect of Phytic Acid on the In-Vitro Rate of Digestibility of Rapeseed Protein and Amino Acids

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ABSTRACT

Phytic acid in rapeseed flour was reduced by pH adjustment to 5.15 with subsequent dialysis or by phytase treatment at pH 5.15 with subsequent dialysis. The effect of phytate reduction on the rate and extent of protein and amino acid digestibilities was then determined using an in vitro pepsin-pancreatin proteolysis method with simultaneous dialysis of reaction products. A 51% reduction in phytic acid increased the rate of release of many essential amino acids but a further 89% reduction in phytic acid did not enhance that effect. Phytate removal did not improve the protein digestibility.

INTRODUCTION

THE ADVERSE EFFECTS of phytic acid [myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate)], which constitutes 1–3% by weight of many cereals and oilseeds have been attributed to its ability to interact with essential minerals and proteins leading to the formation of insoluble complexes which are physiologically unavailable (Erdman, 1979). Its effects on mineral absorption have been well documented (Erdman and Forbes, 1977; Erdman, 1979), and have led to the development of methods aimed at removing phytic acid from vegetable protein systems (deRham and Jost, 1979; Gillberg and Tornell, 1976; Okubo et al., 1975; Serraino and Thompson, 1984; Thompson and Cho, 1984).

Current interest particularly lies with rapeseed, for its high levels of essential amino acids as well as its importance as an oilseed crop, render it a very attractive raw material for the production of protein foods for human consumption. Defatted rapeseed flour and protein concentrates, however, contain about 5–6% phytic acid (Ohlson and Anjou, 1979) the nutritional implications of which have yet to be fully assessed.

Phytic acid binds to protonated basic residues of proteins at acidic pH forming a binary protein-phytate complex. At alkaline pH, in the presence of cations, phytic acid forms a ternary protein-mineral-phytate complex (Cheryan, 1980). Such complex formation is believed to obstruct or inhibit the enzymatic degradation of the protein (O'Dell and De Boland, 1976; Singh and Krikorian, 1982). Others who use nitrogen solubility as a criteria for assessing digestibility (Iacobucci et al., 1973) believe that during protein hydrolysis, phytate forms peptide-phytate complexes that are insoluble, thus reducing the production of soluble nitrogen, which in turn appears to give a lower rate of hydrolysis.

Phytic acid has also been implicated in the reduced rate of starch digestion and lower blood glucose response in humans observed with certain starchy foods such as legumes (Yoon et al., 1983). This may be beneficial to health as slow rate of digestion and nutrient absorption are believed to reduce metabolic stress (Jenkins, 1981). By forming complexes with proteins and/or peptides, phytic acid may in fact be slowing the rate of protein digestibility due to the action of some specific amino acids involved in complex formation but this remains to be investigated.

An in vitro pepsin-pancreatin proteolysis with continuous removal of digestion products by dialysis allowed to measure directly the availability of individual amino acids (Savoie and Gauthier, 1985; Gauthier et al., 1982, 1985). This method overcomes the principal limitation of most other in vitro methods, namely the inhibition of proteolysis resulting from the accumulation of digestion products in the medium and hence, best mimics in vivo digestion. The objective of this study was to examine the effect of phytic acid reduction in rapeseed flour on the rate of protein and amino acid digestibility.

MATERIALS & METHODS

Sample preparation

Dehulled, solvent extracted, nonheat treated rapeseed flour (RF) was kindly provided by Dr. J.D. Jones, Food Research Institute, Ottawa, Canada. Two low-phytate RF were prepared based on methods previously reported by Serraino and Thompson (1984). Low-phytate RF (RF-D) was prepared by dialyzing a 5% (w/v) aqueous dispersion of RF at pH 5.15 against distilled water at 4°C for 3 days followed by freeze-drying. Phytase treated low-phytate RF (RF-PD) was prepared by incubation of 5% (w/v) dispersion of RF with phytase (3g/100g RF) (Sigma Chemical Co, St. Louis, MO) at pH 5.15 at 57°C for 2 hr followed by dialysis and freeze-drying as for RF-D.

Samples were analyzed at least in duplicate for nitrogen using AOAC (1980) methods and phytic acid according to the methods of Latta and Eskin (1980). Amino acids were measured using an amino acid analyzer (Beckman Model 6300) after acid hydrolysis of the sample under vacuum with 6N HCl for 24 hr at 110°C.

In vitro digestion

In vitro digestion of protein, carried out in a dialysis system previously described by Gauthier et al. (1982), and modified by Savoie and Gauthier (1985), involves a two-step hydrolysis carried out at 37°C. Two hundred fifty mg protein (N×6.25) were first digested with 1.0 mL of pepsin (1.0 mg/mL) (hog mucosa 1:60,000, Sigma Chemical Co.) in 16 mL 0.1N HCl at pH 1.9 for 30 min (Gauthier et al., 1985). The digestion was stopped by raising the pH to 7.5 with the addition of 1N NaOH, and volume was adjusted to 22.0 mL with deionized water. This sample was then transferred to a dialysis bag (inner part) (Spectrapor 6, molecular weight cut off 1000, Spectrum Medical Industries Inc., Los Angeles, CA) of the digestion cell (Savoie and Gauthier, 1985). To the sample was added 1 mL pancreatin (hog pancreas 5X, ICN Nutritional Biochemicals, Montreal, Canada) solution (10 mg pancreatin/mL 0.01M sodium phosphate buffer pH 7.5). As hydrolyzed, digestion products diffused to the outer cylinder of the digestion cell and were removed through the continuous circulation of a sodium phosphate buffer (0.01M, pH 7.5) at a flow rate of 1.4 mL/min at 37°C. Digestion was allowed to proceed for a total of 8 hr, with dialyzed samples collected for analysis at 2-hr intervals. Thimerosal (0.8 mg/16 mL 0.1N HCl) (ICN Nutritional Biochemical Co.) was used as the antimicrobial agent.

Dialysates were analyzed in duplicate for nitrogen using an autoanalyzer and method No.329-74 WB of Technicon Industrial Systems (Tarrytown, NY).

Digestibility data were mean values of three experiments and were subjected to one-way analysis of variance using the Hewlett-Packard (HP-41C) programmable calculator.

One mg of dialyzed protein (N × 6.25) was evaporated to dryness

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and submitted to 6N HCl hydrolysis, under vacuum, as described earlier. Values were calculated after each 2 hr interval and for the total 8 hr period. Results were expressed by the following equations:

$$\text{Protein digestibility (\%)} = \frac{\text{N dialysate (mg)}}{\text{N sample (mg)}} \times 100$$

$$\text{Amino acid digestibility (\%)} = \frac{\text{Amino acid dialysate (mg)}}{\text{Amino acid sample (mg)}} \times 100$$

$$\text{Amino acid availability index} = \frac{\text{Amino acid digestibility (\%)}}{\text{Protein digestibility (\%)}}$$

Retentates were collected, freeze-dried, weighed and analyzed for phytic acid to determine recovery as follows:

$$\text{Phytic acid recovered} = \frac{\text{Retentate (g)} \times \text{Phytic acid (\%)}}{\text{Sample (g)} \times \text{Phytic acid (\%)}} \times 100$$

RESULTS & DISCUSSION

TWO LOW-PHYTATE FLOURS (RF-D and RF-PD) were tested in this study to differentiate between the effects of phytate reduction per se, and those of the dialysis procedure used to prepare the flours. The protein and phytic acid composition of the RF, RF-D and RF-PD are given in Table 1. Dialysis of RF at pH 5.15 for 3 days with or without prior phytase treatment (Serraino and Thompson, 1984) resulted in a 51% and 89% loss in phytic acid, respectively. The concentration of protein was higher in the low-phytate flours (RF-D and RF-PD) probably as a result of losses in compounds such as sugars, minerals, pigments, phenolics and small peptides in addition to phytate during the dialysis. The increase in protein concentration with concurrent phytate reduction resulted in a three-fold and twelve-fold increase in protein to phytic acid ratio for RF-D and RF-PD, respectively (Table 1). Neither method of phytic acid removal significantly affected the amino acid composition of the flours, as all three were comparable in this respect (Table 2).

The rate of nitrogen digestibility in RF, RF-D and RF-PD is given in Table 3. Removal of phytic acid by dialysis did not affect the rate as no significant difference ($p < 0.10$) was observed between RF and RF-D. The small difference between the two is primarily due to the nonprotein nitrogen present in RF that had already been dialyzed out of the RF-D. This nonprotein nitrogen is released in the initial 2–4 hr and most likely accounts for the slightly higher total nitrogen digestibility value obtained for the RF control at the end of the 8-hr digestion period.

Removal of phytic acid by dialysis combined with phytase treatment (RF-PD) resulted in a significantly lower ($p < 0.05$) rate of nitrogen digestibility compared with the control (RF). No significant difference ($p < 0.10$) was observed between RF-PD and RF-D and between RF and RF-D; hence the enhanced decrease in digestibility in RF-PD cannot be attributed to the effect of phytic acid per se. Since the only varying factor between RF-D and RF-PD is that the RF-PD was phytase treated at 57°C for 2 hr prior to dialysis, perhaps phytase is in some way inhibiting the enzymatic activity of pancreatin or its major components (chymotrypsin, trypsin, or carboxypeptidase A and B). Phytase has been seen to inhibit the activity of salivary amylase (unpublished data), although the mechanism of this inhibition remains unclear. As the activity of phytase is not phytate specific, phytase can hydrolyze a number of phosphoesters (Lolas and Markakis, 1977). Hence if there are any phosphate groups associated with the digestive enzymes that may influence enzyme activity, phytase may inactivate the enzymes by dephosphorylating them. As yet, however, there is no evidence to support that hypothesis. Phytase may also inhibit enzymatic activity by forming a protein-protein type of interaction with either the amino acids of rapeseed protein or of the digestive enzymes. More research regarding the nature of phytase activity and its effects on digestive enzymes is required.

Another factor which may have contributed to the lower overall nitrogen digestibility observed with the low-phytate flours was the method of processing. Both low-phytate flours were dispersed in water adjusted to pH 5.15, dialyzed and freeze-dried during preparation, and changes in the conformational structure of the protein during phytate removal and freeze-drying may have occurred. Those changes may either reduce the digestibility of the protein or the solubility of nitrogen or both.

Relative rates of amino acid release (amino acid availability index) after 2, 4, 6, and 8 hr pancreatin digestion were calculated for RF (Table 4), RF-D (Table 5) and RF-PD (Table 6). Amino acids of the crude rapeseed protein (RF) were not enzymatically released at the same rate (Table 4). Some of them (tyrosine, phenylalanine) were readily available at the first 2–4 hr of digestion and their relative release decreased as the digestion proceeded. Some others, also fast-released, either diminished (alanine, aspartic acid, leucine) or increased (isoleucine, valine) their rate of release. Except for proline, the hydrolysis of slow-released amino acids increased as the digestion proceeded.

The rates of release of amino acids in RF-D (Table 5) and RF-PD (Table 6) were similar but both differed from that of RF. In the first 2 hr of digestion, the rates of release of methionine, histidine, phenylalanine, leucine, isoleucine, valine, lysine, arginine, tyrosine, and serine were significantly faster ($p < 0.05$) while rates of release of aspartic acid and glutamic acid were significantly slower ($p < 0.05$) for RF-D and RF-PD than for untreated RF. Many of these differences disap-

Table 1—Protein and phytic acid contents and protein/phytic acid ratio of untreated (RF), dialyzed (RF-D) and phytase treated and dialyzed (RF-PD) rapeseed flour

Sample	Protein (N × 6.25%)	Phytic acid %	Protein/Phytic acid Ratio
RF	41.30	5.79	7.13
RF-D	56.30	2.81	20.04
RF-PD	55.20	0.63	87.62

Table 2—Amino acid composition (g/16gN) of untreated (RF), dialyzed (RF-D) and phytase treated and dialyzed (RF-PD) rapeseed flour

Amino acid	RF	RF-D	RF-PD
Essential[a]			
Lys	6.26	6.45	6.38
Thr	4.70	4.62	4.58
Val	3.83	4.01	3.96
Met	2.30	2.41	2.37
Ile	3.08	3.20	3.13
Leu	7.77	7.89	7.66
Phe	4.27	4.27	4.08
Tyr	3.17	3.22	3.03
Nonessential			
Asp	8.04	7.47	7.67
Ser	5.22	5.27	5.17
Glu	21.39	20.94	21.47
Pro	9.21	9.35	9.59
Gly	5.70	5.85	5.86
Ala	5.12	5.10	5.08
His	3.05	3.12	3.15
Arg	6.92	6.86	6.87

[a] Tryptophan and cysteine not analyzed.
Tyrosine and cysteine are not normally considered to be essential amino acids though they replace the requirement levels for phenylalanine and methionine, respectively. Histidine is considered essential for human infants.

Table 3—Nitrogen digestibility of untreated (RF), dialyzed (RF-D) and phytase treated and dialyzed (RF-PD) rapeseed flour[a]

	N dialyzed (%)				
Sample	2 hr	4 hr	6 hr	8 hr	total
RF	11.2	14.0	10.8	11.9	47.9
RF-D	10.1	12.0	10.6	11.8	44.5
RF-PD	9.6	10.1	10.7	10.7	41.1

[a] Means of three determinations

Table 4—Amino acid availability index after 2, 4, 6 and 8 hr of digestion of untreated rapeseed flour

Amino acid	2 hr	4 hr	6 hr	8 hr
Tyr	2.28	1.80	1.64	1.40
Phe	1.74	1.57	1.43	1.22
Ala	1.28	1.11	1.15	1.15
Asp	1.22	1.12	1.05	0.96
Leu	1.21	1.11	1.09	1.01
Ile	1.19	1.32	1.41	1.39
Val	1.11	1.28	1.34	1.33
Thr	1.08	0.98	1.03	0.98
Arg	1.00	0.96	0.96	1.00
Met	0.96	1.02	1.20	1.17
Ser	0.90	0.94	0.99	1.07
His	0.85	0.98	1.07	1.13
Glu	0.82	0.83	0.93	0.98
Gly	0.80	0.79	0.92	0.96
Lys	0.79	0.84	0.97	1.03
Pro	0.26	0.32	0.27	0.37

Table 5—Amino acid availability index after 2, 4, 6 and 8 hr of digestion of dialyzed rapeseed flour

Amino acid	2 hr	4 hr	6 hr	8 hr
Tyr	2.43	1.96	1.64	1.38
Phe	2.17	1.71	1.44	1.22
Leu	1.38	1.18	1.11	1.05
Ile	1.30	1.40	1.35	1.31
Val	1.21	1.29	1.24	1.26
Ala	1.20	1.22	1.17	1.13
Arg	1.12	1.22	0.96	1.24
Met	1.11	1.05	1.27	0.96
Asp	1.08	1.02	1.01	0.97
Thr	1.03	0.95	0.98	0.94
Ser	0.92	0.97	1.01	1.02
His	0.92	1.12	1.15	1.21
Lys	0.87	0.93	0.96	0.99
Gly	0.79	0.83	0.90	0.90
Glu	0.61	0.80	0.95	1.04
Pro	0.31	0.32	0.29	0.34

Table 6—Amino acid availability index after 2, 4, 6 and 8 hr of digestion of phytase treated and dialyzed rapeseed flour

Amino acid	2 hr	4 hr	6 hr	8 hr
Tyr	2.56	1.94	1.67	1.43
Phe	2.20	1.67	1.47	1.25
Leu	1.40	1.16	1.14	1.07
Ile	1.34	1.37	1.35	1.33
Val	1.26	1.28	1.26	1.27
Ala	1.19	1.22	1.20	1.16
Met	1.11	0.99	1.30	0.99
Arg	1.07	1.20	0.93	1.28
Asp	1.05	0.99	0.96	0.92
Thr	1.04	0.98	1.00	0.94
Ser	0.97	1.21	1.03	1.05
His	0.91	1.15	1.13	1.20
Lys	0.89	0.93	0.98	1.02
Gly	0.80	0.90	0.90	0.91
Glu	0.62	0.79	1.03	1.03
Pro	0.28	0.32	0.27	0.31

peared by the fourth hour of digestion, with only methionine being released significantly faster ($p < 0.05$) and aspartic acid and glutamic acid being released significantly slower ($p < 0.05$) for RF-D and RF-PD than for RF. This result suggests the short term effect of phytic acid on amino acid digestion, the physiological implications of which remain to be determined.

The method used to reduce phytic acid levels in RF-D, namely pH adjustment to 5.15 prior to dialysis, would not be expected to affect any binary complexes that might exist between the basic amino acids (which are positively charged at this pH) and negatively charged phytic acid (Serraino and Thompson, 1984). Ternary complex formation, on the other hand, requires the binding of cations to protein and phytic acid, and since the major site for cation binding appears to be the imidazole group

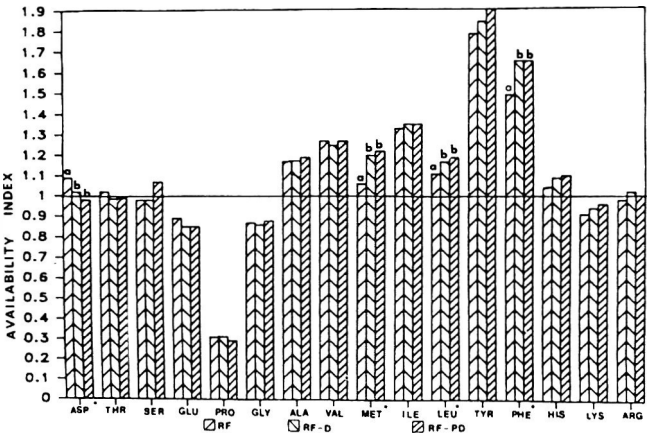

Fig. 1—Cumulative amino acid availability index after an 8 hr in vitro digestion of untreated (RF), dialyzed (RF-D) and phytase treated and dialyzed (RF-PD) rapeseed flour. [Means with different notations are significantly different ($p < 0.01$).]*

of histidine with pK of 6 to 7 (Cheryan, 1980), dissociation of the ternary complex is expected below this pH, i.e., at pH 5.15. As phytate is removed from the system by dialysis, then histidine, and perhaps any neighboring amino acids on the peptide chain, may become more accessible to enzymatic attack and consequently released faster. In addition, the increase or decrease in the rate of release of those amino acids may, as in the case of total nitrogen digestibility, be related to change in protein conformation during sample preparation. As the imidazole group of histidine becomes protonated at pH 5.15 and freed from its interaction with cations and phytic acid, it may form protein-protein type linkages with some of the negatively charged amino acids, i.e., aspartic acid and glutamic acid, thereby changing the conformation of the protein.

There are no marked differences between the rates of release of amino acids in RF-D and RF-PD, despite the fact that the former contains four times the phytic acid of the latter. Phytase treatment hydrolyzed most of the phytic acid present. Thus, if phytic acid reduction was increasing the rates of release of the amino acids originally involved in complex formation with phytate, greater increase in those rates and more amino acids would have been expected with the RF-PD. Perhaps, again, the effects of phytase inhibition of the digestive enzymes are confounding the effects of phytate reduction. The fact that the patterns of amino acid release were similar for RF-D and RF-PD suggested that enzyme inhibition may be occurring; for with such an inhibition, it is the actual amount of amino acid released that would be affected rather than the pattern of amino acid release.

The cumulative availability index of each amino acid after 8 hr pancreatin digestion (Fig. 1) can provide further insight on the effect of phytic acid reduction on the overall availability of the amino acids. With the RF-D, eight amino acids (arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, methionine), were more available according to their availability index, four were less available (aspartic acid, threonine, glycine, valine) and three were of similar availability (serine, alanine, proline) in comparison with the control (RF). With the RF-PD, eleven amino acids (arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, methionine, alanine, glycine, serine) were more available according to their availability index, while four (aspartic acid, threonine, glutamic acid, proline) were less available and one (valine) had similar availability compared to the control. Of those observations, however, only the increase in the cumulative availability index of phenylalanine, methionine and leucine and decrease in the availability of aspartic acid in RF-D and RF-PD were significantly different ($p < 0.01$) from RF. Further-

Table 7—Phytic acid recovery (%) in the retentate collected after 8 hr pancreatic digestion at pH 7.5

Sample	Phytic acid recovery (%)
RF	79
RF-D	57
RF-PD	44

more, no significant differences existed between the RF-D and RF-PD. The reason for the insignificant differences between those two samples is not clear in view of the four-fold difference in their protein-phytate ratio. Nonetheless, it should be noted that a statistical significance does not always imply a significant physiological effect.

In the in vitro system used in the study, amino acids and small peptides bound to phytic acid can theoretically pass through the digestion membrane and hence be considered "available." While it has been postulated that insoluble peptide-phytate complexes occur during hydrolysis (Iacobucci et al., 1973), the "availability" of such peptides remains uncertain (Cheryan, 1980). Phytic acid determination of dialysis retentates collected after the 8 hr digestion period (Table 7) indicated that some phytic acid did pass through the membrane during the pancreatin digestion. That phytate may have been in the form of soluble complex with minerals or with certain amino acids or small peptides. The latter is more probable as many of metal-phytate complexes are relatively insoluble at alkaline pH (Jackman and Black, 1951). Thus, while the in vitro system serves to examine the rate of amino acid release, it makes no direct implication regarding physiological availability or absorption of those amino acids.

In conclusion, phytic acid removal in rapeseed did not improve the in vitro rate of nitrogen digestibility but did change the rate of release of many amino acids.

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Improved Nutritional Value in Wheat Bread by Fortification with Full-Fat Winged Bean Flour (*Psophocarpus tetragonolobus* L.DC)

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ABSTRACT

The nutrient composition of winged bean (*Psophocarpus tetragonolobus* L.DC) full-fat flour (WBFF), wheat flour (WF) and WBFF-fortified breads was studied. When WBFF fortification was increased from 0 to 20%, there was an increase of 63% in protein, 153% in fat, 130% in calcium, 97% in phosphorus, and 105% in iron contents. WBFF contained greater amounts (g/16g N) of histidine and lysine compared to WF. Except methionine which decreased, all other essential amino acids increased when WBFF was substituted for WF from 0 to 15%. The protein efficiency ratio for 10%-WBFF bread (1.62) was found to be significantly greater ($P<0.05$) than that of 5%-WBFF bread (1.40) and wheat bread (1.11).

INTRODUCTION

THE WINGED BEAN (*Psophocarpus tetragonolobus* L.DC) is a large-seeded legume, identified as a potential crop for humid tropics and sub-tropics during an extensive survey by the U.S. National Academy of Sciences in 1974 (U.S. NAS, 1975). The mature seeds of winged bean (WB) contain about 34% protein and 17% oil, which is similar to soybean (U.S. NAS, 1975) and, hence, are very good sources of these nutrients (Delumen and Salamat, 1980; Garcia et al., 1979; Garcia and Palmer, 1981; Sajjan and Wankhede, 1981; Heldebrand et al., 1981). The amino acid composition of WB is similar to soybean except that lysine is slightly higher in WB (Gillespie and Blagrove, 1978), thus increasing its value in supplementing cereal proteins.

Cerney et al. (1971) reported that at 10% protein, the protein efficiency ratio (PER) and net protein utilization (NPU) of the WB determined with rats, were superior to those of peanuts. They also showed that WB and soybean gave similar PER and NPU values. Further, they found that an experimental diet (3 parts of corn to 2 parts of WB) resulted in PER and NPU that was comparable to that of skim milk. Jaffe and Korte (1976) observed that supplementation of autoclaved WB seeds with 0.3% methionine greatly increased PER values.

Bread is a convenient item to use in protein fortification of the diets of young children suffering from protein-energy malnutrition, particularly in less developed countries. It is convenient since once purchased, it requires no further preparation. The consumption of bread and bakery products shows an increasing trend in most developing countries (Pulle and Ino, 1975). One problem encountered by nonwheat growing less-developed countries is the dependence on wheat imports. One way of reducing wheat imports is to use indigenous nonwheat flours in making bread.

The purpose of this study was to investigate the nutritional qualities of bread made from wheat flour fortified with winged bean full-fat flour and to evaluate its protein quality (PER).

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MATERIALS & METHODS

Wheat flour (WF)

WF (extraction rate, 74% and protein content, 12% d.b.) used in this investigation was a blend of hard red spring and winter wheat milled by Prima Flour Milling Corporation, Sri Lanka.

Winged bean full-fat flour (WBFF)

Mature, dried winged bean seeds were obtained from Dodangolla Agricultural Research Farm of the University of Peradeniya, Sri Lanka. These were soaked in a 1% sodium bicarbonate solution overnight at room temperature (28 ± 2°C) to facilitate the softening of seed hulls. To reduce odor and to improve texture, 1% citric acid was added to the soaking solution (Citroreksoko, 1981). The soaked beans were washed several times with water, boiled in water for 20 min and dehulled (grain dehuller, The Baur Bros. Co., U.K.). The dehulled beans were steam blanched (100°C, 5 min), oven dried (90°C, 2 hr), roasted (60°C, 5 min), ground in a micro pulverizer grinder (U.S. Filter Corporation, New York) and sieved through a 60-mesh screen.

Preparation of WBFF-WF blends

Blends containing 5, 8, 10, 12, 15, and 20% of WBFF replacing WF were prepared by gradual mixing of WBFF into WF in an Artofex electric mixer (Artofex Engineering Works Ltd., Middlesex, England).

Baking of WBFF-WF breads

The WBFF-WF blends were baked using the straight-dough method (Method 10-10, AACC, 1976). Only white pan bread was prepared. The amount of water needed to make the dough was estimated from the farinograph absorption and from the feel of the dough during mixing. The formula and baking conditions are given in Table 1. Sodium stearoyl 2-lactylate (Emplex, Patco Products Co., Kansas City, MO) was used as dough improver.

Proximate analysis

Protein, fat and ash contents of the WBFF, WF and WBFF-WF breads were determined using AACC Methods 46-11, 30-10, and 08-16, respectively (AACC, 1976). Fiber was determined using the acid detergent method (Anon., 1971).

Table 1—Baking formula[a] and conditions[b] for winged bean full-fat flour-wheat flour bread

Wheat flour	100%
Salt	2%[a]
Shortening	1.5%
Sugar	2%
Yeast (compressed)	2.5%
Nonfat dry milk	4%
Water	variable
Potassium bromate	15 ppm
Sodium stearoyl 2-lactylate	1%
Fermentation	2.5 hr, 38°C, 80% RH
Scaling	480-g
Intermediate proofing	10 min, 38°C
Final proofing	1 hr, 71°C
Baking	25-30 min, 260°C

[a] Ingredients listed as percent of flour.
[b] AACC method 10-10 (AACC, 1976).

Amino acid analysis

Fat-free samples of the WBFF, WF and the WBFF-WF breads were obtained using petroleum ether as extracting solvent. They were analyzed for amino acid contents as outlined in the Technicon Manual (Technicon Instruments Corporation, 1978). Protein hydrolysates of the samples were prepared according to the method of Spitz (1973). Chemical score for amino acids was calculated by dividing grams of amino acid in the test protein by the grams of the same amino acid in the reference protein (whole egg protein) and expressed as percent (FAO/WHO, 1973).

Mineral analysis

Fat-free samples of WBFF-WF samples were analyzed for selected minerals by atomic absorption spectrophotometry (method 40-70, AACC, 1976). Wet ashing of the samples used concentrated nitric acid and perchloric acid (AOAC, 1980). Standard solutions of the minerals were prepared as in AACC method 40-70 (AACC, 1976). A Varian Techtron atomic absorption spectrophotometer (model AA6, Varian Instruments Co., Palo Alto, CA) was used. Phosphorus was analyzed colorimetrically (Method 40-15, AACC, 1976) using a Varian Techtron spectrophotometer (model 635, Varian Instruments Co., Palo Alto, CA).

Biological evaluation of protein quality (PER)

AOAC (1980) procedures for PER were followed. Weanling male albino rats (Sprague-Dawley), 28 days of age, were randomized into four groups. Each group (10 rats) was fed a different assay diet, one of which was the casein-containing reference diet. Rats were housed in individual cages at 25–26°C and 60% RH. Assay diet and water were supplied *ad libitum*. The animals were weighed and food intake was measured every third day. The total period of the test was 28 days. The following diets (10% protein) were used:
Group A: Reference diet containing ANRC casein (U.S. Biochemical Corp., Cleveland, OH); Group B: Diet containing wheat bread; Group C: Diet containing 5% WBFF-fortified bread; Group D: Diet containing 10% WBFF-fortified bread.

All the ingredients added to the bread were added in the same quantities to the reference diet. From the weight gain and food intake data, PER values were calculated. Analysis of variance and Duncan's Multiple Range tests (Steel and Torrie, 1960) were performed on the actual mean PER values before and after standardizing to 2.5.

RESULTS & DISCUSSION

Proximate analysis of WBFF and WF

WF contained 11% protein while WBFF contained 37% protein (moisture content of WF and WBFF were 8% and 10%, respectively). Thus, based on protein content, WBFF was suitable for fortification of WF to increase the protein content of the resulting bread.

WBFF contained greater amounts of fat, fiber and ash than WF. However, WF had more carbohydrates (85.4%) than WBFF (33%) (Table 2). In this study, 74% extraction WF was used and the results indicated that it contained 0.50% ash. WF at the normal extraction rate of 71–74% should contain about 0.45–0.50% ash to produce a good loaf of bread (Zeleny, 1971). The higher the ash content, the lower the grade of flour and the poorer the color of the flour for bread making. WBFF contained 3.3% ash and this was the limiting factor in its substitution.

Amino acid composition and protein quality

The contents of essential and nonessential amino acids in WBFF and WF are summarized in Table 3. For comparison, the amino acid composition of whole egg protein (FAO, 1970) is included. Tryptophan was not determined, since the amino acid was destroyed during acid hydrolysis. The seventeen amino acids analysed accounted for 94.2 (WBFF) and 94.9 (WF) g/100g protein. The amino acid composition of WBFF was similar to that of previously reported figures for winged bean (Cerny et al., 1971; Ekpenyong and Borchers 1978; Garcia, 1979). Methionine and cystine values of WBFF were higher than those reported by Garcia (1979).

WBFF contained greater amounts (g/16g N) of all essential amino acids, particularly lysine and histidine, than WF. The concentrations of methionine and cystine in WBFF were found to be 1.2 and 0.95 g/16g N, respectively. In contrast, the levels of methionine and cystine in WF were 1.50 and 2.20 g/16g N, respectively. The blending of a legume protein (WBFF) with that of cereal (WF) improves the protein quality of the bread, because the relatively high lysine content in winged bean (7.60 g/16g N) complements lysine in WF (2.70 g/16g N). The higher contents of sulfur-containing amino acids (methionine and cystine) of WF complement the lower contents of methionine and cystine in the WBFF.

WBFF was found to contain higher amounts of lysine and histidine than the reference protein, when the amino acids were expressed as g/16g N (Table 3). The concentrations of phenylalanine, threonine and isoleucine were also adequate in WBFF with respect to the content of these amino acids in the reference protein. However, WBFF was deficient in sulfur-containing amino acids (methionine and cystine).

Protein quality was evaluated in terms of chemical score, defined as the percent of each amino acid compared with the percent of the same amino acid in an "ideal" protein (FAO, 1970). The "ideal" protein used in this study was the FAO/WHO provisional amino acid score (FAO/WHO, 1973). Chemical scores of the essential amino acids, except for tryptophan, for WBFF and WF are given in Table 3. The sulfur-containing amino acids (methionine and cystine) were the first limiting amino acids for WBFF. This result is in agreement with Okezie and Martin (1980). Lysine was found to be the first limiting amino acid in WF. Threonine was the second

Table 2—Proximate analysis[a] of winged bean flour, wheat flour, winged bean full-fat flour-fortified breads and wheat-bread (g/100g dry matter)

Flours and breads	Protein ± S.D.	Fat (ether extract ± S.D.	Fiber ± S.D.	Ash ± S.D.	Carbohydrates[b] ± S.D.
Wheat flour	12.25 ± 0.13[c]	1.14 ± 0.07	0.62 ± 0.04	0.50 ± 0.03	85.40 ± 0.85
Winged bean full-fat flour	40.59 ± 0.68[d]	19.52 ± 0.59	4.05 ± 0.16	3.32 ± 0.29	32.52 ± 0.90
Winged bean full-fat flour-fortified bread					
0% WBFF[e]	10.22 ± 0.10[d]	1.52 ± 0.09	0.87 ± 0.10	2.90 ± 0.19	84.49 ± 0.45
5% WBFF	12.46 ± 0.10[d]	1.76 ± 0.09	0.99 ± 0.11	3.23 ± 0.17	81.56 ± 0.66
8% WBFF	13.73 ± 0.09[d]	2.62 ± 0.16	1.18 ± 0.14	3.45 ± 0.09	79.02 ± 0.85
10% WBFF	14.37 ± 0.17[d]	2.74 ± 0.06	1.37 ± 0.13	3.70 ± 0.09	77.82 ± 0.85
12% WBFF	14.69 ± 0.20[d]	2.95 ± 0.15	1.66 ± 0.09	3.91 ± 0.12	76.79 ± 0.89
15% WBFF	15.25 ± 0.14[d]	2.97 ± 0.21	1.84 ± 0.08	4.22 ± 0.15	75.72 ± 0.74
20% WBFF	16.69 ± 0.13[d]	3.84 ± 0.11	2.12 ± 0.12	4.52 ± 0.12	72.83 ± 0.80

[a] Mean of three determinations.
[b] Calculated by difference.
[c] N × 5.70
[d] N × 6.25
[e] 0% winged bean full-fat flour-substitution = Wheat-bread.

Table 3—Amino acid composition[a] (g/16g N) of wheat flour, winged bean full-fat flour, winged bean full-fat flour-fortified breads and reference protein

Amino acid	Amino acid (g/16g N)		Chemical score[c]		WBFF[b] – substituted bread				Reference protein[e]
	WBFF[b]	Wheat flour	WBFF[b]	Wheat flour	0%[d]	5%	10%	15%	
Essential									
Isoleucine	4.54	3.80	113.5	95.0	5.8	5.9	6.1	6.1	6.3
Leucine	8.90	6.46	127.1	92.3	8.7	8.8	9.0	9.4	8.8
Lysine	7.60	2.70	138.2	49.1[g]	2.8	3.4	4.4	5.0	7.0
Methionine	1.20	1.50	61.4[f,g]	105.7[f]	2.2	2.2	2.3	2.0	3.4
Phenylalanine	4.89	4.60	159.3[h]	130.8[h]	7.9	8.0	8.4	8.3	5.7
Threonine	4.29	2.90	107.3	72.5[i]	4.8	4.9	5.0	5.0	5.1
Valine	4.89	4.30	97.8[i]	86.0	5.6	5.6	5.8	5.9	6.8
Histidine	3.20	2.10	n.d.[j]	n.d.	2.5	2.8	3.9	4.3	2.4
Nonessential									
Aspartic acid	11.17	4.14	n.d.	n.d.	3.6	3.7	3.8	3.5	9.6
Serine	4.89	5.44	n.d.	n.d.	3.8	3.9	4.0	3.8	7.6
Glutamic acid	13.52	30.50	n.d.	n.d.	19.0	17.5	16.0	15.0	12.7
Proline	6.06	10.70	n.d.	n.d.	9.0	8.5	8.2	7.8	4.2
Glycine	3.32	3.22	n.d.	n.d.	3.4	3.5	3.6	3.7	3.3
Alanine	3.94	2.78	n.d.	n.d.	2.2	2.3	2.4	2.0	5.9
Cystine	0.95	2.20	n.d.	n.d.	3.8	3.8	3.6	3.5	2.4
Tyrosine	4.67	3.25	n.d.	n.d.	3.4	3.5	3.6	3.4	4.2
Arginine	6.17	4.30	n.d.	n.d.	4.9	5.0	5.2	5.2	6.1

[a] Mean of two determinations.
[b] Winged bean full-fat flour.
[c] Calculated using provisional scoring pattern (FAO/WHO, 1973).
[d] Wheat-bread.
[e] Whole egg protein (FAO, 1970).
[f] Methionine and cystine.
[g] First limiting amino acid.
[h] Phenylalanine and tyrosine.
[i] Second limiting amino acid.
[j] Not determined.

limiting amino acid in WF. However, valine was found to be the second limiting amino acid in WBFF.

Mineral composition

WBFF contained greater amounts of all minerals analysed than WF (Table 4). This is due to the higher contents of ash in WBFF (Table 2).

Calcium, phosphorus, magnesium, and potassium were present in greater quantities in WBFF. These results are in agreement with Okezie and Martin (1980). Due to the high phytate content in winged bean (Cerny, 1978), calcium and phosphorus may be unavailable.

WBFF contained greater amounts of iron (16 mg/100g dry matter) compared to WF (0.2 mg/100 g dry matter). Ekpenyong and Borchers (1980) reported that winged bean seeds contain good sources of iron and processing has no effect on the iron content. In most developing countries, iron deficiency anemia has been well documented (WHO Chronicle, 1972). Thus, WBFF could supplement the iron in WF when used in bread making. The bioavailability of iron in WB is questionable. More research is needed to determine the bioavailability of iron and other minerals in WB flour. However, in a hemoglobin repletion assay, it has been reported that iron was well utilized by rats from mature winged bean flour (Hettiarachchy and Erdman, 1984).

Proximate composition of WBFF-fortified bread

Protein, fat, fiber and ash contents increased with increasing WBFF-substitution, while the carbohydrate content decreased (Table 2). An increase of 63% in protein, 153% in fat, 144% in fiber, 59% in ash and a 14% decrease in carbohydrates occurred when WBFF-substitution was increased from 0 to 20%. Thus, nutritionally, the WBFF-fortified breads not only provide additional proteins, but also extra calories. Hence, WBFF-fortified breads could be used to alleviate the protein-energy deficiency that prevails in the less developed world.

Increases in protein content with 5, 10, 15, and 20% WBFF-fortified bread compared to wheat bread (0% WBFF-substitution) were 22%, 41%, 49%, and 63%, respectively.

Amino acid composition of WBFF-fortified bread

The amino acid compositions of breads made with 5, 10, and 15% WBFF-fortification are shown in Table 3. With the exception of methionine, all essential amino acids increased from 0 to 15%. The greatest increase was observed in lysine and histidine [79 and 72% compared to control (wheat bread),

Table 4—Mineral composition[a] of winged bean full-fat flour, wheat flour and winged bean full-fat flour-fortified breads (mg/100g dry matter)

Minerals	WBFF[b]	Wheat flour	WBFF[b] – substituted breads				
			0%[c]	5%	10%	15%	20%
Calcium	396	34	49.70	66.10	82.50	92.50	114.10
Magnesium	261	15	31.70	40.10	50.10	57.20	63.40
Potassium	758	126	99.80	108.10	177.00	204.50	228.70
Sodium	41	2	332.80	408.30	447.10	527.00	643.00
Phosphorus	637	86	112.30	125.30	181.10	193.50	221.70
Iron	16	0.2	1.26	1.42	1.71	1.92	2.58
Zinc	6	0.5	0.42	0.49	1.05	1.77	2.48
Manganese	30	0.9	1.17	1.37	1.83	2.08	2.34
Copper	1.1	0.4	0.32	0.32	0.39	0.53	0.53

[a] Mean of two determinations.
[b] Winged bean full-fat flour.
[c] Wheat bread.

respectively]. Therefore, blending this legume protein with cereals should improve protein quality, since the higher content of lysine in WB complements the lower content of lysine in WF. By doing so, the lysine deficiency in WF could be corrected and the bread protein might be better utilized metabolically. However, methionine and cystine decreased from 2.2 g/16g N to 2.0 g/16g N and from 3.8 g/16g N to 3.5 g/16g N, respectively, when the substitution of WBFF was increased from 0 to 15%. Deficiency of sulfur amino acids (methionine and cystine) was reported by Jaffe and Korte (1976), who showed that supplementation of WB with 0.3% methionine improved the PER.

When compared to the reference protein (whole egg protein) the 15% WBFF-substituted bread contained greater amounts (g/16g N) of histidine, leucine and phenylalanine (Table 3). However, all other essential amino acids in the 15% WBFF-bread were lower than those in the reference protein. Breads made with 15% WBFF were also found to contain greater amounts (g/16g N) of glutamic acid, proline and glycine than the reference protein. Even though the methionine content was low (2 g/16g N) in the 15% WBFF-fortified bread compared to the reference protein (3.4 g/16g N), the cystine content of 15% WBFF-fortified bread (3.5 g/16g N) was greater than that of the reference protein (2.4 g/16g N).

Of the essential amino acids in 15% WBFF-fortified bread, lysine and arginine were found to be the first and the second limiting amino acids, respectively, on the basis of chemical score calculated from Table 3. In the chemical score calculations, methionine and cystine were considered together as sulfur-containing amino acids.

Mineral composition of WBFF-WF bread

The mineral contents of the WBFF-fortified breads are given in Table 4. When WBFF-fortification was increased from 0 to 20%, there was an increase of 130% in calcium, 100% in magnesium, 129% in potassium, 93% in sodium and 97% in phosphorus content. The increase in iron and manganese contents of the bread at 20% WBFF-fortification were 105 and 100%, respectively.

Biological evaluation of protein quality (PER) of WBFF-fortified breads

The results of PER studies of wheat bread and 5% and 10% WBFF-fortified bread are given in Table 5. At 10% protein, both the 10% and 5% WBFF-fortified breads showed significantly greater PER values ($P<0.05$) than the wheat bread (0% WBFF-substitution) diet. Significant differences were observed between the PER values of 5% and 10% WBFF-fortified bread diets.

The percent increase in body weight gain of rats over that of the wheat bread diet was 49.4 for 10% WBFF-fortified bread diet and 46.2 for the 5% WBFF-fortified bread diet. The percent protein increase with 10% WBFF-fortification and the amino acid content also showed similar results when compared to wheat bread (Tables 2 and 3).

It is known that raw mature WB do not promote growth of weanling rats used in PER studies (Jaffe and Korte, 1976). This is largely due to the presence of trypsin and chymotrypsin inhibitors in the raw seeds of WB (Delumen and Salamat, 1980; Hafez and Mohamed, 1983). However, in this study, these anti-nutritional factors were inactivated while processing the WB seeds into flour.

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Table 5—Growth and protein efficiency ratio (PER) for rats fed with winged bean full-fat flour (WBFF)-fortified bread

Dietary source of protein	Total wt gained (g)	Total feed consumed (g)	Total protein[a] consumed	PER[b] Actual ± S.E.	PER[b] Adjusted
Casein (ANRC)[c]	62.2	268.8	24.2	2.57 ± 0.05ad	2.50c
10% WBFF-fortified bread	34.6	229.6	20.7	1.67 ± 0.08b	1.62b
5% WBFF-fortified bread	25.6	197.8	17.8	1.44 ± 0.07c	1.40c
Wheat-bread (0% WBFF – substitution)	17.5	170.0	15.3	1.14 ± 0.08d	1.11d

[a] AACC Method 46-11 (AACC, 1976).

[b] PER (protein efficiency ratio) = weight gain/protein intake. Adjusted = ANRC adjusted/ANRC actual X actual (bread); 10 male weanling rats per group, Sprague-Dawley strain. SE = standard error.

[c] Casein (U.S. Biochemical Corporation, Cleveland, OH).

[d] Means within columns followed by the same letter are not significantly different ($P < 0.05$) from each other. Each mean represents 10 determinations.

Degradation of Wheat Starch in a Single-Screw Extruder: Mechano-Kinetic Breakdown of Cooked Starch

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ABSTRACT

Wheat starch was processed in a 19 mm diameter split-barrel laboratory extruder under varying conditions of barrel temperature (79–121°C), screw speed (50, 100 rpm) and moisture content (25, 30%). Final product and samples removed from along the barrel length were examined for changes in molecular size by dilute solution viscometry. Final products were also examined for paste viscosity and estimated degree of cook. A mathematical model was developed which relates the residence time of the starch in the barrel, the nominal shear stress acting on the starch and the degree of cook of the product to the extent of molecular degradation of the starch. Solution viscometry was found to be more rapid than gel permeation methods previously used to determine the extent of molecular changes.

INTRODUCTION

CURRENT EFFORTS in extrusion research are aimed at the development of comprehensive models that describe the complex physical and chemical changes occurring within the extruder. While several authors have been working on the mechanics of the process (Bruin et al., 1978; Harper, 1978,1981), others have been trying to relate the physicochemical changes occurring during extrusion to the process conditions. Lawton et al. (1972) investigated the effects of fifteen extruder variables on the physical changes occurring during the extrusion of corn starch. These changes were characterized by determining the degree of gelatinization of the starch using an amylograph. Mercier and Feillet (1975), Mercier et al. (1980), and Colonna and Mercier (1982) have shown that the physical characteristics of the end products depend mainly upon the extrusion temperature, screw speed, screw design and moisture content of the feed. Similarly, macromolecular degradation, as measured by changes in the intrinsic viscosity of the samples, was also found to be a function of the same extrusion parameters.

A mechano-kinetic model, developed to predict the extent of degradation of wheat starch under specific conditions (Davidson et al., 1984), indicated that amylopectin degradation was due primarily to mechanical shear. Davidson et al. used a very tedious gel permeation chromatographic technique to measure the extent of wheat starch degradation in extrudates obtained in the temperature range 121–177°C at moisture contents of 20 and 25%. In the present study dilute solution viscometry was used to study wheat starch degradation in the temperature range 79–121°C and moisture contents of 25 and 30%, since these conditions approximate those in some commercial extrusion processes. More importantly, the use of lower operating temperatures reduced the thermal degradation of starch, so that the mechano-chemical effects could be more readily observed.

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MATERIALS & METHODS

COMMERCIAL WHEAT STARCH, donated by Industrial Grain Products Ltd., Montreal, was used for all experiments.

The 19 mm internal diameter, single-screw extruder described by Timbers et al. (1976) was used throughout these experiments. The extruder design was similar to the C.W. Brabender Model 2002 plastics extruder but further modified to allow the barrel to be opened along its horizontal axis to expose the screw (Paton et al., 1980). The inside surface of the barrel was rifled with eight grooves, each one 0.32 cm wide by 0.08 cm deep, running parallel to the screw axis and equally spaced around the barrel circumference.

A single-flight, uniform pitch screw with a 3:1 compression ratio, a feed depth of 0.38 cm and a blunt tip was used in all cases. The metering zone at the end of the screw had a volume of 12.7 cm^3, and a 6.3 mm (i.d.) circular die was used throughout. In order to allow feeding of starch at moisture levels higher than 25%, the working distance between the paddle wheel and the inner casing surface was increased to ensure a complete filling of the feed section of the screw.

Extruder operations

The extruder operating conditions were selected from factorial combinations of the following parameters: (i) screw speed: 50, 100 rpm; (ii) barrel temperature: 79°, 100°, 121°C; (iii) moisture content of the feed: 25, 30%. The barrel and die were held at the same temperature. The starch was preconditioned by spraying distilled water onto the powder while it was agitated in a Patterson V-blender; the mixture was blended overnight at 5°C to achieve equilibrium. The extruder was operated at steady state for each set of conditions. Attainment of steady state was judged by a constant torque required to turn the screw and by a steady extrusion rate.

During the extrusion cooking process, torque, output rate and pressure readings were recorded. Final products were collected for each of the conditions studied. At the end of each run, the barrel was quickly split open and the position of the transition zone was noted and photographed. Samples were taken at discrete points along the extruder barrel for analysis.

Residence time distributions (RTD)

When the extruder was operating under steady-state conditions a tracer pellet of 1% MnO_2 in starch was injected into the feed section of the barrel. The residence time distribution was measured for each run by determining the concentration of manganese in the product as a function of time according to the procedure described by Davidson et al. (1983). The time of the highest manganese concentration was used to determine the time spent by the material in the active volume of the extruder according to the relationship:

$$\frac{\text{Active volume}}{\text{Total volume}} = \frac{\text{Time in active volume}}{\text{Time to peak } MnO_2 \text{ conc}}$$

Melt apparent viscosity (η)

The apparent viscosity of the melt in the active volume was calculated using the equation of Harper et al. (1971):

$$\eta = 78.5\,(\dot{\gamma})^{-0.49} \exp(2500/T) \exp(-7.9\,M)$$

where $\dot{\gamma}$ = mean shear rate, $[\pi DN/H]$, sec^{-1}; N = screw speed, rpm; D = barrel diameter; H = average screw channel depth over the active volume; T = barrel temperature; and M = moisture fraction of the feed.

The measured barrel temperature rather than the starch temperature was used. Since in the active zone the melt temperature is not significantly different from the barrel temperature, this approximation has a negligible effect.

Shear stress (τ)

The mean shear stress was calculated as the product of the melt apparent viscosity and the mean shear rate:

$$\tau = \eta\dot{\gamma}$$

where η = melt apparent viscosity, Nsm^{-2}, and $\dot{\gamma}$ = nominal shear rate.

Intrinsic viscosity of samples ([η])

In an effort to reduce the time required for analysis, solution viscometry was used to estimate the size of polymer molecules. Samples were solubilized according to the technique of Colonna and Mercier (1982) in 1.0N KOH at 4°C. Solutions were then prepared in a concentration range of 0.05–0.2 g/dL in 0.2N N KOH. An Ostwald capillary viscometer was then used for the measurement of intrinsic viscosities.

Paste viscosity of extruded products

The Ottawa Starch Viscometer (OSV), as described by Voisey et al. (1977), was used to measure the characteristics of paste viscosity and to calculate the degree of cook according to the procedure developed by Paton and Spratt (1981). For each extrudate, the difference in area under the cooling and heating curves was measured, and the amount of cooked starch was read from a calibration curve. The latter was constructed by recording the pasting curves of known mixtures of unprocessed and precooked wheat starch and plotting this as a function of cooking and heating curve differences.

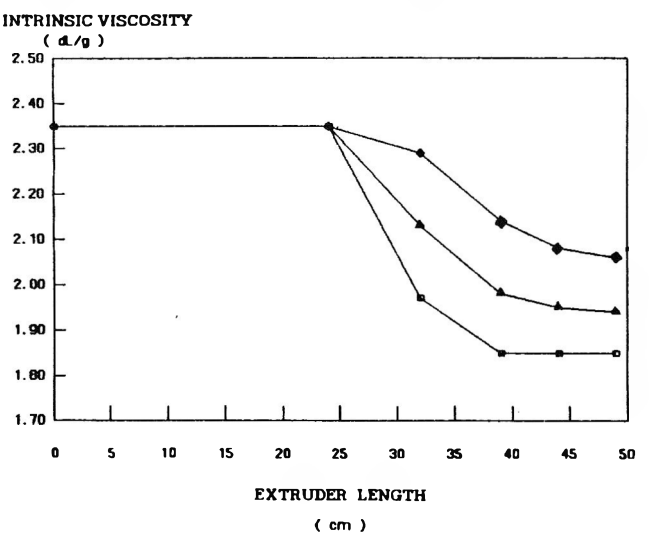

Fig. 1—Variation of intrinsic viscosity along the extruder barrel. Runs were performed at 25% moisture, 50 rpm ■ 79°C, ▲ 100°C, □ 121°C.

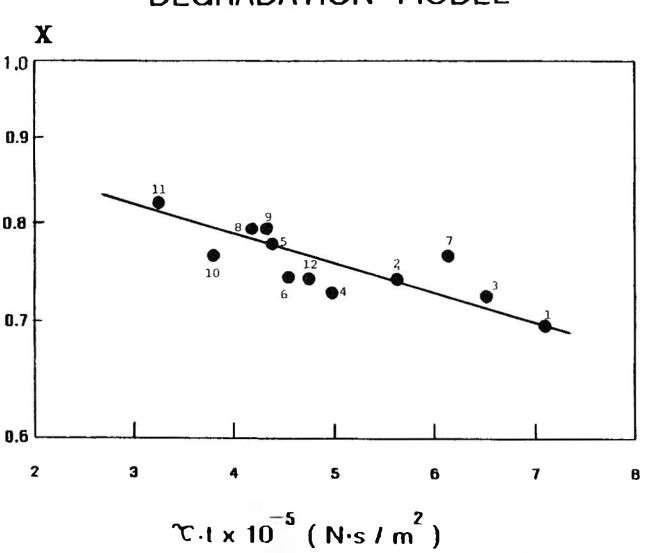

Fig. 2—Degradation Model: plot of τt against the log of X at the following conditions: 1. 25%, 50 rpm, 79°C; 2. 25%, 100 rpm, 79°C; 3. 25%, 50 rpm, 100°C; 4. 25%, 100 rpm, 100°C; 5. 25%, 50 rpm, 121°C; 6. 25%, 100 rpm, 121°C; 7. 30%, 50 rpm, 79°C; 8. 30%, 100 rpm, 79°C; 9. 30%, 50 rpm, 100°C; 10. 30%, 100 rpm, 100°C; 11. 30%, 50 rpm, 121°C; 12. 30%, 100 rpm, 121°C.

RESULTS & DISCUSSION

Active volume

Davidson et al. (1984), defined, on an empirical basis, the active volume of the extruder as the barrel volume between the beginning of the transition zone and the barrel end, that is, the zone where shear effects are high. This assumption was examined. Results from sampling along the extruder barrel show that, indeed, the degradation starts in the transition zone and continues at a much decreased rate to the die (Rosen, 1983). Fig. 1 illustrates this trend and is only one of a family or similar curves obtained for each of the extrusion runs. No significant further degradation occurred in the die as can be seen in Table 1. Although the length of the transition zone never exceeded 15% of the total length of the screw it was apparent from Fig. 1 that molecular degradation of starch was extensive within this zone. Table 2 shows the value for the active volume as a fraction of the total extruder volume.

Degradation model

Davidson et al. (1984) showed that the mechano-kinetics of

Table 1—Intrinsic viscosity measurements

Conditions	Final product		At die	
Moisture/temp./ screw speed,%/°C/rpm	[η](dL/g)	Std. error	[η](dL/g)	Std. error
25/79/50	2.03	0.07	2.03	0.02
25/79/100	2.00	0.04	1.93	0.02
25/100/50	1.90	0.04	1.99	0.04
25/100/100	1.80	0.03	1.85	0.04
25/121/50	1.85	0.02	1.93	0.03
25/121/100	1.54	0.05	1.76	0.05
30/79/50	2.15	0.03	2.16	0.02
30/79/100	2.06	0.03	2.06	0.03
30/100/50	2.06	0.05	2.07	0.06
30/100/100	1.93	0.05	1.94	0.02
30/121/50	1.95	0.04	1.87	0.10
30/121/100	1.79	0.06	1.85	0.04

Table 2—Length and location of the active volume and time spent in the active volume

Conditions Moisture/temp/ screw speed %/°C/rpm	Active length[a] (cm)	Active volume[b] (Fraction of total vol)	Time in active volume (sec)
25/79/50	15.88	0.16	8.35
25/79/100	12.70	0.13	4.51
25/100/50	21.59	0.25	11.84
25/100/100	15.24	0.16	6.08
25/121/50	18.41	0.20	11.44
25/121/100	19.05	0.21	8.49
30/79/50	12.06	0.11	10.44
30/79/100	10.80	0.10	4.98
30/100/50	15.24	0.15	11.03
30/100/100	13.34	0.13	6.88
30/121/50	16.51	0.17	12.90
30/121/100	19.65	0.22	10.56

[a] Active length is the distance between the beginning of the transition zone and the entrance to the die.

[b] Active volume was calculated based on the average height of the flights in the active zone.

the extrusion process could be represented by a first order reaction model in which the extent of molecular degradation of starch was related to the product of shear stress and average resistance time of the starch within the active zone. The model can be written in the form:

$$\ln X = -k'(\tau t) + b$$

where X = extent of molecular degradation of the amylopectin component of starch; k′ = kinetic constant ($m^2N^{-1}s^{-1}$); τ = mean shear stress ($N\ m^{-2}$); t = time spent in the active zone (sec); and b = constant. The two main assumptions in this model are that mechanical effects prevail over thermal effects and all extruded particles have been equally affected in the process. However, if not all the starch is cooked, the second assumption of the Davidson model becomes invalid. A comprehensive model must account for the effect of distribution of cooked and uncooked starch fractions in the active and inactive zones of the extruder barrel. The low temperature conditions used in the present study were deliberately chosen to ensure that mechanical effects would predominate.

Tables 2 and 3 show the active volume, residence time, shear rate, and calculated shear stress of all the extrudates as functions of moisture, barrel temperature and screw speed. Table 4 lists the calculated values for intrinsic viscosity, final paste viscosity and degree of cook of the extruded products under the same extrusion conditions. The paste viscosity curves were greatly altered by the extrusion process. A correlation of 0.8 was obtained between the final paste viscosity and the intrinsic viscosity of the extruded products as shown in Table 4. These data confirmed that the functional behavior of extruded starch was related to its structure. Table 4 also shows that there was wide variability in the degree of cook obtained by the starch during extrusion. Since the starch in the extruder consisted of both unprocessed and fully cooked granules, the observed extent of final product degradation may be considered to be a combination of effects on two independent components, only one of which was predominantly susceptible to shear degradation.

Table 3—Calculated values of shear rate and shear stress

Conditions Moisture/temp/ screw speed, %/ °C/rpm	Average Shear rate ($\dot{\gamma}$) (sec^{-1})	Shear stress ($\tau = \dot{\gamma}\eta$) × 10^{-4} Nm^{-2}
25/79/50	38	8.46
25/79/100	80	12.35
25/100/50	34	5.35
25/100/100	77	8.11
25/121/50	36	3.86
25/121/100	71	5.46
30/79/50	40	5.87
30/79/100	81	8.39
30/100/50	39	3.85
30/100/100	80	5.57
30/121/50	38	2.65
30/121/100	70	3.66

Table 4—Viscosity characteristics and degree of cook of extrudates

Conditions Moisture/temp/screw speed %/°C/rpm	[η] (dL/g)	Final paste viscosity N.m × 10^{-6}	Degree of cook (%)
25/79/50	2.03	172	50
25/79/100	2.00	147	65
25/100/50	1.90	162	59
25/100/100	1.80	123	78
25/121/50	1.85	123	96
25/121/100	1.54	93	100
30/79/50	2.15	221	40
30/79/100	2.06	147	61
30/100/50	2.06	162	61
30/100/100	1.93	162	71
30/121/50	1.95	118	89
30/121/100	1.79	88	98

If the assumption is made that the cooked and uncooked components have different intrinsic viscosities, the intrinsic viscosity can be approximated by the relationship:

$$[\eta]' = f[\eta]_i + (1\text{-}f)[\eta]_{rs}$$

where $[\eta]'$ = intrinsic viscosity of the final product; $[\eta]_{rs}$ = intrinsic viscosity of raw (uncooked) starch fraction; $[\eta]_i$ = intrinsic viscosity of the fully cooked starch fraction; f = fraction of the starch which is fully cooked. Thus X, the extent of molecular degradation can be redefined, in terms of intrinsic viscosity as:

$$X = \frac{[\eta]_i}{[\eta]_{rs}}, \text{ where } [\eta]_i = \frac{[\eta]' - (1\text{-}f)[\eta]_{rs}}{f}$$

Plotting X as a function of shear stress and residence time confirms the linear relationship with the experimentally determined constants $k' = 0.39 \times 10^{-6}\ m^2N^{-1}s^{-1}$ and $b = -0.08$. The correlation coefficient, r^2, was 0.88. The value for k′ is of the same order of magnitude as the value obtained by Davidson et al. (1984) of $1.7 \times 10^{-6}\ m^2N^{-1}s^{-1}$.

Accordingly, the model for determining the overall extent of reaction during extrusion may then be written as:

$$X_T = f.e^{-k'(\tau t)+\ b} + (1\text{-}f)$$

where X_T = extent of polymer modification defined in terms of viscosity as $[\eta]'/[\eta]_{rs}$ and k′ and b are the experimental constants.

CONCLUSIONS

A MODEL that can predict the structural changes in extruded wheat starch on the basis of feed moisture, extrusion temperature and extruder screw speed has been developed. A mechano-degradation model describes the behavior of the system in the temperature range 79–121°C, moisture levels of 25 and 30%, and screw speeds of 50 and 100 rpm. From the extrusion experiments, a number of specific conclusions may be drawn. Analysis of samples obtained from the split extruder barrel indicated that the degradation of starch started in the transition zone; no further significant changes occurred in the die. Only fully cooked starch was susceptible to shear degradation.

The degradation was found to be almost exclusively due to shear. The model accounted for the effects of shear stress, time spent in the active zone, and extent of irreversible damage suffered by the starch granules as expressed by the degree of cook. The model fitted the data with a correlation coefficient ≥ 0.8. The value of the rate constant k′ was determined to be $0.39 \times 10^{-6}\ m^2N^{-1}s^{-1}$ for the system.

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Effect of the Extraction of a Hemagglutinin on the Nutritive Value of *Amaranthus leucocarpus* Seeds

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ABSTRACT

Hemagglutinins present in *Amaranthus leucocarpus* seed meal were extracted with saline buffer and nutritive value was estimated by PER, NPR as well as Digestible Energy Consumption Evaluations. Protein digestibility was determined both *in vitro* and *in vivo*. Trypsin inhibitor activity was also determined. Saline-extracted Amaranth meal had significantly higher PER and NPR values than whole meal ($p<0.05$). There were no significant differences in digestible energy (Kcal/day/animal) and protein consumption (g/day/animal) between diets ($p<0.05$). *A. leucocarpus* improves in nutritive value after the saline extraction procedures and has a good potential as a complementary food due to its lysine content. In addition purified agglutinin may be a useful biochemical.

INTRODUCTION

THE NEED for diversification of protein sources is especially imperative in developing countries. Many plants which are being rediscovered are valuable in human nutrition because of the high quality of their protein (Inglett, 1977; Desborough et al., 1981; Hill and Rawate, 1982; Kidwal and Zain, 1969; Mugerwa and Bwabye, 1974; Sugimoto et al. , 1981). One example is *Amaranthus*, a genus of particular interest for the role that it played during the religious ceremonies of the ancient Aztecs in Mexico (Early, 1977; Ruttle, 1976; Branch, 1978; Sánchez Marroquín, 1980). The Indians used the plant in a variety of forms (Early, 1977; Sánchez Marroquín, 1980) and the popped seeds are still used to prepare a confection very popular in some parts of the country.

The seed of *Amaranthus leucocarpus* (syn. *hypochondriacus*) is rich in protein and other elements important in human or animal diets (Sánchez Marroquín, 1980). However, it also contains an extractable protein that binds specifically to N-acetyl-D-galactosamine-containing structures and induces the aggregation of red cells in suspension (Zenteno and Ochoa, 1984). This protein belongs to a group of biological macromolecules called lectins (Goldstein et al., 1980; Lis and Sharon, 1977, Goldstein and Hayes, 1978). Since the presence of lectins in edible seeds has been related with low digestibility and toxic effects (Turner and Liener, 1975; Jaffé and Gaede, 1959), the aim of this study was to determine the nutritional value of a saline extracted meal of *Amaranthus leucocarpus* and compare it with that of the whole ground meal.

MATERIALS & METHODS

FIVE kg of finely ground seeds (Mill: Arthur H. Thomas Co., PA, USA Mesh #40) were suspended in 10 parts (w/v) 0.9% NaCl solution and stirred 2 hr at 4°C. The pH was then adjusted to 4 with 4M acetic acid and the mixture allowed to stand overnight at 4°C. The sediment obtained by centrifugation (DAMON, IEC Division, JEC-HN-S centrifuge, Needham Hts, MA) at 3000 rpm for 15 min was washed three times with one part (5L) 0.2M acetate buffer pH 4. The supernatants were pooled and concentrated using Amicon filtration (Amicon, Lexington, MA) with a PMIO Diaflo membrane having pores of 10 Kda and/or freeze dried (FTS Systems, Inc. Stone Ridge, NY).

To test the agglutination capacity of the crude extract, a 2% suspension of human erythrocytes in PBS (Phosphate Buffer Saline, 0.02M KH_2PO_4, 0.9% NaCl, pH 7.4), was employed following the two fold serial dilution procedure in microtiter plates with an aliquot of the extract corresponding to 25 μg protein. Accordingly, the hemagglutination titer of the saline crude extract was defined as the reciprocal of the maximum dilution factor showing visible hemagglutination capacity.

Amino acid analyses were carried out on an automatic amino acid analyzer Durrum D-500 (Dionex, CA, USA) hydrolyzing 3 mg flour with about 500 μL HCl at 110°C in sealed tubes for 48 hr (Moore and Stein, 1963).

The trypsin-inhibitor capacity of the saline extract was estimated spectrophotometrically according to Kasell (1970), determining the rate of hydrolysis of a synthetic trypsin-substrate, N-benzoyl-DL-arginine-p-nitroanilide (BAPA) (Sigma, St. Louis, MO), at 405 nm (Spectronic 2000, Bausch and Lomb, Orange, CA). One inhibitor unit was defined as 0.1M BAPA not hydrolyzed by 20 μg trypsin in 5 min. The specific inhibition of activity was defined as the number of inhibitor units per mg of protein in the crude extract. Protein concentration for this assay was determined colorimetrically by the method of Lowry et al. (1951).

Whole grain flour and the extracted meal of *Amaranthus* were analyzed for protein (N × 6.25), fat, carbohydrates and ash according to AOAC (1980) procedures. Gross energy was determined using an adiabatic calorimeter (Parr Instruments, Co. Moline, IL).

Protein quality was assayed *in vivo* by means of the protein effi-

Table 1—Basal diet composition[a]

Ingredient[b]	%
Corn Oil	8.0
Vitamin Premix[c]	1.0
Mineral Premix[d]	5.0
Cr_2O_3	0.2
Cellulose	1.0
Water	5.0
Protein Source to make 10% of diet	
Starch and Dextrose to make 100% of diet	

[a] Corn oil, minerals premix, cellulose and water were adjusted after proximate analysis of ingredients sample was calculated as (1.6% N in sample) × 100, according to AOAC method 43.212, which is applicable to materials with %N above 1.8.

[b] All the ingredients except corn oil were from Bioserv, Inc. (New Jersey).

[c] The vitamin premix supplied the following g/kg of diet: ascorbic acid 0.45, biotin 0.0002, calcium pantothenate 0.03, choline 0.633, folic acid 0.0009, inositol 0.05, menadione 0.02, niacin 0.04, PABA 0.05, pyridoxine 0.01, riboflaviin 0.01, thiamin 0.01, Vitamin A 9,000 IU, Vitamin B-12 0.01mg, Vitamin D 1,000 IU, Vitamin E 25 IU.

[d] Mineral premix supplied the following g/kg of diet: aluminium 0.0005, calcium 11.0865, chlorine 4.7935, copper 0.0175, fluorine 0.0027, iodine 0.0030, iron 0.385, magnesium 0.3812, manganese 0.0055, phosphorus 2.5305, potassium 5.8820, sodium 1,3690, sulphur 0.1162, zinc 0.0637.

Table 2—Composition of Amaranthus *seed meal before and after saline extraction*

	Components (%)			
Sample	Protein (N × 6.25)	Fat	NFE[a]	Ash
Whole flour	14.69	8	74.04	3.27
Extracted flour	13.25	8.5	76.58	1.67

[a] Determined by difference. Only traces of crude fiber were found.

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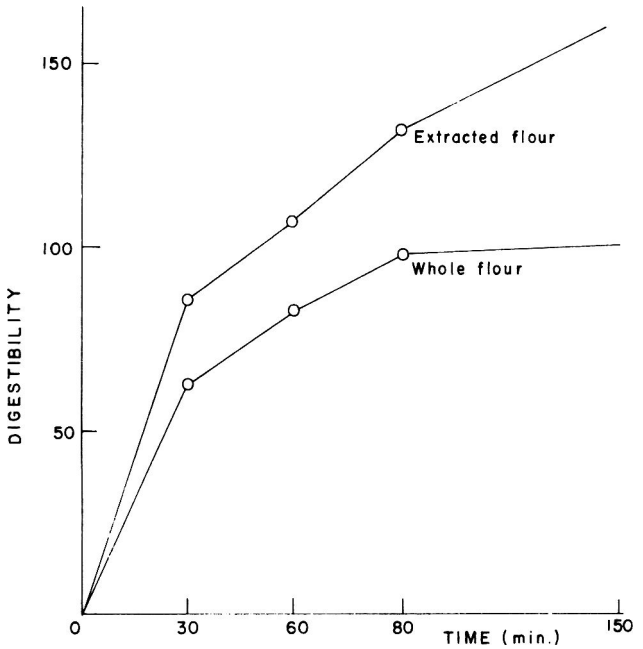

Fig. 1—In vitro digestibility of extracted and whole seed flour of Amaranthus leucocarpus *seeds as determined by the liberation of α-amino acid N following trypsin hydrolysis.*

ciency ratio (PER) according to AOAC (1980) procedures. Thirty weanling Sprague Dawley rats were randomly assigned into three groups and each fed either a diet containing saline extracted *Amaranthus* meal, whole meal or reference protein (ANRC Casein) for 28 days. The basal diet is presented in Table 1. All diets contained 10% protein and were of equal caloric densities. The experimental animals were housed individually in stainless steel cages held under controlled environmental conditions, at 22 ± 1°C, 55–65% relative humidity under a 12 hr light-dark cycle. Water and food were provided *ad libitum*.

Table 3—Essential amino acid composition of whole and extracted Amaranthus *flour*[a]

Amino Acid	Whole flour g/16g N	Egg ratio %	Extracted flour g/16g N	Egg ratio %
Lysine	3.65	47	3.19	41
Threonine	2.69	55	2.22	45
Valine	4.21	59	2.97	42
Methionine	2.40	75	1.83	58
Isoleucine	3.61	61	2.63	45
Leucine	5.81	67	4.54	52
Phenylalanine	4.27	78	3.45	63
Tryptophane[b]	-		-	
Chemical score[c]	47 (Lys)		41 (Lys)	
Essential amino Acid index[d]	62		49	

[a] Essential amino acids for the human adult.
[b] Was not determined.
[c] Mitchell and Block (1946).
[d] Calculated as the geometric means of the egg ratios of the essential amino acids (Oser,1951).

Under the same experimental conditions, NPR and estimated NPU were determined according to Bender and Doell (1957). Apparent protein digestibility and digestible energy were determined according to Valencia et al. (1979) and Edwards and Gillis (1959) respectively, utilizing a Cr_2O_3 marker, fed throughout the experiment and collecting fecal samples during the last 10 experimental days.

The experimental arrangement was a randomized design. Common variance was tested previous to the selection of parametric or non-parametric procedures, namely t test and Man-Whitney (Zar, 1974) respectively.

In vitro digestibility was determined according to Marquez and Lajolo (1981) with trypsin, measuring the α-amino nitrogen produced (Kabat, 1961). A sample of *A. leucocarpus* seed meal containing 5 mg protein/mL in 0.05M phosphate buffer pH 7.0 was incubated with an enzyme solution (1 mg/mL) in 10^{-3}M HCl at 37°C. The enzyme:substrate ratio was maintained at 1:40 and at different incubation intervals aliquots of 5 ml were taken and mixed with trichloroacetic acid (TCA) to a final TCA concentration of 5%. After standing period of 1 hr, the precipitated undigested material was separated by centrifugation at 3000x *g*, and the liberated amino acids present in the supernatant were assayed colorimetrically with ninhydrin. Leucine was used as a standard. Digestibility was expressed as mg of amino acid liberated per gram of protein.

In vitro digestibility with trypsin of both whole and the extracted flours was tested to estimate growth depressing effects due to the presence of only trypsin inhibitors (Turner and Liener, 1975).

RESULTS & DISCUSSION

THE TRYPSIN inhibitor activity of *A. leucocarpus* crude extract was 3.3 specific units. However, unextracted trypsin inhibitors may be responsible for differences in α-amino nitrogen liberation provoked by the trypsin digestion of the meals, as suggested by Fig. 1.

As shown in Tables 2 and 3 there were few differences in chemical composition between the whole flour and the saline-extracted flour.

The essential amino acid composition of the whole and extracted *Amaranthus* flour showed a decrease in all amino acids after the extraction procedure which is reflected in an Essential Amino Acid Index change from 62 to 49 in whole and extracted four, respectively. Lysine was the limiting amino acid in both cases (Table 3). These methods are not very good indicators of protein quality and did not agree with the biological evaluation.

No significant differences ($p<0.05$) were found in apparent digestible energy or protein consumption between the saline extracted and the whole Amaranth meal (Table 4), thus the biological methods utilized reflected only the effect on protein quality. The analysis of the Amaranth meals showed significant differences ($p<0.05$) between the whole and extracted meals by means of the PER, NPR and estimated NPU, where an increase in protein quality after the saline extraction was observed (Table 4).

The results suggest that the presence of agglutinating activity in *Amaranthus leucocarpus* might have a possible negative effect in protein quality ($p<0.05$), that could possibly be due to the binding of agglutinins to receptor sites on the surface of the intestinal epithelial cells, interfering with the absorption of nutrients across the intestinal wall. This type of mechanism has been suggested by Jaffé and co-workers who reported that a decrease in *in vivo* digestibility in rats was observed when

Table 4—Biological evaluation of the whole and saline extracted Amaranthus leucocarpus *flours*

Source of diet	Apparent digestible energy consumption[a] (Kcal/day/animal)	PER[a]	PER[a] corrected	NPR[a]	NPU[a] estimated (%)	Apparent protein digestibility (%)	Apparent digestible[a] protein consumption (g/day/animal)
Casein[c] (ANRC)	46.37	3.43	2.50	5.22	84.83	90.81	1.27
Extracted flour	43.96[a]	2.95[a]	2.15[a]	4.57[a]	74.14[a]	79.29	1.10[a]
Whole flour	39.91[a]	2.68[b]	1.95[b]	4.06[b]	66.23[b]	77.79	1.00[a]

[a] Means with different superscript are significantly different ($p<0.05$).
[c] Bioserv. (New Jersey).

agglutinins from *P. vulgaris* were added to diets containing casein (Jaffé et al., 1955; Jaffé and Camejo, 1961).

CONCLUSIONS

It appears that the presence of hemagglutinating activity in *Amaranthus leucocarpus* seeds affected protein quality. Measured agglutinating activity was considerable whereas trypsin inhibitor activity in the same saline extract was extremely low. Additionally, no relationship was found between the protein quality evaluated by the biological methods and the amino acid composition; the saline-extracted flour showed higher values in PER and NPR and had a generally lower Essential Amino Acid Index and Chemical Score. Finally, the extraction procedure can yield a potentially useful biochemical and a residual flour with higher nutritive value.

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Calculation of Required Hold Time of Aseptically Processed Low Acid Foods Containing Particulates Utilizing the Ball Method

ROBERT DAIL

ABSTRACT

Analytical solutions for conduction heat transfer into infinite slab and infinite cylinder were manipulated to give heating equations for a cube and a finite cylinder. Calculated values of j and f_h from these equations were substituted into Ball's formula to calculate required hold time for low acid foods containing particulates. This simplifies the calculations normally involved and eliminates the requirement of a computer. However, the method has a limited range of application due to assumptions made in the derivation of Ball's Formula. The assumptions of Ball's Formula relative to aseptic systems are discussed, and a sample calculation is included.

INTRODUCTION

TWO METHODS presented in the literature for calculating the microbial lethality imparted during continuous aseptic processing of foods containing particulates are: (1) the General Method (de Ruyter and Brunet, 1973; Newman and Steele, 1978), and (2) Stumbo's integrated lethality (Manson and Cullen, 1974). These methods are tedious or complicated and require the use of a computer. It would be desirable to have a simplified method of calculating required hold tube times for different particle shapes and sizes that could be done without the use of a computer and would be usable by a greater number of people in the food industry. The recent issuance of guidelines for aseptic processing and packaging systems in meat and poultry plants allows the use of lethality from the cooling portion of the process as long as specified conditions are met (USDA, 1984). The objective of this study is to employ Ball's Formula Method (Ball and Olson, 1957), which incorporates cooling lethality, to calculate required hold tube time. This simplifies process calculations and eliminates the requirement of a computer.

Since foods containing particulates are best processed in aseptic systems utilizing scraped surface heat exchangers (SSHE) for heating and cooling product, this discussion will be limited to processing in SSHE equipment.

FORMULAE DEVELOPMENT

HEAT TRANSFER to homogeneous foods in scraped surface heat exchangers occurs by convection. When heterogeneous foods are processed in SSHE equipment, heat transfer to the fluid portion is still by convection, but heat transfer into the particulates occurs by conduction. When heterogeneous foods are processed in cans by conventional retorting, the heat transfer rate into the particulate is determined by impaling the particulate on the end of a thermocouple and monitoring temperature with a potentiometer. This is not possible in aseptic processing systems. However, the heat transfer rate for food particulate in the shape of a cube or a finite cylinder can be approximated by manipulating the theoretical solutions for conduction heat transfer into an infinite slab and an infinite cylinder. The analytical solutions for heat transfer into an infinite slab and an infinite cylinder are given by the Fourier series:

$$Y_{slab} = \frac{T_{RT} - T}{T_{RT} - T_{IT}} = \sum_{m=1}^{\infty} \frac{2(-1)^{m+1}}{m} \cdot \cos\left(\beta_m^2 \cdot \frac{2x}{L}\right) \cdot \exp\left(\frac{-4\beta_m^2 \alpha t}{L^2}\right) \quad (1)$$

$$Y_{cyl} = \frac{T_{RT} - T}{T_{RT} - T_{IT}} = 2 \sum_{n=1}^{\infty} \frac{J_o(\beta_n \cdot \frac{r}{R}) \cdot \exp\left(-\beta_n^2 \cdot \frac{\alpha t}{R^2}\right)}{\beta_n \cdot J_1(\beta_n)} \quad (2)$$

(Carslaw and Jaeger, 1959).

Cube

The series solution for infinite slab can be manipulated to give an equation for heat transfer into a cube by taking the first term of the series and cubing it (assumptions about series convergence will be addressed in a following section). Letting m = 1 to obtain the first term of the series and setting x = 0 for temperature at the geometric center gives the "long time" solution for heat transfer into a infinite slab:

$$\left(\frac{T_{RT} - T_c}{T_{RT} - T_{IT}}\right)_{slab} = \frac{4}{\pi} \cdot \exp\left(\frac{-\pi^2 \alpha t}{L^2}\right) \quad (3)$$

Cubing Eq. (3) gives the heating equation for a cube. Rearranging to solve for time and converting to common logarithms yields:

$$t = \frac{2.303 L^2}{3\pi^2 \alpha} \cdot \log\left\{2.07\left(\frac{T_{RT} - T_{IT}}{T_{RT} - T_c}\right)\right\} \quad (4)$$

Finite cylinder

If the Fourier series solutions for infinite slab and infinite cylinder are multiplied together, they yield the series solution for heat transfer into a finite cylinder:

$$Y_{FC} = \frac{T_{RT} - T}{T_{RT} - T_{IT}} = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{2(-1)^{m+1}}{\beta_m} \cdot \cos(\beta_m \cdot \frac{2x}{L}) \cdot \frac{2J_o(\beta_n \cdot \frac{r}{R})}{\beta_n J_1(\beta_n)} \times \exp\left\{-\left(\frac{\beta_n^2}{R^2} + \frac{4\beta_m^2}{L^2}\right) \alpha t\right\} \quad (5)$$

Letting m and n equal one to obtain the first term of the

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series, setting x and r equal to zero for temperature at the geometric center, rearranging to solve for time, and converting to common logarithms gives the "long time" heating equation for a finite cylinder:

$$t = \frac{2.303}{(\frac{5.78}{R^2} + \frac{\pi^2}{4L^2})\alpha} \cdot \log \{2.04(\frac{T_{RT} - T_{IT}}{T_{RT} - T_c})\} \quad (6)$$

An examination of Eq. (4) and (6) reveals that they are similar in form to Ball's equation for calculating required process time for conventionally retorted products:

$$B_b = f_h \cdot \log \{j(\frac{I}{g})\} \quad (7)$$

If like terms are equated from the three equations:

$$B_b = t$$

$$f_h = (\frac{2.303L^2}{3\pi^2\alpha})_{cube} \text{ or } \left(\frac{2.303}{(\frac{5.78}{R^2} + \frac{\pi^2}{4L^2})\alpha}\right) \text{ finite cylinder}$$

$$j = (2.07)_{cube} \text{ or } (2.04)_{finite\ cylinder}$$

$$I = T_{RT} - T_{IT}$$

$$g = T_{RT} - T_C$$

The similarity of Eq. (4), (6), and (7) suggest that required hold tube time can be determined by using a calculated f_h value with a commensurate j value, and letting the temperature of the fluid portion of the food serve as "retort temperature" in Ball's formula method.

Series convergence

Using only the first term in the Fourier series solutions for heat transfer into infinite slab (Eq. 1) and finite cylinder (Eq. 5) assumes that process time (in this case, hold tube time) is long enough to allow for series convergence. Series convergence is dependant on particle size, time spent in the heating medium, and the thermal diffusivity of the food. For smaller food particles and/or particulate composed of a food material that diffuses heat rapidly, series convergence will be quite rapid. Conversely, for larger food particles and/or particulate composed of a food material that does not diffuse heat rapidly, series convergence will occur more slowly. If hold tube time is not long enough to allow adequate time for series convergence, the prediction of the particle center temperature at the end of the hold tube will not be accurate. Therefore, a method for determining the time required for series convergence is needed.

Series convergence occurs at the time when the temperature at the particle center goes from curvilinear to semi-log straight-line heating. This time can be defined by plotting the series. However, this is complicated and would require the use of a computer. A simpler method would be to use the Fourier number equation rearranged to solve for time:

$$t_l = \frac{F_N L^2}{\alpha} \quad (8)$$

$$t_l = \frac{F_N R^2}{\alpha} \quad (9)$$

Eq. (8) and (9) will define the minimum time required for series convergence of a cube and a finite cylinder, respectively. For cube-shaped particles, series convergence can be expected for Fourier number values of 0.03. For cylindrically shaped particles, series convergence can be expected for Fourier number values of 0.1.

If the required time for series convergence were longer than the hold time required for sterilization, Ball's Formula Method could not be used for calculating required hold tube times. However, use of Eq. (8) or (9) will show that the condition where calculated required hold tube time will be exceeded by the time required for series convergence will only be when a very low required F_o value is used in the calculations.

f_h Calculation

To calculate f_h, a thermal diffusivity value is needed. Diffusivity values for various foodstuffs are available in the literature (Singh, 1982; Morely, 1972). However, the substitution of a thermal diffusivity value from the literature into the f_h equations would assume negligible surface resistance to heat transfer. Since negligible surface resistance occurs only in cases of heating by condensing steam or with well agitated liquids having extremely low viscosities, a thermal diffusivity value from the literature can rarely, if ever, be used. In most cases, the liquid portion of the food being processed will be viscous enough to create a conduction heating layer around the food particle that will significantly slow the heat transfer rate. Not accounting for this condition will result in underprocessing.

A method that may be used to obtain a thermal diffusivity value is to determine f_h of a larger sized particle (e.g., a "long" cylinder or square rod) using long thermocouples. The use of long thermocouples will eliminate error caused by thermocouple conduction. Once f_h is determined for the long cylinder or square rod, a thermal diffusivity can be determined by substituting the f_h value into one of the following equations:

$$\alpha_a = \frac{0.398R^2}{f_h} \quad (10)$$

$$\alpha_a = \frac{0.467a^2}{f_h} \quad (11)$$

If the thermal diffusivity value is determined in the actual liquid matrix that the particulate is going to be processed in, the value obtained will reflect the influence of the boundary conditions. Since the value is different than if there were no boundary layer, the value obtained may be termed the "apparent" thermal diffusivity value. This value reflects the combined heat transfer resistance of the particle material and the surrounding fluid boundary layer, and, therefore, must be determined in a nonmoving liquid. Otherwise, the Biot number would be required. If this condition is met, the apparent thermal diffusivity value determined by using either Eq. (10) or (11) can then be substituted into the f_h equation for finite cylinder or cube, respectively.

Eq. (10) and (11) are derived by rearranging the f_h analogues for infinite cylinder or square rod to solve for thermal diffusivity. The determination of the thermal diffusivity may be done at a lower temperature (e.g., 200°F) and used in Eq. (10) and (11) on the assumption that the thermal diffusivity will remain constant over the 200–275°F temperature range. Newman and Steele (1978) were able to show good agreement between experimentally obtained values of f_h and f_h values calculated with thermal diffusivity values (negligible surface resistance). The calculated values tended to be slightly larger than experimentally obtained values. The differences were attributed to variation of thermal diffusivity over the time/temperature curve or particle shrinkage upon cooking. However, the error introduced is on the side of microbiological safety, and therefore, the assumption of a constant thermal diffusivity value is safe.

Another method for determining f_h is to obtain it directly by heat penetration testing on the particle size that will be processed. However, this method has an inherent disadvantage in that extremely fine thermocouple wires must be used to

eliminate thermocouple conduction error whereas, in the previous method, commercially available thermocouples may be used. If f_h is obtained directly by heat penetration, the determination can be performed at a lower temperature and extrapolted to higher temperatures for the same reason cited for the previous method.

An example of this calculation is included in the sample process calculation at the end of the text.

Initial temperature

The use of Ball's Formula to calculate required hold tube time requires: (a) knowledge of the center temperature of the food particle as it enters the hold tube, and (b) that there be a difference of at least 80°F between the particle center temperature and the fluid portion of the food as the mixture enters the hold tube.

Ball's formula (particle heating equation) cannot be used to determine particle center temperature gain (RT-g) in the heat exchanger because it assumes a constant temperature heating medium. A series solution for obtaining particle center temperature gain in scraped surface heat exchangers has been derived by de Ruyter and Brunet (1973) for spherical shaped particles. This solution can also be applied to particles in the shape of a cube or cylinder, with conservative error, by enlarging the diameter of the sphere to enclose the dimensions of a cube or cylindrically shaped particle. However, the application of this solution may not always be necessary. Using a C-diagram, Milton (1970) was able to show that, under certain conditions, particle residence time can be as little as 0.2 of the bulk average residence time. In small heat exchangers, it can be assumed then that a certain percentage of the food particles gain no temperature at the geometric center prior to entering the hold tube. In this case, the initial temperature of the particle as it enters the heat exchanger should be used in the calculation of required hold tube time.

For larger heat exchangers, the series solution derived by de Ruyter and Brunet (1973) for calculating particle temperature gain in the heater can be applied. As long as an eighty degree temperature differential between the particle center and the fluid exists as the mixture enters the hold tube, the formula method may be used. It will be found, however, that for processing in large heat exchangers, the Formula Method can only be used for the largest slowest heating particulate (e.g., meatballs) because the required temperature differential will not exist. Alternately, the temperature gain in the heater can be forgone to simplify calculations.

It should be noted here that by using the particle center temperature as it enters the heat exchanger as initial temperature, a conservative error is introduced. Ball's heating equation assumes a constant temperature throughout the particle at the start of heating before being suddenly immersed in a constant temperature heating medium. Consequently, lethality will be slightly underestimated because time spent on the curvilinear portion of the heating curve will not be as large as assumed. The error introduced will be greater in larger heat exchangers, because more time is given for heat penetration.

Cooling

In the development of his formula, Ball assumes that the slope of the cooling curve is equal to that of the heating curve. Also, at the end of heating, sudden immersion of the particle into a constant temperature cooling medium is assumed.

Food particles being cooled in a SSHE are not suddenly exposed to the final temperature of the fluid portion of the food, but instead are exposed to a temperature gradient. However, for the fastest moving particle through a small heat exchanger, the error introduced by assuming sudden immersion is not great and is on the conservative side. The fastest moving particle represents the worst case from the standpoint of accumulated lethality (Milton, 1970), and should be used for process calculations in any case. The assumption of sudden immersion for the fastest moving particle might also be made for food in larger heat exchangers. However, the particles would have to be large, slow heating, and the error introduced becomes larger due to the extra time allowed for transferring heat out of the particle.

DISCUSSION

BALL'S FORMULA METHOD assumes that the lethality integration starts below the lethal range. To accomplish this, Ball started the integration at eighty degrees below the heating medium temperature assuming that this would be sufficient to start the integration below the lethal range in all cases. For conventional retorting, the assumption is correct and presents no problems. However, at low acid food aseptic processing temperatures, the assumption represents somewhat of a limitation. Since significant lethality starts accumulating at 195°F for *Clostridium botulinum* (Z = 18), the upper temperature limit for the fluid portion of the food in the hold tube would be 195°F + 80°F = 275°F. Practically, however, this is no real limitation because severe overprocessing of the fluid portion will result at temperatures higher than this. In addition, very high temperature-short time processes may not ensure enzyme inactivation when larger particulate sizes are being processed (Leonard et al., 1964).

The assumption that f_c is equal to f_h is conservatively valid for the fastest moving particle going through the cooling exchanger. However, if the particulate matter is fragile and breakup due to mechanical forces occurs, the assumption will not be valid, because more rapid cooling will occur in the smaller, broken particulate. If particle breakage is observed, lethality from cooling should be eliminated using the rho factor (Ball and Olson, 1957). Required process time can then be adjusted with a few trial and error calculations.

Of extreme importance in the determination of f_h or thermal diffusivity is that the test be carried out in the actual liquid matrix that the particulate is going to be processed in so that the boundary layer is most accurately accounted for. Limited experimentation using one-inch hot dog segments has shown that f_h can increase 40–50% in an oil bath as compared to an f_h determined in water (Dail, unpublished, 1984)

Also, the f_h or thermal diffusivity value should be determined in a nonmoving media since this relationship represents the worst case for heat transfer through the boundary layer. Otherwise, a method for a Biot number calculation is required.

CONCLUSION

THE RECENTLY ISSUED USDA guidelines for aseptic systems in meat and poultry plants allows the use of lethality from the cooling portion of the aseptic process as long as certain conditions are met. It was found that Ball's Formula Method could be used to calculate required residence time in the hold tube within certain limitations. In these instances, the ability to apply Ball's Formula greatly simplifies process calculations, eliminating the requirement of a computer. Generally, the method is best used for foods processed through small heat exchangers because of the assumption of sudden immersion into both the heating and cooling mediums inherent in the Formula Method. However, the method may be used for foods with large, slow heating particulate processed through large heat exchangers if one is willing to trade accuracy for convenience.

NOMENCLATURE

a = Width of square rod
x = ½ Thickness of slab
L = Length
R = Radius of cylinder

r = Inner radius of hollow cylinder
β_m = Root of cosine function = $\pi/2$ for m = 1
β_n = Root of Bessel function = 2.405 for n = 1
J_o = Zero order Bessel function = 1 for $J_o(0)$
J_1 = First order Bessel function = 0.5191 for $J_1(2.405)$
α = Thermal diffusivity of food
α_a = Apparent diffusivity
t = Time
t_l = Time spent on the curvilinear portion of the heating curve
T_{RT} = Temperature of heating medium
T_{IT} = Initial temperature of food
T = Temperature after time t
T_c = Temperature at particle center
B_b = Required process/hold tube time (minutes)
f_h = Negative reciprocal of particle heating curve slope
f_c = Negative reciprocal of particle cooling curve slope
j = Particle heating curve lag factor
I = Heating medium temperature minus particle center temperature entering the hold tube
g = Heating medium temperature minus particle center temperature at end of heating period
F_o = Number equivalent to minutes at 250°F when Z = 18.
F_n = Fourier number
Z = Negative reciprocal of thermal death time curve slope

SAMPLE PROCESS CALCULATION

(1/2 in. × 1/2 in. × 1/2 in. Meat cube in gravy sauce)

f_h Calculation

$$f_h = \frac{2.303L^2}{3\pi^2\alpha} = \frac{2.303(1.61\cdot10^{-4}m^2)}{3\pi^2(1.18\cdot10^{-7}m^2/s)^a}$$

$$= 106.2s = 1.77 \text{ min}$$

Required hold time calculation

Process factors:

j = 2.07
f_h = 1.77
Gravy temperature in hold tube = 270°F
Particle center temperature entering hold tube = 180°F
Required F_o = 15
F_i = 0.077

$$\frac{f_h}{U} = \frac{f_h}{F_oF_i} = \frac{1.77}{1.155} = 1.532$$

Log g = 0.144[b]

Calculation:

$$B = f_h \quad \log \{(jI) - \log g\}$$
$$= 1.77\ (2.270 - 0.114) = 3.8 \text{ min}$$

Required time for series convergence

$$t_l = \frac{L_2F_N}{\alpha} = \frac{(1.61\cdot10^{-4})(0.03)}{1.18\cdot10^{-7}} = 40.9s = 0.68 \text{ min}$$

0.68 < 3.8 therefore, calculation is valid.

[a]Assumed value
[b]Source – American Can Co. (1967)

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Role of Consumer Expectancies in the Acceptance of Novel Foods

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ABSTRACT

Six experiments were conducted to examine factors affecting the consumer acceptance of novel foods. Variables included for analyses of their effects were: (1) preparation variables; (2) product name and type of serving vessel; (3) brand labels and packaging; (4) availability of product information; (5) nature and quantity of product information; and (6) degree of familiarity of the user with the product. Results of these experiments were interpreted within a theoretical framework that postulates that the hedonic response to food is a function of the degree to which expectancies about the food are matched by subsequent experiences with it. Based on the theory of cognitive dissonance, this theoretical framework is proposed as a useful analytic tool for predicting consumer responses to novel foods.

INTRODUCTION

MORE THAN 50,000 food items are currently marketed within the United States. This wide variety of items reflects our cultural diversity, the omniverous nature of human beings and the enterprising nature of the American food industry. Although many of these food items are consumed by a wide cross-section of the population, others, such as ethnic, regional and gourmet foods, are consumed by only small sub-groups. The foods falling within the latter category are often placed under the rubric of "novel" foods, where "novel" is defined by Webster's dictionary as "new, recent, strange or unusual." Examples of these foods include squid, venison, halavah, tofu, grits and kiwi fruit. Others include those prepared and manufactured for special applications in clinical dietetics, in space flight, or for the military; or those that are the result of new processing technologies, e.g. restructured meats, dehydrated foods, and alternative protein products.

Although the primary factors controlling purchase and consumption of most foods are availability, cost, hedonic preference, and nutritional value, additional factors play an important role in the selection or nonselection of novel foods. In fact, many consumers avoid novel foods simply due to lack of familiarity with them. This food neophobia may be due to past regional nonavailability, idiosyncracies in familial food practices or religious and/or cultural taboos.

In order to induce consumers to try previously untried foods, researchers have examined a variety of advertising and product-image variables. Woodward (1945) studied the effects of motivational appeals, such as "American," "money," "variety" and "nutritional value" on the consumption of soy dishes. He found an increase in consumption of some soy products when a combination of "American," "money," and "variety" appeals were used and a decrease in consumption when only "nutritional" appeal was used. Greaves and Ramsey (1980) studied the effects of person-to-person discussions, demonstrations and advertising on the acceptance of textured vegetable protein, but found unpredictable variation in consumers' responses to these techniques. Other factors that may increase the likelihood of self-exposure to novel foods include (1) product name, (2) packaging, (3) nutritional information, (4) cost information, (5) usage information, and (6) presentation of the product in an appealing form or recipe.

Although food habits are notoriously resistant to change (Seaton and Gardner, 1959; Peryam, 1963), several studies have demonstrated an effect of simple exposure on subsequent consumption of novel foods. For example, Rozin and Schiller (1980) have demonstrated that repeated exposure to the novel and aversive taste of chili peppers will reduce negative hedonic responses to this foodstuff. Similarly, mere exposure has been shown to increase consumption and acceptance of foods, independent of their hunger-reducing value (Hollinger and Roberts, 1929; Torrance, 1958; Capretta and Rawls, 1974; Domjan, 1975, 1976; Kalat and Rozin, 1973). The latter effect is strongest when exposure occurs early in development (Kuo, 1967; Capretta et al., 1975). Zajonc (1965, 1968) has reviewed similar positive effects of mere exposure on the acceptance of a wide variety of stimuli, including music and paintings, and Harrison (1976) has provided a cogent review of more recent data and their theoretical implications.

Unfortunately, the positive effect of exposure to a food applies only within limits. Beyond these exposure limits, decrements occur in acceptance due to monotony (Kamen and Peryam, 1961; Siegel and Pilgrim, 1957; Zelmer, 1970). Thus, acceptance is an inverted-U function of exposure (Berlyne, 1970; Stang, 1975), with the major gains to be achieved during initial presentations of the product.

The U.S. Armed Forces and N.A.S.A. frequently require the development of "novel" foods for use in extreme environments. As a result, our laboratory has conducted a series of studies to assess the factors affecting the acceptance of novel foods. The results of several of these studies have implicated consumer expectancies as a determining factor in the acceptance of these foods. The explanatory power of a model of acceptance based on the hedonic consequences of the confirmation or disconfirmation of expectancies has been suggested previously by Aronson and Carlsmith (1962) and Carlsmith and Aronson (1963). Their model is based on the psychological concept of "cognitive dissonance" (Festinger, 1957), which postulates that psychological discomfort will arise from any situation in which behavioral or perceptual events are incongruent with an individual's expectations (Carlsmith and Aronson, 1964). The objective of the present set of studies was to assess the effect of a variety of variables on the acceptance of novel foods. The variables of concern were: (1) preparation variables; (2) product name and type of serving vessel; (3) brand labels and packaging; (4) availability of product information; (5) nature and quantity of product information; and (6) familiarity of the user with the product.

MATERIALS AND METHODS

Experiment 1 — Preparation variables

Samples. Six batches of ground beef were extended by 20% by the addition of different soy protein ingredients that were chosen to maximize sensory differences among the samples. Information on these

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ingredients, including the results of a sieve test to determine particle size distributions, are shown in Table 1.

Appearance differences were induced in the samples through the use of colored and uncolored soy ingredients. Flavor character was manipulated by the use of both soy concentrates and flours. Texture was varied through the use of ingredients with differing particle size distributions that were previously shown to produce significant differences in the texture of 20% extended patties (Cardello et al., 1983). In addition, an unextended sample of ground beef served as a control.

The 20% extended products were produced by mixing choice chuck (3/4" grind plate) with one of the six soy ingredients, each of which had been rehydrated to 18% protein. Beef fat or fat-free muscle tissue was added to the beef to obtain a 22% fat level, producing extended products with a fat level of 17.5%. The control sample was adjusted to 17.5% fat. Each treatment batch was passed through a 1/8" grind plate. A Hollymatic Press (model 52) was used to produce 2 oz patties. The prepared patties were frozen to −18°C and stored for 3 months.

In the first test, samples were prepared and cooked as patties. Half of each lot of patties was thawed at room temperature, placed on a 177°C, preheated, flat grill and cooked to an internal temperature of 71°C (internal temperature probe), turning once at 66°C. All samples consisted of one-half patty served on a heated dish without condiments.

In the second test, samples were prepared and cooked as chili (Armed Forces Recipe #L-28, adjusted to make 50 servings). Chili was chosen for its spicy flavor, dark color and noncohesive texture, all of which would likely minimize flavor, color and texture differences among the samples. On the day prior to the test, the remaining lots of patties were thawed at room temperature and mixed with the other recipe ingredients. The chili was cooked, allowed to cool, and stored overnight at 4°C. Prior to the test, samples were heated to 65°C. Samples consisted of 50g chili served in a heated bowl.

Panelists and test environment. Two random samples, each consisting of 50 male and female panelists, were drawn from a 460-member volunteer, consumer panel consisting of civilian and military personnel employed at Natick Laboratories. Approximately 90% were civilian. Sixty-six percent were males. The age of members varied from 17 to 70 years, with a mean age of 42.4 years. As part of the membership requirements, all panelists were instructed not to discuss specific aspects of panel tests with other members. In addition, panelists were not informed of the nature of the test when recruited to avoid panelist bias and self-selection for participation.

Testing was conducted in light-controlled testing booths. Samples were presented to panelists through serving hoods and were accompanied by distilled water for rinsing. An interstimulus interval of 90 seconds was maintained throughout all tests.

Procedure. Each test consisted of two sessions. During each session panelists were asked to evaluate three extended samples and the control sample. The order of presentation of samples was random. Panelists were asked to judge the (1) overall acceptability, (2) acceptability of texture, (3) acceptability of flavor, and (4) acceptability of appearance of the products. A nine-point, labeled hedonic scale was used to make all judgments (Peryam and Pilgrim, 1957).

Experiment 2 — Product name and serving vessel

Samples. Samples consisted of flavored dental liquids and commercial, canned soup. The dental liquid products, developed by the Food Engineering Laboratory at NRDC for consumption by jaw-fracture patients were prepared by pulverizing cooked, freeze-dehydrated meat, into a particulate, viscous liquid that can be consumed through a straw. Chicken cacciatore and beef stroganoff diets were used in these tests. Cream of mushroom soup (Campbell Soup Co.) was used as a counterpart test sample to the liquid diets, because it is a moderately viscous, flavorful liquid food that can be consumed in the same manner as the liquid diets. The canned mushroom soup concentrate was mixed with water and prepared according to manufacturer's directions. Both the dental liquid diets and the soup were heated to 68°C and maintained at that temperature in a water bath during testing.

Panelists and test environment. Two tests were conducted. Each used sixty, randomly chosen volunteer consumer panelists. In this test and all subsequent ones, the selection of panelists was totally random (with replacement) from the pool of 460 volunteers. Thus, some small percentage of panelists may have participated in Experiment 1 (or in subsequent experiments). However, since each experiment reported in this paper involved a different set of independent variables and different test samples, negligible carry over effects would be expected.

For each test, thirty panelists were assigned to a "bowl and spoon" condition, and thirty were assigned to a "glass and straw" condition. In the "bowl and spoon" condition, samples were presented in opaque, ceramic bowls with a soup spoon. In the "glass and straw" condition, samples were presented in a transparent glass with a plastic straw.

Procedure. In Test 1, the dental liquid and the soup were labeled "soup" in both conditions. All panelists judged the acceptability of the flavor, acceptability of the texture and overall acceptability of the product on the same nine-point, labeled, hedonic scale used in Experiment 1. Order of presentation of samples was randomized.

Test 2 was conducted in the same manner as Test 1. However, only those samples presented in the "bowl and spoon" condition were labeled "soup." The samples in the "glass and straw" condition were labeled "dental liquid diet."

Experiment 3 — Brand label and packaging

Samples. Test samples consisted of individual serving-size packages of commercial instant coffee (Maxwell House brand; General Ford Corp., White Plains, NY), commercial powdered non-dairy creamer (Coffee-mate brand; Carnation Co., Los Angeles, CA), commercial grape jelly (Smucker's brand; J.M. Smucker Co., Orrville, OH), and commercial salted crackers (Monarch brand; PYA/Monarch, Inc., Greenville, SC). The commercial package for each of these items can be seen on the left side of Fig. 1. Four military counterparts to these commercial products were also tested: military-pack instant coffee (Tenco; Linden, NY), military-pack, powdered, non-dairy creamer (Beatrice Foods Co., Madison, WI), military-pack grape jelly (Kern Foods, Inc., Industry, CA) and military-pack salted crackers (American Pouch Foods, Inc., Willowbrook, IL). All of the latter items were prepared and packaged according to military ration specifications, and are shown on the right side of Fig. 1.

A factorial design was used, in which both the commercial and military-pack items were tested in both the commercial and military packages. To accomplish this, one-half of each set of packages were emptied and their contents replaced with their counterpart items.

Panelists and test environment. Forty different, randomly selected consumer panelists were used in each of the four conditions. Panelists were chosen from the same pool of panelists used in Experiments 1 and 2, and the testing environment was the same.

Procedure. Conditions were run on each of the four consecutive days. Panelists were given written instructions at the start of the ses-

Table 1—Description of soy additives used in Experiment 1

Soy Protein	Color Added	Product #	Manufacturer	Density g/mL	% Retained on sieve Sieve opening size 12.7mm	9.52mm	4.76mm	2.38mm	1.41mm	9.25mm	Bottom
Textured soy concentrate	No	4400	Central Soy	0.24	0.01	0.00	21.01	47.47	17.41	12.48	1.46
Textured soy concentrate	No	7240	Staley	0.26	0.00	0.00	1.87	65.78	19.07	12.04	1.03
Textured soy concentrate	Yes	7241	Staley	0.30	0.00	0.00	1.03	40.68	31.05	25.47	1.66
Granular soy concentrate	No	2060	Staley	0.58	0.00	0.02	0.01	0.03	0.38	98.13	1.25
Textured soy flour	No	165-118	ADM	0.40	0.00	0.00	0.51	41.44	36.21	20.86	0.83
Textured soy flour	Yes	165-210	ADM	0.34	24.93	60.39	13.10	0.05	0.04	0.49	0.76

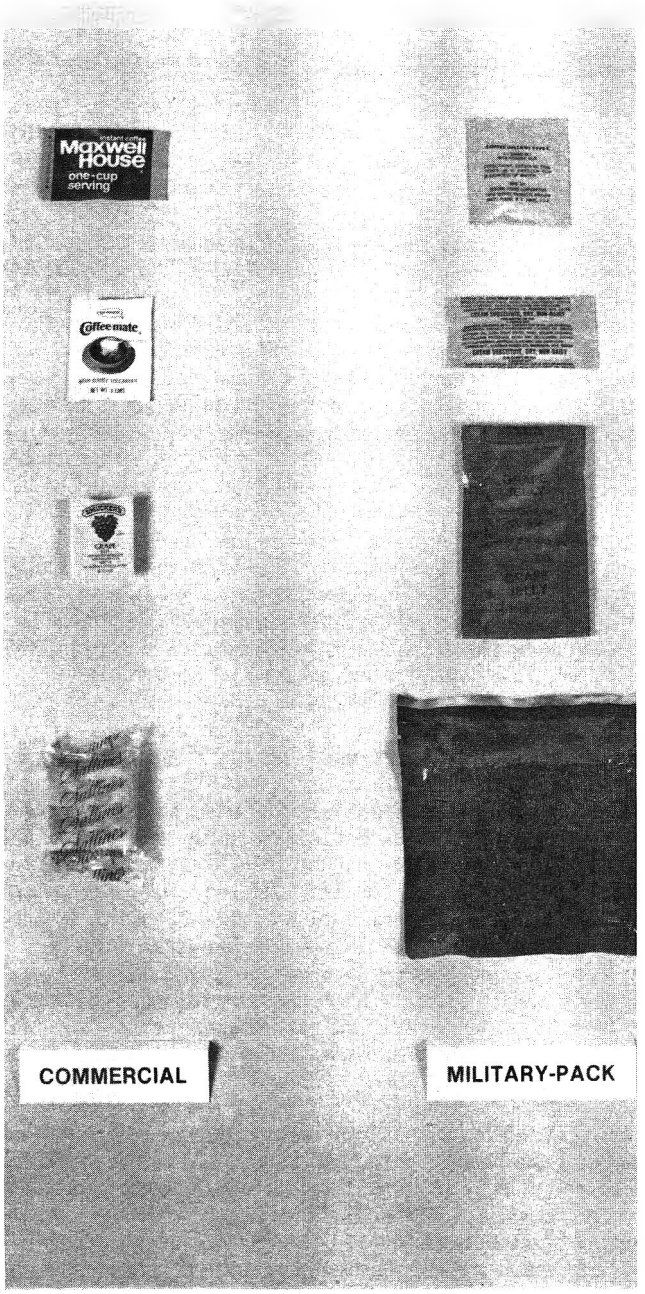

Fig. 1—Photograph showing commercial and military packaging used in Experiment 3.

sion and no mention was made of the relevance of the packaging. Panelists were told that they were participating in an acceptance test of the items and that the packages had been pre-opened for their convenience.

The four test items were presented in fixed order. The package of instant coffee was presented first, placed label-side-up and facing the panelist on a saucer. It was accompanied by an 8-oz ceramic cup of hot water and spoon. Panelists were instructed to place the entire contents of the package into the cup of hot water, stir and taste. After tasting, panelists rated the coffee for acceptability using the 9-point hedonic scale used in Experiments 1 and 2. After passing their rating card back through the serving hood, the package of powdered nondairy creamer was presented, label-side-up in a small transparent cup. The panelist was instructed to pour the entire contents of the package into the cup of coffee. After tasting and rating the acceptability of the creamer, the coffee cup, saucer, etc. were returned through the serving hood.

The opened package of grape jelly was presented next, label-side-up on a small plate with a spoon. Panelists were instructed to taste one teaspoonful of the jelly and rate it for acceptance. After returning the jelly and rating card, the package of crackers was presented, label-side-up, for evaluation.

Experiment 4 — Availability of product information

Samples. Two tests were conducted, one using soybean curd (tofu) as a test product and the other using squid (*Ilex illecebrousus*) as a test product. Each was prepared and cooked in two different ways to expand upon the effects of preparation variables examined in Experiment 1.

Samples of tofu were prepared from firm tofu (Nasoya Foods, Leominster, MA), cubed into small portions (approximately 2.5 cm × 2.5 cm × 2.5 cm) and pressed for 25 min to remove excess water. Half of the portions were placed into a heated (68°C) chicken broth (RJR Foods Inc., NC) for 20 min and served to panelists in ceramic bowls with spoons. The other portions were fried in vegetable oil (Wesson) at 177°C for approximately 10 min, until the tofu had a crisp outer skin. Fried samples were served with chicken broth sauce made from 1 can chicken broth (RJR Foods, Inc., NC), 4 tablespoons margarine and 4 tablespoons flour and simmered in a frying pan for 20 min. Fried samples were served on a heated plate with knife and fork.

Samples of squid were prepared from pre-frozen squid mantles (Pier 12 brand, Aslanis Fisheries; Pappas International Foods, Boston, MA). After cleaning, mantles were separated into two batches. Samples in one batch were prepared by placing four whole mantels in an uncovered casserole dish along with 1/4 cup water, 2 teaspoons lemon juice and 1/4 teaspoon salt. The mantles were cooked for 4 minutes in a conventional microwave oven. After cooking, mantles were cut across their width into 3 cm sections and served in heated bowls with a fork and knife.

The second batch of mantles were sliced into 2 cm wide circular rings. The rings were dipped into a batter consisting of eight large eggs, 2 teaspoons oregano, 1 teaspoon salt and 1/2 teaspoon black pepper, placed in a plastic bag with flour, shaken until coated, and deep fried in corn oil (191°C) until golden brown. Fried samples were served on heated ceramic dishes with a fork and knife.

Panelists and test environment. The squid and tofu samples were evaluated separately by sub-samples of the same consumer population used in Experiments 1–3. Panelists were first screened for food preferences using a food preference survey consisting of 34 novel food names. Respondents were asked to indicate *how much* they liked each food item using a 9-point labeled hedonic scale, and *how often* they ate each, using a 7-point labeled frequency scale. A "never tried" category was also available. Approximately 200 members of the consumer panel completed the survey, and the subject pool for the experimental sessions consisted of those respondents who had "never tried" soybean curd (or squid). Thirty-two male and female panelists were used in each experimental condition. The test environment was the same as used in the previous experiments.

Procedure. Three information conditions were evaluated. In the first condition (low level of information), the two samples of tofu (or squid) were presented sequentially, in random order, to panelists. Subjects were merely told that they were receiving "oriental tidbits" (or "seafood tidbits") for evaluation. No other information was provided. Each of the 32 panelists rated each sample for overall acceptability using a 9-point labeled hedonic scale and responded "yes" or "no" to four purchase and use questions; i.e., "would you prepare this food at home," "would you order this food in a restaurant," "would you serve this food to guests" and "would you serve this food to your family."

In the second condition (intermediate level of information), the panelists were given nominal information about the nature of the test product. They were told that they were receiving "soybean curd (tofu)" (or "squid"), and no additional information. The rating scales were the same as described above.

In the third condition (high level of information), panelists were told that they were evaluating "soybean curd (tofu)" (or "squid"). In addition each panelist was also given an information sheet that provided explicit information about the product, including its sensory qualities, nutritional value, cost and suggested uses.

Experiment 5 — Nature and quantity of product information

Samples. Nonfat dry milk was chosen as the test product, because it is a familiar product that varies greatly in use and acceptance by consumers. Two batches of instant, nonfat dry milk (Carnation Co., Los Angeles, CA) were prepared according to manufacturer recom-

mendations. One cup chocolate syrup (Hershey Co., Hershey, PA) per two quarts liquid milk was added to one of the batches in order to assess the effect of differences in preparation. Samples were chilled to 4°C and held at 4 ± 0.5°C during testing. Samples were served in 1 oz transparent glasses.

Panelists and test environment. Consumer panelists were drawn from the same panel population used in Experiments 1-4. Six groups of thirty-six (36) panelists each participated in one of six experimental conditions. All panelists were chosen randomly and without consideration for their prior use of nonfat dry milk.

Procedure. Six test sessions were conducted over a 2-wk period. Conditions differed only with regard to the nature and quantity of the information presented to panelists. In the first condition, panelists were given no information other than that the samples consisted of "nonfat dry milk." This information was posted at the entrance to the test booths. In conditions 2-5 panelists were given written, one-paragraph, product information statements concerning nonfat milk. These statements contained information on cost, flavor properties, versatility of use, or nutritional value, for conditions 2-5, respectively. In the last condition, all four information paragraphs used in conditions 2-5 were presented to panelists.

To ensure that the information that was presented was actually read, a written quiz was administered to all panelists in conditions 2-6. The questions covered facts provided on the information sheets and were administered to panelists before they received their first test sample.

Order of presentation of the two test samples was randomized. Panelists were requested to rate each for acceptability, using the same 9-point hedonic scale used in Experiments 1-4, and to respond to the same four "purchase and use" questions used in Experiment 4.

After completing their evaluation of the two test samples, panelists were given a short usage survey to complete. The survey asked (1) whether the panelist had ever tried non-fat dry milk (YES/NO), (2) if yes, when was the last time (six category choices ranging from "within the past month" to "within the past 20 years or more.") and (3) if yes, how often they currently drink nonfat dry milk (seven category choices ranging from "never" to "more than once per week.")

Experiment 6 — Familiarity with the product

Samples. Test samples were three familiar food items (mashed potatoes, peaches and baked beans) and three similar, but unfamiliar (novel) food items (grits, kumquats and black-eyed peas). The mashed potatoes (Stop & Shop brand, Stop & Shop Co., Chicago, IL) and the grits (Quaker Oats Co., Chicago, IL) were "instant" variety. Both products were made according to manufacturer recommendations and served hot in 4 oz ceramic bowls. The baked beans (brick oven) were canned (Friend's Brand, W.M. Underwood Co., Westwood, MA), as were the black-eyed peas (N.K. Hurst Co., Indianapolis, IN). Both were removed from the can, heated to 80°C and served in 4 oz ceramic bowls. The peaches (Libby, McNeill and Libby Co., Chicago, IL) and the kumquats (Oriental Pioneers Industry Corp., Taipei, Taiwan) were both canned in syrup. They were removed from the can and served at room temperature in ceramic bowls.

Panelists and test environment. Panelists consisted of 432 consumers drawn from the same panel population described previously. Panelists were assigned randomly to one of twelve test groups. No attempt was made to screen panelists on the basis of prior use or familiarity with the test items. However, it was presumed that the choice of test samples would ensure that the average consumer population would show a large variation in both familiarity and use for the two groups of foods. In this way, any observed effects could be generalized beyond a restricted user population. Test environment and other general conditions of testing were the same as described previously.

Procedure. Twelve tests were run on consecutive days. Two conditions were run for each of the six test foods. Panelists were first asked to complete a short survey on their use of 20 food items. Embedded within this list were the six test items.

In the first of the two conditions, panelists were presented with no information other than product name. In the second condition, panelists were presented with a product information sheet upon entering the test booth. The information sheet consisted of a single paragraph summarizing the nutritive value, cost, market availability and versatility of the product. Panelists were requested to rate the sample for acceptability using the same 9-point scale used in previous experiments. Questions concerning use were the same as used in Experiment 5. Two-way ANOVA's and Chi-Square tests were applied to the data to assess the effect of information and food type (novel vs familiar) on acceptance and intended purchase and use.

RESULTS & DISCUSSION

Experiment 1 — *Preparation variables*

Analyses of variance conducted on the ground beef patty data showed statistically significant differences among the samples on all four judged attributes ($F's = 2.30, 5.48, 2.26$ and 3.73; $df = 7, 245$; $p<0.05$, <0.001, <0.05, and <0.001 for appearance, flavor, texture and overall acceptability, respectively). For the chili data a significant effect was found only on judgments of flavor acceptability ($F = 2.12$; $df = 7,259$; $p<0.05$).

Although Neuman-Keuls contrast tests showed no significant differences among the extended ground beef patties on judgments of appearance, two of the samples extended with uncolored textured soy concentrates (#4400 and #7240) were rated significantly different in appearance from the unextended, all-beef control ($p<0.05$). Similarly, on judgments of flavor and overall acceptability, the granular soy concentrate (#4400) was rated significantly less acceptable ($P<0.05$) than, the unextended control and significantly less acceptable ($P<0.05$) than all of the other samples, with the exception of the rating of flavor acceptability for textured soy concentrate #7241, for which there was no significant difference. On judgments of texture, textured soy concentrate #7240 was rated significantly less acceptable ($P<0.05$) than the all-beef control.

For the chili data, only one significant difference was found. The flavor of the textured soy flour ingredient (#165-210) was rated significantly less acceptable ($P<0.05$) than the all-beef control.

The theory of cognitive dissonance postulates that experiences that match prior expectancies result in greater satisfaction than do experiences that fail to match expectancies. In keeping with this postulate, Nichoff (1967) has argued that one of the most important factors in the acceptance of any novel food is that its characteristics be similar to those of the product it is replacing. In the present study, considerably more differences were found between the extended and unextended patty samples than between the extended and unextended chili samples. These results provide data to support Nichoff's argument, demonstrating that novel ingredients/products that are used within the context of multi-ingredient recipes are less likely to be differentiated from the unadulterated forms of the products that they are replacing. Although Nichoff's postulate is seemingly trite, the importance of it to the present paper is that it is consistent with an interpretation based on the confirmation or disconfirmation of consumer expectancies. That is, the reason that the acceptance of a novel food is dependent on its similarity to a more familiar item can be directly attributed to an increased likelihood that the perceptual experience with the novel product matches the perceptual expectancy set by experience with the familiar product that it is replacing. The technique of using preparation variables (alternative recipes) as a means for reducing the perceptual differences between such products is only one of several that can be effectively used by investigators attempting to introduce novel products to the market.

Experiment 2 — *Product name and serving vessel*

Analyses of variance were conducted to assess the effects of sample (dental liquid vs soup) and serving vessel/mode of consumption (bowl and spoon vs glass and straw). In Test 1, a significant main effect ($F = 7.83$; $df = 1,57$; $p<0.01$) of samples was found on judgments of the acceptability of the texture. The mushroom soup sampled was rated significantly more acceptable than the dental liquid diet. No other effects were significant.

In Test 2 there was again a significant main effect ($F = 7.50$; $df = 1,57$; $p<0.01$) of sample on judgments of the acceptability of the texture. However, there was also a significant interaction effect ($F = 5.18$; $df = 1,57$; $p<0.05$) of sample x serving ves-

sel/mode of consumption, such that the acceptability of the texture of the mushroom soup was greater than that for the dental liquid in the "bowl and spoon" condition, but less than that of the dental liquid in the "glass and straw" condition.

Although the effects of product name have often been investigated for traditional foods (Bachrach, 1970; Schutz et al., 1972; Rucker et al., 1973; Kincaid, 1975), only two published reports bear directly on the problem of novel foods (Seaton and Gardner, 1957; Wolfson and Oshinsky, 1966). Seaton and Gardner (1958), working with meat-flavored liquid foods, showed that the descriptive name of "Air Force meat-flavored liquid foods" significantly increased the acceptance of these products over the non-descript name "experimental samples." Similarly, Wolfson and Oshinsky (1966) found that the descriptive label "space food" produced significantly higher acceptance ratings for liquid foods than did the label "unknown." For the effects of serving vessels and/or modes of consumption, only the study by Seaton and Gardner (1959) is directly relevant. Here the investigators either served the liquid diets in cups for normal drinking or in opaque bottles for sipping through a tube. Significantly higher acceptance ratings were found in the bottle condition and were attributed to the masking of the negative aspects of the appearance, odor and temperature of the products.

In contrast to the results of Seaton and Gardner (1959), the data from Test 1 suggest that serving vessel/mode of consumption had no effect on the acceptance of either novel dental liquids or soup. However, Seaton and Gardner (1959) only observed their effect in the condition in which liquid diets were labeled as "experimental samples." They postulated that, in this condition, the novelty of sipping the samples from opaque bottles may have increased subjects' interest in the samples. However, an alternative explanation, based on the hedonic consequences of the confirmation or disconfirmation of expectancies (Aronson and Carlsmith, 1962; Carlsmith and Aronson, 1963), can account for both Seaton and Gardner's results, as well as the present data.

In most studies of food acceptance the expectancy of the subject for the food is sometimes set by the sample label and sometimes by its serving vessel. For example, if a subject is told that he/she will evaluate roast beef, an expectation is generated about how the sample should look, smell, taste, etc. If this expectation is not met, cognitive dissonance results, with accompanying negative effect. On the other hand, if little or no information is given about the product, then subjects' expectancies may be set by other cues, such as the mode of presentation of the sample, its serving vessel, etc. In the descriptive label condition of Seaton and Gardner (1958), expectancy was set by the label. The label generated an expectation for a food with novel flavor, texture, appearance, etc. The serving vessel would not have been anticipated to have had an effect on this expectancy, since the subjects would have had no pre-conceptions about how this food should be served. The expectancy for a novel food was undoubtedly met by the subsequent "taste" of the meat-flavored liquids. However, in the nondescriptive label condition, expectancies were likely set by the serving vessel. The conventional "glass cup" condition would have established an expectancy for a conventional beverage. Meat-flavored liquid would not have met this expectancy. The opaque bottle condition would have led to an expectancy for something novel; an expectancy that was met by the meat-flavored liquids.

The results of the present study can be similarly explained. Since all samples in Test 1 were labeled "soup," an expectancy for soup-like products was established. The texture and flavor of the mushroom soup met normal consumer expectations for "soup;" whereas, the texture and flavor of the dental liquids did not, since their texture was more viscous than soup, with suspended particulate matter and a novel, very heavy, spicy flavor. As in the "Air Force meat-flavored liquid food" condition of the Seaton and Gardner (1959) study, serving vessel/mode of consumption had no significant effect on acceptance judgments in Test 1. However, a significant effect of sample was observed. The mushroom soup was rated significantly more acceptable than the dental liquid, the former confirming the "soup" expectancy, the latter disconfirming it.

The "expectancy" explanation is further supported by the results of Test 2. The only difference between this test and Test 1 was that the label "soup" was changed to "dental liquid" in the "glass and straw" condition. Results in the "soup" condition were identical to the results of Test 1 in which samples were labeled as soup. However, when the label in the "glass and straw" condition was changed to "dental liquid," the effect was reversed. The dental liquid was rated more acceptable than the mushroom soup. The change in expectancy, caused by the change in label, made the soup more dissonant and resulted in a reduced affective response.

Experiment 3 — *Brand label and packaging*

The data were statistically analyzed across foods to test the null hypothesis of "no difference in acceptance ratings within package conditions." A t-statistic calculated on the difference scores between military and commercial items showed no significant difference from zero in the military package condition, but a significant difference in the commercial package condition ($t = 2.78$, $df = 159$, $p < 0.01$), indicating significantly higher acceptance ratings for the commercial items in the latter condition (Fig. 2). The data from this experiment are consistent with the hypothesis that established brand labels and packages create an expectancy for a "quality" product. This expectancy was met by the commercial items, but not by the military-pack items. The military labels and packages, on the other hand, create a less stringent expectancy. This expectancy was adequately met by both the commercial and military-pack items.

Experiment 4 — *Availability of product information*

Fig. 3 (tofu) and 4 (squid) show the relevant data from this experiment. A two-way analysis of variance conducted on the acceptance ratings for tofu showed a significant main effect of information condition ($F = 5.17$; $df = 2,93$; $p < 0.01$). Newman-Keuls contrast tests showed that the acceptance ratings for the fried samples in the "high" and "intermediate" information conditions were significantly ($p < 0.05$) greater than the ratings in the "low" information condition. No significant differences were found for the samples served in broth. The results of Kruskal-Wallis tests applied to the four "purchase

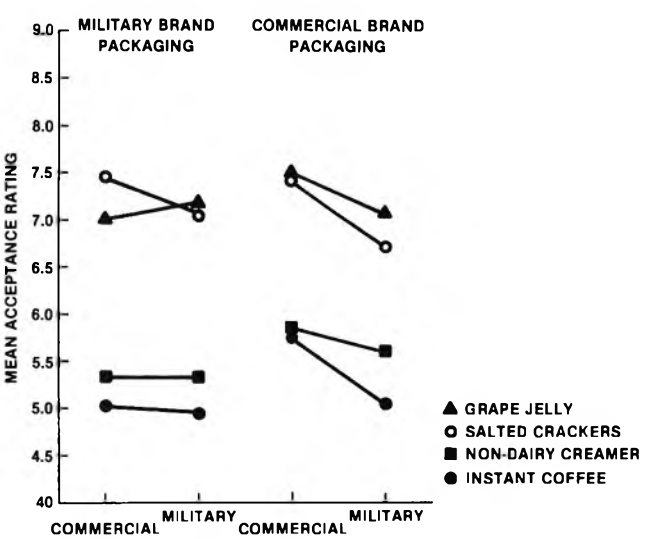

Fig. 2—Plot of the mean acceptance ratings as a function of product type (military vs commercial, designated on abscissa) and product packaging.

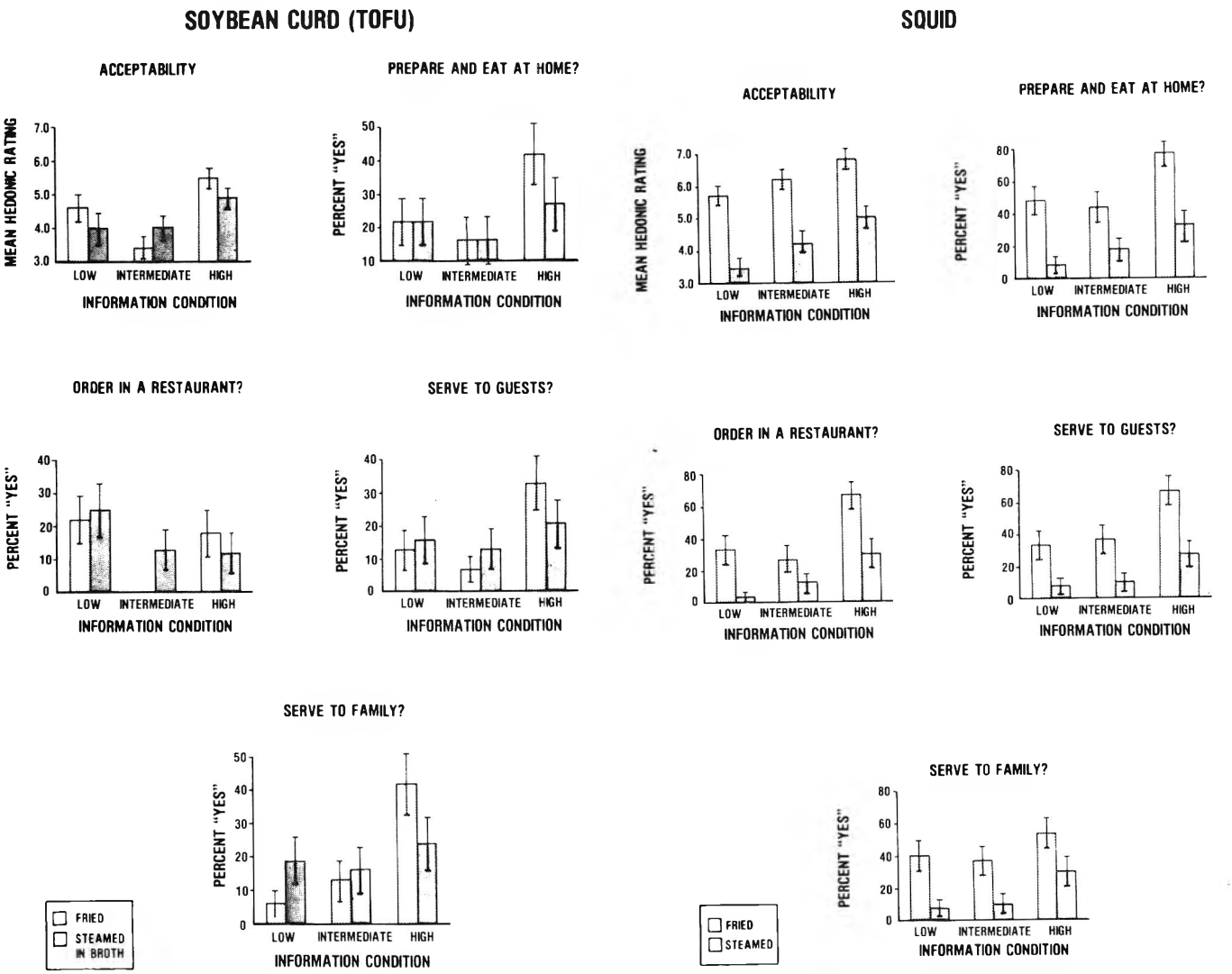

Fig. 3—Plots of the mean acceptance ratings for tofu and the percentage of "YES" responses to each of the purchase and use questions for the three test conditions of Experiment 4.

Fig. 4—Plots of the mean acceptance ratings for squid and the percentage of "YES" responses to each of the purchase and use questions for the three test conditions of Experiment 4.

and use'' questions were similar to the results for the acceptance data. Question 1 (would you prepare and eat it at home?), Question 3 (would you serve it to guests?), and Question 4 (would you serve it to your family?) showed significant main effects of condition for the fried samples ($H = 8.02$, $df = 2$, $p<0.05$; $H = 10.57$, $df = 2$, $p<0.01$; and $H = 12.79$, $df = 2$, $p<0.01$, respectively). No significant effects were found for the samples served in broth. The ''high information'' condition elicited a greater proportion of ''yes'' responses to these questions than did either the ''low'' or ''intermediate'' information conditions.

The two-way ANOVA on the acceptance ratings of squid showed significant main effects of treatment ($F = 4.78$; $df = 2,87$; $p<0.05$) and sample ($F = 190.14$; $df = 1,87$; $p<0.001$). The Newman-Keuls tests showed that only the difference between the ''high'' and ''low'' information conditions was significant ($p<0.05$), but that the difference was significant for both the fried and steamed samples. The Kruskal-Wallis tests conducted on the ''purchase and use'' questions showed a significant treatment effect on Question 1, Question 2 (would you order it in a restaurant?) and Question 4. Moreover, for Question 1 and 2 the effects were found for both the fried and steamed samples (Question 1, fried: $H = 8.99$, $df = 2$, $p<0.05$; Question 1, steamed: $H = 7.71$, $df = 2$, $p<0.05$; Question 2, fried: $H = 9.88$, $df = 2$, $p<0.01$; Question 2, steamed: $H = 8.39$, $df = 2$, $p<0.05$). On Question 4, the effect was only significant for the steamed sample ($H = 7.21$, $df = 2$, $p<0.05$). Again, the ''high'' information condition elicited a greater proportion of ''yes'' responses than did either of the other two conditions.

If the hypothesis put forth by Aronson and Carlsmith (1962) is correct, namely that the judged acceptability of foods can be explained on the basis of relative matches and mismatches between consumer expectancies and actual experiences, then one should be able to minimize such mismatches by providing the consumer with product information, so that the consumer's expectancies of the new product are modified to better match the actual product characteristics. By reducing the degree of mismatch between expectations and experience, information should have the effect of increasing the acceptance of the food item.

The present data both support this contention and extend the effect from mere acceptance ratings to consumers' stated intentions of purchase and use. These data also confirm the importance of sample preparation on acceptance, as found in Experiment 1. This latter effect was seen most clearly in the squid data, where the fried samples were preferred to the steamed samples under all conditions. A similar trend in preferences for the fried samples over the samples served in broth were seen in the tofu data.

Experiment 5 — *Nature and quantity of product information*

Performance on the quizzes indicated that all groups of panelists receiving information sheets read and understood the factual contents. Over 97% of panelists in tests 2-5 made one error or less. Over 97% of the panelists in Test 6, who received 12 test questions, made three or less errors.

Data obtained from the different treatment groups were first analyzed by grouping the data across panelists. A two-way ANOVA was conducted to assess the effect of treatments (different product information groups) and the effect of flavoring (white vs chocolate milk). Results of the ANOVA showed a significant effect of flavoring ($F = 56.04$; $df = 1,209$; $p<0.001$) on acceptability judgments (chocolate milk was rated more acceptable than white milk), but no treatment effect. Kruskal-Wallis tests conducted on the responses to the "purchase and use" questions also showed no significant treatment effects suggesting that the information manipulations used in the present study were not differentially effective in modifying expectations.

In order to assess the effect of prior or current use of nonfat dry milk on judgments of acceptance, Kendall rank-order correlation coefficients were calculated for the association between acceptance ratings and responses for (1) the last time the panelist had tried nonfat dry milk and (2) how often the panelist currently drinks nonfat dry milk. In both cases, those panelists who had never previously tried nonfat dry milk were placed into the "never" category. This category was treated as the most chronologically remote "use" category and the most infrequent "current use" category. While the correlation coefficients were low for the purpose of predictability (Kendall's tau = 0.13 and 0.16 for past and current use questions, respectively), they were both statistically significant ($p<0.01$) and indicated that the more recent or the more frequent the use, the greater the judged acceptance of the product.

Although the above correlational analyses may simply reflect the fact that people who like nonfat dry milk consume it more frequently and more recently than people who dislike it, they raise the question of whether those people who have never tried it are more susceptible to the effects of product information than are those people who have tried it previously. It might be argued that product information would only have a beneficial impact on product acceptance for products that are truly novel to the individual, since expectations would still be maliable and not constrained by prior experiences with the product, as would be the case with products with which the individual has had some prior familiarity. Therefore, the data were reanalyzed by separating and comparing panelists according to whether or not they had ever previously consumed non-fat dry milk.

A two-way ANOVA conducted on the acceptance ratings of panelists who had previously tried nonfat dried milk showed a significant main effect of sample type ($F = 32.09$; $df = 1,155$; $p<0.001$), but no effect of information. Similarly, Kruskal-Wallis tests on the purchase and use questions showed no significant effects of information condition.

For panelists who had never tried non-fat dry milk there was a significant main effect of sample type ($F = 29.48$; $df = 1,48$; $p<0.001$), but no effect of information condition on acceptance ratings. However, a significant effect of information condition was found on the two purchase and use questions concerning "ordering the food in a restaurant" and "serving it to guests." (t statistic = 13.74, $df = 5$, $p<0.05$ and 12.48, $df = 5$, $p<0.05$, respectively). In both cases the information concerning "versatility of product use" had the greatest positive effect.

Although these results are not conclusive, the significant effects for non-users suggest that product information may have beneficial effects on purchase and use of untried (novel) foods, but relatively little effect on purchase and use of familiar foods. This finding supports the notion that product information functions by increasing the likelihood that a consumer's expectations of a product match his/her experiences with it. For novel foods, where expectancies may be quite different from the actual product, information has the potential to play this role. However, for familiar foods, expectations are likely to have reached a state of consolidation, and additional information does not add significantly to the likelihood that expectancies will match experiences.

Experiment 6 — *Familiarity with the product*

Table 2 shows the result of the usage surveys given to panelists. As can be seen, there are large differences in all three aspects of usage between the "familiar" and "novel" foods. All three 'familiar" foods had been consumed at least once by over 97% of all test populations and were eaten at least once per year *and* within the past year by over 80% of all panelists. Although some of the "novel" foods, such as grits, were tried previously by as many as 75% of the test panelists, less than 39% had tried any one of these foods within the past year and less than 25% ate any as frequently as once per year. Thus, familiarity with the two sets of food samples differed greatly.

Fig. 5 shows the mean acceptance ratings obtained for each food under the "information" and "no information" conditions. Consumer product information greatly increased the acceptance ratings of the three novel food items (grits, kumquats, black-eyed peas). For the familiar food items (mashed potatoes, peaches, baked beans) there was no appreciable effects of information. A two-way ANOVA confirmed a significant main effect of food type ($F = 31.27$; $df = 5,11$; $p<0.001$), a significant main effect of information ($F = 22.86$; $df = 1,11$; $p<0.001$), and a significant food type × information interaction ($F = 4.49$, $df = 5,11$; $p<0.001$). Collapsing the data across "novel" foods and "familiar" foods resulted in a significant main effect of novelty ($F = 100.92$; $df = 1,2$; $p<0.001$), a significant main effect of information ($F = 14.75$; $df = 1,3$; $p<0.001$), and a significant novelty × information interaction ($F = 12.93$; $df = 1,3$; $p<0.001$).

The percentages of panelists responding "yes" to the four purchase and use questions for each food and for each information condition are shown in Fig. 6. The data closely parallel the data in Fig. 5. Information increased the stated likely purchase and use of the novel foods. The effect on familiar foods

Table 2—Results of the usage survey for the panelists participating in each of the twelve tests (6 foods × 2 information conditions) of Experiment 6[a]

	Have tried the item previously		Have eaten the item within the past year		Eat the item at least once per year	
	Information	No Information	Information	No nformation	Information	No Information
Potatoes	100%	100%	100%	94%	89%	92%
Peaches	100%	100%	94%	94%	89%	97%
Baked Beans	100%	97%	89%	83%	83%	86%
Grits	75%	67%	39%	22%	25%	19%
Kumquats	53%	36%	28%	0%	11%	11%
Black-eye Peas	56%	50%	19%	14%	25%	11%

[a] Entries are the percentages of panelists in each group falling into the three usage categories. Percentages are based on a total of 36 panelists in each group.

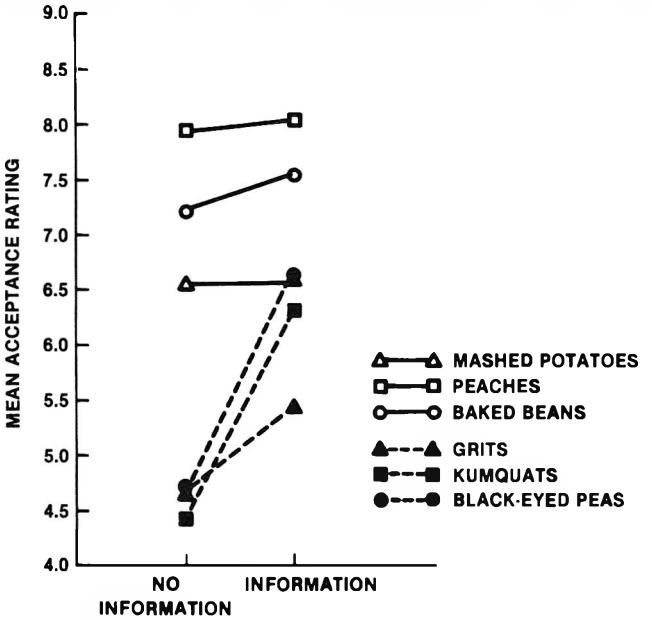

Fig. 5—Plot of the mean acceptance ratings for each of the six test foods used in Experiment 6 as a function of information condition.

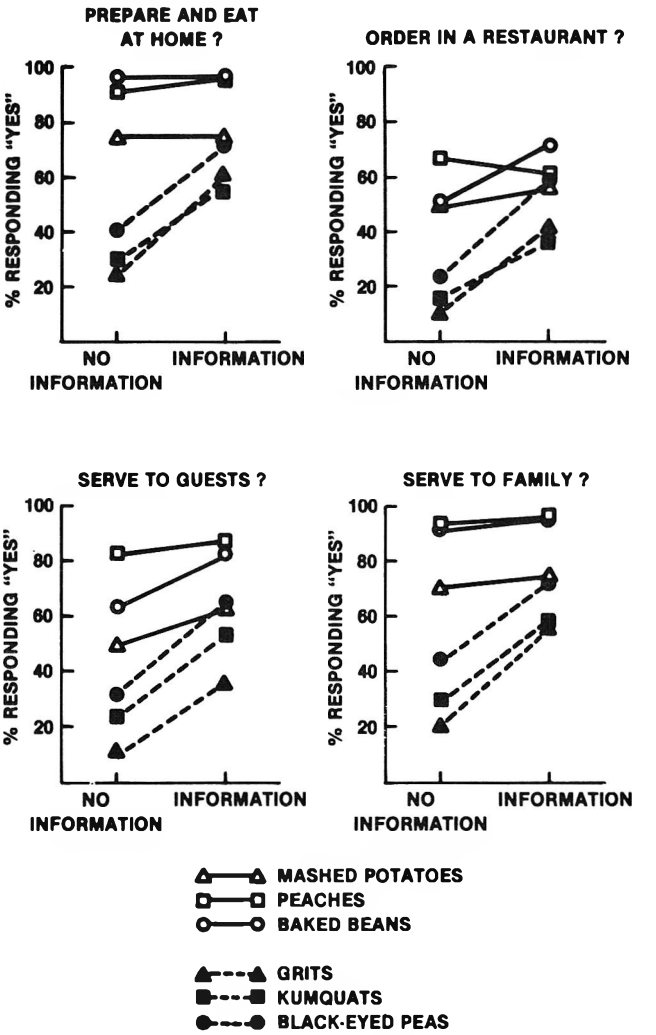

Fig. 6—Plot of the percentage of "YES" responses to each of the purchase and use questions as a function of information condition for the six test foods used in Experiment 6.

was minimal, with the exception of the question on "serving to guests," where the effect of information occurred for both food classes. The apparent differences in Fig. 6 were confirmed by Chi-Square analyses. Significant differences ($p<0.001$) were found on all four purchase and use questions between the information and no information conditions for the novel foods (Chi Squares = 19.49, 18.40, 17.17, and 18.05 (df's = 1) for questions 1-4, respectively). For the familiar foods, only the responses to question 3 were significantly different (Chi Square = 3.91; df = 1; $p<0.05$) between the two information conditions.

Pearson product-moment correlation coefficients between responses to each of the three usage questions and each of the five dependent variables are shown in Table 3. All coefficients are statistically significant at the 0.001 probability level. There was a positive correlation coefficient between each of the five dependent variables and responses to the questions "have you ever tried the food before" and "how often do you eat the food." In contrast, there was a negative correlation between each of the five dependent variables and responses concerning "the last time the food was eaten." These data all suggest a strong association between product usage (familiarity), acceptance ratings and stated intentions of purchase and use.

The results of this experiment provide unambiguous evidence that product information has greater facilitative effects on consumer acceptance (and likely purchase and use) for novel foods than it does for familiar foods. It is proposed that this is due to product information reducing the discrepancy between expectancies and experiences for novel foods, but having minimal effect on familiar foods.

Table 3—Pearson product-moment correlations among the five dependent measures and the three measures of prior usage in Experiment 6. All r values are significant at the 0.001 probability level

	"Have you ever tried the food before?"	"How often do you eat the food?"	"When was the last time you ate it?"
Acceptance Rating	+0.43	+0.44	−0.43
"Prepare and eat at home?"	+0.40	+0.43	−0.44
"Order in a restaurant?"	+0.28	+0.31	−0.27
"Serve to guests?"	+0.30	+0.30	−0.33
"Serve to family?"	+0.38	+0.42	−0.43

CONCLUSIONS

WHENEVER A FOOD is presented for consumption, expectancies are generated about what the sensory qualities of the item "should" be. These expectancies are based upon information that may be as simple as the name of the item, the manner in which it is packaged, its shape, the vessel in which it is served, the utensils provided for its consumption, etc. The results of the present set of studies show that a wide variety of such variables can affect consumer acceptance and stated likely purchase and use of novel foods, but that many of these variables do not have commensurate effects on familiar foods. These results are consistent with a theoretical model that delegates importance to the role of cognitive dissonance in determining consumer acceptance of foods. The greater the degree to which a consumer's experience with the product matches his/her pre-established expectancies of it, the greater is his/her liking of the product. The larger the discrepancy between the two, the greater the disliking. It is suggested that the multiplicity of variables controlling consumer expectancies about novel foods be exploited during the marketing phase of any new or novel product, in order to optimize acceptance by consumers. Moreover, future applications of the theoretical model of cognitive dissonance and the confirmation or disconfirma-

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A Collaborative Study to Develop a Standardized Food Protein Solubility Procedure

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ABSTRACT

A colaborative study was conducted to develop a rapid, simple and reliable procedure for determining the solubility of food protein products, e.g., spray-dried whey protein concentrate, sodium caseinate, egg white protein and soy protein isolate. The procedure was developed by modifying the nitrogen solubility index (NSI) procedure. Protein content and soluble protein were determined by micro-Kjeldahl or biuret procedures with standard deviations of ±0.83–4.12 for all proteins except caseinate which had a value of ± 13.95. Although the biuret and micro-Kjeldahl procedures generally provided comparable accuracy and precision for protein content and solubility of certain proteins, the biuret procedure exhibited considerable error and variability for other proteins.

INTRODUCTION

COMMERCIAL food protein products are important functional ingredients in a number of formulated food products (Kinsella, 1976; Pour-El, 1979; Cherry, 1981; Morr, 1982). These protein products, which are customarily spray-dried, must in most cases provide a high degree of solubility in order to be useful and functional. The solubility of these protein products is dependent upon the physicochemical state of their protein molecules, which are either favorably or adversely affected by heating, drying and other processing treatments during their manufacture and storage.

Protein solubility is commonly expressed as water-soluble nitrogen, nitrogen solubility index, water-soluble protein or protein dispersibility index (Wolf and Cowan, 1975). These latter methods utilize different dispersion media and procedures and different experimental conditions for recovering and quantitating the soluble proteins. These procedures are generally tedious and time-consuming and are unsatisfactory for routine work. Thus, researchers commonly resort to short-cut solubility methods which frequently fail to correlate well with the physicochemical and functional properties of the protein products (Shen, 1976).

This collaborative study was undertaken to develop a rapid, convenient and reliable protein solubility procedure that would be suitable for characterizing large numbers of protein products. A major objective underlying the development of this procedure was to identify and eliminate sources of variability in the results between laboratories. The approach used was to make appropriate modification in the widely accepted nitrogen solubility index procedure (AOCS, 1982). Reference food protein products were distributed to all collaborators and results were pooled and analyzed for accuracy and reproducibility over a four-year period. Modifications were made throughout the study to improve the accuracy and variability of the procedure. Only data collected during the final year of the study are included in this paper.

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MATERIALS & METHODS

Reference food proteins

Spray-dried commercial food protein products included: whey protein concentrate (WPC) prepared from sweet cheese whey by ultrafiltration to contain 35% protein (Land O' Lakes, Minneapolis, MN); Alanate 110 sodium caseinate (SC) prepared from edible casein to contain 94% protein as N × 6.38 (New Zealand Milk Products, Inc., Petaluma, CA); Supro 620 soy protein isolate (SPI) to contain 92.5% protein as N × 6.25 (Ralston Purina Company, St. Louis, MO); and egg white protein (EWP) containing ≤ 0.1% sodium dodecylsulfate (Ballas Egg Solids Div., Zanesville, OH). Each reference protein was divided into sublots, packaged in plastic containers and distributed to each of the collaborators. The proteins were stored at ≤ 5°C.

Protein determination

The protein content of reference food protein products was determined by both micro-Kjeldahl (AOAC, 1980) and biuret (Gornall et al., 1949) procedures. The micro-Kjeldahl procedure was conducted on 10–40 mg of dry protein product and protein content was computed using nitrogen conversion factors of 5.71 for soy protein, 6.38 for milk proteins, and 6.25 for egg protein.

The biuret procedure was performed by accurately weighing about 100 mg of protein sample into a 10 mL beaker and adding 6 mL of 1N NaOH solution in small increments with stirring to provide a smooth paste that was free of lumps. The protein dispersion was transferred to a 10 mL volumetric flask, diluted to the mark with 1N NaOH solution and mixed by inverting and swirling several times. Appropriate aliquots of the dispersion (0.1–1.0 mL) were pipetted into separate spectrophotometer cuvettes and distilled water was added to bring the volume to 1.0 mL. Four milliliters of biuret reagent were added, and the cuvettes were vortexed. The absorbance of the solutions was determined at 540 ηm after holding for 15–30 min for color development. Protein contents were determined from a standard curve of absorbance at 540 ηm vs protein concentration prepared with bovine serum albumin (A-7638, Sigma Chemical Company, St. Louis, MO).

Protein solubility

About 500 mg of dry protein product were accurately weighed into separate 150 mL standard beakers and several aliquots of 0.1M NaCl solution were added with stirring to form a smooth paste. Additional 0.1M NaCl solution was then added to bring the total volume of the dispersion to about 40 mL. The beaker was placed on a magnetic stirrer that was covered with a 5 cm × 5 cm square of open-mesh plastic sink matting (Fisher Scientific, Atlanta, GA) to insulate the beaker and prevent heating during the subsequent stirring period. A 2.5 cm smooth, plastic-coated stir bar was added and the dispersion was stirred at a rate that just failed to form a vortex. The pH of he dispersion was immediately determined and adjusted to 3.0 or 7.0 with 0.1N HCl or NaOH solution. The dispersion was stirred for a total of 1 hr under these conditions and the pH was intermittently monitored and maintained at the prescribed value throughout the stirring period. The dispersion was then transferred into a 50 mL volumetric flask, diluted to the mark with additional 0.1M NaCl solution and mixed by inverting and swirling. An aliquot of the dispersion was centrifuged 30 min at 20,000 × *g* and the resulting supernatant fraction was filtered through Whatman No. 1 filter paper. The protein content of the filtrate was determined by either micro-Kjeldahl or

Table 1—Composition of food protein products[a]

Food protein product	Ash		Soxhlet fat		Roese-Gotlieb fat	
	Mean of replicate values (%)	Standard deviation	Mean of replicate values	Standard deviation	Mean of replicate values (%)	Standard deviation
Whey protein concentrate	6.62	±0	0.11	±0	5.36	±0.09
Sodium caseinate	3.64	±0.02	0	±0	1.33[b]	±0.01
Soy protein isolate	3.74	±0.04	0.38	±0.01	6.93[b]	±1.22
Egg white protein	6.10	±0.11	0.04	±0.04	0.83[b]	±0.07

[a] AOAC (1980). Values are means of duplicate determinations.
[b] HCl hydrolysis prior to fat extraction.

Table 2—Protein content of reference food protein products

Food protein product	Pooled replicate protein content from all labs				Lab mean protein content[a]		
	Number of replicate values	Range of replicate values (%)	Mean of replicate values (%)	Standard deviation	Number of labs	Range of lab mean values (%)	Standard deviation
Micro-Kjeldahl procedure							
Whey protein concentrate	17	33.88-35.77	35.15	±0.58	6	34.61-35.58	±0.40
Sodium caseinate	14	89.05-95.64	91.56	±2.13	5	89.69-95.16	±0.53
Soy protein isolate	18	73.45-80.85	77.93	±1.70	6	76.65-79.70	±1.10
Egg white protein	18	79.12-84.19	80.72	±1.21	6	79.87-82.27	±0.72
Biuret procedure							
Whey protein concentrate	6	65.3 -76.4	70.62	±5.38	2	65.7 -75.5	±0.68
Sodium caseinate	6	87.4 -93.9	89.46	±2.46	2	88.0 -90.9	±1.86
Soy protein isolate	6	87.9 -94.7	91.20	±2.48	2	89.2 -93.2	±1.50
Egg white protein	6	79.1 -84.2	79.18	±4.87	2	75.6 -86.9	±2.21

[a] A minimum of three replicate values for each mean.

Table 3—Solubility of food protein products by the micro-Kjeldahl procedure[a]

Food protein product	Pooled replicate solubility from all labs				Lab mean solubility data[b]		
	Number of replicate values	Range of replicate values (%)	Mean of replicate values (%)	Standard deviation	Number of labs	Range of lab mean values (%)	Standard deviation
pH 3							
Whey protein concentrate	9	83.1-86.5	85.0	± 1.21	3	84.1-85.6	± 0.81
Sodium caseinate	9	5.2-16.4	10.0	± 4.12	3	5.5-14.7	± 4.60
Soy protein isolate	9	4.9- 9.7	8.5	± 1.52	3	7.3- 9.2	± 1.09
Egg white protein	9	91.5-99.2	96.0	± 2.87	3	92.6-98.8	± 2.87
pH 7							
Whey protein concentrate	9	89.6-94.2	91.4	± 1.71	3	90.4-93.4	± 1.69
Sodium caseinate	9	70.4-99.9	89.3	±13.95	3	70.8-98.8	±16.0
Soy protein isolate	9	17.3-19.7	18.7	± 0.83	3	18.2-19.2	± 0.50
Egg white protein	9	94.2-99.2	97.0	± 1.72	3	95.3-99.0	± 1.88

[a] Protein content and soluble protein were both determined by micro-Kjeldahl procedure. Protein solubility was calculated using the mean protein content values determined in each researcher's own laboratory.
[b] A minimum of three replicate values for each mean.

biuret, using appropriate aliquot volumes. The solubility of the protein product was calculated as:

$$\text{Protein solubility (\%)} = \frac{\text{Supernatant protein conc (mg/mL)} \times 50}{\text{Sample wt (mg)} \times \frac{\text{Sample protein content (\%)}}{100}} \times 100$$

Mineral and fat content of food protein

Ash was determined by heating dry samples of the protein at 550°C in an electric furnace (AOAC, 1980) and fat was by Soxhlet petroleum ether extraction and the Roese-Gotlieb mixed solvent extraction procedures, with and without prior HCl hydrolysis (AOAC, 1980).

RESULTS & DISCUSSION

Composition of food protein products

The composition of the four food protein products is given in Table 1. These protein products contained from 3.6 to 6.6% ash and up to 0.38% fat by Soxhlet extraction. Whey protein concentrate and soy protein isolate contained 5.36 and 6.93% fat, respectively, by the Roese-Gottlieb alcohol-ether extraction procedure, which presumably includes polar lipids in addition to neutral triglycerides.

The four food protein products exhibited a wide range of protein contents (Table 2). Mean micro-Kjeldahl protein content ranged from about 35% for whey protein concentrate to 91.5% for sodium caseinate. Pooled replicate and laboratory mean values exhibited standard deviation values of under ± 2.1 (Table 2), indicating fair and acceptable reproducibility.

Pooled replicate biuret protein content data (Table 2) showed poorer reproducibility than by micro-Kjeldahl. Standard deviations for lab mean protein content by biuret were only slightly greater than those by micro-Kjeldahl. However, an even greater problem was the lack of agreement between biuret and micro-Kjeldahl data (Table 2). For example, mean biuret protein content of whey protein concentrate was 70.6% compared to a mean value of 35.15% by micro-Kjeldahl. A similar discrepancy was observed for soy protein isolate, where the mean biuret protein value was 91.2% compared to only 77.9% by

Table 4—Solubility of food protein products by the combination of micro-Kjeldahl and biuret procedures[a]

Food protein product	Pooled replicate solubility from all labs: Number of replicate values	Range of replicate values (%)	Mean of replicate values (%)	Standard deviation	Lab mean solubility data[b]: Number of labs	Range of lab mean values (%)	Standard deviation
Micro-Kjeldahl protein content and biuret soluble protein procedures, pH 3							
Whey protein concentrate	9	73.4-153	116	±33.52	3	74.7-151	±38.5
Sodium caseinate	12	2.1- 19.2	8.9	± 5.21	4	5.0- 17.6	± 5.81
Soy protein isolate	12	6.1- 13.2	9.2	± 2.11	4	6.9- 10.6	± 1.72
Egg white protein	9	89.3-110	97.8	± 9.06	3	90.0-109	±10.1
Micro-Kjeldahl protein content and biuret soluble protein procedures, pH 7							
Whey protein concentrate	9	98.1-189	138	±39.51	3	98.8-188	±45.4
Sodium caseinate	9	79.0- 98.8	88.2	± 8.43	3	78.4- 97.8	± 9.70
Soy protein isolate	12	17.4- 22.2	19.8	± 1.40	4	18.2- 20.9	± 1.29
Egg white protein	9	92.7-116	100.9	±10.83	3	93.3-115	±12.53

[a] Protein solubility was calculated using the mean protein content values determined in each researcher's own laboratory.
[b] A minimum of three replicate values for each mean.

Table 5—Solubility of food protein products by the biuret procedure[a]

Food protein products	Pooled replicate solubility from all labs: Number of replicate values	Range of replicate values (%)	Mean of replicate values (%)	Standard deviation	Lab mean solubility data[b]: Number of labs	Range of lab mean values (%)	Standard deviation
Biuret protein content and soluble protein procedures, pH 3							
Whey protein concentrate	3	70.4- 71.9	71.0	± 0.78	1	71.0	±47.7
Sodium caseinate	6	5.6- 18.6	9.5	± 5.15	2	5.9-13.1	± 5.09
Soy protein isolate	6	8.6- 56.4	30.1	±23.11	2	9.2-51	±29.6
Egg white protein	6	84.7-101.9	95.9	± 6.13	2	95.7-99.4	± 2.62
Biuret protein content and soluble protein procedures, pH 7							
Whey protein concentrate	6	66.6- 89	78.2	±11.21	2	68.0-88.4	±14.42
Sodium caseinate	6	81.5-100.7	92.1	± 8.61	2	84.5-99.6	±10.68
Soy protein isolate	6	14.7- 19.3	17.2	± 1.78	2	15.5-18.1	± 1.84
Egg white protein	6	94.5- 99.9	98.3	± 2.01	2	97.8-98.8	± 0.78

[a] Protein solubility was calculated using the mean protein content values determined in each researcher's own laboratory.
[b] A minimum of three replicate values for each mean.

Table 6—Comparison of mean replicate protein solubility (%) values obtained by different procedures[a]

Food protein product	pH 3: M/M[b]	B/M[c]	B/B[d]	pH 7: M/M[b]	B/M[c]	B/B[d]
Whey protein concentrate	85.0	116	71.0	91.4	138	78.2
Sodium caseinate	10.0	8.9	9.5	89.3	88.0	92.1
Soy protein isolate	8.5	9.2	30.1	18.7	19.8	17.2
Egg white protein	96.0	97.8	95.9	97.0	100.9	98.3

[a] A minimum of six replicate determinations from all labs.
[b] Protein content and soluble protein by micro-Kjeldahl procedure.
[c] Protein content by micro-Kjeldahl and soluble protein by biuret.
[d] Protein content and soluble protein by biuret.

micro-Kjeldahl. It is interesting to note that the two proteins with the greatest discrepancies between their biuret and micro-Kjeldahl values contained the highest fat contents (Table 1). In both cases the biuret values were greater than the micro-Kjeldahl values, suggesting that fat may have contributed to these discrepancies by interfering with protein dissolution or by contributing turbidity during the spectrophotometric determination of the biuret procedure.

It was concluded that the micro-Kjeldahl protein determination should be used as the reference method and that the biuret procedure should only be used after first establishing that it provides reliable protein values that agree with micro-Kjeldahl values.

Solubility of food proteins

Protein solubility results based upon micro-Kjeldahl and biuret data for protein content and soluble protein are in Table 3. A range of mean solubility values from about 8 to 96% at pH 3 and from 19 to 97% at pH 7 was observed. These data were highly reproducible, except for sodium caseinate at pH 7 where considerable difficulty was experienced in obtaining complete protein dispersion.

Protein solubility results based upon biuret data for protein content and soluble protein (Tables 4 and 5) were generally inconsistent and exhibited greater standard deviations among replicate and laboratory mean data than by micro-Kjeldahl. In spite of this general lack of agreement and reproducibility for biuret protein solubility values, fairly good agreement was obtained for mean protein solubility data determined by the three combination procedures for protein content and soluble protein (Table 6). Exceptions were noted for whey protein concentrate at both pH 3 and 7 and for soy protein isolate at pH 3. It was concluded that the micro-Kjeldahl procedure is most reliable for routine protein solubility evaluation and should be used until the biuret procedure has been tested and shown to provide reliable results.

The difficulty experienced by researchers in obtaining accurate and reproducible protein solubility data is believed to be due to a consequence of both the complexity of the food protein and more importantly the minor differences in the use of analytical methods in each of the laboratories. This fact

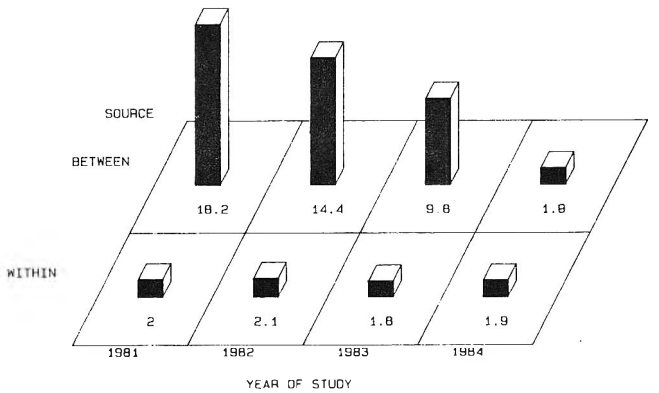

Fig. 1—Summary of variance component analyses on the variability between and within laboratories over the duration of the study to develop the protein solubility procedure. Shown are standard deviations for representative data performed (SAS, 1982).

became apparent during the initial phases of this study. Variance component analyses were performed on representative results over the duration of the study and these findings are summarized in Fig. 1. These results graphically demonstrate the nature of the problem in developing methods for determining protein solubility and functionality. While the magnitude of the variation within each laboratory did not change over the course of the study, interlaboratory variability was drastically reduced. Even at the initial stages of the study where results revealed major discrepancies between laboratories, precision of results within laboratories was quite high. The low standard errors in analyses of solubility reflected the ability of individual researchers to repeat errors consistently rather than eliminate them. A long-term improvement in the design of the methodology was necessary to reduce the variability between laboratories.

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tion of consumer expectancies should uncover other potential variables for facilitating the acceptance of "novel" foods.

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Rapid Field Screening Method for Flat Sour Spoilage

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ABSTRACT

A rapid field test based on bacterial catalase was developed to detect flat sour spoilage of shelf stable canned meats. Containers of canned corned beef were spoiled by inoculating with spores of *Bacillus stearothermophilus* and/or *B. coagulans* and incubating at 55°C. Spore outgrowth was evident and vegetative cells were usually recovered at levels of at least one order of magnitude above the original spore load. Although neither abnormal odors nor significant pH shifts were detected, catalase was consistently demonstrated in all spoiled samples.

INTRODUCTION

FLAT SOUR SPOILAGE is a descriptive term used for categorizing microbial spoilage of commercially canned foods. It is frequently associated with sporeforming bacteria that spoil a product without producing a significant amount of gas. Consequently, flat-soured products usually have a slight sour odor and a reduced pH while the containers remain flat (externally normal).

Sporeforming bacteria resembling *Bacillus stearothermophilus* and *Bacillus coagulans* are commonly associated with flat sour spoilage (Fields, 1970; Feig and Stersky, 1981; Stumbo, 1973). Other strains, including *B. subtilis* and *B. macerans*, may also produce this type of problem (Segner, 1979). Contamination of products with spores of these *Bacillus* species may or may not result in spoilage.

The true incidence of flat sour spoilage is difficult to assess due to the lack of external signs and obvious detection difficulties. Thermophilic flat sours are no longer a significant problem of domestic canned goods but still sporadically plague imported items such as canned meats. Currently, flat sour spoilage of canned meats is monitored by the Food Safety and Inspection Service (FSIS) field inspectors who rely on detecting abnormal or sour odors to identify this type of problem. At import stations detecting a single can of flat soured product may result in import refusal of the entire production lot on the basis of adulteration. Such severe consequences accent the difficulties associated with these subjective odor evaluations. Consequently, this study was directed at developing a simple, field-compatible test to use in conjunction with the sensory method for confirming flat sour spoilage.

MATERIALS & METHODS

Spore production

Spores of *Bacillus stearothermophilus* ATCC 12016 were prepared using a modified protocol of Labbe (1979). Log phase *B. stearothermophilus* was inoculated into 22 ml of trypticase soy broth (TSB) and shaken for 16–18 hr at 55°C. Two milliliters of the broth were spread onto large (15 × 140 mm) petri dishes of nutrient agar fortified with 0.3 μg/mL of $MnSO_4$ and 0.52 μg/mL Na_2EDTA. The cultures were incubated at 55°C and examined at 48 and 72 hr under phase contrast for maximum spore production. The spores were harvested and washed in sterile Butterfield's phosphate buffer (Speck, 1976). Following an additional wash, the spores were suspended in sterile buffer containing 0.1 mg/mL lysozyme and incubated at 37°C for 2 hr. After an additional 8–10 washings the spores were suspended in sterile 50% ethanol in buffer and stored at ambient temperature (ca 22°C).

Spores of *Bacillus coagulans* ATCC 7050 were prepared by inoculating 25 mL of TSB with a log phase culture and shaking it for 16–18 hr at 55°C. Two milliliters of the broth were spread onto thick plates (15 × 140 mm) of Plate Count Agar (PCA). The plates were incubated at 37°C and examined for maximum spore production at 24 and 48 hr. The spores were harvested and washed as previously described. The spore crops were examined for purity and quantitated, after heat shock, on trypticase soy agar. Two different experimental lots of corned beef were prepared to reproduce a flat sour spoilage condition. In lot A, approximately 1 kg of commercially canned corned beef was inoculated with spores of both *B. stearothermophilus* and *B. coagulans* to a concentration of 10^5/g and 10^4/g, respectively. The meat was thoroughly mixed and aseptically packed into sterile 99.2g (3.5 oz) glass jars. These were pasteurized in flowing steam for 10 min and capped with self-sealing screw-on lids. The jars were cooled at 37°C and incubated at 55°C for 30 days. Ten noninoculated control jars were similarly prepared and handled. The second experimental lot (B) of corned beef was repackaged into sanitary cans. Canned corned beef was inoculated with 2 × 10^3 spores/g of either *B. stearothermophilus* or *B. coagulans* or 2 × 10^3 spores/g of each in combination. The spores were mixed into the corned beef in a sanitized bowl cutter. Fifteen sterile No. 303 sanitary cans were aseptically packed from each inoculated lot. The cans were sealed on an electric sealer and pasteurized in a retort at 121°C, 6.8 kg pressure. Internal temperatures were monitored with two additional samples equipped with Ecklund needle type thermocouples. The internal temperatures were allowed to reach 68.3°C (155°F). The cans were cooled to 54.4°C (130°F), transferred to a 55°C incubator and held for 3 wk. Six control cans were repacked directly from a commercial No. 10 can of corned beef. These were otherwise handled as above.

After 3–4wk of incubation at 55°C the samples were cooled to room temperature and examined from loss of vacuum or swelling. The containers were aseptically opened for culturing. The cans were opened with a Bacti-Disc cutter. The top portion of each sample was discarded and the remaining contents mixed. Fifty gram sample portions were blended and plated in duplicate with Plate Count Agar (PCA) as described in the Microbiology Laboratory Guidebook (USDA, 1974). A parallel dilution series in bromcresol purple dextrose broth (USDA, 1974) was also inoculated. Duplicate analyses were incubated at 35°C and 55°C and examined at 48 hr and after 1 wk. Spores were estimated by heat shocking the primary dilution at 80°C for 10 min and culturing as described. Representative isolates were presumptively identified on the basis of Gram morphology, growth at 55°C and 35°C, growth on Sabouraud's dextrose agar and PCA with 0.02% sodium azide. The samples were further characterized by direct microscopic examination, pH, and odor.

Catalase assay

Catalase activity was determined on the basis of bubbling following the addition of 3% hydrogen peroxide. Each sample was thoroughly mixed and a small portion (about 5–10g) was transferred to a clean

Table 1—Characteristics of the flat-sour spoilage in Lot A of canned corned beef after 1 month at 55°C

Agent	*B. coagulans*	*B. stearothermophilus*	Control
Spore inoculum	10^4/g	5×10^5/g	-
Mean recovery	10^6/g	10^7/g	< 100/g
Viable spores	10^4/g	10^5/g	< 100/g
pH	6.2–6.4		6.3–6.4
Catalase reaction	Positive		Negative

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Table 2—Characteristics of the flat-sour spoilage in Lot B of canned corned beef after 3 wk at 55°C

Agent	*B. coagulans*	*B. stearo-thermophilus*	*B. coagulans & B. stearothermophilus*	Control[a]
Spore inoculum	2×10^3/g	2×10^3/g	4×10^3/g	—
Recovery range	$100–10^4$/g	$100–10^3$/g	$100–10^4$/g	<100/g (10^7/g)
Microscopic	Numerous vegetative bacilli with few refractive spores.			Few cells
pH	6.4–6.5	6.4–6.5	6.4–6.5	6.4–6.5 (6.0)
Catalase reaction	Positive	Positive	Positive	Negative (+)

[a] One control contaminated with 10^7/g *B. coagulans*, results in parentheses.

16 × 100 mm test tube. Approximately 8–10 mL of 3% hydrogen peroxide was added to the sample to half fill the tube. The mixture was very gently stirred with an applicator stick or stirring rod to insure full contact with the reagent. These mixtures were examined for bubbling. The absence of either a foam or production of gas bubbles after one minute was considered a negative reaction.

RESULTS & DISCUSSION

IN THE FIRST ATTEMPT to reproduce thermophilic flat sour spoilage in canned corned beef, a heavy inoculum of both *B. stearothermophilus* and *B. coagulans* spores was used. The second group was prepared with single and combined strains at a spore load reduced at about 2000/g. In both experiments germination and growth occurred. Vegetative cells were usually recovered at levels of at least one log above the inoculated spore levels. In the second group the weakest growth was recovered from the *B. stearothermophilus* inoculated samples. Growth and subsequent die-off was indicated by the range of recovered viable cells in comparison to the numerous bacilli microscopically observed in all of the samples. This characteristic might be associated with a period of cool storage in which the samples were shipped between laboratories after they had been incubated. This cool-down period may or may not have compounded the frequently observed process of autosterilization in which numerous strains of *Bacillus* fail to sporulate at reduced oxygen levels and consequently die off.

Despite the bacterial growth that was evident in both experimental groups, no abnormal odors were detectable over the strong normal aromas. The pH of the samples from both lots varied less than ± 0.2 from the control average of 6.4 and was not considered significant.

A number of enzyme systems were screened using the first group of samples to find a potentially suitable field test to confirm spoilage. These included activity tests for alkaline phosphatase, nuclease, protease, amylase, and catalase. Screenings were performed using crude aqueous extracts of the spoiled product. Catalase was the only enzyme consistently detected in all of these samples. Catalase activity was subsequently detected in all of the spoiled samples of the second experimental lot as well. These included samples containing only *B. stearothermophilus* and only *B. coagulans*. It is interesting that although none of the experimental cans developed classic flat sour spoilage conditions, one control can of the second group did so. The product used for the control apparently contained dormant spores that were activated by the heat of pasteurization and incubation temperature. The pH of the spoiled product was 6.0, or 0.4 below normal and the product was sloppy with a slight abnormal odor. *B. coagulans* was recovered at 10^7/g and the contents were strongly catalase positive.

On the basis of these results, a simple and inexpensive field test to confirm flat sour spoilage in canned meats is proposed. The few supplies required include 3% hydrogen peroxide, active baker's yeast, clean test tubes or small plastic "Whirl-Pak" (NASCO, WI) bags and stirring rods. The procedure as applied to canned corned beef requires a thorough mixing of the sample and removal of a small portion (5-10g) to either a test tube or small plastic bag. The hydrogen peroxide solution is added to approximately 2–3 times the sample volume and gently stirred for full contact with the sample. In using plastic bags, after adding the reagent, the air from the bag is expelled and the bag sealed. A positive catalase reaction starts to bubble within 15 sec. Strong reactions produce a heavy foam within 1 min at ambient temperature and cause the plastic bag to distend. A few grains of baker's yeast are separately used as a positive reagent control to insure hydrogen peroxide activity.

Catalase measurements have been applied to various processes in the food industry to monitor microbial as well as innate enzyme activity associated with native animal and plant tissue (Willits and Babel, 1965; Belica, 1973; Frank and Hertkorn-Obst, 1980; Gannon and Bennett, 1981; Dodds et al., 1983). In this respect, a positive reaction with the proposed catalase test can indicate other serious production problems besides flat sour spoilage. In commercially sterile canned foods the proper processing inactivates enzyme systems otherwise naturally present in raw food materials, including catalase. Consequently, after processing any catalase detected in such a product indicates a problem which could arise not only from flat sour spoilage but other product defects including leakage and underprocessing. This test is, then, recommended as a companion for sensory evaluations to detect and identify flat sour spoilage in the shelf stable canned meats. Questions concerning the long term stability of catalase and degree of enzyme inhibition caused by nitrite (Cassens et al., 1979; Cantoni and Menaggia, 1981), however, still need to be examined. A number of *B. stearothermophilus* strains are also reported not to produce catalase (Gordon et al., 1973) and a few instances of flat sour spoilage caused by catalase negative formers resembling *B. macerans* have been documented over the last few years (Segner, 1979).

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Isolation, Purification, and Characterization of Lipase Isoenzymes from a Technical *Aspergillus niger* Enzyme

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ABSTRACT

A qualitative screening revealed the occurrence of lipase, esterase, protease, amylase, endo-1,4-β-D-glucanase, xylanase, pectinmethylesterase, polygalacturonase, catalase, β-D-glucosidase and β-D-galactosidase activities in the technical *Aspergillus niger* enzyme under study (Lipase 2212 D, Röhm). The isolation and purification of lipolytic activities were performed by combination of DEAE-Trisacryl M ion exchange chromatography, Sephadex G 50 gel filtration and hydrophobic chromatography using Phenylsepharose CL-4B. The individual purification steps were checked by specific enzyme visualization in ultrathin agar gels after ultrathin-layer isoelectric focusing (UIEF). Two UIEF homogeneous lipase isoenzymes (I and II) were isolated and characterized by the following parameters: isoelectric points (I: 4.0; II. 3.5); molecular weights (I: 31000 daltons; II: 19000 daltons); carbohydrate contents (I: 6%; II: 9%) and compositions; pH optima (I, II: 5-6); substrate specificities and various effectors.

INTRODUCTION

INCREASED ATTENTION being paid in the past to biotechnological processes led to the use of various technical enzymes including microbial lipases in food processing (Rutloff et al., 1979; Präve et al., 1982). Different authors described the application of fungal lipases for the formation of milk- and butter flavors in food products (Farnham, 1957; Engel et al., 1970; Farnham et al., 1970; Nelson, 1972; Buhler et al., 1972; Fenaroli, 1977; Kanisawa et al., 1982), whereas others recommended the use of microbial lipases in cheesemaking processes (Feldman and Dooley, 1975; Huang and Dooley, 1976; Kosikowski and Jolly, 1976; Kinsella and Hwang, 1976; Moskowitz, 1980; Thomasow, 1980) and yoghurt production (Seitz, 1974). However, as technical enzymes generally contain a number of various enzyme activities and, qualitatively and quantitatively, differ in lipolytic and other activities, the problems of their controlled and systematic technological use are not totally solved as yet. Depending on the composition of the technical enzyme and on the processing conditions often greatly differing results were obtained (Mick, 1982). The knowledge of the properties of the enzymic activities to be used for biotechnological purposes therefore has to be the fundament of all studies. It was the aim of this work to isolate, purify and characterize the lipolytic activities of a commercial technical *Aspergillus niger* lipase in order to create the basis for subsequent technological studies.

MATERIALS & METHODS

Enzyme

Lipase 2212 D was kindly provided by Röhm GmbH, Darmstadt.

Protein determinations

Protein determinations were carried out according to Lowry et al. (1951) with crystalline serum albumine as standard. Protein concentrations in column effluents were estimated from the absorption at 278 nm.

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Qualitative enzyme assays

Except for the assays of β-D-glucosidase (Bailey and Nevalainen, 1981), β-D-galactosidase (Kuby and Lardy, 1953), esterase (Jacks and Kircher, 1967), and catalase (Hale and Renwick, 1981) qualitative enzyme assays were performed using agar diffusion plate tests (Habermann and Hardt, 1972) as already described in detail elsewhere (Höfelmann et al., 1983).

Quantitative determinations of lipase activity

With tributyrin as substrate: to a solution of 8g gum arabic in 100 mL 0.02M succinic acid buffer (pH 6.0) 8g tributyrin was added. Twenty five mL of the emulsified (ultraturrax) substrate solution were heated at 30°C and adjusted to pH 6.03–6.05 with 0.1N NaOH. After addition of enzyme solution the reaction was checked by pH control: each time pH 5.95–6.00 was reached by enzymic liberation of free fatty acids, the pH value was adjusted to 6.03–6.05 by adding 250 μL 0.02N NaOH. The time intervals and the consumption of 0.02N NaOH were recorded. Blank tests were carried out using thermally inactivated (100°C, 10 min) enzyme solution. 1 U/mL = formation of 1 μM free fatty acids per mL enzyme solution.

For substrate specificity control nitrophenyl esters were used as substrates, i.e. o-nitrophenyl butanoate, p-nitrophenyl acetate, -hexanoate, -octanoate, -decanoate and -dodecanoate, respectively. To 9.9 mL 0.01M phosphate buffer (pH 7.0) 0.1 mL methanolic nitrophenyl ester solution (50 mM) was added. The assay was started by adding 50 mL enzyme solution to 3 mL substrate solution. ΔE/min was determined graphically from absorption measured at 405 nm (30°C) for 20 min. Blank tests were carried out using 50 μL buffer instead of enzyme solution. 1 U/mL = hydrolysis of 1 μM substrate per mL enzyme solution.

Purification procedures

Crude enzyme solution. Four grams Lipase 2212 D (Röhm, Darmstadt) were solved under stirring (4°C) in 100 mL 0.06M Tris/HCl buffer (pH 7.0) and centrifuged at 12000 × *g* for 15 min (4°C). Eighty mL of the supernatant were ultrafiltered and adjusted to 50 mL with the same buffer.

DEAE-Trisacryl M ion exchange chromatography. The adsorbent was equilibrated in 0.06M Tris/HCl buffer (pH 7.0) and packed into a column (2.6 × 55 cm). Forty mL ultrafiltered crude enzyme solution (cf. above) were applied. Ten mL fractions were collected (4°C) first washing (85 mL/h) with 800 mL 0.06M Tris/HCl buffer (pH 7.0) and then eluting (70 mL/h) with 1800 mL of a linear Tris/HCl (pH 7.0) gradient (0.06 M – 0.23 M) as well as 600 mL 0.5M Tris/HCl buffer (pH 7.0), respectively.

Sephadex G 50 gel filtration. The combined lipase active fractions obtained by DEAE-Trisacryl M ion exchange chromatography (no. 150-180) were ultrafiltered to 8 mL, equilibrating with 0.05M acetate buffer (pH 5.0). Seven mL of the concentrated enzyme solution were applied to a column (1.6 × 82 cm) packed with Sephadex G 50, which had been equilibrated with 0.05 M acetate buffer (pH 5.0). The flow rate was 5 ml/h and 2 ml fractions were eluted. Estimation of molecular weights was achieved using a mixture of protein markers (MS II, Serva).

Hydrophobic chromatography. Phenylsepharose CL-4B was equilibrated with 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0) and a column (1.6 × 41 cm) was packed.

Lipase I (first run). The fractions no. 32-37 of gel filtration were combined (12 mL) and, after separation of 1 mL for enzyme assays, adjusted to 1M $(NH_4)_2SO_4$ and applied to the column. Stepwise washing and eluting (14 mL/h) was carried out with 50 mL 1M $(NH_4)_2SO_4$,

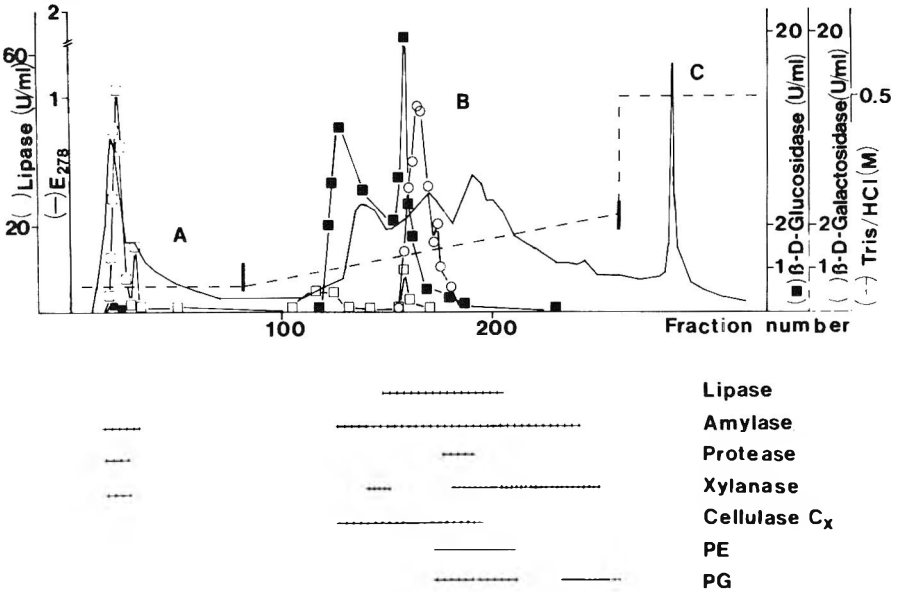

Fig. 1—Ion exchange chromatography on DEAE-Trisacryl M of ultrafiltered crude enzyme (2212 D, Röhm). Column, 2.6 × 55 cm; 0.06M Tris/HCl buffer (pH 7.0). Sample 40 mL. Flow rates, 85 mL/h (A); 70 mL/h (B,C). 10 mL-fractions. Elution, Tris/HCl buffer (pH 7.0), 0.06M (A); linear gradient 0.06–0.23M (B); 0.5M (C). + + + + = Zones of enzyme activities detected by agar diffusion plate tests (PE = pectinmethylesterase; PG = polygalacturonase).

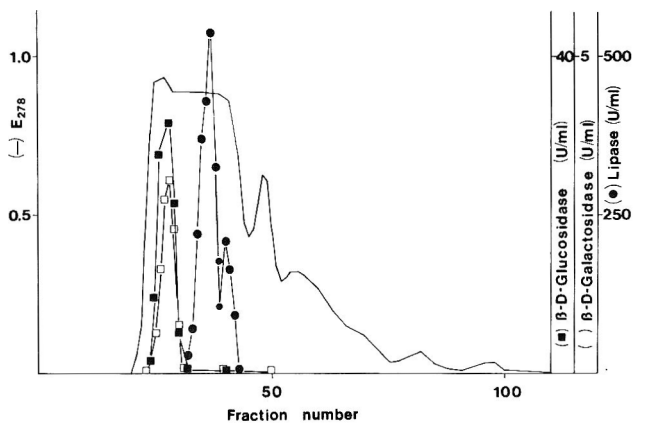

Fig. 2—Gel filtration on Sephadex G 50 of lipase active fractions obtained by DEAE-Trisacryl M chromatography (150–180; cf. Fig. 1). Column, 1.6 × 82 cm; 0.05M acetate buffer (pH 5.0). Sample, 7 mL; flow rate, 5 mL/h. 2 mL-fractions.

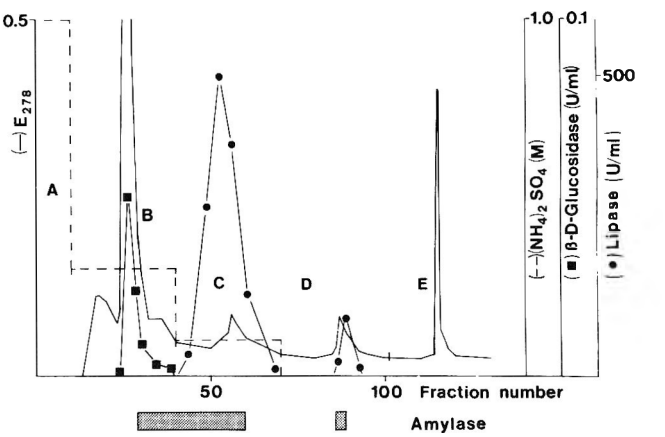

Fig. 3—Phenylsepharose CL-4B hydrophobic chromatography of lipase active fractions 32-37 obtained by gel filtration (lipase I, first run). Column, 1.6 × 41 cm; 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Sample, 11 mL; flow rate, 14 mL/h; 5 mL-fractions. Washing (A), 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Eluting, 0.3M $(NH_4)_2SO_4$ (B), 0.1M $(NH_4)_2SO_4$ (C), 0.01M $(NH_4)_2SO_4$ (D), each in 0.01M phosphate buffer (pH 6.0), respectively; demineralized water (E).

150 mL 0.3M $(NH_4)_2SO_4$, 150 mL 0.1M $(NH_4)_2SO_4$, and 150 mL 0.01M $(NH_4)_2SO_4$, each in 0.01M phosphate buffer (pH 6.0), respectively, as well as finally 100 mL demineralized water. Five mL fractions were collected.

Lipase I (rechromatography). The fractions no. 41-73 of the first run were combined and ultrafiltered to 15 mL. After adjusting to 1M $(NH_4)_2SO_4$ 14.5 mL were applied to the column (cf. above). Rechromatography was carried out by stepwise eluting (14 mL/h with 50 mL 1M $(NH_4)_2SO_4$, 100 mL 0.1M $(NH_4)_2SO_4$, each in 0.01M phosphate buffer (pH 6.0) collecting 5 mL fractions as well as employing then 180 mL of a linear $(NH_4)_2SO_4$ gradient (0.1M–0M) in 0.01M phosphate buffer (pH 6.0) sampling 2 mL-fractions. Lipase I active fractions 47–100 were combined, equilibrated with 0.01M phosphate buffer (pH 6.0) and ultrafiltered to 4 mL.

Lipase II (first run). Fractions no. 39–43 of gel filtration were combined (10 mL) and, after separation of 2 mL for enzyme assays, adjusted to 1M $(NH_4)_2SO_4$; 8 mL were applied to the column (cf. above). Stepwise washing and eluting was performed with 50 mL 1M $(NH_4)_2SO_4$, 150 mL 0.03M $(NH_4)_2SO_4$, each in 0.01M phosphate buffer (pH 6.0) as well as 200 mL 0.01M phosphate buffer (pH 6.0) and 100 mL demineralized water. The flow rate was 14 mL/h and 5 mL fractions were collected.

Lipase II (rechromatography). Fractions 57–59 of the first run were combined and after adjusting to 1M $(NH_4)_2SO_4$ applied to the column. Stepwise washing and eluting (14 mL/h) was carried out using 50 mL 1M $(NH_4)_2SO_4$ and 100 mL 0.1M $(NH_4)_2SO_4$, each in 0.01M phosphate buffer (pH 6.0), collecting 5 mL fractions as well as subsequently 180 mL of a linear $(NH_4)_2SO_4$ gradient (0.1M–0M) in phosphate buffer (pH 6.0) sampling 2 mL fractions. Lipase II active fractions 151–161 were combined and ultrafiltered to 2 mL after equilibration with 0.01M phosphate buffer (pH 6.0).

Ultrathin-layer isoelectric focusing (UIEF). UIEF was carried out using 50 μm polyacrylamide gels (Radola, 1980). The experimental details for the preparation of ultrathin polyacrylamide and agar gels (for enzyme visualization) have been previously described (Höfelmann et al., 1983).

Ultrafiltrations

Ultrafiltrations were performed at 4°C and 3 bar N_2 using a TU-AN 4045-380 membrane (8000 D, Kalle) in an Amicon cell.

Analysis of carbohydrates

Total sugar content in the purified lipases was determined by phenol-sulfuric acid method (Dubois et al., 1956). The individual carbohy-

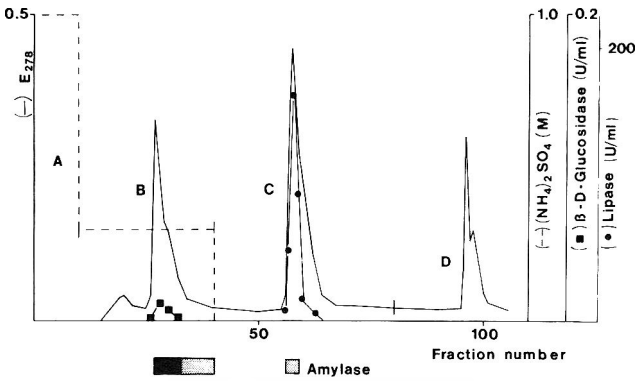

Fig. 4—Phenylsepharose CL-4B hydrophobic chromatography of lipase active fractions 39-43 obtained by gel filtration (lipase II, first run). Column 1.6 × 41 cm; 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Sample, 8 mL; flow rate, 14 mL/h; 5 mL-fractions. Washing (A), 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Eluting, 0.03M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0) (B); 0.01M phosphate buffer (pH 6.0) (C); demineralized water (D).

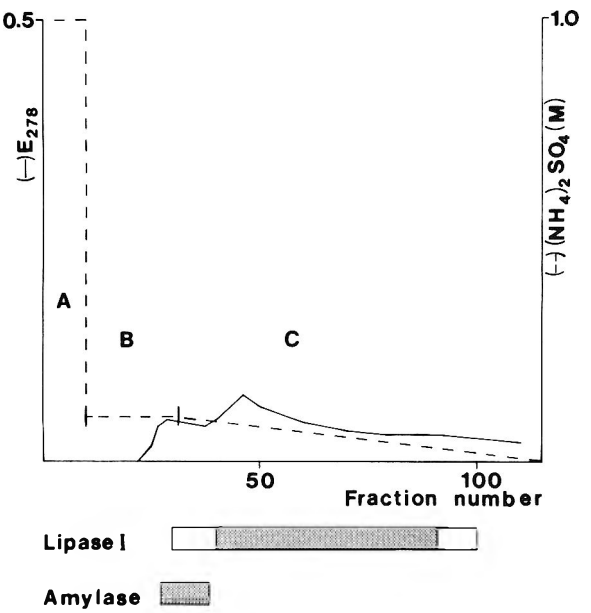

Fig. 5—Phenylsepharose CL-4B rechromatography of lipase I (cf. Fig. 3). column, 1.6 × 41 cm; 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Sample, 14.5 mL; flow rate, 14 mL/h; 5 mL- (A) and 2 mL-fractions (B,C). Washing (A), 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Eluting, linear gradient 0.1M–0M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0).

drates were analyzed as trimethylsilyl derivatives by capillary gas chromatography after hydrolysis by modified methanolysis according to Zanetta et al. (1972).

RESULTS & DISCUSSION

Isolation and purification of lipase isoenzymes

The technical *Aspergillus niger* enzyme under study (Lipase 2212 D, Röhm) contained 55% protein. The qualitative investigation of enzyme activities in the crude enzyme revealed that lipase, esterase, protease, amylase, endo-1,4-β-D-glucanase, xylanase, pectinmethylesterase, polygalacturonase, catalase, β-D-glucosidase, and β-D-galactosidase activities were present in the preparation.

Quantitative determinations of lipase activity were carried out with tributyrin as substrate using a modified pH-Stat ap-

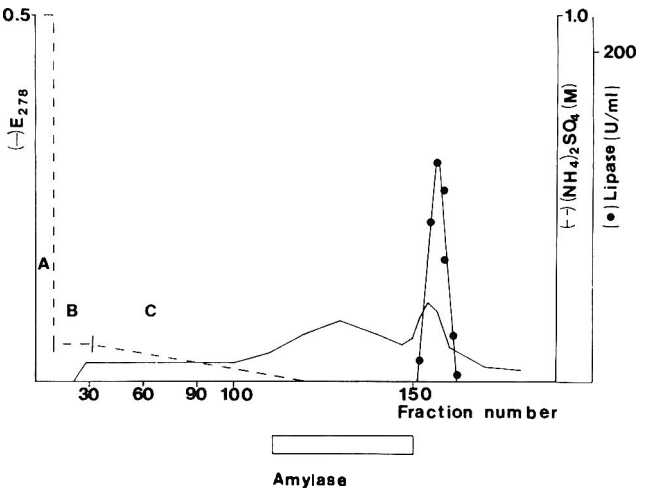

Fig. 6—Phenylsepharose CL-4B rechromatography of lipase II (cf. Fig. 4). Column, 1.6 × 41 cm; 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Sample, 15 mL; flow rate, 14 mL/h; 5 ml-(A) and 2 mL-fractions (B,C). Washing (A), 1M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0). Eluting, linear gradient 0.01M–0M $(NH_4)_2SO_4$ in 0.01M phosphate buffer (pH 6.0).

proach. Tributyrin was chosen instead of olive oil or other mixed triglyceride substrates, as fungal lipases are known to catalyze predominantly the hydrolysis of triglycerides containing short-chain fatty acids (Macrae, 1983).

The isolation of lipase activities from the technical preparation was performed starting with DEAE-Trisacryl M ion exchange chromatography of the ultrafiltered crude enzyme solution (Fig. 1). Fractions 150–180 were found to contain lipase activity, which could be purified 3.6-fold by this chromatographic step (Table 1). These fractions were combined and concentrated by ultrafiltration before further study on gel filtration. As outlined in Fig. 2 two lipase activities (I and II) with different molecular weights were separated by gel filtration on Sephadex G 50. With this procedure β-D-glucosidase and β-D-galactosidase activities could be removed. Remaining impurities in lipase I (fractions 32–37) and lipase II (fractions 39–43) were separated by hydrophobic chromatography on Phenylsepharose CL-4B (Fig. 3 and 5) and rechromatography using the same gel under varied conditions (Fig. 4 and 6). After these procedures lipases I and II were found to be homogeneous in ultrathin-layer isoelectric focusing (UIEF) (Fig. 7 and 8). As summarized in Table 1, using the combination of these above-mentioned chromatographic procedures, the lipases were purified 68-fold and 13-fold, respectively.

Properties of lipase I and II

Isoelectric point (IEP). Using UIEF procedure isoelectric points (IEP) of 4.0 and 3.5 were determined for lipase I and II, respectively (Fig. 7, 8). In the literature, only a few results have been reported on IEP of fungal lipases. Tsujisaka and Iwai (1984) found 4.3 (*Aspergillus niger* and *Geotrichum candidum*), 4.9/4.1 (*Penicillium cyclopium*), and 7.3/8.2 (*Rhizopus delemar*), whereas Grimpe (1977) in his study of *Candida cylindracea* lipases determined IEP in the range 2.1–5.3.

Molecular weight. Estimations of molecular weights of lipase I and II by gel filtration revealed 31000 and 19000 daltons, respectively. These values correspond to data previously published for microbial lipases. Macrae (1983) described molecular weights for fungal lipases between 20000 and 60000 daltons; lipases from *Aspergillus niger, Geotrichum candidum, Penicillium cylopium* and *Rhizopus delemar* showed molecular weights between 27000 and 54000 daltons (Tsujisaka and Iwai, 1984). Furthermore, values of 24500 and 29000 daltons were

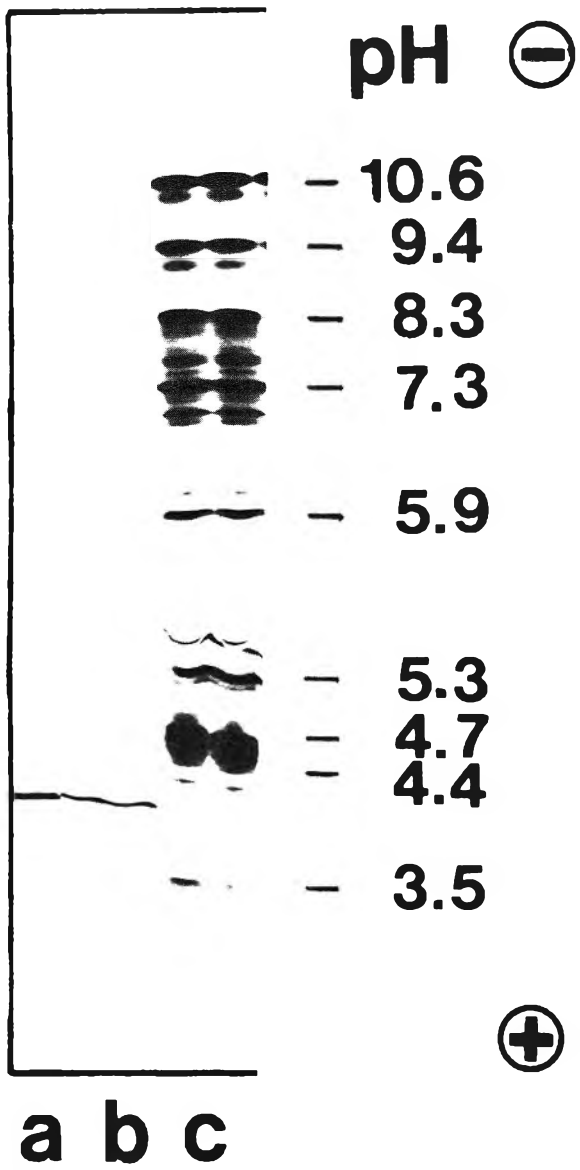

Fig. 7—Ultrathin-layer isoelectric focusing of lipase I after Phenylsepharose CL-4B rechromatography (ultrafiltered fractions 47-100; cf. Fig. 5). a = visualization of lipase with tributyrin as substrate; b = protein staining; c = protein staining of marker proteins. Sample, 5 μl. Ampholytes, mixture (1:1), of pH 2-11 and 4-9 Servalyt T (Serva, Heidelberg). Prefocusing, 400 V (30 min). Focusing, 400 V (15 min); 1000 V (15 min); 1500 V (30 min).

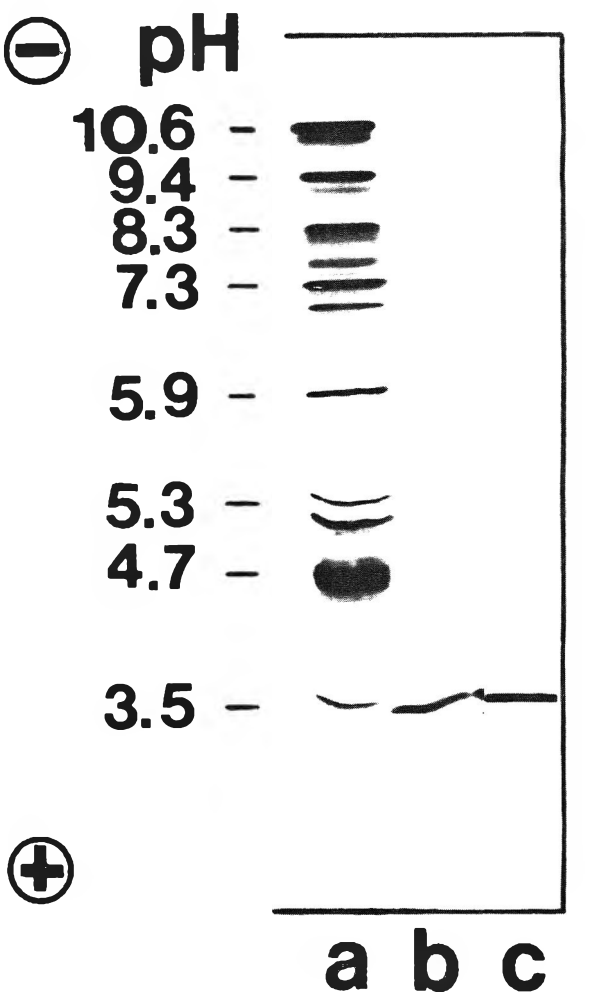

Fig. 8—Ultrathin-layer isoelectric focusing of lipase II after Phenylsepharose CL-4B rechromatography (ultrafiltered fractions 155-170; cf. Fig. 6). a = protein staining of marker proteins; b = protein staining; c = visualization of lipase with tributyrin as substrate. Sample, 5 μL. Ampholytes, mixture (1:1) of pH 2-11 and 4-9 Servalyt T (Serva, Heidelberg). Prefocusing, 400 V (30 min). Focusing, 400 V (15 min); 1000 V (15 min); 1500 V (30 min).

found for *Mucor lipolytica* lipases (Nagaoka and Yamada, 1973), whereas 43000 daltons was determined for a *Rhizopus arrhizus* enzyme (Benzonana, 1974).

Chemical composition. The carbohydrate content of the purified lipases I and II was estimated to be 6% and 9%, respectively. In the hydrolyzate of the preparations the following carbohydrates were found by capillary gas chromatography: mannose, galactose, glucose, fucose, arabinose and a hexosamin in relative amounts of 26:7:5:3:3:2 (lipase I) as well as mannose, glucose, fucose, arabinose and a hexosamin in the relative quantities of 20:10:14:2:13 (lipase II). The high mannose content of microbial lipases is known from previous studies, e.g., Tombs and Blake (1982) detected 12 mannose and 2 galactose and 2 N-acetylglucosamin units in an *Aspergillus niger* lipase.

Substrate specificity. It is well known that microbial and pancreas lipases differ in their substrate specificities (Harper, 1957; Kornacki et al., 1979), but among the various fungal lipases differences have been also observed. Lipases from *Aspergillus niger* have been found to catalyze the hydrolysis both of short- and long-chain fatty acids from triglycerides (Harper, 1957; Iwai et al., 1964). A *Candida paralipolytica* lipase has been detected showing highest activity against triglycerides of C8-fatty acids (Ota et al., 1972), whereas *Mucor lipolyticus* enzymes have been described catalyzing predominantly the hydrolysis of triglycerides of fatty acids with chain-lengths between C4-C12 (Nagaoka and Yamada, 1973).

In our study, employing agar diffusion plate tests lipases I and II did not show any difference in activity against triglycerides with C4-C18 fatty acids as substrates, but a distinct effect of chain-length of fatty acids on the enzymic hydrolysis of monoesters was found. Using nitrophenyl esters of C2-C12 fatty acids, high hydrolysis rates of esters of C6-C12 fatty acids were determined for lipase I. Conversely, lipase II predominantly catalyzed the hydrolysis of esters of short-chain fatty acids (Table 2).

Effect of pH on the activity. Lipases I and II showed a broad maximum of activity between pH 5 and 6 and were still active at pH 7-8. These values correspond to data found with microbial lipases (Macrae, 1983).

Table 1—Purification steps of lipase isoenzymes from the technical enzyme 2212 D (Röhm)

Purification step[b]	Volume (mL)	Protein		Lipase activity[a]		Purification	Yield (%)
		mg/mL	total mg	U/mL	U/mg protein		
Crude enzyme 4/100 (w/v)	100	22.0	2200	108.0	4.9	1.0	100
C 80/50, UF	40	30.0	1200	177.0	5.9	1.2	66
DEAE-Trisacryl M							
F 150-180	310	1.4	434	25.0	17.8	3.6	72
UF	8	36.6	293	780.0	21.3	4.3	58
Sephadex G 50							
Lipase I (F 32-37)	12	6.4	76.8	240.0	37.5	7.6	27
Lipase II (F 39-43)	10	6.1	61.0	90.0	14.7	3.0	8
Phenylsepharose CL-4B							
Lipase I, first run (F 41-73; UF)	15	0.36	59.4	110.8	307.8	62.8	15
rechromatography (F 41-100; UF)	4	0.39	42.1	129.4	331.7	67.7	5
Lipase II, first run (F 57-59)	15	0.65	9.8	32.0	49.2	10.0	5
Rechromatography (F 151-161)	22	0.40	8.8	26.3	65.8	13.4	5

[a] Tributyrin as substrate.
[b] C = centrifugation; UF = ultrafiltration; F = fractions (cf. Fig. 1-6).

Table 2—Effect of chain-length of fatty acids (C2-C12) on the hydrolysis of nitrophenyl esters catalyzed by lipase I and II

Substrate	Lipase I U/mg protein	Lipase II U/mg protein
p-Nitrophenyl acetate	0.08	0.19
o-Nitrophenyl butanoate	0.09	0.19
p-Nitrophenyl hexanoate	1.40	0.12
p-Nitrophenyl octanoate	2.60	0.11
p-Nitrophenyl decanoate	5.80	0.03
p-Nitrophenyl dodecanoate	3.70	0

Table 3—Influence of effectors on the activity of lipase I and II[a]

Effector	Lipase I		Lipase II	
	U/mg protein	% activity	U/mg protein	% activity
None	0.08	100	0.19	100
$HgCl_2$(10^{-4}M)	0.07	87	0	0
EDTA-Na(10^{-4}M)	0.07	87	0.18	95
$AgNO_3$(10^{-4}M)	0.04	50	0	0
SDS(10^{-4}M)	0.05	62	0.14	74
Iodoacetamide(10^{-4}M)	0.04	50	0.16	84
NaCl(10^{-2}M)	0.07	87	0.18	95
$MgCl_2$(10^{-2}M)	0.11	137	0.18	95
$MnCl_2$(10^{-2}M)	0.07	87	0.19	100
$CaCl_2$(10^{-2}M)	0.11	137	0.19	100

[a] Spectrophotometric determinations at 405 nm (30°C) with p-nitrophenyl acetate as substrate

Effect of temperature on the activity and stability. Both lipases I and II were stable below 40°C, but the activities were partly lost (40%) after 10 min incubation at 60°C and totally destroyed at 80°C.

Influence of effectors on the activity. The results of our studies of the influence of effectors on the activity of lipases I and II are summarized in Table 3. The determinations were carried out with p-nitrophenyl acetate as substrate and the effectors were selected according to Chopra et al. (1980). This spectrophotometric method using a soluble substrate was much easier to perform than the pH-Stat approach employed for the determination of lipase purification (cf. Table 1). Additionally, due to the sensitivity of the method, only small amounts of sample were needed. Semi-quantitative agar plate diffusion tests with tributyrin as substrate showed that the results taken from nitrophenyl ester hydrolysis were coincident with those obtained with the triglyceride system. As outlined in Table 3 lipase II was found to be more sensitive against Hg^{2+} and Ag^{+} than lipase I, which could not be totally destroyed under the assay conditions. The greatest inhibitory effects for lipase I were observed with Ag^{+} and iodoacetamide.

These results supplement previously reported findings on the influence of various effectors on microbial lipase activities. Thus, Chopra et al. (1980) described Na^{+}, Ca^{2+}, Mg^{2+} and Mn^{2+} as activators of *Aspergillus wentii* enzyme, whereas Moskowitz et al. (1977) in their study of *Mucor miehei* lipase found inhibition by NaCl and activation by $CaCl_2$.

CONCLUSIONS

THE FINDINGS obtained during this work show that besides enzymic "side activities" expected in a technical enzyme preparation also main activities differing in their properties may be present. First attempts to use lipases I and II in technological processes for flavor formation led to differences in flavor composition. In further studies, the influence of enzyme properties in relation to processing conditions will be studied.

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Rinsing Behavior of Deposited Layers Formed on Membranes in Ultrafiltration

KAZUHIRO NAKANISHI and HEINZ-GERHARD KESSLER

ABSTRACT

Skim milk and aqueous milk protein suspensions were ultrafiltered in a tubular module under controlled conditions (temperature, pressure, flow rate) and were subsequently rinsed with water also under controlled conditions. The effect of rinsing on the deposited layer was followed by observing the flux with time. Ultrafiltration and rinsing should be carried out at high flow rates and at low pressures. Increasing the pressure led to an undesirable increase in the compactness of the deposit. Theoretical considerations of the mechanism of transport in and at the deposited layer made it possible to obtain data on the thickness of the deposits, on mass transfer coefficients and velocity profiles within deposits. The experimental rinsing curves could be well explained by the calculated results. The results should help to optimize the performance and cleaning of UF modules.

INTRODUCTION

A NUMBER of experimental and theoretical studies have been reported on the performance and mechanisms of ultrafiltration (UF) (Blatt et al., 1970; Porter, 1972; Gernedel, 1980; Rautenbach and Albrecht, 1981). Particularly, great attention has been paid to the mechanism of UF with colloidal suspensions such as skim milk and whey because of a growing practical importance (Glover et al., 1978; Kessler, 1981). In a practical operation, however, cleaning of membranes after UF operation, i.e., removal of deposits formed on the membrane surface during UF is also a very important problem, since the cleaning efficiency is closely related to the total productivity of the system. At present, choice of operating conditions in the cleaning process is largely based on experience. Quantitative information on the selection of cleaning conditions, such as cleaning period, flow velocity, pressure difference across the membrane, and temperature is scarce (Glover et al., 1978). Factors which make the deposited layer more compact might have a considerable influence on the cleaning behavior of UF membranes as well as on the permeation during UF.

Kessler et al. (1982) determined the specific resistance of the deposited layer during UF of dilute skim milk and aqueous protein suspension under different conditions, with an UF batch cell in which the liquid in contact with the membrane is stationary, and clarified various factors affecting the compactness of the layer. The purpose of this research was to investigate the kinetics of rinsing experimentally and theoretically as a first step towards a complete description of the cleaning process of an UF apparatus. In most cases, skim milk was ultrafiltered and then the membrane with a deposited layer was rinsed under different conditions.

MATERIALS & METHODS

THE EXPERIMENTS were carried out with fresh, pasteurized skim milk (supplied from the Bavarian Dairy of Weihenstephan) with a protein concentration of around 3.3%. The milk was stored at 4°C and used within 2 days. An aqueous protein suspension without lactose and milk salts which was prepared by ultrafiltering skim milk and then washing the concentrate by repeated dilution with distilled water and subsequent UF-concentration was also used (Gernedel, 1980).

Apparatus

The apparatus used for ultrafiltration and rinsing experiments, diagrammatically shown in Fig. 1, was similar to that described by Gernedel (1980). The tubular module was 24 mm in diameter, 588 mm long and was in most cases equipped with a cellulose acetate membrane (CA-30 cut-off value of 30,000, Kalle Co., Ltd.). Polyamide membranes (E-10 cut-off value of 25,000, Kalle Co., Ltd.) were used in experiments at temperatures up to 60°C.

Procedures

Every experiment was started with a clean membrane. Tap water was used to set the experimental conditions (temperature, pressure difference across the membrane, flow rate). When the flux (permeation rate) of water, $\dot{m}_w$, had been determined, the apparatus was switched to fresh pasteurized skim milk. A deposit formed on the membrane as soon as ultrafiltration of skim milk was started. Flux measurements indicated that the thickness of the deposit remained constant after 20 min. After 60 min of UF, the apparatus was switched to rinsing with tap water. Changes of flux with time were determined by measuring the quantity of permeate. The temperatures, pressures and flow rates were in some cases the same as those used during the UF, but in other cases they were changed. To eliminate the effects of lactose and milk salts, several experiments were carried out with an aqueous protein suspension.

Some other rinsing experiments were designed to eliminate the effect of the convective transport of solids to membrane due to per-

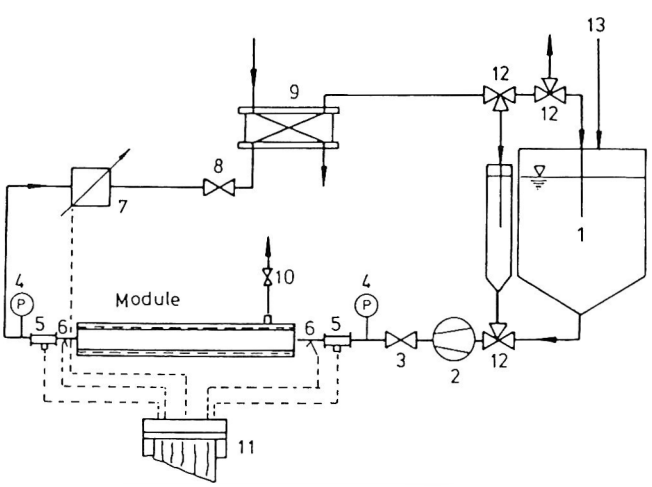

Fig. 1—Diagrammatic representation of the experimental arrangement:

1–Tank
2–Centrifugal pump
3–Throttle valve
4–Pressure gauge
5–Pressure controller
6–Thermocouple
7–Inductive velocity controller
8–Back-pressure valve
9–Plate heat exchanger
10–Valve for permeate
11–Recorder
12–Threeway-valve
13–Tapwater.

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meation. For this purpose the annular permeate collecting space was filled with permeate and closed with valve 10 (Fig. 1) so that the pressure difference across the membrane was reduced to zero. Valve 10 was opened only at certain intervals to measure the flux and was then immediately closed.

Most experiments were carried out at 14°C (using a cellulose acetate membrane), at pressure differences across the membrane, Δp, of up to 3.35 bar and at average velocities along the tubular module, $\bar{v}$, ranging from 1.5 to 5.85 m/sec. The UF module was rinsed for up to 3 hr.

RESULTS & DISCUSSION

ALL THE RESULTS are given in graphs in which the relative permeation flux, $\dot{m}_{rel}$, is plotted against UF time and rinsing time, respectively. The use of $\dot{m}_{rel}$ eliminates effects due to varying properties of the membranes.

$$\dot{m}_{rel} = \frac{\dot{m}}{\dot{m}_w} = \frac{\text{Actual flux during UF or rinsing}}{\text{Initial flux of water through the clean membrane}}$$

Effect of flow rate

Figures 2 and 3 show the effect of the average velocity, $\bar{v}$, along the tubular UF module on flux at a constant pressure difference across the membrane of 3.35 bar. After the beginning of rinsing the $\dot{m}_{rel}$ values rose steeply but the curve flattened out after 60 min, the flux seemed to asymptotically approach a final value which, however, never reached the value of "one" (the initial flux of water before UF). The average velocity has a marked effect on $\dot{m}_{rel}$ in UF as well as during rinsing. When the velocity (or resp. the shear stress) is high, high $\dot{m}_{rel}$ values can be achieved, i.e., the deposit will be largely removed.

Figure 3 shows the results of experiments in which the UF conditions were kept constant (pressure difference = 3.35 bar; $\bar{v}$ = 2.5 m/sec). As in previous rinsing experiments, increasing $\bar{v}$ increased $\dot{m}_{rel}$. However, the effect of $\bar{v}$ was not as marked when a high $\bar{v}$ during rinsing followed a low $\bar{v}$ during UF as when both UF and rinsing were carried out at the same high $\bar{v}$. The reason was that in the former case a thicker deposit accumulated than in the latter. On the other hand, when the change was from a $\bar{v}$ of 2.5 m/sec during UF to one of 1.5 m/sec for rinsing, higher fluxes during rinsing were obtained than when both UF and rinsing were carried out at the same low $\bar{v}$ of 1.5 m/sec (Fig. 2). The reason here is that the high $\bar{v}$ during UF resulted in a thinner deposit. This result demonstrated the great influence of the deposit on flux during UF and rinsing.

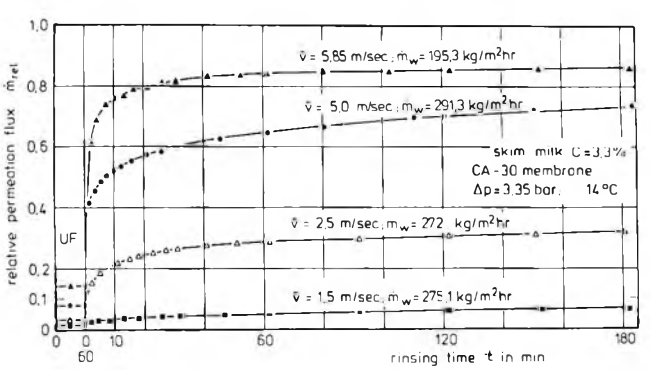

Fig. 2—Effect of velocity of flow on rinsing behavior.

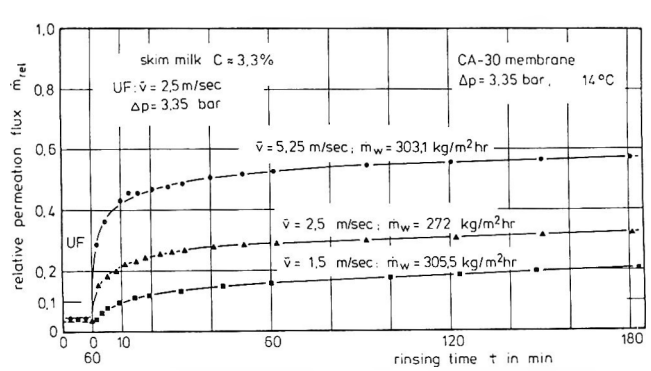

Fig. 3—Effect of velocity of flow on rinsing behavior (same UF conditions in all experiments).

Effect of pressure difference across the membrane

Figure 4 shows the effect of the pressure difference on the rinsing behavior of the deposited layer at a constant average velocity of $\bar{v}$ = 2.5 m/sec. Reducing the pressure difference considerably increased the rate of removal of the deposited layer. It should, however, be noted from Fig. 2, 3 and 4 that the effect of the pressure difference on UF is small, although the flux of water, $\dot{m}_w$, increased proportionally with Δp. The actual flux during UF given by the product of [$\dot{m}_{rel}$ (UF) · $\dot{m}_w$] varied by only 10%, when Δp increased from 1 to 3.35 bar at $\bar{v}$ = 2.5 m/sec. It is advisable therefore to use small Δp values in practice as long as the permeation flux is not reduced too much.

Effect of convective transport to the membrane due to permeation

Removal of the deposited layer in the absence of convective transport was investigated by closing valve 10 (Fig. 1). Figures 5 and 6 show the effect of the presence and absence of convective transport on flux during rinsing and demonstrate that this type of transport influences deposit removal considerably. When convective transport is eliminated, the only factors affecting deposit removal are diffusion and axial movement of the deposit. A comparison of deposits formed from skim milk (Fig. 5) and from an aqueous milk protein suspension (Fig. 6) shows clearly that there are differences in compactness between the two deposits, i.e., the mass transfer coefficient of the deposit from the skim milk is considerably smaller than that from the protein suspension. As shown in a previous study (Kessler et al., 1982), the calcium ions affect not only the resistance but also the rheological properties of the deposited layer formed during ultrafiltration of skim milk. During the measurements by opening valve 10 (Fig. 1), the decreasing $\dot{m}_{rel}$ indicates in Fig. 5 that the deposited layer which swelled under nonconvective transport condition, again contracted under pressure during the flux measurement. On the other hand, the milk protein molecules deposited from the aqueous protein suspension were removed almost completely by diffusion in the absence of convective transport as shown in Fig. 6.

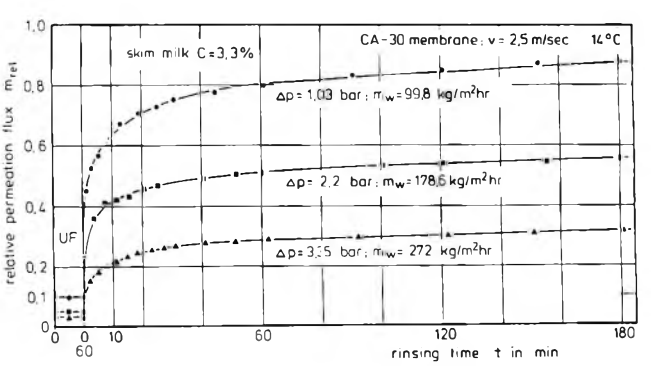

Fig. 4—Effect of pressure difference across the membrane on rinsing behavior.

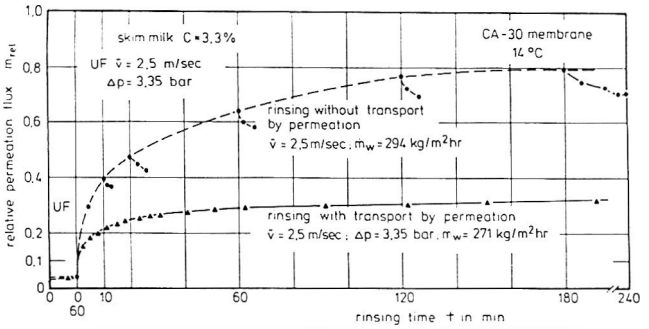

Fig. 5—Effect of convective transport to the membrane by permeation on rinsing behavior after UF of skim milk. (The additional points not on the curve mark the spots where samples were taken.)

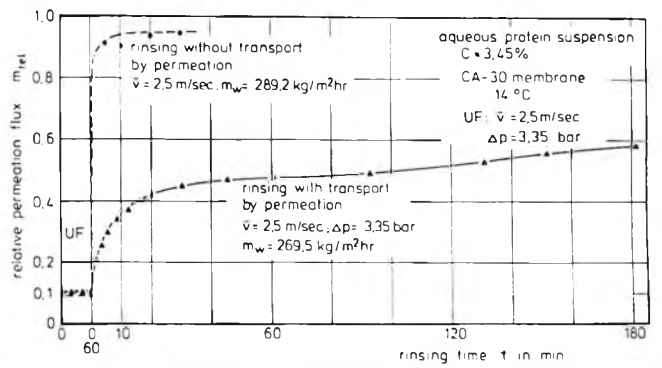

Fig. 6—Effect of convective transport to the membrane by permeation on rinsing behavior after UF of aqueous milkprotein suspension.

Thickness of the deposited layer

From the measurement of fluxes, $\dot{m}$, for water and skim milk or aqueous protein suspension, the resistance of the membrane and deposited layer, ξ_M and ξ_g, are obtained using the Hagen-Poiseuille law for the permeation flux described by the following equation:

$$\dot{m} = \frac{\Delta p}{32 \cdot \nu_p} \frac{1}{\xi_M + \xi_g} \qquad (1)$$

The thickness of the deposited layer, y_g, is expressed as:

$$y_g = \frac{\xi_g}{\xi_g^* \cdot \rho_g \cdot C_g} \qquad (2)$$

The mean specific resistance of the deposited layer, ξ_g^*, in Eq. (2) was previously determined by ultrafiltering dilute skim milk and aqueous protein suspension with an UF batch cell (Kessler et al., 1982). Thus the thickness of the deposited layer during UF and rinsing can be estimated from Eq. (1) and (2).

Table 1 lists such estimates for a number of experiments. Immediately after the start of UF, deposits began to form, the thickness of which depended less on Δp and more on $\bar{v}$ (or shear stress at the wall). Higher $\bar{v}$ resulted in thinner layers. The thickness of all of these layers was, however, less than 10% of that of the laminar sublayer which can be calculated from the following expression (Schlichting, 1955):

$$\delta_{lam} = 5 \nu \sqrt{\rho / \tau_w} \qquad (3)$$

Rinsing, especially at low Δp and at high $\bar{v}$ removed a large part of the deposit. Considering that the diameter of casein micelles ranged from 0.026 to 0.14 μm, the calculated thickness of the deposit remaining corresponded to about one micellar diameter. On the other hand, rinsing at high Δp and low $\bar{v}$ caused a large amount of deposit to remain on the membrane

Table 1—Thickness of deposited layer under various conditions

	Ultrafiltration			Rinsing			
Run	Δp bar	v̄ (m/sec)	$y_{g,o}$ μm	Δp bar	v̄ m/sec	y_g (180 min) μm	$\frac{y_g}{y_{g,o}}$ (%)
	Skim milk						
1	3.35	1.5	11.3	3.35	1.5	3.2	28.3
2	3.35	2.5	5.7	3.35	2.5	0.49	8.6
3	3.35	5.0	2.1	3.35	5.0	0.08	3.8
4	1.03	2.5	4.5	1.03	2.5	0.09	1.9
5	3.35	2.5	4.8	3.35	1.5	0.83	17.4
6	3.35	2.5	4.9	3.35	5.25	0.16	3.3
7	3.35	2.5	4.9	0	2.5	0.08	1.6
	Aqueous protein suspension						
8	3.35	2.5	6.0	3.35	2.5	0.52	8.7
9	3.35	2.5	6.5	0	2.5	n.d.	< 0.7

surface as shown in Table 1. A large amount of deposit remained in the case of UF at high Δp and low $\bar{v}$, followed by rinsing also at high Δp and low $\bar{v}$. These findings indicated that the pressure difference Δp during rinsing as well as UF affected the degree of compactness of the deposit and its thickness.

There was nearly no difference in the thickness of the deposited layer formed from UF of skim milk and aqueous protein suspension when permeation took place under a pressure difference. In the case of rinsing without convective transport, i.e., $\Delta p = 0$, salts especially Ca ions influenced the structure of the residual deposit of skim milk as described previously, while the deposited layer of the aqueous protein suspension disappeared almost completely after rinsing for 10–20 min.

Effect of temperature

After skim milk had been ultrafiltered at 14°C, 3.35 bar and 2.5 m/sec with a polyamide membrane (E-10), the UF module was rinsed with tap water at 50–60°C at 3.35 bar and 2.5 m/sec. The effectiveness of rinsing decreased compared with that at 14°C. The thickness of the deposited layer was estimated as 2.7 μm in the case of rinsing at 50°C after 60 min, whereas it was about 0.5 μm at 14°C as shown in Table 1. In every case increasing the temperature led to decrease of rinsing effectiveness. The reason for this difference might be that the compactness of the deposited layer increased by precipitation of Ca ions from the tap water used for rinsing at high temperatures or by acceleration of the reaction rate of coagulation between Ca ions and casein molecules with higher temperatures.

Theoretical consideration

The rinsing behavior observed experimentally was analyzed, based on the model which took into consideration the diffusional and axial transports of the deposited layer of proteins.

Permeation rate during ultrafiltration

To simplify matters, the thickness of the deposited layer was assumed to be constant over the length of the membrane during UF. The movement of material on the membrane surface and changes in concentration in the boundary layer are depicted schematically in Fig. 7. The protein was deposited onto the membrane surface due to the permeation flux of the solvent, but it did not permeate the membrane. The deposited protein moved along the membrane surface due to shear stress as described later. Thus, the rate of deposit of protein on the membrane, $\dot{m}_{pr}$, is given by Eq. (4) in a way similar to the gel polarization model (Blatt et al., 1970; Porter, 1972), assuming constant ρ and D as:

$$\dot{m}_{Pr} + \dot{m} = k \cdot \rho \ln \frac{C_g - \dot{m}_{Pr} / (\dot{m}_{Pr} + \dot{m})}{C_K - \dot{m}_{Pr} / (\dot{m}_{Pr} + \dot{m})} \qquad (4)$$

where $k (= D/\delta)$ is the mass transfer coefficient of the protein.

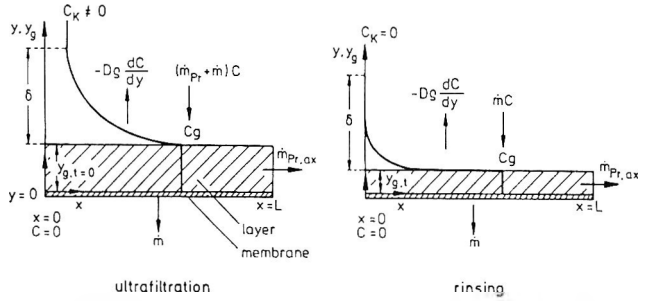

Fig. 7—Changes in (protein) concentration in the boundary layer.

Blatt et al. (1970) and Gernedel (1980), in their studies of the steady state permeation flux during UF, not only dealt with the convective transport of material to the membrane and its return transport by back diffusion, but also took into account the axial movement of the layer produced by shear stress at the membrane surface. In the absence of a deposited layer the velocity profile in a laminar sublayer is expressed as follows (Schlichting, 1955):

$$v_y = \frac{v_\tau^2}{\nu} y \tag{5}$$

In the presence of a deposited layer, however, the velocity distribution inside this layer might be changed due to its rheological properties. A previous study on the property of the deposited layer with an unstirred UF-cell (Kessler et al., 1982) showed that the deposited layer consisted of at least two layers, i.e., a loose, easily removable layer and a strongly compacted one. Considering such change in structure, the following expression is assumed for its velocity profile:

$$v_y = \frac{v_\tau^2}{\nu} \frac{y_g}{(n+1)} \left(\frac{y_g}{y_{g,o}}\right)^n \tag{6}$$

The value of n indicates the degree of reduction of the velocity due to the changed hydrodynamic properties inside the layer. In the case of n = 0, Eq. (6) corresponds to Eq. (5).

By means of Eq. (6) an expression can be obtained for the total axial transport of the protein, $\dot{m}_{Pr,ax}$, in a module of length L:

$$\dot{m}_{Pr,ax} = \frac{(\rho_g C_g - \rho C_K)}{L} \int_o^{y_{g,o}} \frac{v_\tau^2}{\nu} \frac{y}{(n+1)} \left(\frac{y}{y_{g,o}}\right)^n dy \tag{7a}$$

$$\approx \frac{\rho_g \cdot C_g \cdot v_\tau^2 \cdot y_{g,o}^2}{L \cdot \nu \cdot (n+1)(n+2)} \tag{7b}$$

Permeation rate during rinsing

Back diffusion, convective transport of suspended material to the membrane and axial transport of the deposited layers also affected permeation during rinsing. Assuming that the deposited layer was removed at uniform rate from every part of the module surface, the rate of reduction in thickness of the layer can be expressed as follows:

$$-\frac{dy_g}{dt} = V_{ax} + V_{dif} \tag{8}$$

When t = 0, then $y_g = y_{g,o}$. V_{ax} is the rate of reduction in thickness of the layer due to axial transport and V_{dif} is that due to net diffusion (back diffusion minus convective transport to the membrane). V_{ax} as obtained from Eq. (7b) is:

$$V_{ax} = \frac{v_\tau^2}{\nu} \frac{y_g^{n+2}}{y_{g,o}^n} \frac{1}{(n+1)(n+2)L} \tag{9}$$

The contribution of diffusion to the rate of reduction in thickness is determined as follows. The diffusion equation to be applied within the boundary layer at any time, t', during rinsing is given by the following differential equation, assuming that the concentration in the bulk flow is zero:

$$\frac{\partial C}{\partial t'} = D \frac{\partial^2 C}{\partial y^2} + \frac{\dot{m}}{\rho} \frac{\partial C}{\partial y} \tag{10}$$

$$\text{at } t' > 0 \text{ and } y = y_{g,t} + \delta,\ C = 0 \quad \text{and } y = y_{g,t},\ C = C_g \tag{11}$$

As it is very complicated and not practical for the present study to solve exactly Eq. (10), the following simplifications were adopted. As seen in the experiments, the permeation rate of water increased relatively slowly during rinsing. Therefore, it can be assumed that the steady state concentration gradient within the boundary layer is formed after only a very short interval during the rinsing period and that the pseudo steady state approximation holds with respect to the concentration profile. To obtain the steady state solution for the net diffusion flux of protein from a deposit for a very short time interval, the following hypothetical initial conditions are assumed to exist:

$$\text{at } t' = 0: \qquad y_{g,o} < y < y_{g,o} + \delta,\ C = 0 \tag{12}$$

If one assumes here that the permeation rate $\dot{m}/\rho$ is constant, Eq. (10) to (12) can be solved using the Laplace transformation method. The result is the following expression for the transport of the protein due to net diffusion.

$$\dot{m}_{Pr,dif} = -D\frac{dC}{dy}\Big|_{y=y_g} - \frac{\dot{m}}{\rho} C_g = k \cdot \rho_g \cdot C_g \left[\gamma \coth(\gamma) - 1\right] + \sum_{k=1}^{z} \frac{2(k\pi)^2 \exp\left\{-\frac{k}{\delta} t' \left[(k\pi)^2 + \gamma^2\right]\right\}}{\left[(k\pi)^2 + \gamma^2\right]} \tag{13}$$

When t' is as large as a few seconds, the first term in the bracket of Eq. (13) has a predominating influence and Eq. (14) is obtained:

$$V_{dif} = \frac{\dot{m}_{Pr,dif}}{\rho_g \cdot C_g} = k\left[\gamma \coth(\gamma) - 1\right], \tag{14}$$

where $\gamma = \dot{m}/2\rho k$. Eq. (14) represents a pseudo-steady-state solution applicable to cases where the convective transport ($\dot{m}/\rho$) cannot be neglected, when the limiting conditions are those expressed in Eq. (11). V_{dif} decreases sharply as $\dot{m}/\rho$ increases. In the case of no flow through the membrane, i.e., $\dot{m}/\rho = 0$, the following expression is derived:

$$\dot{m}_{Pr,dif} = k \cdot \rho_g (C_g - o) \tag{15}$$

The rate of reduction in the thickness of the deposit (Eq. 8) can therefore be described by Eq. (9) and (14).

Simulation of the rinsing curves

It is possible to simulate rinsing curves ($\dot{m}_{rel} = f(t)$) from experimental data using Eq. (1), (2), (4), (8), (9), and (14), provided the value of n in Eq. (9) and the mass transfer coefficient k in Eq. (14) are known. The following method was used to determine these values. The value of n, i.e., in Eq. (7b) or (9) was obtained from curves calculated from Eq. (8) and (9) for the case of $V_{dif} = 0$, which fitted experimentally derived rinsing curves during the later stage of rinsing. The effect of diffusion can be neglected in this stage or rinsing. Figure 8 shows the velocity profile in the deposited layer at different values of n calculated from Eq. (6). Using the determined value of n, the mass transfer coefficient k (for ultrafiltration conditions) was then calculated from Eq. (4) and (7b). The value of the diffusion coefficient D can be obtained, for instance, from the following equation (Deissler, 1955):

$$Sh = 0.023 \cdot Re^{0.875} \cdot Sc^{0.25} \tag{16}$$

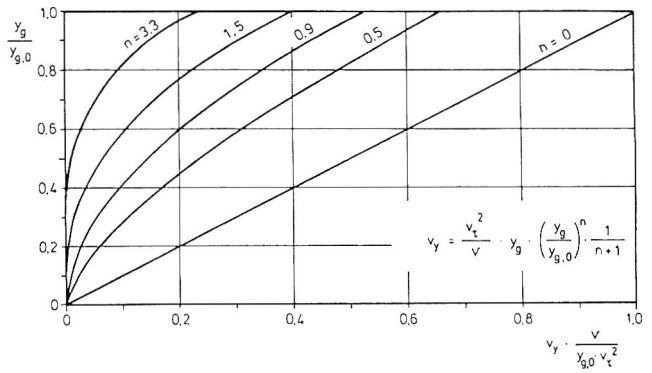

Fig. 8—Velocity profile in the deposited layer.

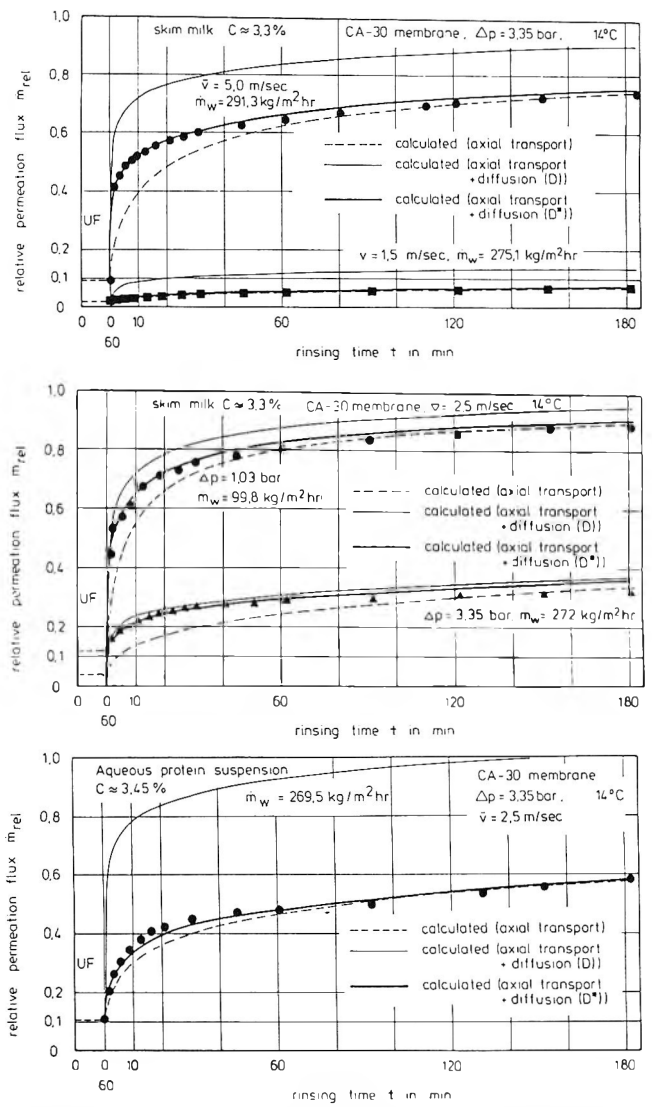

Fig. 9a-c—Comparison of experimental and calculated rinsing curves. Axial transport and diffusion are taken into account (values of n, D and D from Table 2).*

The mass transfer coefficient obtained from ultrafiltration conditions was adjusted to fit the conditions during rinsing (e.g., by adjusting the density and viscosity in the Reynolds and Schmidt numbers) and was used to compute the data for the rinsing curves. In Fig. 9a, b and c, rinsing curves calculated as described above and using the values of n and D, are compared with experimentally obtained ones (plotted by points from Fig. 2, 4 and 6). In the broken line curves only axial transport was taken into account, whereas both axial transport and diffusion (diffusion coefficient D) were accounted for in the thin solid line curves. In all cases the value of the diffusion coefficient, D, obtained from UF experiments seemed too high to be used for obtaining rinsing curves. Diffusion coefficients, D*, the values which best fitted the experimental rinsing curves, were therefore determined using Eq. (1), (2), (8) and (14) in conjunction with the previously established values of n (thick solid lines). As shown in Fig. 9a–9c, the experimental results are in good agreement with calculated ones using the D* values. Table 2 gives a list of n, D and D* values. The differences between D (from UF experiments) and D* (from rinsing experiments) are probably due to differences in the structure of the deposit surface. Deposits formed during ultrafiltration have a surface consisting of a loose layer of proteins. This would make mass transfer easier during UF; and the removal of the loose layer during rinsing would make mass transfer in the remaining deposit layer more difficult, since the deposited layer near the membrane surface is much more compact due to the interaction between proteins and Ca ions under high pressure (Kessler et al., 1982). Here, the diffusion coefficient was determined based on Eq. (16) using a constant viscosity for bulk solution. The viscosity, however, might vary markedly in the boundary layer above the deposited layer. This may affect the value of D more or less.

CONCLUSION

IMMEDIATELY after beginning of UF of skim milk or aqueous protein suspension in a tubular UF module, deposits, mainly composed of milk proteins, formed on the membrane surface. Flux measurements indicated that the thickness and the resistance of the deposit remained constant after 20 min. The thickness depended less on the pressure difference, Δp, but more on the average velocity or shear stress along the membrane. Δp had only a small effect on the flux, but higher velocity increased the flux considerably. During rinsing, high velocities and low pressure differences had a marked effect in removing the deposit, i.e., more than 98% compared with only 70–80% in the case of low velocities and high Δp.

The mathematical model which took into consideration axial movement of the deposited proteins parallel to the membrane with a changed hydrodynamic velocity profile and net diffusion transport of the proteins vertical from the membrane helped to explain the physical phenomena that occurred during rinsing. At the beginning of rinsing diffusional transport dominates. During the latter stage the effect of diffusion can be neglected. In addition, the compactness of the surface structure of the deposited layer formed during UF was different from that of the layer appeared during rinsing.

NOMENCLATURE

C	kg/kg	Protein concentration
C_g	kg/kg	Protein concentration in the deposited layer
C_K	kg/kg	Protein concentration in the bulk solution above the membrane
D	m^2/sec	Diffusion coefficient calculated from data of flux during UF
D*	m^2/sec	Diffusion coefficient calculated from the rinsing curve
d	m	Diameter of the tubular UF module
k	m/sec	Mass transfer coefficient of the proteins ($k = D/\delta$)
L	m	Axial length of the UF module
$\dot{m}$	kg/m^2·sec	Flux or permeation rate during UF or rinsing
$\dot{m}_{Pr}$	kg/m^2·sec	Rate of deposit of protein onto the membrane
$\dot{m}_w$	kg/m^2·sec	Water flux through the clean membrane

Table 2—Values of parameters determined: D—Diffusion coefficients calculated from the data of flux during UF; D—Diffusion coefficients calculated from the rinsing curve*

Run	Δp bar	$\bar{v}$ m/sec	τ_w kg/m·sec²	$y_{g,o}$ μm	n —	D m²/sec	D* m²/sec
	Skim milk						
1	3.35	1.5	8.1	11.3	3.3	$4.0 \cdot 10^{-12}$	$1.7 \cdot 10^{-12}$
2	3.35	2.5	19.8	5.7	1.4	$5.7 \cdot 10^{-12}$	$5.0 \cdot 10^{-12}$
3	3.35	5.0	66.7	2.1	0.9	$9.4 \cdot 10^{-12}$	$6.2 \cdot 10^{-12}$
4	1.03	2.5	19.8	4.5	0.5	$6.2 \cdot 10^{-12}$	$5.1 \cdot 10^{-12}$
	Aqueous protein suspension						
8	3.35	2.5	19.8	6.0	1.5	$24.6 \cdot 10^{-12}$	$8.1 \cdot 10^{-12}$

$\dot{m}_{rel}$	—	Relative permeation flux ($\dot{m}_{rel} = \dot{m}/\dot{m}_w$)
n	—	Value for characterization of the changed hydrodynamic properties inside the deposited layer
Δp	kg/sec²m = 10^{-5} bar	Pressure difference across the membrane
Re	—	Reynolds number ($Re = \bar{v}\, d/\nu$)
Sc	—	Schmidt number ($Sc = \nu/D$)
Sh	—	Sherwood number ($Sh = kd/D$)
t	sec	Time
v	m/sec	Rate of reduction in thickness of the deposited layer
$\bar{v}$	m/sec	Average velocity along the tubular UF module
v_τ	m/sec	Friction velocity
v_y	m/sec	Axial velocity at a distance y of the membrane surface
x	m	Axial distance at the membrane surface
y	m	Distance from the uncovered membrane surface
y_g	m	Thickness of the layer
$y_{g,o}$	m	Thickness of the layer at time t = 0
δ	m	Thickness of the concentration boundary layer above the deposited layer
δ_{lam}	m	Laminar sublayer
ν	m²/sec	Kinematic viscosity of the solution
ν_p	m²/sec	Kinematic viscosity of the permeate
ξ_g	m⁻¹	Mean resistance of the deposit
ξ_g^*	m/kg	Mean specific resistance in unit mass
ξ_M	m⁻¹	Mean resistance of the membrane
ρ	kg/m³	Density of the solution
ρ_g	kg/m³	Density of the deposited layer
τ_w	kg/sec²m	Shear stress at the wall

Indices

ax	Characterization of the axial protein transport parallel to the membrane (due to shear stress)
dif	Characterization of the net protein transport vertical from the membrane (considering back diffusion minus convective transport)

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Comparison of Media for Enumeration of Clostridium perfringens in Foods

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ABSTRACT

Iron Milk Medium (IMM), Tryptose Sulfite Cycloserine (TSC) and Shadihi-Ferguson-perfringens (SFP) agar were compared for enumeration of *Clostridium perfringens* in four typical foods. The overall recovery in 160 samples tested at two inoculum range levels (10^2–10^3 and 10^4–10^5 per mL of sample homogenate) indicated that any of the three media could be used for most routine analyses. There were no significant differences in recovery abilities of the three media used for clams, oysters and turkey meats. In 15 naturally contaminated samples, recoveries of *C. perfringens* were not different in IMM compared to TSC. Therefore, the iron milk medium which is inexpensive, rapid and simple to prepare can be used as an alternative method for routine analyses for *C. perfringens* in foods such as shellfish and lean meats.

INTRODUCTION

THE MOST COMMON solid media used for the isolation and enumeration of *C. perfringens* in foods include tryptose-sulfite-cycloserine (TSC) (Harmon et al., 1971), tryptose-sulfite-neomyxin (TSN) (Marshall et al., 1965), Shahidi-Ferguson perfringens (SFP) (Shahidi and Ferguson. 1971) and sulfite-polymixin-sulfadiazine (SPS) (Angelotti et al., 1962). The selectivity of these media is based on the ability of *C. perfringens* to reduce sulfite to sulfide with production of black colonies. Other selective agents, such as antibiotics, are added to inhibit any competitive organisms also capable of reducing sulfite. These procedures require preparation of plates with an agar overlay, anaerobic incubation, isolation and biochemical identification of isolates. All these time-consuming steps can limit the number of samples processed.

Rapid screening procedures (Erickson and Diebel, 1978; St. John et al., 1982; Abeyta, 1983) have been developed for the enumeration of *C. perfringens* in water, soil and foods. The rapid perfringens medium (RPM), developed by Erickson and Diebel (1978), consists of fortified litmus milk broth with inhibitory agents. In a comparison with SPS and TSC plate media, the RPM was superior in selectivity and sensitivity, detecting low to moderate numbers of *C. perfringens*. The method developed by St. John et al. (1982) utilizes pasteurized whole milk supplemented with 2.0% iron powder in the direct enumeration of *C. perfringens*. The selectivity of this medium is based solely on the rapid growth of *C. perfringens* at 45°C and the typical stormy fermentation reaction within 18 hr. Results obtained by St. John et al. (1982) showed no significant differences between iron milk medium and the solid agar medium for isolating *C. perfringens* from environmental samples. Foods were not tested. This study was designed to determine if iron milk medium could be used for a direct enumeration of *C. perfringens* in foods.

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MATERIALS & METHODS

Bacterial strains

Eight strains of *C. perfringens* FD-1, FD-2, FD-21, FD-29, FD-37, S-34, S-45, and CDC-1861 were obtained from S.M. Harmon (FDA, Center for Food Safety & Applied Nutrition, Washington, DC). All cultures were obtained as spore suspensions and were stored at −72°C in equal parts buffered trypticase peptone glucose yeast broth (TPGY) and glycerol salt solution. Before testing, vegetative cells of the eight strains were grown in cooked meat medium (Difco) for 18–24 hr at 35°C.

Media

Three media, Iron Milk Medium (IMM), Tryptose Sulfite Cycloserine (TSC) agar, and Shahidi-Ferguson-Perfringens (SFP) agar were compared for the enumeration of viable cells of *Clostridium perfringens* in four different foods: beef gravy, turkey, clams, and oysters.

The iron milk medium (St. John et al., 1982) was prepared by placing 10 mL homogenized pasteurized milk and 0.2g iron powder in 16 × 150 mm screw-cap tubes. The medium was sterilized at 10 psi pressure, 116°C for 10 min and used on the day of preparation. The three-tube Most Probable Number (MPN) procedure was used. The two plating media were prepared according to manufacturer's directions; 0.1 mL portions of serial decimal dilutions were spread over the surface of the medium with sterile glass rods as specified in the official AOAC method (AOAC, 1980).

Sample preparation

Sample homogenates were prepared by adding 25-g portions of each food into a sterile 800 mL Mason jar and bringing the total volume to 250 mL with sterile 0.1% peptone water. Samples were homogenized with an Oster blender at low speed for 1 min.

Inoculation of food samples

Broth cultures (cooked meat medium) of the test organisms were diluted in 0.1% peptone water and aseptically added to the food homogenates to give final concentrations of approximately 10^2 and 10^4 organisms per mL of food. Inocula levels were tested by plating on Tryptic Soy Agar (TSA). Five sample of four foods were each inoculated with eight strains. A total of 160 analyses were performed at the two concentrations for each medium (960 subsamples). Single MPN determinations were performed for IMM and duplicate plates were observed at the appropriate dilution for the SFP and TSC media.

Testing for *Clostridium perfringens*

Immediately following inoculation of food homogenates, serial decimal dilutions were prepared and inoculated into the three-tube IMM-MPN series. One-tenth milliliter portions of each dilution were surface plated onto TSC and SFP media. All inoculated plates were overlayed immediately with 5–10 mL of basal medium and incubated in anaerobic jars at 35°C. The MPN tubes were incubated at 45°C in a water bath. Positive and negative controls consisting of each sample homogenate were run along with inoculation studies. After incubation, the black (sulfite reducing) colonies were counted and representative isolates were picked for confirmation in motility-nitrate and lactose-gelatin media as specified in the official AOAC method (AOAC, 1980).

Naturally contaminated seafoods

A total of 15 samples of dried sliced cuttlefish imported from Korea were collected from a routine regulatory investigation, samples were

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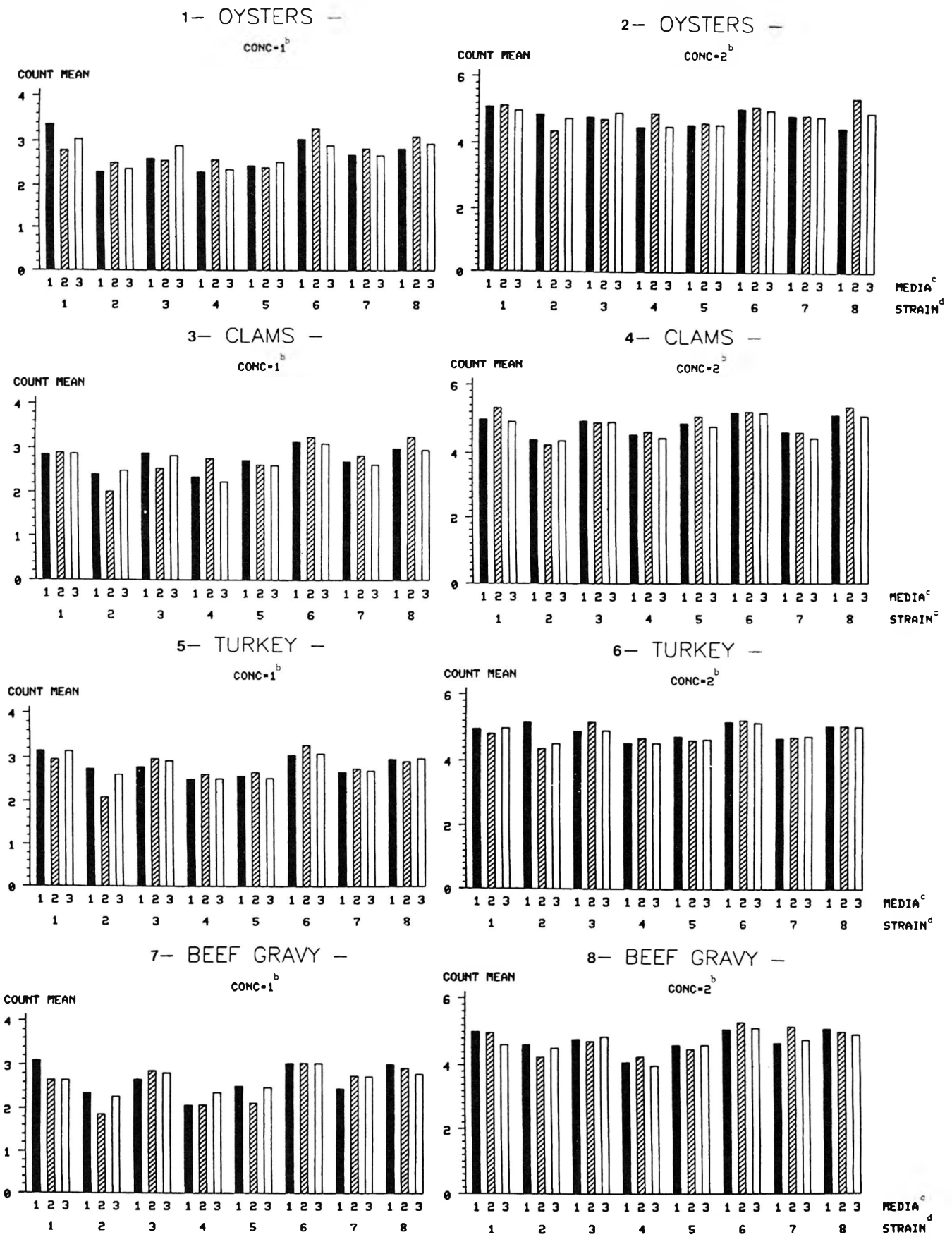

Fig. 1–8—Recovery of viable cells of eight Clostridium perfringens strains from homogenates of four different foods on three media (IMM, SFP, and TSC) at high and low population levels.

[a] *Mean of $\log_{10}$ count per mL of food homogenate as calculated from data with five replicate determinations per strain.*

[b] *Concentration: Conc #1 = 10^2–10^3 per mL of food homogenate; Conc #2 = 10^4–10^5 per mL of food homogenate.*

[c] *Media: 1. Sulfite-Ferguson-Perfringens (SFP) Agar; 2. Iron Milk Medium (IMM); 3. Tryptose Sulfite Cycloserine (TSC) Agar.*

[d] *Strain: (1) FD-1, (2) FD-37, (3) S-45, (4) FD-29, (5) FD-21, (6) S-34, (7) CDC-1861, and (8) FD-2.*

Table 1—Geometric mean per gram for Clostridium perfringens observed on SFP, IMM, and TSC media by concentration and product

Product	Conc.	Geometric Mean / GM SFP	IMM	TSC	Percent Difference Compared to IMM SFP	TSC
Clams	Low	550 (80)[a]	570 (40)	500 (80)	−3.5	−12.3
	High	60,000 (80)	75,000 (40)	53,000 (80)	−20.0	−29.3
Oysters	Low	480 (80)	550 (40)	500 (80)	−12.7	−9.1
	High	56,000 (80)	73,000 (40)	62,000 (80)	−23.3	−15.1
Turkey	Low	620 (80)	590 (40)	630 (80)	+5.1	+6.8
	High	74,000 (80)	65,000 (40)	62,000 (80)	+13.8	−4.6
Beef Gravy	Low	440 (78)	340 (40)	450 (74)	+29.4	+32.4*
	High	52,000 (80)	56,000 (40)	45,000 (80)	−7.1	−19.6

[a] Number of observations in parentheses. Duplicate plate counts were observed for SFP and TSC samples.
* Significant at the $\alpha = 0.05$ level.

Table 2—Tests of significance for media comparison

Product	Conc	Degrees of freedom		F-Ratio
Clams	Low	2,	90	0.49
	High	2,	89	1.64
Oysters	Low	2,	91	0.60
	High	2,	90	2.34
Turkey	Low	2,	92	0.24
	High	2,	91	1.12
Beef Gravy	Low	2,	90	3.85*
	High	2,	96	2.38

* Significant at the $\alpha = 0.05$ level.

Table 3—Estimates of replicate error for plate count methods

Product	Conc.	Replicate error — SFP	DF[a]	Replicate error — TSC	DF
Clams	Low	0.00226	40	0.00160	40
	High	0.00287	40	0.00351	40
Oysters	Low	0.00219	40	0.00211	40
	High	0.00293	40	0.00196	40
Turkey	Low	0.00245	40	0.00139	40
	High	0.00222	40	0.00325	40
Beef Gravy	Low	0.00228	39	0.00224	37
	High	0.00289	40	0.00487	39
		0.00251	319	0.00261	316

[a] Degrees of freedom

submitted to the FDA/Microbiological Laboratory, Seattle, WA, and analyzed as described in Abeyta (1983) except that the alternative pour plate procedure was used with TSC agar instead of surface plating.

Biochemical confirmation tests

Isolates from iron milk and plate media were tested for motility, nitrate reduction, gelatin liquefaction and lactose fermentation as outlined in the official AOAC method (AOAC, 1980).

Statistical analysis

An analysis of variance was performed for each of the eight food and concentration combinations. Thus, for each of the three media there were 40 samples (8 strains × 5 subsamples). The SFP and TSC media were prepared as agar plates and colony forming units were counted on the plates. The *C. perfringens* were enumerated by MPN and IMM. The statistical analysis was modified to compare the media results since the MPN and plate counting variance were unequal. All counts were converted to $\log_{10}$ count to assure normality. Components of variance (Graybill, 1961) and the appropriate degrees of freedom were estimated to compare media means. These means were based on

Table 4—Comparison of Iron Milk Medium (IMM) and Tryptose Sulfite Cycloserine (TSC) methods for enumeration of natural contaminated Clostridium perfringens in dried sliced cuttlefish

Sample	3-Tube IMM MPN/g	TSC cells/g
1	< 3	< 10
2	3.6	10
3	23	20
4	3.6	10
5	3.6	10
6	23	20
7	9.1	10
8	43	10
9	9.1	30
10	23	< 10
11	23	20
12	9.1	20
13	3.6	10
14	9.1	< 10
15	3.6	< 10
Count range/g	< 3 - 43	< 10 - 30
Geometric mean[a]	8.2	7.0
No. of positive Samples (%)	14 (93.3)	11 (73.3)

[a] The counts < 3 and < 10 were set equal to 1.0.

single MPN determinations (40 measurements) and duplicate plates per dilution for plate counts (80 measurements). Samples where no growth was reported were deleted from the analysis (8 observations for turkey). Tests of significance were performed at the $\alpha = 0.05$ level.

RESULTS & DISCUSSION

THE COUNT as geometric mean per gram is listed in Table 1 for all three media. Estimates of percent difference compared to IMM results are also shown. The SFP values ranged from 23% lower to 29% higher than IMM counts, and TSC values ranged from 29% lower to 32% higher than IMM counts. The IMM value of 7.5×10^4/g although higher than the TSC and SFP recoveries for clams, was not significant. This mean was 29% higher than the TSC mean of 5.3×10^4/g. In only one instance was *C. perfringens* recovery from IMM significantly lower (32% lower compared to TSC). This occurred at a low inoculum in beef gravy (Table 1).

There were differences among strains and a relative difference in recovery among media for each strain (Fig. 1–8). As an example, Fig. 7 shows the $\log_{10}$ count mean at the low inoculum levels for beef gravy. The recovery for SFP was higher for strain 1 (FD-1) while recovery was about equal for strains 3 and 6 (S-45 and S-34). Overall, highest recoveries occurred in IMM followed by SFP and TSC. There were no dramatic strain differences where one or more solid media failed to recover *C. perfringens*.

The comparison of mean recoveries for each medium could not be computed by pooling error estimates for each media mean. An estimate based on components of variance for each mean was computed since IMM results were based on MPN counts, and the SFP and TSC values were recorded as plate counts. The weighted error for the eight analyses (4 foods times 2 concentrations) ranged from 0.06058 to 0.18605. An MPN experimental error is typically ~0.100. Thus, larger differences among media means were required (i.e., 32% between TSC and IMM for beef gravy) before significance was detected at the $\alpha = 0.05$ level. Table 2 shows the F ratios for the comparison among means. A Duncan's multiple range test (Kramer, 1956) was used to determine which means differed from each other. The variation among means of strains ranged from 0.54090 overall recovery in oysters to 1.70116 in beef gravy.

A part of the components of variance was due to the duplicate plate count observations. These replicate errors for the plate count procedures ranged from 0.00139 to 0.00487 (Table 3). All of the estimates were within the 0.00500 limit generally

reported for replicate plate counting error. The current AOAC method (1980) requires TSC agar. This medium and SFP have been studied previously (Harmon et al., 1971) and the present data confirmed this work.

From naturally contaminated dried cuttlefish containing a mixed microflora, results showed the geometric mean of 15 samples in IMM was 8.2/g and in TSC 7.0/g (Table 4). The microbial count range was similar in both media with < 3-43/g obtained in IMM and < 10-30/g in TSC. The percentage of positive samples by the IMM and TSC method was 93.3% and 73.3%, respectively. The percent positives did not differ significantly at the $\alpha = 0.05$ level. These data suggested that the IMM MPN procedure was comparable to the TSC plate medium for the enumeration of *C. perfringens*. In fact, a recent study by Abeyta (1983) demonstrated that the IMM MPN method was superior to the direct SFP plate method in sensitivity and selectivity for the isolation and enumeration of *C. perfringens* from other naturally contaminated seafoods.

A presumptive result for *C. perfringens* on TSC and SFP was indicated by its ability to reduce sulfite and produce lecithinase. However, other *Clostridium* species also can give the same response on plating media (SFP and TSC) (Harmon and Kautter, 1978). Because TSC and SFP allowed the growth of other sulfite-reducing clostridia, biochemical confirmation of these organisms was necessary for a positive identification. The confirmation required picking a representative number of colonies for isolation followed by biochemical testing.

Stormy fermentation in iron milk at 45°C, one of the confirmatory tests for *C. perfringens*, was highly indicative of the presence of this organism (St. John et al., 1982; Abeyta, 1983). The stormy fermentation is defined as the production of an acid curd with subsequent disruption of the curd by large volumes of gas. The acid clot is produced by the acidification of milk (lactic acid fermentation) to a pH of 4.5 (MacFadden, 1980). St. John et al. (1982) have isolated from environmental samples, some *Bacillus* species and *Escherichia coli* capable of producing both a curd and a digestion on the surface of the milk. These reactions are similar but not identical to a weak stormy fermentation. Abeyta et al. (1984) found that *C. perfringens*-like strains (*C. barati, C. perenne, C. absonoum*, and *C. paraperfringens*) will also grow rapidly at 45°C and demonstrate stormy fermentation in iron milk after 48 hr. Results indicated best results for *C. perfringens* enumeration were obtained up to a maximum time of 16–18 hr incubation. In fact, a rapid positive response occurred after only 2 and 3 hr incubation of IMM inoculated with pure cultures at 10^8 and 10^7 cells/mL, respectively. This observation has been previously noted by Harmon and Kautter (1978).

The use of IMM for enumeration of *C. perfringens* increased the selectivity and sensitivity in the presumptive identification of this pathogen in foods. Experience in our laboratory as well by other investigators (Erickson and Diebel, 1978; Wekell et al., 1980; St. John et al., 1982; Abeyta, 1983) showed the identification of *C. perfringens* in Iron Milk Medium could be made without confirmation; however, confirmation was recommended.

CONCLUSION

WHILE A NUMBER of solid agar media have been used for enumeration of *C. perfringens* in foods, most of these formulations incorporate antibiotics or other compounds which can result in a variable inhibition of the organism. To circumvent the potential inhibition of *C. perfringens* and subsequent underestimation of their numbers in foods and to avoid laborious, costly, and time-consuming preparation of plates, the use of pasteurized whole milk supplemented with 2.0% iron powder has been used in the direct enumeration of *C. perfringens*. In this study, four typical foods were used in the comparison of IMM to solid agar medium for enumerating of*C. perfringens* at two concentrations. Our results showed no significant differences when *C. perfringens* were enumerated by the IMM method compared to TSC and SFP agar plates with the exception of beef gravy at an inoculum of 10^2 cells. Though the MPN is far less precise than anaerobic plate counts, IMM is inexpensive, rapid, simple to prepare and can be used as an alternative method for enumeration of the pathogen *C. perfringens* in foods.

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Simplified Direct Plating Method for Enhanced Recovery of *Escherichia coli* in Food

J. M. DAMARÉ, D. F. CAMPBELL, and R. W. JOHNSTON

ABSTRACT

A refined plating medium designed to enumerate *E. coli* in food within 24 hr was developed. The medium combined tergitol 7 with the fluorogenic substrate, 4-methylumbelliferyl β-D-glucuronide. *E. coli* colonies were detected by fluorescence under long-wave UV light. The new agar allowed superior recovery of heat and freeze-stressed cells and produced *E. coli* counts from meat and poultry samples at levels generally equivalent to or better than those of the standard 5 tube Most Probable Number method. This method provides a simple and rapid alternative to traditional techniques.

INTRODUCTION

THE ENZYME β-glucuronidase has been examined by several researchers as an indicator of viable *E. coli* (Kilian and Bulow, 1976, 1979; Feng and Hartman, 1982; Alvarez, 1984; Robison, 1984). Its high degree of correlation with the majority of *E. coli* strains (96–97%) and otherwise limited distribution among enterics (excepting some *Salmonella* and *Shigella* strains) provides potential as an alternative indicator to the traditional lactose based systems (Kilian and Bulow, 1976; LeMinor, 1979; Feng and Hartman, 1982). Rapid assay methods for *E. coli* based on the fluorogenic glucuronidase substrate, 4-methylumbelliferyl β-D-glucuronide (MUG), were described recently by Feng and Hartman (1982). The specificity of these methods for detecting *E. coli* in foods within 24 hr was confirmed by Alvarez (1984) and Robison (1984). Limitations associated with endogenous glucuronidase in certain foods, such as oysters, were described by Koburger and Miller (1985).

The objective of this study focuses on developing a selective plating medium for *E. coli* designed to enhance glucuronidase detection and improve recovery of stressed cells. A Peptone Tergitol Glucuronide (PTG) agar is described, compared with three other coliform plating procedures for recovering impaired *E. coli* and evaluated using meat and poultry samples.

MATERIALS & METHODS

Media

Peptone Tergitol Glucuronide agar was prepared using 5g proteose peptone #3 (Difco), 3g yeast extract (Difco), 0.3g dipotassium phosphate, 0.1 mL tergitol 7 (Berg Chem. Co., Bronx, NY), 15g agar, 50 mg 4-methylumbelliferyl β-D-glucuronide (Sigma Chem. Co., St. Louis, MO) dissolved in a liter of distilled water and sterilized at 121°C for 15 min. The pH was adjusted to 7.2 before autoclaving. Tryptone Bile Agar (TBA) was prepared as described by Delaney et al. (1962). Other media were obtained commercially from Difco Laboratories (Detroit, MI).

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Samples

Samples of chicken meat, water from poultry carcass chill tanks and retail samples of pork chitterlings were examined. The samples were shipped frozen and then partially thawed prior to analysis.

Analyses

Meat or poultry samples (50g) were blended and serially diluted in Butterfield's phosphate buffer (Speck, 1976). Comparative analyses for *E. coli* were performed using PTG agar and the standard 5 tube Most Probable Number (MPN) procedure (APHA, 1965). PTG agar plates were inoculated in triplicate with 0.1 mL portions using the spread plate technique. In the case of water samples, 0.5 mL portions of undiluted samples were also plated. The plates were incubated at 35°C and examined over a 16–24 hr period. Fluorescent reactions were observed under long-wave ultraviolet light as described by Feng and Hartman (1982) using a Transilluminator, Model C-63 (UV Products Inc., CA). Representative fluorescent colonies, demonstrating MUG hydrolysis, were picked to EMB agar and streaked for purity onto tryptic soy agar. These isolates were characterized by the IMViC method of Powers and Latt (1977) using a multipoint inoculator (Lovelace and Colwell, 1968) and multiwell culture dishes (Falcon, 3047). Atypical isolates were further characterized by the API-20E (Analytab Products) and conventional biochemical tests.

The relative recovery of stressed cells on PTG agar was compared to that of three other rapid plating techniques for *E. coli*. These included: (1) prepoured violet red bile (VRB) agar plates over-layered with 5 mL of tempered VRB agar and incubated 24 hr at 35°C; (2) the Anderson and Baird-Parker (1975) TBA method using cellulose acetate membranes and incubation at 44.5°C for 24 hr; and (3) the resuscitation modification of Rayman et al. (1979) using an initial 4 hr incubation on Tryptic Soy Agar (TSA) at 35°C followed by membrane transfer to TBA and incubation at 44.5°C for 24 hr. Tryptic Soy Agar pour plates were used for viable reference levels.

A strain of *E. coli* (8-17) isolated from meat was subjected to heat and freeze stress. A two ml aliquot of stationary phase broth culture was blended into 198g of pureed cooked ham using a stomacher. The sample was split, with one portion frozen at −10°C for 1 wk and the other heated at 55°C for 30 min. The samples were prepared as above, plated in triplicate and tested three times by the various methods.

RESULTS & DISCUSSION

THE PTG AGAR FORMULATION is an outgrowth of Chapman's (1947) Tergitol 7 Agar that has been modified specifically for the glucuronidase assay. The fluorogenic substrate, MUG, provides a rapid and specific indicator of *E. coli* using a sensitive technique proposed by Dahlen and Linde (1973). Lactose was omitted and the buffering capacity of the formulation was slightly increased. The acidification of the agar surrounding *E. coli* colonies in lactose based media negatively affected the discrimination of the MUG hydrolyzing colonies. This is consistent with the findings of Dahlen and Linde (1973) who obtained the most pronounced fluorescence from MUG hydrolysis at alkaline pH. The lack of lactose in PTG agar renders it unsuitable for general coliform enumeration but allows reduction of the MUG concentration from 100 μg/mL (Feng and Hartman, 1982) to 50 μg/mL in PTG agar. This level still provides approximately twice the minimum concentration needed for *E. coli* recognition (unpublished data). *E. coli* isolates form colonies of 1–2 mm diameter on PTG agar after 18 hr at 35°C and are easily discriminated under long-wave UV by fluorescence.

Fig. 1 provides a comparison of the data of the PTG agar method and the standard 5 tube MPN technique for determining *E. coli* levels in 30 meat or poultry samples. The relative recoveries of the methods are depicted as log values about the theoretical line of equality. A correlation coefficient of 0.96 was obtained by linear regression analysis with the line of best fit expressed as log y = 0.59 + 0.84 log x. No statistically significant difference between the methods was found at a 90% confidence limit using the paired t-test (P(t) = 0.09).

The specificity of β-glucuronidase detection as a method of identifying *E. coli* from these samples was confirmed in 222 of 224 isolates. This coincides with the 90–95% reported range of confirmation for *E. coli* in foods using MUG hydrolysis in other types of enteric media (Feng and Hartman, 1982; Alvarez, 1984; Robison, 1984). The two false-positive (nonconfirmed MUG-positive) isolates were presumptively identified as *Enterobacter agglomerans* and *Hafnia alvei*. False-positives were noted by Robison (1984) at about 4.8% from foods analyzed using MUG supplemented Lauryl Sulfate Broth. These were presumptively classified as streptococci. In this study a few isolates identified as *Micrococcus* and *Staphylococcus* spp. were observed to fluoresce on PTG agar after 48 hr incubation. These same genera were also reported by LeChevallier et al. (1983) to lead to false coliform reactions on another newly developed tergitol 7 based coliform agar. On PTG agar, however, these MUG hydrolyzing isolates were not encountered within a 24 hr incubation period but served to indicate the potential limitations of the medium and glucuronidase specificity. The performance and value of MUG as an indicator cannot be separated from the selective aspects of the medium particularly since glucuronidase activity among heterotrophic bacteria is not unique to *E. coli* (Dahlen and Linde, 1973; Littel and Hartman, 1983; Gadelle et al., 1985). Among the enterics, however, the fact that 40–50% of *Shigella* and 17–30% of *Salmonella* strains are the only other principal members of the *Enterobacteriaceae* that also hydrolyze MUG, (Kilian and Bulow, 1976; LeMinor, 1979; Feng and Hartman, 1982) does not significantly alter or seriously compromise the intended public health significance of using MUG as an *E. coli* based sanitary index. This was earlier pointed out by Feng and Hartman (1982).

A drawback associated with supplementing standard coliform media with MUG is the poor recovery of injured cells associated with the normal selective agents such as bile salts and deoxychclate (Busta, 1976; Ray, 1979; Hurst, 1980). The recoveries of heat and freeze-stressed *E. coli* on PTG agar compared favorably to the three other rapid plating assays for *E. coli* (Fig. 2). In fact, the PTG assay method yielded recoveries that were comparable to the more complicated resuscitation technique of Rayman et al. (1979). These data not only further substantiate the recovery problems associated with bile salts and elevated temperature regimes (44.5°C) but also the relative gentleness of tergitol 7 for sublethally injured cells. LeChevallier et al. (1983) determined the optimum concentration of tergitol 7 for recovering injured cells (92%) was 0.01%, confirming Chapman's (1947) original T-7 agar concentration.

The results of this study indicated that PTG agar, by combining a less stringent selective agent (tergitol 7) with a more specific indicator (MUG) than commonly used in standard coliform media, can provide an effective medium for rapidly (<24hr) enumerating *E. coli* in food samples. Although only a limited number of meat and water samples was tested, the medium appears to be particularly suited for quality control monitoring of processed and frozen foods in which injured *E. coli* can be expected (Ray, 1979; Hurst, 1980) and resuscitation techniques deemed too expensive. The only specific equipment required is a long-wave UV light source; portable hand held units (Spectroline ENF-24, Spectronics Corp., Westbury, NY) are performing satisfactorily.

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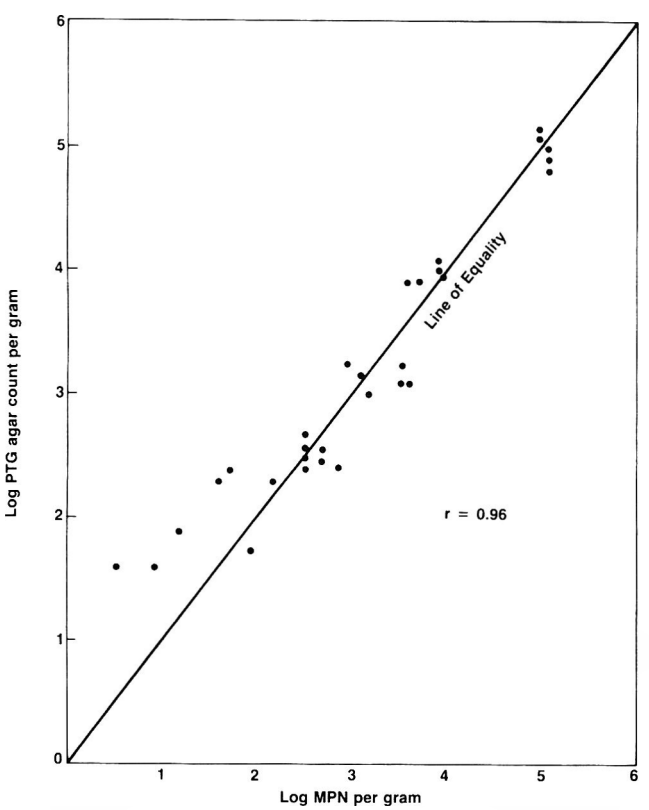

Fig. 1—Comparison of E. coli *recoveries from meat and poultry samples by the standard 5 tube MPN technique and the PTG agar method.*

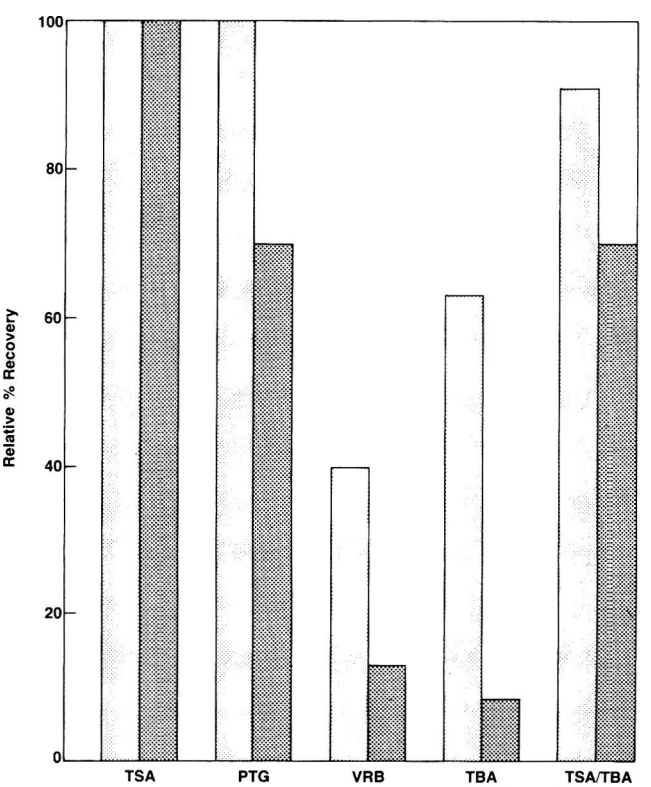

Fig. 2—Percentage mean relative recoveries of heat (light grey) and freeze (dark grey) stressed E. coli *using: Tryptic Soy agar (TSA) for viable reference levels, Peptone Tergitol Glucuronide agar (PTG), Violet Red Bile agar (VRB), Tryptone Bile agar (TBA), and a resuscitation procedure using TSA followed by TBA (TSA/TBA). See text for details.*

Cross-Linking of α_{s-1}Casein by Sodium Hypochlorite

TERUYOSHI MATOBA, TAIICHI SHIONO, and MAKOTO KITO

ABSTRACT

α_{s-1}Casein was polymerized in the presence of sodium hypochlorite (NaOCl). The polymerization was suppressed by succinylation of the protein. Dityrosine and α-aminoadipic acid were detected in the hydrolysate of the reacted proteins. A carbonyl group was detected in the reaction product of acetyl lysine methylester and NaOCl. The Schiff base formation between lysine and α-aminoadipic δ-semialdehyde residues, and dityrosine formation may clarify the mechanism of polymerization. The chemical modification of proteins by NaOCl is expected to be useful for improving the functional properties of food proteins, since polymerization by NaOCl could occur under mild conditions (NaOCl conc.,<0.05%; time, <5 min; at 37°C and pH 7–9).

INTRODUCTION

SODIUM HYPOCHLORITE (NaOCl) is widely utilized as a germicide for sterilizing not only vegetable and fruits but also packages and apparatus in food processing industries. NaOCl also has been known to be very effective in destroying aflatoxins (which have hepatotoxic and carcinogenic properties) during the preparation process of protein isolates from peanuts (Natarajan et al., 1975a).

NaOCl is a very potent oxidizing agent and its reaction with proteins is expected to cause their physicochemical changes. Natarajan et al. (1975b) indicated that the reaction of peanut protein isolates with NaOCl caused changes in the color, viscosity, solubility, amino acid composition and molecular size (polymerization) of the proteins. Of these changes, polymerization may be the most significant in affecting the functional properties of food proteins. However, the mechanism of the polymerization of proteins by the reaction with NaOCl is not clearly understood.

The purpose of the present study is to elucidate the mechanism of polymerization by using α_{s-1}casein as the model protein. There have been several publications concerning cross-linkings of proteins by enzymatic and nonenzymatic oxidative reactions. Such cross-linkings have been suggested to be caused by formation of Schiff base and dityrosine (Fowler et al., 1970; Siegel and Mortin, 1970; Aeschbach et al., 1976; Stahmann and Spencer, 1977a, b; Zaitsu et al., 1981; Verweij et al., 1982; Cooper and Varner, 1983; Matheis and Whitaker, 1984). In the present paper, we focus on the modification of lysine and tyrosine residues in α_{s-1}casein to investigate the mechanism of polymerization of proteins by NaOCl.

MATERIALS & METHODS

Materials

Sodium hypochlorite (NaOCl) and α-aminoadipic acid were purchased from Nakarai Chemicals Ltd, Kyoto. α_{s-1}Casein was prepared according to the procedure of Zittle and Custer (1963). Hammarsten casein was used instead of acid casein. Polyacrylamide gel electrophoresis was carried out in the presence of 7M urea to confirm purity (more than 90%). Succinylation of α_{s-1}casein followed the method of Franzen and Kinsella (1976). Dityrosine was obtained using the procedure of Malanik and Ledvina (1979). TSK G 3000 SW column was obtained from Toyo Soda Manufacturing Co., Ltd, Tokyo. Disposable column PD-10 (packing Sephadex G-25) was purchased from Pharmacia Fine Chemicals, Uppsala. Pre-coated TLC plates silica gel 60F-254 and Hammarsten casein (Art. 2242) were obtained from Merck, Darmstadt. 2, 4-Dinitrophenylhydrazone of n-hexanal was synthesized according to the procedure of Shriner et al (1956). Acetyl lysine methylester-HCl and acetyle tyrosine ethylester were purchased from Peptide Institute, Inc., Osaka.

Reaction system

Reaction of protein with NaOCl. α_{s-1}Casein or succinylα_{s-1} casein was incubated at 37°C in 5 mL 0.1M phosphate (pH 6.0 or pH 7.0) or 0.1M borate (pH 9.0) containing NaOCl (final conc, 0–0.5%). The pH of the mixture was adjusted to the desired value with 1N NaOH or 1N HCl. After incubation, the reaction mixture was passed through a disposable column PD-10 (packing Sephadex G-25). The resulting protein fraction, which had been lyophilized, was used for the subsequent experiments. Fluorescence measurement was carried out with a spectrofluorometer (Hitachi MPF-4). The nitrogen content of the samples was determined by elemental analysis.

Reaction of amino acid derivatives with NaOCl. Acetyl tyrosine ethylester (63 mg) was incubated at 37°C for 20 hr in 10 mL 60% aqueous ethanol solution containing NaOCl (final conc 0.5%). After incubation, the reaction mixture was evaporated and the residue was dissolved in 1N HCl. The product was extracted with ethyl acetate. The organic layer was washed with water, dried with Na_2SO_4, and then evaporated. After acid hydrolysis of the residue (6N HCl, 110°C, 24 hr), high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) analyses were carried out as described below.

Acetyl lysine methylester, HCl (100 mg) was incubated at 37°C for 24 hr in 10 mL 0.1M borate (pH 9.0) containing NaOCl (final conc 0.5%). After incubation, a part of the reaction mixture was analyzed by TLC (solvent system: n-butanol/acetic acid/water, 4:1:1). The remaining mixture (2 mL), to which 1M $Na_2S_2O_3$ (400 μL) was added, was allowed to react with 2,4-dinitrophenylhydrazine according to the method of Lappin (1951). The resulting mixture was subjected to a spectrophotometric analysis (Shimadzu UV-240).

Gel filtration by HPLC

Gel filtration on TSK G 3000 SW (0.75 × 70 cm) was carried out by HPLC (Hitachi 638-30) and detection was with an UV detector (Hitachi 638-0410) at 210 nm and with a spectrofluorometer (Jasco PF-110) at an emission wavelength of 400 nm with an excitation wavelength of 350 nm. The eluting buffer was 25 mM phosphate (pH 6.8) containing 0.1% SDS and 0.2M NaCl. The flow rate was 0.5 mL/min. The sample solution was prepared by incubating the protein in 25 mM phosphate (pH 6.8) containing 2% SDS and 0.2M NaCl at 100°C for 5 min.

Determination of dityrosine

HPLC determination of dityrosine was carried out according to the procedure of Zaitsu et al. (1981) using a column (4 × 250 mm) packing Hitachi gel 3011-N (10 μm). Chromatography was performed with apparatus described above in "Gel filtration."

Amino acid analysis

Enzymatic method. The sample was digested with pepsin and pancreatin, followed by aminopeptidase-prolidase hydrolysis according to the method of Matoba et al. (1982, 1984). The resulting hydrolysate

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was subjected to amino acid analysis. The quantitative amino acid determination was carried out with an amino acid analyzer (Hitachi 835). γ-Amino-n-butyric acid was used as the internal standard.

Acid and alkali hydrolyses. The sample was hydrolyzed with redistilled 6N HCl at 110°C for 24 hr in an evacuated tube. The sample was also hydrolyzed with 3.75N NaOH at 110°C following the method of Neumann (1967) γ-Amino-n-butyric acid was used as the internal standard.

RESULTS

Changes in molecular sizes of proteins

Changes in the molecular sizes of α_{s-1}casein and succinyl α_{s-1}casein after the reaction with NaOCl were examined by HPLC gel filtration on porous silica gel column (TSK G 3000 SW) in SDS aqueous solution. This chromatography has been reported to be highly effective in separation of proteins at high speed (Kato et al., 1980). Fig. 1 shows the chromatogram of the reacted proteins under various conditions. When α_{s-1}casein was allowed to react with NaOCl (final conc 0.1%) for 5 min at pH 6, 7 and 9, the polymerization of the protein at pH 9 was extensive (Fig. 1, A-D). The chromatograms after the reaction for 2 and 24 hr were the same as those after the reaction for 5 min, suggesting that the reaction with NaOCl proceeded rapidly.

When α_{s-1}casein was allowed to react with different concentrations of NaOCl at pH 9.0 for 5 min, the polymerization proceeded extensively as the concentration of NaOCl increased (Fig. 1, E-H). Fluorescence intensity of the peak on the chromatogram increased as polymerization proceeded (Fig. 1, A-H).

Succinylation of α_{s-1}casein was carried out to suppress the reactions for which amino groups are responsible. The degree of polymerization of the succinyl protein was significantly low compared with that of α_{s-1}casein, although fluorescence intensity of the peaks on the chromatograms was detected (Fig. 1, I-L). From these results it appears that the ε-amino group of lysine residues takes part in the polymerization.

Amino acid analysis

The amino acid composition of α_{s-1}casein after the reaction with NaOCl is shown in Table 1. Lysine, tyrosine, methionine and arginine residues were damaged considerably during the reaction. The damage to methionine residue was also determined after alkali hydrolysis (3.75N NaOH), since methionine sulfoxide has been known to revert largely to methionine by 6N HCl hydrolysis (Keutmann and Potts, 1969), indicating that methionine residue was not damaged after the reaction in the presence of 0.05% NaOCl, but was mostly oxidized after the reaction in the presence of more than 0.1% NaOCl (data not shown). After the reaction at more than 0.05%, an unknown peak on the chromatogram was detected as shown in Fig. 2. This peak was identical to that of authentic α-aminoadipic acid.

The amino acid composition of succinyl α_{s-1}casein after ex-

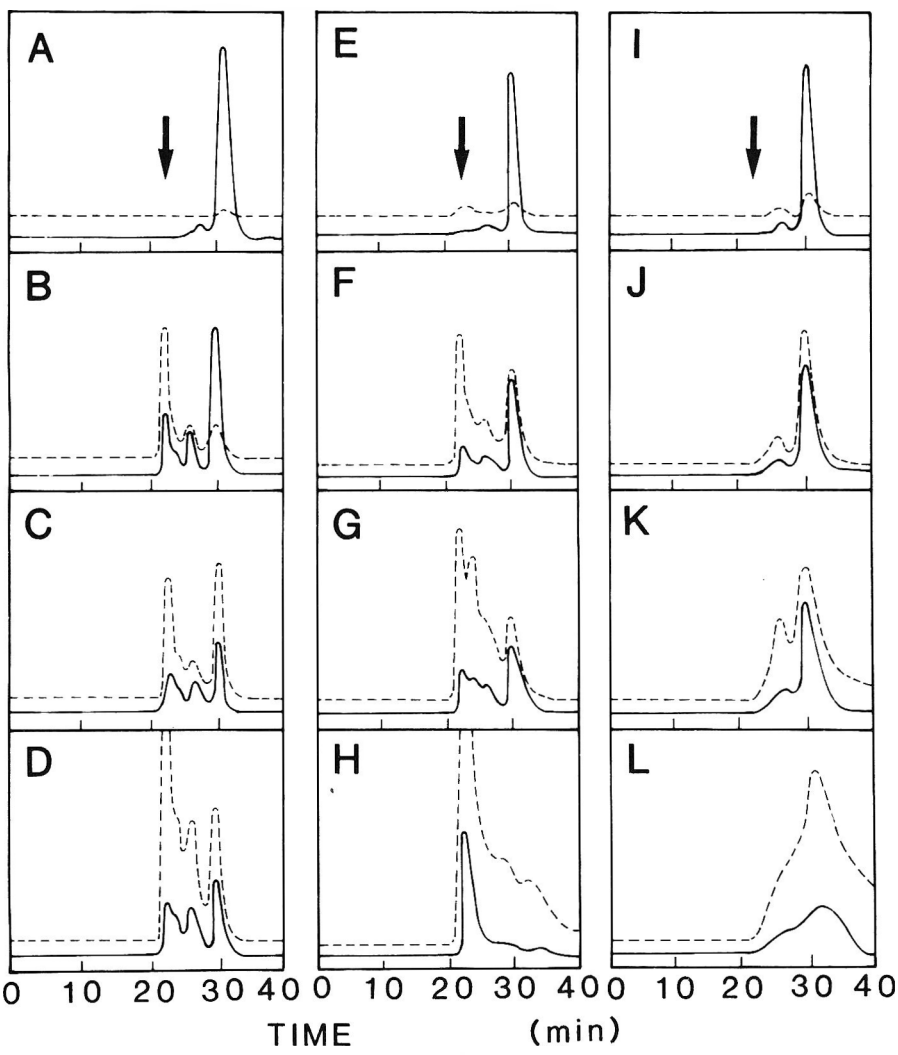

Fig. 1—HPLC on TSK G 3000 SW of α_{s-1} casein and succinyl α_{s-1} casein following reaction with NaOCl. (A) control α_{s-1} casein; (B-D) α_{s-1} casein was allowed to react with NaOCl (0.1% conc) at pH 6.0 (B), pH 7.0 (C) and pH 9.0 (D); (E-H) α_{s-1} casein was allowed to react with various concentrations of NaOCl (E, 0.005%; F, 0.05%; G, 0.1%; H, 0.5%) at pH 9.0; (I-L) succinyl α_{s-1} casein was allowed to react with various concentrations of NaOCl (I, 0.005%; J, 0.05%; K, 0.1%; L, 0.5%) at pH 9.0. Blue dextran was eluted at the position marked with an arrow. ———— absorbance at 210 nm. ---- fluorescence at an emission wavelength of 400 nm with an excitation wavelength of 350 nm.

Table 1—Amino acid composition of α_{s-1} casein following reaction with NaOCl[a]

	mmoles/16g N				
	NaOCl (%)				
	0	0.005	0.05	0.1	0.5
Asp	59.1	59.5	60.7	66.4	64.6
Thr	19.8	19.8	20.0	16.5	17.7
Ser	52.9	53.1	53.1	55.2	55.6
Glu	160.3	160.9	160.1	164.3	164.9
Pro	82.9	82.9	83.2	82.4	78.1
Gly	37.2	37.1	36.9	34.7	35.8
Ala	38.0	38.1	38.2	36.3	38.1
Val	45.3	45.4	45.5	43.9	45.2
Met	17.4	17.5	16.8	9.3	8.4
Ile	43.1	43.3	43.3	38.9	41.3
Leu	70.9	71.4	71.2	75.8	71.4
Tyr	42.6	42.3	38.9	37.5	15.4
Phe	33.4	33.9	33.4	34.0	33.3
Lys	62.9	62.9	60.9	56.7	33.1
His	20.8	21.1	20.4	18.7	18.3
Arg	26.5	26.8	25.8	26.2	19.9

[a] α_{s-1} Casein was allowed to react with NaOCl (conc 0–0.5%) at pH 9.0 and 37°C for 5 min. Quantitative determination was carried out after 6N HCl hydrolysis.

Table 2—Amino acid composition of succinyl α_{s-1} casein following reaction with NaOCl[a]

	mmoles/16g N				
	NaOCl (%)				
	0	0.005	0.05	0.1	0.5
Asp	62.4	61.6	62.4	62.3	57.6
Thr	21.3	20.6	19.0	15.2	17.4
Ser	53.0	51.5	51.5	51.0	50.5
Glu	168.6	175.4	166.8	158.4	157.8
Pro	85.7	83.9	85.1	83.1	82.6
Gly	38.2	37.1	36.7	33.1	33.2
Ala	42.1	40.7	37.4	35.0	40.1
Val	50.3	50.0	49.3	50.4	51.9
Met	17.7	16.4	14.6	8.2	6.0
Ile	46.5	44.3	44.7	39.1	45.1
Leu	74.3	72.4	73.0	74.0	73.2
Tyr	46.6	44.5	42.4	33.9	2.9
Phe	34.9	34.0	34.2	33.0	36.0
Lys	64.1	62.7	64.1	64.0	71.9
His	22.1	21.1	21.0	19.3	10.8
Arg	28.1	27.6	27.3	24.2	10.2

[a] Succinyl α_{s-1} casein was allowed to react with NaOCl (conc 0–0.5%) at pH 9.0 and 37°C for 5 min. Quantitative determination was carried out after 6N HCl hydrolysis.

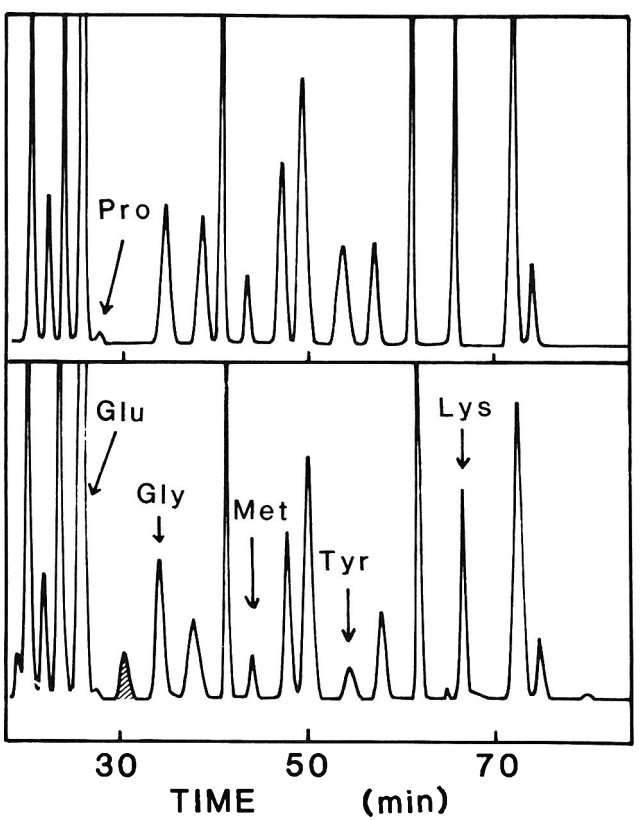

Fig. 2—Amino acid chromatogram of HCl hydrolysate of α_{s-1} casein following reaction with NaOCl.
Upper figure, control α_{s-1} casein; lower figure, reacted α_{s-1} casein. α_{s-1} Casein was allowed to react with NaOCl (0.5%) at pH 9.0 and 37°C for 5 min. α-Aminoadipic acid was eluted at the position indicated by shading.

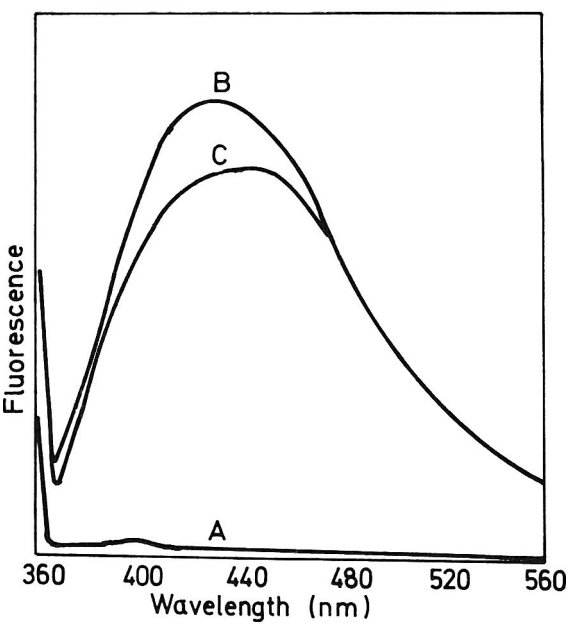

Fig. 3—Fluorescence emission spectra of α_{s-1} casein and succinyl α_{s-1} casein following reaction with NaOCl.
α_{s-1} Casein and succinyl α_{s-1} casein were allowed to react with NaOCl (0.5%) at pH 9.0 and 37°C for 5 min. (A) unreacted α_{s-1} casein or succinyl α_{s-1} casein; (B) reacted α_{s-1} casein; (C) reacted succinyl α_{s-1} casein. Concentration of each protein was 0.75 mg in 1 mL 0.1M borate (pH 10.0).

posure to NaOCl is shown in Table 2. The damage to amino acid residues was very similar to that of α_{s-1}casein (Table 1), except that no damage was found to the lysine residue of the reacted succinyl protein. No α-aminoadipic acid was detected in the hydrolysate of the succinyl protein. This suggests that the α-aminoadipic acid was derived from the lysine residue in α_{s-1}casein. The lysine content in the succinyl protein reacted in the presence of 0.5% NaOCl was higher than that found in other concentrations. This may be due to the overlapping of unidentified materials with the peak of lysine on the chromatogram.

As seen in Fig. 1, the reacted α_{s-1}casein and succinyl α_{s-1} casein showed a fluorescence. Fluorescence emission spectra of both proteins after the reaction with NaOCl were determined (Fig. 3). Both proteins exhibited fluorescence with an emission maximum of about 430 nm, when excited with light of 330 nm. Dityrosine seems to be present in the caseins after exposure to NaOCl, because the fluorescence spectra of the caseins were similar to those exhibited by proteins containing dityrosine residues (Verweij et al., 1982).

The contents of α-aminoadipic acid and dityrosine of α_{s-1} casein and succinyl α_{s-1}casein were measured after the reaction (Table 3). The content of dityrosine increased with a decrease in the content of tyrosine in both proteins. The content of α-aminoadipic acid, which was detected only in the α_{s-1}casein, increased as the reaction progressed.

Table 3—Amino acid composition of enzymatic hydrolysate of α_{s-1} casein following reaction with NaOCl[a]

	mmoles/1.6g N				
	NaOCl (%)				
	0	0.005	0.05	0.1	0.5
Asp	25.6	25.5	25.9	18.8	17.7
Thr + Gln	60.5	57.7	54.5	61.2	38.2
Ser + Asn	45.9	47.4	49.5	39.7	36.2
Glu	83.2	82.9	86.5	79.2	63.1
Pro	74.9	73.7	75.8	41.4	46.5
Gly	32.2	32.5	33.6	32.9	19.9
Ala	33.3	34.5	34.5	34.1	24.6
Val	38.3	38.4	39.4	44.1	33.6
Met	18.4	18.1	18.6	t	t
Ile	35.9	35.6	37.3	34.2	30.0
Leu	66.5	65.6	66.5	75.8	50.0
Tyr	39.4	39.5	41.0	35.7	5.0
Phe	32.8	31.8	31.8	36.8	28.5
Lys	58.7	56.8	57.8	54.2	20.7
His	19.6	19.2	19.3	21.1	10.2
Arg	23.9	23.0	23.7	24.7	12.5

[a] α_{s-1} Casein was allowed to react with NaOCl (conc 0–0.5%) at pH 9.0 and 37°C for 5 min.

Table 4—Change in tyrosine and lysine content of α_{s-1} casein and succinyl α_{s-1} casein following reaction with NaOCl[a]

NaOCl	mmoles/16g N			
(%)	di-Tyr*	Tyr	AAacid**	Lys
α_{s-1} casein				
0	0	42.6	0	62.9
0.005	0.2	42.3	0	62.9
0.05	1.1	38.9	t	60.9
0.1	3.1	37.5	1.3	56.7
0.5	3.7	15.4	19.9	33.1
Succinyl α_{s-1} casein				
0	0	46.6	0	64.1
0.005	0.2	44.5	0	62.7
0.05	0.7	42.4	0	64.1
0.1	1.3	33.9	0	64.0
0.5	1.7	2.9	0	71.9

* di-Tyr, dityrosine; **AAacid, α-aminoadipic acid.
[a] Reaction conditions with NaOCl were the same as those given in Tables 1 and 2.

Digestibility by proteolytic enzymes

In vitro digestibility of α_{s-1}casein after the reaction with NaOCl was examined using the following hydrolytic processes: pepsin-pancreatin digestion, followed by aminopeptidase-prolidase hydrolysis. The amino acid liberated from the reacted protein is shown in Table 4. The degree to which amino acid was liberated from the protein in the presence of a low concentration of NaOCl (<0.05%) was the same as that when NaOCl was absent, but the amino acid liberation was considerably decreased in the presence of NaOCl (>0.1%).

Reaction of amino acid derivatives with NaOCl

This experiment was carried out to examine qualitatively the reaction products of tyrosine and lysine residues after exposure to NaOCl.

After acetyl lysine methylester was allowed to react with NaOCl, the reaction mixture was analyzed by TLC. A spot, revealed that dityrosine was contained in the mixture. TLC analysis of the mixture showed that a fluorescent spot appeared at the position of the same Rf value as that of authentic dityrosine. However, unidentified products other than dityrosine were also observed by both analyses.

After acetyle lysine methylester was allowed to react with NaOCl, the reaction mixture was analyzed by TLC. A spot, which reacted with 2,4-dinitrophenylhydrazine-HCl solution to produce a yellow color, was detected at R_f value 0.73. The absorption spectrum of 2,4-dinitrophenylhydrazone of the reaction mixture was compared with that of the hydrazone of n-hexanal. Both spectra were very similar, having a maximum

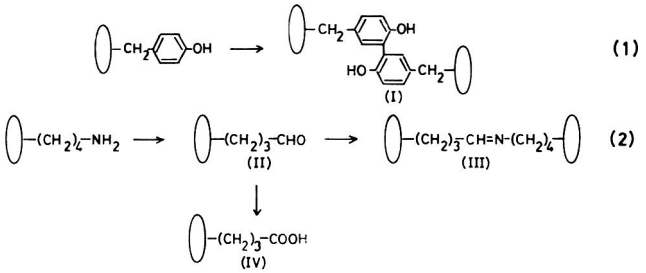

Fig. 4—Possible mechanism of cross-linkings of proteins during the reaction with NaOCl.

at around 450 nm (data not shown). This result suggests that the reaction of acetyl lysine methylester with NaOCl resulted in the formation of a carbonyl group. After HCl hydrolysis of the reacted acetyle lysine methylester, α-aminoadipic acid was detected by an amino acid analyzer.

DISCUSSION

Natarajan et al. (1975b), using SDS polyacrylamide gel electrophoresis, observed that peanut proteins were polymerized after reaction with NaOCl. We assumed that this polymerization was caused by the formation of covalent bonds other than disulfide bonds, and we determined changes in the molecular sizes of proteins with HPLC, since this method has the advantage of determining various sizes of proteins sensitively and quantitatively. From evidence described above, at least two types of cross-linking events lead to polymerization of proteins after the reaction with NaOCl, as illustrated in Fig. 4. One may be the Schiff base formation (III) between the ε-amino group of lysine residue and α-aminoadipic δ-semialdehyde (allysine) residue (II) which was derived from lysine residue oxidized by NaOCl [Fig. 4, (2)]. The production of α-aminoadipic acid residue (IV) in the reacted α_{s-1}casein and acetyl lysine methylester may be explained by the further oxidation of allysine residue by NaOCl. The other cross-linking may be the dityrosine (I) formation [Fig. 4, (1)]. Succinylation of α_{s-1}casein sufficiently suppressed the polymerization (Fig. 1, I-L), suggesting that the contribution of dityrosine formation to the polymerization (inter-molecular cross-linking) is less than that of the Schiff base formation. The cross-linking formation between allysine and lysine residues has been found in biological tissues (Fowler et al., 1970; Siegel and Mortin, 1970; Stahmann, 1977; Stahmann and Spencer, 1977a). The presence of dityrosine residue in proteins has also been demonstrated (Aeschback et al., 1976; Zaitsu et al., 1981; Verweij et al., 1982; Cooper and Varner, 1983).

Metheis and Whitaker (1984) stated that the enzymatic modification of food proteins by polyphenol oxidase and peroxidase is useful for changing their functional properties. Therefore, chemical modification by NaOCl may have the potential of improving the functional properties of food proteins, since NaOCl can form the same types of cross-linkings as those caused by polyphenol oxidase and peroxidase as described above under mild conditions (NaOCl conc, <0.05%, within 5 min, 37°C, pH 7–9).

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Metabolites of *Eurotium* Species, Their Antioxidative Properties and Synergism with Tocopherol

YUKIHIRO ISHIKAWA, KYOZO MORIMOTO, and TAKASHI HAMASAKI

ABSTRACT

Natural antioxidants and synergists for tocopherol (Toc) have been isolated as acetone or ethyl acetate extracts from mycelial mats or culture broth of *Eurotium* species. The acetone extract, eluted stepwise with benzene and ethyl acetate by silica gel column chromatography, gave fractions that were synergistic with Toc. However, the benzene fraction appeared better because it was highly soluble in oil and did not change the color and taste of the oil. The metabolites in the benzene fraction differed in the various *Eurotium* species in their composition in respect to known metabolites with antioxidative activity.

INTRODUCTION

FLAVOGLAUCIN, a metabolite of *Eurotium chevalieri*, was found to be as active as tocopherol (Toc) in inhibiting the autoxidation of lard and to act as an excellent synergist for Toc (Ishikawa et al., 1984). Little work has been done on antioxidants and synergists of microbial origin, although microorganisms may offer great possibilities for the isolation of potent antioxidants and synergists. Meisinger et al. (1959) isolated 2-(6-hydroxy-2-methoxy-3,4-methylenedioxyphenyl)benzofuran as an antioxidant from years. Zaika and Smith (1975) demonstrated that extracts of *Aspergillus niger* had antioxidative activities and synergistic effects in lard. Recently, Nakakita et al. (1984) isolated antioxidative phenolic compounds like methylenebis(*tert*-butyl-cresol) from the culture filtrate of *Penicillium janthinellum*. During screening of *Penicillium* species, *P. herquei* IFO 7904 produced a potent antioxidant which was synergistic with Toc (Ishikawa et al., 1983). Brooks and Morrison (1972) isolated herqueinone and its derivatives from the mycelium of *P.herquei*. An antioxidant extracted from the culture broth of *P.herquei* IFO 7904 was assumed to be a derivative of herqueinone, but its structure has never been determined.

Safe natural antioxidants and syngergists are required as food additives because carcinogenic activity of some synthetic antioxidants has been questioned. Flavoglaucin is not mutagenic to *Salmonella typhimurium* TA98 and TA100 (Hamasaki et al., 1981) according to the modified Ames method (Nagao et al., 1977). Flavoglaucin has been known as one of the common metabolites of the *A. glaucus* group (genus of *Eurotium*) (Turner and Aldridge, 1983), some of which have been used in the manufacture of Katsuobushi (dried bonito), a traditional marine product in Japan. *P.charlesii* is also known to produce flavoglaucin and related substances (Yoshihira et al., 1972).

In the previous paper (Ishikawa et al., 1985), *Eurotium* species was screened for the isolation of synergists for Toc. *Eurotium* species has been known to produce several derivatives of flavoglaucin (Inoue et al., 1977; Hamasaki et al., 1980, 1981). The objectives of this study were to isolate metabolites from the *Eurotium* species and investigate their antioxidative activities and synergism with Toc.

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MATERIALS & METHODS

Culture of molds

The species of *Eurotium* used in this study were purchased from the Institute of Fermentation, Osaka, Japan. Each mold was grown in surface culture (6L) at 24°C on a medium containing per liter of tap water: malt extract (Nakarai Chem., Kyoto, Japan), 20g; glucose, 30g; polypepton (Daigoeiyo Chem., Osaka, Japan), 3g; and sodium chloride, 50g. Mycelial mats harvested after 2 wk were dried in vacuo and repeatedly extracted with acetone. The combined extracts were concentrated in vacuo to give the acetone extract.

Isolation of metabolites

The acetone extract (ca. 6.5g) was suspended in benzene and transferred to a silica gel (five times the amount of each acetone extract; Merck Kieselgel 60, 70-230 mesh) column. The column was then eluted stepwise with 500 mL each of benzene, ethyl acetate and ethanol to give the respective solvent fractions. One hundred milligrams of the benzene fraction, dissolved in a small amount of chloroform, was repeatedly separated by Sephadex LH-20 gel chromatography (column size, 4 cm × 50 cm) using chloroform as solvent. The effluent was monitored with a single beam spectrophotometer (Model 100-10 of Hitachi Co., Tokyo, Japan) at 300 nm. The composition of metabolites in fractions collected by a fraction collector was checked by TLC and HPLC.

Chromatography

TLC plates of pre-coated 0.2 mm silica gel 60 F_{254} (aluminum sheets) were purchased from E. Merck. All plates were developed with hexane/chloroform (6/8 vol). Identification of spots was by the color of each metabolite.

HPLC analysis was performed with a micro pump (Model KHD-94 of Kyowa Seimitsu Co., Tokyo, Japan) and a double beam spectrophotometer (Model 100-50 of Hitachi Co., Tokyo, Japan) set at 280 nm. Mobile phase was hexane with 5% ethyl acetate at a flow rate of 1 mL/min. The column used (4 mm × 25cm) was packed with Merck LiChrosorb SI 60, 5μm.

Simulated active oxygen method (AOM)

A chloroform solution containing 2.8 mg each metabolite was placed into a test tube (18 mm × 16 cm) and the solvent was removed by blowing nitrogen gas. Then, 7g lard with or without 0.04% d-Toc concentrate (Eisai Co., Tokyo, Japan, 80% purity) was added and kept at 100°C. Air filtered through silica gel, was passed through the oil at a flow rate of 30 mL/min. At varied time intervals, ca. 150 mg samples were withdrawn and measured for peroxide value. The induction period was expressed as time (hr) required for peroxide value to reach 100 meq/kg.

NMR and IR spectra

Samples dissolved in deuterated chloroform, deuterated acetone or carbon tetrachloride were analyzed with a Hitachi R-24 NMR spectrometer (Hitachi Co., Tokyo, Japan) at 60 MHz by using tetramethylsilane as an internal standard. IR spectrum was recorded with a Hitachi Model 260-30 infrared spectrophotometer on samples prepared as potassium bromide discs.

RESULTS

Fractionation of the acetone extract by column chromatography

In a simulated AOM test, lard became turbid on addition of the acetone extract. When used as food additives, preferably

the extract should be concentrated by chromatography to yield substances which dissolve freely in oil. Therefore, the acetone extract from one of the *E. repens* species (IFO 4041) was subjected to silica gel column chromatography. Fractions eluted stepwise with benzene, ethyl acetate and ethanol gave a yellow powder, a brown sticky liquid and a dark brown sticky liquid, respectively. On TLC with hexane/chloroform (6/8 vol), the benzene fraction showed the presence of four major spots, but the ethyl acetate and ethanol fractions remained at origin.

Table 1 shows the antioxidant activity of the three fractions and their combined mixture and their synergism with Toc. The amounts were added in proportion to the original amounts of the acetone extract. The benzene and ethyl acetate fractions showed antioxidation and synergism with Toc; the activities of the acetone extract were almost restored by the combination of both benzene and ethyl acetate fractions, which dissolved more quickly in lard. The ethanol fraction showed no activity and was not appreciably soluble in lard. The yields of the three fractions were compared with those of *E. chevalieri* IFO 4086 and *E. echinulatum* IFO 5862; Table 2 indicates that the composition of these fractions varies greatly with *Eurotium* species.

Metabolites in benzene fraction and their structures

HPLC of the benzene fraction showed the presence of several kinds of metabolites. The metabolites from *E. chevalieri* IFO 4086 and *E. echinulatum* IFO 5862 were compared with those from *E. repens* IFO 4041 (Fig. 1).

Fractions corresponding to peaks 1 to 4 for *E. repens* IFO 4041 (Fig. 1) were separated and rechromatographed on Sephadex LH-20. A compound, recrystallized from fraction 1 as orange needles from methanol, had IR and NMR spectra ($CDCl_3$) identical with those of physcion (Yoshihira et al., 1972). Compounds, recrystallized from fractions 2 and 3 as yellow needles from hexane, gave NMR spectra (CCl_4) in agreement with those of tetrahydroauroglaucin (Yoshihira et al., 1972) and dihydroauroglaucin (Hamasaki et al., 1981), respectively. The compound in fraction 4 was auroglaucin (Hamasaki et al., 1981) according to the NMR spectrum (acetone-d_6) and HPLC. Fractions 5 and 6, which were poorly resolved and therefore, contaminated, were separated into two compounds on preparative TLC (Merck Kieselgel 60 F_{254}, 1 mm thickness) with hexane/chloroform (6/8 vol) and recrystallized from hexane as yellow needles. The NMR spectra of the compounds from fractions 5 (CCl_4) and 6 ($CDCl_3$) were identical with those of flavoglaucin (Yoshihira et al., 1972) and isodihydroauroglaucin (Hamasaki et al., 1980), respectively. The structures of the metabolites are shown in Fig. 2.

Synergism of metabolites with tocopherol

Lard with 0.04% Toc and 0.04% each metabolite was autoxidized via the simulated AOM (Table 3). Flavoglaucin was found to be an excellent antioxidant and synergist, followed by auroglaucin. Dihydroauroglaucin exhibited very little activity and the other compounds had no effect.

Table 1—Antioxidative activity of fractions derived from the acetone extract of Eurotium repens *IFO 4041 and their synergism with tocopherol*

Fractions and their combination	Induction period (hr)	
	No Toc	With Toc
Benzene fraction (B)	26	69
Ethyl acetate fraction (A)	29	58
Ethanol fraction (E)	7	43
B + A	73	114
B + E	35	80
A + E	34	59
B + A + E	77	133
Acetone extract	85	121
Control	6	36

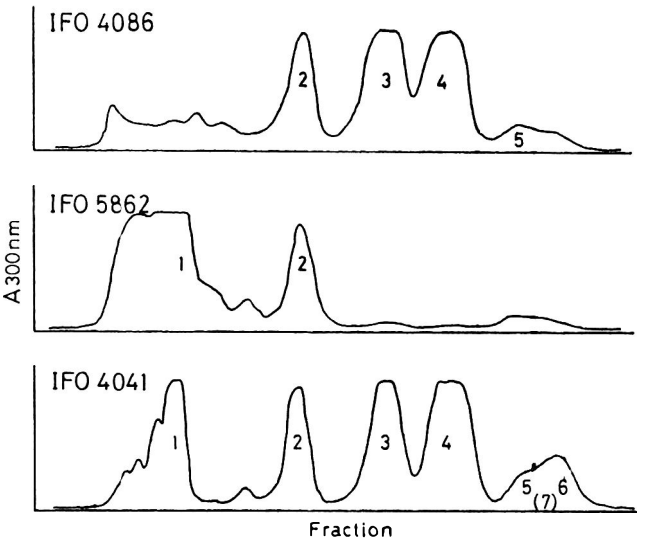

Fig. 1—Sephadex LH-20 gel chromatography of the benzene fractions derived from Eurotium *species:* E. chevalieri *IFO 4086;* E. echinulatum *IFO 5862;* E. repens *IFO 4041. The metabolites isolated and purified from fractions 1 to 6 were separated on HPLC. Their respective retention times were 18.2, 9.0, 10.6, 14.0, 8.9, and 13.5 min.*

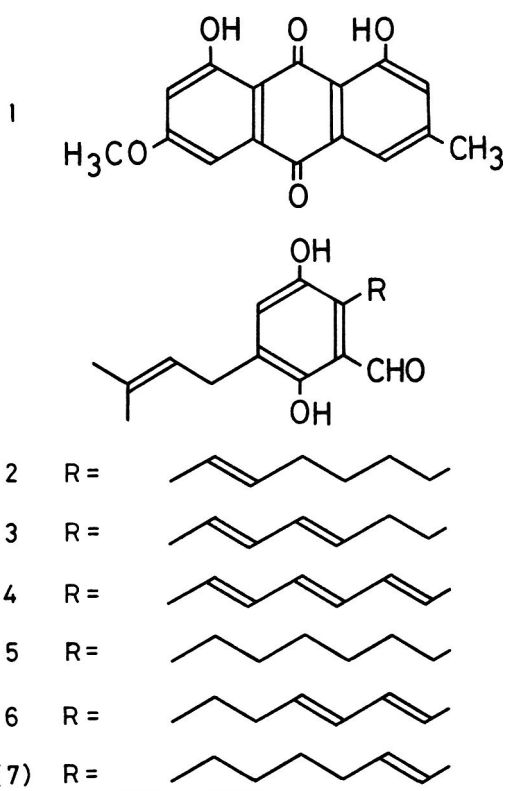

Fig. 2—Structures of metabolites produced by Eurotium *species. Figures correspond to peak numbers 1 to 7 as shown in Fig. 1.*

Table 2—Fractionation of the acetone extracts from Eurotium *species on silica gel column chromatography*

Fraction	*E. chevalieri* IFO 4086	*E. repens* IFO 4041	*E. echinulatum* IFO 5862
Benzene fraction	45.4	42.8	7.7
Ethyl acetate fraction	32.7	20.3	53.7
Ethanol fraction	13.2	28.5	31.3

Figures show weight proportion (%) to the initial weight of the acetone extract.

DISCUSSION

TOCOPHEROL is one of the most useful natural antioxidants. Therefore, it is reasonable to search for substances that would act synergistically with Toc in inhibiting the oxidation of edible oils and fats. Extracts which act synergistically with Toc have been isolated from many Eurotium species (Ishikawa et al., 1985). With silica gel column chromatography of acetone extracts of three *Eurotium* species, fractions eluted stepwise with benzene, ethyl acetate and ethanol differed from one another in their composition (Table 2). These results suggested that the composition of other species may likewise be different. The yellow benzene fraction was desirable as a synergist because it was highly soluble in lard and did not change the color and taste of the fat. The data in Fig. 1 show that the composition of the metabolites in the benzene fraction also differed within the *Eurotium* species. One mold would not necessarily produce all possible metabolites. The possibility of the presence of a small amount of isotetrahydroauroglaucin (Hamasaki et al., 1980) (Peak 7, Fig. 1) was indicated by retention times with HPLC and gel chromatography.

The antioxidative activity and synergism with Toc of flavoglaucin and its derivatives are believed to be based on the hydroxy group, which does not form hydrogen bonds with a formyl group in a molecule. As Table 3 shows, however, antioxidation and synergism seem to be largely related to the number and position of double bond in the 7 carbon side chain. In this case, the difference in the effectiveness of each flavoglaucin derivative might be due to the following reasons: (1) the number and the position of the double bond cause instability of a derivative at high temperature; (2) donation of hydrogen atom from the hydroxy group is directly affected by the structure of the side chain. Auroglaucin seemed to be more stabilized by a conjugated double bond than the other metabolites except for flavoglaucin. The NMR data of physcion show that it has no antioxidative effect since the two hydroxy groups in the molecule from hydrogen bonding with an aromatic carbonyl group.

The mutagenicities of metabolites 2 to 6 (Table 3) were tested on *Salmonella typhimurium* TA98 and TA100 (Hamasaki et al., 1981). These metabolites were nonmutagenic with or without S-9 mix except that auroglaucin was weakly mutagenic to TA100 without S-9 mix at rather high concentration. As flavoglaucin is most effective (Table 3), *Eurotium* species producing a large amount of flavoglaucin and no auroglaucin will be desirable to use for their metabolites as antioxidants and synergists with Toc.

Table 3—Antioxidative activity of metabolites from Eurotium *species and their synergism with tocopherol*

Metabolite		Induction period (hr)	
		No Toc	With Toc
Physcion	(1)[a]	6	35
Tetrahydroauroglaucin	(2)	8	36
Dihydroauroglaucin	(3)	12	48
Auroglaucin	(4)	20	52
Flavoglaucin	(5)	35	70
Isodihydroauroglaucin	(6)	9	38
Acetone extract		85	118
Control		6	35

[a] Figures in parentheses as shown in Fig. 2.

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A Research Note

Enzymatic Proteolysis of Milk Proteins, in a Salt Environment, with a *Bacillus subtilis* Neutral Protease Preparation

E. EL MAYDA, D. PAQUET, and J.P. RAMET

ABSTRACT

An industrial preparation of *Bacillus subtilis* neutral protease offers interesting halophylic properties. The proteolysis of the different caseins in 10% sodium chloride was inhibited by no more than 20% while whey proteins proteolysis was inhibited by 50%. This behavior of the protease allows considering its further utilization for food protein hydrolysates in salt media.

INTRODUCTION

THE INHIBITORY EFFECT of sodium chloride (NaCl) at high concentration on casein hydrolysis of proteolytic enzymes, especially chymosin, during cheese ripening, has been known for many years. β-Casein hydrolysis by chymosin is heavily inhibited by a concentration of 10% NaCl at pH 5.2 and 6. αs-Casein hydrolysis is optimal at a NaCl concentration equal to 5%; it is still high with a NaCl content equal to 20% (Fox and Walley, 1971). These observations are supported by other studies on Cheddar cheese ripening (Pearce, 1982, Phelan et al., 1973). The effect of NaCl concentration on the nature of hydrolysates has been discussed (de Jong and de Groot-Mostert, 1977; Mulvihill and Fox, 1978; 1979). Phenomena resulting from this proteolysis (flocculation and nitrogen solubility changes) develop in two stages: an activation with a small amount of salt, and then, beyond a certain point which seems to be specific for each tested protease, a gradual inhibition (Hamdy and Edelsten, 1970, Ramet, 1984).

The NaCl inhibitory effect on β-casein proteolysis probably results from the induction of a substrate transconformation. This might occur together with a modification of the enzyme molecule, due to a salting out effect at high ionic strength (Fox and Walley, 1971). The structure and the properties of the reactants are completely altered, especially because of the modifications of the hydration and the ionisation states of the environment (Gal and Bankay, 1971; Guerts et al., 1974; Gal, 1975; Hardy and Steinberg, 1984).

From a technological point of view, these interactions induce negative effects on the color of the milk when previously salted (Ramet et al., 1983), and also on the rheological behavior of the gels that appear during coagulation and cheese syneresis (Hamdy and Edelsten, 1970; Ramet et al., 1983; Ramet, 1984).

The subject of our study more particularly concerns the effect of sodium chloride concentration on the behavior of a *Bacillus subtilis* neutral protease preparation, during hydrolysis of milk proteins.

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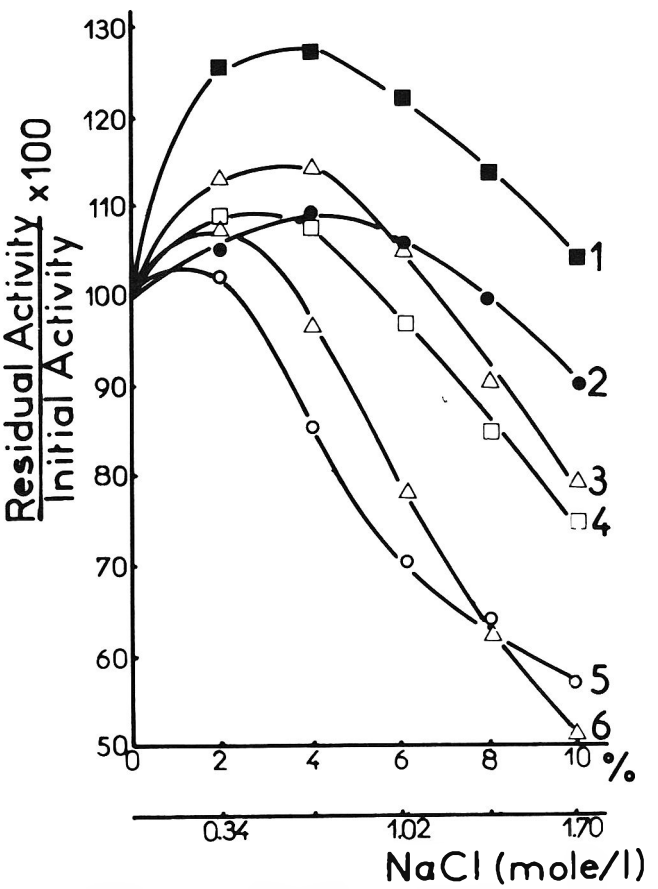

Fig. 1—Influence of sodium chloride concentration on the proteolytic activity of Bacillus subtilis neutral protease on caseins and whey proteins at 35°C. (E/S = 0.37% and pH = 6.5 for caseins; E/S = 1.8% and pH = 8.5 for whey proteins). (1) β-Casein, (2) κ-Casein, (3) whole casein, (4) αs-casein, (5) whole whey proteins, (6) β-Lactoglobulin.

MATERIALS & METHODS

Enzyme and substrates

The enzyme is a commercial preparation of *Bacillus subtilis* neutral protease, supplied by Gist Brocades (Delft, Netherlands). Whole casein was extracted from raw skim-milk by isoelectric precipitation at pH 4.6; it was then fractionated by ion-exchange column chromatography, according to the method of Mercier et al. (1968).

The whey proteins were obtained from the supernatant of the isoelectric precipitation of caseins after dialysis and lyophilization.

β-Lactoglobulin was obtained after treatment of the same supernatant with trichloroacetic acid (TCA) according to the method of Fox et al. (1967).

Enzymatic hydrolysis

The substrates were dissolved at a concentration of 2% in 0.1M sodium phosphate buffer, pH 6.5 for caseins and pH 8.5 for whey

proteins. Proteolysis was performed at 35°C for 15 min with an enzyme/substrate (E/S) ratio equal to 0.37% for caseins and 1.8% for whey proteins. Hydrolysis was stopped by addition of 24% TCA (V:V) and nonprotein nitrogen (NPN) was then determined by the micro-Kjeldahl method. Results were the mean of two replicated studies.

RESULTS

THE EFFECT of sodium chloride concentration on the hydrolysis of the various milk proteins is shown in Fig. 1. Hydrolysis of caseins by *Bacillus subtilis* neutral protease was optimal at 3–4% sodium chloride. The highest activation occurred with β-casein hydrolysis. At pH 8.5 hydrolysis of whey proteins was less activated and the optimum was observed with 2% NaCl. With 10% NaCl, β-casein hydrolysis occurred at a level similar to that in the unsalted medium. Under the same conditions the proteolysis of κ-casein as well as whole casein and αS-casein (a mix of αS1 and αS2) decreased, respectively, by 10, 20 and 25%. Inhibition was stronger and reached about 45% for whey proteins. We also noticed that a residual activity can be measured over the range of 10% NaCl (not shown in Fig. 1); indeed, at 20% NaCl the whole casein proteolysis decreased to 50% and that of whey proteins to 30%.

CONCLUSION

THESE FIRST RESULTS show an original halophylic behavior of the enzymatic preparation towards caseins and whey proteins.

Development of applications of the halophilic property of the enzymatic preparation to food technology other than in the dairy field are of interest and under development in our laboratories.

From a fundamental standpoint, further investigations are being carried out on the enzyme specificity for each particular protein fraction.

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A Research Note

Screening of *Eurotium* Species for Synergists of Tocopherol

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ABSTRACT

Screening of *Aspergillus glaucus* group (genus of *Eurotium*) for the isolation of synergists for tocopherol (Toc) was carried out by growing molds in malt medium with sodium chloride. The respective acetone and ethyl acetate extracts from mycelial mats and culture broth of *Eurotium* species were used to estimate their synergism with Toc in lard. Especially, the extracts of *E. chevalieri* IFO 5233, IFO 6272 and *E. repens* IFO 4885 contributed to inhibiting the autoxidation of lard.

INTRODUCTION

FLAVOGLAUCIN was found to be as active as tocopherol (Toc) in inhibiting the autoxidation of lard and to act as an excellent synergist with Toc (Ishikawa et al., 1984). Flavoglaucin has been known as one of the common metabolites of the *Aspergillus glaucus* group (genus of *Eurotium*) (Turner and Aldridge, 1983). The objectives of this study were to screen and evaluate *Eurotium* species as sources of antioxidants and synergistic agents.

MATERIALS & METHODS

Culture of molds

The species of *Eurotium* used in this study were purchased from the Institute of Fermentation (Osaka, Japan). On screening for ability to produce synergists for Toc, each mold was grown in surface culture at 24°C on a 250 mL medium with 2% malt extract (Nakarai Chem., Kyoto, Japan) and 5% sodium chloride. Quadruplicate mycelial mats and culture broth of each mold were harvested after 2 wk. The mycelial mats, dried below 40°C in vacuo, were repeatedly extracted with acetone, and the combined extracts were concentrated in vacuo to give the acetone extract. The culture broth was repeatedly extracted with ethyl acetate to obtain the ethyl acetate extract.

Simulated active oxygen method (AOM)

Seven grams of lard with 0.04% of Toc (Eisai Co., Tokyo, Japan, 80% purity) was used as the test solution. Twenty milligrams of each extract were added to the test solution. Air, filtered through silica gel, was passed at a flow rate of 30 mL/min through the lard/Toc/extract mixture which was held at 100°C. The induction period was the time in hours required for the peroxide value to reach 100 meq/kg.

RESULTS & DISCUSSION

SYNERGISM of the respective acetone and ethyl acetate extracts from mycelial mats and culture broth of *Eurotium* species with Toc was investigated. The data in Table 1 show that the extracts from many *Eurotium* species reacted synergistically with Toc. The degree of growth differed in *Eurotium* species, and there were great changes in the weight of the extracts. However, the weight of the extracts did not necessarily agree with the degree of synergism. *E. chevalieri* IFO 5233, IFO 6272 and *E. repens* IFO 4885 grew well and their acetone and ethyl acetate extracts contributed to inhibiting autoxidation of lard. These species could be useful as sources of antioxidants and synergists with Toc.

Table 1—Amounts of the extracts from culture broth and mycelial mats of Eurotium *species and their synergistic activity with tocopherol in lard*

Strains (IFO no.)	Broth extract		Mycelial mat extract	
	Wt (mg)	AOM(hr)[a]	Wt (mg)	AOM(hr)[a]
		E. chevalieri Mangin var.		
4086	39	107	316	85
4090	37	141	479	73
4092	37	101	351	74
4298	31	134	326	84
4334	27	101	54	36
4364	136	112	390	140
5233	204	128	806	140
5718	118	103	422	140
5883	70	104	422	125
5322	160	100	263	150
6270	73	108	485	140
6271	122	98	81	127
6272	43	143	806	117
		E. repens de Bary		
4041	303	189	>358	138
4087	252	200	373	148
4332	110	305	328	70
4333	47	100	132	42
4356	154	144	370	110
4884	77	123	114	94
4885	289	225	695	144
5843	35	140	63	45
6005	93	163	431	68
7463	62	142	229	143
7682	105	207	224	140
8914	162	53	98	68
		E. echinulatum Delacroix		
5862	70	158	419	185
		E. amstelodami Mangin		
6667	41	87	107	80
		E. tonophilum		
8157	—	61	—	—
6529	24	106	42	36
Control		36		36

[a] Induction periods determined by a simulated active oxygen method.

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A Research Note

Effect of Salt, Tripolyphosphate and Tertiary Butylhydroquinone on Myoglobin-Lipid Oxidation Indicators in Freeze-Dried Meats

Z. NAKHOST and M. KAREL

ABSTRACT

The previously developed myoglobin oxidation indicators were used to detect the effect of curing agents, sodium chloride and sodium tripolyphosphate (TPP), and also the antioxidant effect of mono tertiary butylhydroquinone (TBHQ) on the oxidation of restructured freeze-dried beef upon storage. Fresh, choice-grade deboned chuck with 15% fat was flaked and treated with salt, TPP, and TBHQ, freeze-dried and stored in the presence of air at 37°C. Upon storage, beef samples were reconstituted and a meat extract was obtained. Myoglobin insolubilization (measured spectrophotometrically at the isobestic point at 525 nm) and oxidation (measurement of α peak intensity of metmyoglobin at 630 nm) in stored samples were determined. Antioxidant-containing samples showed the least myoglobin insolubilization and myoglobin oxidation as compared to control beef (with no additives) and beef treated with salt and TPP.

INTRODUCTION

MEASUREMENT of lipid oxidation, using the classical methods such as thiobarbituric acid (TBA) value and peroxide value do not give adequate results in muscle foods (Melton, 1983; Williams et al., 1983) particularly in freeze-dried meats (Chipault and Hawkins, 1971). In our earlier studies on "model systems" containing metmyoglobin and methyl linoleate a new methodology for detection of lipid oxidation in freeze-dried meats, based on "oxidation-induced changes in myoglobin," was developed (Nakhost and Karel, 1983). Myoglobin oxidation indicators, namely, progressive myoglobin insolubilization (isobestic point, 525 nm); gradual oxidation of oxymyoglobin to metmyoglobin (absorbance at 630 nm); and myoglobin polymerization were found to detect and measure the extent of lipid oxidation in stored freeze-dried beef (Nakhost and Karel, 1984).

Salt and phosphates are commonly added to restructured meat products. These additives contribute to texture and quality improvement of such product via increasing water binding (reducing cooking loss) and consequently enhancing the binding strength between adjacent meat pieces in the restructured product (Siegel et al., 1978, Schwartz and Mandigo, 1976, Krause et al., 1978).

The purpose of this study was to use myoglobin oxidation indicators to measure the effect of curing agents such as sodium chloride and sodium tripolyphosphate (TPP), and antioxidant effect of mono tertiary butylhydroquinone (TBHQ) on the oxidation of freeze-dried restructured beef.

MATERIALS & METHODS

Sample preparation

Flaked and formed beef products were prepared at 4°C as follows: Fresh, choice-grade chuck (deboned) with 15% fat content was flaked and treated with TBHQ (100 ppm, based on final dry weight) and/or sodium chloride (0.5%), sodium tripolyphosphate (0.2%). Each batch was mixed under vacuum (20 inches) for 12 min. The product was then stuffed into polyethylene casings, pressed (at 500 psi) and sliced into 160-g samples. Samples were freeze-dried and packaged in air in flexible pouches and stored at 37°C for 1, 2, 3, and 4 weeks. Control samples (with no additives) were prepared and stored under similar conditions.

"Meat extract" preparation

Upon storage, the freeze-dried meat samples were reconstituted to the original water content of 70%. For myoglobin extraction 1 mL deionized water per gram of reconstitued meat was added and vortexed for 1 min. The samples were stored for 30 min at 4°C, revortexed for 30 sec and centrifuged at 10,000 rpm for 30 min (2°C). The supernatant was filtered (using S&S filter paper #595) and recentrifuged at 10,000 rpm for 10 min (2°C). The clear supernatant (meat extract) was recovered from centrifuge tube and used for our studies.

Myoglobin insolubilization measurement

The extent of myoglobin aggregation and insolubilization in the stored freeze-dried meat was determined by measuring the decrease in the absorbance of "meat extracts" at the isobestic point (525 nm) of natural myoglobin derivatives.

Myoglobin oxidation measurement

Oxidation of oxymyoglobin to metmyoglobin in "meat extracts" was quantified by measuring the α peak intensity of metmyoglobin (absorbance at 630 nm). To obtain this value, % myoglobin insolubilization (loss from the system) of each sample was taken into account.

RESULTS & DISCUSSION

Myoglobin insolubilization

Myoglobin insolubilization in control samples (beef with no additives) increased significantly (30%) after the 1st week of storage and reached 63% upon 4 weeks of storage (Fig. 1). In beef samples containing salt and TPP, myoglobin insolubilization occurred at a slower rate (15% after the 1st week and 33% after 4 weeks of storage). One possible reason for the lower myoglobin insolubilization in samples treated with salt and TPP could be the "salting in" effect of salt and TPP on proteins. Increased meat protein extractability using different concentrations of salt and phosphate has been reported by Fukazawa et al. (1961), Gillett et al. (1977), Siegel et al. (1978), and Theno et al. (1978). Prusa and Bowers (1984) reported that treatment of turkey muscles with salt and TPP resulted in increased concentrations of extracted proteins along with increased number of electrophoretically separated higher molecular weight proteins and increased HPLC peak areas of extracted proteins.

Another possible reason for the lower myoglobin insolubilization is the binding of phosphate anions to proteins which could reduce myoglobin polymerization and aggregation due to free radicals produced during cooxidation of fatty acids and proteins.

Antioxidant-containing samples showed the least myoglobin

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insolubilization (10% after the 1st week and 20% after 4 weeks of storage (Fig. 1).

Myoglobin oxidation

Myoglobin oxidation (conversion of oxymyoglobin to metmyoglobin) can occur as a result of lipid oxidation (Koizumi and Nonaka, 1973; Lin and Hultin, 1977). Oxidation of myoglobin in control samples progressed rapidly after the 1st week (68%) and reached 76% after 4 weeks of storage (Fig. 2). Samples treated with salt and TPP showed 37 and 47% myoglobin oxidation after 1 and 4 weeks of storage, respectively. Antioxidant-containing samples showed the least myoglobin oxidation (11% after the 1st week and 24% after 4 weeks of storage).

In summary, treatment with salt, TPP and TBHQ decreased the rate and extent of myoglobin insolubilization (20% in antioxidant-containing samples versus 63% in control samples) and oxidation (24% in antioxidant-containing samples versus 76% in control samples) in freeze-dried beef samples upon 4 weeks of storage at 37°C.

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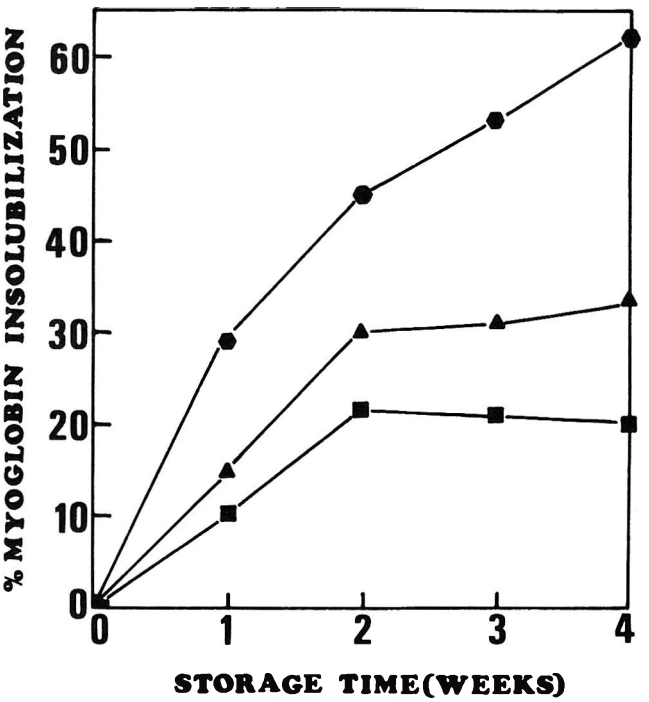

Fig. 1—Comparison of myoglobin insolubilization in "meat extract" of freeze-dried ● control beef (no salt or TPP) vs; ▲ beef + salt + TPP and; ■ beef + salt + TPP + TBHQ, upon storage at 37°C.

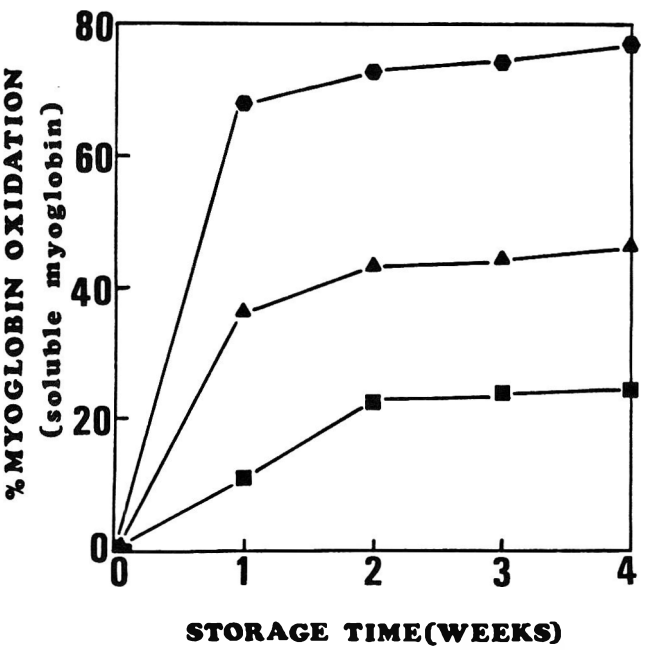

Fig. 2—Comparison of myoglobin oxidation in "meat extract" of freeze-dried: ● control beef (no salt or TPP) vs; ▲ beef + salt + TPP and; ■ beef + salt + TPP + HBHQ, upon storage at 37°C.

A Research Note

Evaluation of Time-Intensity Sensory Responses using a Personal Computer

WILLIAM E. LEE III

ABSTRACT

A method is presented which utilizes a personal computer to measure time-intensity (T-I) sensory responses. The judge uses a game paddle which moves an "X" along a fixed scale appearing on the monitor screen to indicate the attribute intensity at each instant in time. A clicker device on the game paddle can be used to record the occurrence of events such as initial mouth entry and time of swallowing. Data acquisition is continuous with the data stored on discs. This technique has advantages over strip chart recorder methods. Disc storage allows rapid and efficient data analysis. Judges can perform the evaluations virtually unsupervised with only minimal training.

INTRODUCTION

A NUMBER of sensory methods have been developed to measure time-intensity (T-I) responses including variations of the moving chart recorder (Larson-Powers and Pangborn, 1981; Pangborn and Koyasako, 1981) and the "SMURF" (Sensory Measuring Unit for Recording Flux) method (Birch and Munton, 1981). Disadvantages of these methods include the possibility that the judge may see the evolving response curve being traced and the time and effort required to manually extract and tabulate data from the curves for application of statistical analyses. Recent work aimed at reducing these limitations include the utilization of a device to digitize the chart paper curves (Schmitt et al., 1984) and the first real attempt to completely computerize the methodology (Guinard et al., 1985).

The objective of the present work was to develop a method of acquiring T-I responses via a personal computer which would eliminate the disadvantages associated with the non-computer-based techniques.

MATERIALS & METHODS

Hardware and software description

The method was developed for use with a standard Apple computer (monitor with disc storage, 48K memory) although any personal computer would be employed. A clock card capable of reading time to at least 0.1 sec accuracy is required as well as a game paddle, which consists of a dial that turns radially and a "clicker" positioned on the side of the device.

A general flow chart of the program is presented in Fig. 1. Applesoft basic was used in the present work. A fixed line intensity scale labelled "weak" and "strong" at the end points appears on the monitor screen. The judge uses the game paddle to move an "X" along the scale to indicate the perceived attribute intensity. The computer converts the current game paddle position from a 0 to 255 value to a 0 to 10 value with one decimal place. Data acquisition can be as rapid as 10 points per second.

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Sensory procedure

The program illustrated in Fig. 1 was designed to allow the judges to do the entire sensory evaluation with only minimal supervision. First, it requests simple information such as judge identification, sample code, and what attribute is being evaluated (which can be selected from a "menu"). The computer is in the "ready" mode once the information has been collected and the intensity scale has been drawn on the monitor screen.

The method of sample preparation and introduction is decided by the investigator. In this work, "time zero" corresponded to the time when the sample was initially placed in the mouth. Depressing the game paddle clicker at time zero initiates the data collection. The judge proceeds to indicate the instantaneous attribute intensity by turning the dial on the game paddle which moves the "X" on the intensity scale displayed on the monitor. Subsequent clicks of the clicker can be stored as "special event" times, which could include the time of swallowing, times of subsequent mouth entries, or other events of interest. In the present work, the evaluation was complete when the sample was swallowed and the indicated intensity returned to zero. Once the evaluation is complete, the judge can select options from a menu such as performing a replicate run, evaluating a different attribute of the same sample, evaluating a new sample, or ending the session. Following a session for an individual judge, the computer resets itself for the next judge.

Five judges who had been trained in the evaluation of chocolate attributes were used to evaluate the method. Four flavor attributes were chosen for the evaluation: "Fruity-aromatic," chocolate, bitter, and sweet. Samples were introduced into the mouth at time zero. The evaluation ended when the attribute intensity returned to zero following sample swallowing. Each judge evaluated all four attributes in random order at two sessions. The training period averaged about 10 min per judge. The judges felt comfortable with the method after being led through the procedure just once.

Sample description

Commercially available dark chocolate in the form of 2.5 gram squares of approximately 4 mm thickness were evaluated. All samples were allowed to equilibrate at room temperature (25.5°C) prior to sampling.

RESULTS & DISCUSSION

THE RESULTS of the evaluation are presented in Fig. 2. Statistical analysis indicated that the individual response curves were not statistically different from the averaged curves shown in Fig. 2 ($\alpha = 0.10$). The curves indicate the expected response: the volatile "fruity-aromatic" flavor was quickly released followed by the more gradual release of the remaining attritutes. It is speculated that the release is intimately related to the phenomena of fat melting, cocoa and sugar particle dissolving, and emulsion formation (saliva being the aqueous phase).

The data analysis was available approximately 2 hr following the completion of the panels. While not specifically done, it is estimated that the same data analysis performed on data obtained by another method (e.g.: a moving strip chart recorder method) would have required at least an 8-hr day. Thus a significant advantage of the computer method is the ease, speed and accuracy of the post-panelling data analysis. It should be pointed out that disc-stored data are in a format that is very

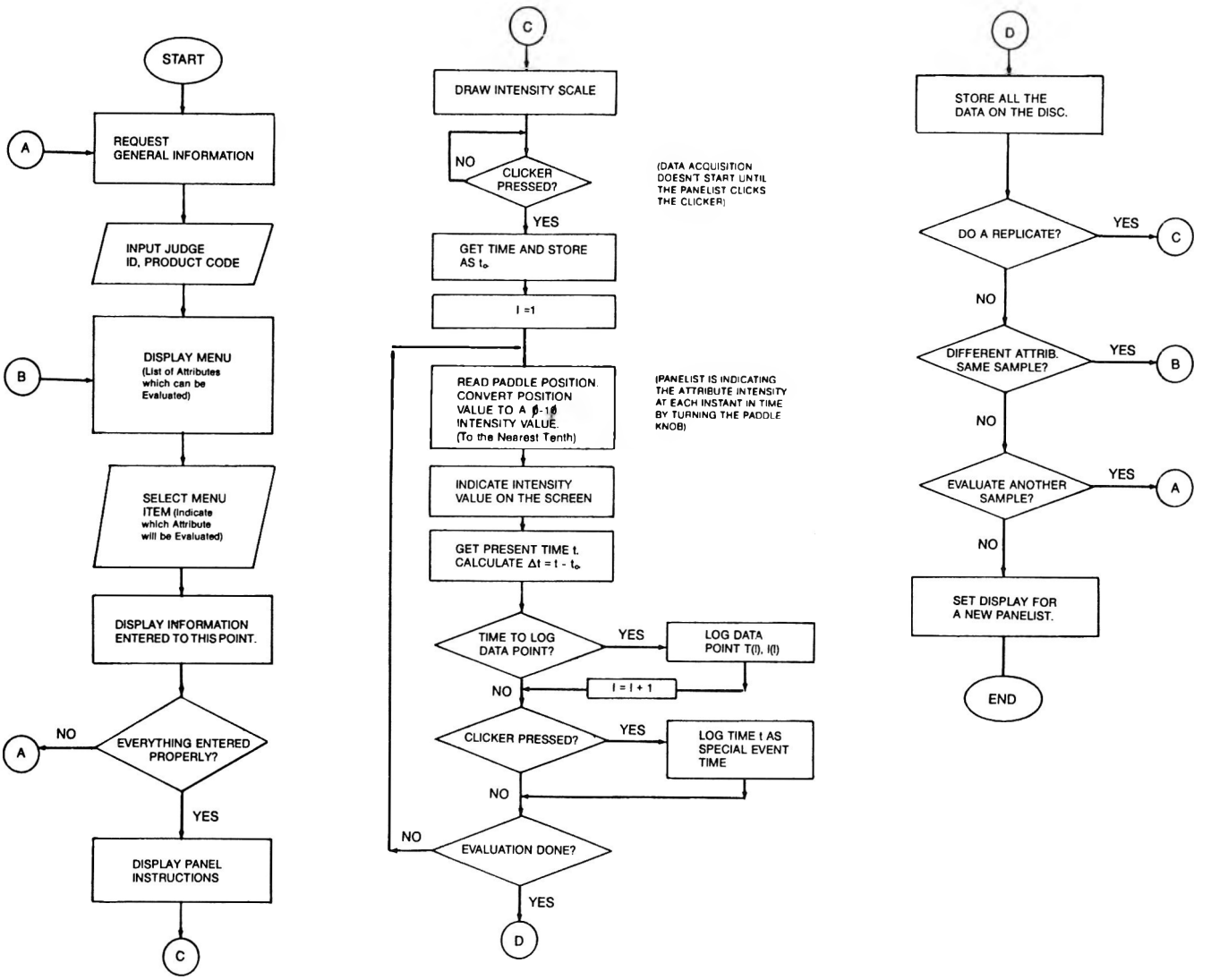

Fig. 1—General flow chart of the computer program.

conducive to statistical analysis and requires much less storage space relative to individual response curves generated on paper formats. Finally, the disc data can be transferred to a larger computer where more powerful software can be accessed.

In a qualitative comparison of other T-I methods to the computer-based method, it was judged that the apparent drawbacks of the noncomputer-based methods had largely been eliminated. Potential biasing resulting from having the entire evolving response curve visible to the judge while the evaluation is in progress is eliminated because the judge can only see the current intensity determination. Having the judge initiate the data collection via a click helped the judge feel more at ease in that he is not trying to get in syncronization with a timer or with a moving chart. Also, the judge can do the evaluation alone. The potential problems often experienced with getting two or more people in the same place at the same time are largely reduced.

These same general conclusions were reached by Guinard et al. (1985) who found that the computer-generated curves did not differ significantly from the chart recorder-generated curves. The present work, done independently of Guinard's work during the same period of time, differs in that all information and T-I data acquisition is done on the monitor screen. Guinard et al. (1985) utilized an external potentiometer with the intensity scale labelled on this external device. Also, the present method software is such (e.g.: the menu-driven features) that judges can perform the evaluations with no super-

—Continued on page 1753

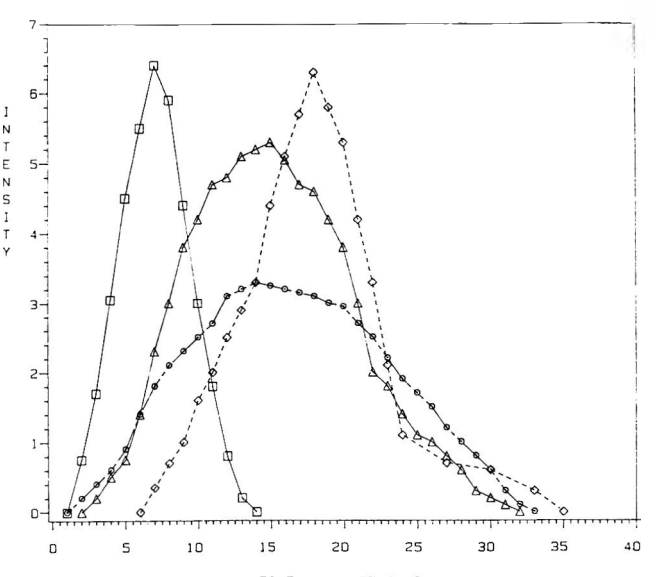

Fig. 2—Evaluation of a dark chocolate by five judges using the computer-based technique. Each curve is the average of the individual response curves (two evaluations per attribute per judge). Legend: "fruity-aromatic;" chocolate; bitter; sweet.

A Research Note

Inhibition of *Clostridium perfringens* by Naphthoquinones

J. L. AULIK and A. J. SIEDLER

ABSTRACT

The minimum inhibitory concentrations (MICs) of several naphthoquinones (NQ) were determined in the presence or absence of nitrite (NO_2^-) against various strains of *Clostridium perfringens*. In fluid thioglycollate medium, MICs ranged from 70–100 ppm for 2-methyl-1,4-NQ (menadione), 200–280 ppm for 1,4-NQ, 180–250 ppm for 1,2-NQ, >500 ppm for several water-soluble derivatives and 100–300 ppm for NO_2^-. Using a type B strain in homogenized meat medium, MICs were 670 ppm for menadione, 620 ppm for 1,4-NQ and 770 ppm for NO_2^-. Nitrite, menadione and 1,4-NQ exhibited comparable and additive rather than synergistic inhibition. Some NQ compounds may have potential as partial nitrite substitutes subject to safety evaluation.

INTRODUCTION

THE USE OF NITRITE in cured meat is under scrutiny due to its involvement with the generation of nitrosamines and some evidence that it may have carcinogenic activity *per se* (National Academy of Sciences, 1981). In cured meat products, nitrite (NO_2^-) is inhibitory to *Clostridium botulinum*, fixes color and prevents rancidity, particularly, the "warmed over flavor" of reheated cooked meat. Naphthoquinones (NQ) are known to have antimicrobial properties (Ambrogi et al., 1970). Other quinones and phenolic antioxidants have antimicrobial including anticlostridial activity (Klindworth et al., 1979; Branen et al., 1980; Raccach and Henningsen, 1981; Reddy et al., 1982).

The objective of this research was to examine and compare the anticlostridial activities of several NQs and NO_2^- using *Clostridium perfringens* as the test organism.

MATERIALS & METHODS

STRAINS of C. *perfringens* were obtained from the American Type Culture Collection (ATCC). The ATCC strain numbers were (3626, 12916, 12917, 12915, 12924, and 14810) (Type A), and the seventh (3626) is a Type B strain (see Table 1). The strains were subcultured in Cooked Meat Medium (Difco, Detroit, MI) at 37°C and stored at 4–7°C.

Inoculum was grown in Fluid Thioglycollate (FT) medium (Difco, indicator-free) overnight at 37°C. One milliliter of culture was added to 9 mL fresh FT and incubated 3–6 hr. Sterile 0.1% peptone water was used to dilute the culture to 0.08–0.14 optical density units at 620 nm and added at 1% of the volume of the medium.

FT and a homogenized meat (HM) medium were used in these studies. The HM medium was made from a meat base consisting of 454g ground beef manually mixed with 22.7g NaCl followed by addition of 680 mL of warm tap water with mechanical stirring. This was heated to 68°C (155°F), cooled to 63°C (145°F), 0.125% (w/w) of papain (Panol, 285-295 tyrosine units/mg, Griffith Labs, Inc., Alsip, IL) added with mechanical mixing and the mixture incubated in a water bath at 67°C (150°F) for 20 min and then homogenized (two-stage) at 3500/500 psig. The homogenate was heated to 91°C (195°F) to inactivate the papain and frozen prior to use. The medium was formulated as follows: 250 mL thawed meat base; 650 mL water; 5g lecithin (Lecigran 5720, Riceland Foods, Inc., Stuttgart, AR); 5g dextrose; 4g powdered yeast extract (GIBCO, Grand Island, NY); and 0.5g cysteine-HCl. The pH was adjusted to 7.0–7.2 with 20% NaOH and water added to a final volume of 1L. The resultant medium was autoclaved at 121°C for 15 min prior to use.

Menadione (2-methyl-1,4-NQ, vitamin K_3), 1,4-NQ and acetomenaphthone were dissolved in dimethyl sulfoxide (DMSO, Mallinkrodt, reagent Grade) and diluted under asceptic conditions. (Kligman, 1965). The salts of 1,2-NQ-4-sulfonic acid, 1,4-NQ-2-sulfonic acid and menadione bisulfite and sodium nitrite were dissolved in water and filtered through a disposable filter unit. Test compounds and sources were: $NaNO_2$ (J.T. Baker Company, Phillipsburg, NJ); menadione (GIBCO, Grand Island, NY); 1,4-NQ, 97% tech. grade and tech. grade 1,2-NQ-4-Sulfonic acid, Na salt (Aldrich Chemical Company, Milwaukee, WI); 1,2-NQ and acetomenapthone (Pflatz and Bauer, Stamford, CT) and 1,4-NQ-2-sulfonic acid, K salt (Eastman Kodak Company, Rochester, NY).

Capped tubes containing Durham tubes plus 9 mL of HM medium or FT medium were autoclaved, cooled, 10X stock solution of test compound and water added to a final volume of 10 mL. Tests were run at 37°C in triplicate. After 24 hr incubation, the tubes were checked for gas formation. Gas production was considered positive. Nongassy tubes were incubated an additional 12–24 hr to confirm the results.

Table 1—Minimum inhibitory concentration (MIC) of menadione or nitrite for C. perfringens *strains in fluid thioglycollate medium*

Strain	Type	ppm	
		Menadione	NO_2^-
3624	A	80–100[a]	135
3626	A	70–90[a]	135–170[a]
12915	A	100	100
12916	A	100	200
12917	A	100	300
12924	A	80–100[a]	170
14810	B	80–100[a]	135

[a] Ranges are presented when multiple determinations were run.

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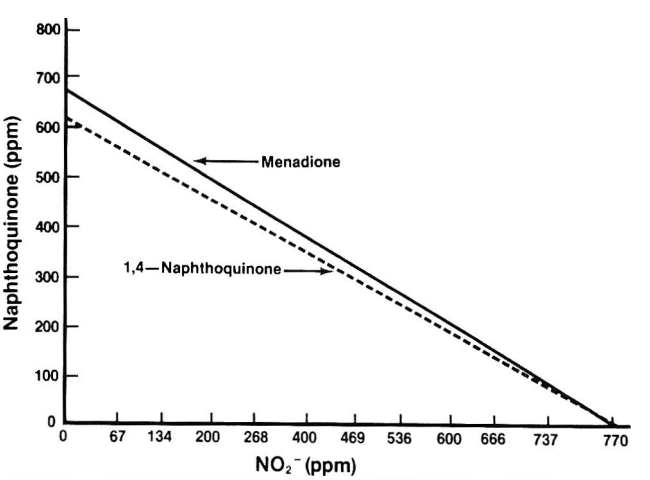

Fig. 1—Linear regression plot of the inhibition of C. perfringens *3626 in homogenized meat medium using various combinations of mnenadione or 1,4-naphthoquinone plus nitrite.*

RESULTS & DISCUSSION

THE MICs for 2-methyl-1,4-NQ (menadione) and NO_2^- in FT medium for several strains of *C. perfringens* are presented in Table 1. Menadione was equally or slightly more effective than NO_2^- on a ppm basis in FT media and approximately fourfold more effective on a molar basis than nitrite. The unsubstituted compounds, 1,4-NQ and 1,2-NQ were also tested against *C. perfringens* 3626 in FT medium. The MICs ranged from 200–280 ppm for 1,4-NQ and 180–250 ppm for 1,2-NQ. These compounds appeared to be slightly less effective than menadione against this strain but more effective than NO_2^- on a molar basis.

The water-soluble naphthoquinone derivatives, 1,4-NQ-2-sulfonic acid K salt; 1,2-NQ-4-sulfonic acid Na salt; menadione sodium bisulfite; and acetomenaphthone were inactive up to 1000 ppm in FT medium when tested against *C. perfringens* 3626. Lack of activity may be due to inability of these compounds to penetrate the cell at the pH used or specific substituent effects.

The MICs versus *C. perfringens* observed when NO_2^- was added after autoclaving the medium were generally lower than those reported by Riha and Solberg (1975) but similar to those published by Gough and Alford (1965). Autoclaving NO_2^- in the medium may increase its anti-clostridial activity (Klindworth et al., 1979; Lee et al., 1978; Perigo et al., 1967).

Combinations of menadione and NO_2^- were assessed for possible additive or synergistic effects. The HM medium was used in these studies to more closely approximate actual conditions of potential use. The combined results of two separate experiments with combinations of menadione or 1,4-NQ plus NO_2^- are presented in Fig. 1. Regression analysis (ANOVA) indicated linearity of the best fit line with a correlation coefficient of 0.97 for menadione and 0.99 for 1,4-NQ. The MIC (Y intercept) was 670 ppm for menadione, 620 for 1,4-NQ and 774 — 770 for NO_2^- (X intercept). Menadione or 1,4-NQ and nitrite had additive inhibitory activity in this system which suggests a common site for the mode of action for these compounds.

Substitution of the methyl group in the 2-position did not affect the inhibitory activity in HM medium as noted using FT medium. Other water soluble naphthoquinones tested were not active at levels up to 1000 ppm.

NQs may have use as model compounds to study the antimicrobial activity of NO_2^-. NQs may also have utility as potential alternatives or partial substitutes for the antimicrobial activity of NO_2^-: however, sufficient toxicological data are not available.

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vision; there is not need for the typing of preliminary information by the panel supervisor before the judge can do the evaluation. As such, the present method is slightly more "user friendly."

In summary, the computer-based method's main advantages are in the ease and speed of the post-panelling data analysis, and in reduction of possible error which could arise during sensory evaluation. Such a method can be set up with a minimum of effort in the training of the judges.

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A Research Note

Resistance of Yeast to Dry Heat

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ABSTRACT

The dry heat resistance of 10 strains of yeast was investigated to develop data useful for the evaluation of aseptic systems for packaging acid products and which sterilize containers with hot air. Although three of the strains tested showed little survival at 110°C, four other strains had $D_{110°C}$ values between 1 and 4 min. *Torulopsis glabrata* had a $D_{126.7°C}$ of 0.78 min. *Saccharomyces* strains showed the highest dry heat resistance, with the most heat resistant strain tested having a $D_{126.7°C}$ of 5 min. The z values for these strains ranged from 9.1° to 13.3°C.

INTRODUCTION

FRUITS, fruit products and their containers generally require only a mild heat treatment to achieve commercial sterility, since the common acid food spoilage organisms such as yeasts and molds are relatively heat sensitive (Miller, 1979; Put et al. 1976; Splittstoesser, 1978). Although it has been determined that microorganisms are more resistant to dry heat than wet heat (Schmidt, 1954), most dry heat studies have dealt with bacterial spores (Angelotti et al., 1968; Brannen and Garst, 1972; Bruch et al., 1963; Winans et al., 1977). Studies on the dry heat resistance of sporeformers were stimulated in part by commercial development in the 1950s of the Dole aseptic canning system, which uses superheated steam to sterilize metal containers and covers (Pflug, 1960). The recent development of new aseptic packaging systems has included one using hot air for sterilizing composite containers used in aseptically packing fruit juices. This has pointed out the need for data on dry heat sterilization of acid food spoilage organisms. For this reason we have investigated the dry heat resistance of several strains of yeast.

MATERIALS & METHODS

THE YEASTS used in these experiments were all isolated from foods. Yeast suspensions in phosphate buffered saline (PBS) were prepared from cells grown for 3–4 days on unacidified potato dextrose agar incubated at 30°C (35–37°C for *Saccharomyces* strains; 24–27°C for strain 84–60). The suspensions were stored at 4°C.

Sterile foil strips measuring approximately 0.5 × 1–2 cm were prepared from a double layer of heavy duty aluminum foil. Yeast suspensions were diluted in PBS to give approximately 10^4–10^5 organisms per strip, assuming a 0.5 to 1 log loss of viability during air drying for 1–2 days in a laminar flow hood.

Strips were heated in a Fisher Isotemp model 349 oven (provided by Boise Cascade, St. Louis, MO) in which the temperature controller and sensing probe had been modified and the thermometer opening enlarged to accommodate the strip holder. The strip holder (Boise Cascade) consisted of a 35-cm long flat metal rod attached to a 10 × 10 cm base plate. Alligator clips for holding the foil strips were attached to the rod. One copper-constantan thermocouple, clipped by the strip holder with the tip resting on a foil strip, was used to measure strip temperature; a second thermocouple was used to monitor oven air temperature.

The strip holder containing the yeast strips and thermocouple was inserted through the opening in the top of the oven and turned so the strips faced the air flow. Temperatures were recorded every 10 sec. Ten strips were heated at each time interval; in general four time intervals were tested for each of four temperatures. At the end of the time interval the holder was removed from the oven and the strips were transferred in a laminar flow hood to tubes of Mycophil broth (1% polypeptone and 4% dextrose autoclaved 15 min at 12 psi). Cultures were incubated for 7–10 days at 30°C (24–27°C for 84–60). Uninoculated strips and unheated strips were used as negative and positive controls, respectively. The number of viable yeast cells per strip was determined by resuspending yeast from unheated strips in 0.1% peptone, followed by dilution and plating. The mean count from 10 strips was used as the initial count in data analysis. D and z values were calculated as described by Scott and Bernard (1982).

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RESULTS & DISCUSSION

Pichia membranaefaciens 67-272, *Rhodotorula rubra* C-46A, and an unidentified yeast isolated from acidified pepper brine (84-60) did not have appreciable dry heat resistance; there was little survival after 5 min at 110°C (the lowest time and temperature tested). *Debaryomyces hansenii* NRRL Y-7268 had a $D_{110°C}$ of 1.25 min; however, there was little survival above that temperature. The dry heat resistance of *Kloeckera apiculata, Lodderomyces elongisporus, Hansenula anomala* and *Torulopsis glabrata* is shown in Table 1.

The most heat resistant yeasts tested were the *Saccharomyces* strains (Table 2). *S. cerevisiae* O-UC-188 had a $D_{126.7°C}$ of over 2 min and a $D_{132.2°C}$ of almost 1 min. This was approximately three times more resistant than *T. glabrata*, the most resistant strain tested other than the *Saccharomyces*. A suspension of *Saccharomyces* sp. ABC 0519 had even higher dry heat resistance, having an average $D_{126.7°C}$ of 5 min. A second suspension of strain ABC 0519 showed heat resistance intermediate to that of strain O-UC-188 and the first suspension of ABC 0519.

Several investigators have studied the heat resistance of yeasts in various solutions such as buffers and fruit juices (Corry, 1976; Gibson, 1973; Juven et al., 1978; Put et al., 1976; Put and De Jong, 1982a, b; Splittstoesser, 1982; Su and Beuchat, 1984). These studies have generally used temperatures between 48°C and 60°C. $D_{60°C}$ values have been reported to range from 0.1 min for vegatative cells to 40 min for some ascospores (Put and De Jong, 1982b), with z values of approximately 4–6.5°C (Put and De Jong, 1982a). Our studies have shown that yeast were much more resistant to dry heat than wet heat. The D values for several strains were established at 110–135°C; z values ranged from 9–13°C. *Saccharomyces* strains were more resistant to dry heat than the other strains tested. Put and De Jong (1976) reported similar findings when testing the wet heat resistance of yeast.

No attempt was made to produce ascospores with the ascomycetous strains, however, it is likely that low numbers of ascospores may have been present (Put and De Jong, 1982b). Put et al. (1976) found that in general asporogenous yeasts were less heat resistant than ascomycetous types. Only three of the yeasts studied here (*Kloeckera, Rhodotorula* and *Torulopsis*) were from asporogeneous species. The *Torulopsis* strain showed higher heat resistance than several ascomycetous strains (*Lodderomyces, Hansenula* and *Pichia*).

Table 1—Dry heat resistance of several strains of yeast

Strain	D value (minutes) 110°C	112.8°C	115.6°C	118.3°C	126.7°C	129.4°C	132.2°C	135°C	z
Kloeckera apiculata NRRL Y-1382	1.80	1.05	0.65	0.31	ND[a]	ND	ND	ND	11.1°C
Lodderomyces elongisporus NRRL YB-4239	2.92	2.56	1.93	0.64	ND	ND	ND	ND	13.3°C
Hansenula anomala 67-455	3.63	ND	0.77	ND	0.13	ND	ND	ND	11.8°C
Torulopsis glabrata GSU-80	ND	ND	ND	ND	0.78	0.59	0.32	0.12	10.1°C

[a] ND = not determined

Table 2—Dry heat resistance of Saccharomyces

Strain	D value (minutes) 126.7°C	129.4°C	132.2°C	135°C	z
O-UC-188[a]	2.36 ± 0.41	1.39 ± 0.31	0.91 ± 0.15	0.49 ± 0.02	12.7 ± 1.3°C
ABC 0519(1)[a]	5.00 ± 0.39	2.93 ± 0.29	1.63 ± 0.18	0.89 ± 0.02	11.1 ± 0.9°C
ABC 0519(2)[b]	4.15	1.95	0.96	0.51	9.1°C

[a] Mean and standard deviation of four experiments

[b] Second suspension of strain 0519; single experiment

Dry heat is considered to be a range of conditions (Angelotti et al., 1968); moisture content or water activity (a_w) of the microorganisms and the relative humidity (RH) of the heating atmosphere are important factors in determining dry heat resistance (Angelotti et at., 1968; Brannen and Garst, 1972; Murrell and Scott, 1966). These factors were not controlled in our experiments, as they would not be in the commercial application of the technology; however, RH in the oven was estimated to be 33–38%. The dried yeast would have equilibrated rapidly to this RH (Angelotti et al., 1968). This RH is within the range (0.2–0.4a_w) where maximal spore resistance is observed (Angelotti et al., 1968; Murrell and Scott, 1966). The RH or a_w at which yeast show maximum heat resistance has not been determined.

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A Research Note

Dietary Fiber and Other Constituents of Some Tongan Foods

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ABSTRACT

Components of dietary fiber (soluble nonstarch polysaccharide, pectin, hemicellulose, cellulose and lignin), water, energy, protein, lipid and starch were measured in banana, breadfruit, cassava, coconut, mango, pawpaw, plantain, sweet potato, taro and Pacific yam, all from the Pacific Island Kingdom of Tonga. The Tongan foods were generally higher in dietary fiber than New Zealand foods, consistent with the hypothesis that dietary fiber is an important factor contributing to a lower incidence of certain gastrointestinal disorders in Tonga, than in New Zealand.

INTRODUCTION

THE DIETARY FIBER hypothesis suggests that there is a negative relationship between the dietary fiber intake of a community and the incidence of a variety of noninfectious gastrointestinal diseases (Burkitt et al., 1972). The South Pacific presents an opportunity to investigate this hypothesis, for many diseases of the gut which are common in the developed Western society of New Zealand are relatively rare in the less developed South Pacific Islands. For instance, over the 11-year period from 1969-1979 the total incidence of cancer of the large bowel, colon and rectum was between 1 and 2 cases per 100,000 per year in Tonga (Stace, 1980) whereas a figure of 49.5 cases per 100,000 per year has been reported for the region of Canterbury, New Zealand, in the five years 1970 to 1974 (Stewart et al., 1979).

However, there appears to have been little published on the composition of foods from the developing Pacific Island Nations. This study was undertaken to obtain information on the content of major constituents in foods from the Kingdom of Tonga, which may assist in future epidemiological studies.

MATERIALS & METHODS

FOODS from the remote Tongan island of Mo'unga'one were collected. This tiny (2 x 1 km) island is far removed from Western influences. Though lacking a well for water supply, the soil is fertile and all fruit, vegetables and nuts commonly eaten in the Kingdom of Tonga are grown in plentiful supply on this island. The foods were all collected in a fresh state suitable for consumption, put into plastic bags (1 kg fresh weight or a minimum of 3 samples of each species) and transported by air to New Zealand. On arrival, the inedible portions were removed (husks etc.), the edible portions were weighed, and a 5g sample taken for water content determination. A subsample of breadfruit was boiled for 15 min. The foods were all stored in plastic bags at −20°C until they were able to be freeze-dried.

Analyses were carried out on pooled, finely ground freeze-dried subsamples of banana (Siane, *Musa* sp.), breadfruit (Mei, *Artocarpus atilis*), cassava (Manioke, *Manihot esculenta*), coconut (Niu, *Cocos nucifera*), mango (*Mangifera indica*), pawpaw (*Carica papaya*), plantain (Hopa, *Musa paradisiaca*), sweet potato (*Ipomoea batatas*), taro (Talo futuna, *Xanthosoma sagittifolium*), taro (Talo Tonga, *Colocasia esculenta*) and Pacific yam, (Ufi, *Dioscorea alata*). Analyses were conducted, in duplicate, on two samples of each food.

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Moisture content was determined by freeze drying, followed by vacuum desiccation over P_2O_5 for 2 days. Total energy was determined using a Gallenkamp bomb calorimeter (Trinkler and Masters, 1932), protein by the Kjeldahl procedure (Varley, 1966) total lipids after methanol:chloroform:water (MCW; 12:5:3) extraction (Sperry and Brand, 1955) and soluble sugars by measurement of reducing sugar in 80% ethanol extracts (Lever, 1972) which had been acid hydrolyzed (5%H_2SO_4, 100°C, 1 hr). Starch was determined by amyloglucosidase digestion of the residue after MCW extraction, followed by glucose oxidase measurement of the released glucose (MacRae, 1971).

Dietary fiber was fractionated into soluble nonstarch polysaccharides (SNSP), pectins, hemicellulose, cellulose and lignin. Freeze-dried sample (200 mg) was MCW extracted, gelatinized in water (2 mL, 1 hr, 100°C) followed by addition of α-amylase in cold buffer (pH 7.0). After incubation (40°C, 1 hr) solids were separated by centrifugation and washed with distilled water. Combined supernatant and washings were made to 80% ethanol and after 1 hr the precipitate (SNSP plus limit dextrins) collected by centrifugation. It was resuspended in sodium acetate buffer pH 4.8 containing β-glucanase-free amyloglucosidase and digested (2 hr, 60°C) until depolymerization of starch products was complete, as indicated by similar treatment of starch standards. Ethanol (4 vol) was added and the resulting precipitate (SNSP) again collected by centrifugation, washed with 80% ethanol and hydrolysed (5% H_2SO_4, 100°C, 2 hr).

Insoluble polysaccharides were fractionated by sequential extraction of the residue from starch + SNSP extraction, with ammonium oxalate (0.5%, 100°C, 2 hr) to remove pectins, and 5% H_2SO_4 (100°C, 2 hr) to remove hemicellulose. The residue (cellulose + lignin) was subjected to Saeman hydrolysis (suspended in 72% H_2SO_4, 3 hr, 20°C, diluted to 5% H_2SO_4, hydrolyzed 2 hr, 100°C). The residue (Klason lignin) was washed with distilled water, ethanol, dried and weighed. Fractions were determined from the sugar content of the hydrolysates by measurement of reducing sugars by the method of Lever (1972). Uronic acids in the SNSP and pectin fractions were measured by the method of Blumenkrantz and Asboe-Hansen (1973).

RESULTS & DISCUSSION

TABLE 1 shows the energy and major nonfiber constituents (protein, lipid, soluble sugars, starch) of the foods analyzed. The foods could be considered in two categories - those with a high soluble sugar and low starch content, typical of ripe fruit (ripe banana, mango, pawpaw), and those with high levels of starch, including storage organs (cassava, sweet potato, taro and Pacific yam) and unripe fruit (green banana, breadfruit and plantain). Breadfruit and green banana occupy an intermediate position in containing relatively high levels of both starch and soluble sugar. Their high levels of starch represent reserves which would ultimately undergo almost complete conversion to soluble sugars during ripening, but which are present in high concentrations when the fruit is immature (Rhodes, 1980). Coconut is alone amongst the foods analyzed here, in containing a high proportion of lipid.

The composition of the fiber (nonstarch) polysaccharides is shown in Table 2. In most cases an appreciable proportion of the fiber polysaccharide is accounted for by the soluble and pectic polymers, which are relative susceptible to digestion in the human gut (Holloway et al., 1981). The physically fibrous nature of mango flesh, compared to that of the other foods, is reflected in its relatively high level of dietary fiber, and a high proportion of hemicellulose, cellulose and lignin in this fiber.

The high starch crops forming a staple energy source for the

Table 1—Energy and nonfiber components of Tongan foods

Food	Water (% fresh wt)	Energy (KJ/100g dry wt)	Component as % dry weight				
			Protein	Lipid	Soluble sugar	Starch	Ash
Banana (green)	70.7	2447	4.4	1.4	23.1	56.4	3.0
(ripe)	70.0	2400	3.7	1.4	78.8	5.8	3.2
Breadfruit (raw)	77.5	2206	4.8	4.5	20.8	42.8	3.5
(cooked)	-	2297	4.1	2.2	21.0	47.5	2.4
Cassava	60.5	2777	1.1	1.2	6.1	84.9	2.1
Coconut (mature)	27.1	11439	7.6	57.2	12.9	0.9	1.9
Mango	88.6	2099	3.9	5.0	46.9	11.4	2.9
Pawpaw	67.0	2313	3.4	1.5	78.8	3.5	3.5
Plantain	66.9	2526	3.2	1.1	5.4	68.5	3.0
Sweet potato	69.0	2464	3.2	1.0	16.0	63.2	3.4
Taro - (Talo futuna)	70.8	2331	4.3	3.3	3.0	73.5	3.9
Taro - (Talo tonga)	63.6	2579	2.4	3.5	7.5	74.4	3.5
Pacific yam - ufi	75.8	2164	9.8	0.8	4.7	79.0	3.6

Table 2—Dietary fiber components in Tongan foods

Food	Polysaccharide fraction (% dry weight)					
	SNSP[a]	Pectin	Hemicellulose	Cellulose	Lignin	Total
Banana (green)	3.4	2.5	1.9	1.2	1.6	10.6
(ripe)	2.2	0.8	0.7	1.1	1.1	5.9
Breadfruit (raw)	6.8	4.7	3.9	4.0	1.7	21.1
(cooked)	6.1	5.4	3.8	6.1	1.7	23.1
Cassava	2.0	5.8	2.7	1.9	1.1	13.4
Coconut (mature)	2.4	0.3	4.6	5.1	3.1	15.5
Mango	4.1	3.9	7.0	11.5	4.2	30.7
Pawpaw	3.5	1.0	0.3	3.6	2.4	10.8
Plantain	4.2	5.5	5.6	1.0	2.1	18.4
Sweet potato	4.4	2.5	3.8	1.9	1.4	14.0
Taro - (Talo futuna)	3.2	4.3	2.5	1.5	0.8	12.3
Taro - (Talo tonga)	3.2	4.5	1.9	1.5	0.8	11.9
Pacific yam - (ufi)	1.9	2.6	3.4	1.6	1.1	10.6

[a] SNSP = soluble nonstarch polysaccaride (polyuronide plus neutral sugar).

Tongans all show relatively low hemicellulose:cellulose ratios (Table 2), typical of noncereal dietary fiber. In contrast, the dietary fiber of the average New Zealand diet, derived to a larger extent from cereal foods, will have a higher hemicellulose:cellulose ratio. The water-holding capacity of dietary fiber appears to correlate positively with its hemicellulose content (Cummings, 1978; Holloway and Greig, 1984), and is also thought to be an important determinant of faecal bulking and intestinal transit times, both parameters being related to a low incidence of gastrointestinal disease (Burkitt et al., 1972). The present results suggest that, on average, the water-holding capacity of fiber in the Tongan diet will be less than that in the New Zealand diet, and that it will therefore have less effect, per unit mass, on the alimentary tract.

However, the total nonstarch polysaccharide content of the Tongan high-starch foods ranged from 8.5% to 21.4%, compared with only 4.84% in potato, 3.2% in white flour and 7.07% in porridge oats (British figures, Englyst et al., 1982). And although the soluble nonstarch and pectin polysaccharides, which are susceptible to degradation by gut bacteria (Holloway et al., 1981) account for a large proportion of dietary fiber in Tongan foods, their utilization will to some extent be offset by an increase in bacterial cell mass, resulting from their availability to the intestinal flora (McBurney et al., 1985).

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A Research Note

Microbiological and Toxicological Aspects of Fermentation of Castor Oil Seeds for *Ogiri* Production

S. AYO ODUNFA

ABSTRACT

The microorganisms involved in the fermentation of castor oil bean for *ogiri* production were isolated and characterized. The most predominant microorganism was *Bacillus subtilis*. Other species were *B. licheniformis*, *B. megaterium* and *B. firmus*. All the *Bacillus* species were proteolytic and were capable of fermenting castor oil seeds and producing the characteristic *ogiri* aroma in pure cultures. The optimum pH for growth of the three major isolates was 7 – 8 while the optimal temperatures were 30°C for *B. subtilis* and *B. megaterium* and 45°C for *B. firmus*. Toxicological evaluation of the fermented product by chicken embryo bioassay showed that the initial toxicity of the beans decreased significantly but was not completely eliminated.

INTRODUCTION

IN WEST AFRICA, there are some oil seeds which are less well known and are important parts of the diet. Such seeds include castor oil seeds (*Ricinus communis*), melon seed (*Citrullus vulgaris*) (Odunfa, 1981a) and sesame seed (*Sesamum indicum*) Odunfa, 1985). These are fermented and ground to make oily pastes called 'ogiri' which are used as flavoring condiments in soups and sauces. The pastes have a very strong putrid odor with some ammoniacal odor. However, they have a pleasant aroma when added to soups and sauces and also contribute to the protein and essential fatty acid intake in West Africa (Chevassus-Agnes and Pascard, 1932; Umoh and Oke, 1974). This is significant especially as protein calorie malnutrition (Atinmo, 1982) and essential fatty acid deficiencies (Naismith, 1973) are widespread.

'Ogiri' from castor oil seeds is consumed in the eastern and the mid-western parts of Nigeria by about 5 million people. The seeds contain 4.3% oil, 19% protein and about 33% carbohydrate (Anosike and Egwuatu, 1981). The protein contains a toxic component, ricin, (Osborne et al., 1905) and other allergenic proteins such as recinine (Merck Index, 1976). Ricin is highly lethal even at low concentration (Kingsbury, 1964; Ishiguro et al., 1964).

With urbanization, the production and consumption of castor oil seed 'ogiri' are decreasing and giving way to imported proprietary bouillon cubes. To sustain and optimize the production process, it is necessary to obtain information about the fermentation. The present study was aimed at providing information on the microorganisisms involved in the fermentation and also at evaluating the toxicity of the boiled unfermented seeds and the fermented product 'ogiri.'

MATERIALS & METHODS

Traditional method of preparing castor oil 'ogiri'

The castor oil seeds were dehulled by breaking the shells lightly with stones on a clean concrete pavement. The cotyledons were then collected and wrapped in small packets (about 10 cm diam) with banana leaves. Holes were punched through the leaves to allow penetration of water into the cotyledons while boiling, and the packets were put into a pot and boiled for 6 hr. After boiling, the packets were removed and put in a warm place (about 32°C) for 4 days to ferment. The fermented seeds were removed from the leaves and ground on a grinding stone to a fine paste called *ogiri*.

Isolation and identification of microorganisms

Ten-g samples of fermenting castor oil seeds were obtained at 24-hr intervals, ground aseptically and serially diluted in sterile tap water. Appropriate dilutions were spread on plates of Nutrient Agar, Plate Count Agar and Rose-Bengal Chloramphenicol Agar, (Oxoid, Basingstoke, U.K.). The plates were incubated at 30°C for 72 hr, and for 5 days for mycological media; half of the agar plates were incubated under anaerobic conditions. The colonies which formed were grouped on a morphological basis, counted and then streaked on agar plates to obtain pure cultures. The isolates were identified following the keys by Gordon (1973) and Buchanan and Gibbons (1974).

Determination of pH and temperature and moisture content

The pH, temperature and moisture changes during the fermentation were determined following the methods described earlier (Odunfa, 1981b).

Toxicology evaluation of the fermented beans

Chicken embryo bioassays were carried out on the unfermented and fermented samples purchased locally. Fertile chicken eggs, obtained from the University of Ibadan Teaching and Research Farm, were candled to eliminate eggs with cracks or with other shell imperfections and to outline the location of air-spaces (AOAC, 1975). The eggs were incubated at 37–38°C and 65% relative humidity. After 5 days the eggs were candled to select healthy embryos and then surface sterilized; 0.5 mL of each of the water extracts of unfermented and fermented castor oil bean, and sterile distilled water were injected through the allantoic route. Twenty-five eggs were used for each treatment. Inoculated eggs were incubated 37–38°C and examined for viability after 5 and 10 days.

RESULTS & DISCUSSION

ONLY BACTERIA were isolated from the fermenting seeds. Almost all were proteolytic *Bacillus* species with strains of *Bacillus subtilis* (Ehrenberg) Cohn. the most predominant; others were *B. licheniformis*, *B. megaterium*, and *B. firmus*. *Bacillus megaterium* was more prominent towards the latter stages of fermentation. Other organisms isolated infrequently and at very low numbers include *Proteus* spp., *Escherichia coli* and *Enterobacter aerogenes*. The highest count was obtained at 72 hr of fermentation; the total viable counts (per g) were: *Bacillus subtilis* 7.8×10^{12}, *B. megaterium*, 6.5×10^{3}, and other *Bacillus* spp, 3.4×10^{6}. The biochemical characteristics of the isolates showed that they were all proteolytic and saccharolytic while only *B. subtilis* was lipolytic. The optimal pH for growth of *Bacillus megaterium* and *B. subtilis* was 7 while that of *B. firmus* was 8. The optimum temperatures were 30°C for *B. subtilis* and *B. megaterium* and 45°C for *B. firmus*.

Of microorganisms isolated, the *Bacillus subtilis* group has frequently been found involved in the fermentation of similar vegetable proteins notably African locust bean and African oil

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bean (Odunfa, 1981b; Odunfa and Oyeyiola, 1985). Gram negative rods were found involved in the fermentation of melon seeds during *ogiri* production (Odunfa, 1981a). *Bacillus megaterium* was found during the fermentation of cocoa (Hansen and Welty, 1968); its appearance in low numbers and towards the end of the fermentation showed that it might not be playing a significant role.

Tests for the ability of the different microbial isolates to ferment sterile castor oil seeds showed that only the predominant *Bacillus* species softened the bean seeds and produced the characteristic aroma of the 'ogiri'. Both strains of *B. subtilis* and *B. licheniformis* produced the strongest aroma. All the species of *Bacillus* which produced even the slightest aroma and softening of the castor oil seed were found to be proteolytic. The fact that Anosike and Egwuatu (1981) found free amino acids in the fermented seeds and none in the unfermented seeds further implies that proteinases are active during the fermentation.

The source of bacteria involved in the fermentation is most probably from the air or banana leaves used for wrapping the castor oil beans during the preparation. This is because the initial step of boiling for 6 hr would have eliminated the natural flora of the seed. The species of *Bacillus* isolated are commonly found in air and in soil hence they can frequently grow on suitable substrates.

The pH of the fermenting mash became alkaline with length of fermentation (Table 1). This is due to the amines and ammonia normally produced during the hydrolysis of proteins, as illustrated by Whitaker (1978). Moisture also increased presumably due to hydrolytic decomposition of the food constituents of the seeds.

Although *Clostridium botulinum* is an obvious hazard in the fermentation of proteinaceous materials at alkaline pH and low oxygen tension, it is unlikely to proliferate in the fermenting beans because of the reported antagonism and toxin inhibition by *B. subtilis* and *B. licheniformis* (Wentz et al., 1967; Mossel, 1982). No case of poisoning due to eating fermented castor oil seeds has been reported.

The chicken embryo test showed that the boiled, unfermented beans contained some toxic constituents (Table 2); this toxicity can not be due to ricin, because studies have shown that the toxicity of ricin and other allergenic proteins are destroyed by heating at 100°C for 1 hr at pH 6.2 (Spies et al., 1962; Jenkins, 1963). The toxicity may therefore be due to other heat stable toxic constituents frequently associated with legumes (Spies et al., 1962). It is important that fermentation brought about a significant decrease in this toxicity although the toxicity was not completely destroyed.

Table 1—Physical changes during the fermentation of caster oil seeds[a]

Time (hr)	Moisture (%)	Temp (%)	pH
0	10.1	30	7.0
24	24.7	32	7.5
48	38.4	32	7.8
72	45.8	30	7.8
96	49.2	30	7.9

[a] Results in each column are the means of three replicate determinations.

Table 2—Toxicological evaluation of fermented castor oil bean (ogiri) using chicken embryo bioassay

Treatment	% of viable eggs	
	After 5 days	After 10 days
Unfermented bean extract	48%[a]	36[a]
Fermented bean extract	84[b]	84[b]
Sterile distilled water	100[b]	100[b]
Uninoculated control	100[b]	100[b]

[a,b] Where superscripts differ, treatments differ significantly, ($P < 0.05$). Figures are cummulative results of duplicate determinations.

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A Research Note

Essential Elements, Cadmium and Lead in Fresh and Canned Corn (*Zea mays L.*)

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ABSTRACT

The content of 16 essential elements and cadmium and lead was determined in fresh and canned corn (*Zea mays L.*) by atomic absorption spectrophotometry. Samples were taken during the canning process to determine where changes in element content occurred. The content of each sample was compared statistically to other samples taken within the process. RDAs and estimated safe and adequate daily dietary intake values as well as percent retention values were calculated. Element retention, excluding chloride and sodium, ranged from 43% to 98%.

INTRODUCTION

PREVIOUS WORK done on the elemental content of corn has included mostly macro-elements such as calcium, iron, phosphorus, potassium, and sodium (Ensminger et al., 1983; Anonymous, 1962; Kramer, 1946; Watt and Merrill, 1963). Work has been published on other elements but little has been done to show the effects of canning on them.

The objectives of this research were (1) to determine and compare the concentration of 18 elements, including cadmium and lead, in fresh and canned corn, and (2) to determine the effects of canning operations on the concentration of these elements in the finished product.

MATERIALS & METHODS

Sampling

Samples of fresh, before blanch, after blanch, and canned corn (*Zea mays L.* NK-199 variety) were obtained at the KMC Corporation plant in Queen Anne, MD. The corn, harvested in Laurel and Harmony, DE in the summer of 1981, was commercially canned shortly after harvesting. Corn was received at the processing plant and conveyed through shakers and blowers to remove silk and loose debris; shucks were also removed (fresh sample taken). The corn was washed, cut off the cob, rewashed and inspected (before blanch sample was taken). It was blanched for 1.5 min at 88°C (190°F) (after blanch sample was taken). Twenty milliliters of filling medium concentrate (NaCl, sucrose, water) were added to each 454g (16 oz, 303 × 406) C-enamel can. Then approximately 283g (10 oz) of corn were added, followed by approximately 140 mL (5 fl oz) of hot tap water, before sealing. The cans were then placed in retorts, processed for 10 min at 125°C (257°F), and cooled in water for 8 min (canned sample taken) before storing. Seven samples were taken at each step of the canning process throughout one day. Sampling from the line was coordinated so that fresh, in-process, and canned samples were from the same batch. Seven samples of filling medium concentrate and two of processing water were taken during the period.

Fresh, before blanch, and after blanch samples weighed 0.5–1 kg each. Six cans of corn were taken for each of the seven canned corn and processed drained liquid samples. The samples were stored at 21°C (70°F) for 19 wk before being opened, drained and mixed. Seven samples were chosen to keep the variation around 15%. This was determined by running preliminary analyses on three canned corn samples and finding the number of samples needed to give 15% variation for a paired "t" test. The data were analyzed using Duncan's test. Analyses of variance were performed at the 1% and 5% levels to test for significant differences.

Samples were prepared for analyses by mixing and pureeing with a Cuisinart food processor, freezing at −8°C (18°F), freeze-drying on a Virtis freeze dryer for 48 hr, grinding in the food processor, and storing in a freezer. Prior to weighing samples, the frozen aliquots were ground with a Waring Blendor, freeze-dried again for 48 hr and stored in a desiccator. The wet ashing procedure of Simpson and Blay (1966) was used for atomic absorption spectrophotometric analyses of all elements except chloride. Duplicate 1.35 ± 0.12g freeze-dried corn samples were analyzed. Samples of plant processing water, filling medium concentrate and processed drained liquid were diluted 1:1 and contained 4% concentrated HNO_3.

Canned corn samples were prepared for chloride determination following AOAC Methods of Analysis 3.071 (AOAC, 1980). Duplicate 1.20 ± 0.04g freeze-dried samples were moistened with 20 mL 5% Na_2CO_3 solution, dried at 90°C for 3 hr and ignited at 450°C overnight. After washing and filtering the ash with hot water, the filter papers were ashed at 450°C for 2.5 hr. The ash was dissolved in 4 mL of HNO_3 (1:4), filtered, added to the first washings and diluted to 100 mL.

Analytical methods

Element analyses were performed with a Perkin-Elmer Model 403 atomic absorption spectrophotometer. A 10 cm (4 in.) burner head and standard air-acetylene flame were used for elemental analyses except for molybdenum and tin for which a nitrous oxide-acetylene burner and flame were used. Single element hollow cathode lamps were used for all elements. The instrument settings and other experimental conditions were in accordance with the manufacturer's specifications.

Appropriate dilutions were used to determine calcium, magnesium, potassium, and sodium in all samples. Samples diluted for calcium and magnesium analyses contained 1% (w/v) lanthanum to overcome potential anionic interferences. Cesium chloride at a final concentration of 2000 ppm cesium was used for potassium and sodium analyses.

Duplicate aliquots of the wet-ashed solutions were used for indirect determinations of phosphorus and silicon. Aliquots of all corn samples, plus water, filling medium concentrate, and processed liquid samples were taken through the procedure described by Parker (1972).

Chloride was also determined indirectly (Parker, 1972). Except for the canned corn, duplicate 0.90 ± 0.07g samples of freeze-dried corn were analyzed. Four mL of the canned corn solutions prepared by the AOAC (1980) method as well as aliquots of the processing water, filling medium concentrate, and processed liquid samples were used for the chloride determinations.

Analyses were done on five replicates of 0.60 ± 0.08g National Bureau of Standards Citrus Leaves (Standard Reference Material #1572). There were analyzed in parallel following the analytical procedures used in this work. Data on the 14 elements analyzed for which NBS standards data were available showed variations of 4.4% or less for iron, magnesium, manganese, potassium, sodium, and zinc; and 14.9%, 18.8%, 18.8%, and 30.8% for calcium, copper, lead, and phosphorus, respectively; cadmium, chromium, molybdenum, and nickel were below the detection limit of the instrument. When standard deviations were considered there were differences of less than 18.2% between the NBS certified values and the values obtained following the procedure used in this work.

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Table 1—Concentration, statistical analyses, and percentage retention of elements in canned corn

Element	*Fresh* (mg/100g)	*Before blanch* (mg/100g)	*After blanch* (mg/100g)	*Canned* (mg/100g)	Retention (%)[a]
Cadmium	<0.01[b]	<0.01[b]	<0.01[b]	<0.005[b]	b
Calcium	5.57 ± 0.66	4.91 ± 0.56[c]	5.56 ± 0.48	5.46 ± 0.45	98
Chloride	27.0 ± 2.0	22.3 ± 1.4	19.9 ± 1.1	281 ± 28[d]	N.A.[f]
Chromium	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	b
Cobalt	<0.04[b]	<0.04[b]	<0.04[b]	<0.03[b]	b
Copper	0.03 ± 0.01[e]	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	67
Iron	0.55 ± 0.07[d]	0.39 ± 0.09	0.40 ± 0.05	0.39 ± 0.05	71
Lead	<0.2[b]	<0.2[b]	<0.2[b]	<0.2[b]	b
Magnesium	19.0 ± 1.2[d]	16.1 ± 2.1	16.1 ± 1.9	10.0 ± 0.5[d]	53
Manganese	0.17 ± 0.03[e]	0.13 ± 0.03	0.14 ± 0.03	0.11 ± 0.01	65
Molybdenum	<0.1[b]	<0.1[b]	<0.1[b]	<0.1[b]	b
Nickel	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]	b
Phosphorus	50.8 ± 2.1[d]	45.0 ± 3.7[d]	36.9 ± 3.2[d]	21.7 ± 1.3[d]	43
Potassium	236 ± 5[d]	217 ± 7[d]	199 ± 6[d]	115 ± 5[d]	49
Silicon	<9[b]	<9[b]	<9[b]	4.84 ± 0.45	b
Sodium	3.37 ± 1.47	2.29 ± 0.19	3.78 ± 0.61	203 ± 20[d]	N.A.[f]
Tin	<1[b]	<1[b]	<1[b]	<1[b]	b
Zinc	0.64 ± 0.04[e]	0.55 ± 0.11	0.55 ± 0.09	0.46 ± 0.05	72

[a] Retention of elements in canned corn, on the basis of element concentration in fresh corn being 100%. Retention = canned/fresh × 100.
[b] Element below the detection limit of the AAS instrument.
[c] Value significantly different ($P \leq 0.05$) from fresh and after blanch values and not significantly different from canned value for this element.
[d] Value significantly different ($P \leq 0.01$) from other values for this element.
[e] Value significantly different ($P \leq 0.05$) from other values for this element.
[f] NaCL was added during canning process.

Table 2—Percentages of recommended dietary allowances and estimated safe and adequate daily dietary intakes per 100g of fresh and canned corn

Element	Fresh corn	Canned corn
Cadmium	a	a
Calcium	0.7[b]	0.7
Chloride	0.8[c]	8.3
Chromium	a	a
Cobalt	a	a
Copper	1.2[c]	0.8
Iron	5.5[b]	3.9
Lead	a	a
Magnesium	5.4[b]	2.9
Manganese	4.5[c]	2.9
Molybdenum	a	a
Nickel	a	a
Phosphorus	6.4[b]	2.7
Potassium	6.3[c]	3.1
Silicon	a	a
Sodium	0.2[c]	9.2
Tin	a	a
Zinc	4.3[b]	3.1

[a] Element below the detection limit of AAS instrument.
[b] Recommended Dietary Allowance as set by the Food & Nutrition Board, National Research Council (Anon., 1980) % RDA in 100g serving for adult male.
[c] Estimated safe and adequate daily dietary intake (%) in 100g serving for adults (Anon., 1980). Percentages are calculated on the average values for the ranges of recommended intakes.

RESULTS & DISCUSSION

ALL DATA are presented on a wet weight basis. Moisture content of the corn samples was determined in duplicate on each of the seven samples. The mean moisture content of fresh corn was 72.56% with a standard deviation of ±0.66; for before blanch, moisture was 73.89% with a standard deviation of ±0.98; for after blanch it was 73.89% with a standard deviation of ±0.93; and for canned corn it was 76.33% with a standard deviation of ±0.38.

The concentration, statistical analyses, and percentage retention of 16 essential elements and cadmium and lead in canned corn are given in Table 1. Percentages of recommended dietary allowances (RDA) and estimated safe and adequate daily dietary intakes per 100g of fresh and canned corn are presented in Table 2. The reported calculated percentages of RDAs and estimated safe and adequate daily dietary intakes assumed 100% availability of the element to provide some measure of the contribution of elements in processed foods to the diet. Availability of iron and zinc to the human body varies with valence, solubility, and chemical species in which the element is present in the food.

Of the elements analyzed which were above the detection limit of the instrument, copper, iron, magnesium, manganese, phosphorus, potassium and zinc decreased significantly in canned corn when compared to the fresh product. These decreases were caused by the elements being leached out during washing of the corn kernels prior to blanching and/or during thermal processing where elements were leached into the liquid. Chloride and sodium increased significantly in the canned product due to the sodium chloride content of the filling medium (14%). Canning did not affect calcium values. Cadmium, chromium, cobalt, lead, molybdenum, nickel, silicon, and tin were below the detection limit of the AAS instrument.

A 100g serving of canned corn supplies less than 4% of each of the established RDAs or estimated safe and adequate daily dietary intakes of the element studied, except for the elements chloride and sodium. The concentration of elements in canned corn ranged from 43% to 98% of the amount present in the fresh product, excluding chloride and sodium which were added to the canned sample during the canning process.

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A Research Note

Formation of Pyrazines by Chitin Pyrolysis

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ABSTRACT

Chitin, poly-β-(1→4)-N-acetyl-D-glucosamine, was heated at 300–500°C for 5–60 min to examine the potential of pyrazine formation from chitin pyrolysis. 2-Methyl-, 2,3-dimethyl- and 2,3,5-trimethylpyrazine were formed at 300°C and additionally, 2,5-dimethylpyrazine was found with increasing pyrolysis temperature up to 500°C. During 60 min of pyrolysis more than twice as much 2,3,5-trimethylpyrazine as 2,5-dimethylpyrazine was developed. Thus, altering time and temperature of chitin pyrolysis could be useful for the development of certain pyrazines that have food flavor applications.

INTRODUCTION

CHITIN, poly-β-(1→4)-N-acetyl-D-glucosamine, a cellulose-like biopolymer, has an unusual combination of properties including toughness, bioactivity and biodegradability (Austin et al, 1981; Muzzarelli, 1977). It possesses unique properties for food applications such as hydrophyllic, interphasic and intermolecular properties, and nutritional significance as dietary fiber (Knorr, 1984; Watkins and Knorr 1983). Chitinous polymers, including chitosan (deacetylated chitin), have been used successfully for biomass recovery and the removal of undesirable substances from liquid systems (Latlief and Knorr, 1983) as well as biodegradable carriers for enzymes, food additives, microorganisms, and cultured plant cells (Knorr, 1983; Knorr, 1985; Rodriguez-Sanchez and Rha, 1981; Vorlop and Klein, 1981). Kroll and Metzger (1978) identified 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose, an important amino sugar used as starting substance for the synthesis of physiologically active oligosaccharides, as the primary product of the thermal degradation of chitin, which was reported to begin at 200°C. Kroll and Metzger (1979) detected acetamide as the main volatile compound resulting during thermal degradation of chitin and indicated that in food systems containing sufficient amounts of water, acetamide is converted into ammonium acetate.

Schlotzhauer et al. (1976) showed that pyrolyzing chitin at 900°C produced pyrazines, heterocyclic nitrogen-containing compounds. They are present in many foods and have been identified as flavor components (Maga and Sizer, 1973a, 1973b; Maga, 1982) especially in heated products (Wang and Odell 1973). Their flavor has often been characterized as "roasted" or "nutty" (Wang and Odell, 1973; Schlotzhauer et al., 1976; Knorr et al., 1976; Maga, 1982) and Bauermeister (1981) and Ziegleder (1982) suggested monitoring the formation of 2,5-dimethylpyrazine during the roasting of cocoa beans as a useful quality control indicator for the roasting process.

Studies by Koehler and Odell (1970), Wang and Odell (1973) and Shibamoto and Bernhard (1976) indicated that reaction temperature and time of sugaramine and amino-hydroxy model systems had a significant effect on pyrazine formation. Consequently, variations in pyrolysis conditions of chitin should result in the formation of different pyrazines of various concentrations.

The objective of this work was to examine the effects of pyrolysis temperature and time on pyrazine formation from heated chitin.

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MATERIALS & METHODS

Materials

Commercially available chitin (Pharmaceutical grade, Bioshell Inc., Albany, OR) was ground to pass a 1.0 mm sieve (Wiley Laboratory Mill, A.H. Thomas Co., Philadelphia, PA). 2-Methyl, 2,5-dimethyl-, and 2,3,5-trimethylpyrazine (Aldrich Chemical, Milwaukee, WI) and 2,3-dimethylpyrazine (Alpha Chemicals, Danvers, MA) were used as reference.

Methods

Preliminary experiments. Five-mg samples of chitin were pyrolyzed in a temperature-controlled tube furnace (Thermcraft Inc., Winston-Salem, NC) at 300 ± 5°C and 400 ± 5°C for 15 minutes. During pyrolysis, nitrogen gas was passed through the tube furnace at a flow rate of 1 liter. min^{-1} then into two traps of 1N sulfuric acid. The samples from the traps were then each placed in a separatory funnel, the pH adjusted to 9.0 with 1N sodium hydroxide and the volatiles were extracted three times with hexane:ether (2:1), following the procedure of Maarse and Schaefer (1978).

Pyrazines in the hexane:ether mixture were analyzed with a gas chromatograph (Model 6A, Shimadzu, Columbia, MD) utilizing dual flame ionization detectors. A glass column (0.5 × 250 cm) (Supelco, Inc., Bellefonte, PA) prepacked with 3% SP 2340 on 100/120 Supelcoport was used. The analyses were performed at a column temperature of 110°C, injector/detector temperature of 160°C, helium flow at 30 $mL.min^{-1}$, hydrogen at 0.2 $kg.cm^{-2}$ and air at 0.6 $kg.cm^{-2}$.

Pyrolysis time/temperature experiments. Ten mg chitin were treated in a coil pyroprobe (CDS 123 Pyroprobe with GC Sample Concentrator, Chemical Data Systems, Inc., Oxford, PA) at setpoint temperatures of 250 to 500°C for 5 to 60 min using helium as carrier gas. The volatiles were collected onto a Tenax-filled trap at room temperature, then backflushed to a gas chromatograph (50 m × 0.25 mm Quadrex SE54 fused silica capillary column with a 60:1 split and an FID). Area data were collected using a Hewlett-Packard 3390-A recording integrater.

RESULTS & DISCUSSION

THE RESULTS of preliminary studies on the effects of temperature of chitin pyrolysis on the formation of 2-methyl, 2,5-dimethyl-, 2,3-dimethyl- and 2,3,5-trimethylpyrazine are given in Table 1. At a pyrolysis temperature of 300°C, 2-methyl-, 2,3,5-trimethyl-, and 2,3-dimethylpyrazine were formed and, additionally, 2,5-dimethylpyrazine was found at 400°C. This finding is in agreement with other authors who reported the formation of 2,5-dimethylpyrazine at the elevated temperatures for roasting of cocoa beans (Bauermeister, 1981; Ziegleder, 1982).

Time dependent pyrolysis experiments (Fig. 1A) revealed an increase in the concentration of 2,5-dimethyl- and 2,3,5-trimethylpyrazine at 400°C setpoint temperature during a 60-min period. More than twice as much 2,3,5-trimethylpyrazine was formed during that period as 2,5-dimethylpyrazine although the concentration of both pyrazines was similar after 10 min

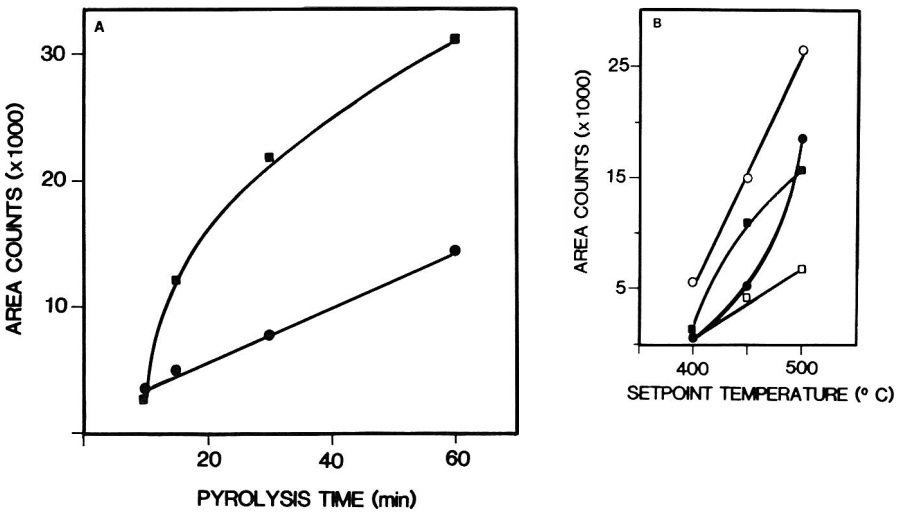

Fig. 1—Time- and temperature-dependent formation of pyrazines: (A) Formation of 2,5-dimethylpyrazine (●) and 2,3,5-trimethylpyrazine (■) at 400°C set point temperature; (B) Pyrazine formation after 5 min of pyrolysis: ○ 2-methylpyrazine; ● 2,3-dimethylpyrazine; □ 2,5-dimethylpyrazine; ■ 2,3,5-trimethylpyrazine.

Table 1—Effect of pyrolysis conditions of chitin on pyrazine formation

Pyrolysis Temperature (°C)	Pyrazine concentration (mg.g^{-1} chitin)[a] 2-methyl-pyrazine	2,5-di-methyl-pyrazine	2,3,5-tri-methyl-pyrazine	2,3-di methyl-pyrazine
300 ± 5	98.20 ± 0.01[c]	0.01[c]	56.88 ± 0.13[c]	8.64 ± 0.01[c]
400 ± 5	86.46 ± 40.41[c]	45.27 ± 0.41[b]	29.64 ± 0.47[b]	10.11 ± 1.97[c]
Standard curves				
2-methylpyrazine:			y = 36,351.87x − 18,088.44	
2,5-dimethylpyrazine:			y = 42,895.06x − 19,251.01	
2,3,5-trimethyipyrazine:			y = 38,833.03x − 11,325.25	
2,3-dimethylpyrazine:			y = 36,461.08x − 1,972.13	

[a] Mean ± standard deviation (dry weight basis)
[b,c] Means with different letters within one column are significantly different ($P < 0.01$)

of pyrolysis. This indicates the potential of using pyrolysis time as a tool for the formation of pyrazine mixtures of various concentrations.

The effect of temperature on the formation of various pyrazines (Fig. 1B) resulted in an increase of all pyrazine concentrations between 400° and 500°C setpoint temperature. The most substantial increase was observed with 2-methylpyrazine and the least with 2,5-dimethylpyrazine.

According to Kroll and Metzger (1979) 75% of chitin is volatile between 200°C and 400°C reaching a maximum between 360°C and 400°C. This is no contradiction to our findings since sample temperatures are expected to be lower than setpoint temperatures of the coil pyroprobe.

Overall, our data confirm that chitin pyrolysis can be used for the formation of pyrazines and that selection of specific pyrolysis conditions can aid the development of different pyrazine mixtures. This could lead to the tailoring of pyrazine mixtures derived from chitin pyrolyzates that have food flavor application. This could also increase utilization and value of chitin-containing food processing wastes and by-products such as fungi, yeasts, and crustacean shells.

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A Research Note

Effects of Processing Treatments on Recovery of Capsaicin in Jalapeno Peppers

MARY KATE HARRISON and NATHOLYN D. HARRIS

ABSTRACT

Capsaicin content in frozen, cooked and canned jalapeno peppers was quantified using GLC analysis and compared to the capsaicin content in raw peper. The frozen peppers were blanched for 3 min and stored at −18°C; canned peppers were blanched for 3 min and processed at 100°C for 50 min; cooked peppers boiled at 100°C for 10 min. Results demonstrated a significant difference (a = 0.05) between each treatment and the raw pepper. Frozen and canned peppers retained approximately one-half of the capsaicinoid compounds that were present in raw pepper.

INTRODUCTION

JALAPENO PEPPERS contribute pungency, flavor, color and nutrients to the diet. Food processors use the oleoresin of peppers to add pungency to a wide variety of products, including meats, candy, beverages and baked goods. Much research has been conducted regarding the composition of the pepper and its chemical and sensory properties. However, information concerning the effect of different processing methods on the pungency of the pepper is limited. In one important study regarding the jalapeno pepper and processing, Huffman et al. (1978) found that thermally processed peppers held at 100°C for 50 min contained significantly higher capsaicin levels than the fresh samples. Their results implied that temperature may be critically important in the processing of peppers. The objective of the present study was to determine the effect of freezing, cooking and canning procedures on capsaicin retention in jalapeno peppers.

MATERIALS & METHODS

THE METHOD chosen for canning followed the recommendations of the National Canners Association. Peppers to be canned were blanched in boiling water at 100°C for 3 min and then rinsed in cold water. Peppers were packed in pint-size jars, and covered with 93°C brine consisting of 2% acetic acid, 2% vegetable oil and 0.2% sodium chloride. The peppers were thermally processed in a 100°C waterbath for 50 min and then stored for 4 days. For freezing, peppers were blanched at 100°C for 3 min and then rinsed 1 min under cold water. The peppers were air-dried at room temperature for 3 min, packed in polyethylene freezer bags and frozen at −18°C. After 3 days peppers were thawed in closed plastic bags with cool water running over the bags for 30 min. The third treatment was cooking. The cooking process involved boiling peppers at 100°C for 10 min. Analysis took place immediately after the peppers drained and cooled for 2 hrs at room temperature.

The peppers were prepared for analysis by chopping into 1/2" slices and drying at 70°C for 6½ hr to 10% of original weight (Todd et al., 1977; Lease and Lease, 1962). After drying, 23 g pepper were ground to a particle size of approximately 1 mm. Capsaicin was extracted 5½ hr from the dried and ground samples using hexane in a soxhlet extractor. Hexane was evaporated under vacuum at 40°C. A 0.1g sample of the extract was combined with 0.7 mg octascosane, an internal standard which was previously mixed with 5 ml of dry tetranhydrofuran. The sample was silylated by adding 1 ml of N,O bistrifloureacetamide (BSTFA), (Todd et al., 1977). The 10 mL volumetric flask containing the sample extract and the internal standard was filled to volume with dry tetrahydrofuran. The contents were mixed by hand and allowed to stand at room temperaure for 5 min before GLC analysis.

A Varian 3700 gas liquid chromatograph (GLC) with a flame inoization detector was used for separation of the capsaicinoid compounds. The GLC was equipped with a polar SP 2100 glass capillary column, 30 m × 0.25 mm i.d. The injection port temperature was regulated at 240°C and the detector temperature was 280°C. A 2 μL sample was injected with the inlet splitter closed and the oven set at 60°C. After 20 sec, the splitter was opened and the oven tmeperature quickly reset to 160°C, the desired column temperature for the start of the GLC program. The temperature programming began at 160°C increased at 4°/min to 240°C and held at the upper limit for 50 min. Nitrogen, the carrier gas, had a flow rate of 1 m/min; hydrogen flow rate was 300 mL/min and air flow rate was 350 m/min.

Compounds were indentified using a Finnigan 4510-GC-EI gas chromatograph-mass spectrometer system. Conditions approximated those used while separating the compounds on the Varian 3700. Ionization was conducted with electrons possessing an energy of 70 ev. Further indentification was accomplished based on retention times of a pure capsaicin standard. The following formula was used to calculate capsaicinoids:

$$\text{Capsaicinoid mg/g} = \frac{(\text{peak ht cap})\ (1)\ (\text{Wt of int std})}{(\text{peak ht int std})\ (\text{Wt of sample})}$$

The concentration of capsaicinoid in each treatment represented the average of duplicate samples from three replicates.

RESULTS & DISCUSSION

A TYPICAL CHROMATOGRAM of the TMS derivatives of nordihydrocapsaicin capsaicin and dihydrocapsaicin is shown in Fig. 1. These capsaicinoids were apparent in every chromatogram but peak height varied depending on the processing treatment. Concentration of the capsaicinoids in the raw, frozen canned and cooked peppers are shown in Table 1. Results of a 2-sample t-test demonstrated a significant difference between each processing treatment and between each treatment and the raw pepper.

The difference between the capsaicinoid concentration of the canned and raw pepper may be attributed to either the blanching, thermal processing, or the storage period for the canned pepper. Contact between the blanched peppers and the surrounding water during cooling after blanching could have resulted in leaching of the capsaicinoids. During the thermal processing, cells are lysed allowing the capsaicin to spread from the pericarp throughout the pepper. Huffman et al. 1978 stated that this reaction apparently made the canned pepper more pungent. In the present study the canned pepper decreased in capsaicin concentration. During the 4-day holding time capsacin may have leached into the brine. Other chemical reactions that can occur during prolonged boiling could involve shortening the fatty acid chain (Nelson, 1919), hydrolysis of the phenolic hydroxy group (Jones and Pyman, 1925) and loss of nitrogen (Newman, 1953). Any of these reactions could have decomposed the capsaicinoid molecule.

The cooked peppers had a slight increase in capsaicin concentration. The 10 min cooking time may have been sufficient

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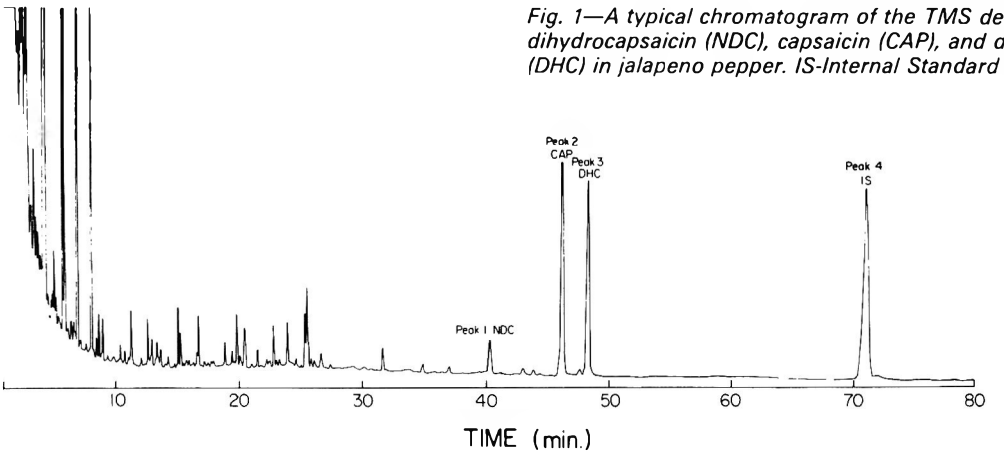

Fig. 1—A typical chromatogram of the TMS derivatives of nordihydrocapsaicin (NDC), capsaicin (CAP), and dihydrocapsaicin (DHC) in jalapeno pepper. IS-Internal Standard (octascosane).

Table 1—Mean values and standard deviations for capsaicinoid content in the jalapeno pepper[a]

	Capsaicin (mg/g)		Dihydrocapsaicin mg/g		Nordihydrocapsaicin mg/g	
	X	SD	X	SD	X	SD
Raw	7.25	0.62	6.33	0.69	1.19	0.10
Frozen	4.00	1.22	3.71	0.95	0.59	0.16
Canned	4.67	0.50	4.53	0.52	0.68	0.11
Cooked	8.62	1.98	7.67	1.76	1.32	0.39

[a] The concentration of capsaicinoids in each treatment was determined by averaging the results of duplicate chromatograms from three replicate samples.

to destory all the enzymes that could decompose the pepper yet short enough to avoid the detrimental reactions mentioned previously. The cooked peppers were analyzed immediately after boiling; thus, excessive contact between the peppers and the water medium was avoided and the risk of leaching was decreased. The short holding time of only 2 hr also decreased the chance for chemical changes that can occur during storage. It is possible that some capsaicin complexed to another compound may have been liberated during cooking.

The frozen peppers retained the smallest concentration of capsaicin. The frozen peppers were blanched, but the heat may not have completely inactivated important enzymes, and cooled in water which may have led to leaching. The freezing treatment may have ruptured cells which could have led to capsaicinoid loss.

Although tissue changes due to processing may have influenced the completeness of capsaicin extraction, the present study shows that different processing treatments can affect capsaicin concentration in the jalapeno pepper. Further research is needed to determine the extent to which capsaicin content is altered by specific processing procedures such as chopping, soaking, heating and freezing.

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A Research Note

Purification and Characterization of α-Galactosidase from Feijão bean *Phaseolus vulgaris*

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ABSTRACT

α-Galactosidase is an important enzyme which degrades galactooligosaccharide in legumes. α-Galactosidase from feijão beans was purified and its characteristics established. The purified enzyme exhibits multiple forms — enzymes I and II with molecular weights of 140,000 and 49,000 daltons, respectively. Optimum pH was 5.5 for enzyme I and 6.0 for enzyme II. Optimum temperature for both was 55°C. Kinetically, enzyme I is more reactive. Heavy metal ions completely inhibited both enzymes I and II. Galactose is a potential inhibitor for both enzymes.

INTRODUCTION

THE INCREASING USE of soybean and other legumes in food as dietary protein supplements has focused attention on the flatulence caused by these legumes. The flatulence results from the presence of raffinose and stachyose in legumes (Rackis, 1975).

α-Galactosidase (α-D-galactoside galactohydrolase, EC.3.2.1.22) hydrolyzes galactooligosaccharides, especially stachyose and raffinose (Cruz and Park, 1982).

The effect of germination on oligosaccharide in soybean was investigated by Pazur et al. (1962) and East et al. (1972). They observed that the concentration of galactooligosaccharides decreased rapidly and that monosaccharides accumulated during germination. This reaction was affected by the α-galactosidase present in the extracts from the germinating beans.

Agrawal and Bahl (1968) reported the presence of α-galactosidase activity in the extracts of Pinto beans (*Phaseolus vulgaris*) and its characterization. Dey and Pridham (1969) also reported some characteristics of α-galactosidase from the broad bean, *Vicia faba*.

Feijão beans (*Phaseolus vulgaris*, L. var. Carioca-80) are one of the main items of the menu in Brazil. Therefore, the objective of this investigation was to isolate the enzyme involved in the degradation of galactooligosaccharides, and to determine some of its characteristics, so that these properties may be applied in the removal of sugars during processing of the beans.

MATERIALS & METHODS

FEIJÃO BEANS, *Phaseolus vulgaris*, L. var. Carioca-80, 1983 crop, were supplied by the Instituto Agronomico de Campinas (IAC). The moisture content of the beans was 9.7%. They were kept at room temperature.

The activity of α-galactosidase was determined as described by Cruz and Park (1982), with some modifications. p-Nitrophenyl-α-D-galactopyranoside (PNPG)-hydrolyzing activity was determined by using PNPG as substrate. A mixture of 2 mL 0.6 mM PNPG in 0.05M McIlvaine buffer, pH 5.0 and 0.1 mL bean extract or enzyme solution was incubated at 50°C for 20 min. The reaction was then stopped by addition of 3 mL 0.1M boric acid-borax buffer, pH 10.7, and p-nitrophenol (PNP) was estimated with a spectrophotometer at 400 nm. One unit of α-galactosidase activity is defined as the amount of enzyme which will liberate 1 μmole PNP per min under the conditions described. Melibiose hydrolyzing activity was determined by incubating the mixture of 0.5 mL enzyme solution, 0.5 mL 0.15M McIlvaine buffer, pH 5.0, and 0.5 mL 10 mM melibiose solution at 50°C for 40 min. The amount of glucose formed was measured by the glucose oxidase-peroxidase chromogen method. Raffinose hydrolyzing activity was determined by incubating the mixture of 0.5 mL enzyme solution and 2 mL 50 mM raffinose in 0.05M McIlvaine buffer, pH 5.0, at 50°C for 60 min. After incubation, 0.5 mL 0.3M $Ba(OH)_2$ and 0.5 mL 0.18M $ZnSO_4$ were added and the mixture centrifuged to separate the precipitate. The amount of galactose formed was measured by the Somogyi method.

To prepare the enzyme extract, 100g feijão bean were powdered and mixed with 500 mL 0.05M McIlvaine buffer, pH 6, and filtered through nylon cloth. Approximately 3 mL saturated lead acetate were added to the filtrate, then centrifuged at 20,000 × *g* for 10 min. Two hundred fifty mL clear supernatant were used as the enzyme extract.

Purification of the enzyme involved four steps: (1) extraction; (2) ammonium sulfate fractionation; (3) chromatography on DEAE-cellulose; (4) Sephadex G 100 gel filtration. The enzyme which was obtained by the addition of 140g ammonium sulfate to the 250 mL enzyme extract (80% saturation) was applied to a column of DEAE-cellulose previously equilibrated with 0.05M McIlvaine buffer, pH 6.0. The column was eluted with a concentration gradient of 0.05–0.3M NaCl. The enzyme fractions from DEAE-cellulose column were further purified by Sephadex G 100 gel filtration. The molecular weight of the enzyme was determined with Sephadex G 100 and G 200 columns which were prepared as described by Andrews (1965).

Polyacrylamide gel electrophoresis was performed as described by Davis (1964). The enzymes I and II were subjected to electrophoresis. After electrophoresis, proteins in the gel were stained with Amido Black 10 B, and periodic acid-Schiff (PAS) technique was applied for the detection of glycoprotein (Zacharius et al, 1969). The α-galactosidase activity in the gel was detected by incubating gels immediately after electrophoresis in 0.25% PNPG in 0.05M McIlvaine buffer pH 6.0 at 50°C.

RESULTS & DISCUSSION

ONE FRACTION which contained α-galactosidase activity was obtained by DEAE-cellulose column chromatography. The fractions of the enzyme were pooled and further fractionated on a Sephadex G 100 gel column. It yielded two separate fractions, both with α-galactosidase activity. The first fraction (Enzyme I) and the second fraction (Enzyme II) were pooled separately. The respective enzymes were recycled separately through a Sephadex G 100 column.

The molecular weight of the enzyme I and II were calculated to be 140,000 and 49,000 datons, respectively.

Electrophoresis of the purified enzyme showed one major band of protein and several minor ones (Fig. 1A); the major band was glycoprotein (Fig. 1B). The major protein band also showed α-galactosidase activity (Fig. 1C). It is clear that α-galactosidase from feijão bean was a multiple enzyme. Previous workers demonstrated that α-galactosidase from pinto bean was a single fraction (Agrawal and Bahl, 1968) whereas α-galactosidase from broad bean exhibited two fractions (Dey and Pridham, 1969).

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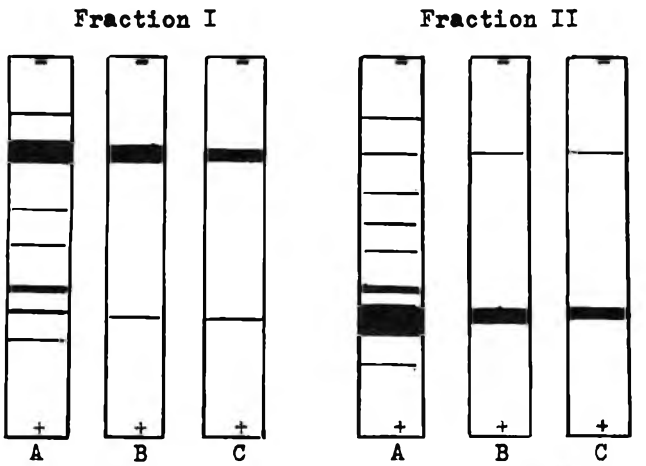

Fig. 1—PAGE of the purified α-galactosidase from feijão bean: (A) stained with Amido Black 10; (B) glycoprotein staining by periodic acid-Schiff technique; (C) Enzyme reaction with ONPG.

Table 1—Kinetic studies of α-galactosidase

	Enzyme I	Enzyme II
Michaelis constant, Km (mM)		
PNPG	0.28	0.71
Melibiose	1.00	2.58
Raffinose	25.21	64.74
V_{max}, (μmole/min/mg of protein)		
PNPG	2.60	26.32
Melibiose	2.87	29.09
Raffinose	2.42	24.52
Activation energy, (Kcal/mol)		
PNPG	7.03	11.58
Melibiose	9.14	15.05
Raffinose	11.25	18.53

The pH-enzyme activity profile of the α-galactosidase was studied with PNPG, melibiose, and raffinose as substrates. Maximum hydrolysis of all substrates occurred at pH 5.5 for enzyme I and pH 6.0 for enzyme II. Optimum temperature for hydrolysis of all substrates was 55°C.

Effect of substrate concentrations on the enzyme activity was investigated with varying concentrations of PNPG, melibiose, and raffinose at optimum pH and temperature. The results were plotted by the method of Lineweaver and Burk (1934). The Michaelis constant (Km) and values of V_{max} are shown in Table 1.

Effect of temperature on the rate of hydrolysis was studied in the range 30–55°C. The Arrhenius plots for the hydrolysis of PNPG, melibiose and raffinose showed linear relationships and were used to calculate the activation energy for substrates by the enzyme. As shown in Table 1, enzyme I was highly reactive to substrates and PNPG was more susceptible to the action of both enzyme I and II than the other two substrates.

The inhibition studies were carried out by incubating mixtures of enzyme-substrate (PNPG) and respective chemical reagents. After incubation, the PNP formed was measured and it was found that heavy metal ions such as Ag^{+} and Hg^{2+} (at 1 mM concentration) completely inhibited both enzymes I and II. Galactose (at 10 mM concentration) also inhibited substantially both enzyme reactions.

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A Research Note

Crude Protein, Minerals, and Total Carotenoids in Sweet Potatoes

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ABSTRACT

Crude protein and mineral content in six sweet potato cultivars and total carotenoids in four orange-flesh cultivars after different storage treatments were determined. On a fresh weight basis, crude protein between cultivars ranged from 1.36–2.13 g/100g, phosphorus from 38–64 mg/100g, potassium from 245–403 mg/100g, calcium from 20–41 mg/100g, magnesium from 13–22 mg/100g and total carotenoids from 5–11.5 mg/100g. Carotenoids slightly increased after curing and short term storage at either 7°, 15.6°, or 26.6°C. Centennial contained the most protein, phosphorus, magnesium, and carotenoids of all cultivars. Travis contained the lowest amount of protein, phosphorus and potassium, but more carotenoids than Jewel or Jasper.

INTRODUCTION

SWEET POTATOES are relatively low in calories and the orange-flesh types provide an excellent source of Vitamin A (Haytowitz and Matthews, 1984). The orange-flesh color is due to carotenoid pigments, primarily beta-carotene (Purcell, 1962) which serves as a precursor for Vitamin A (RDA, 1980). No information is available on the carotenoid contents of the currently grown orange-flesh cultivars under different post-harvest temperatures and limited information exists on the nutritional value of different cultivars.

The objective of this study was to compare crude protein and mineral content of four currently grown orange-flesh cultivars (Centennial, Jasper, Jewel, Travis) and two white-flesh cultivars (Rojo Blanco, Whitestar) and carotenoid content of the four orange-flesh cultivars. The effect of storage temperature on carotenoid content was also determined.

MATERIALS & METHODS

SIX SWEET POTATO CULTIVARS were grown on a silt loam soil at the LSU Hill Farm in Baton Rouge, LA in 1983 following commercially recommended cultural practices (Montelaro et al., 1966). All roots were harvested in mid-October and samples were analyzed the day of harvest for crude protein and minerals and after curing (10 days at 32°C, 90% RH) plus various intervals of storage at different temperatures for total carotenoids. All carotenoid data were calculated back to the weight at harvest to eliminate any concentrating effect due to moisture or dry matter loss.

Five replications of six No. 1 grade roots per replicate were used at each analysis time. Roots were longitudinally cut in half and finely grated uniformly over the entire inner surface to a depth of about 3 mm. Duplicate 10.00-g samples of randomly mixed grated tissue from each of the six roots per replicate were dried at 70°C for 48 hr in a forced air oven for dry matter determinations. Dried samples were ground by a Wiley mill to pass through a 40-mesh screen and analyzed for mineral content according to AOAC (1980) procedures. Potassium, Ca, and Mg were determined by flame atomic absorption spectrophotometry; P by colorimetry; and N by the Kjeldahl method. Crude protein was calculated by multiplying Kjeldahl N × 6.25. Total carotenoids were determined on duplicate samples from each replicate using a slight modification of the procedure of Reddy and Sistrunk (1980). Exactly 0.100g tissue was extracted until colorless with 10 mL hexane using a Thomas E-40 tissue grinder. (A.H. Thomas Co., Philadelphia, PA). Samples were filtered through Whatman #1 paper and the absorbance read at 440 nm and compared to a beta-carotene standard curve.

Statistically significant differences ($P < 0.05$) between cultivars or storage treatments in the amount of each component (i.e. crude protein, minerals, carotenoids) were determined by analysis of variance and means were separated by Duncan's multiple range test (Snedecor and Cochran, 1967).

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RESULTS & DISCUSSION

LARGE DIFFERENCES in % dry matter existed between cultivars, making interpretation of the results dependent on whether the data were expressed on a dry or fresh weight basis. Percent dry matter of each cultivar at harvest was: Whitestar – 31.6%; Rojo Blanco – 29.2%; Centennial – 26.3%; Jewel – 23.4%; Jasper – 22.7%; Travis – 17.7%. Nutritional value comparisons between cultivars should be based on fresh weight since it compares equal weights of the same amount of edible portion.

Crude protein

Centennial contained the most crude protein while Jasper and Travis had the least on a fresh weight basis (Table 1). The quantitative amounts of protein in Centennial, Jewel, and Whitestar were very similar to those reported by Purcell et al. (1972) of North Carolina grown sweet potatoes.

Consumed in moderation, sweet potatoes are not a major contributor of protein to the diet. The minimum recommended dietary allowance (RDA) of protein for adult males is 56g and adult females is 44g (RDA, 1980). Consumption of a 150-g serving size of Travis after baking would have provided 2.0g protein, Jewel – 2.2g, and Centennial 3.2g.

Minerals

Centennial had the highest P on a fresh weight basis and Travis the lowest (Table 1). Scott and Bouwkamp (1974) and Hammett et al. (1984) reported slightly lower P concentrations for Centennial and Jewel roots than the values herein. Current RDA of P for adults is 800 mg (RDA, 1980). Consumption of a 150-g serving from this study would have provided from 57 mg P (Travis) to 96 mg P (Centennial).

Whitestar contained the most potassium (K) and Travis the least K on a fresh weight basis (Table 1). Rojo Blanco and Centennial contained more K than Jasper or Jewel. Scott and Bouwkamp (1974) found somewhat higher K concentrations in Centennial and Jewel than the values reported here. Sweet potatoes are a good source of K and consumption of 150g from this study would have provided from 368 mg K (Travis) to 605 mg K (Whitestar). The daily estimated safe and adequate levels of K for adults range from 1875 – 5625 mg (RDA, 1980).

Whitestar contained the highest amounts of Ca and Mg on a fresh weight basis — more than twice the amounts of Ca than Jasper and Rojo Blanco (Table 1). Centennial had more Mg but similar amounts of Ca compared to Jewel. Scott and Bouwkamp (1974) found similar Ca values to those reported herein for Jewel and Centennial. They also found a similar amount of Mg in Centennial, but slightly more Mg in Jewel.

Table 1—Crude protein and mineral content in roots of six sweet potato cultivars

Cultivar	Crude protein g/100g[z]	Phosphorus mg/100g[z]	Potassium mg/100g[z]	Calcium mg/100g[z]	Magnesium mg/100g[z]
Jasper	1.38 a	55 b	341 c	20 a	13 a
Travis	1.36 a	38 a	245 a	35 c	19 cd
Centennial	2.13 c	64 d	365 d	26 b	22 de
Jewel	1.44 ab	52 b	321 b	26 b	16 ab
Rojo Blanco	1.50 ab	59 c	374 d	20 a	17 bc
Whitestar	1.59 b	54 b	403 e	41 d	22 e

[z] Fresh weight basis.

[y] Means within a column followed by different letters are significantly different ($P < 0.05$).

Table 2—Total carotenoid content of four sweet potato cultivars at harvest and after different storage treatments

Storage treatment	Total carotenoids (mg/100g)[z] Centennial	Travis	Jasper	Jewel
At Harvest	9.5	6.3	5.4	5.0 a[y]
Cured[x] + 4 wk 15.6°C	10.5	7.4	6.3	6.0 abc
Cured + 16 wk 15.6°C	11.5	8.0	6.5	6.8 c
Cured + 5 wk 26.6°C	10.6	8.8	7.0	5.5 ab
Cured + 4 wk 7°C + 1 wk 15.6°C	11.2	8.6	6.8	6.5 bc
	N.S.[w]	N.S.	N.S.	

[z] Fresh weight basis. All data were corrected for weight loss during storage and expressed on an at harvest weight basis.

[y] Means within a column followed by different letters are significantly different ($P < 0.05$).

[x] Cured = 10 days at 32°C, 90% RH.

[w] N.S. = no significant treatment differences ($P > 0.05$).

The adult RDA is 800 mg Ca and 300–350 mg Mg (RDA, 1980). Consumption of a 150-g serving would have provided from 30 mg Ca (Rojo Blanco, Jasper) to 62 mg Ca (Whitestar); and from 20 mg Mg (Jasper) to 33 mg Mg (Whitestar, Centennial).

Comparing the mineral content in roots of all cultivars, K was the element in greatest concentration followed by N, P, Ca, and Mg, respectively. Equal fresh weight comparisons between cultivars indicated Centennial had the most crude protein and P; Whitestar the most K, Ca, and Mg (along with Centennial); while Travis had the lowest amount of crude protein, P, and K. Compared with Jewel, Centennial provided a better source of crude protein, P, K, and Mg and equal Ca.

Total carotenoids

Carotenoids were higher, although generally not significantly, after curing and short term 7°, 15.6°, or 26.6°C storage than at harvest in all four cultivars (Table 2). However, the 26.6°C storage treatment resulted in increased weight loss, sprout initiation, and internal pithiness in Jasper, while the 7°C treatment resulted in external chilling injury, increased decay and reduced shelf-life. Recommended commercial storage temperature is 15.6°C. Centennial contained the most carotenoids of the cultivars tested, followed by Travis, and Jasper and Jewel. Previous workers had found carotenoids to increase during curing and storage after several months in some cultivars while no change was found in others (Ezell and Wilcox, 1952; Ezell et al., 1956).

The RDA of Vitamin A for adults is 1000 retinol equivalents for males and 800 for females (RDA, 1980). Vitamin A nutritional value calculations assume an 8% loss of carotenoids during baking (Watt and Merrill, 1963); 90% of the total carotenoids are composed of beta-carotene and the remaining carotenoids are Pro-Vitamin A carotenoids (Ezell and Wilcox, 1952; Purcell, 1962) using the formula: μg beta-carotene ÷ 6 + μg other ProVitamin A carotenoids ÷ 12 = retinol equivalents (RDA, 1980) and only using beta-carotene to calculate Vitamin A value [1 IU of Vitamin A = 0.6 μg beta-carotene (RDA, 1980)]. Consumption of a 150-g baked root after curing plus 4 wk at 15.6°C would have provided the following retinol equivalents and Vitamin A value (international units): Centennial – 2295, (21,735); Travis – 1617, (15,320); Jasper – 1376, (13,040); Jewel – 1311, (12,420). The standard cultivar grown in the 1950's and 1960's, Porto Rico, had about 12,000 IU Vitamin A for a 150-g serving (Watt and Merrill, 1963). The major cultivars grown in the U.S. today contain similar amounts (Jewel, Jasper) more (Travis) or substantially higher amounts (Centennial) of Vitamin A value.

Combining crude protein, mineral content, and total carotenoids, Centennial was the superior cultivar nutritionally of the six tested.

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A Research Note

Physical and Chemical Properties of Randomly Interesterified Blends of Corn Oil and Tallow

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ABSTRACT

The sodium methylate-catalyzed random interesterification of corn oil-tallow blends was explored in order to develop plastic fats of varying physical properties. Lipase hydrolysis of the randomized fats showed that with 0.5% catalyst, interesterification was completed within 30 min at 80°C. Interesterification decreased the melting point and solid fat index of the randomized fats. The *trans*- fatty acid level and fatty acid profile of the rearranged fats did not show any change upon interesterification. The oxidative stability of the fats decreased after random interesterification, but addition of 0.01% citric acid and 0.01% butylated hydroxyanisole produced a fat blend of comparable stability to commercial margarine oils.

INTRODUCTION

IN A PREVIOUS STUDY (Lo and Handel, 1983) soybean oil was interesterified with edible beef tallow to produce plastic fats suitable for use as margarine oils. Interesterification has been proposed as an alternative to partial hydrogenation to avoid the formation of positional and *trans*-isomers (List et al., 1977). In this study, corn oil was evaluated as the liquid fraction in an interesterification mixture with edible tallow.

MATERIALS & METHODS

THE CORN OIL (CO) and corn oil tub margarines were commercial products and were purchased locally. The beef tallow (T) was an undeodorized institutional shortening that contained 3% cottonseed oil. All fats and oils were stored below 0°C until used.

Interesterification was carried out at 80°C for 30 min under a nitrogen atmosphere using 1 mL 0.5% sodium methylate in xylene as catalyst (Lo and Handel, 1983) with a yield of 95.8%. Samples were water washed and dried under vacuum prior to analysis. They were not deodorized. AOCS Official Methods (1973) were used to determine melting point (Cc 1-25), solid fat index (SFI) (Cd 10-57) acid value (Cd 3a-63), iodine value (Cd 1-25), *trans*- fatty acid content (Cd 14-61) and peroxide value (PV) (Cd 8-53). Cholesterol was determined by an enzymatic/colorimetric test (No. 139050, Boehringer Mannheim, Indianapolis IN). Determination of the extent of randomization by analysis of fatty acids at the *sn*-2 position, measurement of fatty acid profiles and determination of oxidative stability during accelerated storage at 60°C were carried out as previously described (Lo and Handel, 1983).

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RESULTS & DISCUSSION

Randomization of corn oil and tallow proceeded in essentially the same way as soybean oil and tallow (Lo and Handel, 1983) being complete after 30 minutes.

Physical properties

As expected, decreasing the proportion of corn oil in a mixture increased the melting point (Table 1). Interesterification caused a decrease in the melting point of a mixture due to the formation of additional triunsaturated triacylglycerols upon rearrangement. The 45 T:55 CO interesterified blend had a melting point between those of the tub margarine oils. Solid fat index data are presented in Table 2. SFI curves (not shown) of the corn oil blends were flatter than those of the commercial margarine oils and the soybean oil blends of the previous study (Lo and Handel, 1983); therefore, the choice of corn oil blend would involve a compromise at one end of the temperature scale or the use of a third fat or oil in the blend. In this study, the 45 T:55 CO blend was chosen for further study of chemical properties and stability.

Chemical properties

The chemical properties of the corn oil-tallow blends (Table 1) were similar to those of soybean-tallow blends (Lo and Handel, 1983). Interesterification did not alter fatty acid composition; however, acid values (Table 1) were reduced from the original value as a result of saponification and fatty acid removal during water washing. Similar results were obtained by Anand and Vasishtha (1978). Interesterification did not have any effect on the cholesterol content of the oil blends (Table 1). The amount of cholesterol found in the blend (42.6 mg/

Table 1—Properties of corn oil, beef tallow and margarine oils

Oil	Melting point[a] (°C)	Acid value (mg KOH/g)	Iodine value (g I_2/100g)	P/S ratio[b]	trans (%)	Cholesterol (mg/100g)
Corn	—	0.05	124.2	4.59	nd[c]	nd
Tallow	47.0	0.46	44.0	0.21	9.0	94.6
20T: 80 CO interesterified[d]	36.0	0.19	110.2	2.57	1.8	18.9
30T: 70 CO interesterified	38.8	0.19	98.7	1.99	2.7	28.4
40T: 60 CO interesterified	39.8	0.19	89.1	1.55	3.6	37.8
45T: 55 CO mixture	42.3	0.22	86.3	1.36	4.1	42.6
45T: 55 CO interesterified	40.8	0.20	86.4	1.36	4.1	42.6
50T: 50 CO interesterified	43.2	0.20	82.1	1.20	4.5	47.3
Tub margarine A	39.8	0.02	107.0	2.34	18.9	nd
Tub margarine B	41.3	0.03	92.5	1.53	23.6	nd

[a] Capillary method.
[b] Calculated from fatty acid data.
[c] nd — not detected.
[d] T = beef tallow, CO = corn oil.

Table 2—Solid fat indices of margarine oils

Oil	Solid fat index (°C)			
	10.0	21.1	26.7	33.3
20T: 80 CO interesterified[a]	5.2	3.5	2.9	1.2
30T: 70 CO interesterified	7.5	4.7	4.0	2.7
40T: 60 CO interesterified	10.5	6.7	6.0	4.1
45T: 55 CO mixture	15.4	10.2	9.1	6.4
45T: 55 CO interesterified	15.0	9.8	8.9	6.6
50T: 50 CO interesterified	17.4	11.4	10.3	6.9
Tub margarine A (corn)	13.8	9.6	6.8	2.5
Tub margarine B (corn)	17.4	11.0	7.8	3.4
40T: 60 SBO interesterified[a]	12.3	5.5	4.3	1.9

[a] T = tallow, CO = corn oil, SBO - soybean oil.

100g) was greater than the amount found in commercial margarines but much lower than the amount present in butter (219 mg/100g) (Reeves and Weihrauch, 1979).

Oxidative stability

After 24 days of storage at 60°C, tub margarine oil A with 0.01% citric acid added had the highest peroxide value (PV), at 158 meq/kg, of the samples examined. Tub margarine oil B, with 0.01% citric acid added, had a PV of 132 meq/kg after 24 days at 60°C. Interesterification decreased the stability of a 45 T:55 CO blend as evidenced by a PV of 142 meq/kg for the interesterified blend versus 94 meq/kg for the noninteresterified mixture, after 24 days storage at 60°C. Addition of 0.01% citric acid to the interesterified blend decreased its PV to 123 meq/kg and addition of 0.01% citric acid plus 0.01% BHA decreased the PV further to 110 meq/kg after 24 days storage at 60°C. Lau et al. (1982) were unable to explain exactly why stability decreased after interesterification. The interesterified 45 T:55 CO corn oil blend with added antioxidant had a PV of 110 meq/kg after 24 days of storage at 60°C, and was more stable than the equivalent soybean oil blend evaluated in the previous study (Lo and Handel, 1983) where a 60% soybean oil-40% tallow blend reached a peroxide value of 140 meq/kg after 17 days.

This study has shown that interesterification of corn oil and edible tallow can be used as an alternative to hydrogenation to produce plastic fats with low levels of *trans*- fatty acids. The final products have acceptable fatty acid compositions and the oxidative stabilities are better than equivalent blends formulated with soybean oil.

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ERRATA NOTICE

J. Food Science 50(5): 1246-1248 + 1256. Effect of Sprout Inhibitors and Nitrogen Fertilization on Nitrate-N Content of Potato Tubers by R. Ponnampalam and N. I. Mondy. On page 1246, line 5 of the Introduction, change the word nitrates to nitrites.

J. Food Science 50(5): 1442-1444. On a Rank Sum Test Due to Kramer by D. N. Joanes. According to the author, the frequency table on page 1442 is incorrect, adding to only 1600, not 4096. The correct figures are printed below. The resulting cumulative probabilities should therefore be:

$$P(R_i \leq 8) = 0.0068$$
$$P(R_i \leq 9) = 0.0205$$
$$PR_i \leq 10) = 0.0498$$
$$P(R_i \leq 11) = 0.1025$$

Nothing else in the paper is affected by this error. Please make corrections accordingly.

Rank Sum	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	TOTAL
Frequency	1	6	21	56	120	216	336	456	546	580	546	456	336	216	120	56	21	6	1	4096

The Institute of Food Technologists

The Institute of Food Technologists is an educational and scientific society of food professionals – technologists, scientists, engineers, educators, and executives – in the field of food technology. Food technologists apply science and engineering to the research, production, processing, packaging, distribution, preparation, evaluation, and utilization of foods. Individuals who are qualified by education, special training, or experience are extended an invitation to join in professional association with the select group of the food industry's scientific and technological personnel who are IFT members. Membership is worth many times its modest cost, reflecting positive benefits, stimulation, and opportunities for the individual in his/her business or profession.

OBJECTIVES

IFT has several major aims: to stimulate investigations into technological food problems; to present, discuss, and publish the results of such investigation; to raise the educational standards of food technologists; and to promote recognition of the scientific approach to food and the basic role of the food technologist in industry. All of these activities have the ultimate objective to provide the best possible foods for mankind.

ORGANIZATION AND PROGRESS

Organized July 1, 1939, at Cambridge, Mass., with a membership of less than 100, the Institute has grown to more than 22,000. It is worldwide in scope with more than 3,000 of its membership overseas.

QUALIFICATIONS FOR MEMBERSHIP

Professional Members. Any person who meets the following minimum requirements: (1) Bachelor's degree or higher from a college or university with a major in one or more of the sciences or branches of engineering associated with food technology; and (2) Five years of professional experience in food technology, for which a master's degree may be presented as the equivalent of one year's experience; a doctor's degree, the equivalent to three year's experience.

Members. Any person active in any aspect of the food industry and who evidences interest in supporting the objectives of IFT. Recent graduates who are completing their experience requirement for Professional Member status.

Student Members. Any person who is registered as a full-time student in an educational institution and who is pursuing candidacy for an associate degree or higher in one or more of the sciences or branches of engineering associated with food technology shall be eligible for membership as a Student Member. After graduation, a student may convert automatically to Member without obligation for the higher Member dues until the following calendar year.

DUES

Professional Members and Members – $45 a year; includes subscription to *Food Technology* and *IFT World Directory & Buyers' Guide;* option to subscribe to the *Journal of Food Science* at members' special rate of $15. **Student Members** – $15 a year; includes subscription to one IFT journal, *IFT World Directory & Buyers' Guide,* automatic membership in the Student Association, and option to subscribe to the other journal at $7.50. **Emeritus Members** – no dues; includes *IFT World Directory & Buyers' Guide,* option to subscribe to either or both journals at $7.50 each.

PUBLICATIONS

The Institute publishes two journals. *Food Technology,* issued monthly, is the official journal of the Institute; *Journal of Food Science,* issued bimonthly, is devoted to basic and applied research papers on fundamental food components and processes. In addition, an *IFT World Directory & Buyers' Guide* is published annually.

REGIONAL SECTIONS

Where 25 or more members live within commuting distance of a given geographic area, a regional section may be established. Presently, there are 50 regional sections.

DIVISIONS

Where 50 or more members of the Institute have a common interest in a particular broad-based discipline of food technology, they may form a division. There are presently 12 divisions serving the areas of Biotechnology, Carbohydrates, Food Engineering, Food Packaging, Foodservice, Food Microbiology, Muscle Foods, Nutrition, Quality Assurance, Refrigerated and Frozen Foods, Sensory Evaluation, and Toxicology and Safety Evaluation.

SPECIALIZED TECHNOLOGY GROUPS (STGs)

When 25 or more members have a common interest in a rather narrow, product-oriented or similar special area, they may form a technology group to serve the needs of this specialized area. There are currently five STGs – Citrus Products, Dairy Products, Extension, Fruit and Vegetable Products, and Seafood Products.

STUDENT ASSOCIATION

All Student Members of IFT are automatically members of the Student Association, which provides special services and activities for students. This Association, which is run by and for the students, also provides the organizational mechanism for giving students a voice in IFT affairs.

STUDENT CHAPTERS

An IFT Student Chapter certificate may be granted to a group of students enrolled in the food science and technology curriculum in a particular school who have organized to form a student club. There are 38 student chapters.

AFFILIATE ORGANIZATIONS

Affiliate certificates may be granted to food technology organizations outside the U.S.A. There are currently 15 chartered Affiliate Organizations.

ANNUAL MEETINGS

An Annual Meeting of the Institute provides a specially organized technical program, awards program, and an exposition (FOOD EXPO) of equipment, services, processes, and ingredients. The program is designed to emphasize current trends and technological developments.

AWARDS

The Institute presents the following awards, usually annually:

Nicholas Appert Award. Purpose of this award (medal furnished by the Chicago Section, and $5,000 by IFT) is to honor a person for pre-eminence in the contributions to the field of food technology.

Babcock-Hart Award. Purpose of this award ($3,000 furnished by the Nutrition Foundation and a plaque by IFT) is to honor a person for contributions to food technology that have improved public health through some aspects of nutrition or more nutritious food.

IFT International Award. Purpose of this award (plaque and $3,000 furnished by IFT) is to recognize an IFT Member for promoting international exchange of ideas in food technology.

IFT Food Technology Industrial Achievement Award. Purpose of this award (plaque to company or companies involved) is to recognize and honor the developers of an outstanding new food process and/or product representing a significant advance in the application of food technology to food production, successfully applied in actual commercial operation.

Wm. V. Cruess Award for Excellence in Teaching. Purpose of this award (medal furnished by the Northern California Section and $3,000 by IFT) is to recognize excellence in university teaching in food science and technology.

Samuel Cate Prescott Award for Research. Purpose of this award ($3,000 and a plaque furnished by IFT) is to recognize a research scientist 36 years of age or younger who has demonstrated outstanding ability in food science or technology.

Fellows Awards. Any Professional Member who has been active for at least 10 years and who has outstanding contributions to the field of food science and technology is eligible to be elected a Fellow of the Institute.

Carl R. Fellers Award. Recognizes individual members of IFT and Phi Tau Sigma who have served and brought honor and recognition to the profession of food science and technology. Winner receives $1,000 from Phi Tau Sigma and a plaque from IFT.

Donald K. Tressler Award. Honors an IFT member for pre-eminence in food science and technology and the ability to communicate with both the scientific community and the public. Winner receives $3,000 from the Tressler Memorial Fund and an engraved plaque from IFT.

SCHOLARSHIP/FELLOWSHIP PROGRAM

To attract and encourage students in the field of food science and food technology, the Scholarship/Fellowship program is offered to worthy and deserving students, primarily on the basis of scholastic ability.

IFT sponsors 10 $500 Freshman and 10 $750 Sophomore awards.

In addition, three Freshmen, two Sophomore, and 51 Junior/Senior as well as 17 Graduate Fellowships, ranging in amount from $500 to $6,000 annually, are sponsored by various food companies and IFT and administered by IFT. Details are available in the booklet. *IFT Administered Fellowship/Scholarship Program – 1986-1987,* available from IFT's Scholarship Department.

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Provide sufficient detail of the method and equipment to allow the work to be repeated. Accepted methods should be cited by adequate references. In reporting procedures, the active voice is preferred.

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Use lower case letters a, b, c, etc., following the year to distinguish among different publications of the same author(s) in any one year.

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Anonymous. 1982. Tomato product invention merits CTRI Award. Food Technol. 36(9): 23.

(Book)

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Weast, R.C. (Ed.). 1981. "Handbook of Chemistry and Physics," 62nd ed. The Chemical Rubber Co., Cleveland, OH.

(Bulletin, circular)

Willets, C.O. and Hills, C.H. 1976. Maple syrup producers manual. Agric. Handbook No. 134, U.S. Dept. of Agriculture, Washington, DC.

(Chapter of book)

Hood, L.F. 1982. Current concepts of starch structure. Ch. 13. In "Food Carbohydrates," D.R. Lineback and G.E. Inglett (Ed.), p. 217. AVI Publishing Co., Westport, CT.

(Journal)

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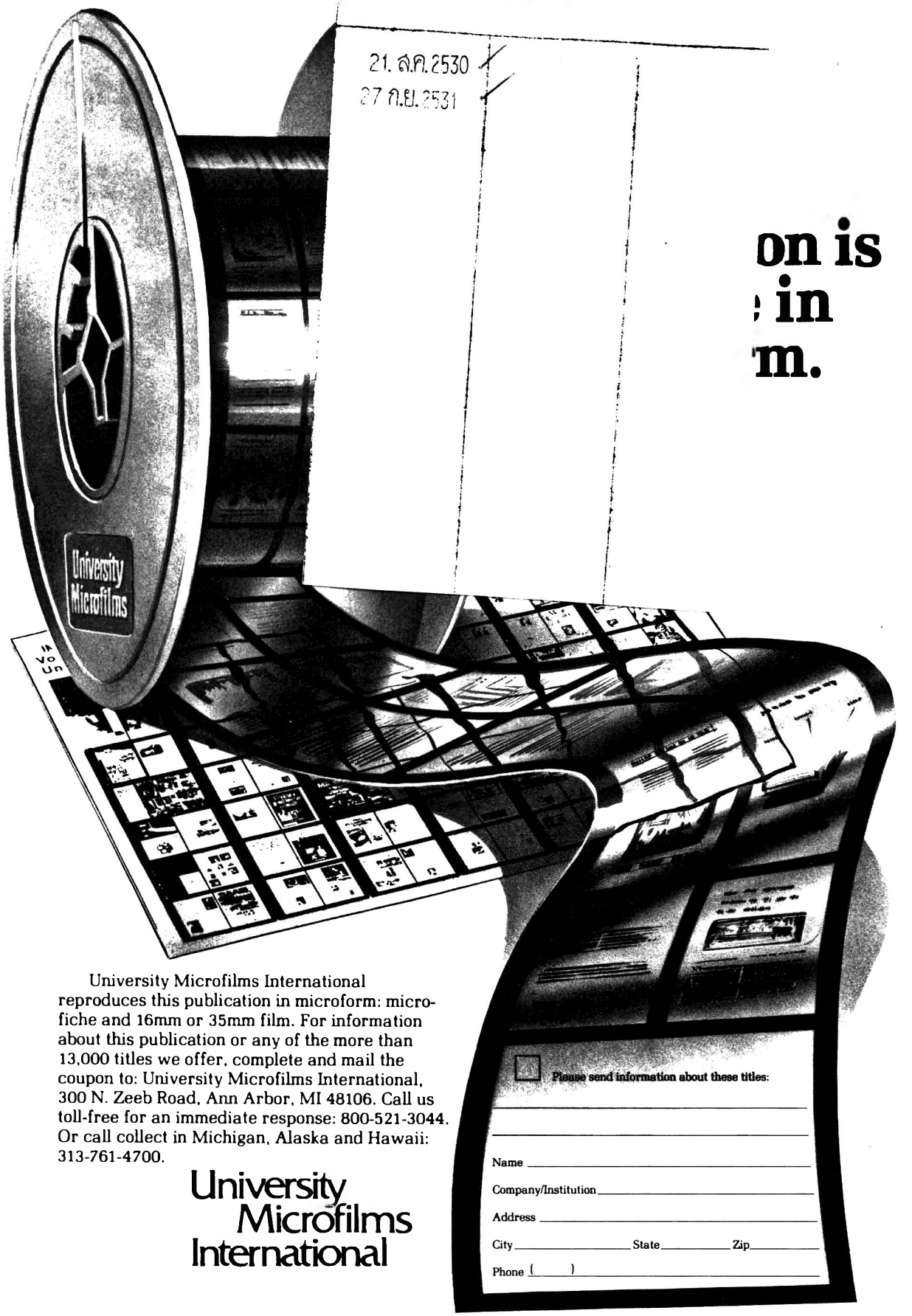

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